



Analytical Challenges in Emergency Response to Chemical Contamination Events in Foods

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Outline

- Introduction
- FCC's Approach to Chemical Contamination Events
- FCC Casework: Analytical Challenge Examples
- Melamine in Pet Food
- Gulf Oil Spill
- Arsenic in Food



History of the Forensic Chemistry Center

Began as the
Elemental
Analysis
Research Center
(EARC)

1979

Federal Anti-
Tampering Act
passed by
Congress

1982

1983

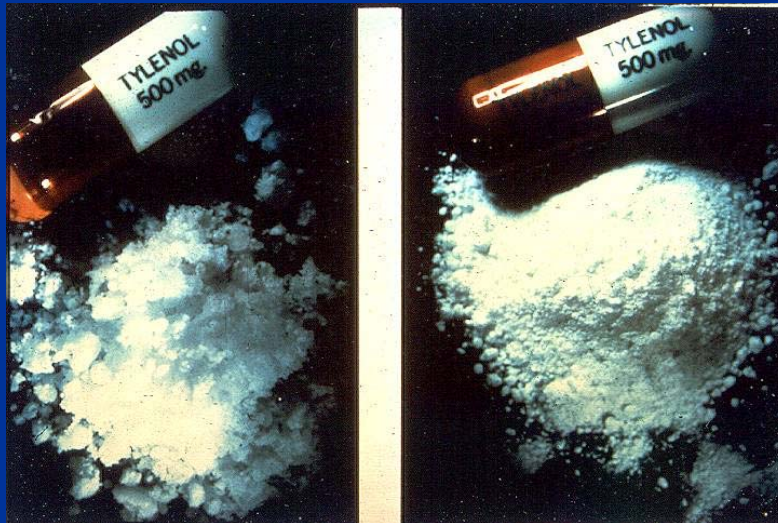
Chilean grape crisis
results in expansion of
capabilities and staff
and establishment of
the Forensic Chemistry
Center (FCC)

1989 1994

2002



Tylenol®
tampering incident
results in 7 deaths
caused by cyanide
poisoning



Sept. 11 terror attacks
prompt addition of staff
and instrumentation for
counterterrorism initiatives





FCC Functions

- Forensic casework serving FDA's Office of Criminal Investigations (OCI)
- Laboratory support in traditional FDA investigations as needed
- Research and method development related to product tampering, counterterrorism, counterfeiting, and fraud
- Special health instances and emergencies (national and international)
- Provide assistance to other regulatory and law enforcement agencies as needed

Investigational Information



Analytical Team
Problem Solvers

Separations
HS/GC/MS
GC/MS
LC/MS
HPLC

Molecular
Spectroscopy
FT-IR/RAMAN/UV

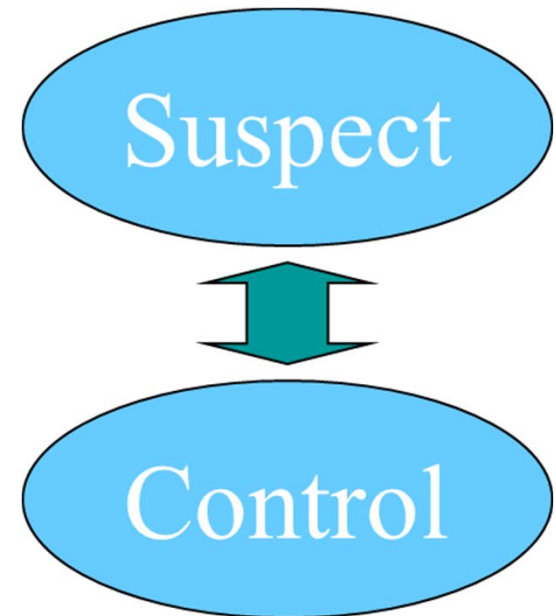
Elemental
Analysis
ICP-AES(MS)

Mixed Threat Triage
BSL-3

Optical
Characterization
Microscopy (SEM)
Image Analysis

FCC Casework

- Chemical Contamination/Product Tampering/Terrorism
 - Unintentional vs. Intentional (includes economic adulteration)
 - Timely Response
 - Develop Supporting Evidence
 - Method Development & Research
- Counterfeit Foods and Pharmaceuticals
 - Finished Dosage and API
 - Establish Physical & Chemical Profiles
 - Differentiate by Lot, Manufacturer, etc.
- Unapproved Drugs
 - Dietary Supplements
 - Identification (Qualitative Analysis) and Quantitation



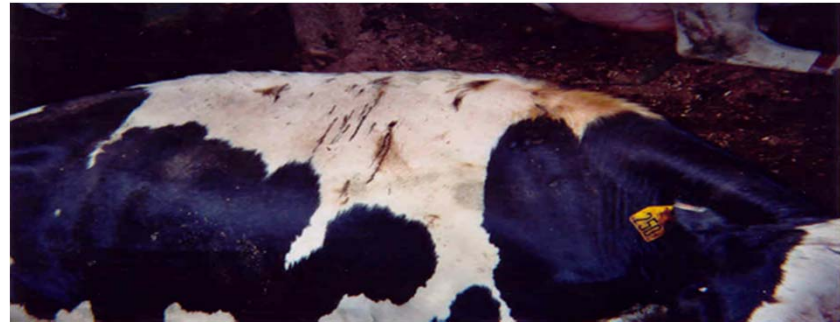
Baby Food Tampering

July 2004



- GC/MS screen – identified unusual component (*ricinoleic acid*)
- Lateral Flow Device (ricin) – positive for ricin
- ELISA –positive for ricin
- LC/MS – positive for ricinine
- Optical microscopy identified botanical material consistent with castor bean
- western blot – positive for ricin
- capillary LC/TOF –confirmed ricin

Suspected Tampering with Dairy Cattle: Identification of unknown substance



- **Washington State Dairy Farm 2004**

Ten cows at large dairy farm (400 head) sick / some deceased
18 with spots on the hide and neck/hide was hard "leathery"

Suspected Tampering with Dairy Cattle

- WSDA verifies through contact with the dairy owner that milk from affected animals left the dairy and was processed
- WSDA contacts FDA for assistance
 - unknown substance / commerce
- Is there a public health concern?
 - potentially greater than 10,000 gallons milk from dairy farm affected
 - potentially nearly 700,000 gallons of dairy products contaminated nationwide

Suspected Tampering with Dairy Cattle

- FDA's Forensic Center received samples of hide, tissue, organs, from cow necropsies as well as ear tags
- milk samples received (from both affected and non-affected animals)



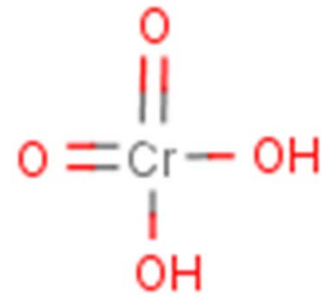
Analysis of Material Scraped from Contaminated Ear Tag

- HPLC/MS poison screen - negative
- GC/MS poison screen - negative
- initial FTIR microscopy - inconclusive
- pH – neutral

- ***Spot test for oxidizing agents – positive***
- ***ICP/AES – high level of chromium (no counterion identified)***
- ***FTIR and Raman spectra consistent with chromic acid.***

Agroterrorism Investigation

- Milk from animals that came into direct contact with the substance found to contain < 1 ppb Cr
- Farmer's relative surfaced as possible suspect:
 - Works with Chromic Acid
 - 1992 Arrest by FBI for illegal storage of Chromic Acid in Montana
 - Admitted storing chromic acid at farm years earlier



Dietary Supplement Analysis for Active Pharmaceutical Ingredients (APIs)

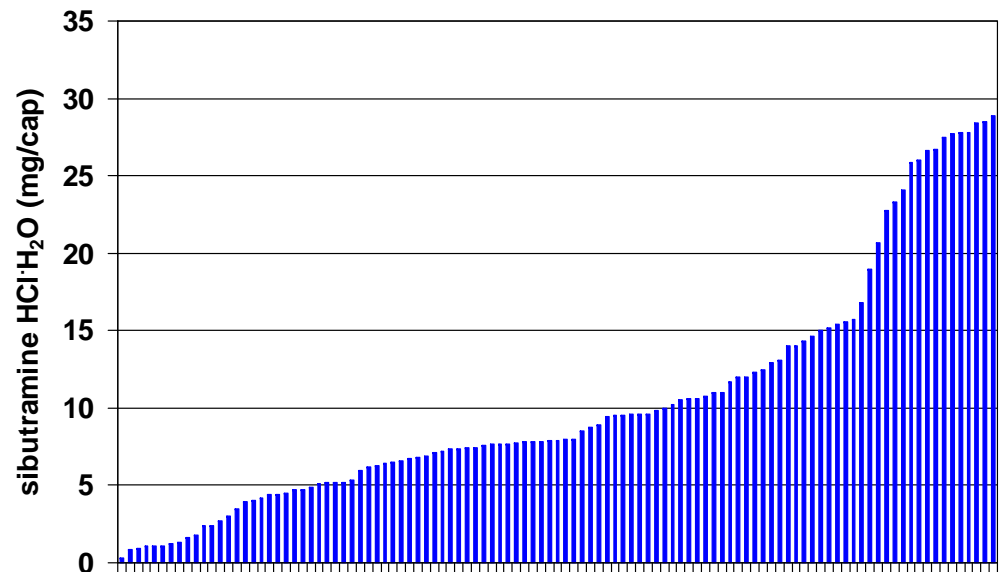
- Typical Supplement Targets
 - Weight Loss (Sibutramine, *etc.*)
 - Improved Sex Life (PDE-5 Inhibitors)
 - Improved Physical Condition (steroids)
- Analytical Challenges
 - Analogs
 - Multiple APIs
 - No standards available for many analogs
- Typical Analyses
 - Qualitative Analysis (ID) using GC-MS, LC-MS, *etc*
 - Quantitative Analysis of active using UV-HPLC





Sibutramine Quantitative Analysis

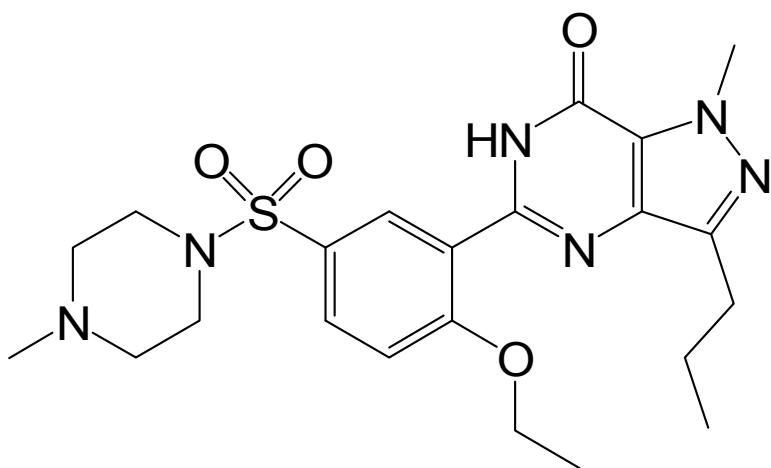
- Dangerous for those with a history of cardiovascular disease
 - Elevated blood pressure
 - Stroke
 - Heart attack
- For healthy people...
 - Anxiety
 - Nausea
 - Heart palpitations
 - Racing heart
 - Insomnia



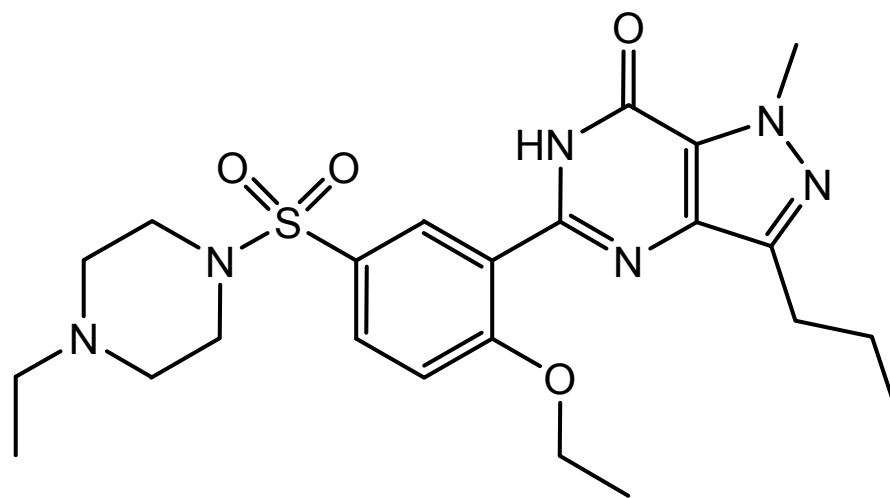
107 samples

What is an analog?

In simple terms, the word analog or analogue is used to describe a substance that has major chemical structures in common with another chemical.



sildenafil



homosildenafil



Synthetic Drug Analogs

➤ Challenges

- Difficult to detect without a target
- No standards
- Unambiguous identification?
- Legal issues
(*i.e. classification of substance as an analog*)

➤ Dangers

- No clinical evaluation
- Pharmacology often unknown
- Safety unknown



Evolution of an Illegal Dietary Supplement Product

- FDA OCI submitted multiple samples of this product to FCC over a 16 month time period.
- Product labeling identical (other than product lot # and Exp.) for each submission

1st Submission: sildenafil and homosildenafil

2nd Submission: homosildenafil

3rd Submission: acetildenafil

4th Submission: Piperadino vardenafil

5th Submission: vardenafil related compound

Is it an analog?

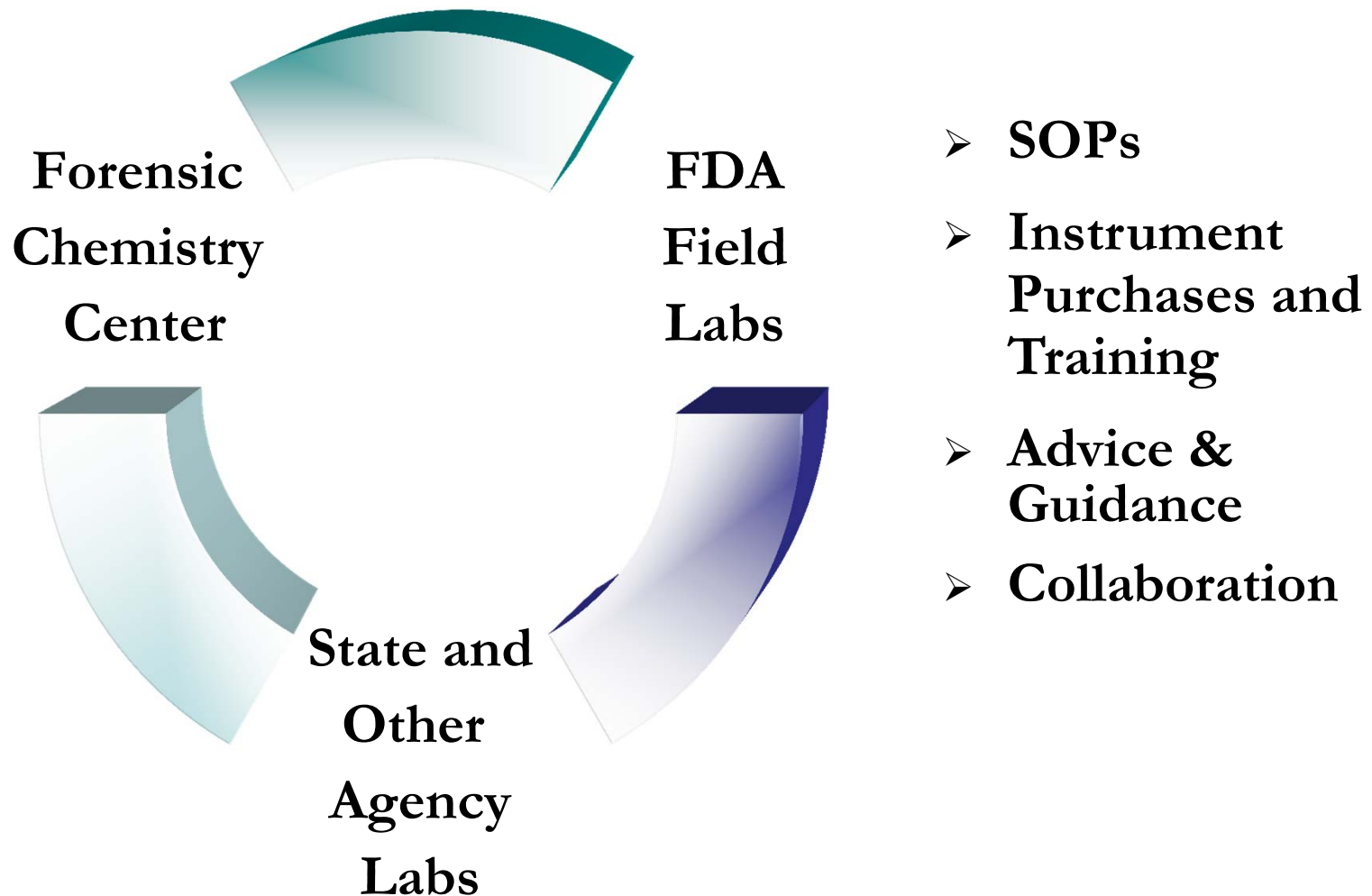
Is it a hydrolysis product?

Is it active?

Partial synthesis of vardenafil



Technology Transfer





MISSION

- Integrate the nation's food-testing laboratories for the detection and identification of threat agents in food at the local, state, and federal levels.
- Includes biological, chemical, and radiological threat agents.
- Full range of food commodities



Chemistry Cooperative Agreement Program (cCAP)

- FDA currently has funded 14 state laboratories through cooperative agreements (beginning 2005)
- Be fully equipped and trained in FERN chemical screen methodologies including LC/MS, GC/MS, ICP/MS, ELISA methods
- Participate in FERN chemical surveillance activities
- Provide method development and validation support for FERN chemistry methods and

2007 Pet Food Crisis

February

Consumer complaints- cat illness

March 6, 7

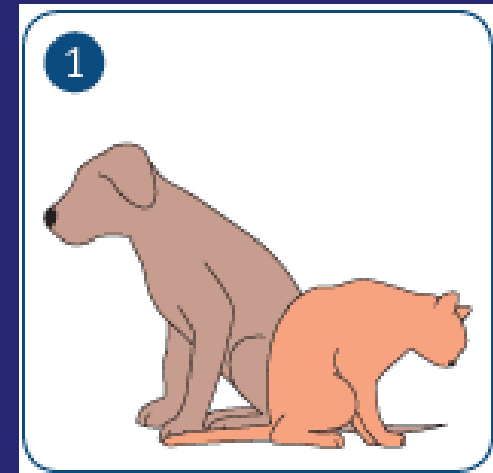
Feeding study- cat illness and deaths

Tests negative for heavy metals, DEG,
Pesticides, toxins

March 12

Focus on wheat gluten – change in supplier

Tests negative



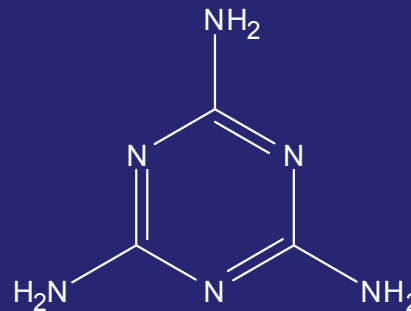
2007 Pet Food Crisis

March 22

- Samples arrive at FCC for additional testing
- DART- Ion Trap-FTICR and GC-MS screen detects **melamine** in pet food.



So What? Low Toxicity



2007 Pet Food Crisis

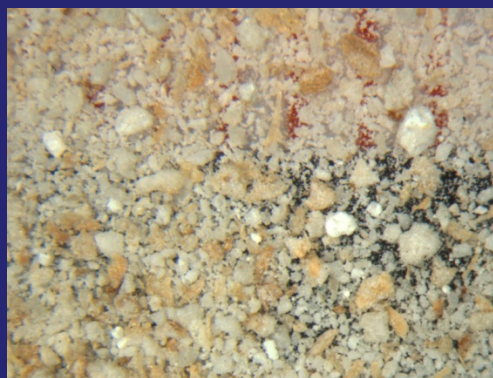
March 26

DART- Ion Trap-FTICR analysis of a crystal isolated from wheat gluten detects:

melamine
cyanuric acid
ammeline
ammeline

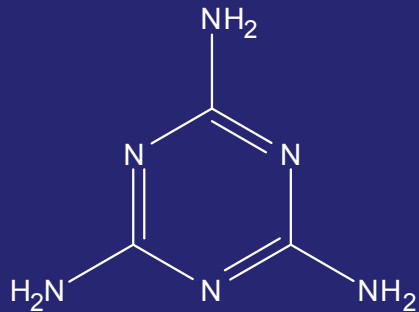


Comparison
Wheat Gluten

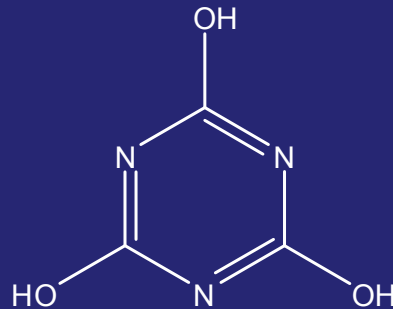


Suspect
Wheat Gluten

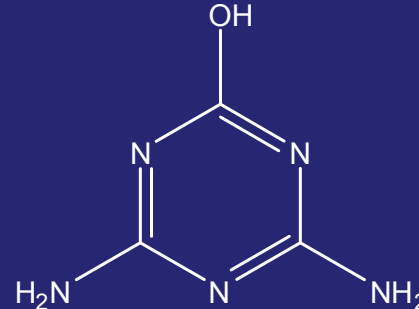
Why Melamine?



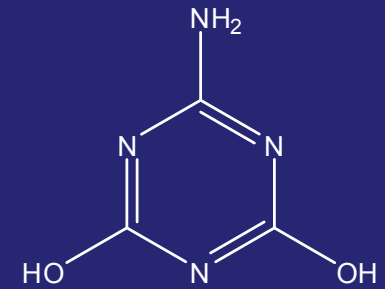
Melamine
MW 126.1



Cyanuric acid
MW: 129.1



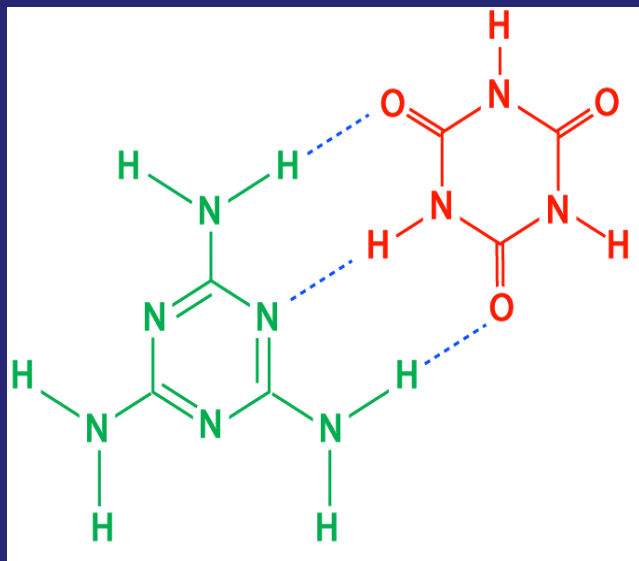
Ammeline
MW: 127.1



Ammelide
MW: 128.1

- Melamine: precursor in production of plastic resins, fertilizer
- Low cost and high nitrogen content leads to use in economic adulteration by increasing the apparent protein content (total nitrogen used as a measure of protein)

- GC-MS Analysis
Acetonitrile/Water/DEA (50:40:10)
Extraction
 - Solvent system disrupts hydrogen-bonded complexes and allows dissolution of all target analytes



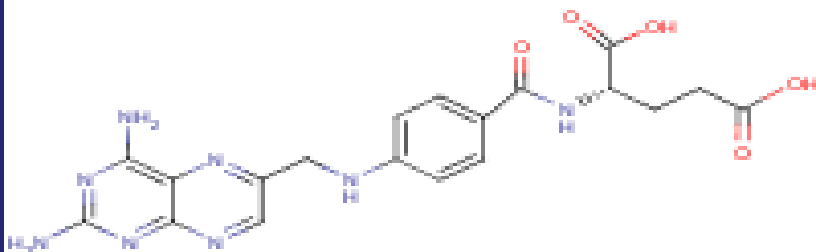
**Melamine-Cyanuric
Acid Complex**

Analytical Challenge

March 23

(AP) - Rat poison has been found in pet food blamed for the deaths of at least 16 cats and dogs, a spokeswoman for the...

Reported concentration:
~ 40 ppm Aminopterin



FDA collected and analyzed the same lot of unopened cans of pet food.

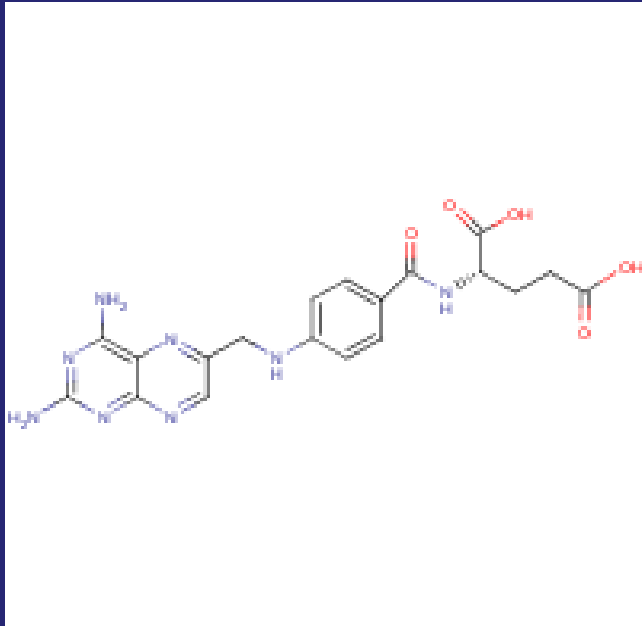
Did not detect aminopterin

Analyzed portion of samples sent to FDA from original testing lab.

Did not confirm the presence of aminopterin

FDA did stability studies on aminopterin in the same lot of pet food.

Aminopterin was stable for at least 15 days

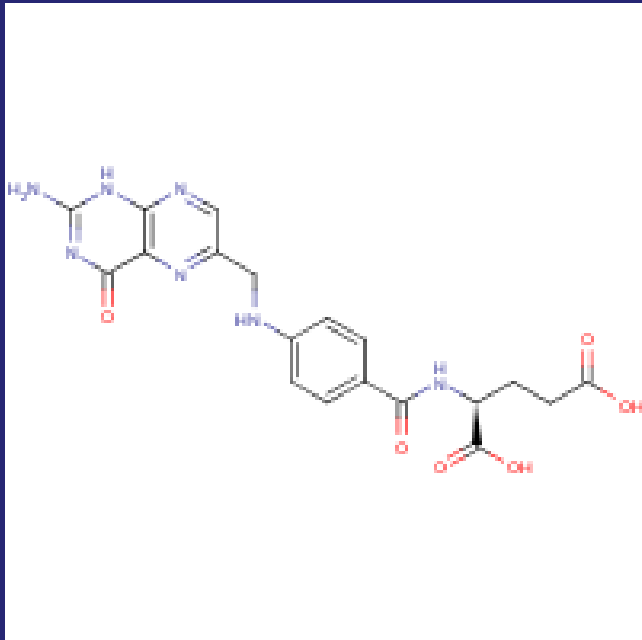


Aminopterin

MW 440.4

MS/MS product ion

294.4

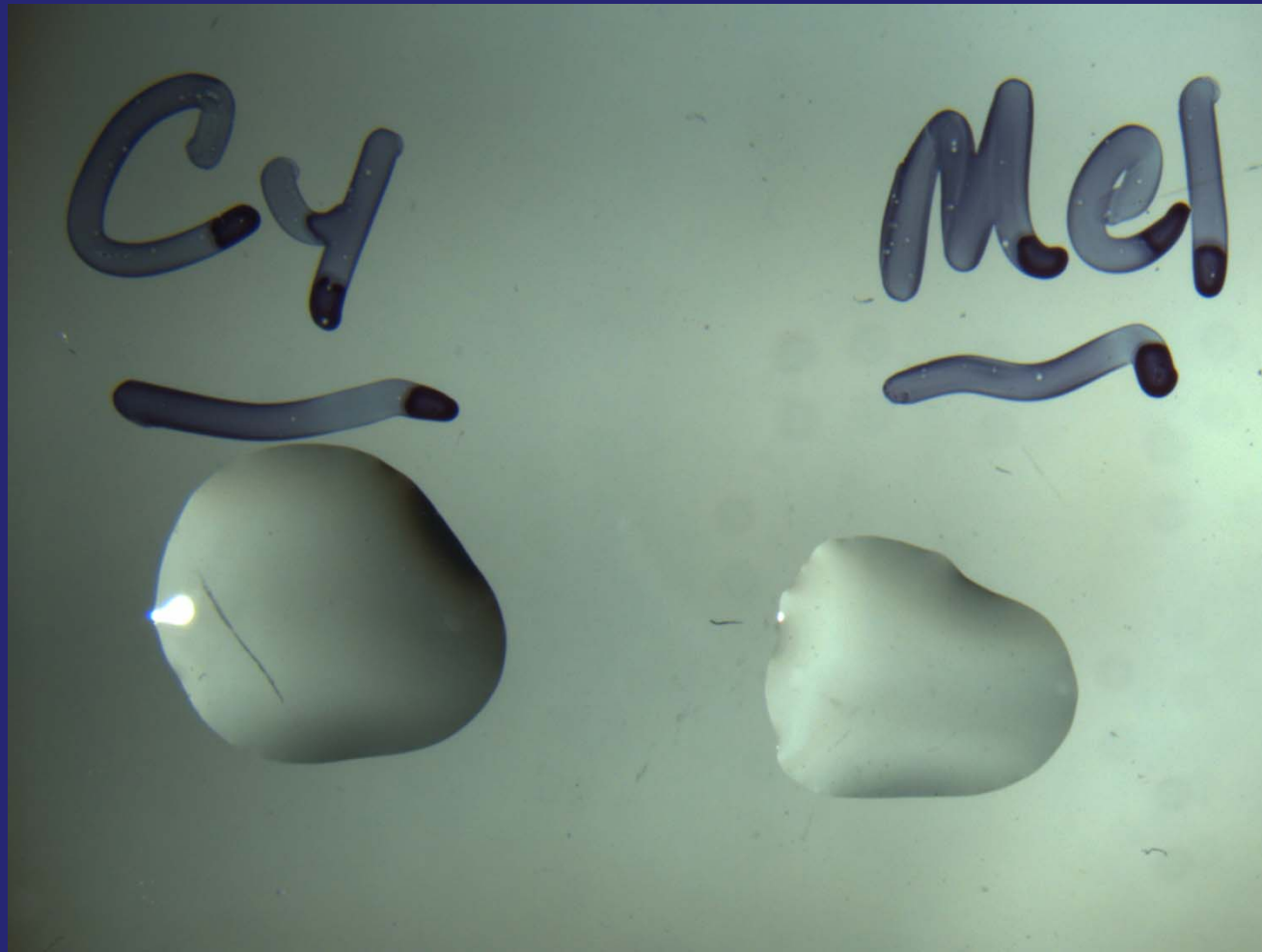


Folic Acid (additive to some
pet foods)

MW 441.4

MS/MS product ion

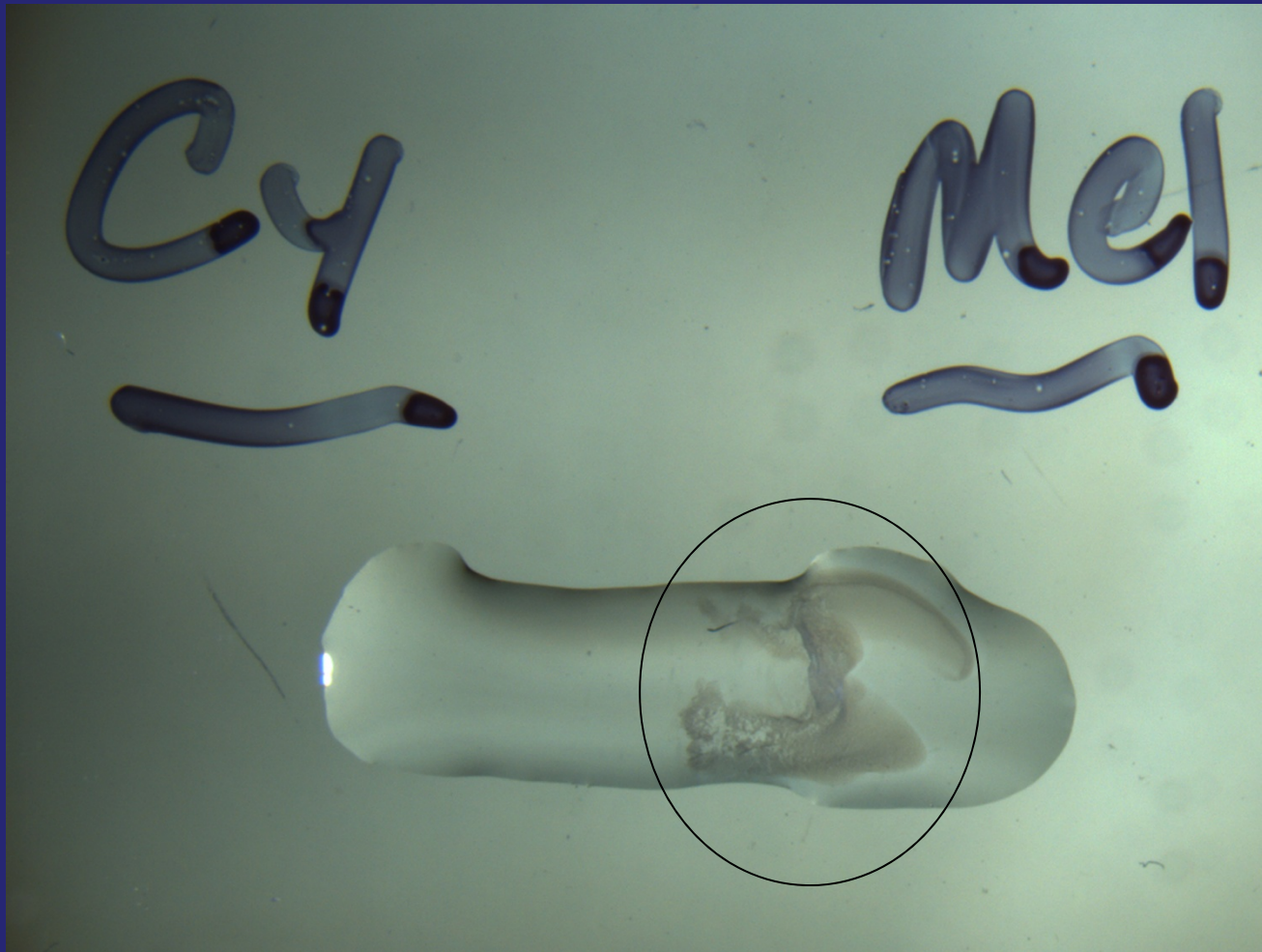
295.8



Cyanuric acid in water

Melamine in water

So what about that toxicity issue?



Spontaneous crystal formation upon mixing both solutions

Deepwater Horizon Oil Spill

The Beginning....



April 20, 2010 - an explosion and fire occurs on the Deepwater Horizon (DWH) oil drilling platform.

Rig capsizes and sinks on April 22 spilling millions of gallons of oil into the Gulf over the following months.

July 15, 2010 - Leak stopped.

What are the concerns?

- **How oil can make seafood unfit for consumption:**
 - Potential to taint seafood with flavors and odors caused by exposure to hydrocarbon chemicals
 - Composed of many chemicals, but it is the carcinogenic PAHs which are of greatest concern because they can be harmful if consumed in sufficient amounts over a prolonged period of time
- **Dispersant Use**
 - Low potential to bioaccumulate in seafood and are low in human toxicity, therefore there is likely little public health risk associated with consuming seafood that has been exposed to them

State Reopening Protocol Outline

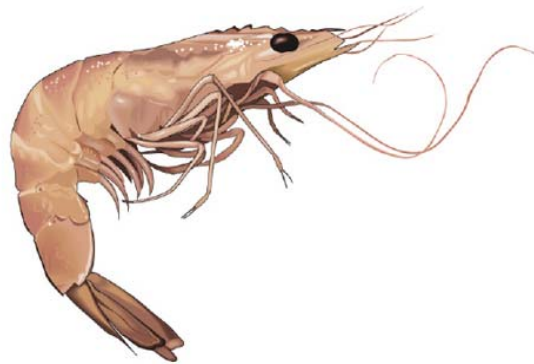
“Protocol for Interpretation and Use of Sensory Testing and Analytical Chemistry Results for Re-Opening Oil-Impacted Areas Closed to Seafood Harvesting Due to The Deepwater Horizon Oil Spill”

- Tiered Approach:
 - Waters are Examined Visually.
 - State submits reopening proposal to CFSAN (State decides which areas and types of seafood).
 - Samples Chosen and Evaluated by State Sensory Screeners.
 - Panel of Organoleptic Experts examines Seafood Sample.
 - ***Chemical Analysis for Polycyclic Aromatic Hydrocarbons (PAHs)***
 - <http://www.fda.gov/Food/ucm217601.htm>

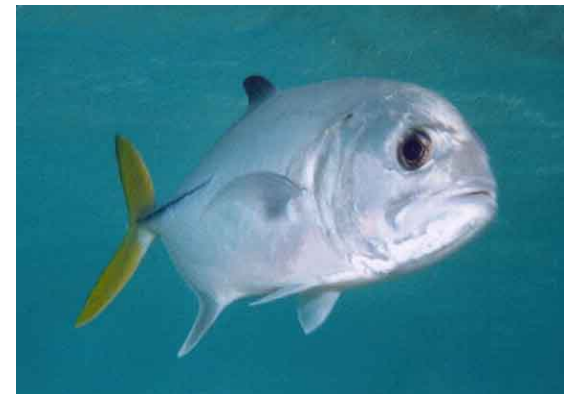
Priority of Seafood Types for Risk

Greatest to Least Risk for contamination

- Oysters
- Crab
- Shrimp
- Finfish



deepwater shrimp



PAH Levels of Concern for Seafood Safety

For PAH with cancer end points estimates of contamination levels and consumption rates that, if sustained for period of 5 years, may result in consumer lifetime cancer risk of 1×10^{-5}

CHEMICAL	LEVELS OF CONCERN (ppm)			BASIS
	13 g/day (shrimp and crab)	12 g/day (oysters)	40 g/day (finfish)	
Naphthalene	123	133	32.7	Non-cancer EPA RfD; 80kg bw
Fluorene	246	267	65.3	Non-cancer EPA RfD; 80kg bw
Anthracene/Phenanthrene	1846	2000	490	Non-cancer EPA RfD; 80kg bw
Pyrene	185	200	49.0	Non-cancer EPA RfD; 80kg bw
Fluoranthene	246	267	65.3	Non-cancer EPA RfD; 80kg bw
Chrysene	132	143	35.0	Cancer 0.001 BaP equivalent
Benzo(k)fluoranthene	13.2	14.3	3.5	Cancer 0.001 BaP equivalent
Benzo(b)fluoranthene	1.32	1.43	0.35	Cancer 0.001 BaP equivalent
Benz(a)anthracene	1.32	1.43	0.35	Cancer 0.001 BaP equivalent
Indeno(1, 2, 3-cd) pyrene	1.32	1.43	0.35	Cancer 0.001 BaP equivalent
Dibenz(a,h)anthracene	0.132	0.143	0.035	Cancer 1.0 BaP equivalent
Benzo(a)pyrene	0.132	0.143	0.035	10^{-5} Cancer risk = (0.110 ug/person/day(78/5)

Includes alkylated homologues, for example C-1, C-2, C-3, C-4 naphthalenes, fluorenes, anthracenes, fluoranthenes, pyrenes and chrysenes. Alkylated homologues are assumed to have similar toxicities to the parent compounds.

NOAA Analytical Methodology

- The NMFS-NWFSC-59: *Extraction, Cleanup, and Gas Chromatography/Mass Spectrometry Analysis of Sediments and Tissues for Organic Contaminants*
 - **Confirmation method.**
 - Reliable (used 20+ yrs by NOAA for programs such as MusselWatch).
 - Withstood Legal Challenges.
 - Validated Method.
 - Flexibility with matrices - can be applied to seafood, water, soil, etc.
 - Very sensitive, thorough analysis of parent and alkyl PAH homologs.
 - Complicated & Labor Intensive.

Issues with NOAA PAH method

- Sample throughput time (7-8 days initially, 3-5 days if lab familiar with method and run multiple shifts).
- Sensitive to environmental contamination.
- Familiarization time needed.
- Acquisition of equipment issues (ASE).
 - **Screening method needed!**

PAH Screening Method

- *Screen for the Presence of Polycyclic Aromatic Hydrocarbons in Select Seafoods Using LC-Fluorescence*
 - **Forensic Chemistry Center, FDA- Lead Laboratory.**
 - Department of Analytical Chemistry, CT Agricultural Experiment Station.
 - Laboratory Services Division, MN Dept. of Agriculture.
 - Method uses QuEChERS sample prep with LC-FL detection.
 - FDA Gulf Coast Seafood Lab (GCSL) produced incurred samples used to develop method.
 - Posted 7/27/2010

Method Throughput Comparison

- NOAA (8 labs)
 - Turnaround Time
 - 3-7days
 - Each Lab
 - 20 samples/week
 - Eight Labs
 - 160 samples/week
- Screening Method (18 labs)
 - Turnaround Time
 - 24 – 36 hours
 - Each Lab
 - 20 samples/day
 - Eighteen Labs
 - 2500 samples/week

Testing to Re-open Waters

June – November 2010

- 2,824 specimens collected from state waters and tested by organoleptic and chemical analyses
- 5,387 specimens collected from federal waters and tested by organoleptic and chemical analyses
- ~20% of all PAH screening tests verified by confirmatory method
- 50% of specimens subjected to testing for dispersant residue

Results from Testing to Re-open Waters

June – November 2010

- Polycyclic aromatic hydrocarbon (PAH) levels in all test samples found to be 100 to 1000 times below levels of concern
- Dispersant (DOSS) levels in test samples found to be below LOD in majority of samples and > 1000 times below level of concern in the few samples in which it was detected
- Results are available at www.fda.gov > More Public Health Focus > Gulf of Mexico Oil Spill Update

Primary Routes of Exposure to Inorganic Arsenic

- Drinking Water
 - US EPA limit: 10 ppb total As
 - Mostly inorganic As
 - Method of removal affected by speciation
- Dietary Sources
 - Wide variety of species present in fish, shellfish and seaweed
 - Mostly iAs and methylated species in terrestrial-based foods



Figure 1.4 An arsenic victim.
(Photograph courtesy of Professor Dipankar Chakraborti.)





Why Arsenic Speciation?

Highly Toxic and Carcinogenic

Arsenite (AsIII)
Arsenate (AsV)

Possible Cancer Promoters

Monomethylarsonic Acid (MMA)
Dimethylarsinic Acid (DMA)

Lower Toxicity/Less Understood

Arsenobetaine (AsB)
Arsenocholine
Arsenosugars

LD₅₀ (mg/kg, rat)

AsIII 4.5

AsV 4-18

MMA 1800

DMA 1200

AsB 10,000

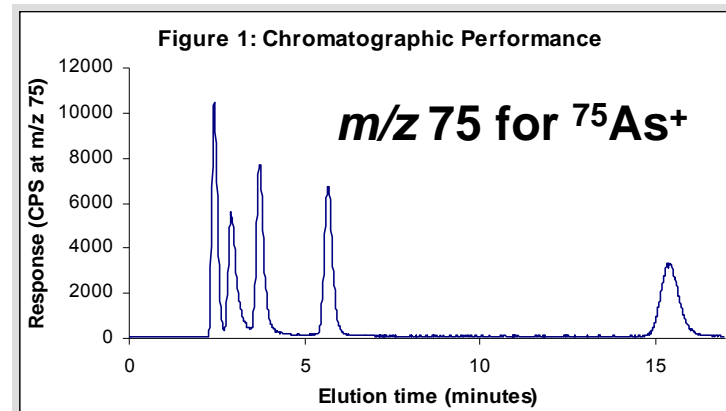
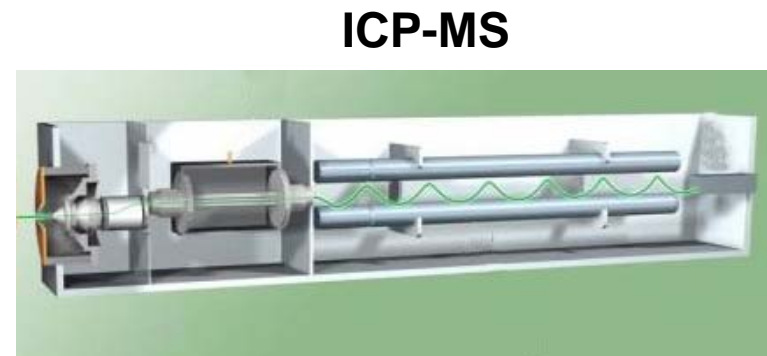
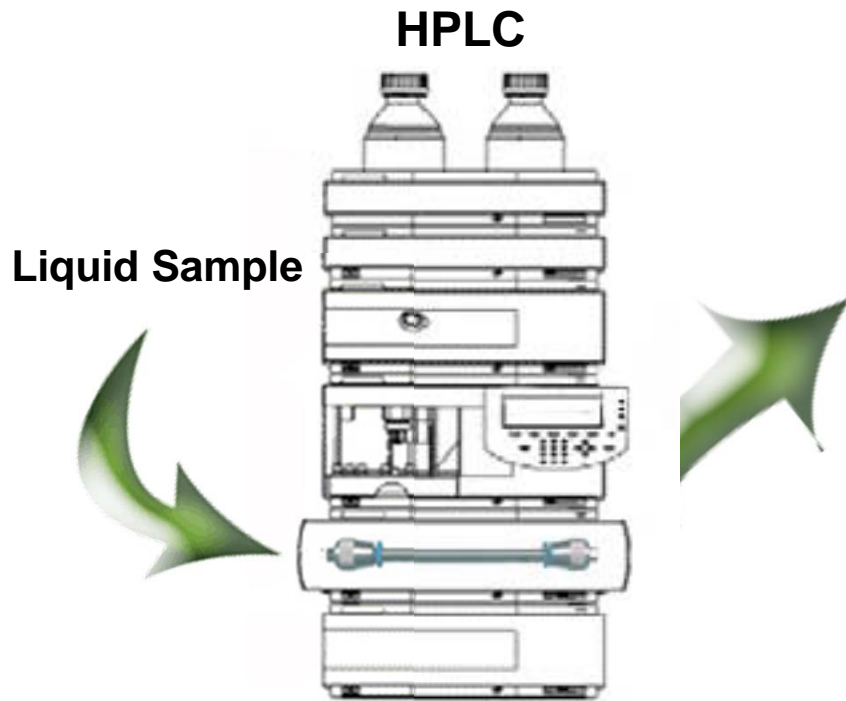
AsC 6000

arsenosugars ??



Arsenic Speciation

Coupling HPLC to ICP-MS





Basis for FDA Interest in Arsenic in Rice

- Sampling results from FDA's Total Diet Study
 - Rice is high in total arsenic relative to most foods
- Speciation data on inorganic arsenic in rice, especially data that became available in 2009

Testing Finds Arsenic in Apple Juice | Food & Water Watch - Windows Internet Explorer

http://www.foodandwaterwatch.org/pressreleases/testing-finds-arsenic-in-apple-juice/

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• *The Dr. Oz Show, Sept 12, 2011*



TESTING FINDS ARSENIC IN APPLE JUICE

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July 21st, 2011

Testing Finds Arsenic in Apple Juice

Groups Call on FDA to hold juice to same minimum standard as drinking water, better monitor imports from countries with serious food safety problems like China

Washington, D.C. - Today, consumer advocacy organizations Empire State Consumer Project and Food & Water Watch called on the Food and Drug Administration to take long overdue action on contamination of apple juice with heavy metals such as arsenic. The groups sent a letter to the agency that contained new data from testing commissioned by the Empire State Consumer Project, which revealed levels of arsenic contamination in one juice sample that was more than five times higher than what the Environmental Protection Agency would allow in drinking water.



The group arranged for testing of different brands of apple juice and apple sauce, and one sample of Mott's Apple Juice registered 55 parts per billion of arsenic. The EPA's tolerance level for arsenic in drinking water is 10 parts per billion, while the FDA does not have a tolerance level for juice.

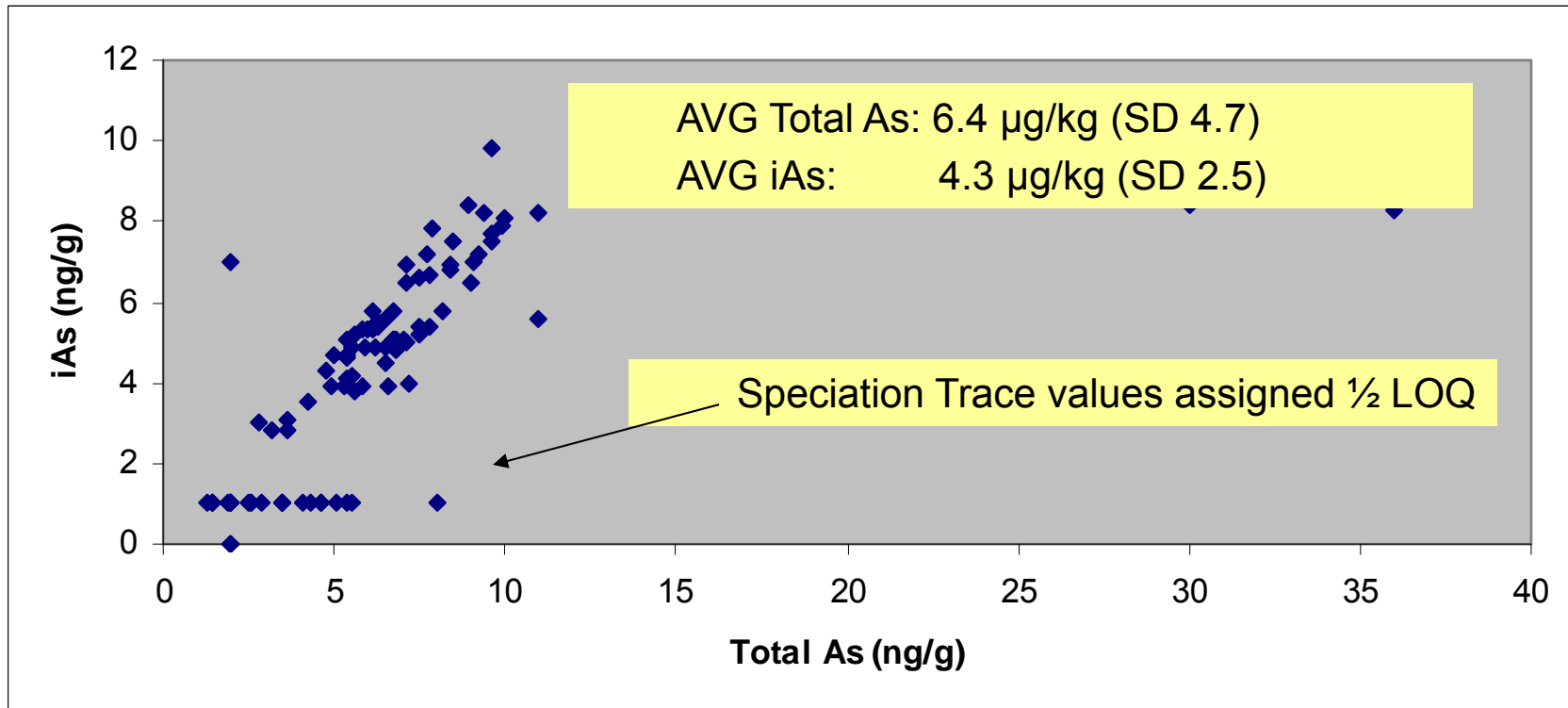


Arsenic Speciation in Pear Juice

- 2008 HHE conclusion: chronic exposure of pear juice products containing over 23 ppb inorganic arsenic would represent a potential health risk.
- Single strength pear juice ≥ 23 ppb total arsenic requires further testing for iAs using HPLC-ICP-MS.
- 40 samples of concentrate analyzed by HPLC-ICP-MS,
 - 16 contained > 23 ppb iAs (calculated as RTD)
 - MMA ranged from < 2 ppb to 513 ppb
- Samples are evaluated on a case-by-case basis.
- 2008-2011: 23/141 samples (includes above) found to contain ≥ 23 ppb iAs, subjected to FDA regulatory action (warning letter, refused entry, recalls).



Summary of Results: Apple Juice Assignment, 2011



- 19 Brands from retail and distribution centers
- 95% (90/94) samples: total As levels < 10 ppb
- 100% samples: iAs levels < 10 ppb
- DMA only present at trace level
- MMA mostly less than LOD except for 3 samples: 4.4, 19, 20 µg/kg



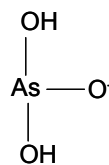
Summary: Arsenic in Apple Juice

- FDA remains confident in the overall safety of apple juice consumed in the U.S.
- FDA currently considers test results for inorganic arsenic in fruit juice on a case-by-case basis.
- Regulatory enforcement action is taken as appropriate. (recalls, refusing entry of imports, etc)
- FDA EAM 4.10 As Speciation in Fruit Juice is available at www.fda.gov
- **On July 12, 2013, FDA proposed an “action level” for inorganic arsenic in apple juice of 10 ug/kg (ppb).**
 - 60-day comment period was extended

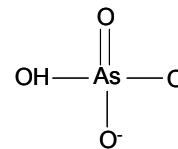


Arsenic Speciation in Rice and Rice Products

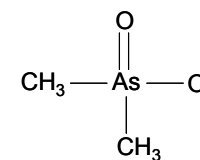
- Why is FDA interested?
- Method description
- Example results



AsIII



AsV



DMA



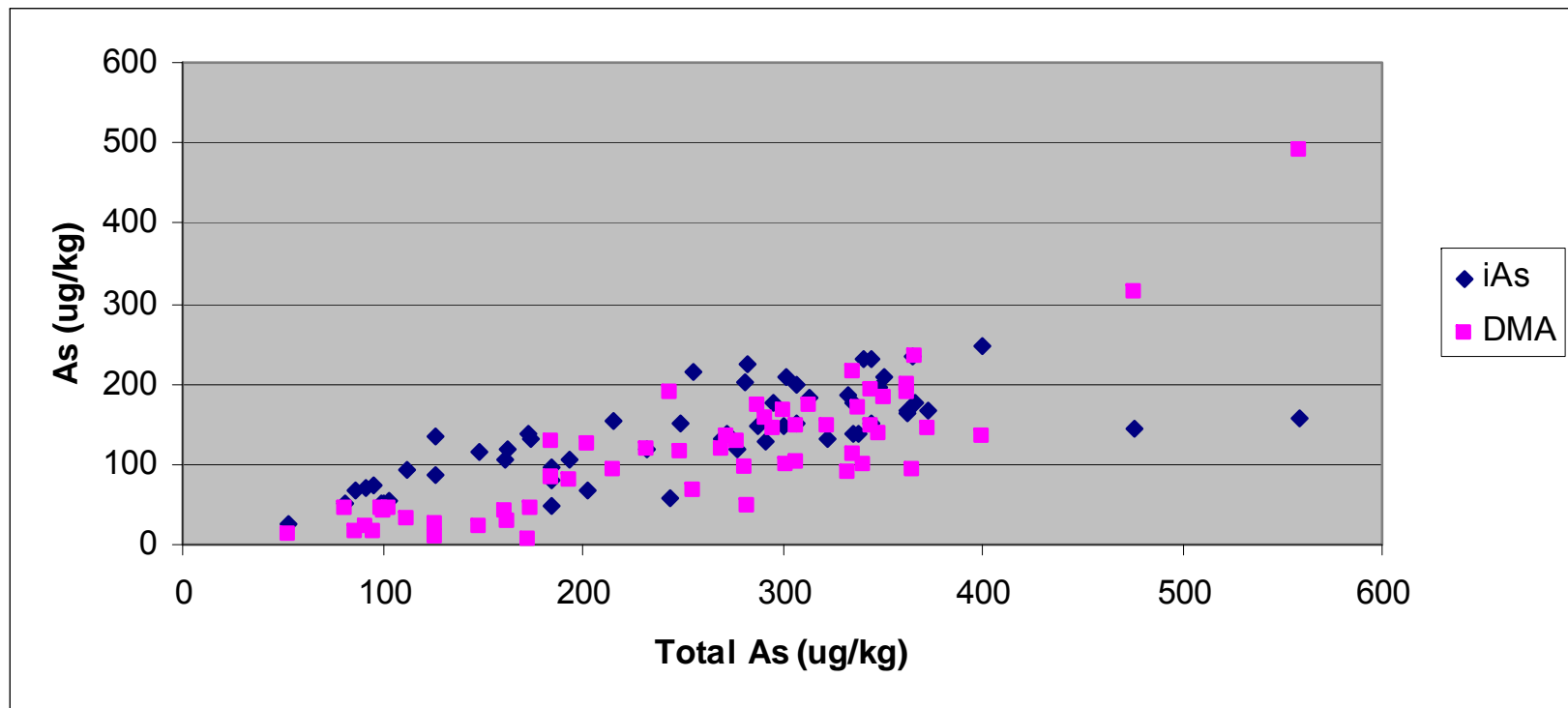
www.food.change.org



www.ehow.org



Rice Samples: DFPG Assignment 12-08





Product Category	Product Subcategory	Average Inorganic Arsenic mcg/serving	Number of Samples
Rice	Basmati	3.5	53
Rice	Brown	7.2	99
Rice	Instant	2.6	14
Rice	Jasmine	3.9	13
Rice	Other (incl wild rice ⁵ , carnaroli, mixed types)	5.6	6
Rice	Parboiled	5.1	39
Rice	White, long grain	4.6	149
Rice	White, medium grain	3.6	91
Rice	White, short grain	3.5	23

September 2013 Results for >1300 samples of rice and rice products:

- Rice
- Bakery mixes and pudding
- Beverages (beer, rice wine)
- Cereals (infant & toddler)
- Grain-based Bars
- Other (infant formula)
- Snacks (rice cakes, cookies)

Available at www.fda.gov



FDA Statement on Testing and Analysis of Arsenic in Rice and Rice Products

The levels FDA found in its testing are too low to cause immediate or short-term adverse health effects. FDA's work going forward will center on long-term risk and ways to manage it with a focus on long-term exposure.



US Food and Drug Administration
Office of Foods and Veterinary Medicine

**Guidelines for the Validation of Chemical
Methods for the FDA Foods Program**

Version 1 2/28/2012

Available at www.fda.gov



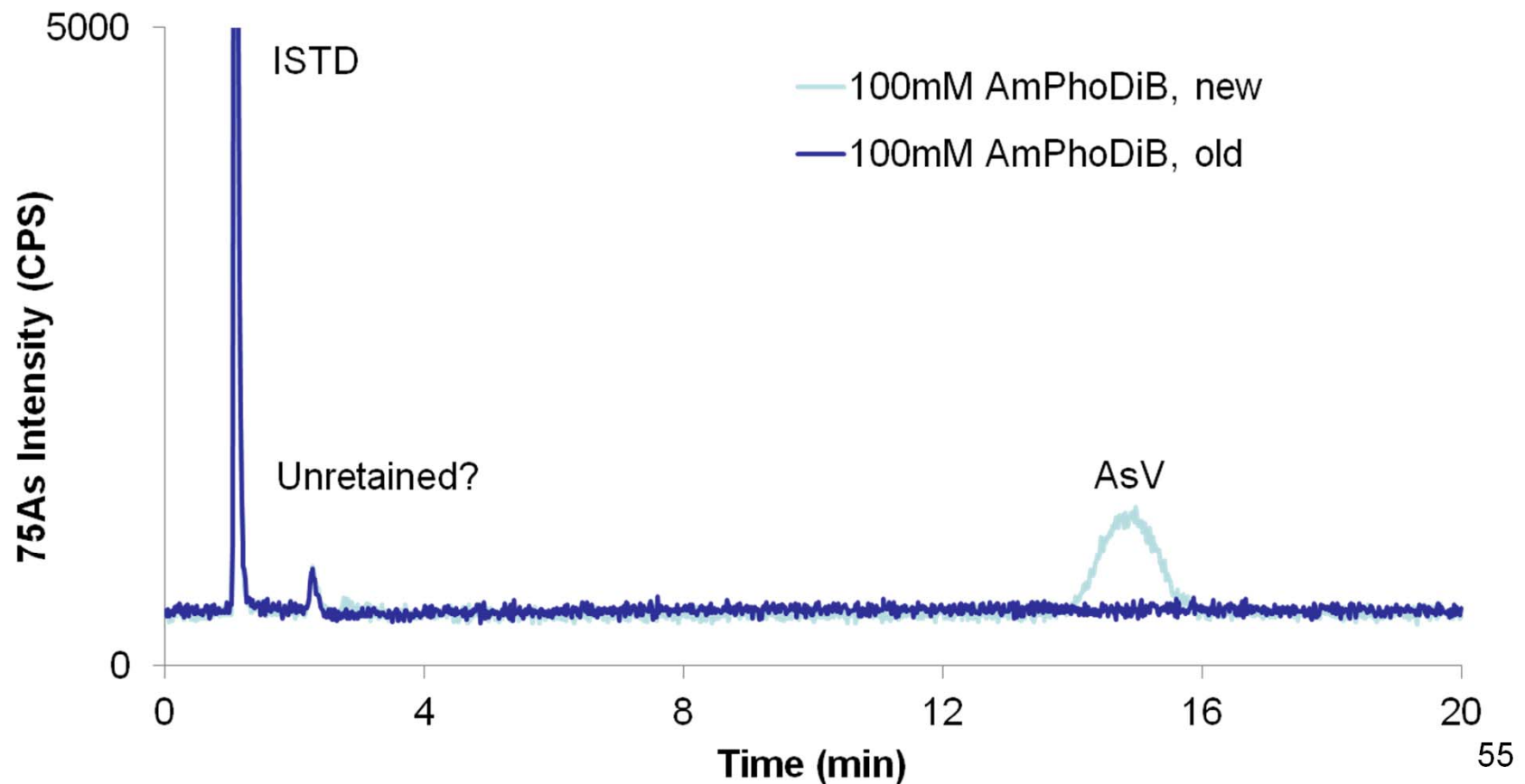
Multi-lab validation/verification

- Currently, 7 laboratories
 - 2 state labs
 - 3 FDA ORA labs
 - 1 FDA CFSAN lab
 - 1 contract laboratory
- 2 rice and 1 rice cereal sample
 - Triplicate
 - 3 spike levels in each in duplicate
- 3 CRM rice flour samples
- Method blanks (spikes at LOQ + low)



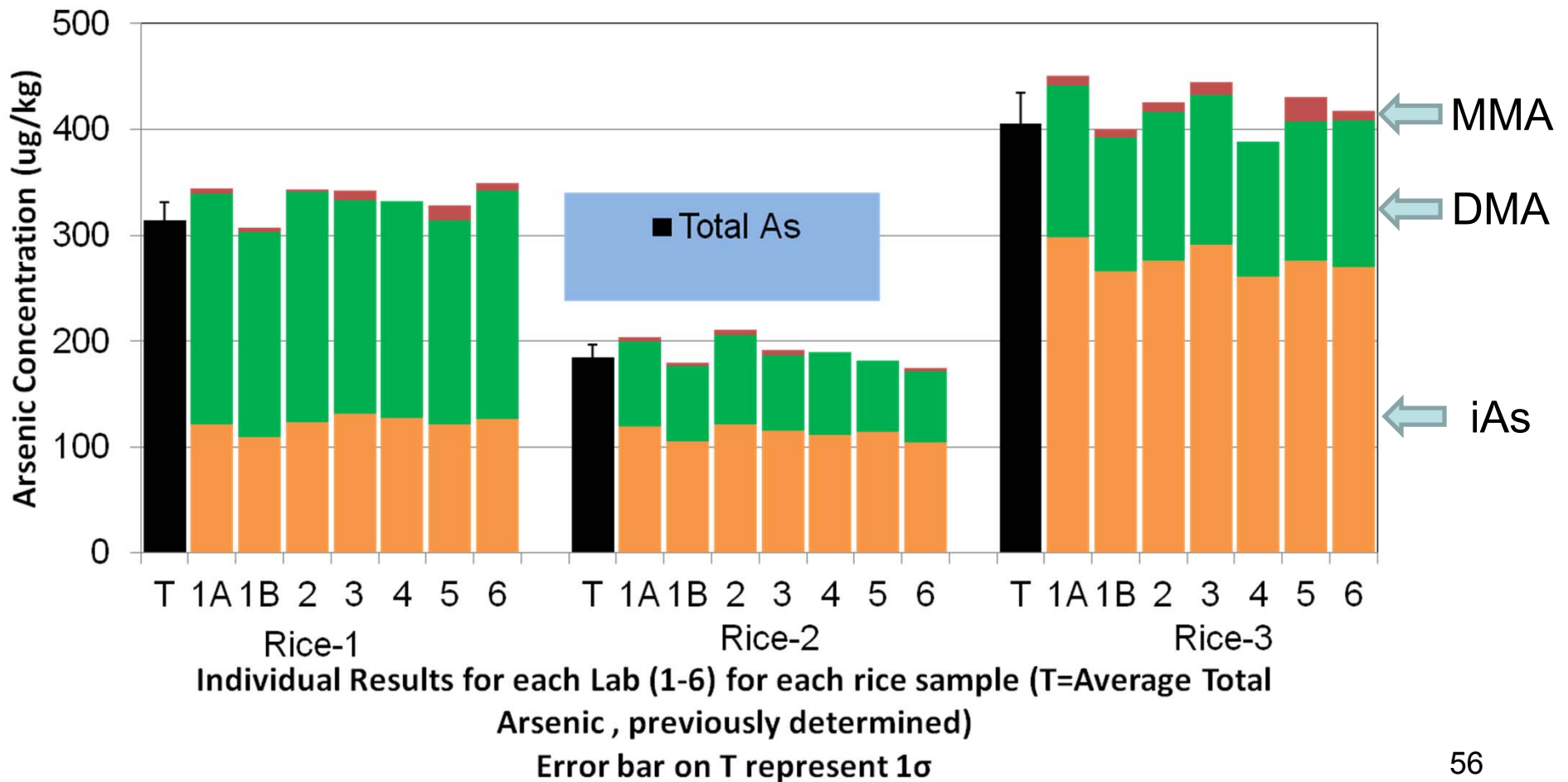
Unexpected Peaks in the Method Blanks

AsV contamination of ammonium phosphate dibasic used in MP



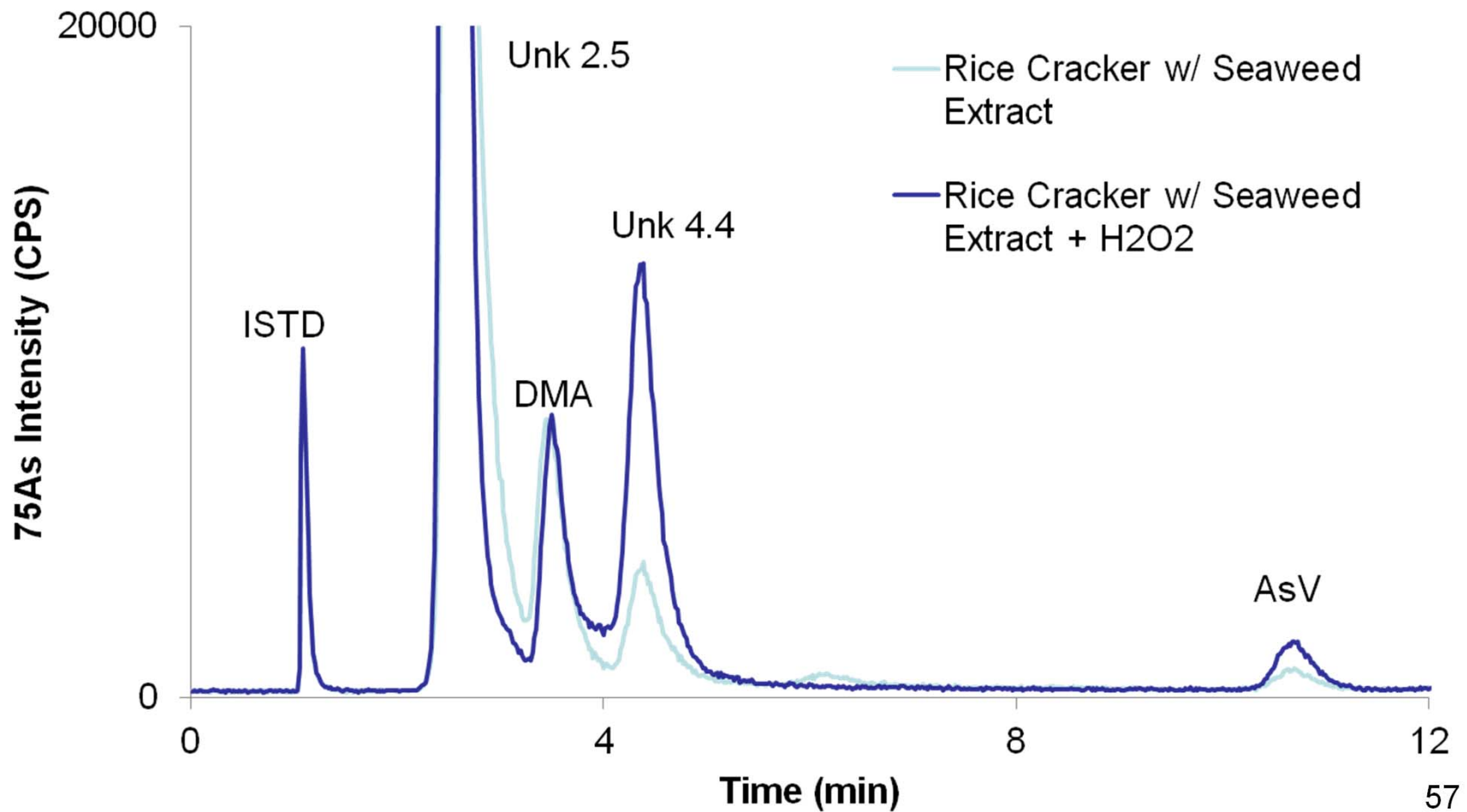


EAM 4.1 Multi-Lab Validation Results

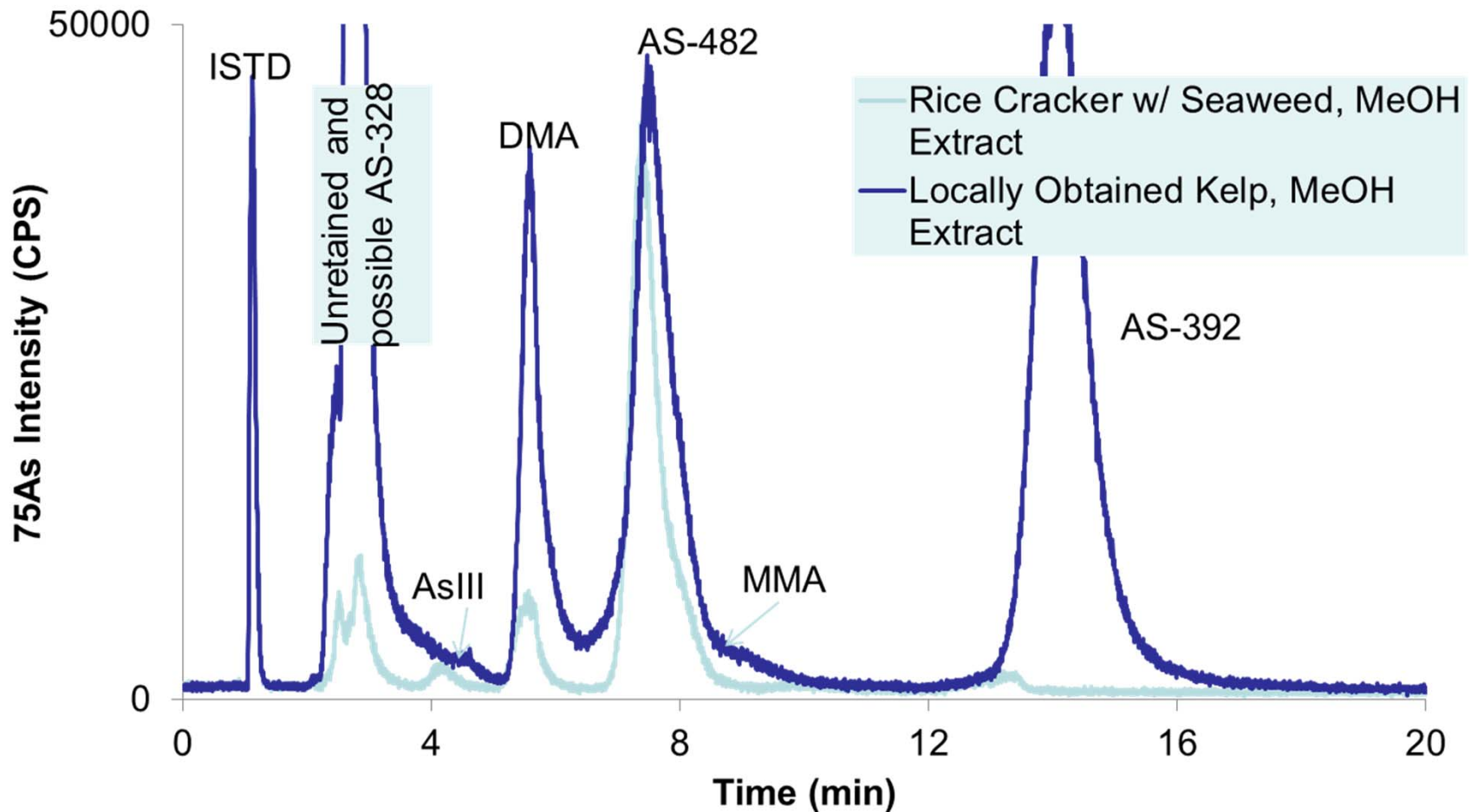




Unknown Peaks in Rice Cracker Sample



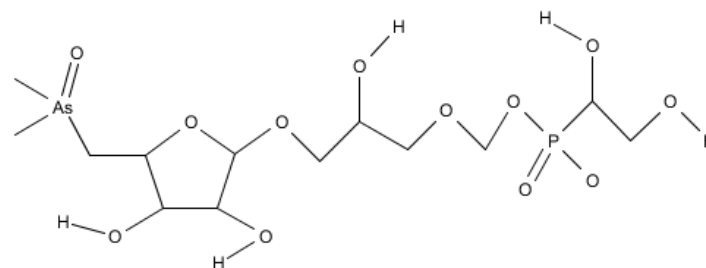
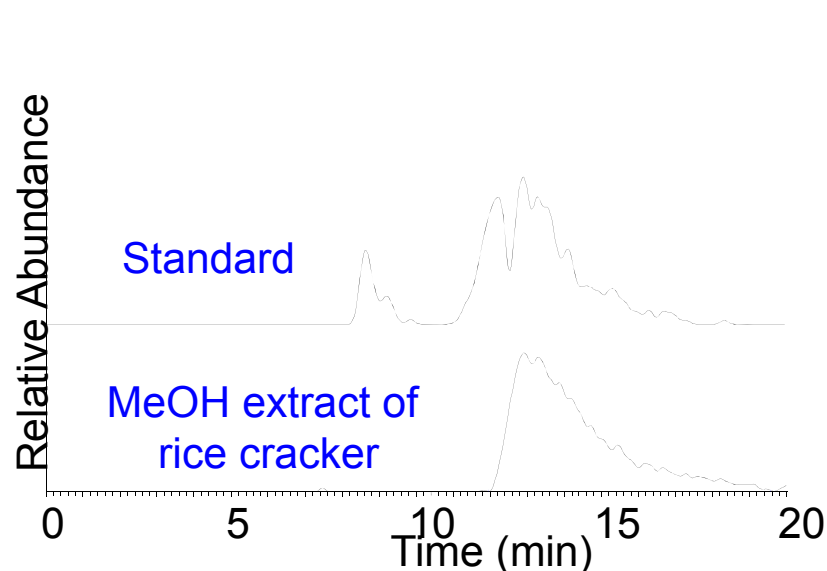
EAM 4.11 HNO₃ extraction may be altering arsenosugars – try a milder extraction



Mobile Phase was 5 mM (NH₄)₂CO₃ in DIW, pH 9.0

HNO₃ has been reported to degrade AS-328 and AS-482 to a cationic arsenosugar referred to as AS-254 (which would be unretained using EAM 4.11)

ID of Additional Peaks in Rice Crackers Using HPLC-ESI-MS/MS



AS-482

Theoretical Mass – 483.06071 (M+H)

Exact Mass – Standard

483.06094 ($\Delta m=0.476\text{ppm}$)

Exact Mass – Rice Cracker Extract

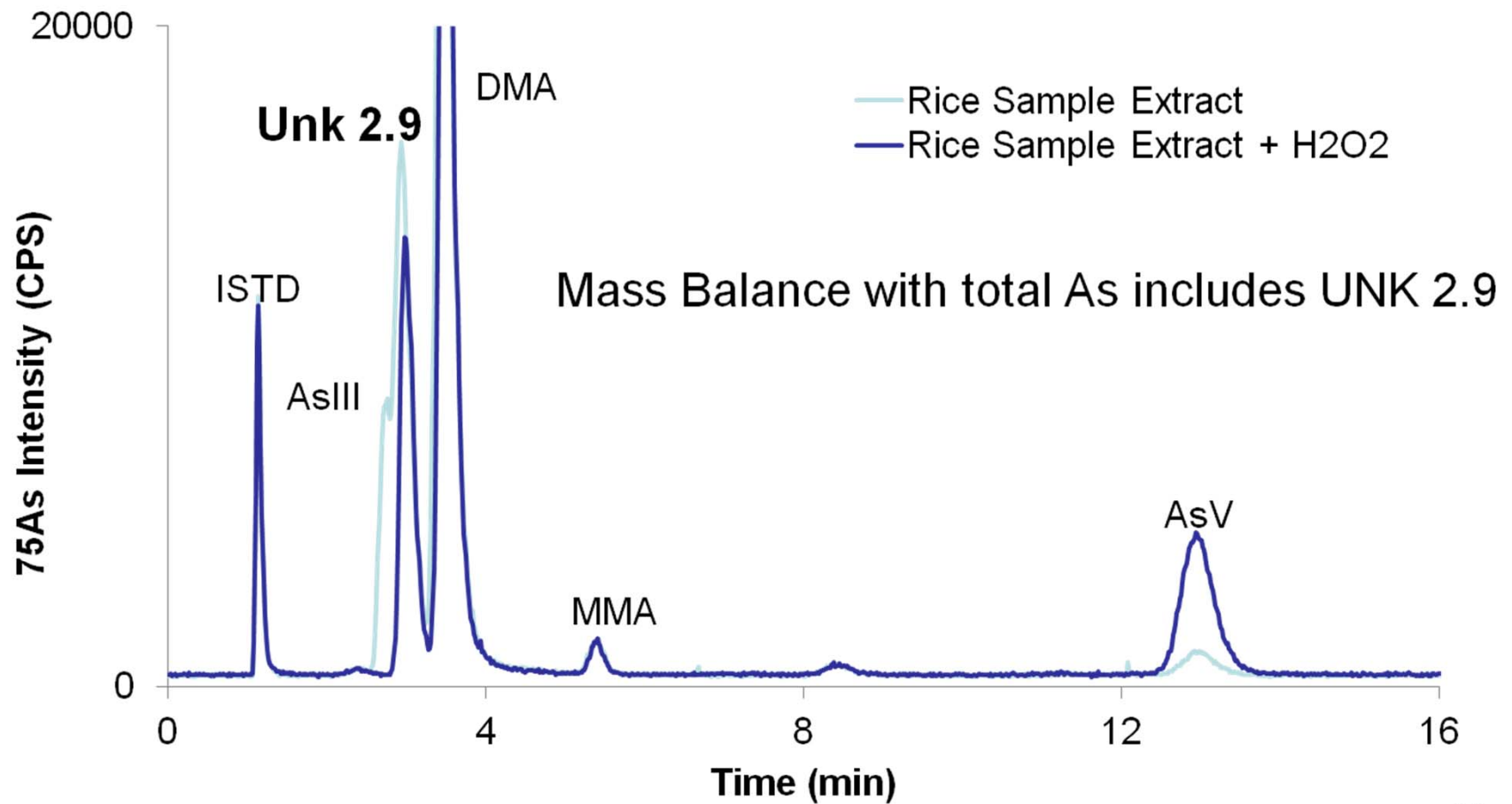
483.06098 sample ($\Delta m=0.538\text{ppm}$)

MS fragmentation pattern consistent with literature

HPLC-ESI-MS/MS conditions, Thermo Orbitrap
Zic-HILIC (2.1x150mm, 5 μ m)
MP – a) 15mM ammonium formate
b) ACN with 0.1% Formic Acid
90% B to 40% B, over 17 min, to 90%B at 20 min
10 μ L/min



Unknown Peak in Selected Rice Samples





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- Dr. Duane Satzger, Director FCC
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- ORA Lab staff
- FERN NPO
- FERN cCAP Lab staff