Melamine

Pet Food, Infant Formula, and More

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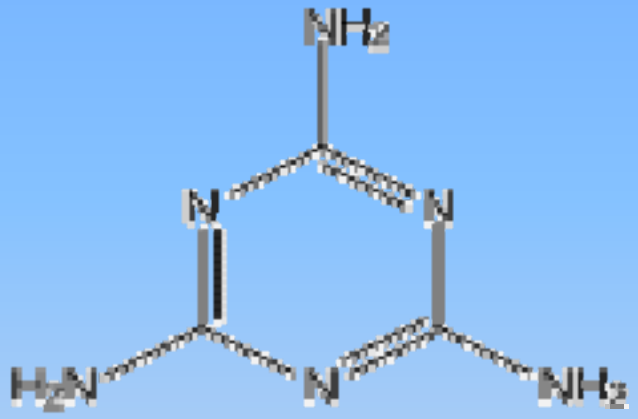
Amy Arcus-Arth

Office of Environmental Health Hazard Assessment, Cal/EPA

Melamine

- Mugshot:



- triazine ring with three amine groups
- $C_3H_6N_6$
- 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, 203°F, 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

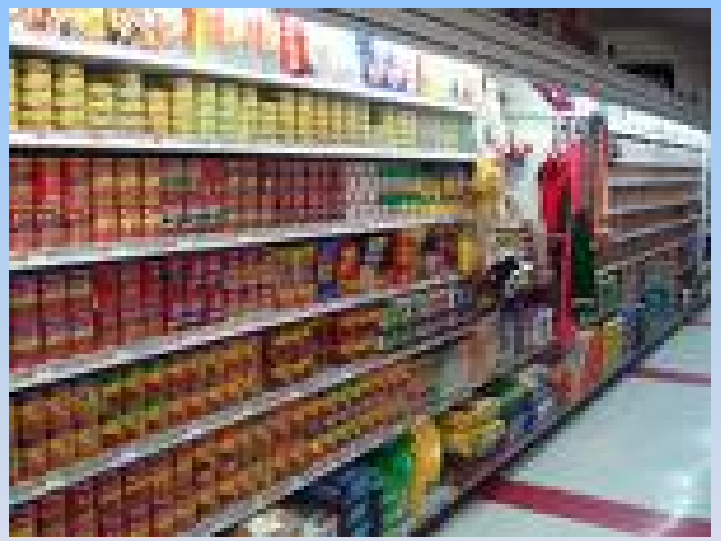
- North America 2007, dogs and cats
- Acute renal failure within hours of consuming pet food

- 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



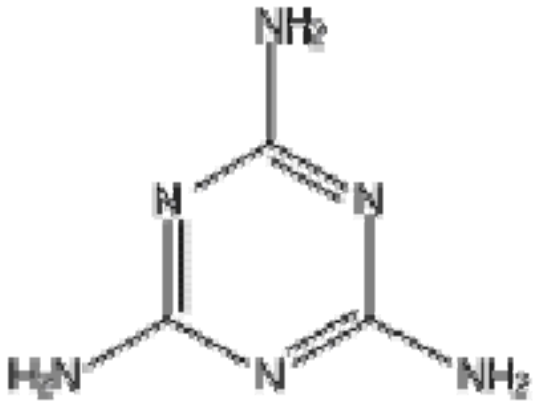
The Great Pretender

- 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

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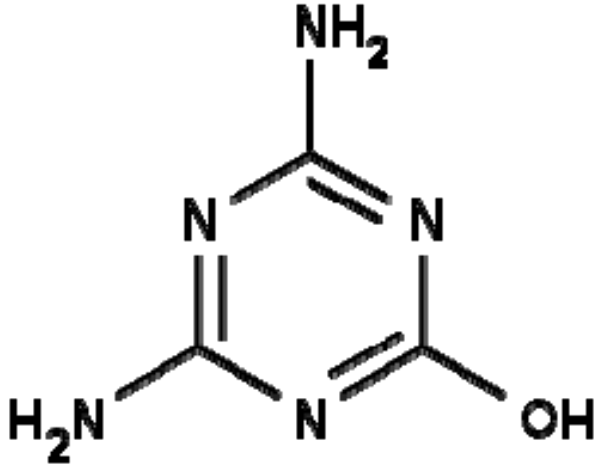

Ammeline

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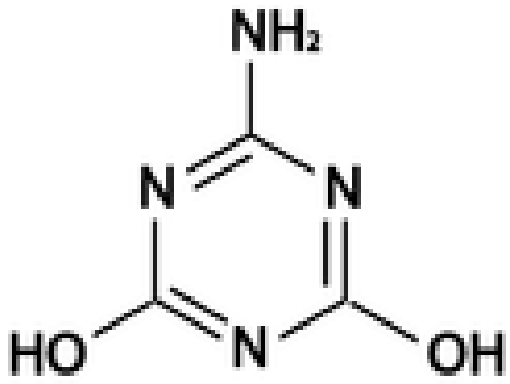

Ammelide

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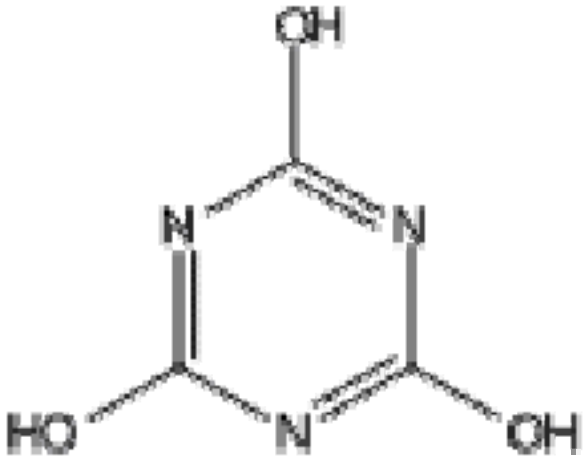
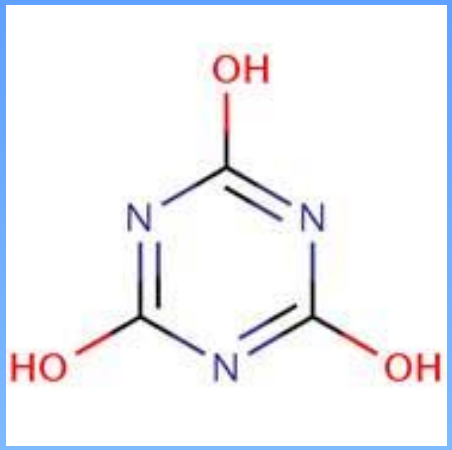


Cyanuric Acid

Toxicity due to one of melamine analogues?

- Ammeline and ammelide
  - Little toxicity data
  - Used with other chemicals in polymers, etc

Cyanuric acid



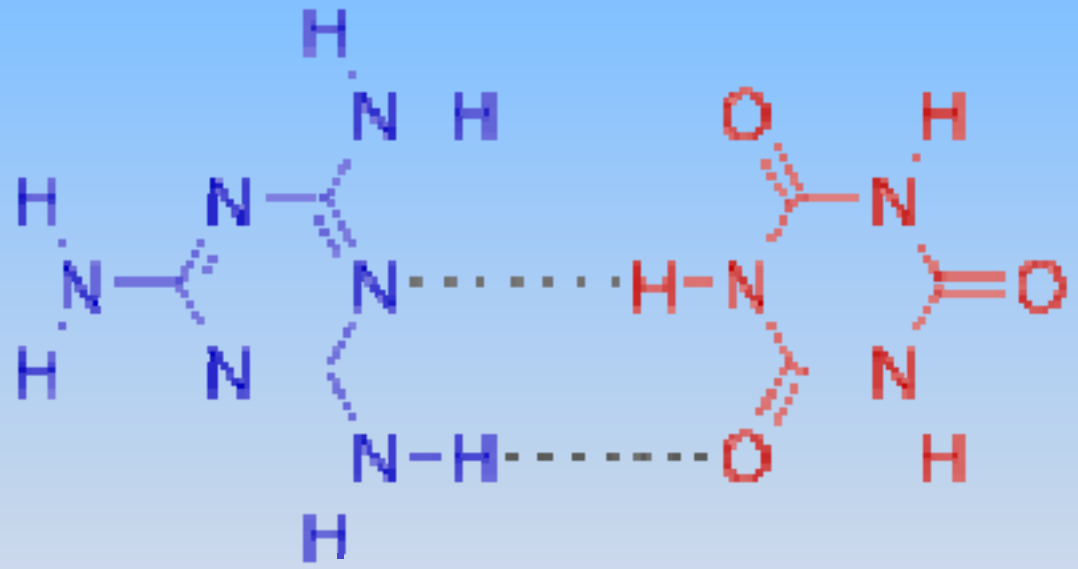
- Tox studies in rats, mice, dog
  - Results similar to melamine
  - Acute renal effects only at very high doses
  - Subchronic and chronic exposures, high doses resulted in bladder stones
- Potential exposure
  - Dichloroisocyanurates - used as disinfectant in swimming pools
  - Dissociates to cyanuric 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

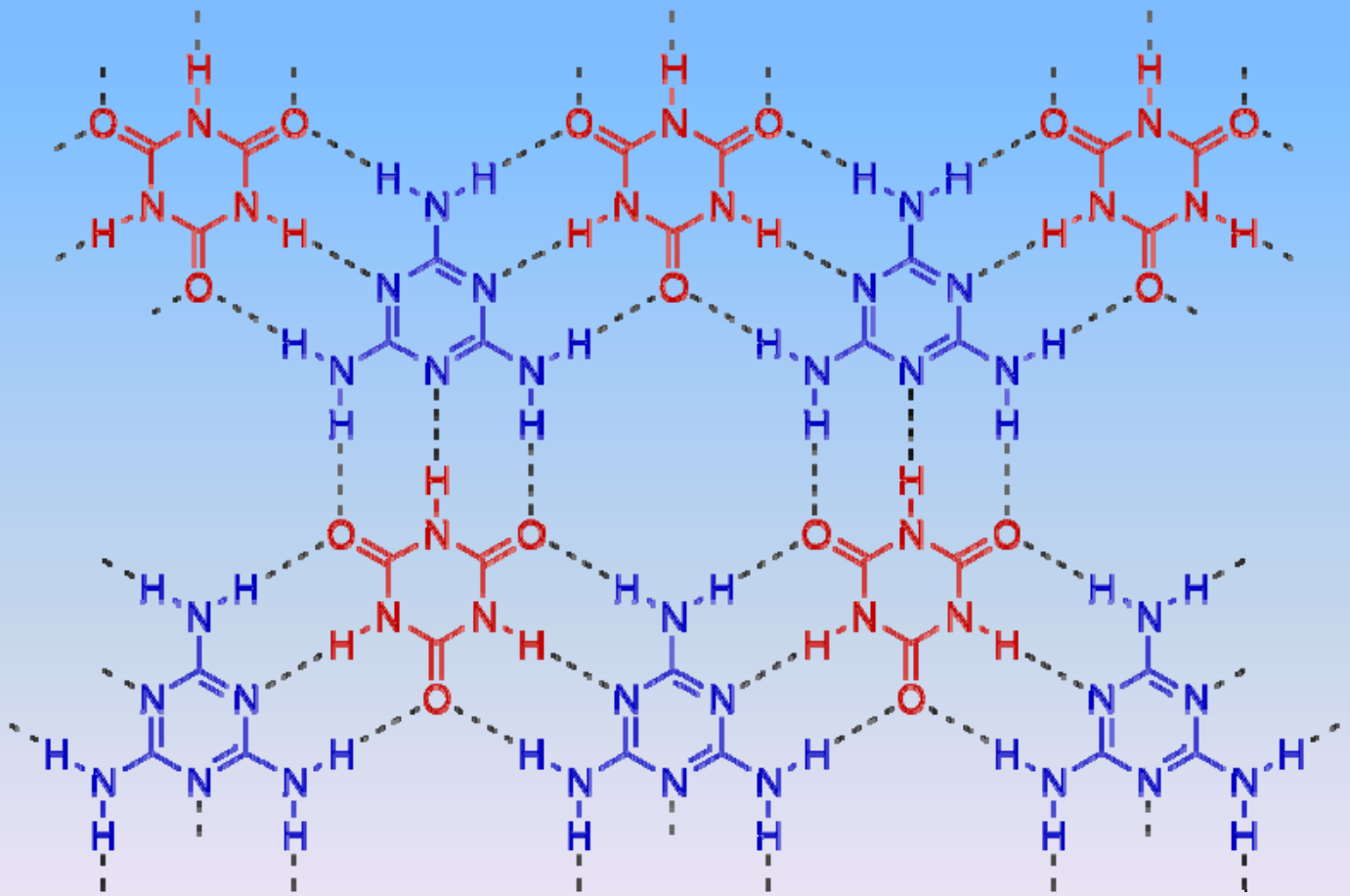
- Melamine forms hydrogen bonds with cyanuric acid to form melamine cyanurate



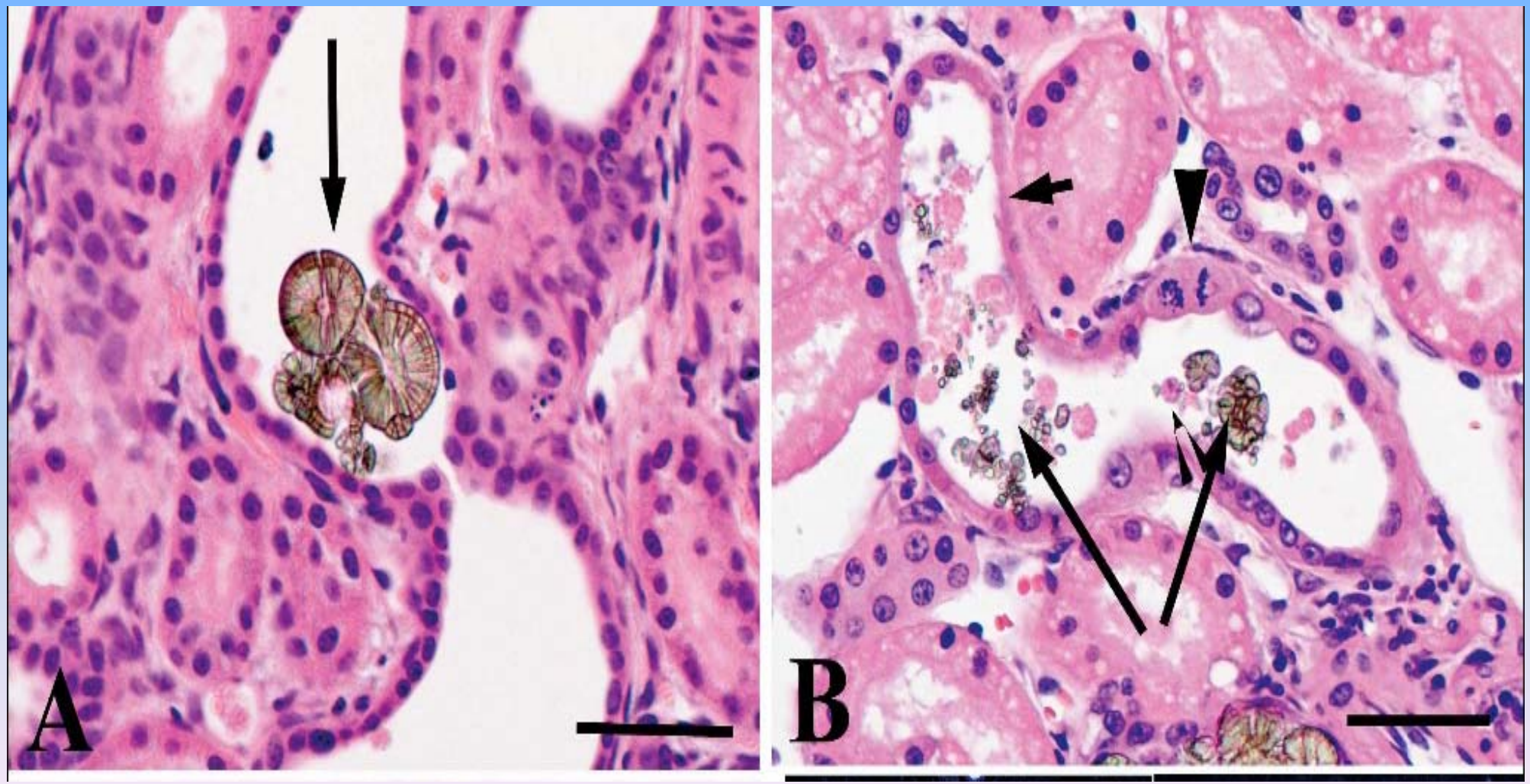
- Note: still available amine group, carbonyl group

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

Melamine	Cyanuric acid	Melamine cyanurate
3.1 g/L	2 g/L	0.01 g/L

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

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)

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

Chinese infants ill from tainted formula

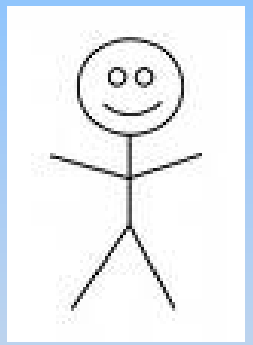
- Guan et al. (2009) identified risk factors for renal stones
  - Preterm
  - Higher levels melamine in formula
- Sun et al. (2008) examined composition of stones (14 stones examined)
    - **3:2** molar ratio **uric acid to melamine**
    - cyanuric acid, ammeline, ammelide – **not** detected

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

	Serum	Urine
Human		
Infant		86
low-birthwt neonate	5.8	
low-birthwt 11-mos age	6.0	
Child	2.0 – 6.5	
Adult	3.6 – 7.3	45 +/- 18
Male	5.1	
Female	4.3	
Cats	0.0 – 0.7	6.3
Dogs	0.0 – 1.0	~2 - 12

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

Age	29-33 wks	38-40 wks	5-9 yrs	adults
Serum uric acid (mg/dL)	7.7	1.7	3.7	5.1 (males) 4.3 (females)
Fractional excretion uric acid (%)	61%	38%	10%	7%
Urine uric acid (mg/dL)		86		45

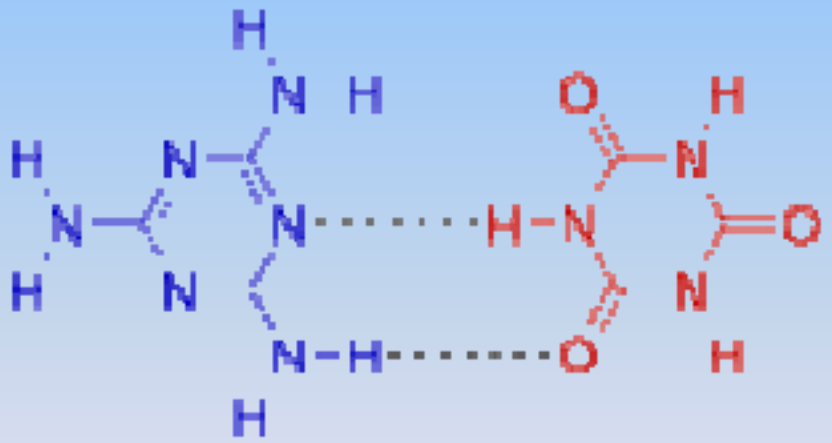
Infant susceptibility (cont'd)

- 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

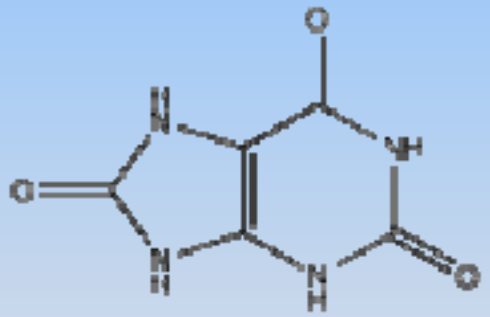
Other melamine co-crystals

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

ammeline acid



melamine cyanurate



uric

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?