Toxic effects of inorganic salts
Metals and other inorganics

Why are they of such concern?

• Widely distributed in the natural environment
• Non-biodegradable and persistent in the environment
• Neither created nor destroyed by humans
• Concentrated due to industrial use
• Global dispersion due to human use
Inorganic Arsenic in Drinking Water
“Natural Contamination”

Human Industry and Environmental Metals: Lead in Greenland Ice

(Modified From: Clarkson et al)
What is a Metal?

• Physical properties:
  – electrical conductivity
  – thermal conductivity
  – luster
  – deformed without cleavage under stress

• Chemical properties:
  – tendency to donate electrons (cationic)
  – formation of basic oxides
Types of “Metals”

Periodic Table of the Elements

- Alkali metals
- Alkaline earth metals
- Metalloids
- Other metals
- Transition (heavy) metals
- Inner transition metals (rare earths)
Essential Metals

• Examples of essential metal nutrients: Cu, Fe, Zn

• Examples of metal functions that are essential to life:
  – regulation of gene expression
  – DNA synthesis and repair
  – enzyme activity and structure
  – oxygen transport
Metals/Metalloids as Toxic Agents

- Essential metals have intentional accumulation, transport and storage mechanisms to prevent cellular damage
- Examples:
  - metallothionein for copper or zinc storage
  - transferrin and ferritin for iron transport and storage
Metals as Toxic Agents

• Exposure to toxic metals/metalloids generally results in disruption of enzyme systems
  – High affinity for sulfhydryl residues
    • Cysteine residues
  – Acute doses can result in disruption of ATP synthesis at the cellular level and ultimately cause death
Highly Toxic Inorganics

- Metals considered highly toxic include:
  - arsenic, beryllium, cadmium, chromium, lead, mercury, and nickel
- Many are potent neurotoxins (acute and chronic exposure)
  - e.g., lead
- Some inorganics are considered human carcinogens
  - Chronic exposure
Carcinogenic Metals/Metalloids

- Known human carcinogenic metals:
  - arsenic (skin, bladder, lung, liver)
  - beryllium (lung)
  - cadmium (lung)
  - chromium (lung, sino-nasal cavity)
  - nickel (lung, sino-nasal cavity)
Uptake of metals: Kidney as an example

Diagrammatic representation of putative mechanisms involved in the uptake of heavy metals (Cd, Hg and Pb) along the nephron. In the proximal tubule, many transporters of essential metals such as Zn2+ transporters may be involved in the uptake of the free form of toxic metals: zinc transporter 1 (ZnT1), Zrt/Irt-like protein (ZIP) and ATP-binding cassette protein (ABC protein). Reabsorption of metal conjugated with metallothionein (MT) and glutathione (GSH) could also participate in the renal uptake of Cd, Hg and Pb in this segment by endocytosis of the complexed conjugates or by transport of Cys-conjugates through the Na + -amino acid cotransporter after the degradation of GSH by the brush-border enzyme γ-glutamyltransferase. In the loop of Henle, the main transporter of metals is probably the divalent metal transporter 1 (DMT1), a divalent metal cotransporter coupled to proton transport. Paracellular pathways may be also involved in heavy metal transport along the proximal segment and the loop of Henle. In terminal segments (distal tubule and connecting ducts), the DMT1 and the stretch-activated channels (SACs) could play an important role in the uptake of ionized forms of Cd, Hg and Pb. Barbier et al. (2005)
General Mechanisms of Metal Toxicity

• direct binding to cellular components:
  • direct binding leading to dysfunction
    – enzyme inhibition, DNA adduction, etc.
  • direct binding leading to aberrant function
    – gene activation, receptor activation, etc.
  • direct binding through mimicry leading to displacement of essential metal:
    – adverse effect of released essential metal
    – disrupted homeostasis
General Mechanisms of Metal Toxicology

• disruption of normal cellular metabolism
  • leading to aberrant metabolism or altered homeostasis
  • frequently occur through atomic or molecular mimicry
  • examples:
    – disruption of essential metal metabolism
    – depletion of cofactors (e.g., S-adenosyl methionine)
    – depletion of GSH (could result in altered cellular redox status)
    – etc.
General Mechanisms of Metal Toxicology

• indirect attack on cellular components:
  • generation of radicals that attack cellular components
    – directly with redox active metals (eg. Ni, Cr, Cu, etc)
    – indirectly with metals that displace redox active essential metals (eg. Fe, Cu)
  • adverse effects of radical attack:
    – disruption of protein conformation leading to dysfunction
      » diminished or enhanced
      » oxidative DNA damage or base modification leading to aberrant gene expression or mutation
      » lipid peroxidation and membrane disruption
Metals as Toxic Agents

• Toxic metals often follow essential metals
  – Metabolic pathways
  – Transport pathways for cellular entry

• This “molecular mimicry” can
  – Occur with the ionic form
    • e.g., Cd$^{2+}$ cellular uptake via Ca$^{2+}$ channels or Zn$^{2+}$ transporters
  – In combination with an organic molecule
Molecular Mimicry

Bridges et al., Toxicol. Appl. Pharmacol., (2005), 204: 274-308
Molecular Mimicry with Metals: Uptake of Ionic Cadmium

![Bar graph showing cellular cadmium uptake with treatments](image)

- **Control**
- **NEM**
- **KCN**
- **Zinc**

![Diagram illustrating cellular uptake](image)

- **Cell**
- **Cd (+2)**
- **Zn (+2)**

**ATP**

**SH**
Molecular Mimicry with Metals: Uptake of Organomercurials

**Chemical Reaction:**

\[
\text{CH}_3\text{Hg}^+ + \text{S-CH}_2\text{-CH-COO}^- + \text{NH}_3^+ \rightarrow \text{CH}_3\text{Hg-S-CH}_2\text{-CH-COO}^- + \text{NH}_3^+
\]

**Diagram:**

- **Cell**
  - HgCH₃
  - Neutral amino acid carrier
  - Methionine

**Equation:**

- **Methylmercury + Cysteine**
- **Methylmercury-cysteine complex**
Cadmium

- Relatively rare metal present in the earth’s crust
- Occurs in only one valency state Cd$^{2+}$
- Used as
  - Protective coating on steel
  - Colored pigments in paints and plastics (bright yellow, orange and red)
  - Rechargeable nickel-cadmium batteries
  - Byproduct of burning fossil fuels (esp. coal)
- Exposure
  - workplace, food, cigarette smoke (1-2 µg/cigarette)
    - plants accumulate Cd in leaves
Cadmium

- accumulates in body over time - increases with age
  - 50 years of age kidney Cd concentrations
    - Smoker: 25 µg/g
    - Non-smoker: 12 µg/g
- Targets
  - Kidney – more on mechanism
  - Lung-emphysema
  - Bone
    - exposure associated with ↑ risk of osteoporosis, height loss, bone fractures
    - Cd interacts with osteoblast (bone forming cells) and increases bone resorption (maybe indirect effect on osteoclast)
    - Not accumulated in bone to any major extent
itai-itai disease

• itai-itai (“ouch-ouch”)
  – Cd contamination in Jinzu river basin by mining company
  – Atrophic kidney
    • Renal tubular dysfunction
      – ↑excretion of glucose, protein, β2-microglobulin and amino acids
      – Progresses to renal failure
  – Osteomalacia
  – Multiparous, postmenopausal women
Metallothionein

- small (6-7 kDa), cysteine rich, metal binding proteins
- major intracellular zinc binding proteins
  - zinc and copper homeostasis
- highly inducible
  - Cd, Zn, Cu
- sequestration of Cd
  - overwhelming induction in cells exposed to Cd
Factors Influencing Metal Toxicity: Metal-Binding Proteins

![Graph showing proportional survival (%) vs cadmium dose (nM) for metallothionein producing cells and metallothionein deficient cells.]
Metallothionein and Renal Toxicity

- Cd absorbed into body and initially accumulates in liver
- Cd-GSH complexes excreted into bile (subject to enterohepatic cycling)
- MT-Cd complexes formed and slowly leak into systemic circulation
- MT-Cd complexes then accumulate in kidney
- MT is protective but has a threshold or protection limit
  - Protects through sequestration of Cd within cells
  - Also causes accumulation
  - Rapid lysosomal degradation of Cd-MT complex
- Massive concentrations of Cd released -- toxicity
Mercury

- Exists in three chemical forms
  - Elemental (Hg$^0$) (liquid at RT, vapor)
  - Inorganic (Hg$^{1+}$, Hg$^{2+}$)
  - Organic (methyl, ethyl and phenyl mercury)
- Conversion of inorganic to methylated by anaerobic bacteria in soil/water
- Elemental – as a solid not readily absorbed at gut (0.01%), vapor can cross lung tissue
- Inorganic forms- 7-15% absorption
- Hg(CH$_3$)$_2$ – 90-95% absorbed
Mercury Exposure Sources

- Non-anthropogenic sources highest
  - Natural degassing of earth’s crust
- Burning of fossil fuels
- Pulp and paper mill effluent
- Mining
- Dental amalgam
- Organic mercury highly lipid soluble
  - bio-concentrates in food chain
  - especially marine
Mercury Poisoning Case

- Industrial discharge of mercury into water fairly common
  - Thought to be innocuous
  - Sink and remain bound to sediments
    - Methyl mercury produced by microorganisms...bioaccumulation

- Minamata disease
  - Japan: chemical company in Minamata Bay used inorganic Hg compound in a chemical synthesis
  - Unaware that process resulted in production of organomercurial, discharged into bay-bioaccumulation in marine animals consumed regularly by local population
Minamata Disease

- Degenerative neurological disorder, characterized by burning or tingling sensations, poor articulation of speech, and the loss of coordination and peripheral vision.
- 900 people died (1956) and ~2 million people affected
- Fetal nervous system had extra susceptibility to the toxic effects
  - mental retardation, cerebral palsy, seizures, death
Elemental and Inorganic Mercury Toxicity

- Hg$^0$ vapor
  - Bronchitis, interstitial pneumonitis
  - Contact with tissue results in oxidation to mercuric ion Hg$^{2+}$

- Hg$^{2+}$ and Hg$^+$
  - Severe intestinal upset
  - Kidney toxicity
    - Acute tubular necrosis
    - Immunologic glomerulonephritis
    - Nephrotic syndrome
  - CNS
    - Chronic exposure, permanent damage
    - “Mad as a Hatter”
      - Workers exposed to mercury nitrate


**Valence:** Pb$^{2+}$, Pb$^{4+}$

- Sulfides not easily absorbed
- Oxides are
Lead Absorption and Distribution

- GI absorption
  - Adults 5-15%, retain 5%
  - Children 40%, retain 32%
    - Pb crosses enterocyte membrane through Ca (and Fe\(^{2+}\)) uptake systems
    - Ca\(^{2+}\) uptake mechanisms upregulated during growth
- Children tend to be exposed to higher levels of Pb due to ↑ “hand to mouth” contact
- Pulmonary
  - ~90% of Pb in outdoor air small enough to enter alveoli
- 90% of Pb absorbed is distributed to the red blood cells (half-life 30 days)
- Eventually redistributes to bone (half-life 30 years)
Progressively lower levels of lead in blood post-leadèd gasoline ban in US population

- Current mean blood lead levels around 2-3 µg/dL
Current Sources of Lead

Despite reduced blood levels due to unleaded gasoline—still toxicity issues esp. for children

- Food
- Drinking water from pipes with Pb solder (especially if pH<6.5)
- Paint in houses built before 1978
- Dishes and crystal
- Soil and air near factories which use Pb
- Vinyl toys
- Mini-blinds
- Playground equipment
Chronic Toxic Effect of Lead

- Gastrointestinal: affects smooth muscle, anorexia, muscle discomfort, constipation, intestinal spasm, severe abdominal pain, or *lead colic*
- Renal: proteinuria, hematuria, and casts in the urine, histologically lead nephropathy has characteristic nuclear inclusion body


- Hypertension? (adults)
Chronic Toxic Effect of Lead

- Neurotoxicity
  - Most common in children
  - >80 µg/dl severe brain edema, ataxia, convulsions, death
  - 50-70 µg/dl ↓ cognitive abilities-intelligence, speech, language processing----persistent symptoms
    - Possibly related to interference with neurotransmission
    - Fetal brain appears more susceptible
      - Pb mobilized from bone during pregnancy and lactation
Hematological Effects

- Basophilic stippling (ribonucleic acid accumulation)
- Hypochromic microcytic anemia

\[ \text{Succinyl CoA + Glycine} \]
\[ \xrightarrow{\delta\text{-aminolevulinate synthase}} \]
\[ \delta\text{-Aminolevulinate (\(\delta\text{-ALA}\))} \]
\[ \xrightarrow{\delta\text{-aminolevulinate dehydratase}} \]
\[ \text{Porphobilinogen} \]
\[ \xrightarrow{\text{porphobilinogen deaminase}} \]
\[ \xrightarrow{\text{uroporphyrinogen III cosynthase}} \]
\[ \text{Uroporphyrinogen III} \]
\[ \xrightarrow{\text{uroporphyrinogen decarboxylase}} \]
\[ \text{Coproporphyrinogen III} \]
\[ \xrightarrow{\text{coproporphyrinogen oxidase}} \]
\[ \text{Protoporphyrin IX} \]
\[ \xrightarrow{\text{ferrochelatase} + \text{Fe}^{2+}} \]
\[ \text{Heme} \]

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<th>Action produced by lead:</th>
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<tr>
<td>Inhibition</td>
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<td>Postulated inhibition</td>
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Arsenic

- Acute doses commonly associated with homicide
- Naturally distributed in the environment (soil, air, water)
  - predominantly found in inorganic forms:
    - arsenite (As\text{\textsubscript{III}})
      - More reactive (high affinity for thiol groups)
      - Acute toxicity through inhibition of enzymes, GSH depletion
    - arsenate (As\text{\textsubscript{V}})
      - Mimics phosphate, uncouples oxidative phosphorylation

Figure 4
Proposed pathways of transporters for uptake and efflux of arsenites and enzymes responsible for arsenic excretion into extracellular space in hepatocytes. iAs\textsubscript{III}, inorganic arsenite; MMeAs\textsubscript{III}, monomethylarsonous acid; As(SG)\textsubscript{3}, arsenite trithiokollathione; MMeAs(SG)\textsubscript{2}, monomethylarsonic dithiothiolate; Cyt19, arsenic methyltransferase; γGCS, γ-glutamylcysteine synthase; GSTs, glutathione S-transferases; GSH, glutathione; AQP9, aquaglyceroporin 9. Proteins (green) are regulated by Nrf2.

Arsenic

- Metabolized to organic forms: monomethyl and dimethylated forms
  - Originally thought to be detoxification products but trivalent forms more toxic than even \( \text{As}^{3+} \)
- Human exposure occurs throughout the world
  - Latin America and Asia
- Common natural contaminant of drinking water
  - most important exposure route

- Bangladesh (and other countries)
  - attempt to provide “safe” drinking water tube wells: ↓microbial contamination ↑As contamination
- present in most foods but concentrations are low or poorly absorbed/easily excreted forms
  - High levels of arseno sugars in shellfish, shrimp
  - Excreted unchanged in urine
Anthropogenic Arsenic Sources

• Released during smelting of copper, zinc, lead
• Used in pesticides and herbicides
• Coal burning
• Computer chips
• Semi-conductors
• Pressure treated lumber

Clinical Use of Arsenic

• neoplasia
  – acute promyelocytic leukemia
• protozoal infections
  – African trypanosomiasis (sleeping sickness)
• syphilis
• psoriasis
Arsenic Carcinogenicity

- An established human carcinogen
- Multi-target
  - tumors of skin, lung, bladder, liver, kidney
- Inorganic arsenic medicinal use linked to skin cancer as early as 1888
- Mechanism still unclear
  - Complex and multiple
Chronic Arsenic Exposure….NOT Only a Carcinogen

- **Cardiovascular**
  - Hypotension
  - Congestive heart failure
  - Cardiac arrhythmias
  - Peripheral vascular disease
    - gangrene of the extremities (especially of the feet)
    - often referred to as blackfoot disease
  - Myocardial damage

- **Gastrointestinal**
  - Watery diarrhea gradually progresses to bloody diarrhea
    - Capillary effects and inhibition of normal cellular proliferation

- **Skin**
  - High thiol content of keratin—binding and retention of As
  - Diffuse spotted hyperpigmentation
  - Hyperkeratosis on palms and soles
  - Cutaneous vasodilation
  - Eventually leads to skin tumors

- **Kidney**
  - Action on renal capillaries, tubules, and glomeruli may cause severe renal damage.
  - Tubular necrosis and degeneration
    - Oliguria with proteinuria, hematuria, and casts

- **Liver**
  - Fatty infiltration, central necrosis, and cirrhosis
  - Mild to severe (death)
  - Injury is generally to the hepatic parenchyma
  - But may closely resemble occlusion of the common bile duct
Chromium

**Cr(III)**
**Cr(VI)**

Chromium

- Reactive oxygen species
  - Lipid peroxidation
  - Direct DNA damage
  - Mutation
    - Oncogene expression
    - Up-regulation of growth factors
    - Excess cell growth
    - Cancer

Chromium VI

1. Incomplete Conversion to Cr III in Stomach
2. Absorption
   - Plasma/RBC Levels
3. Some Uptake into Tissues
4. Leakage from Tissues (Reservoir)
5. Excretion - Prolonged Plasma and Urinary T_{1/2}

Chromium III

1. Cr III in Stomach
2. Minimal Absorption, Minimal Change Plasma/RBC Levels
3. Minimal Uptake into Tissues
4. No Leakage from Tissues (No Reservoir)
5. Excretion - Short Plasma and Urinary T_{1/2}

Toxicokinetic model—hexavalent chromium versus trivalent chromium. Sedman et al. 2006
Figure 6. Formation mechanisms and biological effects of Cr–DNA adducts. Asc, ascorbate; Cys, cysteine; GSH, glutathione; and L, ligand (ascorbate, cysteine, glutathione, or histidine).

A. Dose-Response: Chromate with UVR

B. Chromate Enhancement of UVR

- UV alone
- 0.5 ppm K2CrO4 + UV
- 2.5 ppm K2CrO4 + UV
- 5 ppm K2CrO4 + UV

Average UVR dose = 1.18 kJ/m² / exposure
Treatment for Metal Intoxication

• Intervention to prevent or reverse the adverse effects of metal exposure is sometimes indicated
• Most common class of agents used: Chelators
• Form metal ion complexes that are then excreted
• Ideal agent:
  – specific
  – resistant to biotransformation
  – form non-toxic complexes
  – able to reach metal storage sites
**Common Chelators**

- Calcium disodium EDTA
  - Pb
- Pentetic acid (DTPA)
  - Similar to CaNa$_2$EDTA, higher affinity
- Dimercaprol (BAL)
  - Developed during WWII to protect against arsenic gas
  - Useful for Hg and Pb
- Succimer
  - As, Cd, Hg, Pb
  - Less toxic, less mobilization of essential metals
- Penicillamine
  - Cu (Wilson’s disease), Hg, Pb
Problems With Chelation

• Chelation does not work with all metals
• Can exacerbate toxicity
  – Increased urinary excretion = increased renal exposure
• Major drawback is depletion of essential metals
• All have toxic side effects
• Often only slow progression
  – Multiple doses required