



*Melamine*

*The Anatomy of an Adverse Event*

*Pet food Recall of 2007*

*and the*

*Infant Formula Incident in China 2008*

Renate Reimschuessel VMD PhD  
Center for Veterinary Medicine, FDA

Giesecker, C., Eric Evans, Nick Hasbrouck, Tamara Mayer  
Miller, R.A., Rummel, N., Heller, D., Nochetto, C. de Alwis,  
H.A., Andersen, W.C., Turnipseed, S.B., Karbiwnyk, C.M.,  
Satzger, R.D., Crowe, J., Wilbur, N., Roberts, J., Reinhard,  
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

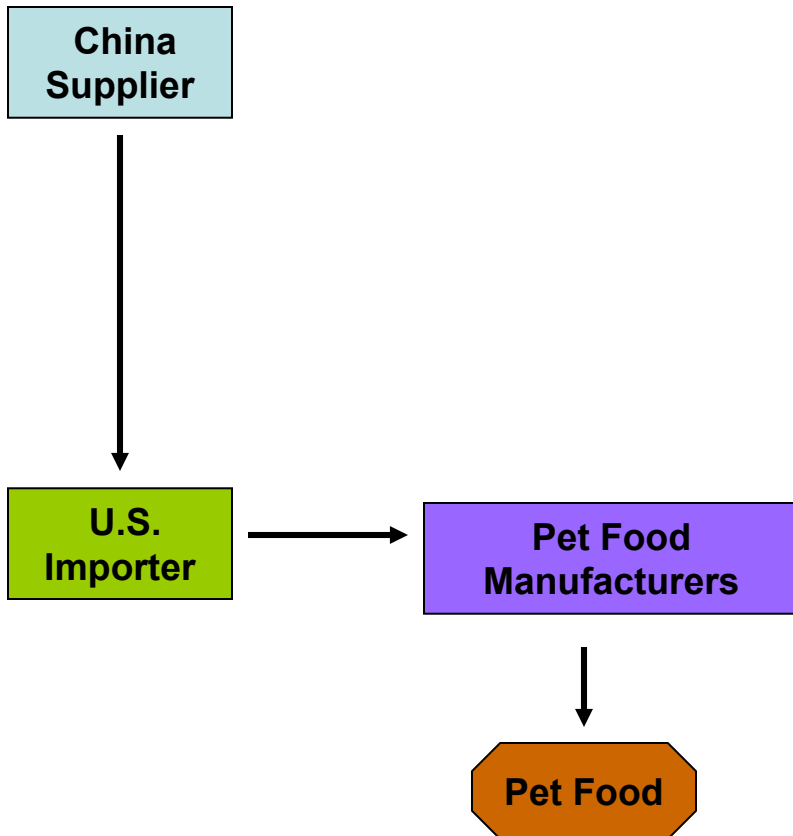




# Contaminated Pet Food/Animal Feed Incident – 2007

## Sequence of Events

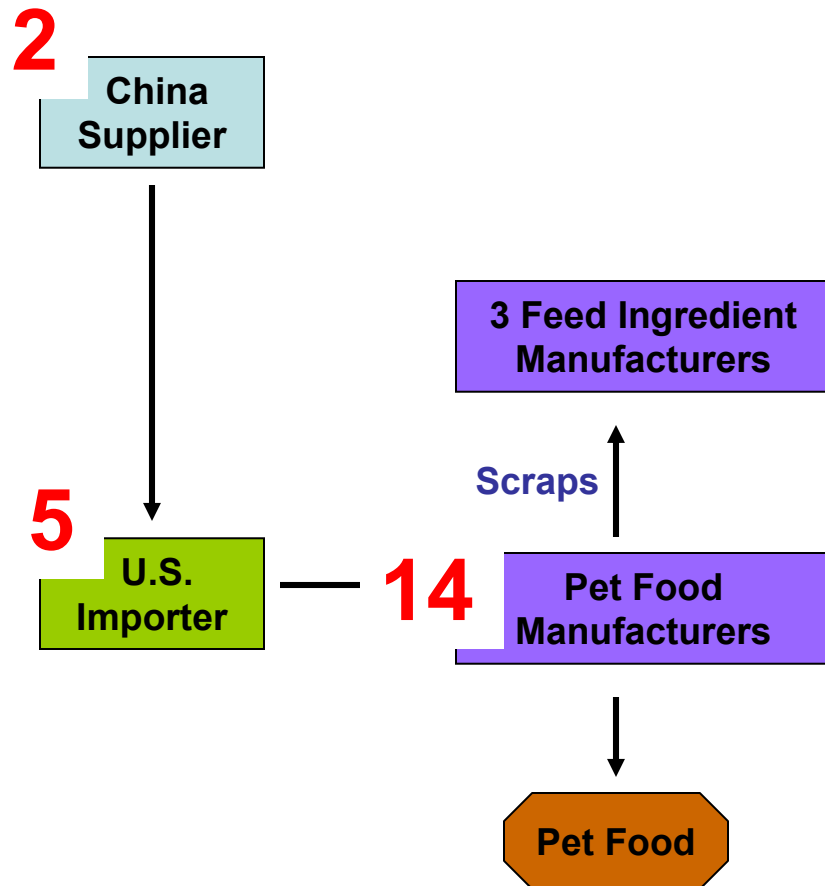
**3/19/07**



# Contaminated Pet Food/Animal Feed Incident – 2007

## Sequence of Events

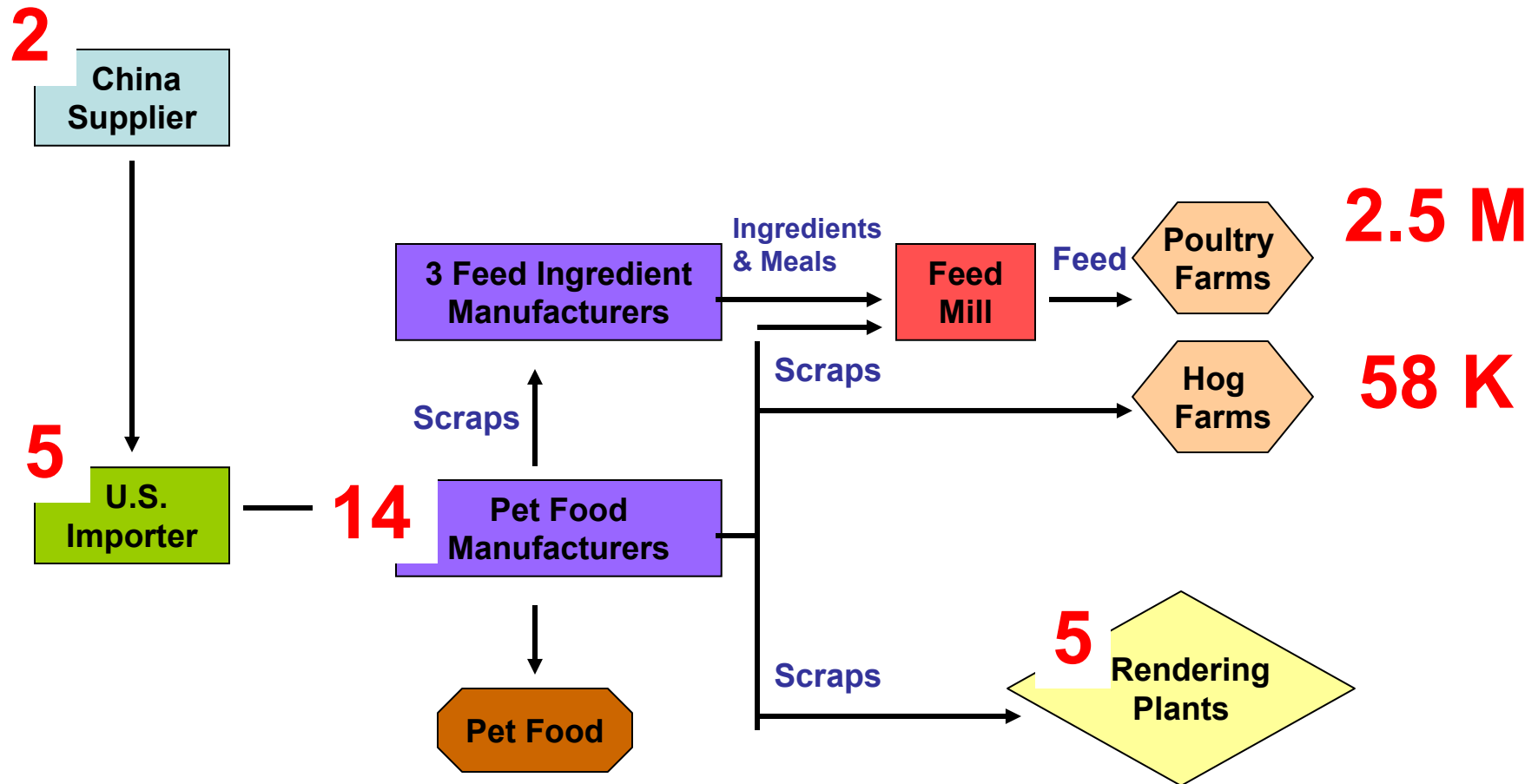
3/19/07    4/11/07



# Contaminated Pet Food/Animal Feed Incident – 2007

## Sequence of Events

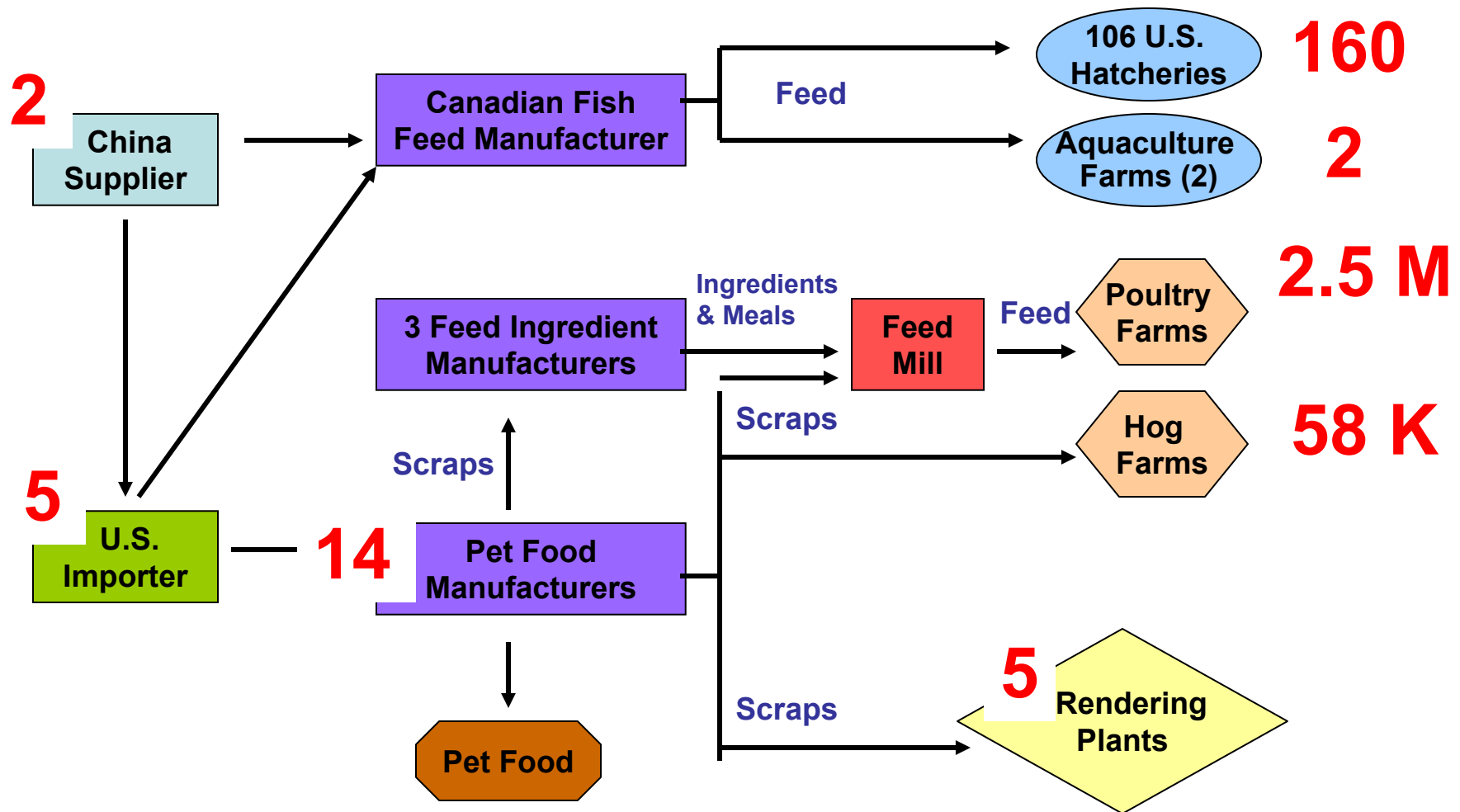
3/19/07    4/11/07    4/12/07



# Contaminated Pet Food/Animal Feed Incident – 2007

## Sequence of Events

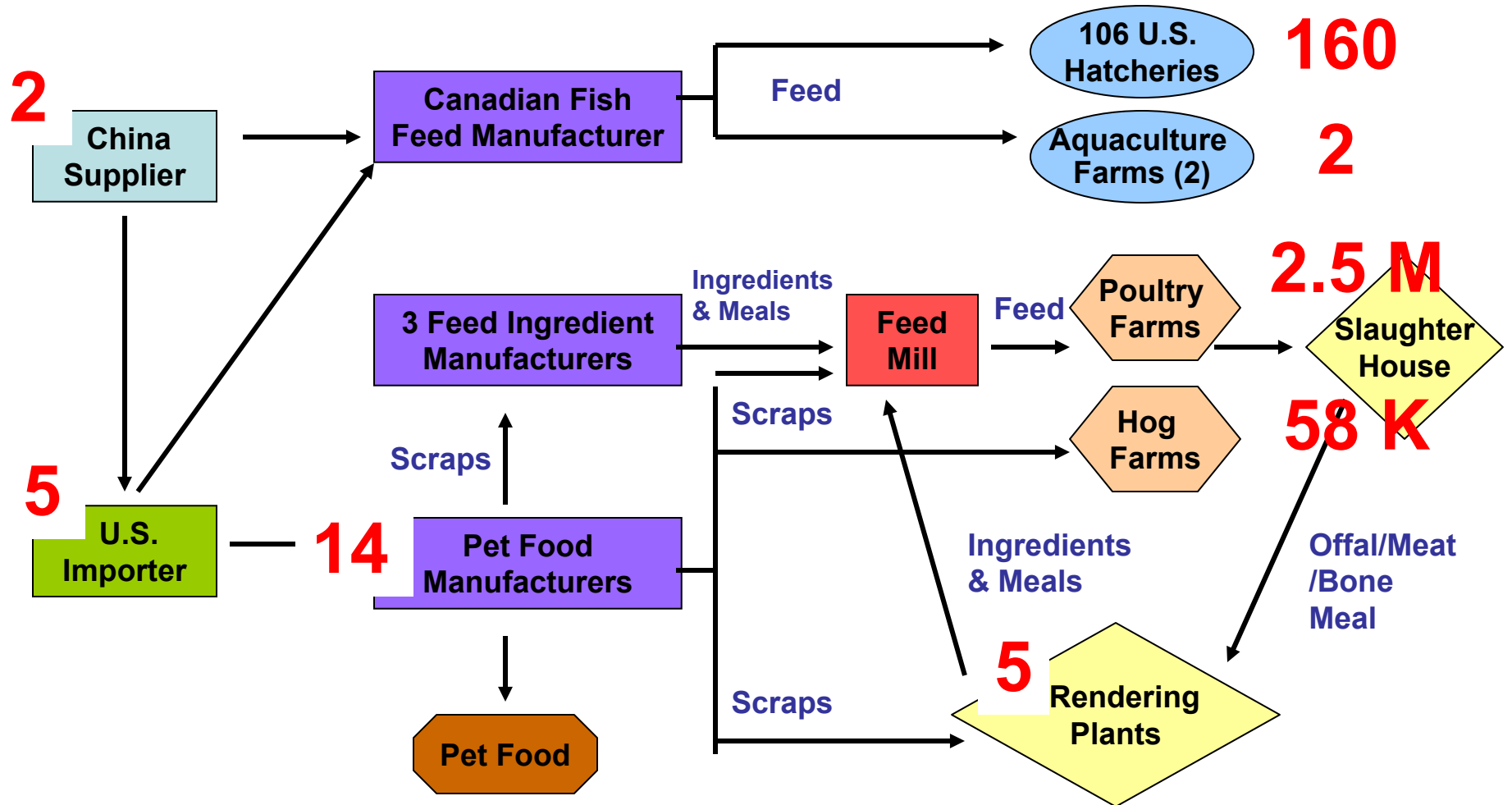
3/19/07    4/11/07    4/12/07    4/13/07



# 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



# 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)**

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

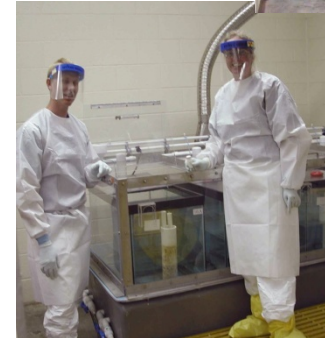
- Hundreds of calls daily
  - often from elderly, asking if their pet food on recall list
  - some said pets already dead
- A few cursed FDA for not doing enough
- Emotionally tough period
  - call volume made it physically tough, too

# 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

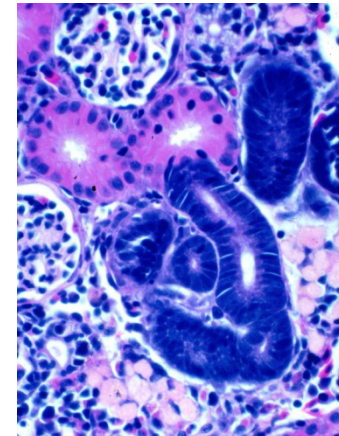
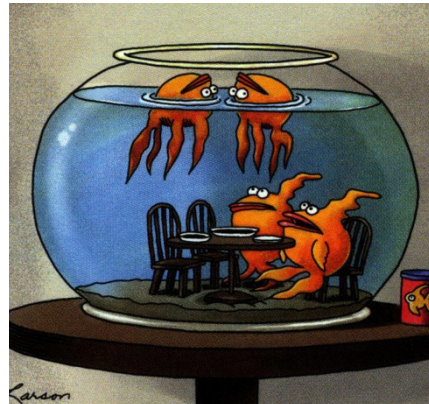


## HELP with pathology reports, interact with veterinary pathologists

**Practicing  
Veterinarian**

**Aquatic Diagnostic Pathology**

**Kidney  
Research**





March 30, 2007

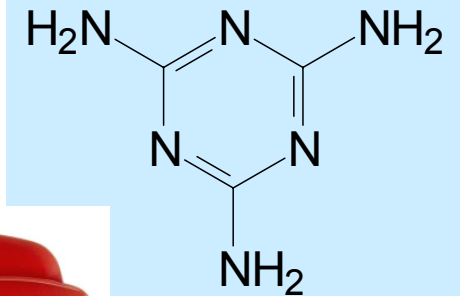
# Melamine

FDA and Cornell University

- Found in pet food
- Found in cat urine

Toxicologists say – NOT really toxic

??? Is it the cause???



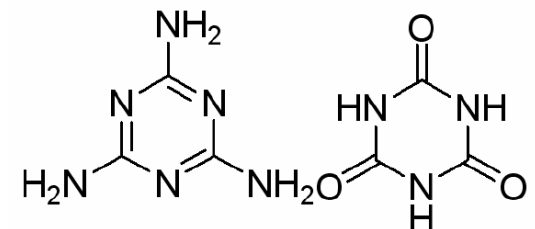
Plastics



Fertilizer



Flame retardant –  
with Cyanuric acid

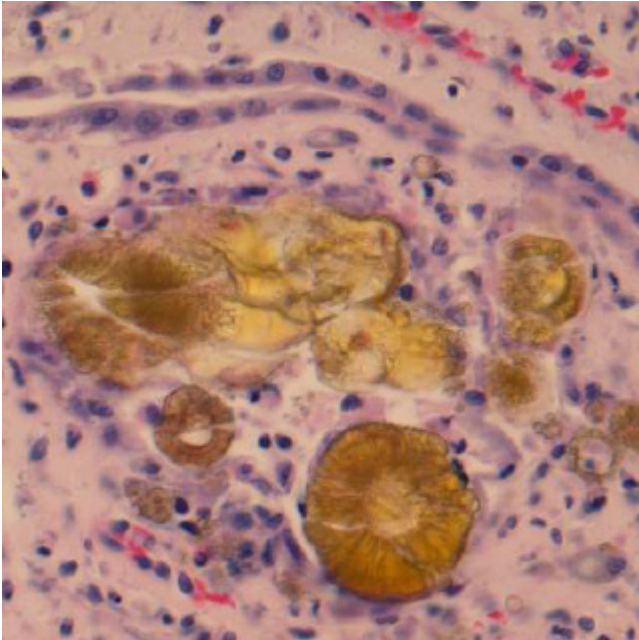


# Conference call on “Unusual Crystals”

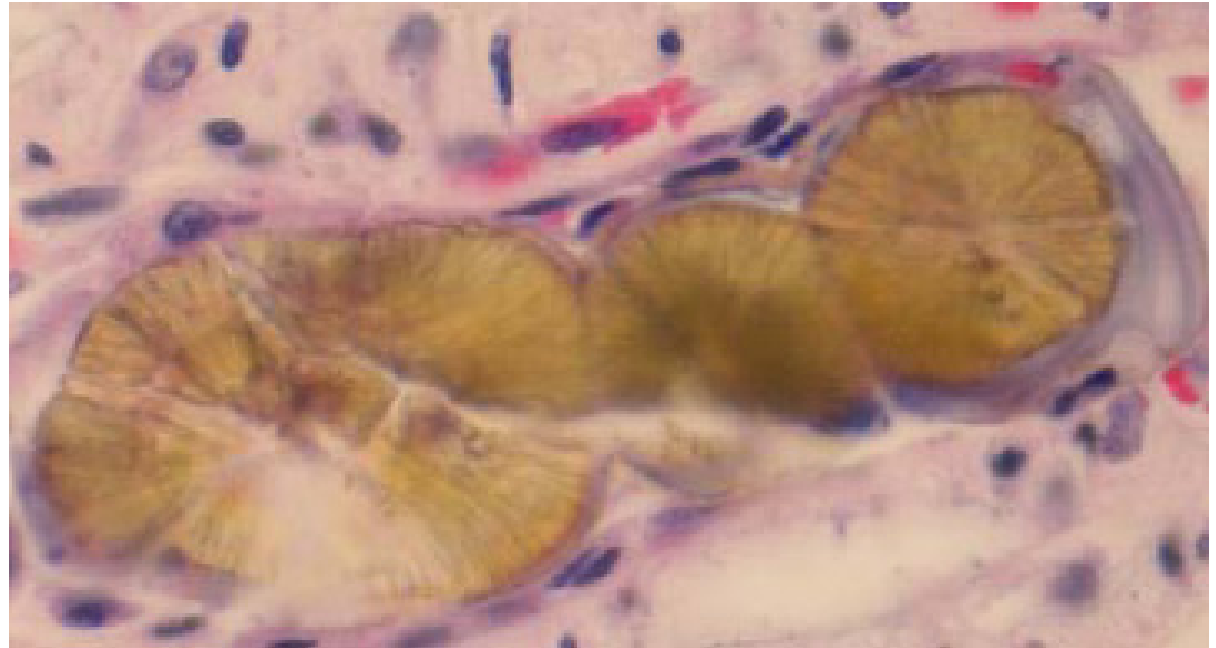
- Vet Pathologists saw **crystals in kidney**  
**but**  
**not enough** to cause clinical signs  
**not enough** to cause pathology
- Pathology photos sent to FDA

Collaborations with AVMA, AAVLD, Veterinary Schools, private Vets

# Crystal Morphology



Michigan State University- Apr 3, 2007



Needle like crystals arranged in a radial sphere

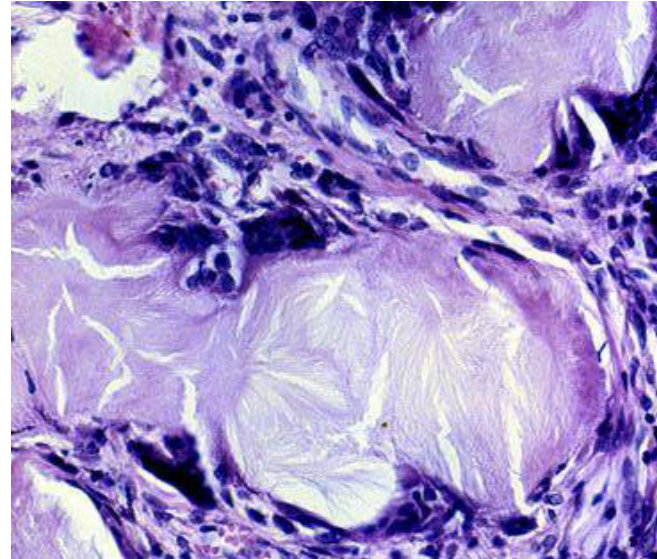
These crystals looked familiar to me



Received April 3, 2007



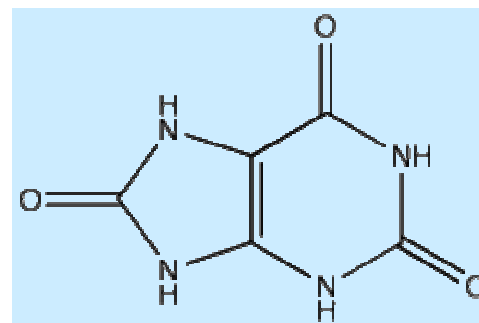
# Human Gout – Uric acid crystals



<http://www.pathguy.com/lectures/gout.jpg>



<http://www.csmc.edu/5546.html>



**URIC ACID**

Needle like crystals, dissolve in formalin



# Uric Acid Spherulites

Human Gout

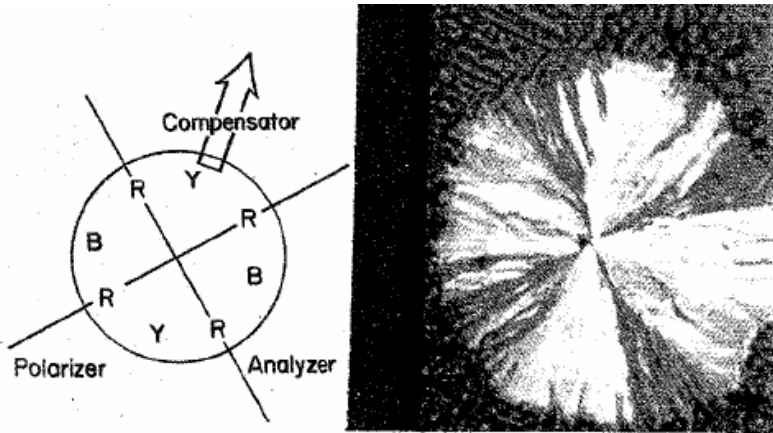
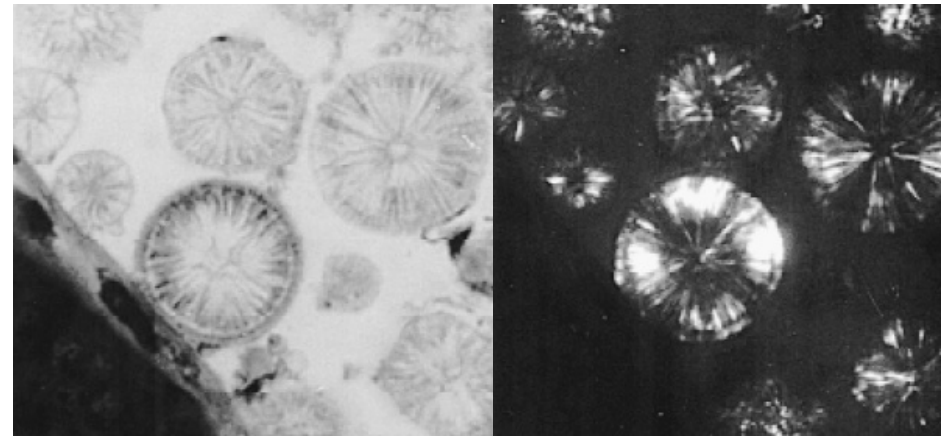


Fig 2.—Monosodium urate monohydrate (MSUM) crystalline spherulite

Ascidian Renal Sac Spherulites



**Urate Spherulite**

Fiechtner et al. JAMA 1961



**Uric Acid Accumulation in the Solitary Ascidian *Corella inflata***

Lambert et al. J. Exp Zool. 282:323–331 (1998)

# Massive literature search on melamine

# 2 year melamine - cancer study

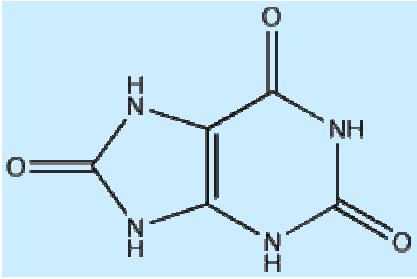
Ogasawara, et al (1995)

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

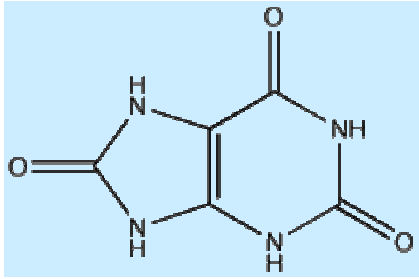
Bladder stones: **Melamine:Uric acid** – 1:1

**Microcrystals** in renal pelvis



# Uric Acid Crystals

- Dissolve in Formaldehyde
- Formaldehyde is routinely used to preserve tissue for pathology
- Could crystals be dissolving?



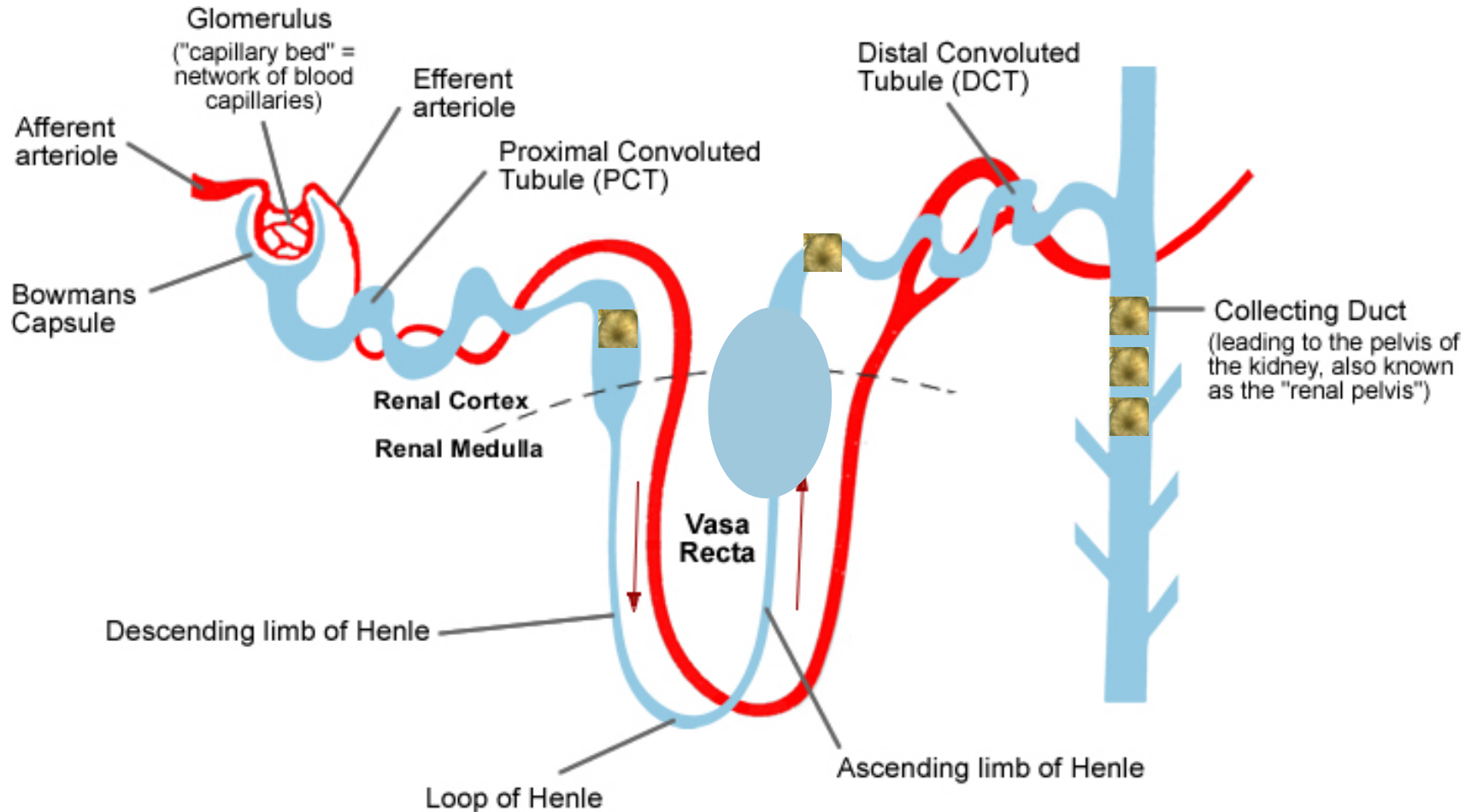
# Uric Acid Crystals

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



[http://www.ivy-rose.co.uk/Topics/Urinary/Kidney\\_Nephron\\_clvyRose.jpg](http://www.ivy-rose.co.uk/Topics/Urinary/Kidney_Nephron_clvyRose.jpg)

**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



**ProPoolSupply**  
Formerly **Poolwarehouse.net**  
One-Stop Shop For All Your Outdoor Needs ☺

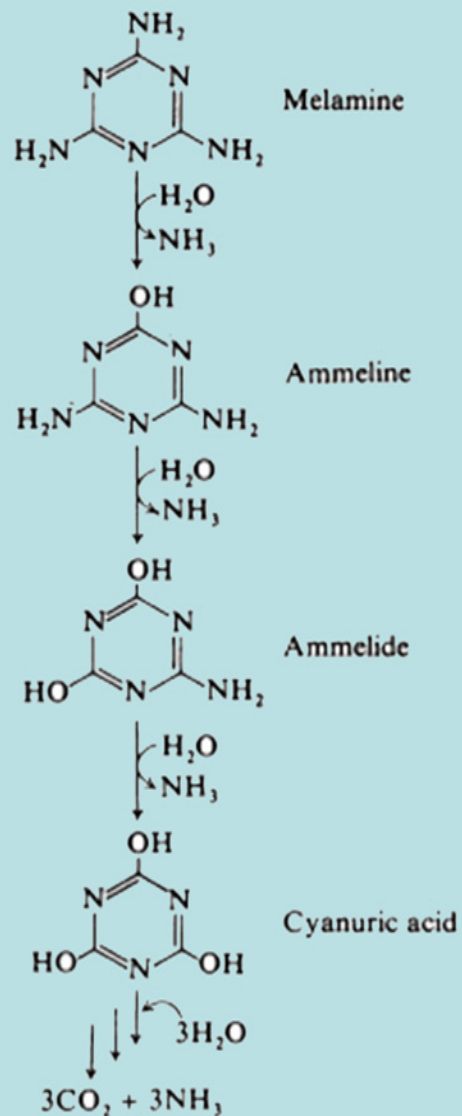


Fig. 3. The degradative pathway of melamine

Jutz -et al Biochem. J. (1982) 208, 679-684

Melamine  
to  
Cyanuric acid  
  
all triazines

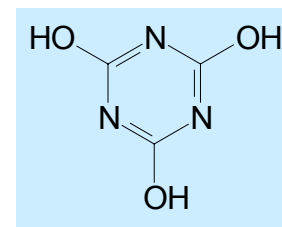
Bottom line – all have high nitrogen

Low Toxicity

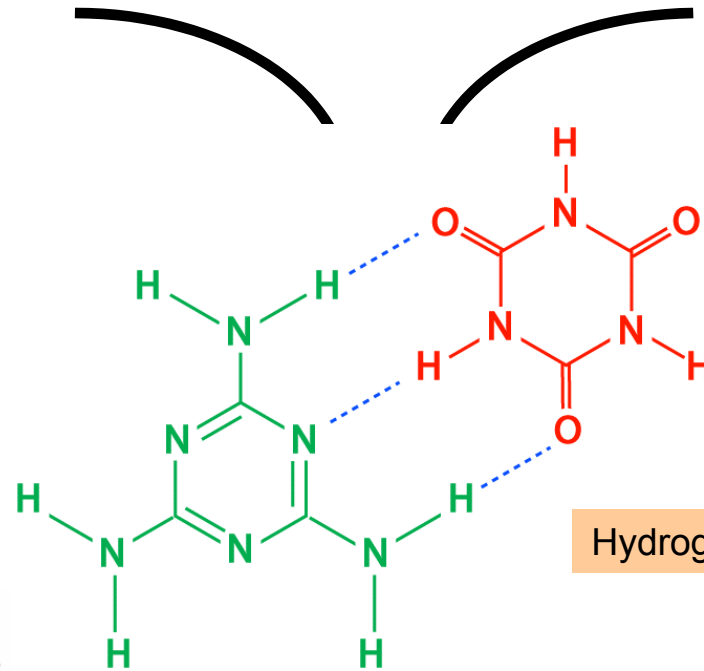


Melamine

Low Toxicity



Cyanuric Acid



Hydrogen bonding- Melamine-cyanurate

Renal crystals

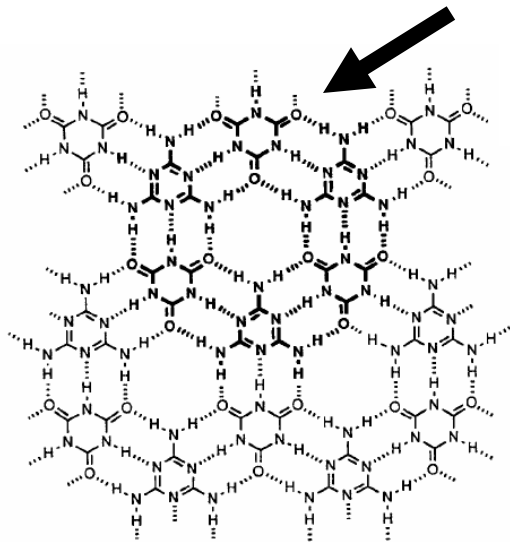
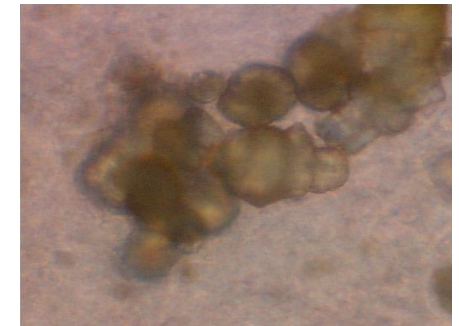
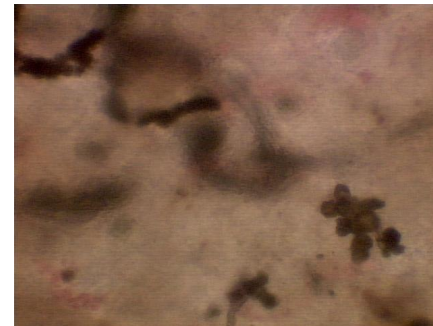
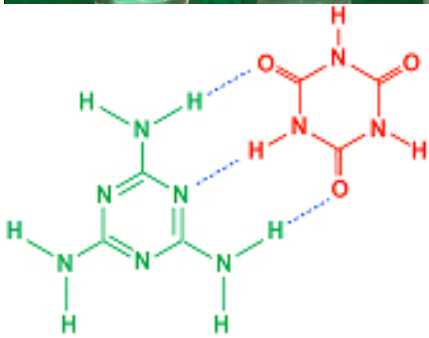


Fig. 6 Cyanuric acid-melamine lattice<sup>12</sup> (cyclic hexamer shown in bold).

Crystal Lattice Structure



# Why does the combination cause crystals?



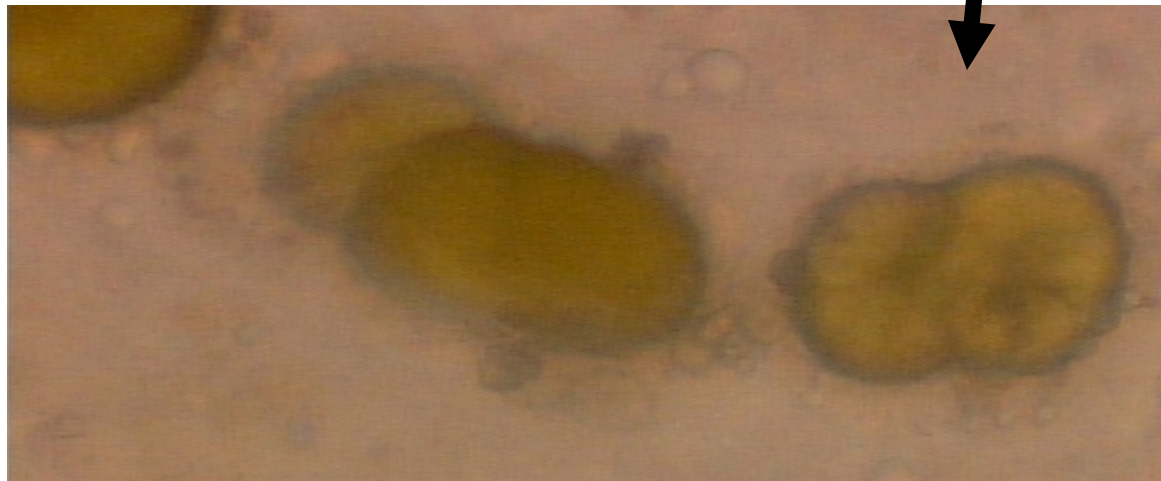
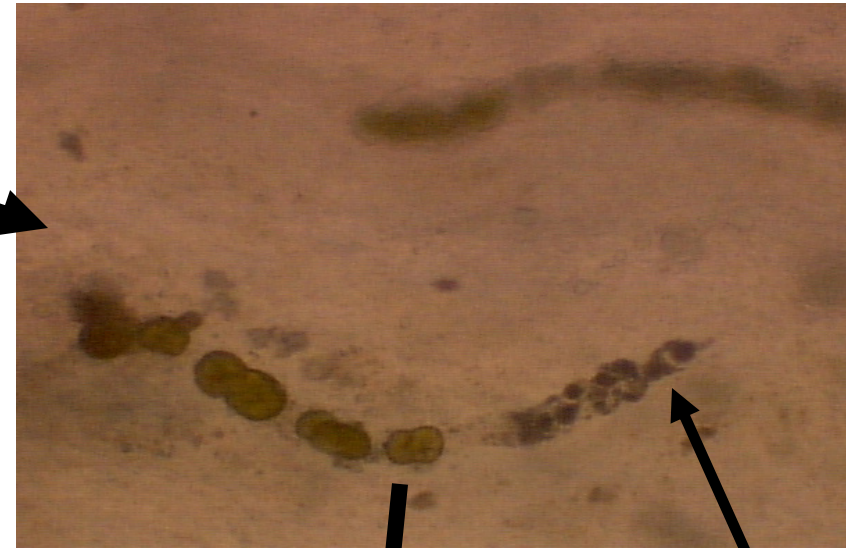


# Test the Hypothesis

## Wet mounts



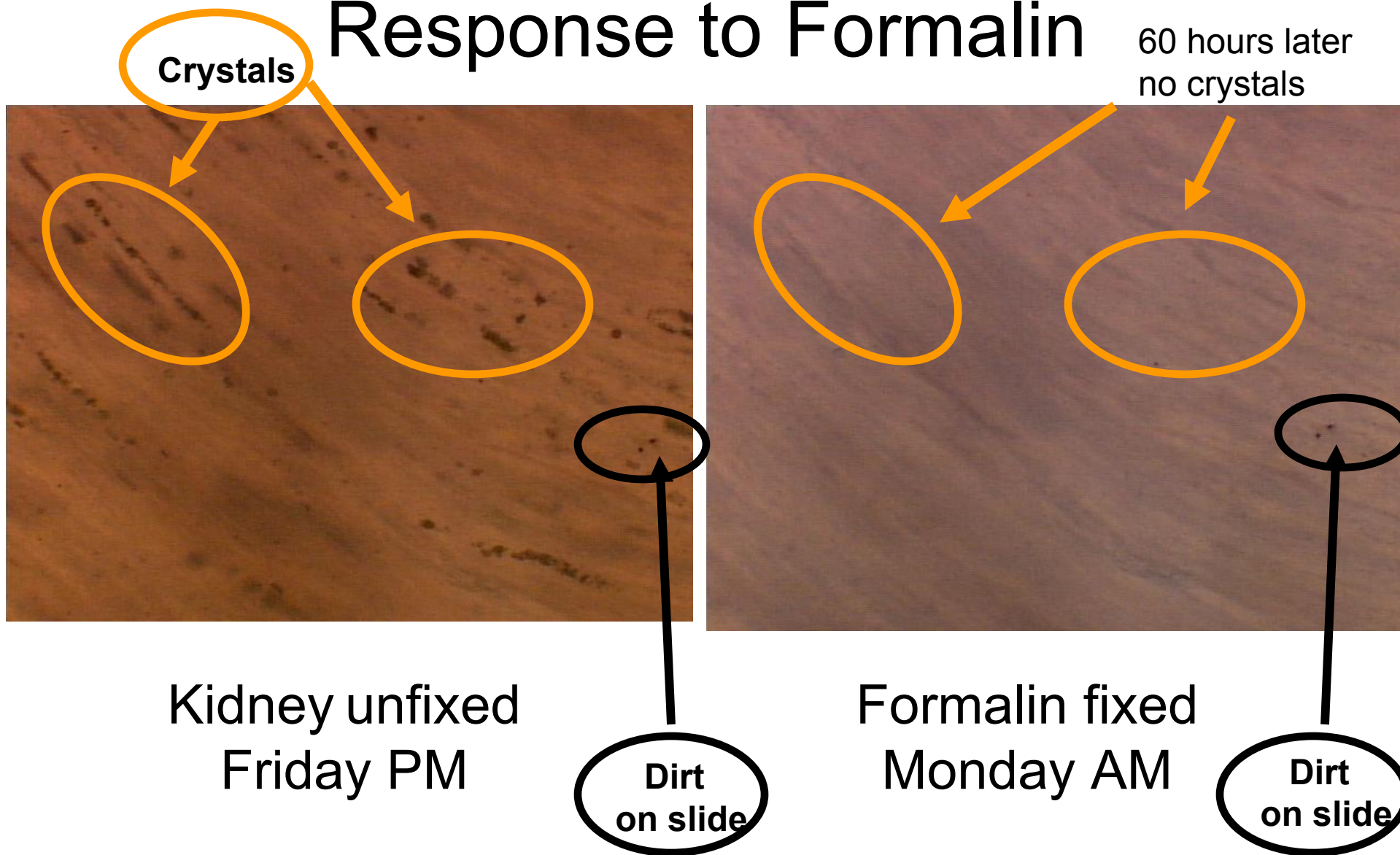
Received April 13, 2007



Debris  
caught in  
tubule  
upstream

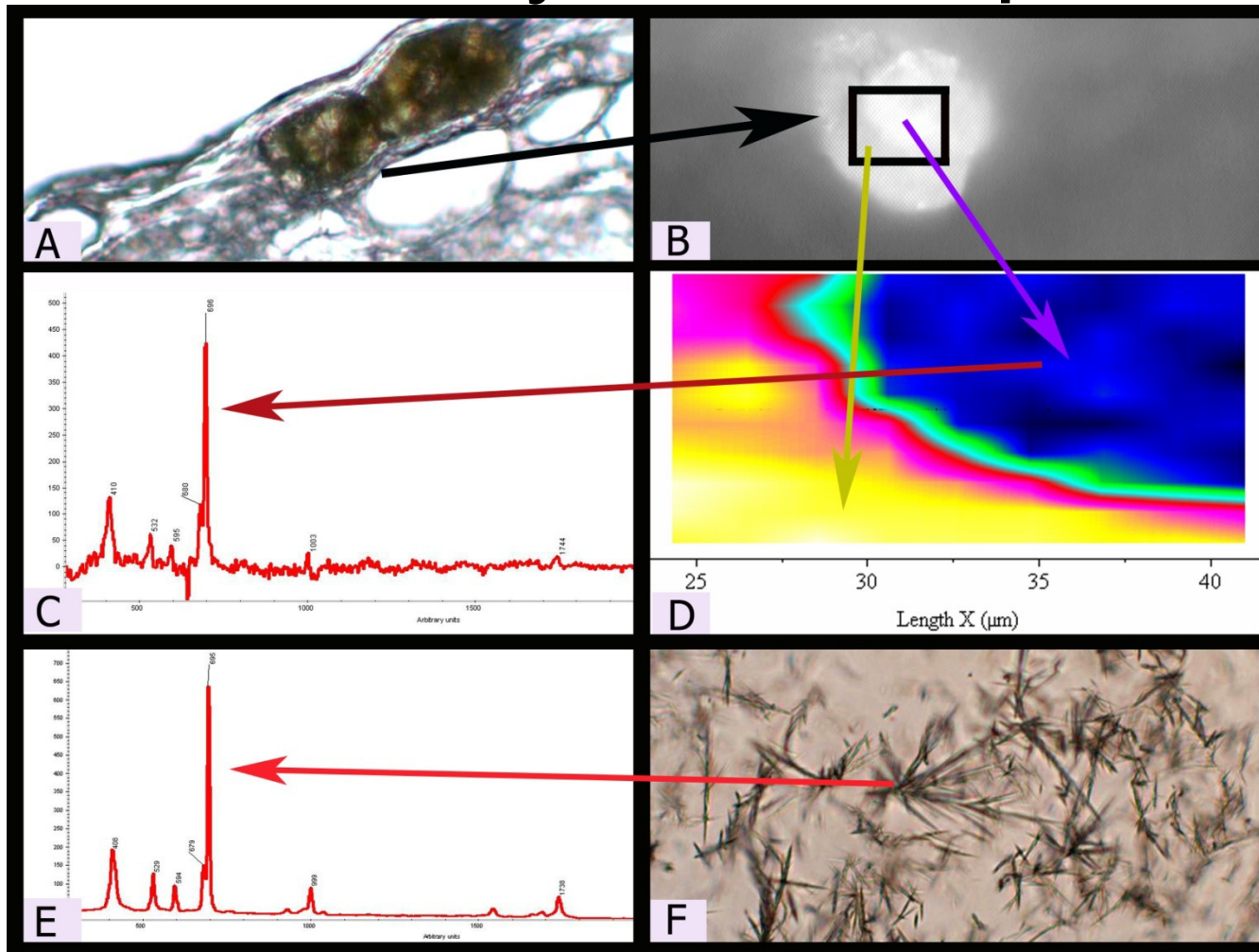


# Response to Formalin



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



Fourier Transform Infrared Spectroscopy

FDA Forensic Chemistry Center



# 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**



# 2007 LIVESTOCK FEEDS ALSO CONTAMINATED

washingtonpost.com

April 27, 2007

## Hogs That Ate Tainted Food to Be Euthanized

About 300 Could Have Already Gotten Into Humans' Food Supply

85,000 HOGS



msnbc

May 1, 2007

## Tainted poultry has entered food supply

Millions of chickens ate feed containing melamine, government officials say



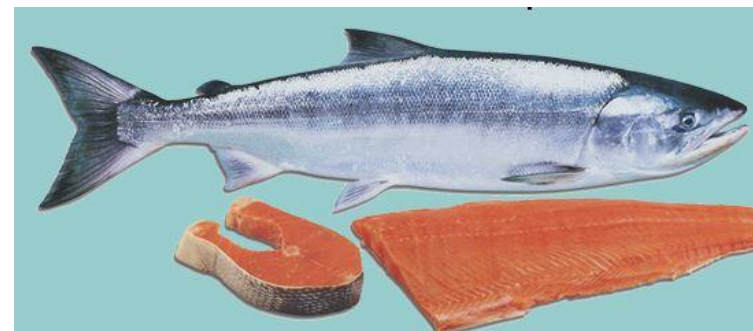
2.5 Million CHICKENS

CNN.com

May 9, 2007

## Fish meant for humans fed tainted food

FISH



# The New York Times

Filler in Animal Feed Is Open Secret in China

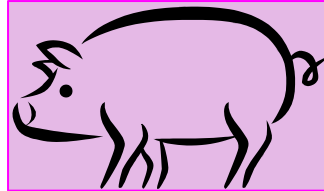


Using “scrap” melamine

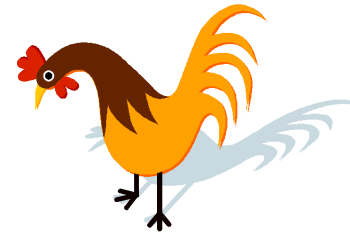
*Nationwide recall* of pet foods –  
*Melamine* and *Cyanuric Acid*

# 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

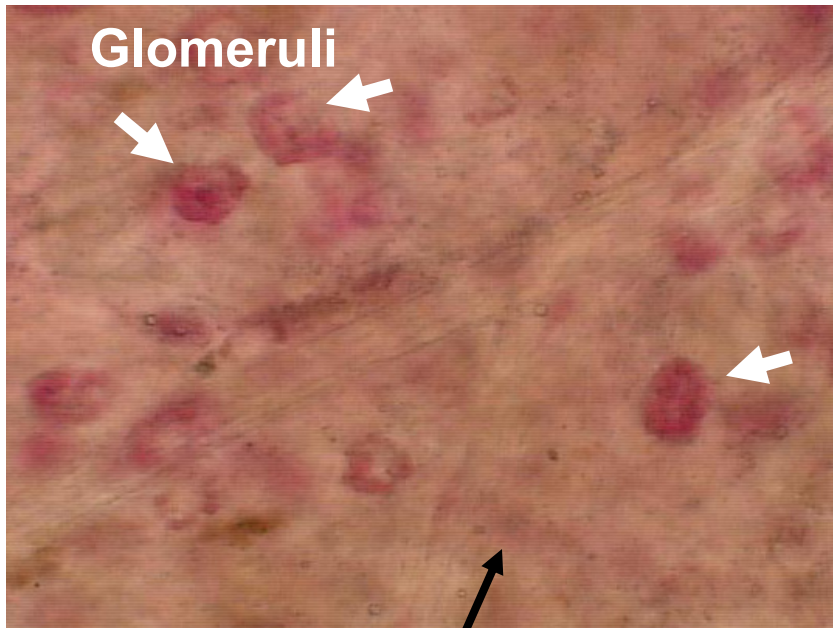


June 2007

Catfish Kidney

MELAMINE+CYANURIC ACID (400 mg/kg bw)

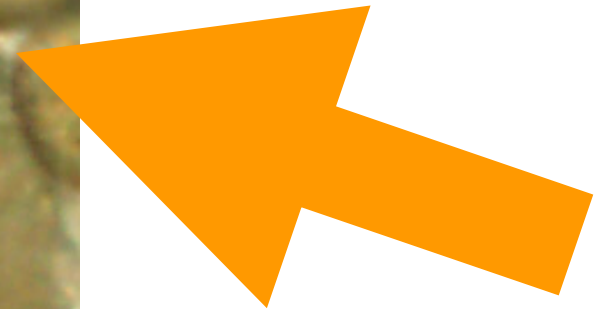
Crystals



CONTROL  
MELAMINE Only  
CYANURIC ACID Only  
(Normal appearance)



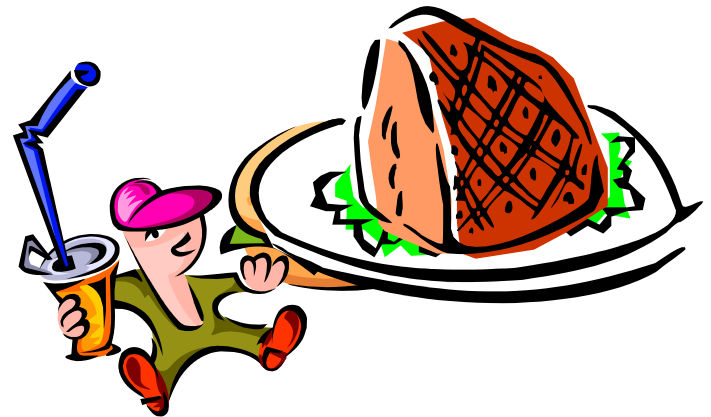
**Our fish data  
later confirmed in  
Pigs and cats**





# 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

SCRAP- 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**



[http://www.latimes.com/news/printedition/asection/rac-mnik\\_kraavync20080917042550,0,4471277.photo](http://www.latimes.com/news/printedition/asection/rac-mnik_kraavync20080917042550,0,4471277.photo)



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)

## **Ogasawara, et al (1995)**

Rats fed melamine for 2 years developed

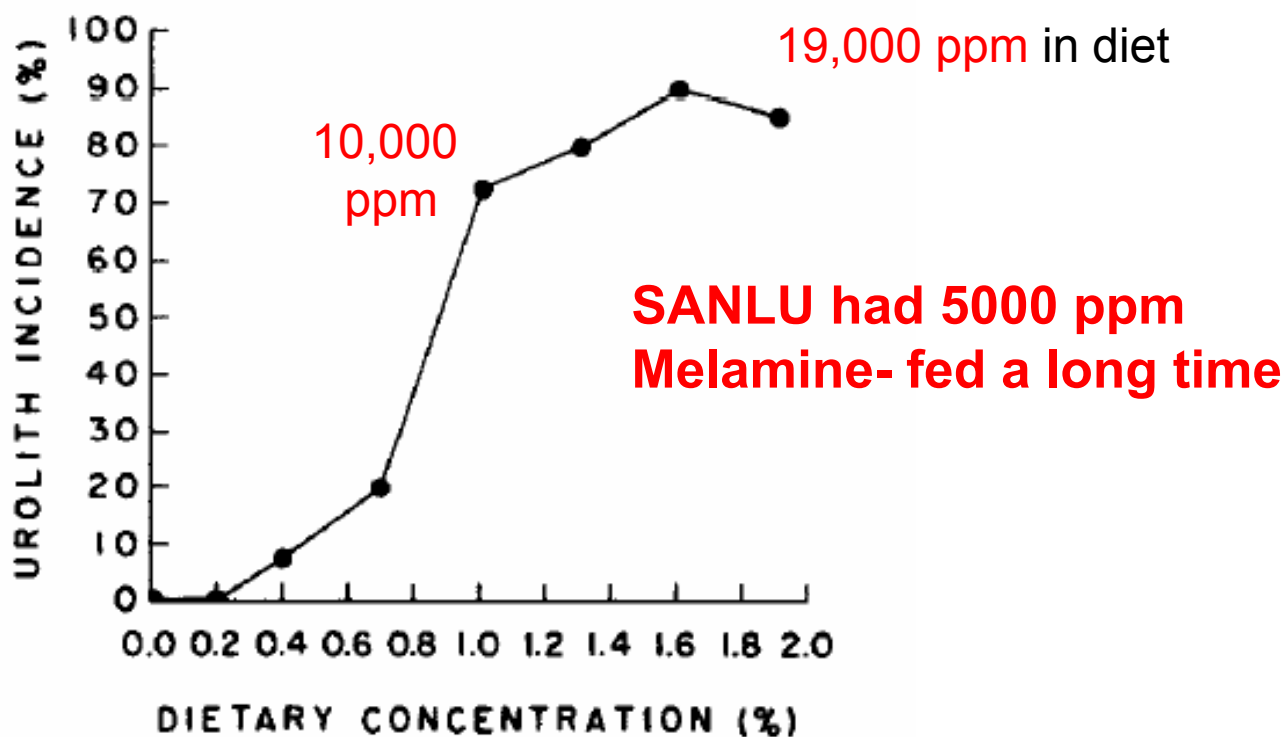
Bladder stones: **Melamine:Uric acid** – 1:1

**Microcrystals** in renal pelvis

# Toxicology: Melamine

Heck and Tyl 1985 - F344 rats (weanling rats)

Bladder stones **AFTER ONLY 4 WEEKS**





# 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 powdered 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 cm<sup>2</sup> sand like calculus

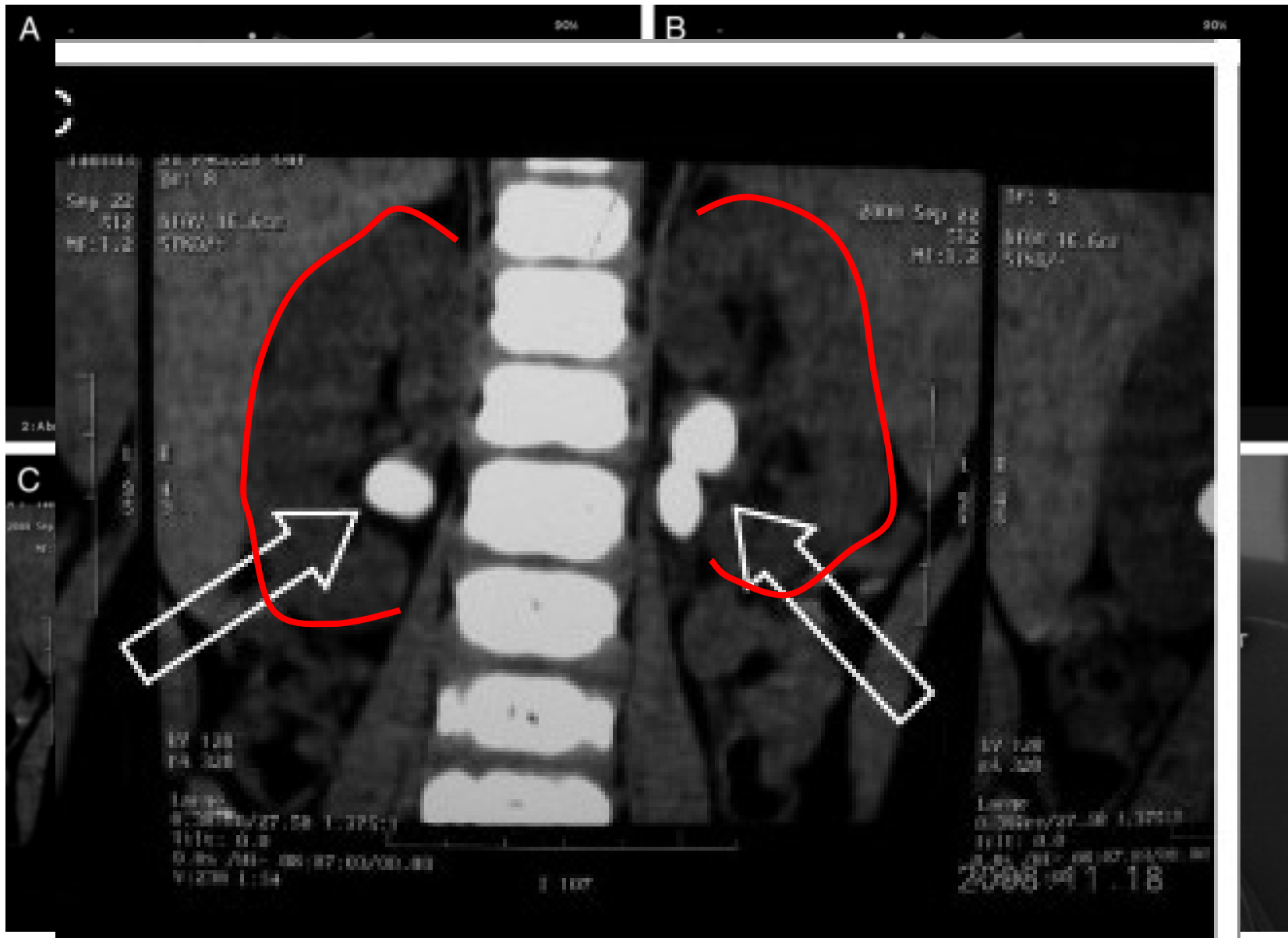


Sun et al 2010 Eur J. Pediatr

# 10 mo male 0.6-0.4 cm<sup>2</sup> 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

# STONES

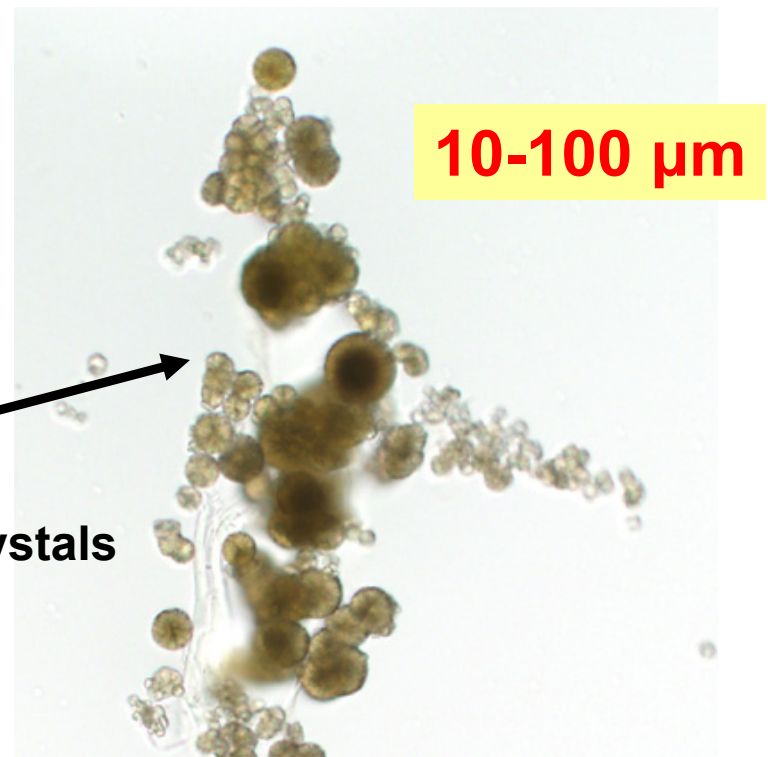
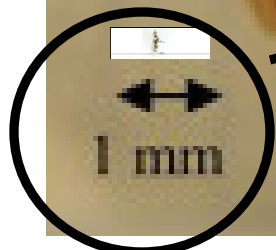
Melamine-Uric Acid

VS

# CRYSTALS

Melamine-Cyanuric Acid

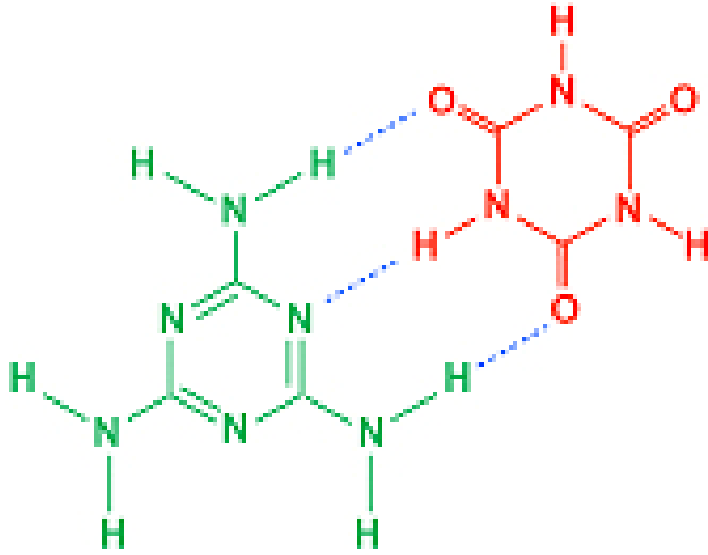
0.7 x 1.2 cm stone



>50 crystals



# Crystal interaction



wikipedia

Melamine-Cyanuric acid interaction to form crystals

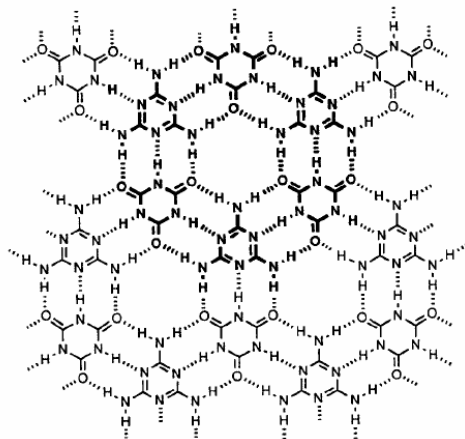
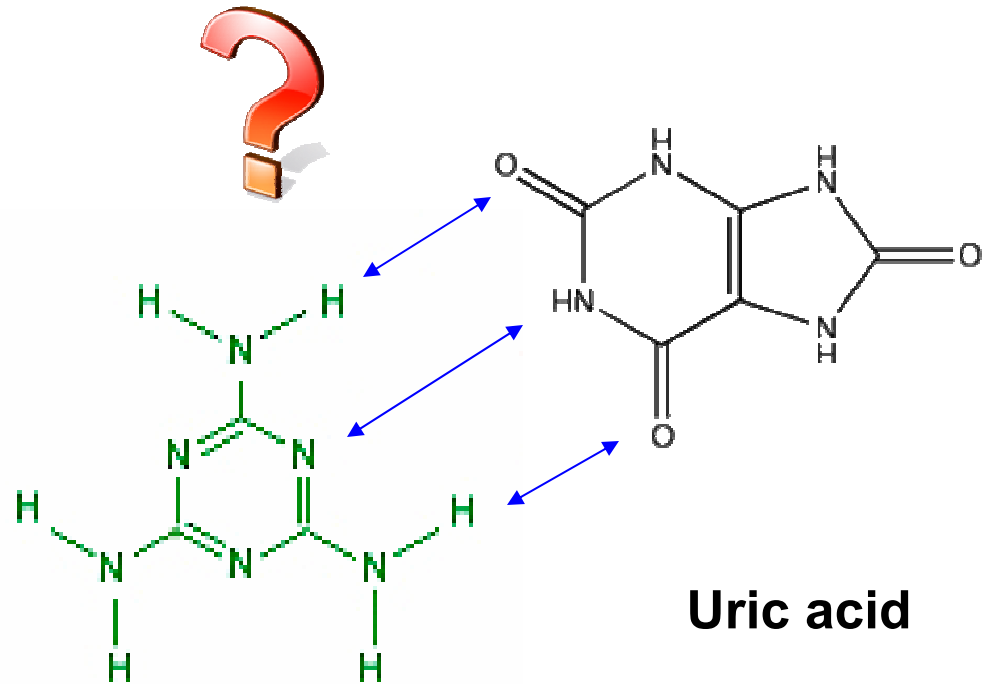


Fig. 6 Cyanuric acid-melamine lattice<sup>12</sup> (cyclic hexamer shown in bold).



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

N=2	MG/KG BW	PPM in feed
Control	0	0
MEL+CYA	1	25
MEL+CYA	3.3	83
MEL+CYA	10	250
MEL+CYA	33	635
MEL+CYA	100	2500
CYA	200	5000
MEL	200	5000



28 day  
study

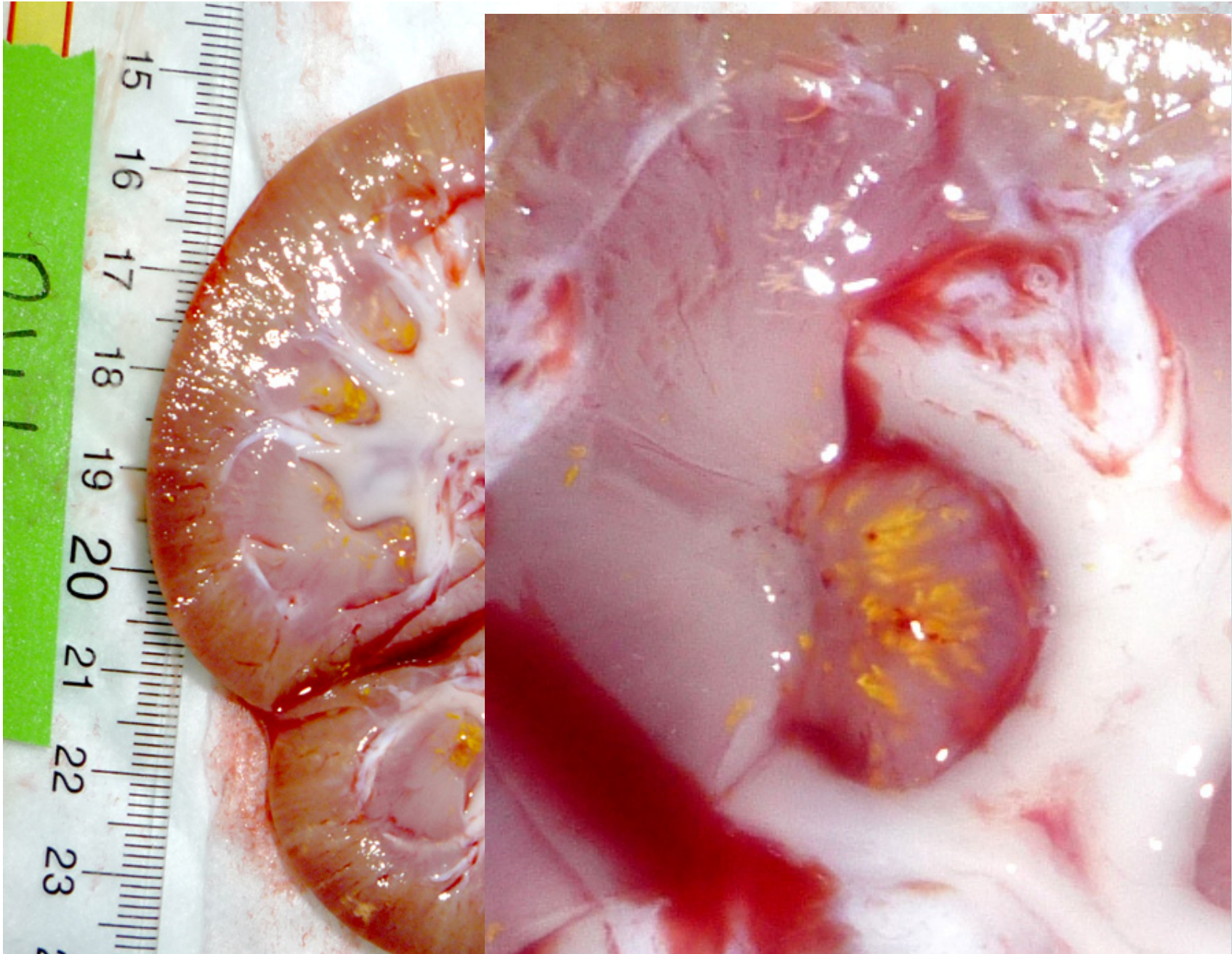


**Match NCTR Rat Study Pilot Dosages**



# Dosing daily as a bolus

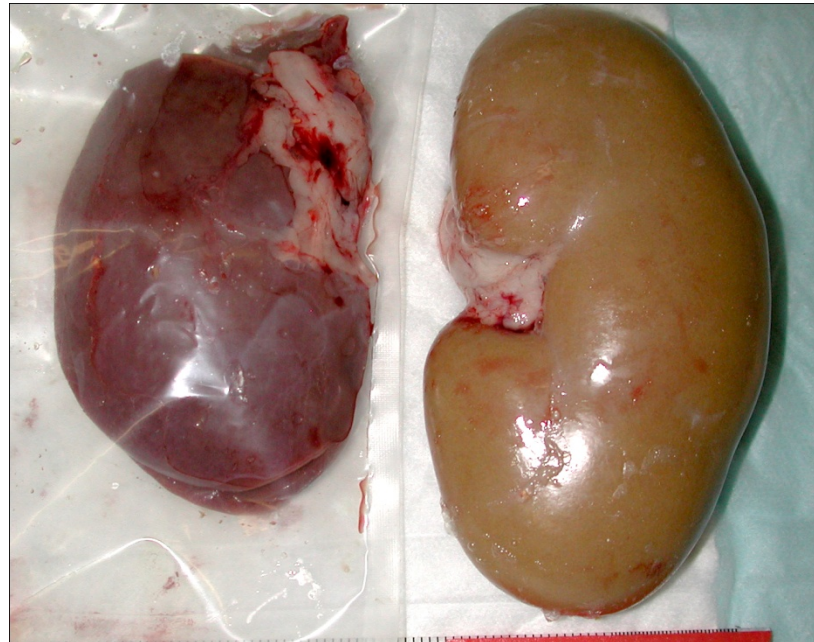




10 mg/kg kidney – yellow crystals readily apparent



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

2007

- Dogs and Cats

SCRAP- MEL (with CYA)

- Kidney Failure-Crystals  
Death

- 100s – 1000s

2008

- Babies

PURE MEL (no CYA)

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

- 300,000



# Melamine in feed was still a concern Nov 1, 2008



[Go to Google News](#)



A market in Xiamen

## Related News

[China: Feed makers defied rule](#)

In addition to scientist

## 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, and to ban melamine from being added to livestock feed.

"The ministry will tighten supervision of those who add melamine to the feed," said a spokesman for the Agriculture Ministry's livestock division.

Melamine, an industrial chemical, was discovered in China's milk in 2008, and has since been added to milk in China, according to a report by 53,000 other people.

The chemical -- which causes kidney stones -- was then discovered in China's food supply, and is now prevalent in China's food supply.

Wang acknowledged that the addition of melamine into



<http://media.wktv.com/images/dairy%20products%20milk%20eggs.jpg>

Dec 2009  
Arrested

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.

Last February, George M. and Robert V. Schuler, owners of Schuler Grain Co., pleaded guilty in the U.S. District Court for the District of Minnesota to three counts of selling adulterated wheat in 1985.

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**

# Situazione attuale della melamina nelle farine di carne

P. Cattaneo - L. Ceriani

Istituto di Ispezione degli Alimenti di Origine Animale - Facoltà di Medicina Veterinaria - Università  
Via Celoria 10 - 20122 Milano - Italia

Melamine contents in animal meals

- 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

Mitt. Gebiete Lebensm. Hyg. 74, 74–79 (1983)

Eingegangen 20. September 1982. Angenommen 14. November 1982

## Nachweis von 2,4,6-Triamino-1,3,5-triazin (Melamin) in Kartoffelproteinen

Determination of 2,4,6-Triamino-1,3,5-triazine (Melamine)  
in Potatoe Proteins

*R. Bisaz und A. Kummer*  
Eidg. Oberzolldirektion, Bern

- Melamine in **potato** meal  
**50%** of samples positive in 1982

n=4

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

# May 29, 2007 Sentenced

[Home](#) > [International](#)

## Ex-China Drug Regulator to Be Executed

China Sentences Former Top Drug Regulator to Death, Installs Food Recal



Undated file photo of Zheng Xiaoyu, former director of China's State Food and Drug Administration (SFDA), during a conference in Beijing. Zheng, 63, was convicted of taking bribes and dereliction of duty, according to the first instance hearing of the Beijing Municipal No. 1 Intermediate Peoples Court. The bribes taken by Zheng, including cash and gifts, were worth more than 6.49 million yuan (about 850,000 US dollar), according to the court. (Newscom)

# July 10, 2007 Executed

WORLD

## China Executes Ex-Food and Drug Chief

- [Hear NPR's Anthony Kuhn on 'All Things Considered'](#)
- [Hear Kuhn and Renee Montagne on 'Morning Edition'](#)



*NPR.org, July 10, 2007* · China executed the former head of its food and drug watchdog on Tuesday for approving untested medicine in exchange for cash, the strongest signal yet from Beijing that it is serious about tackling its product safety crisis.

The execution of former State Food and Drug Administration

Bribery – but melamine didn't help

## 2008 Arrests

### WINDOW OF CHINA

#### Six more arrests over melamine-tainted Chinese dairy products

www.chinaview.cn 2008-10-23 18:53:55

Print

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.

Farmers and producers



### The Province

ter

Search for  in the The Province

Owner of factory at centre of melamine scare arrested

mol

Officials

Nov 2009  
Executed

## 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

[Comments \(72\)](#) | [Add to My Stories](#)

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



# Questions?

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 2,470ppm of melamine





