Melamine
The Anatomy of an Adverse Event
Pet food Recall of 2007
and the
Infant Formula Incident in China 2008

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M.K., Witkowski, M. AND OTHERS
OUTLINE

• History-Tracking the products
• Communications during recall – public/scientist
• Understanding the mechanism of action
• Scientific response – proactive research
• Infant formula event
• Research response for risk assessment/WHO
• Political Implications
• Current Events
Early Information

- Thursday, Mar. 15, 2007

- Menu Foods, Streetsville, Ontario, announced pending recall

- 60 million containers, “cuts and gravy” style food; 100 brands

- Company complaints, death of palatability panel animals
FDA Response

• Inspectors at Emporia, Kansas, plant within 24 hours

• Later to Pennsauken, New Jersey, plant

• Both plants received wheat gluten produced in China
Contaminated Pet Food/Animal Feed Incident – 2007
Sequence of Events

3/19/07

China Supplier

U.S. Importer

Pet Food Manufacturers

Pet Food
Contaminated Pet Food/Animal Feed Incident – 2007
Sequence of Events

3/19/07  4/11/07

2

China Supplier

3 Feed Ingredient Manufacturers

Scrap

14

U.S. Importer

Pet Food Manufacturers

Pet Food
Contaminated Pet Food/Animal Feed Incident – 2007
Sequence of Events

3/19/07  4/11/07  4/12/07

China Supplier

U.S. Importer

Pet Food Manufacturers

3 Feed Ingredient Manufacturers

Feed Mill

Ingredients & Meals

Scraps

Poultry Farms

Hog Farms

2.5 M

58 K

Pet Food

Scraps

Rendering Plants

2

5

14
Contaminated Pet Food/Animal Feed Incident – 2007
Sequence of Events

3/19/07  4/11/07  4/12/07  4/13/07

China Supplier

U.S. Importer

Pet Food Manufacturers

3 Feed Ingredient Manufacturers

Canadian Fish Feed Manufacturer

Feed

Aquaculture Farms (2)

106 U.S. Hatcheries

Poultry Farms

Hog Farms

5 Rendering Plants

Feed

Ingredients & Meals

Feed Mill

Scrap

160

2

2.5 M

58 K

Scraps
Contaminated Pet Food/Animal Feed Incident – 2007
Sequence of Events

3/19/07  4/11/07  4/12/07  4/13/07  4/18/07

2

China Supplier

2.5 M

58 K

160

Canadian Fish Feed Manufacturer

Feed

Aquaculture Farms (2)

Poultry Farms

Hog Farms

Slaughter House

2

3 Feed Ingredient Manufacturers

Ingredients & Meals

Feed

Ingredients & Meals

Feed

Feed Mill

Pet Food Manufacturers

Pet Food

Feed

Scrap

Ingredients & Meals

5 Rendering Plants

Pet Food

Scraps

U.S. Importer

14

5

U.S. Hatcheries

106 U.S. Hatcheries

3 Feed Ingredient Manufacturers

Ingredients & Meals

Pet Food Manufacturers

Scraps
Finding Melamine

- Review of production records showed no obvious problem
- Common thread – wheat gluten
- FDA, University, Industry, “FERN” (Food Emergency Response Network) confirmed melamine in wheat gluten
- Pet food made with adulterated ingredient
Rice Protein Concentrate

- **April 15**, Wilbur Ellis Co., San Francisco, notified FDA of melamine in imports

- Product came from China
  - recall began April 18
  - 155 metric tons
Workload

• FDA investigators went to all manufacturing, warehouse, distributor sites
  – difficult records,
  – uncooperative companies
  – tracked product by lots

• State officials provided significant help
2007 FDA’s Largest Recall

350 people at CVM

FDA’s Pet Food recall page attracted 6.1 million hits

CVM’s Web site attracted 1.3 million hits

18,700 calls in 11 weeks (5-6000/ year)
Consumer Consternation

• Extremely upset, active during recall
• FDA complaint coordinators fielded 18,700 recall phone calls
  – versus no more than 6,000 for typical year
Intensely Emotionally

- Hundreds of calls daily
  - often from elderly, asking if their pet food on recall list
  - some said pets already dead
- A few cursed FDA for not doing enough
- Emotionally tough period
  - call volume made it physically tough, too
Media Response

• Partnered with media
  – for quick dissemination of information
• Twice weekly briefings
  – 14 during by May 30, 2007
• Two dozen press releases
• Radio, TV interviews
Center for Veterinary Medicine’s Aquaculture Research

HELP with pathology reports, interact with veterinary pathologists

Practicing Veterinarian  Aquatic Diagnostic Pathology  Kidney Research
March 30, 2007

Melamine

- Found in pet food
- Found in cat urine

Toxicologists say – NOT really toxic

??? Is it the cause???
Conference call on “Unusual Crystals”

- Vet Pathologists saw crystals in kidney but not enough to cause clinical signs
- not enough to cause pathology

- Pathology photos sent to FDA

Collaborations with AVMA, AAVLD, Veterinary Schools, private Vets
Crystal Morphology

These crystals looked familiar to me

Michigan State University - Apr 3, 2007

Needle like crystals arranged in a radial sphere

Received April 3, 2007
Human Gout – Uric acid crystals

Needle like crystals, dissolve in formalin

http://www.pathguy.com/lectures/gout.jpg
http://www.csmc.edu/5546.html
Uric Acid Spherulites

Human Gout

Ascidian Renal Sac Spherulites

Urate Spherulites in Gouty Synovia

Fiechtner et al. JAMA 1981 245:1533-6

Uric Acid Accumulation in the Solitary Ascidian *Corella inflata*

Massive literature search on melamine
2 year melamine - cancer study

Urinary bladder carcinogenesis induced by melamine in F344 male rats:
Correlation between carcinogenicity and urolith formation.
Carcinogenesis, 16, 2773–2777.

Rats fed melamine for 2 years developed

Bladder stones: \textbf{Melamine:Uric acid} – 1:1

\textbf{Microcrystals} in renal pelvis

Ogasawara et al. 1995
Uric Acid Crystals

- Dissolve in Formaldehyde

- Formaldehyde is routinely used to preserve tissue for pathology

- Could crystals be dissolving?
Uric Acid Crystals

Uric Acid Nephropathy:

Crystals form in humans with purine metabolism abnormalities (chemo)

These cause intratubular obstruction and renal failure
Crystals form in tubules
Plugging the Plumbing

Like Acute Uric Acid Nephropathy in humans
Locations vary depending on species
Hypothesis

Could a combination of melamine with uric acid or cyanuric acid be toxic?

Is the pathogenesis similar to Acute uric acid nephropathy?

Submitted April 6, 2007
April 20, 2007 – CYANURIC ACID

Another high nitrogen compound was found in pet food.
Melamine to Cyanuric acid

all triazines

Bottom line – all have high nitrogen
Melamine Cyanuric Acid

Crystal Lattice Structure

Low Toxicity

Melamine

Cyanuric Acid

Hydrogen bonding - Melamine-cyanurate

Renal crystals

Fig. 6 Cyanuric acid–melamine lattice12 (cyclic hexamer shown in bold).
Why does the combination cause crystals?
Test the Hypothesis
Wet mounts

Debris caught in tubule upstream

Received April 13, 2007
Response to Formalin

Kidney unfixed
Friday PM

Formalin fixed
Monday AM

Same field – photo taken unfixed, then flooded with formalin, left on microscope over weekend. Crystals not visible on Monday (same field).
Cat crystals consistent with Melamine:Cyanurate spectrum
Proctor and Gamble

Investigating effects of Melamine and Triazine analogues in rats

April 13 – contacted CVM to discuss their ideas and research

They had used MEL+3 analogues at 10:1:1:1 ratio

Discussed and shared our ideas:

- uric acid nephropathy mechanism suggested
- DON’t fix in FORMALIN
  try using 1:1 ratio of MEL:CYA

Collaboration on further rat work and with the cat case

Dobson et al. 2008

Best Paper of the Year Award – Soc. Toxicology
LIVESTOCK FEEDS ALSO CONTAMINATED

**2007**

**Hogs That Ate Tainted Food to Be Euthanized**
About 300 Could Have Already Gotten Into Humans' Food Supply

April 27, 2007

**Tainted poultry has entered food supply**
Millions of chickens ate feed containing melamine, government officials say

May 1, 2007

**Fish meant for humans fed tainted food**

May 9, 2007
The New York Times

Filler in Animal Feed Is Open Secret in China

Nationwide recall of pet foods – Melamine and Cyanuric Acid

Using “scrap” melamine
Concerns for human food safety

Hogs
Chickens
Fish
exposed to contaminated feed
WE NEEDED METHODS ASAP!
FDA regulates drugs and feeds for seafood

Use fish for method development
Study crystal formation at the same time

We routinely do incurred residue in fish
Had animals on hand immediately, vs pigs
CVM dosing fish
June 2007
Catfish Kidney

Glomeruli

CONTROL
MELAMINE Only
CYANURIC ACID Only
(Normal appearance)

MELAMINE+CYANURIC ACID (400 mg/kg bw)

Crystals

Glomeruli
Catfish Kidney

Glomeruli

CONTROL

MELAMINE Only

CYANURIC ACID Only

(Normal appearance)

MELAMINE + CYANURIC ACID (400 mg/kg bw)

Crystals

Our fish data later confirmed in Pigs and cats
Risk Assessment

• USDA, FDA developed risk assessment
• Low human food safety risk
  – due to dilution factors
  – 132 lb. person must eat 800 lb. of contaminated meat daily for risk
MELAMINE EVENTS

2007

- Dogs and Cats

SCARP- MEL (with CYA)

- Kidney Failure-Crystals
  Death

- 100s – 1000s
Experiments

• 2007 – Incurred Residue (fish, pigs, chick)
  Demonstrated Crystals MEL+CYA

• 2008 – Depletion Study (fish)
  NOAEL – for crystals (fish)
    (No observable adverse effect level)
  Sequential dosing effects (fish)
Sept 11, 2008
News - Melamine In Formula

- Children on formula a long time
- Bloody urine
- Sandy urine
- Stones – urinary obstruction
- Renal Failure (Obstruction)
- Sanlu and other formula contained MELAMINE

300,000 infants
URGENT NEED FOR INFORMATION
Melamine: Chronic Toxicity

NTP Study
Rats fed 225 mg/kg bw (4500 ppm in diet)

2 years – bladder stones
Melamine:Uric Acid
bladder cancer (related to stones)

Rats fed melamine for 2 years developed
Bladder stones: Melamine:Uric acid – 1:1

Microcrystals in renal pelvis
Toxicology: Melamine

Heck and Tyl 1985 - F344 rats (weanling rats)
Bladder stones AFTER ONLY 4 WEEKS

SANLU had 5000 ppm
Melamine- fed a long time
What was happening?

• Melamine was being added to watered down milk by dairy farmers (arrests with melamine in possession)

• Melamine was being added to watered down milk by producers/distributers. (arrests with melamine in possession)

• Melamine? Added to powered products? (very high levels in some Powdered milk-infant formula no evidence for direct addition)??
2008 INFANT FORMULA

- Melamine in formula as high as 5000 ppm (Sanlu)
- Primary contaminant – MELAMINE – pure (very very little cya)
- Stones: melamine – uric acid
8 mo female 0.15 cm² sand like calculus

Sun et al 2010 Eur J. Pediatr
10 mo male 0.6-0.4 cm²
stone like calculus

Sun et al 2010 Eur J. Pediatr
Wet et al. Journal of Urology
Grases F et al. Urology. 2009
Melamine Urinary Bladder Stone.
11 mo infant girl born Nov 2007

0.7 x 1.2 cm stone

Melamine-Uric Acid

Melamine-Cyanuric Acid

>50 crystals

10-100 µm

>50 crystals
Crystal interaction

Melamine-Cyanuric acid interaction to form crystals

Uric acid

Melamine

Melamine – Uric acid May form a complex, perhaps less stable

Thomas et al. 2007
Risk Assessments needed data fast!

Yi Yongsheng’s deceased infant son with his grandfather
Fish Group starts working on a pig study
## Pilot Dose Range – 7 Days

<table>
<thead>
<tr>
<th>N=2</th>
<th>MG/KG BW</th>
<th>PPM in feed</th>
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<tbody>
<tr>
<td>Control</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>MEL+CYA</td>
<td>1</td>
<td>25</td>
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<tr>
<td>MEL+CYA</td>
<td>3.3</td>
<td>83</td>
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<tr>
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<td>10</td>
<td>250</td>
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<tr>
<td>MEL+CYA</td>
<td>33</td>
<td>635</td>
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<td>100</td>
<td>2500</td>
</tr>
<tr>
<td>CYA</td>
<td>200</td>
<td>5000</td>
</tr>
<tr>
<td>MEL</td>
<td>200</td>
<td>5000</td>
</tr>
</tbody>
</table>

Match NCTR Rat Study Pilot Dosages

28 day study
Dosing daily as a bolus
10 mg/kg kidney – yellow crystals readily apparent
33 and 100 mg/kg pigs
Reduced wt gain
elevated creatinine
yellowish, enlarged, turgid
GLP study

QAU oversight
MELAMINE EVENTS

2008

• Babies

PURE MEL (no CYA)

• Kidney Stones
  No signs, Pain, Death (6)

• 300,000
# MELAMINE EVENTS

<table>
<thead>
<tr>
<th>Year</th>
<th>Description</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>2007</td>
<td>Dogs and Cats</td>
<td>100s–1000s</td>
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<tr>
<td></td>
<td>SCRAP- MEL (with CYA)</td>
<td></td>
</tr>
<tr>
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<td>Kidney Failure-Crystals Death</td>
<td></td>
</tr>
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<td></td>
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</tr>
<tr>
<td>2008</td>
<td>Babies</td>
<td>300,000</td>
</tr>
<tr>
<td></td>
<td>PURE MEL (no CYA)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Kidney Stones</td>
<td></td>
</tr>
<tr>
<td></td>
<td>No signs, Pain, Death (6)</td>
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</tr>
</tbody>
</table>
Melamine in feed was still a concern Nov 1, 2008

China to tighten control of feed industry: state media

18 hours ago

BEIJING (AFP) — China has pledged to tighten supervision of the animal feed industry, state media said Saturday, amid concerns about the widespread injection of the toxic chemical melamine into livestock feed.

"The ministry will tighten supervision of feed and food products," the Ministry of Agriculture Ministry's livestreaming chatroom said Saturday.

Melamine, an industrial chemical, was added to milk in China in the past, killing one person and sickening 53,000 other people.

The chemical -- which can cause kidney stones -- was then discovered in China's ice cream, yogurt and candy, making its way into the country's food chain.

Wang acknowledged that the addition of melamine into feed is "a very serious issue."
Dec. 11, 2009

3 Arrested at Shaanxi dairy for repackaging melamine-contaminated milk powder

• Liu, Miao and Lu repackaged the products on the nights of September 4 and 5 when other workers were off.
• They sold them as well as another five tons of untested products to the Nanning firm on September 6.
Did we learn from History?

Did we miss some warning shots?
SPIKED Wheat – 1985 – USA-urea

Spiked wheat - Schuler Grain Co. uses additive to artificially boost the protein content of its wheat

Judy Folkenberg

Spiked Wheat

A tip from a disgruntled former employee of a large Minnesota grain company led to court-ordered heavy fines and probation for the owners last February.

Schuler Grain Co. in Breckenridge, Minn., was using an additive to artificially boost the protein content of its wheat and thereby increases the price it brought on the market. Ultimately, the Schulers' profit came out of the consumer's pocket—for higher-priced baked goods at the grocery store.

In August 1985, the tipster told the U.S. Department of Agriculture about the firm's illegal activities. USDA in turn notified FDA.

Investigation by FDA's Minneapolis district office confirmed that urea, a chemical used primarily in fertilizer and feed for cattle, was being illegally added to the wheat before being sold to flour companies.


They were fined $250,000 each and placed on three years' probation. Earlier, they had agreed to to pay more than $400,000 to the Pillsbury Co., which had purchased the adulterated wheat.

Need for vigilance – folks are always looking to make a fast buck
Melamine in fish and meat meal

72% of samples positive in 1979
5% pos in 1987

DUE to their Surveillance Program

ITALY

Published in 1988
Melamine in potato meal
50% of samples positive in 1982

Germany

Published in 1983
Political Implications
Congressional Response

• Title 10 of FDA Amendments Act
  – ingredient standards, definitions
  – processing standards
  – labeling standards

• Early warning surveillance, notification
  – identify problem, notify veterinarians, stakeholders during recalls
Congressional Response

• Searchable database of recalled pet foods
• Reportable food registry
  – Food article with reasonable probability of causing adverse health consequence, death
  – to humans or animals
Bribery – but melamine didn’t help
Six more arrests over melamine-tainted Chinese dairy products

HOHHOT, Oct. 23 (Xinhua) -- Police in north China have arrested six men on suspicion of adding fake protein formula to fresh milk supplied to Mengniu, one of the country's biggest dairy companies.
China executes two managers for poisoned milk scandal that killed six children and left 300,000 ill

By MAIL FOREIGN SERVICE
Last updated at 2:57 PM on 24th November 2009

Two people were executed in China today for their role in a tainted milk scandal that killed at least six children.

Nearly 300,000 children fell ill last year after drinking milk intentionally laced with melamine, a toxic industrial compound that can give a fake positive on protein tests.

A total of 21 executives and middle-managers from the now bankrupt Sanlu Group, which sold the milk, were tried and sentenced in January by a court in the northern city
An officer prepares to destroy unqualified milk powder which was confiscated, in Shanghai November 14, 2008.

REUTERS/Stringer
Malaysian authorities supervising the dumping and burying of contaminated biscuits and ammonium bicarbonate into a landfill

 Taiwanese authorities detected melamine in 469 tons of baking ammonia imported from China. Samples tested showed up to 2,470 ppm of melamine.