Melamine
Pet Food, Infant Formula, and More

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Melamine

• Mugshot:

\[
\begin{array}{c}
\text{NH}_2 \\
\text{N} \\
\text{H}_2\text{N} \\
\end{array}
\]

• triazine ring with three amine groups
• \( \text{C}_3\text{H}_6\text{N}_6 \)
• 66% nitrogen by mass
Uses/Exposure

- Melamine used commercially since late 1930s
- In production of polymer resins (plastic) and polymeric agents (e.g., laminates, glues, adhesives, molding compounds, coatings, flame retardant)
- In these products -- melamine in a polymer matrix – user has no contact with melamine by itself
Exposures (cont’d)

• Melamine resin polymer gives durable, semi-heat resistant plastic - popular use tableware

• Tableware tested. Found melamine only leaches out at prolonged high temps and acidic conditions (30 mins, 203⁰F, pH=2-5) – very small amounts leach out
Other potential exposures

• Crop insecticide (Cyromazine)
  • metabolized on plants by microorganisms to melamine
  • only small amounts melamine residue

• Trichloromelamine
  • used as food equipment sanitizer
  • very small amount decomposes to melamine

• Fertilizer
  • melamine added to control the rate that nitrogen seeps into the soil
  • not approved for this use in the U.S.
Melamine Exposure

• Consensus is that exposure to melamine by the consumer and general public is very low.
Pharmacokinetics of melamine

• Numerous animal studies
  – Passes through the body un-metabolized
  – Almost all excreted through the kidneys

• No data are available in humans
  – But no reason to believe also passes un-metabolized through kidneys in humans
Toxicity studies of melamine

- National Toxicology Program (NTP) (1983)
  - Rats and mice
  - Melamine in diet
  - Acute toxicity: very low
  - Subchronic (13 wk) and chronic (103 wk)
    - bladder epithelial hyperplasia and ulceration
    - bladder stones
    - kidney inflammation (chronic only)
  - Cancer (chronic exposure)
    - transitional cell carcinomas (urothelial carcinomas) - bladder
    - only in male rats
    - only at highest dose (4500 ppm in feed)
    - carcinomas statistically associated with stones
Other melamine toxicity studies

- Pigs, sheep, fish
- Findings consistent with NTP study
  - Effects isolated to urinary tract
  - Inflammation, crystals, stones
- Dose dependent
Other tox studies - melamine

- not irritating to skin or eye
- not sensitizing
- not teratogenic
- not genotoxic
General consensus on melamine exposure and toxicity until 2007

• Exposure
  – from monitoring and models
  – general public - considered to be very low

• Toxicity
  – melamine considered to have low toxicity
Pet Food Poisoning Outbreak

- North America 2007, dogs and cats
- Acute renal failure within hours of consuming pet food
- Estimated morbidity in 1000’s, deaths in 100s
- Crystals in urine
- Animals that died: yellowish-brown crystals in renal tubules
Pet Food Poisoning (cont’d)

• Numerous brands pet foods, all traced to one manufacturer who had recently switched to a wheat gluten ingredient from China
• Pet food initially analyzed for mycotoxins, metals, pesticides – nsf
• Then analyzed for small molecules – melamine found
• Largest FDA recall pet food
The Great Pretender

• How did melamine get into pet food??
• Foods - protein levels not directly measured - instead nitrogen level used
• Melamine nitrogen-rich (66% N by weight) so adding melamine to a food will falsely increase apparent protein level of that food
• In China, melamine had been added to gluten and rice protein concentrate to increase apparent protein levels
• Gluten or concentrate used as pet food ingredient
Pet food poisoning question

• Why such high morbidity and mortality in pets when melamine had been considered to have very low toxicity?

• Analysis of pet food and gluten samples also found (in addition to melamine):
  • cyanuric acid
  • ammeline
  • ammelide
Melamine’s Family Tree

Melamine → Ammeline

Cyanuric Acid ← Ammelide
Toxicity due to one of melamine analogues?

• Ammeline and ammelide
  • Little toxicity data
  • Used with other chemicals in polymers, etc
Cyanuric acid

• Tox studies in rats, mice, dog
  • Results similar to melamine
  • Acute renal effects only at very high doses
  • Subchronic and chronic exposures, high doses resulted in bladder stones

• Potential exposure
  • Dichloroisocyanurates - used as disinfectant in swimming pools
  • Dissociates to cyanuric acid
Pet Food Poisonings

• Pet Food Poisoning Asia 2004
  • Clinical signs similar to N.Am 2007
    • Acute renal failure, uremia
  • 6000 dogs, smaller number of cats
  • Had been attributed to mycotoxin
• Both Asia (2004) and North America (2007) incidents
  • Animals that had had renal failure evaluated
  • Crystals and stones found in kidney and bladder
  • But crystals not composed of melamine alone – instead melamine cyanurate
Melamine cyanurate

- Melamine forms hydrogen bonds with cyanuric acid to form melamine cyanurate

- Note: still available amine group, carbonyl group
Melamine cyanurate

• Highly organized lattice crystal structure
Dilated distal tubule contains a cluster of round green melamine/cyanuric acid crystals with radiating spokes and concentric striations (arrow)
Melamine cyanurate

- Toxicity studies
  - Dogs, cats, rats, pigs, fish
  - Mixture of melamine plus cyanuric acid
  - In feed
  - Found to be much more toxic than feeding either melamine or cyanuric acid alone
Melamine cyanurate

- Melamine cyanurate much less soluble in water than either melamine or cyanuric acid alone

<table>
<thead>
<tr>
<th>Melamine</th>
<th>Cyanuric acid</th>
<th>Melamine cyanurate</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.1 g/L</td>
<td>2 g/L</td>
<td>0.01 g/L</td>
</tr>
</tbody>
</table>
Why does melamine cyanurate not precipitate before reaching kidney tubules?

• “Melamine - cyanuric acid complex” identified in food
• Complex stable in gluten and pet food
• Due to low pH of stomach, melamine and cyanuric acid dissociate
• Probable absorption of cyanuric acid in stomach and melamine in small intestine
  – cyanuric acid pKa = 6.9
  – melamine pKa = 5
• Reform complex in renal tubules → crystals
Hypotheses for precipitation in kidney

• critical levels melamine and cyanuric acid needed for precipitation
• increased concentration melamine and cyanuric acid as move down osmotic gradient in kidney
Sources of cyanuric acid in melamine-tainted food

• Hypothesis 1: Melamine in food broken down by microorganisms to cyanuric acid
  • Unlikely since many foods processed at high temps and under hygienic conditions

• Hypothesis 2: Use of impure melamine
  • More likely - melamine produced cheaply from coal -- can result in “melamine scrap” that contains cyanuric acid in addition to melamine

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Infant Formula 2008

- First report, China, September 2008
- Infants: thousands ill, four deaths
- Renal stones, hematuria, uremia, renal failure
- Linked to consumption of infant formula
- Formula found to contain up to 2500 ppm melamine
- It was later found that milk suppliers had diluted milk and added melamine to boost protein content
Melamine in foods

• Do know that melamine added to increase apparent protein level in
  • milk (probably powdered)
  • gluten (corn, wheat)
  • protein concentrate
• These tainted products then used as ingredients for end-product food
## Melamine in foods (cont’d)

<table>
<thead>
<tr>
<th>Food</th>
<th>Max ppm</th>
<th>Probable Source</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infant formula</td>
<td>2563</td>
<td>Tainted powdered milk</td>
<td>Chinese manufacturers</td>
</tr>
<tr>
<td></td>
<td>0.14</td>
<td>Tainted powdered milk or sanitizer</td>
<td>U.S. manufacturer (1)</td>
</tr>
<tr>
<td>Other food products</td>
<td>6.8</td>
<td>Tainted powdered milk</td>
<td>e.g., cookies, ice cream, beverages, crackers, candy</td>
</tr>
<tr>
<td>Ammonium bicarbonate</td>
<td>2470</td>
<td>Probably due to cross contamination in facility that manufactures both</td>
<td>Leavening agent</td>
</tr>
<tr>
<td>Eggs</td>
<td>4.6</td>
<td>Tainted animal feed</td>
<td></td>
</tr>
</tbody>
</table>
Chinese infants ill from tainted formula

- Guan et al. (2009) identified risk factors for renal stones
  - Preterm
  - Higher levels melamine in formula
- Sun et al. (2008) examined composition of stones (14 stones examined)
  - 3:2 molar ratio uric acid to melamine
  - cyanuric acid, ammeline, ammelide – not detected
Why uric acid and not cyanuric acid in formula poisonings?

• Cyanuric acid not in formula?
  • No reports of cyanuric acid detected in (Chinese) formula but not sure if formula tested for cyanuric acid
  • Sun et al. specifically reported cyanuric acid not found in stones
  • So evidence suggests no cyanuric acid in formula but not definitive
Uric acid in humans vs. cats, dogs

• In most mammals uric acid metabolized via uricase to allantoin

• Exceptions:
  • Higher primates, including humans
  • Dalmations
## Uric Acid in mg/dL

<table>
<thead>
<tr>
<th></th>
<th>Serum</th>
<th>Urine</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Human</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infant</td>
<td></td>
<td></td>
</tr>
<tr>
<td>low-birthwt neonate</td>
<td>5.8</td>
<td></td>
</tr>
<tr>
<td>low-birthwt 11-mos age</td>
<td>6.0</td>
<td>86</td>
</tr>
<tr>
<td><strong>Child</strong></td>
<td></td>
<td>2.0 – 6.5</td>
</tr>
<tr>
<td>Adult</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>3.6 – 7.3</td>
<td>45 +/- 18</td>
</tr>
<tr>
<td>Female</td>
<td>5.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.3</td>
<td></td>
</tr>
<tr>
<td><strong>Cats</strong></td>
<td>0.0 – 0.7</td>
<td>6.3</td>
</tr>
<tr>
<td><strong>Dogs</strong></td>
<td>0.0 – 1.0</td>
<td>~2 - 12</td>
</tr>
</tbody>
</table>
Age related susceptibility to melamine?

• Older ages consumed non-formula foods containing melamine – no acute effects
• Why such high morbidity in infants?
• Infants’ increased exposure
  • Greater calories consumed per bodywt
  • Formula is primary or sole source of nutrition for young infants
• Infants’ increased susceptibility
  • Infants have greater urinary uric acid levels relative to older ages (next slide)
# Uric acid excretion by age

<table>
<thead>
<tr>
<th>Age</th>
<th>29-33 wks</th>
<th>38-40 wks</th>
<th>5-9 yrs</th>
<th>adults</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum uric acid (mg/dL)</td>
<td>7.7</td>
<td>1.7</td>
<td>3.7</td>
<td>5.1 (males) 4.3 (females)</td>
</tr>
<tr>
<td>Fractional excretion uric acid (%)</td>
<td>61%</td>
<td>38%</td>
<td>10%</td>
<td>7%</td>
</tr>
<tr>
<td>Urine uric acid (mg/dL)</td>
<td>86</td>
<td></td>
<td></td>
<td>45</td>
</tr>
</tbody>
</table>
Infant susceptibility (cont’d)

- Smaller renal tubular and blood vessel lumens
  - easier irritation of tubular walls
  - occlusion of tubular lumens
  - compression of blood vessels by clumped crystals (stones) – more easily limit blood flow
- Lower glomerular filtration rate vs. older ages
  - takes longer to filter metabolic waste and toxic substances
Other melamine co-crystals

- Co-crystallization of melamine with ammeline or ammelide is not as structurally strong as melamine with uric acid or melamine with cyanuric acid.
Summary

• Exposure via intentionally tainted food – public health measures in place so future outbreaks involving melamine unlikely

• Highlights importance of mixtures
  1. Exposure to an exogenous mixture
  2. In-vivo mixture of exogenous substance (e.g., melamine) and physiological substance (e.g., uric acid)

• What are unique characteristics of subpopulations?
• Expect the unexpected?