INTEGRATING NEUROTOXICOLOGY AND EPIDEMIOLOGY IN EVALUATING HUMAN HEALTH EFFECTS:
MANGANESE AS A CASE STUDY

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OVERVIEW

- The chemistry and biology of manganese (Mn)
  - Biology: Uptake, Distribution and Metabolism
  - Physiological roles: Essentiality and Neurotoxicity
- Health effects of Mn exposure
  - Dietary insufficiency
  - Low grade exposure effects
  - Manganism
- Quantitative risk assessment: challenges and opportunities
  - How much Mn is too much Mn?
  - Current perspectives and opportunities for quantifying risks associated Mn
Manganese in the Environment

- Mn is a transition metal and the 12th most abundant (0.1%) mineral in the Earth’s crust: most commonly as pyrolusite (MnO₂)

- Normal constituent of air, soil, water, and food.

- Reaches the atmosphere and waterways through erosion of rocks and soils.

- Anthropogenic sources include mining and refining activities; industrial wastes, disposal of consumer products (e.g., dry-cell batteries), etc.

- In some countries, automobile exhaust contributes (Methylcyclopentadienyl manganese tricarbonyl (MMT) (CH₃C₅H₄)Mn(CO)₃ used as fuel additive
Production and Common Uses of Mn

- South Africa contributed 6.2 of the 18 million metric tonnes produced globally in 2015
- Common uses for Mn:
  - Manufacturing steel and other alloys
  - Welding rods
  - Dry-cell batteries
  - Fertilizers (e.g., Maneb, Mancozeb)
  - Paints
  - As gasoline additive to improve octane rating (MMT)
  - Medical imaging agent (intravenous contrast agent in MRIs)
  - Cosmetics

Sources of Human Exposure

- **Dietary:** fruits, nuts, oatmeal, cereals and beans, tea, etc.
- **Environmental:**
  - Air – Auto exhaust, mining, steel and alloy production and other industrial operations
  - Water – very low level exposure
  - Soil – very low level exposure
- **Occupational:** manganese miners, miller and refiners, welders, steel and other metal workers
- In the US, 6185 tons of Mn and 73,644 tons of Mn compounds released by industries
- “The inhalation of air contaminated with particulate matter containing manganese is the primary source of excess manganese exposure for the general population in the United States” ATSDR 2012

[http://lpi.oregonstate.edu/mic/minerals/manganese#deficiency](http://lpi.oregonstate.edu/mic/minerals/manganese#deficiency)
The Biology of Manganese

- Commonly enters body via ingestion and inhalation
- Low absorption from the gut (3-5%) but relatively high bioavailability (~40%)
- Ingested Mn: extensive metabolism in bile
- Absorption and excretion controlled in the intestine to maintain homeostasis; Fe affects Mn absorption inversely
- Primary route of elimination: hepatobiliary excretion
- Mn also can travel along olfactory and trigeminal nerves and accumulate in the brain
- Excess Mn starts accumulating in the liver, brain and bone

Adequate Intake (AI) of Manganese

- Daily adult intake ranges from 1 -10 mg/m³
- Insufficient data to set a Recommended Daily Allowance (RDA)
- Adequate Intake (AI) based on the average dietary intake of Mn in the Total Diet Study*
- No overt deficiency state of Mn documented in humans eating natural diets
- Tolerated upper limits, set by FNB, are conservative
- Dietary exposure above TULs may not directly neurotoxic, but may potentiate inhalational exposure

<table>
<thead>
<tr>
<th>Subgroup</th>
<th>AI of Mn (mg/d)</th>
<th>Tolerated upper limit (TUL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men</td>
<td>2.3</td>
<td>11</td>
</tr>
<tr>
<td>Women</td>
<td>1.8</td>
<td>11</td>
</tr>
<tr>
<td>Pregnant women</td>
<td>2.0</td>
<td>-</td>
</tr>
<tr>
<td>Breastfeeding women</td>
<td>2.6</td>
<td>-</td>
</tr>
<tr>
<td>Children 4 - 8</td>
<td>1.5</td>
<td>3</td>
</tr>
<tr>
<td>Infants 1 - 3</td>
<td>1.2</td>
<td>2</td>
</tr>
<tr>
<td>Infants &lt; 1yr</td>
<td>0.6</td>
<td>-</td>
</tr>
</tbody>
</table>

Physiologic Role of Manganese

- Normal ranges of manganese in body fluids:
  - 4–15 µg/L blood
  - 1–8 µg/L urine
  - 0.4–0.85 µg/L serum

- Mn is an essential cofactor for enzymes involved in critical processes:
  - Mitochondrial and cytosolic respiration – Enolase, PEP kinase, etc
  - Carbohydrate, protein and fat metabolism
  - Bone mineralization and skeletal growth
  - Antioxidants – Superoxide Dismutase
  - Wound healing - Prolidase

- Susceptible groups include patients on TPN, IV drug users, IV contrast recipients, premature babies and those with renal or hepatic insufficiency

http://lpi.oregonstate.edu/mic/minerals/manganese#deficiency
Risk Assessment: To determine, quantitatively, exposures at which hazards pose risks to human populations.

Integrating evidence to understand the whole:
- Exposure science (critical to hazard and risk)
- Epidemiology (human studies)
- Toxicology (animal studies)
- Studies of disease mechanism and mode of action

Full value of epidemiology for risk assessment has not been realized.

Each line of evidence contributes to understanding; reduces uncertainties.
# Inhalation Exposure Standards and Guidelines

<table>
<thead>
<tr>
<th>AGENCY</th>
<th>LIMIT</th>
<th>LAST UPDATED</th>
</tr>
</thead>
<tbody>
<tr>
<td>OSHA Permissible Exposure Limit (PEL)</td>
<td>5 mg/m³ Ceiling</td>
<td>2007</td>
</tr>
<tr>
<td>NIOSH Recommended Exposure Limit (REL)</td>
<td>1 mg/m³ TWA 3 mg/m³ STEL</td>
<td>2005</td>
</tr>
<tr>
<td>ACGIH Threshold Limit Value (TLV)</td>
<td>0.02 mg/m³ (respirable) 0.1 mg/m³ (inhalable)</td>
<td>2007</td>
</tr>
<tr>
<td>ATSDR Inhalation Minimal Risk Level (MRL)</td>
<td>0.04 μg/m³ (chronic exposure)</td>
<td>2012</td>
</tr>
</tbody>
</table>

[https://www.osha.gov/dts/chemicalsampling/data/CH_250200.html](https://www.osha.gov/dts/chemicalsampling/data/CH_250200.html)
Health Effects of Mn Exposure

- The brain is the target organ for Mn toxicity in humans
- Regardless of route of exposure, neurotoxicity is the culmination of manganese accumulation in the body, whether due to high exposure or impaired clearance
- Therefore, a large proportion of studies to understand Mn toxicity have center around CNS toxicity
Neurotoxicity – Mode of Action (MoA)

- The mode of action (MoA) for Mn neurotoxicity has been well-characterized based on sufficient data.
- Physiologically based Pharmacokinetic (PBPK) models have been developed based on animal and human data.
- Various exposure states can be modelled.
- However, these are highly complex (see example!)
- Validation in Mn exposed populations needed.

Mn Homeostasis in the CNS

- Half-life of Mn longer in the CSF than in blood
- Physiological levels influenced by increased uptake or reduced elimination
- Mn(II) tightly bound to albumin, Mn(III) to transferrin
- Mn transport in and out of neurons is tightly regulated by several metal transporters in the CNS
- Once homeostasis in plasma is upset, transport increases to accumulation in neurons, leading to neurotoxicity

Molecular effects of Mn in the brain

Pre-synaptic neurons of the basal ganglia

Disruption of ATP synthesis

Impaired apoptosis

Dopamine (DA) auto-oxidation

Impaired DA release in the globus pallidus and putamen

Neurotoxicity and Mn Exposure Concentration

Dr Robinan Gentry’s team adapted Schroeter et al. (2011) primate MoA model to calculate dose to the brain in humans occupationally exposed:

- Compared NOAELs in the occupational cohorts to ambient human exposures
- Estimated the magnitude of the gap between ambient exposures and concentrations associated with health effects.
- Margins of Safety in target tissues:
  - 2500 to 5000 (eye-hand coordination)
  - 6000 to 12000 (hand steadiness)
- Small ambient concentrations of inhaled Mn are not expected to lead to CNS accumulation

<table>
<thead>
<tr>
<th>Respirable dust ( \mu g/m^3 )</th>
<th>Total ( \mu g/g ) in Globus pallidus</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.01</td>
<td>0.4</td>
</tr>
<tr>
<td>0.1</td>
<td>0.4</td>
</tr>
<tr>
<td>1</td>
<td>0.4</td>
</tr>
<tr>
<td>10</td>
<td>0.5</td>
</tr>
<tr>
<td>20</td>
<td>0.6</td>
</tr>
<tr>
<td>50</td>
<td>0.7</td>
</tr>
<tr>
<td>70</td>
<td>0.8</td>
</tr>
<tr>
<td>120</td>
<td>0.9</td>
</tr>
<tr>
<td>150</td>
<td>1.0</td>
</tr>
</tbody>
</table>


Table adapted from Dr RG’s presentation, with permission
## Range of Occupational Exposures and Observed Effects

<table>
<thead>
<tr>
<th>Study</th>
<th>Exp duration (years)</th>
<th>LOAEL (mg/m$^3$ of inhalable air)</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mergler 1994</td>
<td>16.7</td>
<td>0.032</td>
<td>decreased motor function</td>
</tr>
<tr>
<td>Blond and Netterstrom 2007</td>
<td>24</td>
<td>0.07</td>
<td>impaired fast pronation/supination; impaired fast finger tapping</td>
</tr>
<tr>
<td>Lucchini 1999</td>
<td>11.5</td>
<td>0.0967</td>
<td>decreased performance on neuro-behavioural exams</td>
</tr>
<tr>
<td>Iregren 1990</td>
<td>1-35</td>
<td>0.14</td>
<td>decreased reaction time, finger tapping</td>
</tr>
<tr>
<td>Lucchini 1995</td>
<td>1-28</td>
<td>0.149</td>
<td>decreased NB performance, finger tapping, symbol digit, digit span, etc.</td>
</tr>
<tr>
<td>Roels 1992</td>
<td>5.3</td>
<td>0.179</td>
<td>impaired visual time, eye-hand coordination, hand steadiness</td>
</tr>
<tr>
<td>Bouchard 2005</td>
<td>19.3</td>
<td>0.23</td>
<td>impairment in 3/12 cognitive tests, 1/19 neuro-motor tests 1/4 sensory tests</td>
</tr>
<tr>
<td>Bouchard 2007a</td>
<td>15.7</td>
<td>0.23</td>
<td>significantly higher scores for depression, anxiety</td>
</tr>
<tr>
<td>Bouchard 2007b</td>
<td>15.3</td>
<td>0.23</td>
<td>impaired performance on 1/5 NM tests</td>
</tr>
<tr>
<td>Roels 1987</td>
<td>1-19</td>
<td>0.97</td>
<td>altered reaction time, short term memory, decreased hand steadiness</td>
</tr>
<tr>
<td>Chia 1995</td>
<td>1.1-15.7</td>
<td>1.59</td>
<td>postural sway with eyes closed</td>
</tr>
</tbody>
</table>

Manganese Epidemiology: Exposure Response

- Since mid 1950’s, numerous occupational epidemiology studies of Mn exposures among welders, miners, ship builders, steel workers, battery workers, etc. have been published.

- Studies of low to moderate level Mn exposure suggest a continuum of exposure effects, many of which may be arrested or reversed.

- Possible outcomes include a range of subtle neurological defects:
  - cognitive (memory loss, personality changes, neuropsychological test performance)
  - motor (decrease in complex motor activities)

- Mn exposure concentrations ranged from 0.07 – 0.97 mg/m³, while duration of exposure varied between 1 – 24 years.

The precise level(s) and duration(s) of exposure to Mn at which risk of subtle neurotoxic effects increases remain elusive!

Manganese Epidemiology: Manganism

- Manganism is a neuropsychiatric condition that manifests after long-term, high level Mn exposure with rapid onset, stable course and occasionally, partial remission.

- Similar to, but distinct from, Parkinson’s (poor or no sustained response to levodopa).

- Early subjective symptoms include fatigue, anorexia, muscle pain, stiffness, personality changes and emotional lability.

- Progression:
  - Slow speech with loss of tone or inflection, mask-like facies, slow and uncoordinated movements.
  - Hypertonic muscles, tremors and dystonia, characteristic staggering gait (“cock-walk”).
  - Simultaneous bilateral and symmetric motor impairment; low amplitude, rapid tremor.

- Usually irreversible, and clinical symptoms may be progressive, even after exposure ceases.

MoA’s for Mn neurotoxicity have been reasonably identified, based on in-vitro and animal data and PBPK models.

Data available from epidemiology studies establish subtle effects at low exposures, and frank neurotoxicity and manganism at high exposures.

“An actual threshold level at which manganese exposure produces neurological effects in humans has not been established.” (ATSDR 2012)
**Significant Gaps and Challenges Remain**

- **Toxicology:**
  - Multiple routes of exposure – inhalation and ingestion
  - Essentiality vs. neurotoxicity
  - Determining role of dietary levels intake variability on biologically active target tissue dose remains a challenge

- **Epidemiology:**
  - Significant methodological limitations, especially in early studies
  - Cross-sectional studies do not allow estimation of time- and dose-dependent responses
  - Exposure assessment inconsistent across studies and time-periods (especially retrospectively)
  - Health outcomes may be subjective, non-specific, with no clear diagnostic criteria
Challenges (continued)

- Lack of directed therapeutic protocols:
  - No sensitive and specific screening protocol, no therapeutic targets identified

- Few reliable biomarkers
  - Blood levels are good in the short term (previous days to weeks), while hair and nails only suggest exposure in previous months or year
  - Bone – reliable indicator or long term accumulation and toxicity, but difficult to sample

- Non-interventional test biomarkers:
  - fDOPA PET to identify dopaminergic dysfunction not satisfactory
  - Relaxation rate of T1-weighted images are new approaches to identifying CNS accumulation, but have not been validated
  - Neurofunctional tests – inconsistent sensitivity, specificity and reproducibility

Hyperintense signal in the globus pallidus on T1-weighted MRI

Opportunities

- Quantitative evaluation of human health risks associated with moderate to low concentrations of Mn appears to be possible

- Improved indicators – including biomarkers – are increasingly available

- In light of major advancements in neurotoxicology, effects of specific pathways can be isolated and relevant outcomes examined epidemiologically

- Reliable detection of increasingly subtle neurological impacts will strengthen epidemiological validation of mathematical risk models
Concluding Remarks

- Much recent work has helped elucidate pathophysiology of manganese in humans.
- This has paved the way for future investigations of Mn as well as other neurotoxicants.
- In spite of the volume of investigations available on manganese toxicity and human health effects, inconsistencies in the integration and application of this evidence to regulatory policy persist.

Future directions:

- Apply updated methods in exposure characterization (including biomarkers of exposure and enhanced imaging technologies) and sensitive but objectives indicators of neurological response (including biomarkers of exposure) to new epidemiological studies.
- More fully integrate multiple lines of evidence to span remaining gaps and provide a reliable and scientifically sound basis for exposure regulation and controls.
Acknowledgements

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Manganese health effects on neurodevelopment and neurodegenerative diseases
Division of Occupational and Environmental Medicine, Mount Sinai School of Medicine, NY, USA
Sept 25-28 2016
http://events.mountsinaihealth.org/event/manganese2016

Talk by Dr Gentry: “A tissue dose-based comparative exposure assessment for manganese: The importance of homeostatic control for an essential metal”
Selected references - I


THANK YOU