Melamine
Pet Food, Infant Formula, and More

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Melamine

- $\text{C}_3\text{H}_6\text{N}_6$
- 66% nitrogen by mass
- triazine ring with three amine groups
- Mugshot:
Uses

• Melamine used commercially since late 1930s
• In production of polymer resins and polymeric cleaning agents
  • examples: laminates, glues, adhesives, moulding compounds, coatings, flame retardants
  • with formaldehyde in polymer resin plastic

Potential Exposures

• consumer exposure to melamine via these products is only with melamine in a polymer matrix – no contact with melamine alone
Potential exposures

- Melamine in 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, 203ºF, pH=2-5)
Other potential exposures

• Crop insecticide (cyromazine)
  • metabolized by microorganisms to melamine on plants
  • on most crops very little melamine residue
  • melamine residue always less than cryomazine residue (max residue level for cyromazine)
• 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.
Pharmacokinetics of melamine

- Numerous animal studies
- Passes through the body un-metabolized
- Almost all excreted through the kidneys
- No data are available in humans
Toxicity studies of melamine

• National Toxicology Program (NTP) (1983)
  • Rats and mice
  • Melamine in feed
  • Acute: LD$_{50}$ quite high
  • Subchronic (13 wk) and chronic (103 wk)
    • bladder epithelial hyperplasia and ulceration
    • bladder calculi
    • 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)
      • statistically associated with calculi
Other melamine toxicity studies

• Pigs, sheep, fish
• Findings consistent with NTP study
  – Effects isolated urinary tract
  – inflammation, crystals, calculi
• 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

• from monitoring and models:
  – exposure of general public to melamine is considered to be very low

• 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 brand pet foods, all traced to one manufacturer contracted by all
• Manufacturer recently switched to wheat gluten ingredient from China
• Analyzed pet food for mycotoxins, metals, pesticides - nsf
• Analysis for small molecules – melamine identified
• 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 so adding melamine will falsely increase apparent protein level of 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 wheat gluten samples found in addition to melamine:
  • cyanuric acid
  • ammeline
  • ammelide
The Family Tree

Melamine → Ammeline

Cyanuric Acid ← Ammelide

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Toxicity due to one of melamine analogues?

- Ammeline and ammelide
  - Little 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 calculi

• 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 with renal failure evaluated
  • Crystals and calculi 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 study
  - Experimental feeding
  - Mixture of melamine plus cyanuric acid
  - Found to be much more toxic than feeding either melamine or cyanuric acid alone
  - Dogs, cats, rats, pigs, fish
Melamine cyanurate

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

<table>
<thead>
<tr>
<th></th>
<th>Melamine</th>
<th>Cyanuric acid</th>
<th>Melamine cyanurate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration</td>
<td>3.1 g/L</td>
<td>2 g/L</td>
<td>0.01 g/L</td>
</tr>
</tbody>
</table>

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Why does melamine cyanurate not precipitate before reaching kidney tubules?

• “Melamine - cyanuric acid complex” identified in food
• Complex stable in gluten and pet food
• 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 is more likely
  - Melamine produced cheaply from coal -- can result in “melamine scrap” that contains ammeline, ammelide, cyanuric acid
Infant Formula 2008

- First report, China, September 2008
- Infants: thousands ill, four deaths
- Renal calculi, 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 plant 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 calculi
  • Preterm
  • Higher levels melamine in formula
• Sun et al. (2008) examined composition of calculi (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
  - 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>86</td>
</tr>
<tr>
<td>low-birthwt 11-mos age</td>
<td>6.0</td>
<td></td>
</tr>
<tr>
<td><strong>Child</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.0 – 6.5</td>
<td></td>
</tr>
<tr>
<td><strong>Adult</strong></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><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)</td>
</tr>
<tr>
<td>(mg/dL)</td>
<td></td>
<td></td>
<td></td>
<td>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></td>
<td>86</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 not as structurally strong as with uric acid or cyanuric acid

![Molecular structures](image)
Summary

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

• Mixtures
  • Exposure to mixtures
  • In-vivo mixture of exogenous substance (e.g., melamine) and physiological substance (e.g., uric acid)

• What are unique characteristics of subpopulations?
• Mechanism of toxicity and relevance to other chemicals