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HAMNER

INSTITUTES FOR HEALTH SCIENCES

WHERE GREAT MINDS & MEDICINE MEET

The use of PBPK modeling to reduce uncertainty in risk assessment: Example of manganese

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Manganese: An Essential Poison

Manganese (Mn) is an essential trace element necessary for development:

The Estimated Safe and Adequate Daily Dietary Intake (ESADDI) for Mn in adults is 3.0–5.0 mg/day

- corresponding to an absorbed dose of about **0.2 mg/day**

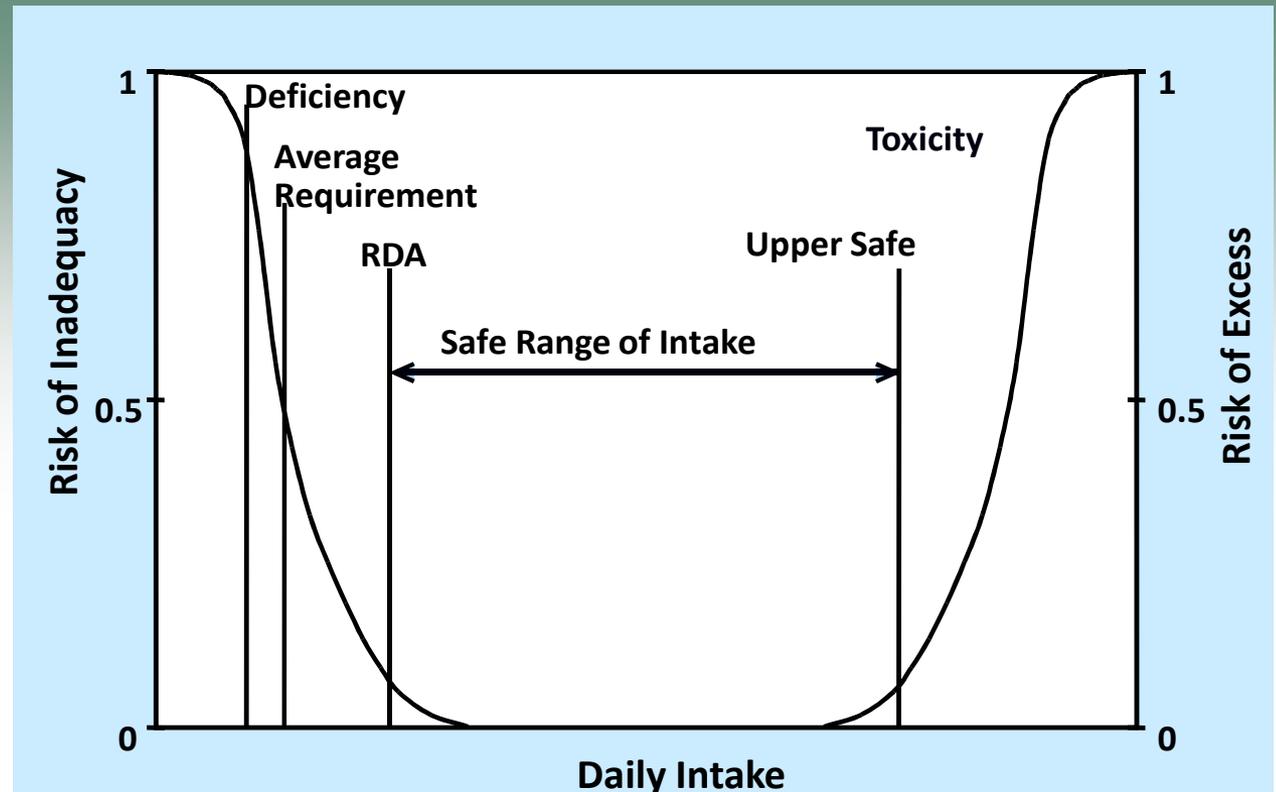
Chronic Mn inhalation has been associated with neurotoxicity:

Parkinsonism-like symptoms in workers inhaling high concentrations of Mn (several mg Mn/m³)

- corresponding to absorbed doses of greater than **10 mg/day**

Question: What's the range of safe and adequate exposures to Mn?

Adequacy and Excess: Pharmacokinetic challenges with essential elements



- What intake rates (*i.e.*, what target tissue levels) are associated with normal function?
- What pharmacokinetic processes are responsible for maintaining manganese tissue concentrations in the body?
- In what manner do dose route and intake rates affect manganese concentrations in target tissues?

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 $[Mn_{\text{midbrain}}]$

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

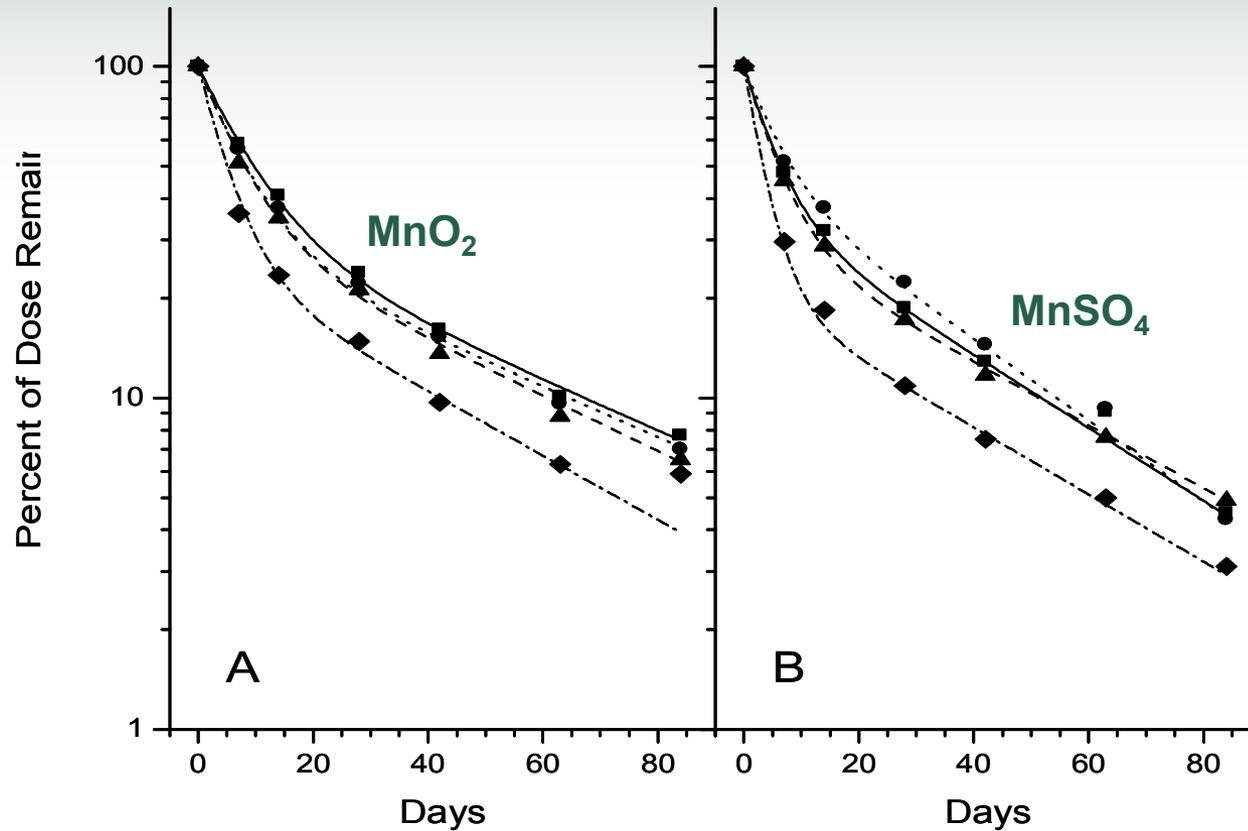
Acceptable exposures would lead to an increase in $[Mn_{\text{midbrain}}]$ of no more than some small percentage of the normal variability.

Available Data for Model Development:

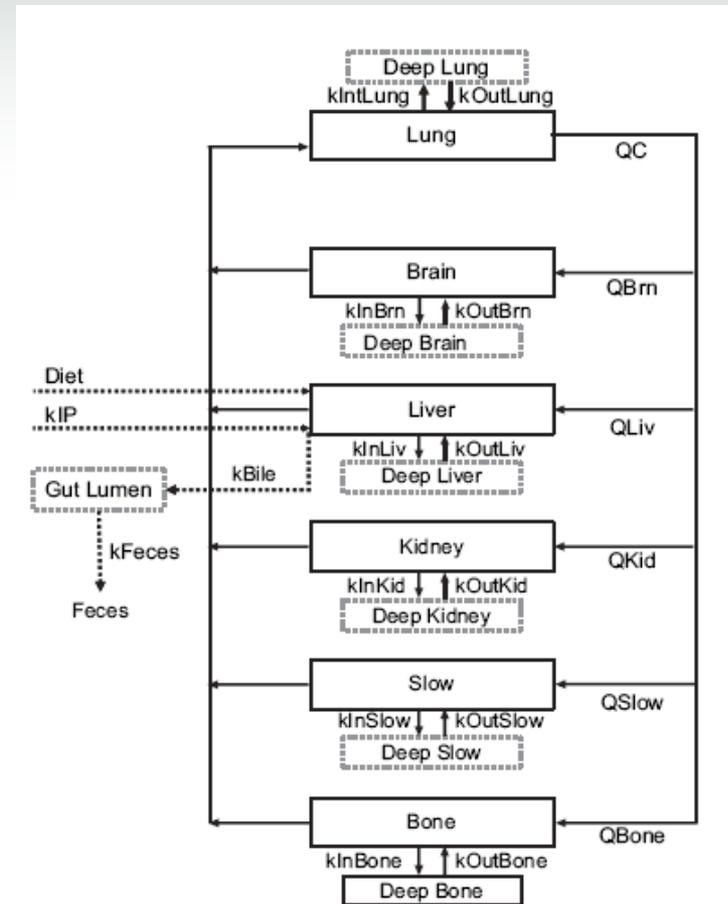
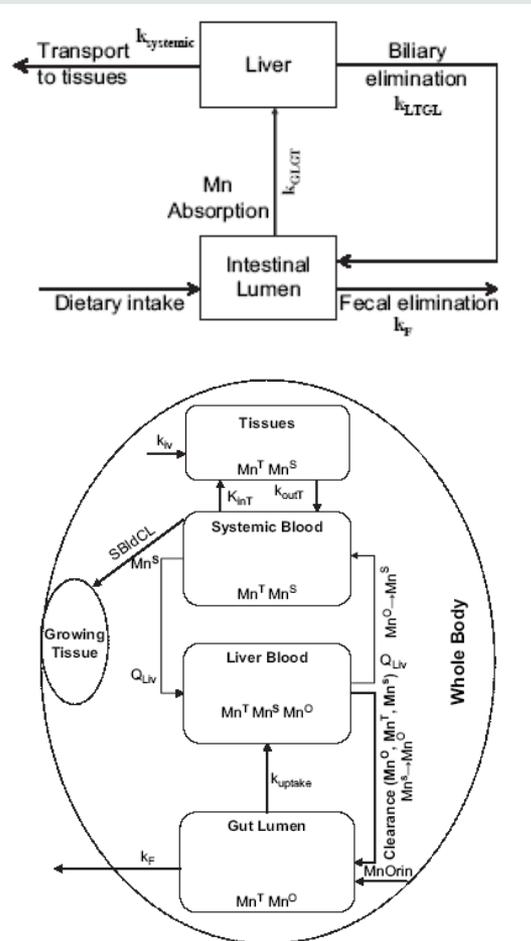
Series of animal studies for inhaled and dietary Mn PK at the Hamner (formerly CIIT):

- Rat fed on different diets (2, 10, 100 ppm Mn)
- ⁵⁴Mn tracer kinetic studies
- Single nasal exposure with occluded nostrils
- Short-term 14-day inhaled exposure (0.03 to 3 mg Mn/ m³)
- Long-term 90-day inhalation exposure (0.01 to 3 mg Mn/ m³)
- Gestational and lactational period exposures
- Primate 90-day period inhalation exposure
- Other data in rats from University of Montreal

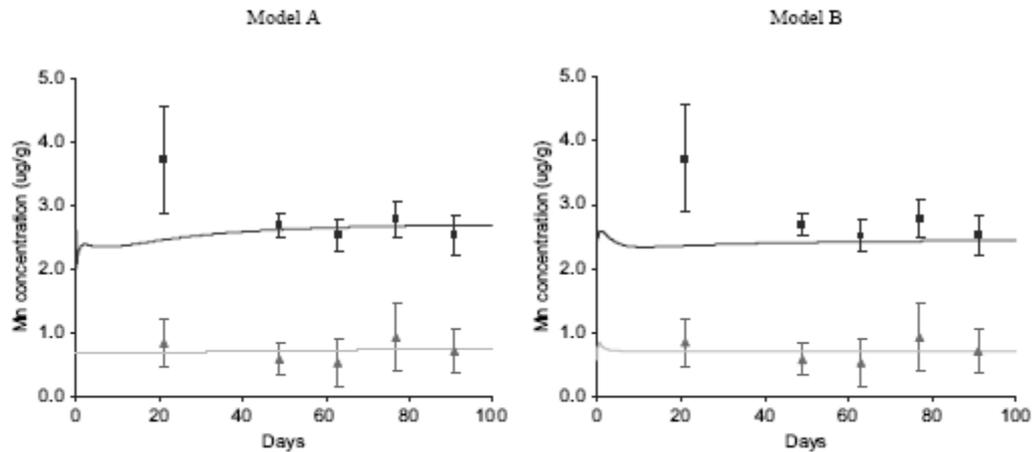
Key Finding: Control of elimination observed for higher dose by inhalation as well as by diet



Initial model development with Mn: linear, intercompartmental transfer rate constants

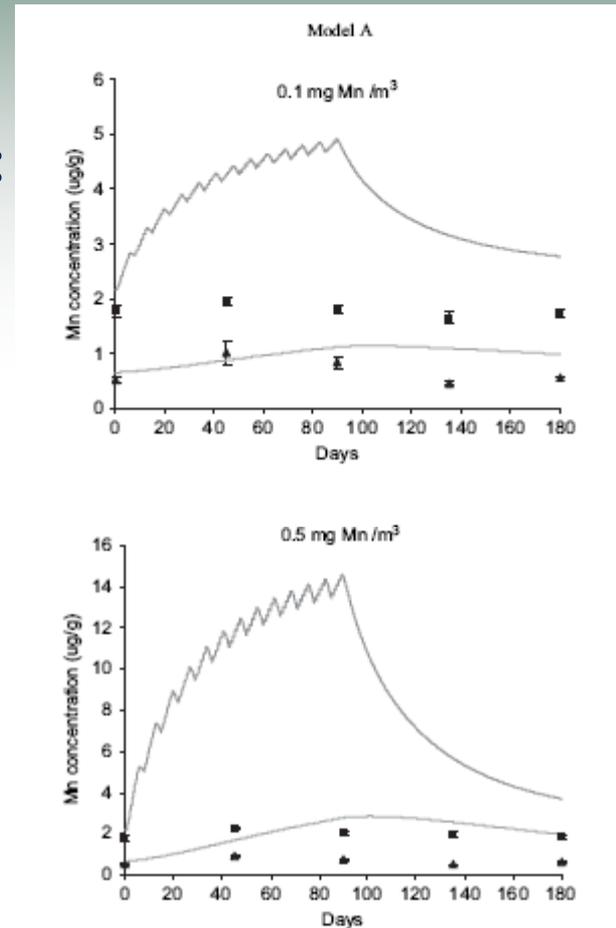


Whatever model used, first parameterized to account for the background tissue Mn and the tracer time courses. (Teeguarden et al., 2007c).



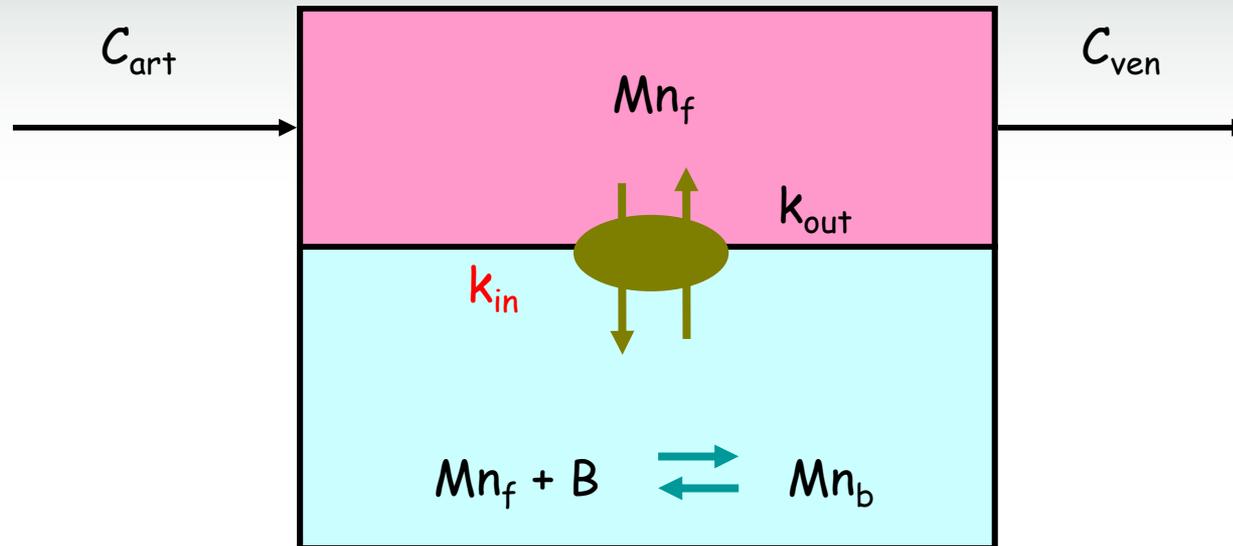
Then applied to the suite of studies:

The linear models could not describe both the 14-day and the 90-day studies. Equilibration and return to pre-exposure steady state were more rapid than expected based on low dose kinetics. New model structure required



Nong et al., (2008).

Model developments: Saturable Tissue Stores and Asymmetric Diffusion



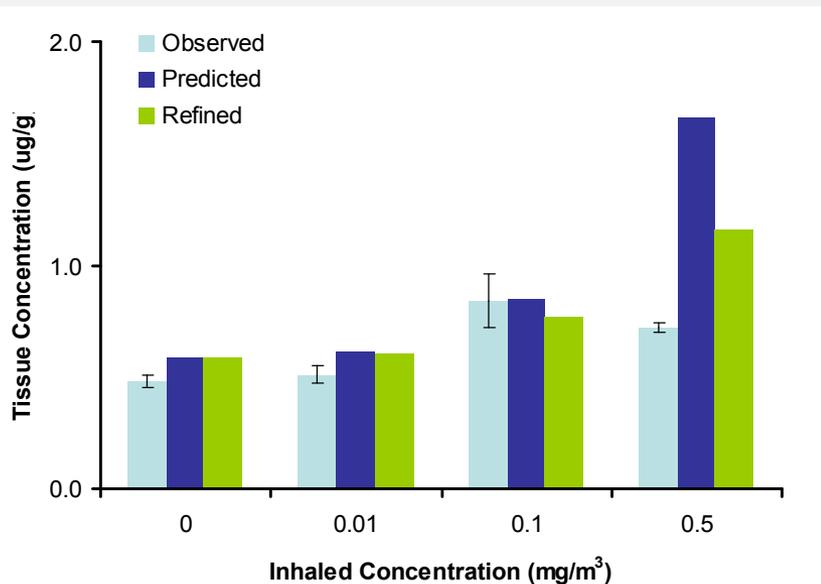
$$Mn_{tot} = Mn_f + Mn_b$$

$$B_{max} = B_f + Mn_b$$

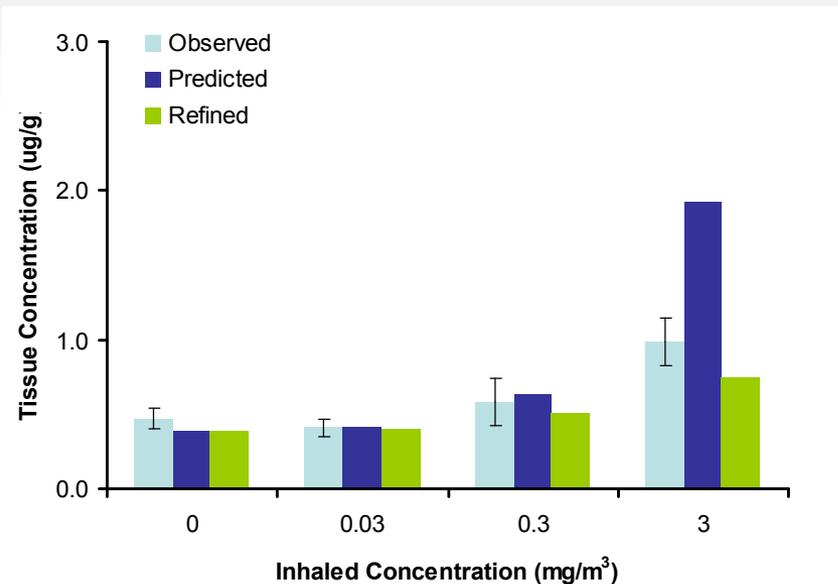
Adult Rat Mn kinetics

Long term exposure (90-day)

Dorman et al. 2001

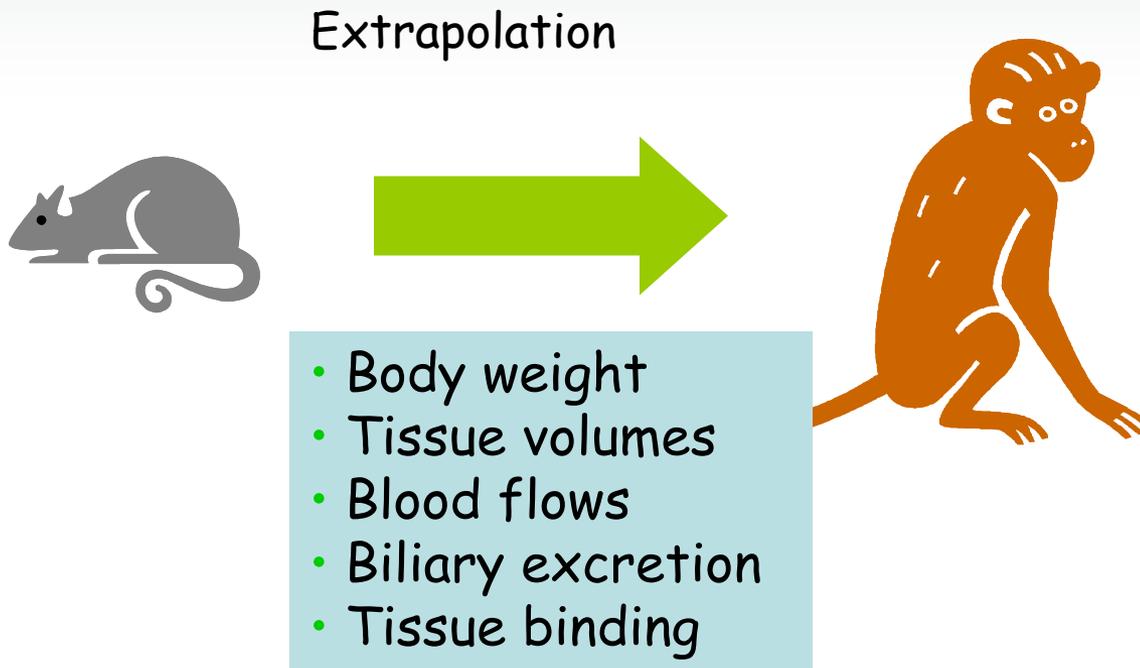


Tapin et al. 2006

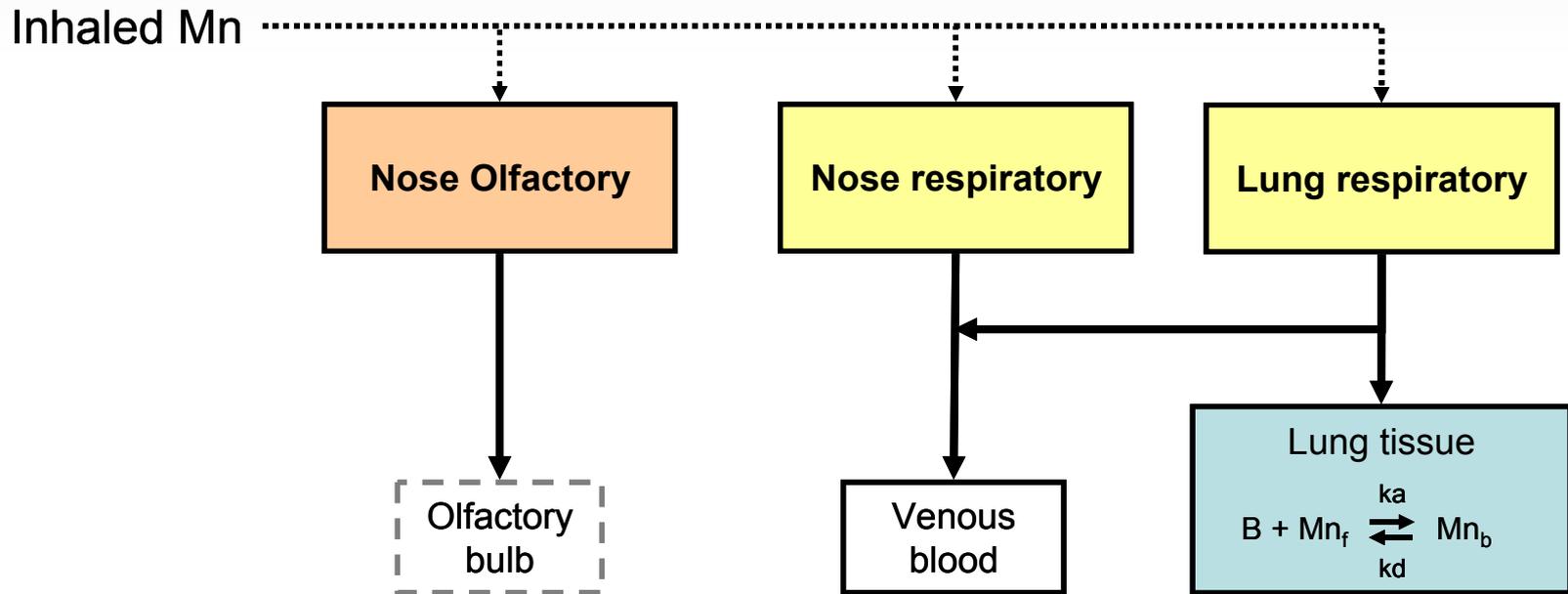


The refinements includes a dose-dependent biliary elimination not required over the course of the 14-day simulation

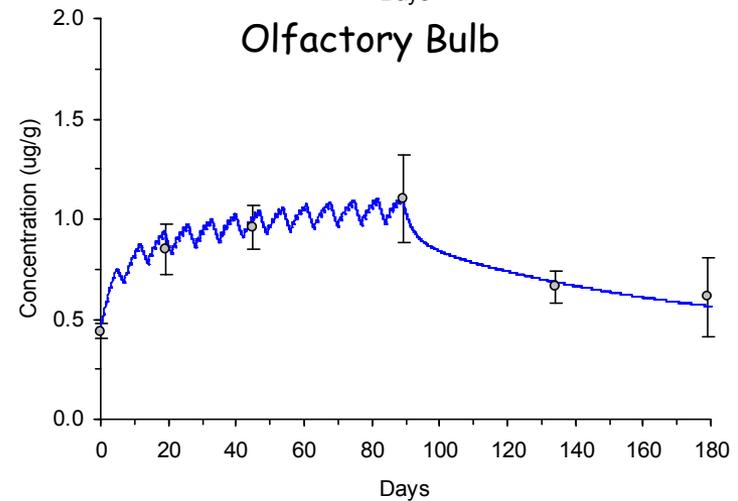
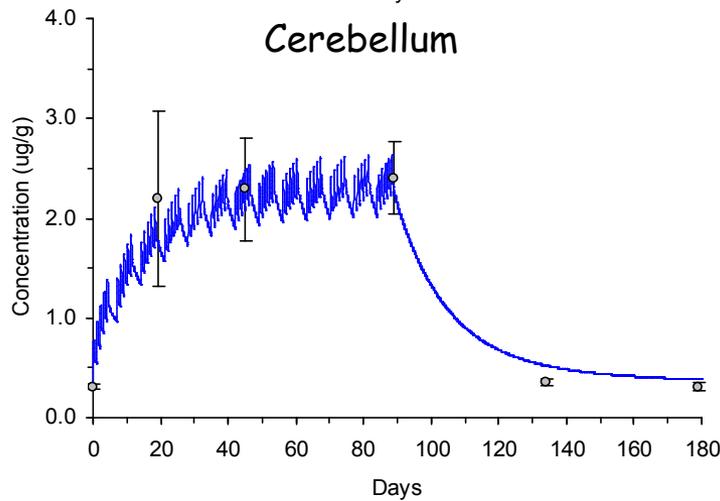
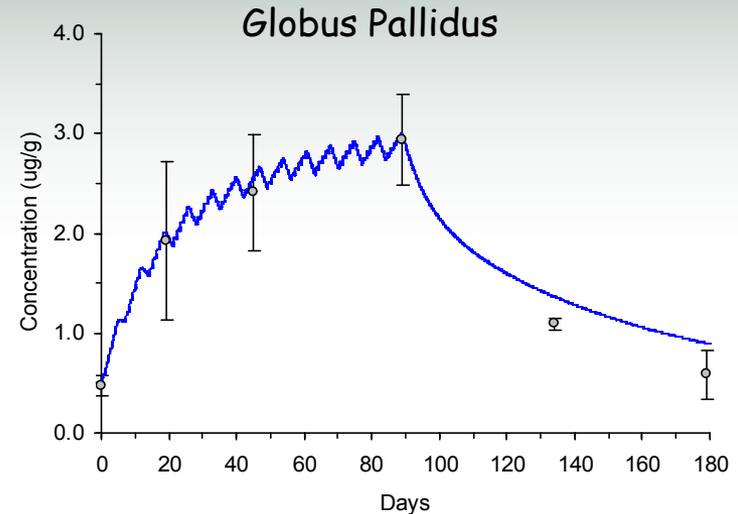
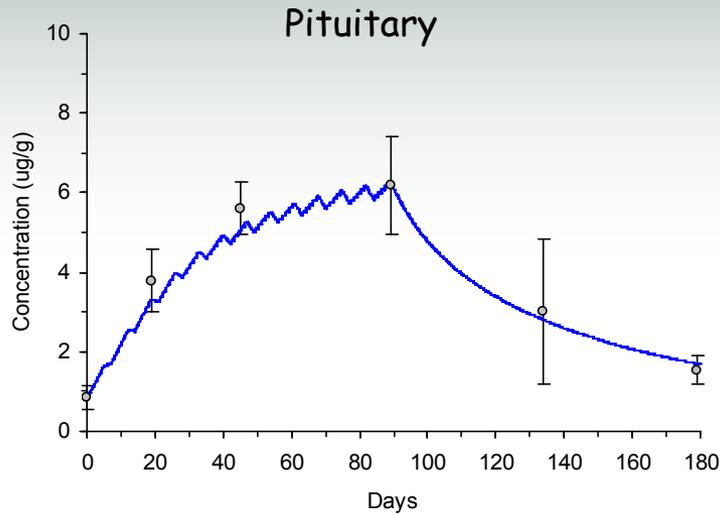
Model extrapolation: rats to monkeys



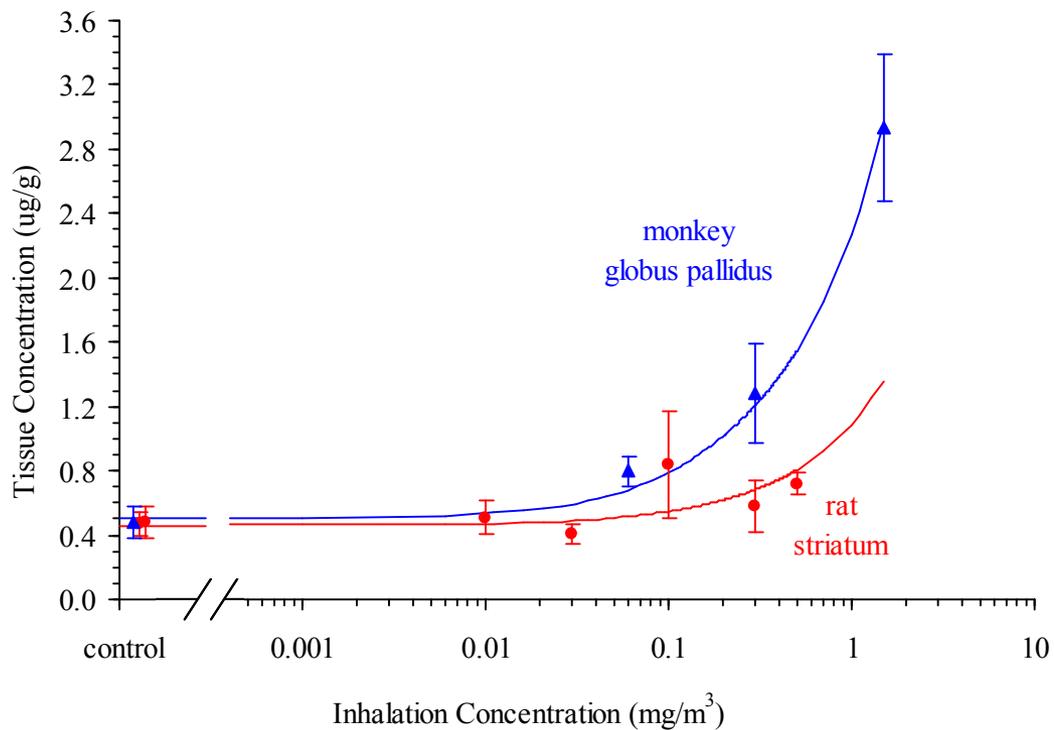
Respiratory/Olfactory structure for monkey



Simulation of different regions in the Brain



Model extrapolation: rats to monkeys



Comparison of end of exposure brain Mn concentration following 90 days

Manganese PBPK Modeling

Human Model Development

Enhancement of the published PBPK model for monkeys to add routes of exposure other than inhalation (oral, IP, IV, subQ)

- Validation against in vivo tracer data

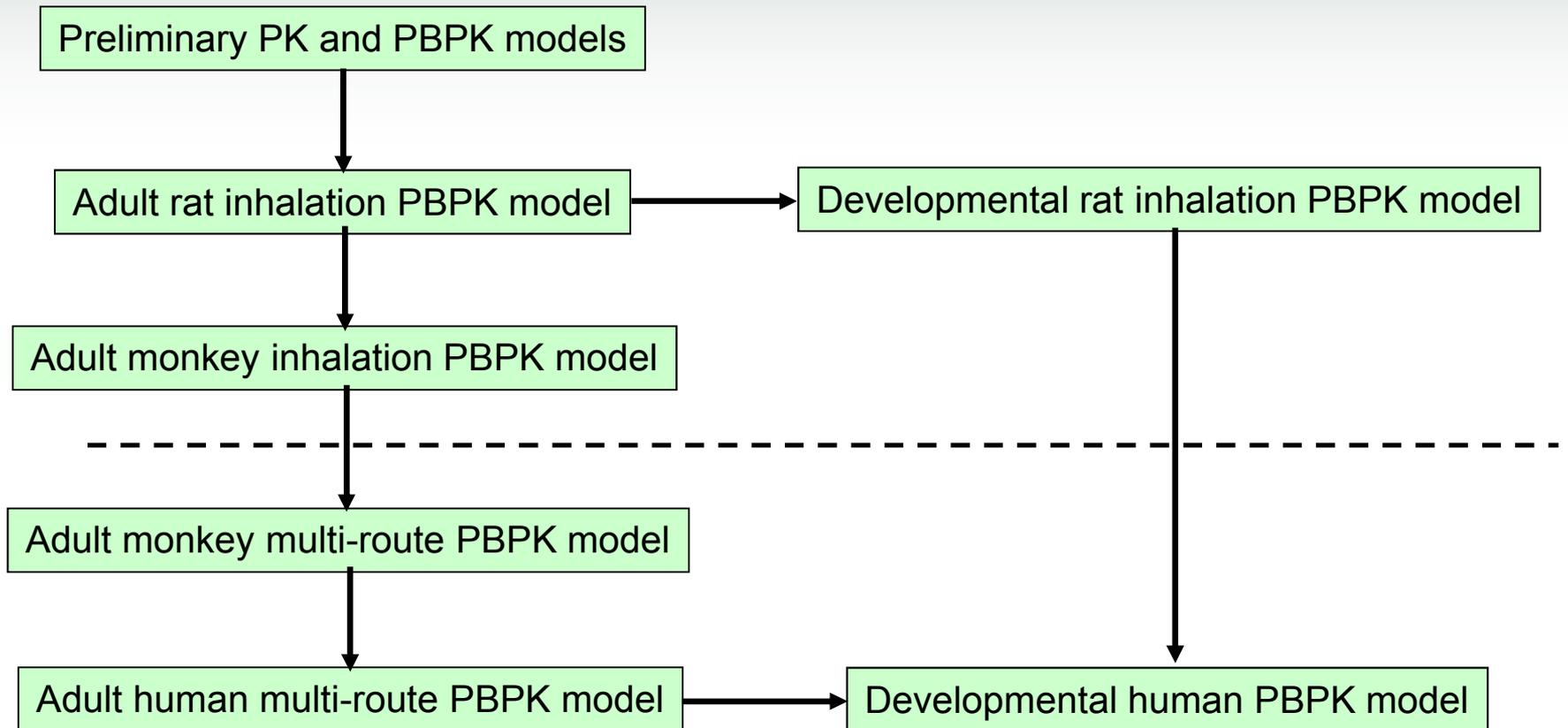
Development of a PBPK model for the adult human based on the multi-route monkey model

- Validation against human tracer data

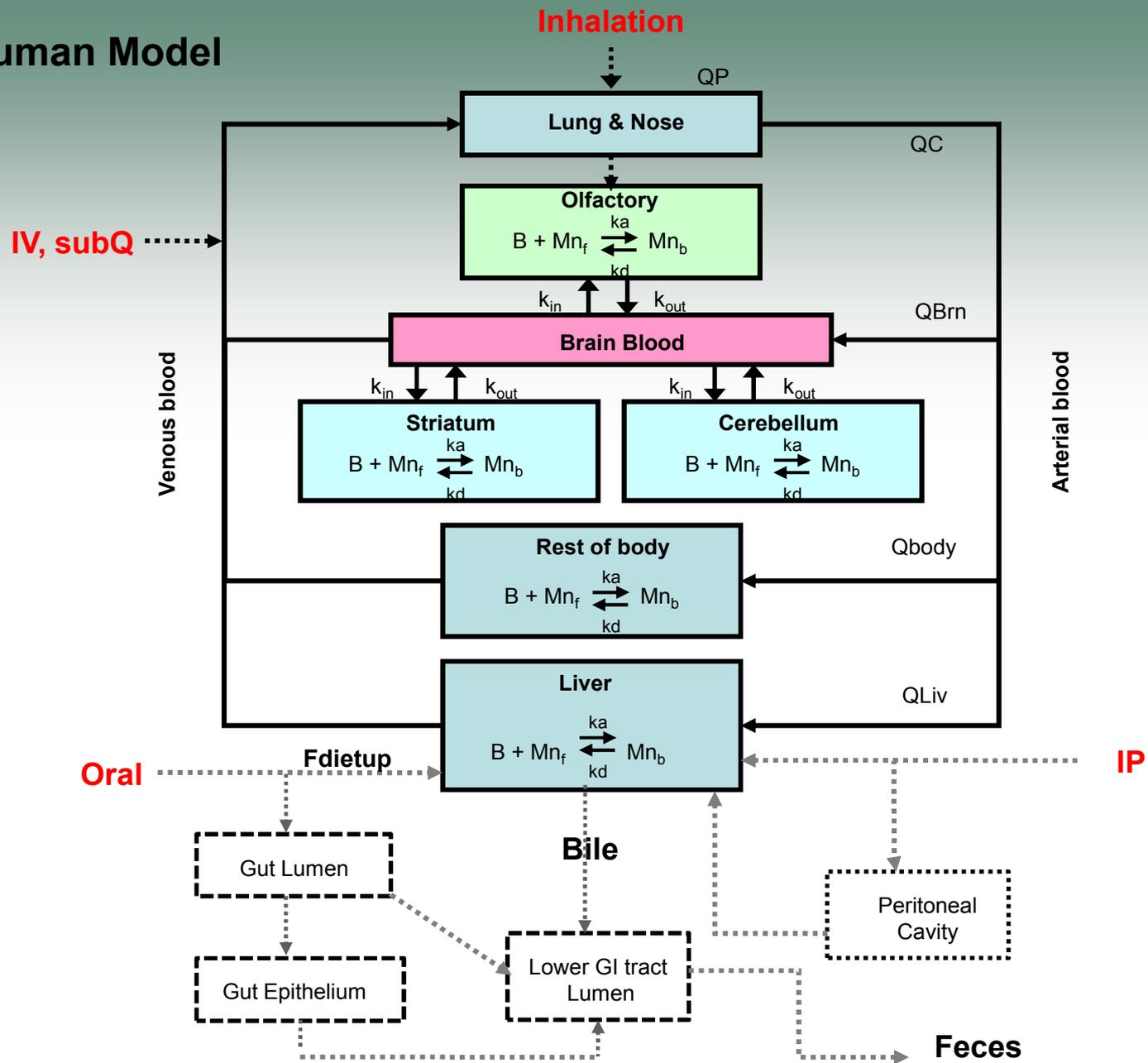
Development of a preliminary PBPK model for human gestation and lactation based on the rat developmental models and human adult model

- Following parallelogram approach used for perchlorate (R. Clewell et al 2008)

Manganese Model Development Information Flow



Mn Human Model



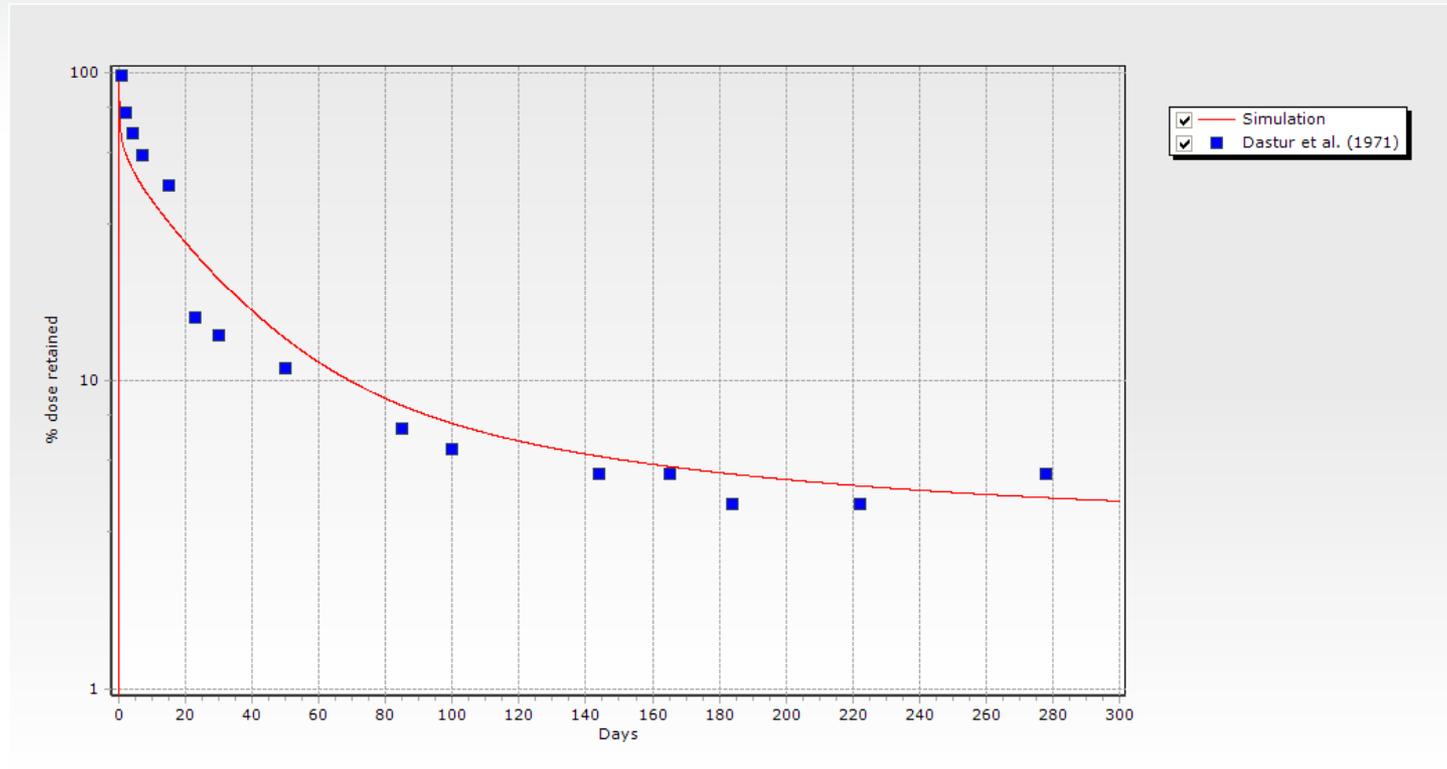
Mn Tracer Kinetics



- Tracer studies permit assessment of overall kinetic behavior of compounds that are maintained in steady-state through continuous dietary intakes.
- Mn PBPK model was modified to include iv, ip, subq exposure routes (in addition to oral and inhalation) of radiolabeled Mn (carrier-free $^{54}\text{MnCl}_2$)
- Model parameters governing dietary absorption and biliary excretion were calibrated to whole body retention and tracer fecal excretion data, while maintaining Mn tissue levels near steady-state levels

Dastur (1971) ip:

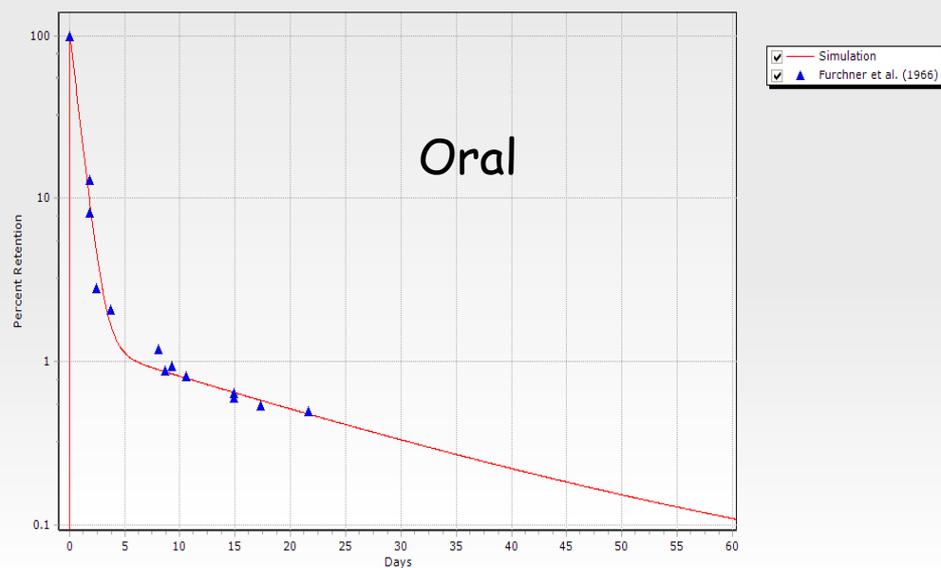
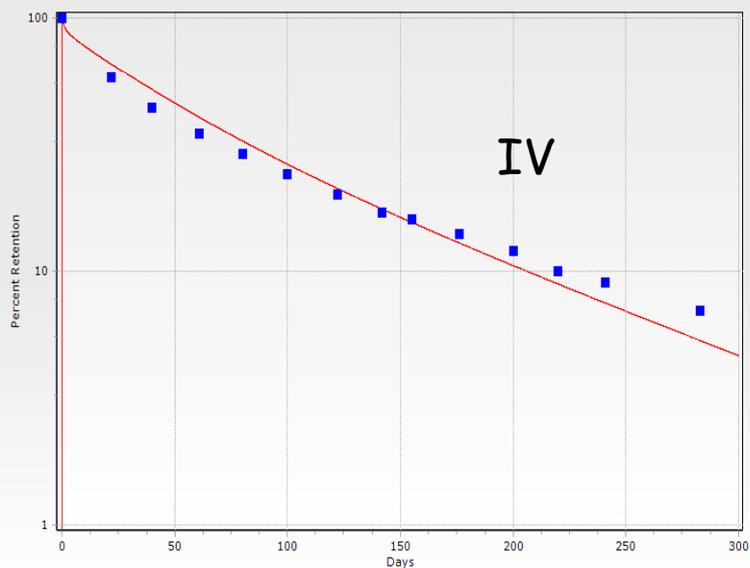
- 12 monkeys (2.5 kg) injected ip with 200 μCi ^{54}Mn
- examined whole-body retention



Whole-body retention after ip administration

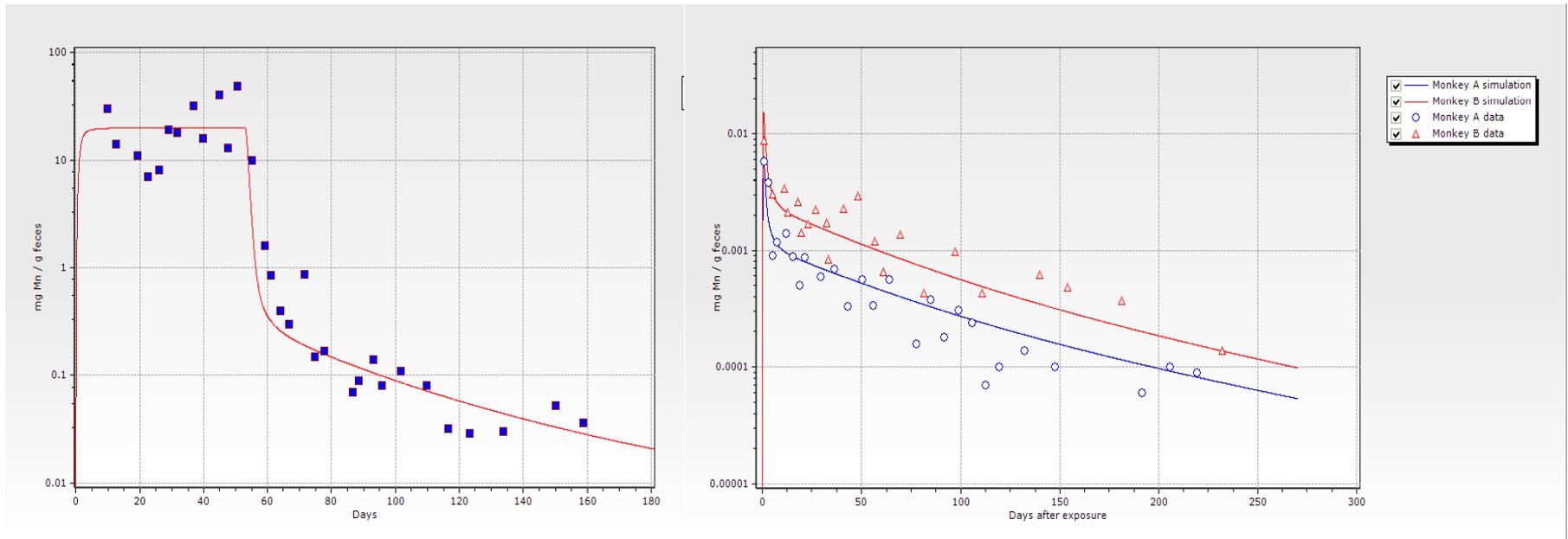
Furchner (1966) - iv vs. oral:

-
- 3 monkeys (8.5 kg) injected iv with $0.6 \mu\text{Ci } ^{54}\text{Mn}$
- 3 monkeys (7 kg) administered ^{54}Mn orally
- examined whole-body retention



Newland (1987) subcutaneous and inhalation:

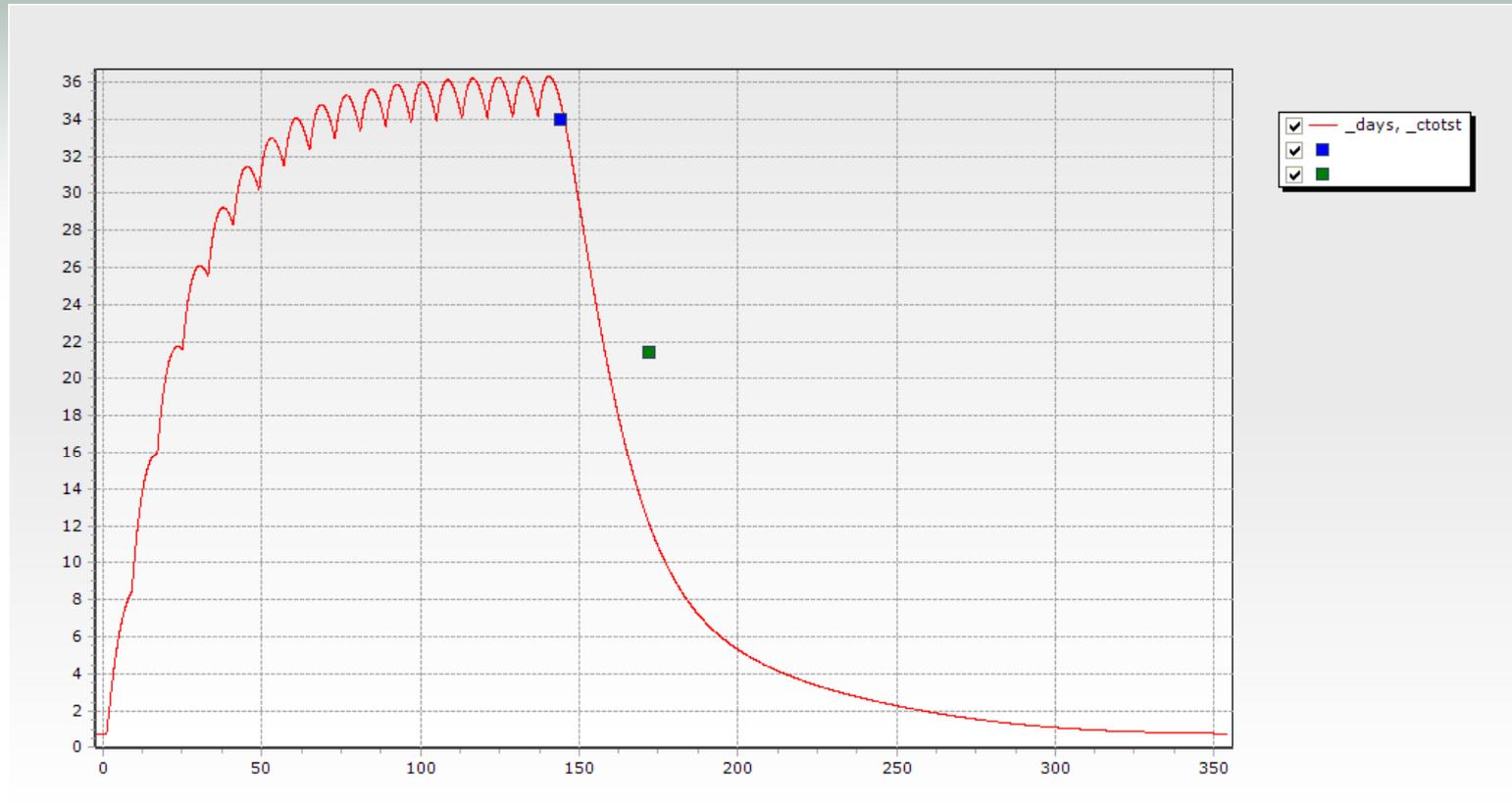
- 1 monkey (5 kg), 6-week continuous exposure
 - 200 μCi ^{54}Mn and 400 mg Mn (MnCl_2 soln.) administered subq
- 2 monkeys endotracheally exposed to carrier-free $^{54}\text{MnCl}_2$ aerosol
- measured fecal activity



PBPK Model Evaluation of Monkey Toxicity Data

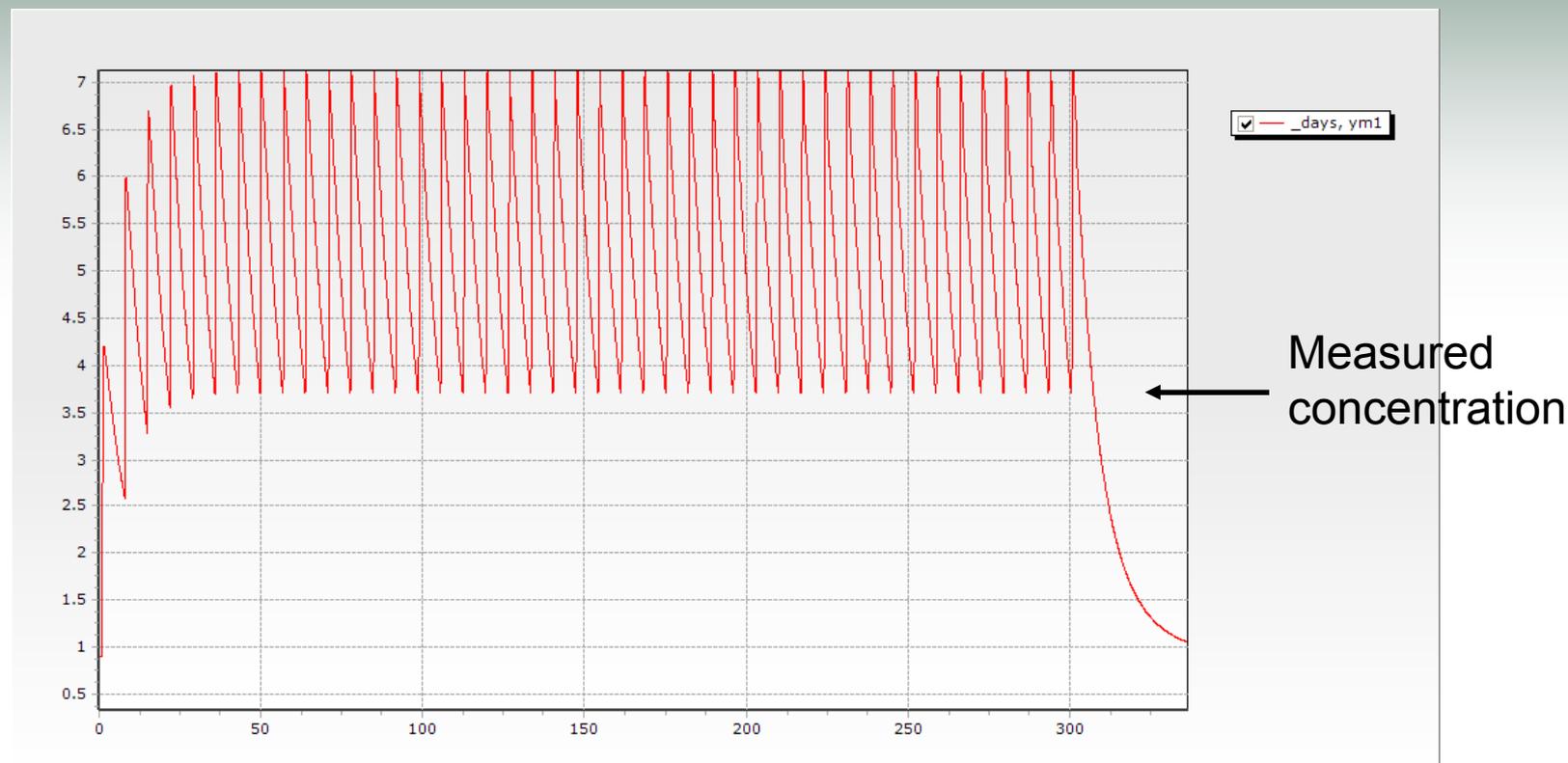
- **Gwiazda et al. 2007:**
 - **“Adequacy and Consistency of Animal Studies to Evaluate the Neurotoxicity of Chronic Low-Level Manganese Exposure in Humans”**
 - Considered all routes of exposure
- **Gwiazda et al. used estimated cumulative absorbed dose as the only metric of exposure for comparison**
 - 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)

Guilarte (2006) – iv dosing

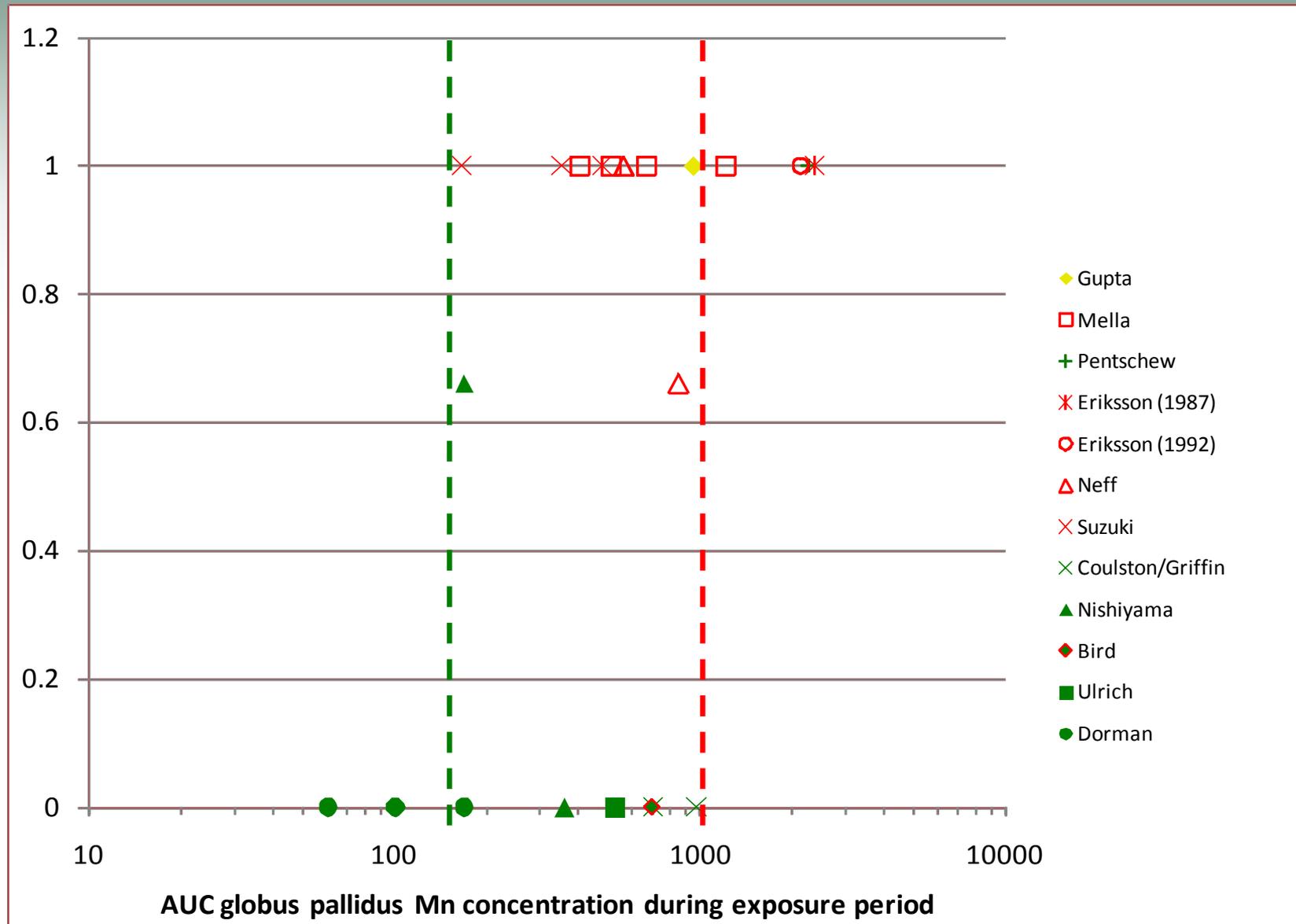


Globus pallidus concentration at lowest exposure:

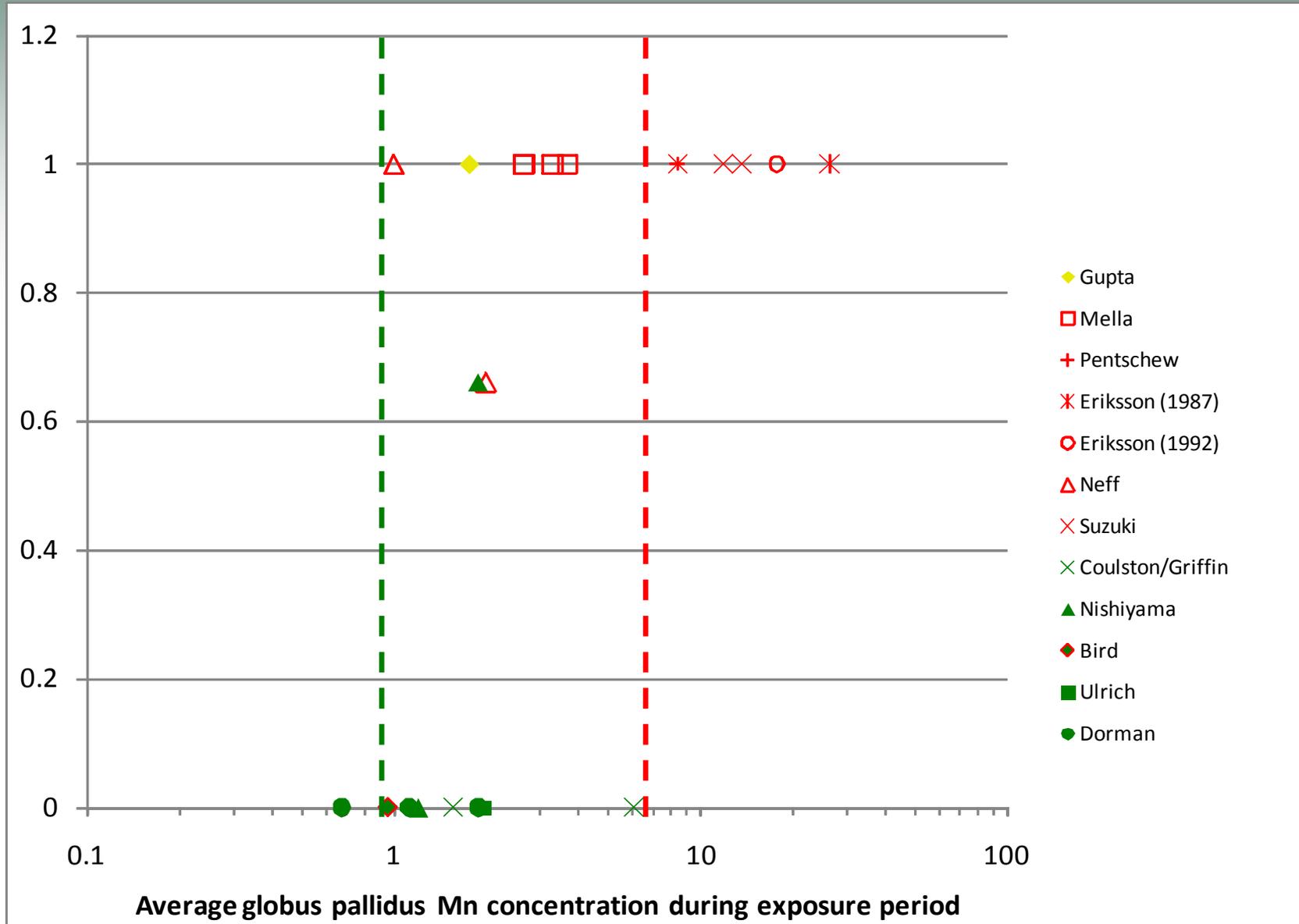
4 mg Mn iv dose of MnSO_4 given once/week for 44 weeks

Predicted blood concentrations ranged from 0.01 to 11 ppm vs ~0.1 measured

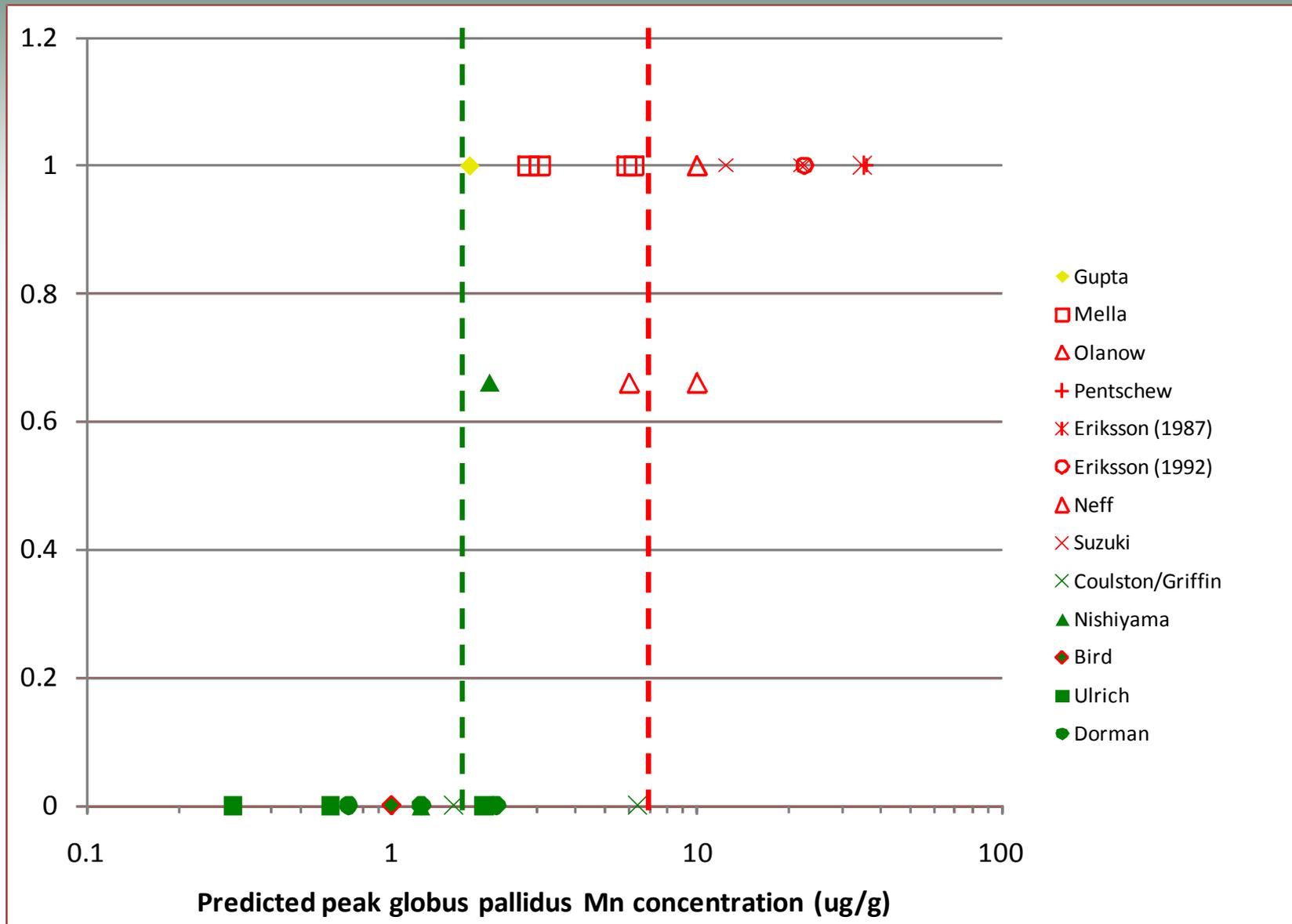
Cumulative Target Tissue Dose during Exposure



Average Target Tissue Concentration during Exposure



Peak Target Tissue Concentration during Exposure



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

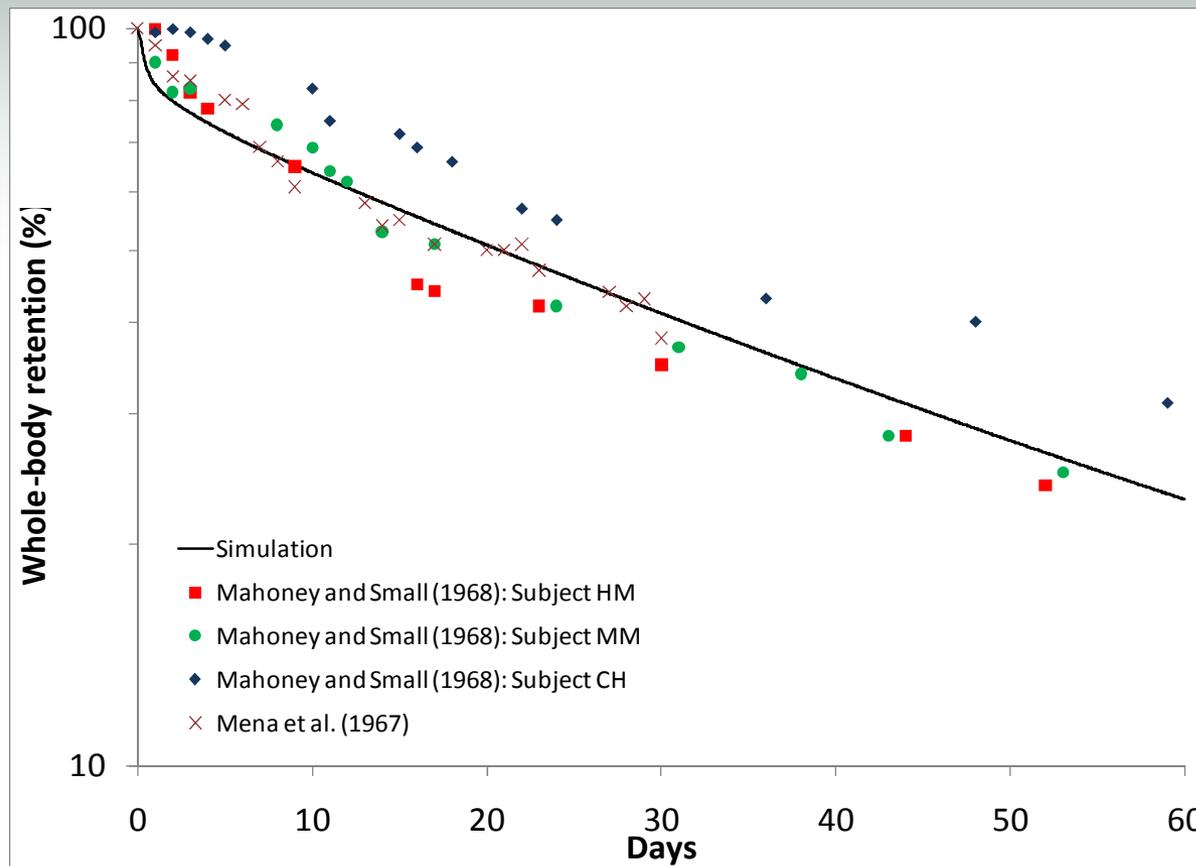
- but estimated peak concentrations produced by the IV dosing are greater than the troughs by factors of 2 and 1000, respectively

Extrapolation to humans



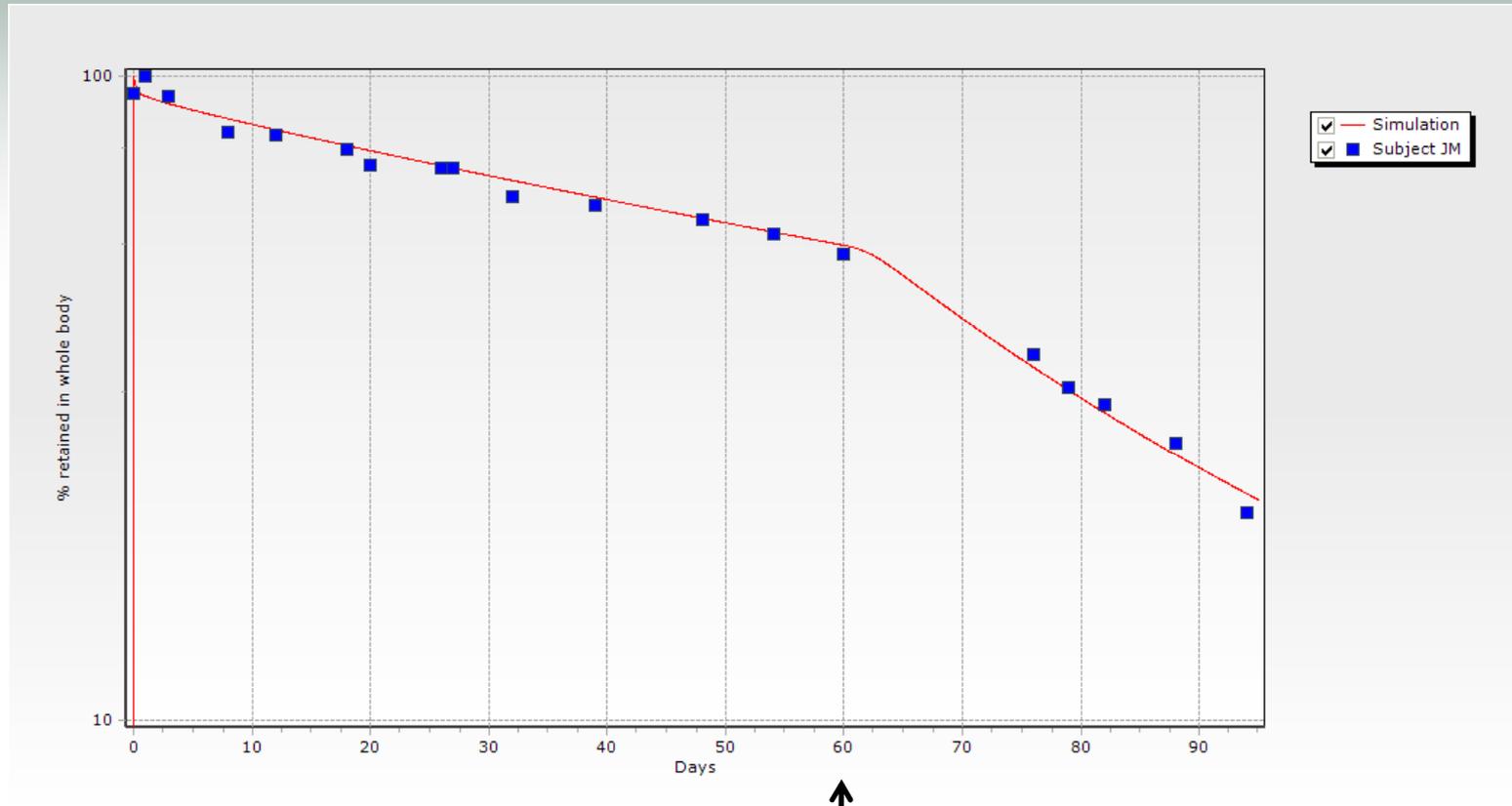
- Use PBPK model structure from the monkey
- Physiological parameters (BW, tissue blood flows, tissue volumes, etc.) either scaled from monkey or obtained from the literature
- Same biliary induction parameters as used in the monkey
- Basal Mn tissue concentrations obtained from cadaver studies
- Assumed typical daily Mn diet: 3 mg/day

Human tracer studies



Whole-body retention in normal subjects

Human tracer studies

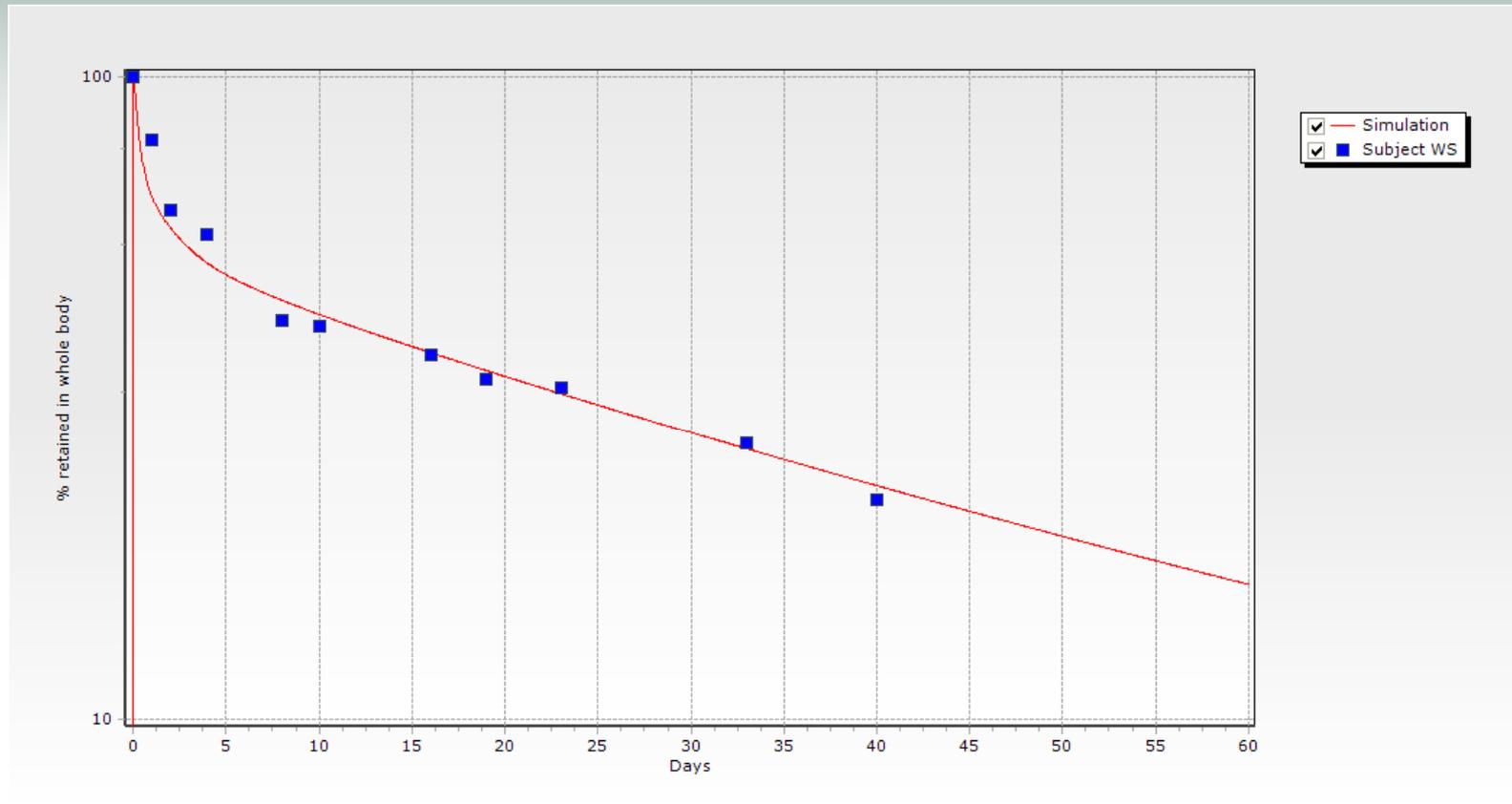


Mahoney and Small (1968)

begin 800 mg/day Mn

Whole-body retention for subject on reduced-calorie diet (800 cal./day)

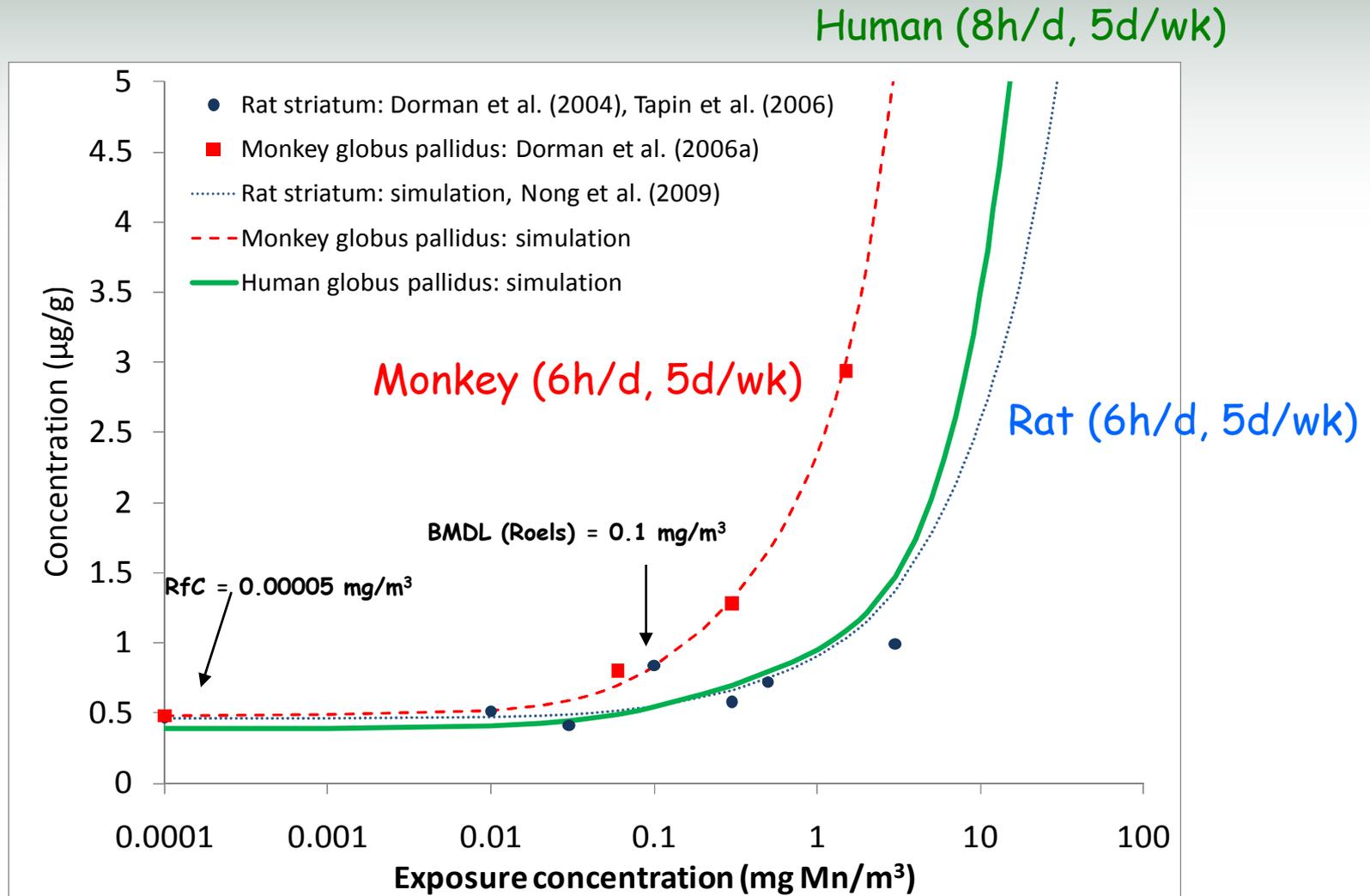
Human tracer studies



Mahoney and Small (1968)

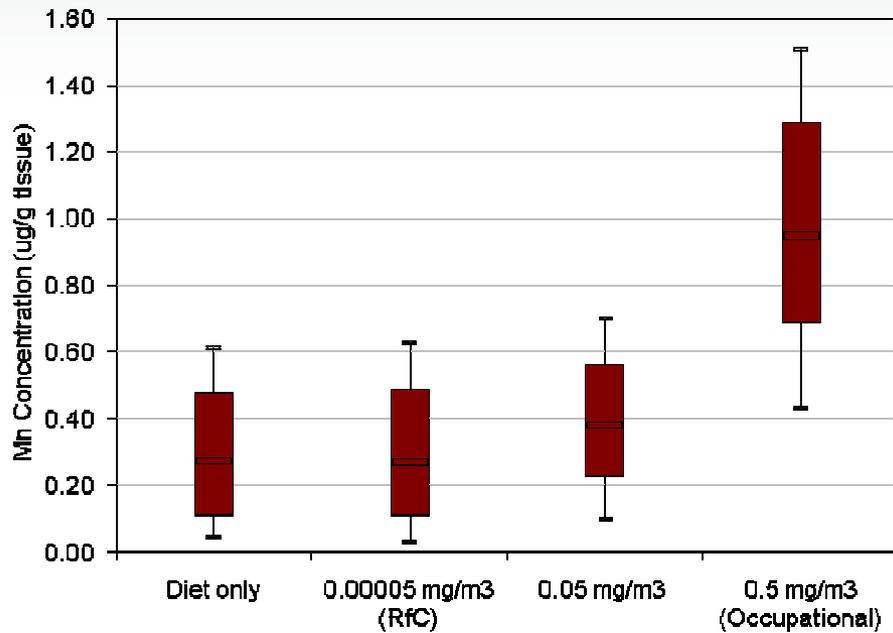
Whole-body retention for subject pre-loaded at 300 mg/day Mn

Mn Inhalation Exposure Across Species

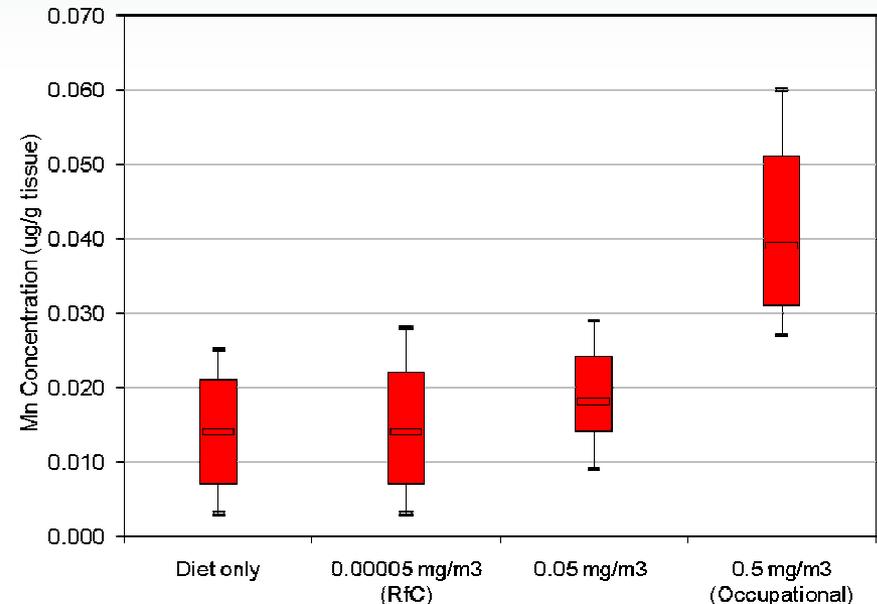


Comparison of Inhalation and Oral Exposure

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



Brain

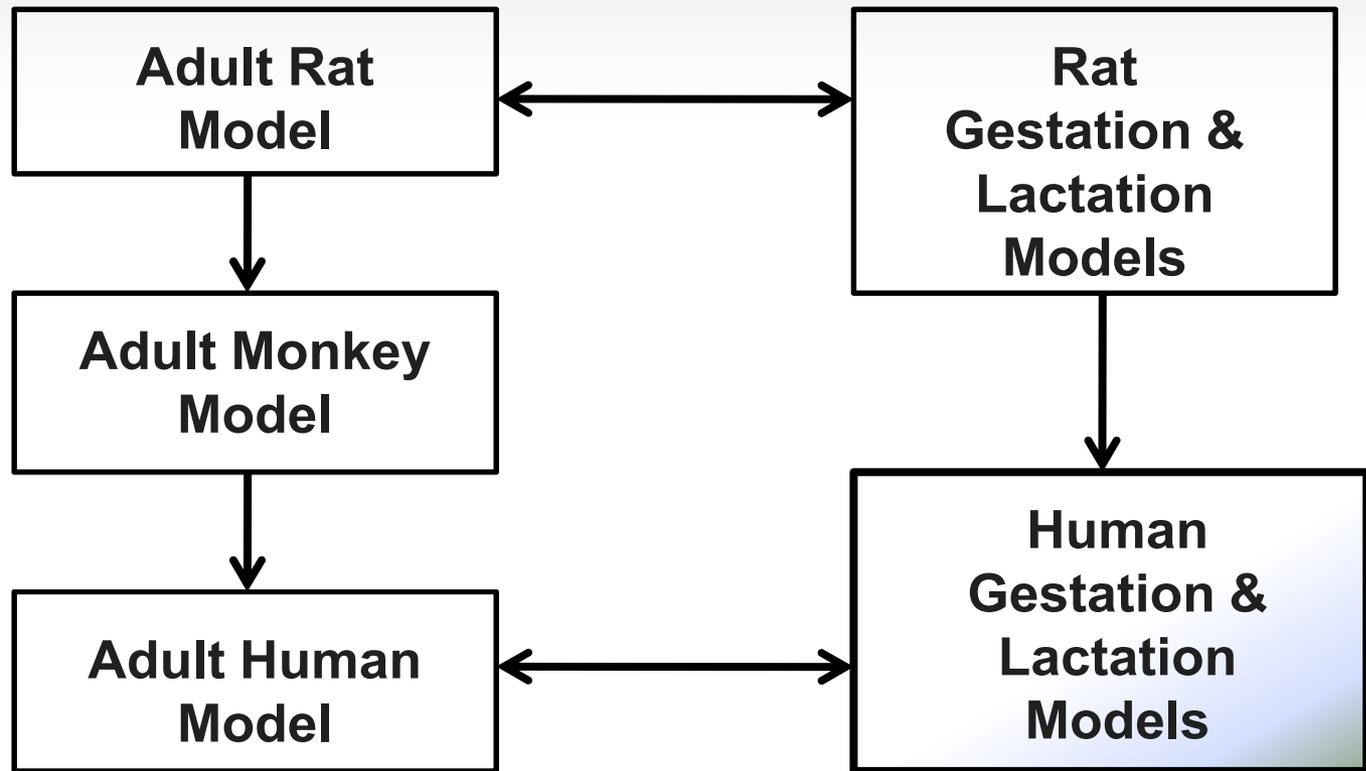


Blood

Summary

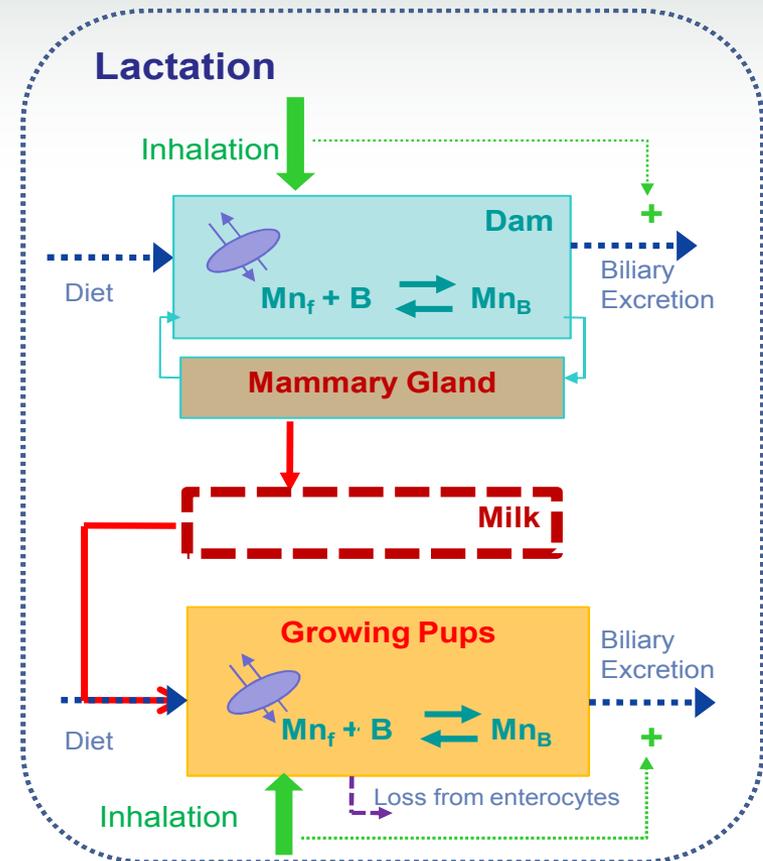
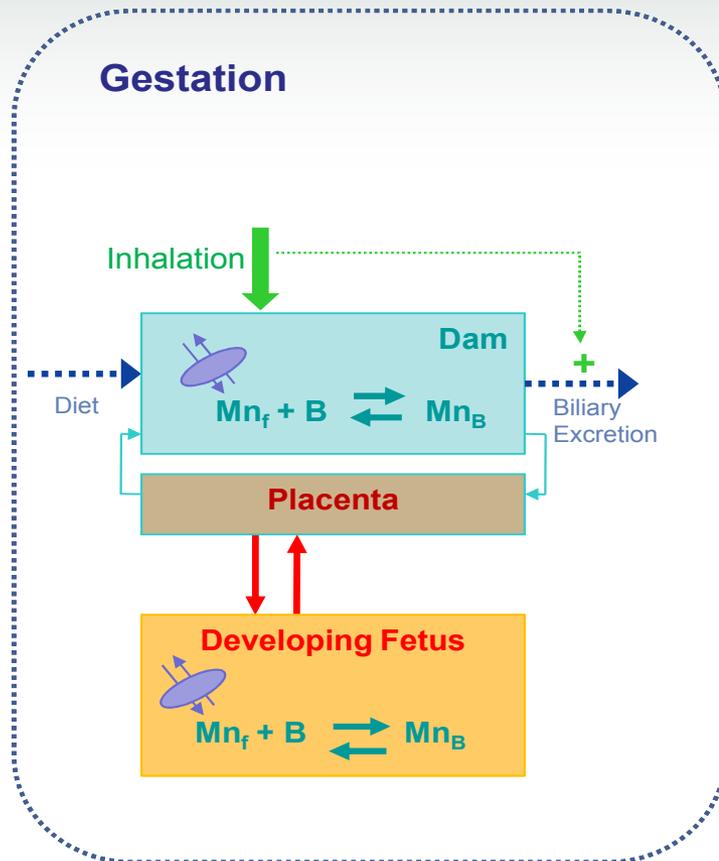
- The monkey PBPK model accurately simulated the fast “free” and slow “bound” elimination phases of Mn tracer using multiple exposure routes
- We were able to assess possible ranges of Mn tissue concentrations due to differences in dietary intake (4-5 fold) using the human PBPK model
- Increases in brain Mn concentration levels occur at inhalation exposures between 0.01 and 0.1 mg/m³ 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

Parallelogram Approach for Developing Mn PBPK model for Human Perinatal Period



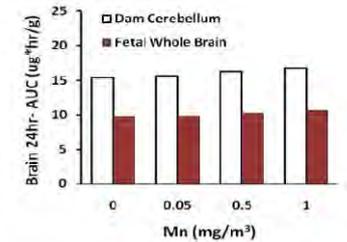
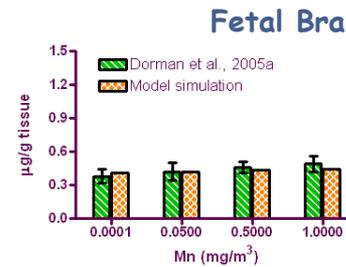
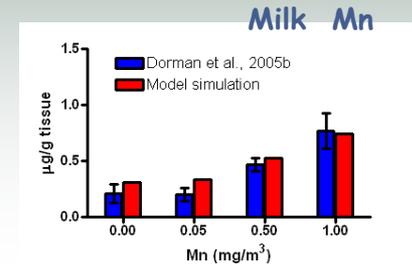
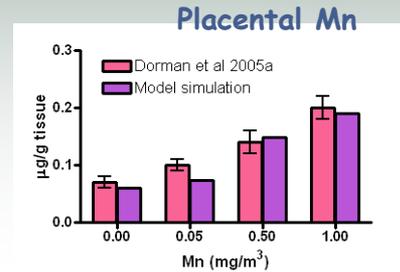
Modified from R. Clewell et al., 2001, Toxicology and Industrial Health

Extending Adult Model to Perinatal Periods: Rat Developmental Models



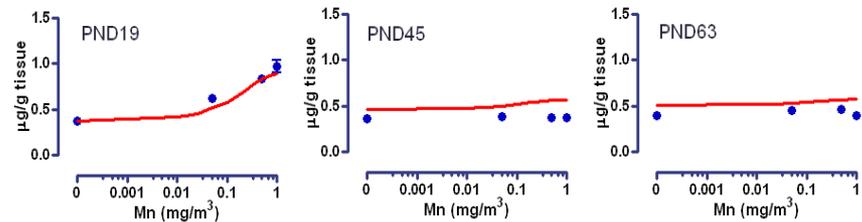
Developmental Model makes it possible...

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



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

Neonatal Striatum Concentration & AUC with Mn inhalation exposures



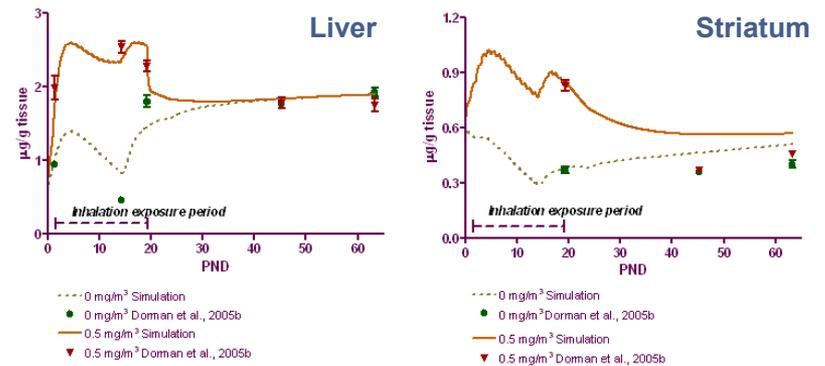
Developmental Model makes it possible...

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

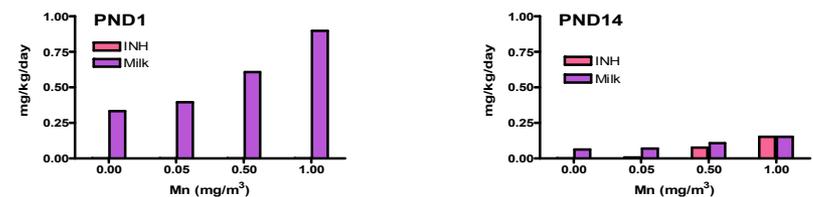
- To compare exposures from different sources of Mn:

- Milk
- Diet
- Inhalation

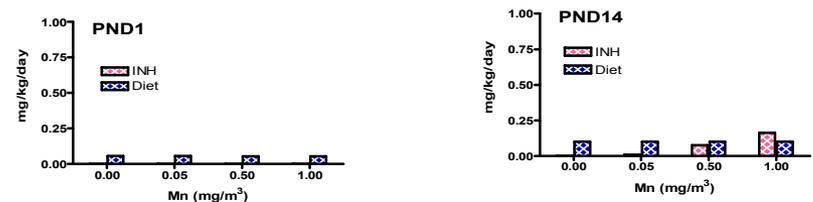
Temporal changes in Mn tissue concentrations during neonatal development



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

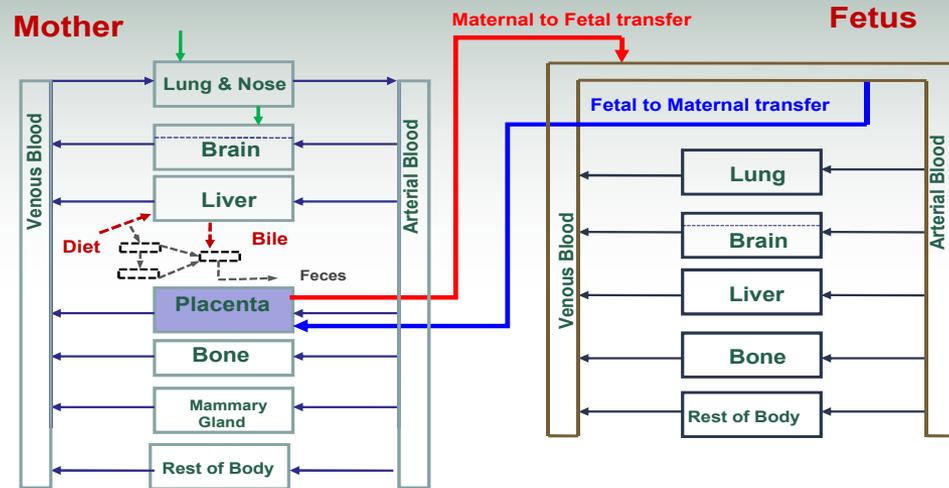
- Drivers for Mn transfer processes from the dam to offspring: Maintaining maternal homeostasis while ensuring adequate Mn to the offspring
- Changes in physiological processes responsible for Mn homeostatic mechanism
 - Mn uptake in gut – higher retention in neonates
 - Biliary excretion – apparently low, but inducible, in neonates

Developing Human Gestation and Lactation Models

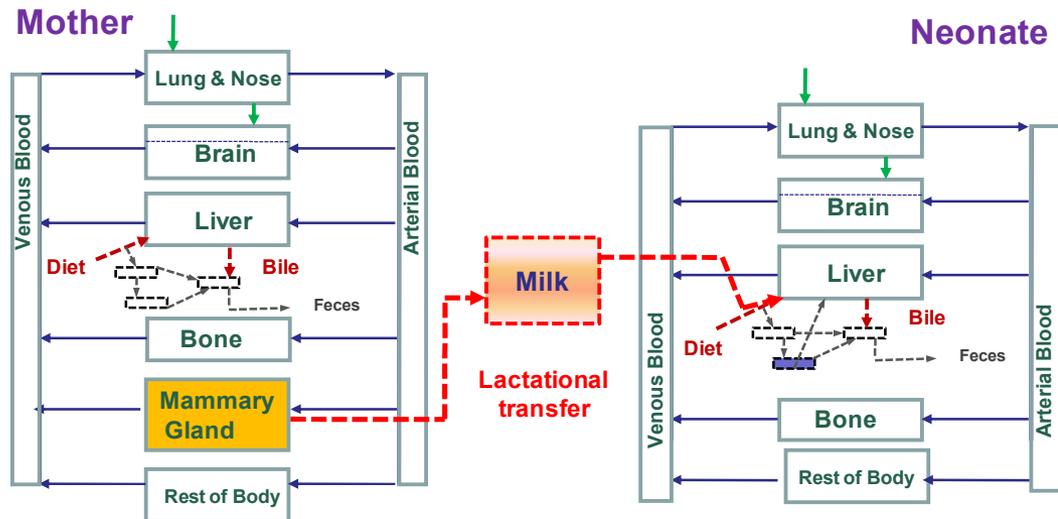
- Features of human model based on successful rat and monkey description, human tissue Mn observations, and the species differences in key processes
- Basic model structure: Rat developmental model based on studies with inhaled Mn exposure for a defined diet
- Extrapolation processes based on
 - Scaling up from adult rats and monkeys to human adults
 - Adults to fetuses/neonates based on
 - Rat developmental modeling
 - Comparative physiology of developmental processes between the rats and humans
- Information on Mn concentration in human tissues including milk and placenta from nutritional studies and autopsy data

Human Developmental Model Structure

Gestation Model



Lactation Model



Key Features of Human Gestation Model

- Efficient transfer of Mn to the fetus based on
 - Fetal tissue Mn similar to adult levels
 - Bone as a storage site for Mn during fetal development
 - while placenta still keeping a role as a barrier for excess Mn transfer to fetus
- Prenatal maturation of human brain compared to the rats
 - 3rd trimester comparable to 1st postnatal week in rats
 - Timing of BBB development in humans

Key Features of Human Lactation Model

- Ability to respond to both low and excess Mn in early ages
 - Neonatal tissue Mn comparable to adults while human milk Mn very low compared to other species
 - Plasma Mn level comparison between breast-fed vs formula-fed infants and TPN infants with or without hepatic dysfunction suggest ability to control excess Mn in infants
 - Homeostasis control at the level of gut uptake and biliary excretion: Very low biliary excretion and higher uptake in the gut during lactation
- Prenatal development of brain and GI compared to the rodents
- Changes in lung structure and ventilation characteristics incorporated to predict particle deposition patterns in different ages

Describing Biliary excretion of Mn in Early Ages

Three-fold higher value for the affinity rate constant for biliary induction required for neonates compared to that of adults

- neonatal blood Mn about 2 – 5 fold higher than maternal or non-pregnant adult blood Mn, while maternal and neonatal serum Mn concentrations were similar
 - Higher erythrocyte Mn responsible for high blood Mn in neonates
 - Serum Mn is assumed to reflect free Mn status in the body for biliary excretion
- The model uses whole blood Mn as a surrogate for free Mn status in the body for biliary excretion/induction
 - affinity constant for this process adjusted in neonate model to account for different RBC/serum ratio

Fetal Tissue Mn Concentration in Humans

	Fetal Mn ($\mu\text{g/g}$) ^a	Adult Mn ($\mu\text{g/g}$) ^c
Liver	0.94 ± 0.35	1.20 ± 0.35
Kidney	0.45 ± 0.10	0.56 ± 0.22
Brain	0.16 ± 0.02 (whole brain)	0.36 ± 0.11 (cerebellum) $0.19 - 0.53$ (different regions) ^b 0.32 (whole brain) ^d
Heart	0.27 ± 0.07	0.21 ± 0.08
Lung	0.22 ± 0.08	0.22 ± 0.09
Muscle	0.13 ± 0.03	0.09 ± 0.05
Bone	0.88 ± 0.21	0.07 ± 0.06

^a Casey et al., 1978: 40 fetuses of 22-43 weeks of gestation, New Zealand

^b Tingey, 1937: newborn to 2 years old, in adult brain, the highest Mn in striatum

^c Sumino et al, 1975: Japanese cadavers

^d Reiman & Minot, 1920

Human Tissue Mn Concentrations during Early Ages

	Birth ($\mu\text{g/g}$) ^a	Neonatal Mn ($\mu\text{g/g}$) (1 – 4 years) ^b	Adult Mn ($\mu\text{g/g}$) ^c
Liver	0.94 ± 0.35	1.42 ± 0.16^b	1.20 ± 0.35^c
Brain	0.16 ± 0.02 (whole brain)	0.64 ± 0.14 (basal ganglia) ^b	0.36 ± 0.11 (cerebellum) ^c $0.19 - 0.53$ (different regions) ^e 0.32 (whole brain) ^d
		0.68 ± 0.14 (cerebellum) ^b	
		0.29 (newborn, whole brain) ^d 0.24 (4yr old, whole brain) ^d	
		$0.17 - 0.25$ (newborn, cerebrum) ^e 0.17 (2yr old, cerebrum) ^e	

^a Casey et al., 1978

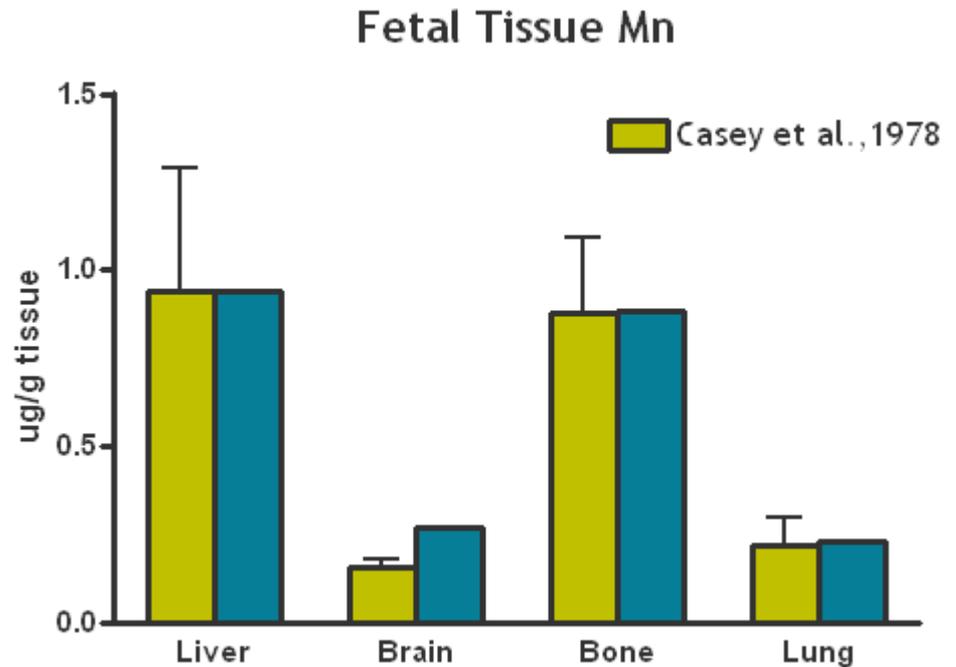
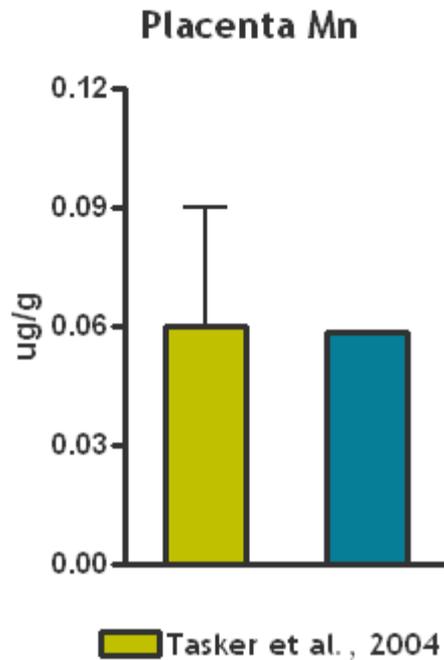
^b Lehmann et al, 1971

^c Sumino et al, 1975

^d Reiman & Minot, 1920

^e Tingey, 1937

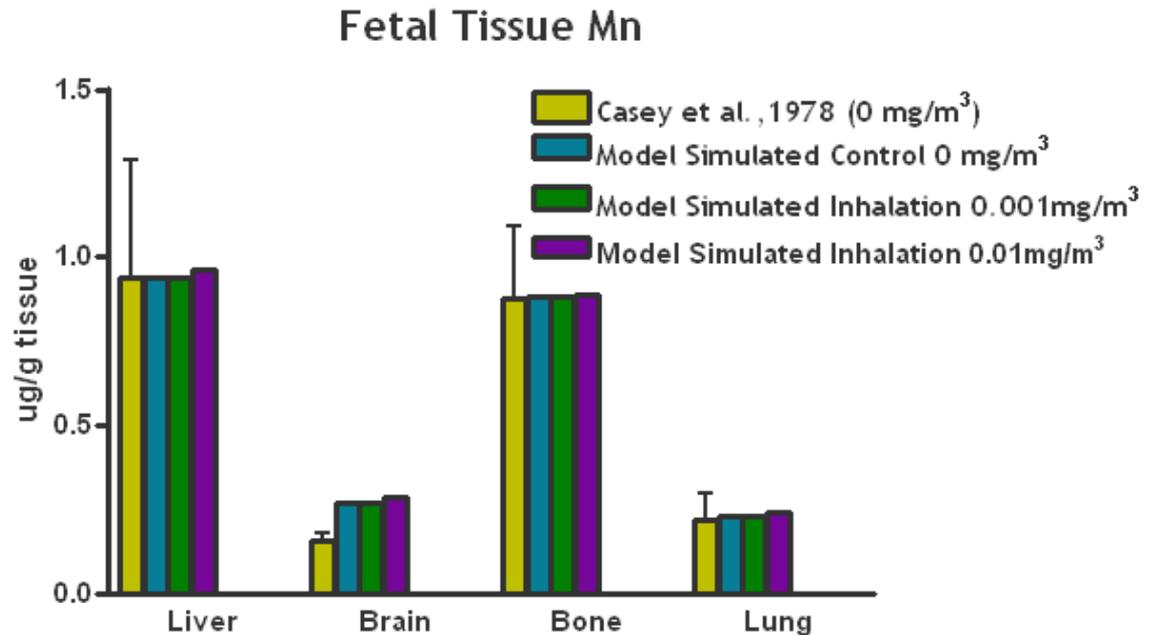
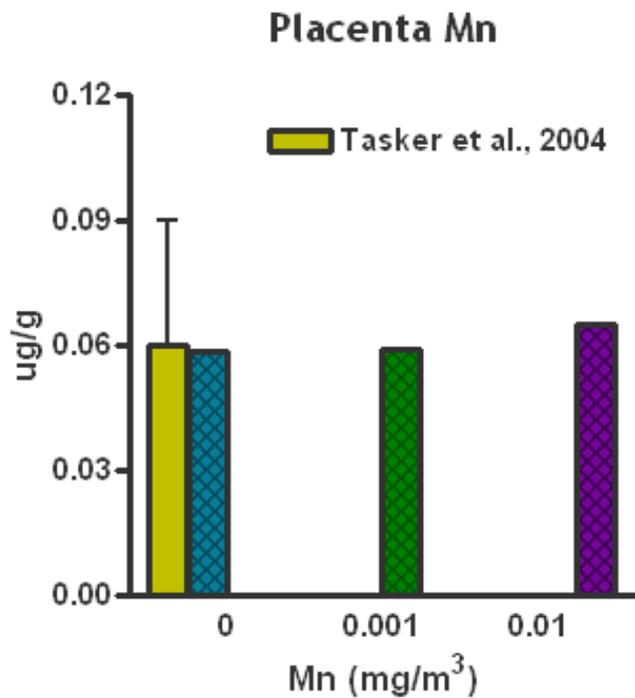
Simulated Placental and Fetal Tissue Mn without Inhalation Exposure



Note: Whole brain mn reported in Casey et al., 1978, while model simulation indicates fetal globus pallidus concentration.

Model simulation for the end of gestation (270days).

Simulated Placental and Fetal Tissue Mn with Inhalation Exposure

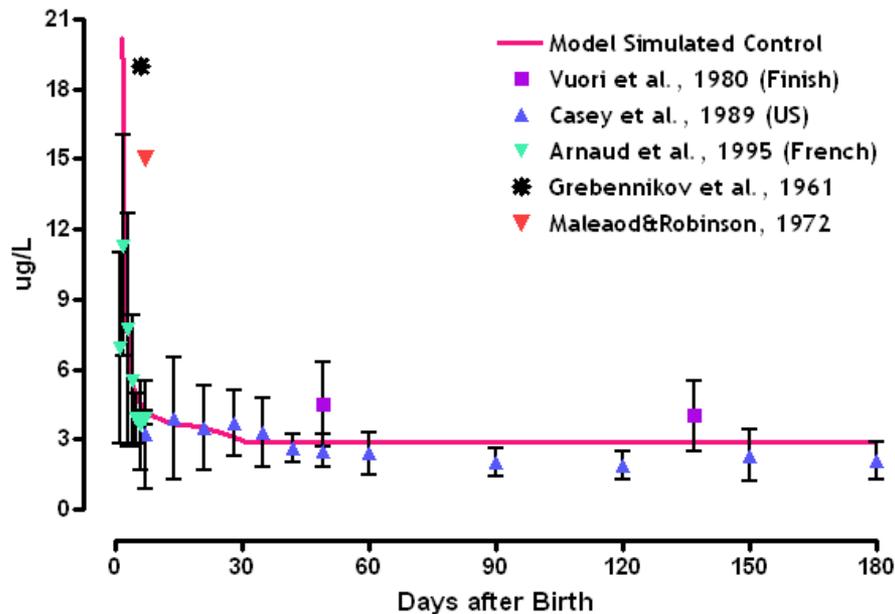


Note: Whole brain Mn reported in Casey et al., 1978, while model simulation indicates fetal globus pallidus concentration.

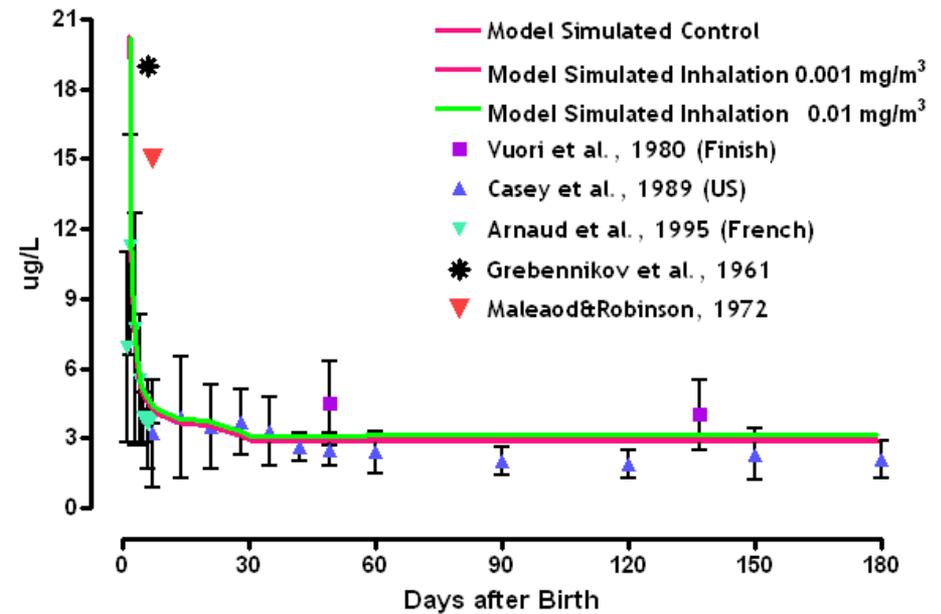
Inhalation of Mn during whole gestation.

Simulated Milk Mn Concentrations in Humans

Diet only



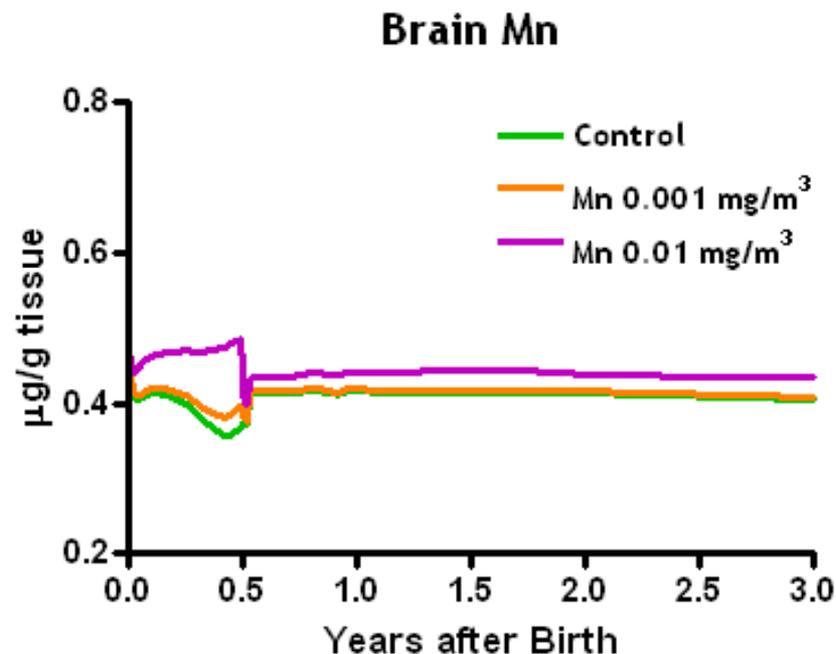
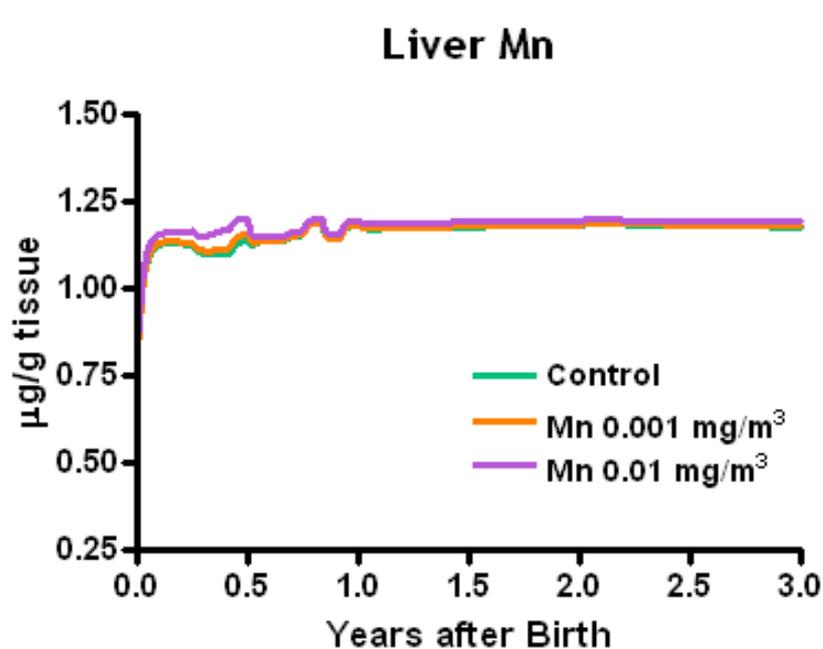
Diet + Inhalation



Exclusive breast-feeding for 6 months was assumed.

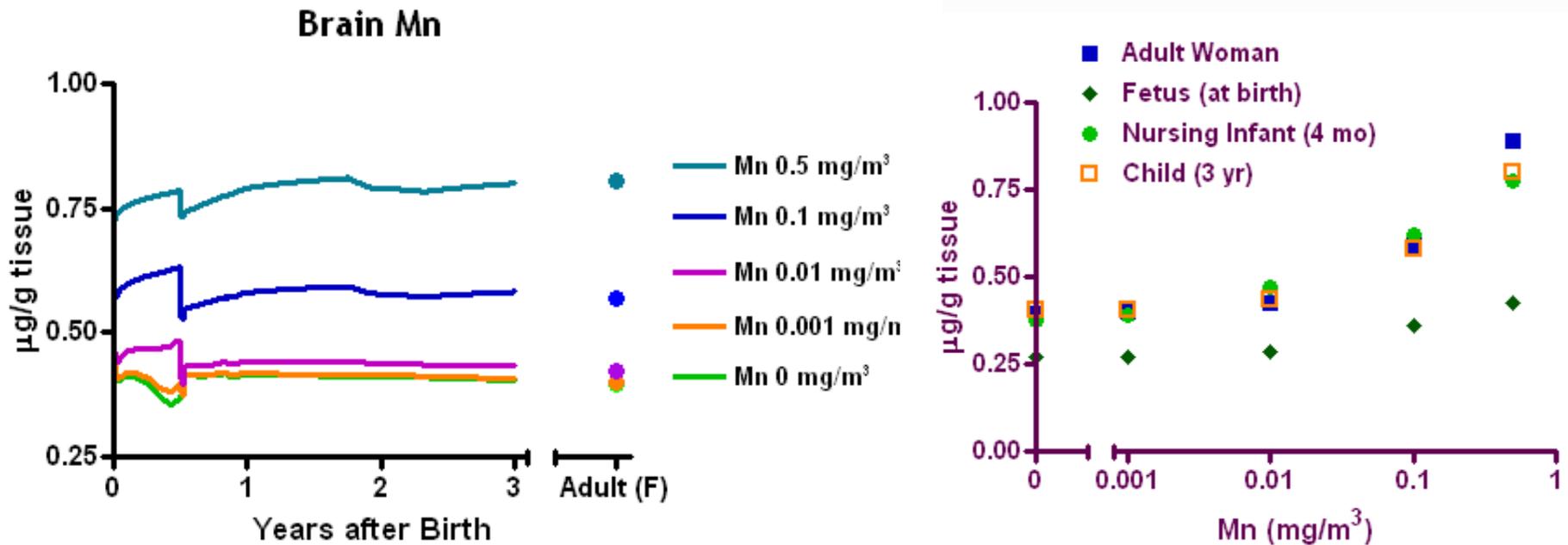
Inhalation during lactation both to the mother and the infant

Changes in Neonatal Tissue Mn Concentrations during Development with or without Inhalation



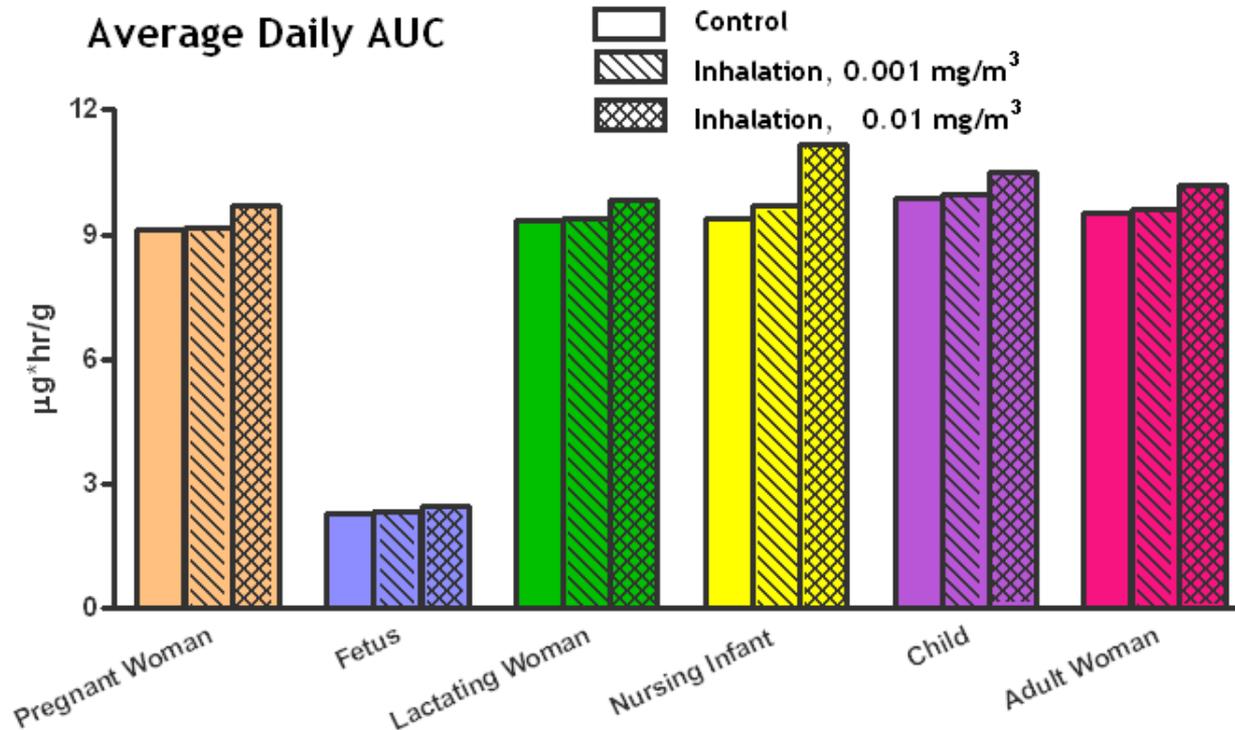
Inhalation was simulated starting before pregnancy and continued through gestation, lactation, and postnatal period.
After 6 months of breast-feeding, dietary Mn intake in children was assumed to be at ESSADI recommended by NAS.

Predicted Brain Mn in Early Ages with High Dose Mn Inhalation Comparable to Occupational Level



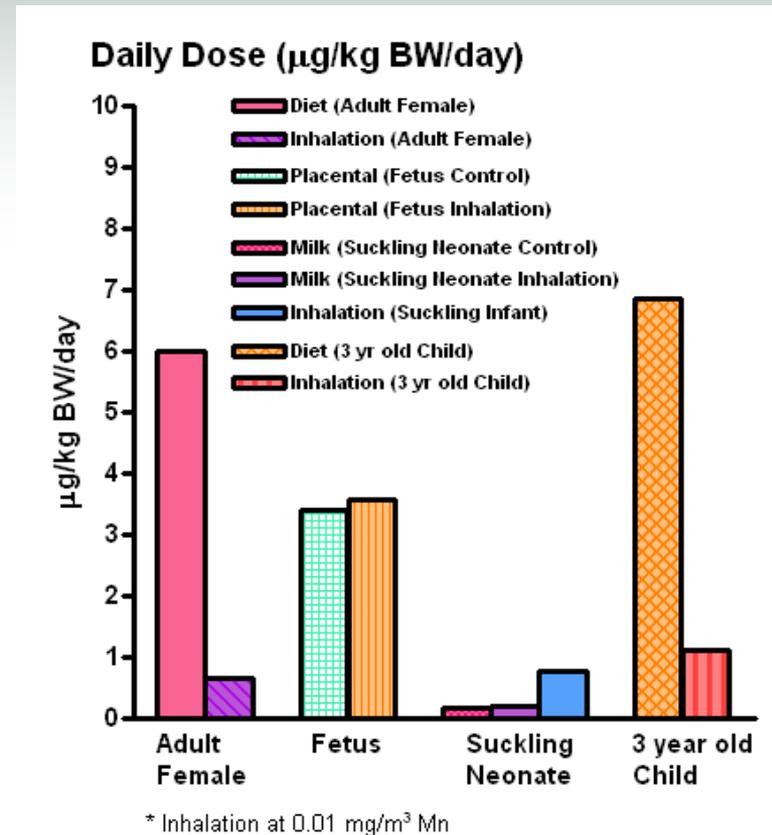
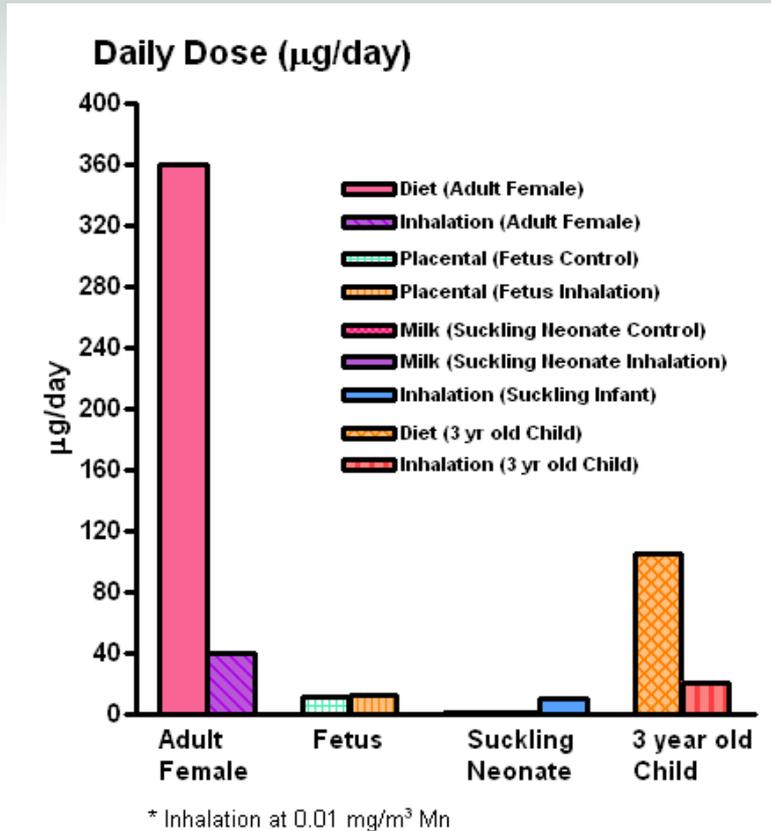
For the adults (female), brain Mn at the target region after 3 years of exposure were plotted.

Comparison of Internal Dose Metrics in Brain



For pregnant mother and fetus, average daily AUCs were calculated during the whole gestation. For lactation mother and nursing infant, values were from averaging AUCs during breast-feeding period (6 months). For the adults and a child (3 years), the AUCs were averaged during the 3 years period after weaning.

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 $0.01 \text{ mg}/\text{m}^3$ of Mn was simulated.

Summary

- Mn PBPK model for human fetus and neonate successfully developed by extrapolating the rat developmental to the human in conjunction with human adult model and comparative physiology between the rat and human
- Model predicted Mn tissue dosimetry in target brain region in fetus and neonate similar to those in the mother or adults.

How Can PBPK Models Help Refine Mn Reference Levels?

Typical role of PBPK models in the derivation of RfCs:

- route-to-route extrapolation
- duration adjustments
- dose extrapolation
- interspecies extrapolation
- intraspecies extrapolation

For Mn, the point of departure will undoubtedly be derived from a human epidemiological study

- The PBPK model can be used to support the application of chemical-specific adjustment factors (CSAFs) instead of default UFs, and to evaluate the impact of reductions in environmental exposure on target tissue dose

Proposed Risk Assessment Approach for Mn

- 1. Determine point of departure ($BMDL_{10}$) based on dose-response for neurological effects associated with human occupational exposure**
- 2. Apply chemical-specific adjustment factor for human variability (sensitive populations) based on PBPK modeling**
- 3. Evaluate need for additional uncertainty factors (to address concerns regarding use of occupational exposures to set environmental guideline) using PBPK model**
 - by comparing brain dosimetry at proposed inhalation guideline with variation due to dietary exposure

How Can PBPK Models Help Refine Mn Risk Assessments?

PBPK models for Mn in rat, monkey and human can be used to:

- Determine the relative contribution of inhaled and ingested Mn to tissue levels in target organs
- Evaluate the movement of Mn throughout the body, including the brain
- Model nasal uptake to the CNS: Mn movement along the olfactory nerves
- Quantify differences regarding tissue delivery due to differences in form and solubility

How Can PBPK Models Help Refine Mn Risk Assessments?

Developmental PBPK models for Mn can be used to:

- Characterize Mn transfer across the placenta and during lactation
- Evaluate lifestage differences in Mn pharmacokinetics
- Compare exposures from inhalation, breast milk, and formula

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