NTP
National Toxicology Program

NTP Research Concept: Melamine/Cyanuric Acid

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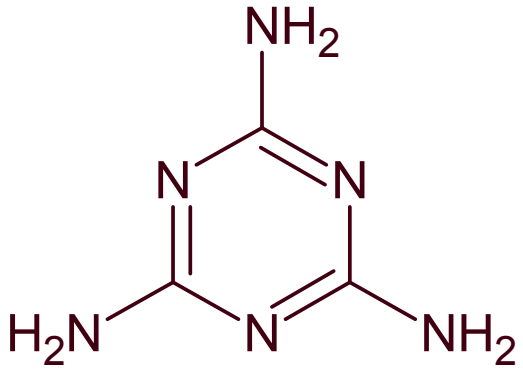
► Melamine

1,3,5-triazine-2,4,6-triamine

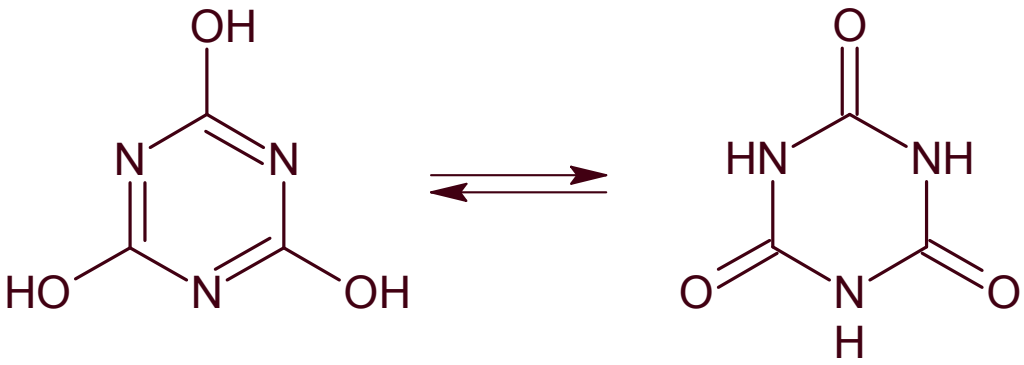


High production volume chemical

- Countertops
- Fabrics
- Glues
- Flame retardants
- Houseware items (cups, plates, mugs...)

► Cyanuric acid

1,3,5-triazine-2,4,6-triol



High production volume chemical

- Herbicides
- Dyes
- Resins
- Antimicrobial agents
- Stabilizer and disinfectant in swimming pool water

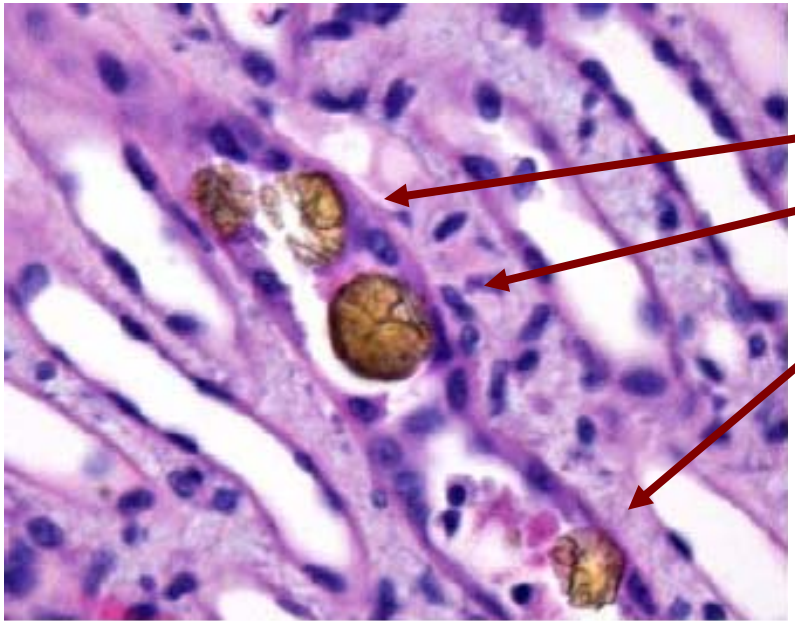
► Pet food toxicity outbreak – Spring 2007

- Sudden illness and kidney failure-related death of a large number of cats (~1950) and dogs (~2200)
- Mass volunteer recall of pet food from the market
- Beginning of largest FDA emergency response in history

Pet food incorporated
imported "wheat gluten" and "rice protein"
adulterated with "scrap" melamine

(melamine, cyanuric acid, ammeline, ammelide...)

► Typical affected cat kidney histology



Melamine cyanurate crystals
(identified by FTIR microscopy)

Proximal/distal tubule lesions

➔ **Acute renal toxicity and crystal formation was later replicated in animals fed melamine and cyanuric acid:**

- **Cats**
- **Aquaculture fish**
- **Hogs**
- **Rats**

➔ **Common mechanism of toxicity**

► Toxicology of melamine and cyanuric acid

Very numerous studies, very low toxicity:

Melamine

$LD50_{oral,rat}$ = 3,161 mg/kg

$LD50_{oral,mice}$ = 3,296 mg/kg

$LD50_{dermal,rabbit}$ > 1,000 mg/kg

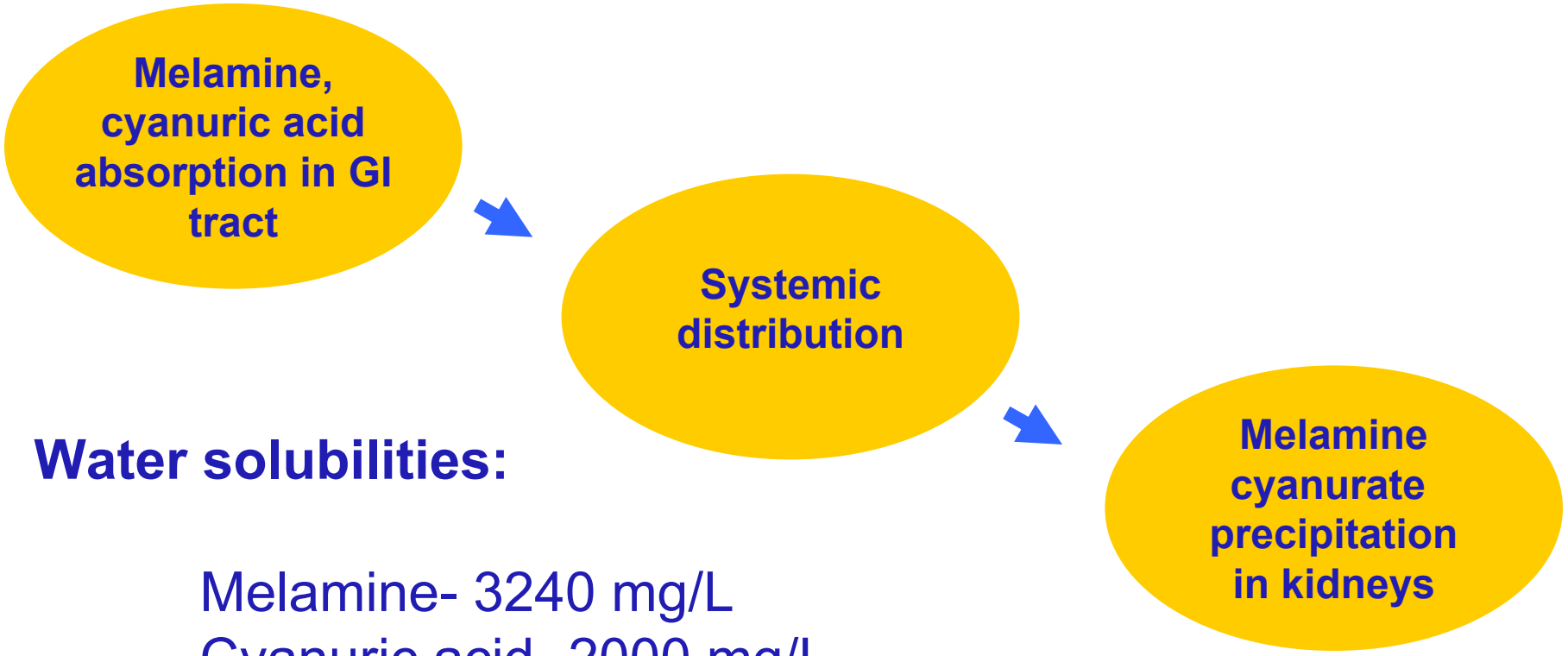
Cyanuric acid

$LD50_{oral,rat}$ > 10,000 mg/kg

$LD50_{dermal,rabbit}$ > 7,940 mg/kg

➔ Toxicity levels comparable to NaCl

► Melamine + cyanuric acid toxicity – why?



Water solubilities:

Melamine- 3240 mg/L
Cyanuric acid- 2000 mg/L
Melamine cyanurate- 2.2 mg/L

→ Synergistic toxicity

Human food chain contamination

- Contaminated gluten and scraps from the pet food industry got incorporated in feed of animals for human consumption:
  - Poultry
  - Swine
  - Aquaculture fish

"Spillage" into the human food chain

- FDA human risk assessment

► Human risk assessment

May 2007 - *Interim Melamine and Analogues Safety/Risk Assessment,* (CFSAN/FDA)

"...the consumption of pork, chicken, domestic fish, and eggs from animals inadvertently fed animal feed contaminated with melamine and its analogues is very unlikely to pose a human health risk."

Currently known synergistic toxicity

Clear need for an updated risk assessment

► Sources of potential human exposure to melamine/cyanuric acid

- **Food/feed contamination**

➔ Recent contamination “spillage” into the human food chain

➔ Literature evidence for previous nephrotoxicity outbreak in pets in Asia in 2004

➔ Literature data shows widespread contamination of fish and meat meal with melamine in Italy in the period 1979-1987
- 72% samples positive, levels up to 1.9% (w/w)

➔ Some food industry facilites formulate both pet and human food
- Poor labeling of bags containing contaminated gluten
- Vegetarian (high-gluten content) diet scenario

► Sources of potential human exposure to melamine/cyanuric acid

- **Other sources**

➔ Some melamine polymer based kitchenware can leach significant amounts of melamine monomer
  - up to 2.5 mg/100 cm^2 with hot acidic foods

➔ Cyanuric acid is an FDA-accepted component of feed-grade biuret, a ruminant feed additive (up to 30% CA and triuret)

➔ Cyanuric acid is a swimming pool stabilizer and disinfectant

  - Recommended 100 ppm (possibly higher in mismanaged pools)
  - Estimated ingestion of water up to 154 mL (children) ~15.4 mg CA

► Key questions

An update on the melamine/cyanuric acid human risk assessment is clearly required

External review panel issued a number of recommendations, including:

- Studies to understand better the pharmacokinetics of melamine;
- Toxicological assessment of the combined exposure to melamine and cyanuric acid in different species;
- Determine the occurence of biomarkers predictive of renal failure upon exposure to melamine/cyanuric acid
- Additional studies addressing the long term effects of a combined exposure to melamine/cyanuric acid

Work proposed

- Based upon the external review panel and FDA interagency group recommendations
- Multi-phase study

Phase 1- Development of analytical methodologies (LC-MS) and range finding study in rats

- Adapt/modify currently existing methods for use with blood and urine samples
- Determine appropriate dosing for the toxicokinetic studies
- Estimate dosing range for the NOAEL studies (3♂, 3♀; 400, 100, 25, 5, and 0 mg/kg; 28 days)

Phase 2- Pharmacokinetic study in a rat model

Absorption and disposition of melamine and cyanuric acid when administered:

- **Individually;**
- **Simultaneously as separate melamine and cyanuric acid;**
- **Simultaneously as a pre-formed compound (melamine cyanurate);**
- **In a time-staggered manner.**

Phase 3- NOAEL determination in a rat model

Determine the NOAEL of a combined gavage treatment with melamine and cyanuric acid in rats for 28 and 90 days using the conditions determined in the previous point as being the most prone to elicit a nephrotoxic response. (12 ♂, 12 ♀; 4 dose levels + control)

Phase 4- Biomarkers of nephrotoxicity

Investigate the occurrence of metabolomic and proteomic early biomarkers of melamine + cyanuric acid-induced nephrotoxicity, obtainable by non-invasive methods (urine)

- Homovanillic acid sulfate
- 4-Hydroxyproline
- NAG
- α- GST *
- μ-GST *
- RPA-1 *
- Clusterin *
- Kim-1
- Urine metabonomic profiling by ^{1}H NMR

* Human nephrotoxicity biomarkers currently undergoing evaluation by the FDA

Phase 5- NOAEL determination and pharmacokinetic study in mini-pigs

Investigate the pharmacokinetics and determine the NOAEL of a combined exposure to melamine and cyanuric acid in a miniature pig model.

This model is considered to be representative of the human kidney anatomy and physiology and as such more appropriate to generate the data required for the human risk assessment.

Phase 6- Possible follow-up studies

Based on the results of the subchronic study consider a chronic study addressing long term systemic toxicity and/or specialized organ systems toxicity of a simultaneous exposure to melamine and cyanuric acid.