

Case studies on method development, validation and use for regulatory action with and without established FDA tolerance limits



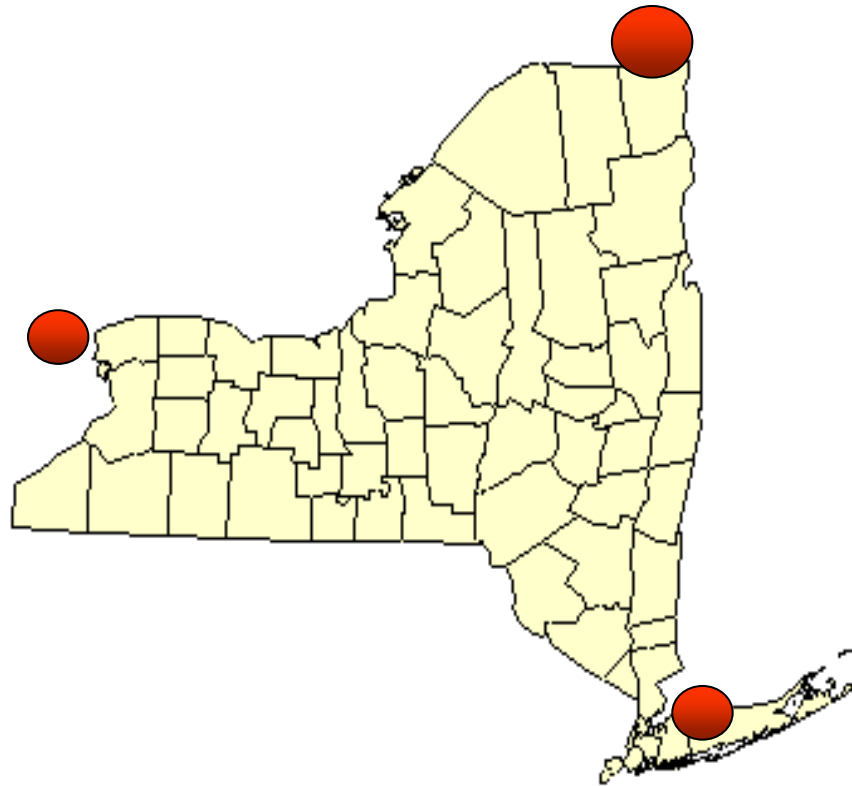
New York State Department of Agriculture & Markets
Food Laboratory

Robert Sheridan

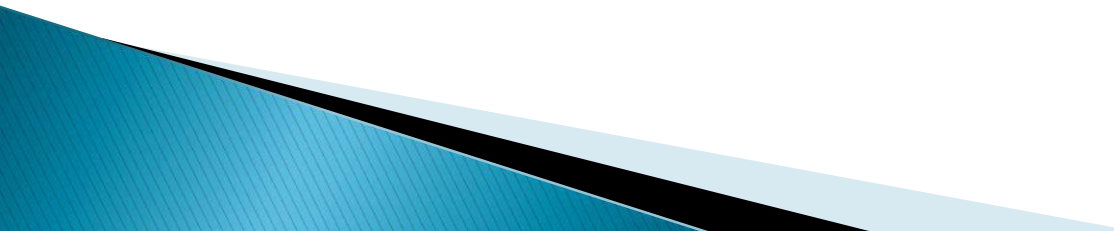
New York State Department of Agriculture & Markets

- ▶ State food and animal feed regulatory agency
 - 116 food inspectors throughout the state
 - Inspect sales establishments
 - Grocery stores
 - Warehouses
 - Bodegas
 - Collect imported and domestic food samples that are suspected to be in violation

33% OF 11,600,000
ENTRIES into US



NYS Dept. Ag& Markets Food Lab testing

- ▶ Average of 23,000 food and animal feed samples analyzed per year
 - ▶ 25 % rate of violation
 - ▶ Typically about 280 recalls issued per year
- 

Violative Samples by Country of Origin

<u>Country</u>	<u># of samples</u>	<u>% of total violations</u>
India	23	21.3%
Vietnam	17	15.7%
China	16	14.8%
Pakistan	8	7.4%
Philippines	8	7.4%
Bangladesh	6	5.6%
Nepal	5	4.6%
Taiwan	1	0.9%
Mexico	1	0.9%
Unknown (likely Asia)	21	19.5%
USA	2	1.9%
Total	108	100%

New York State Department of Agriculture & Markets

- ▶ State food and animal feed regulatory agency
 - 116 food inspectors throughout the state
 - Inspect sales establishments
 - Collect imported and domestic food samples that are suspected to be in violation
 - Food Laboratory
 - Analyzes samples using validated methods
 - When a validated method is not available we investigate the practicality of developing and validating a method
 - Periodically improve existing methods

Unapproved antibiotics in honey

- In 2003 the Food Safety and Inspection Service of our department received a complaint that certain imported honey contained chloramphenicol
- Our lab was asked to develop a test and begin analysis of honey for chloramphenicol



Honey Bee disease

▶ Foulbrood in Honeybees

- Foulbrood – caused by the bacterium *Bacillus larvae*
 - Can destroy entire honey bee hives
 - Until 2005 Oxytetracycline was the only approved antibiotic
 - Foulbrood causing bacterium is becoming Oxytetracycline resistant
 - In 2005 the antibiotic Tylosin received FDA approval for use on bees
- Before this time some honey was found to contain Tylosin residue



Chloramphenicol

- Cheap and effective antibiotic for the control of many bacteria
- Can cause serious health problems in humans including aplastic anemia, a potentially fatal disease
- No safe level or legal tolerance level has been set in any food
- Is still available in many countries and may still be used on a variety of food producing animals including honey bees



Other unapproved drugs

- Enrofloxacin, Ciprofloxacin
 - Fluoroquinolones only approved for human use
- Sulfonamides have been detected in honey by others
 - Sulfonamides
 - Class of 16 antibiotics which can be effective against foul brood
 - Are not approved for use on honey bees
 - Detection requires a more complex sample preparation step to disassociate the sulfonamide drug from the honey sugars

Detection method

- Multiresidue method for Sulfonamides and chloramphenicol at low ppb levels
- LC/MS/MS
- Provides detection with high degree of confirmation confidence
 - Obtains 4 identification points using FDA guidelines for mass spectrometry confirmation
 - Provides FDA with confidence in our data if further action is to be taken



Method Development

- Literature is reviewed
- Analytes are chosen for investigation
 - LC/MS/MS method is developed
- Extraction experiments are conducted
 - Best extraction method chosen
 - Level of quantitation (LOQ) and level of detection (LOD) are chosen for all analytes
- Quantitation method is determined
 - External matrix matched standard
 - Spiking experiments to test recoveries

Method Development

- ▶ During method development it was determined that two extractions were necessary
 - 1 liquid/liquid for fluouoroquinolones and chloramphenicol (did not recover well from SPE)
 - 2 SPE for 14 sulfonamides

Extraction 1 (Chloramphenicol, Fluoroquinolones)

5 grams honey



Extract with acetonitrile

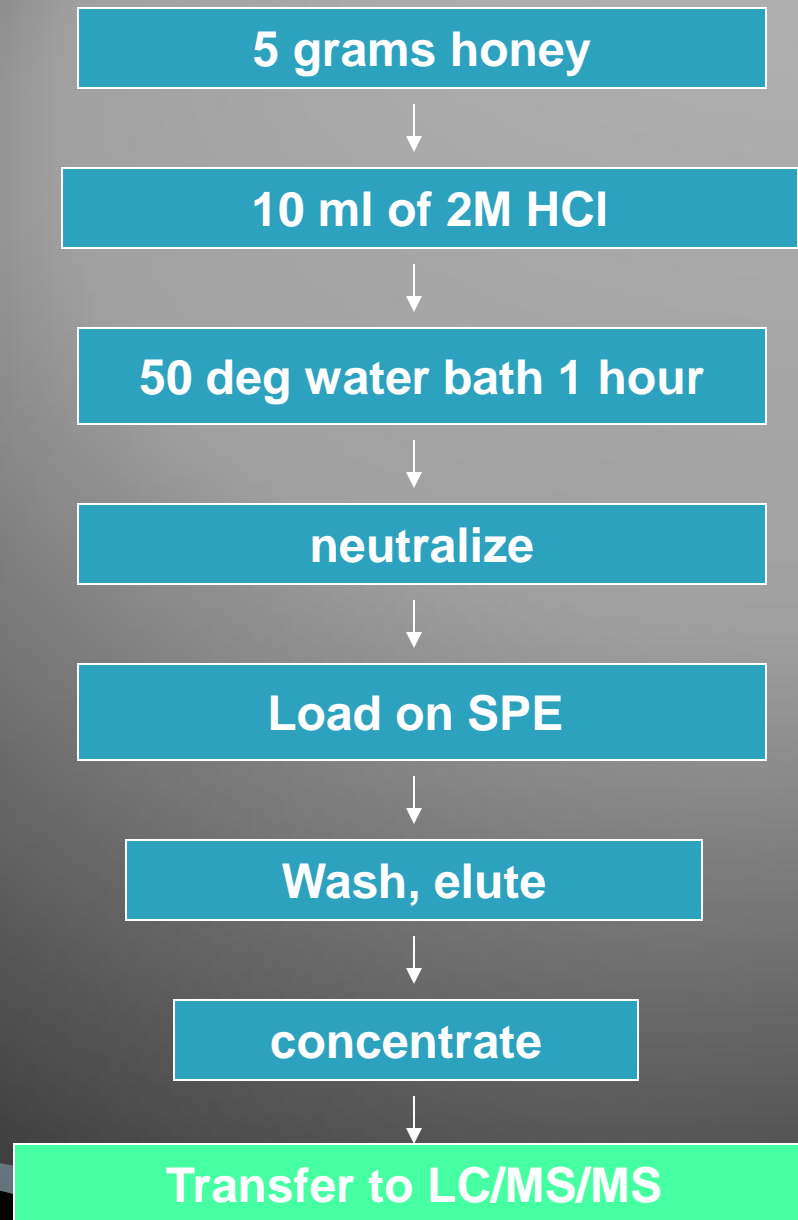


concentrate



Transfer to LC/MS/MS

Extraction 2 (Sulfonamides)



Method Validation

Spiking experiments

- All analytes are added to honey that is free of analytes at
 - LOQ level (3 times each)
 - 5x LOQ level (3 times each)
 - 10x LOQ level (3 times each)
 - LOD level (twice)
 - Blank honey

Method Validation Requirements

➤ Quantitation

- spike recoveries must be 50% < > 150%
- typical for residue level methods

➤ Identification confirmation

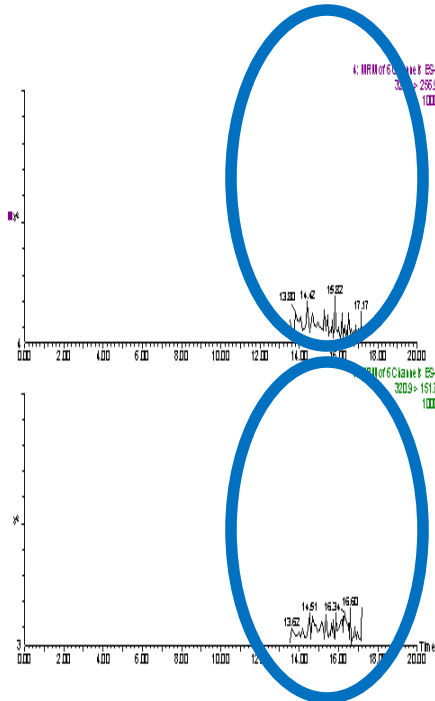
- RT: $\pm 5\%$ from standard
- Ion ratio measurement : $\pm 15\%$ from standard

QC/QA requirements

- ▶ Each batch of samples must include
 - Blank sample
 - No detected analytes
 - Spike of all analytes
 - All analytes detected (requirements of detection met)
 - Recoveries 50% < > 150%
 - FAPAS previously analyzed sample
 - Incurred residues with accepted concentrations
 - Results must fall within acceptable range
 - Particularly important for sulfonamides because of binding with sugars in honey
 - Participation in FAPAS check sample program
 - Required by ISO 17025

RT 16.08

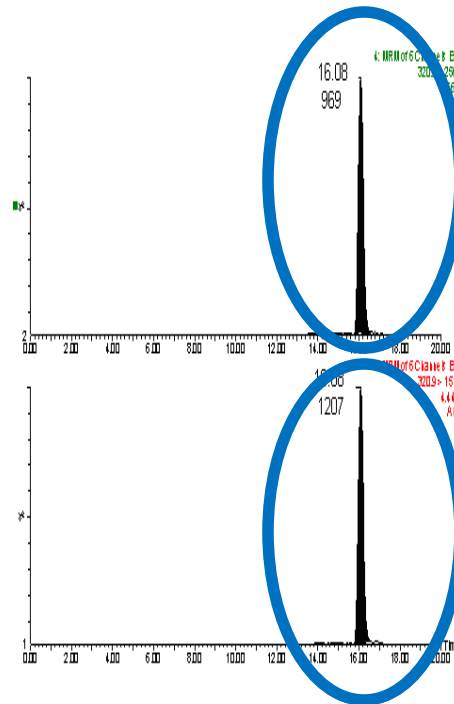
Ion ratio 80%



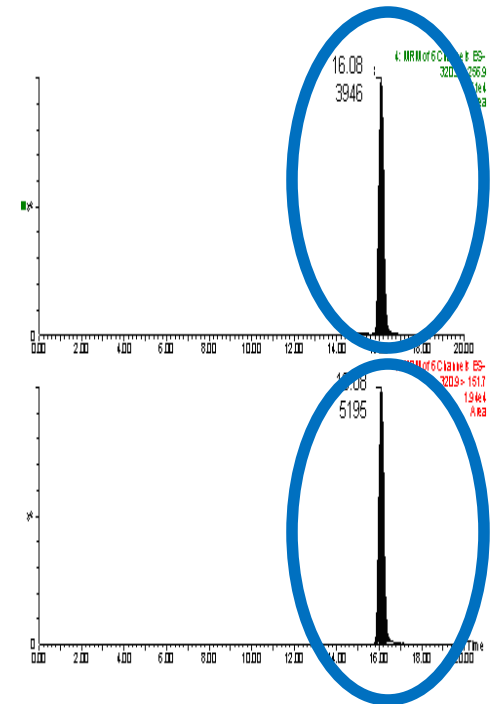
Blank honey

RT 16.08

Ion ratio 76%



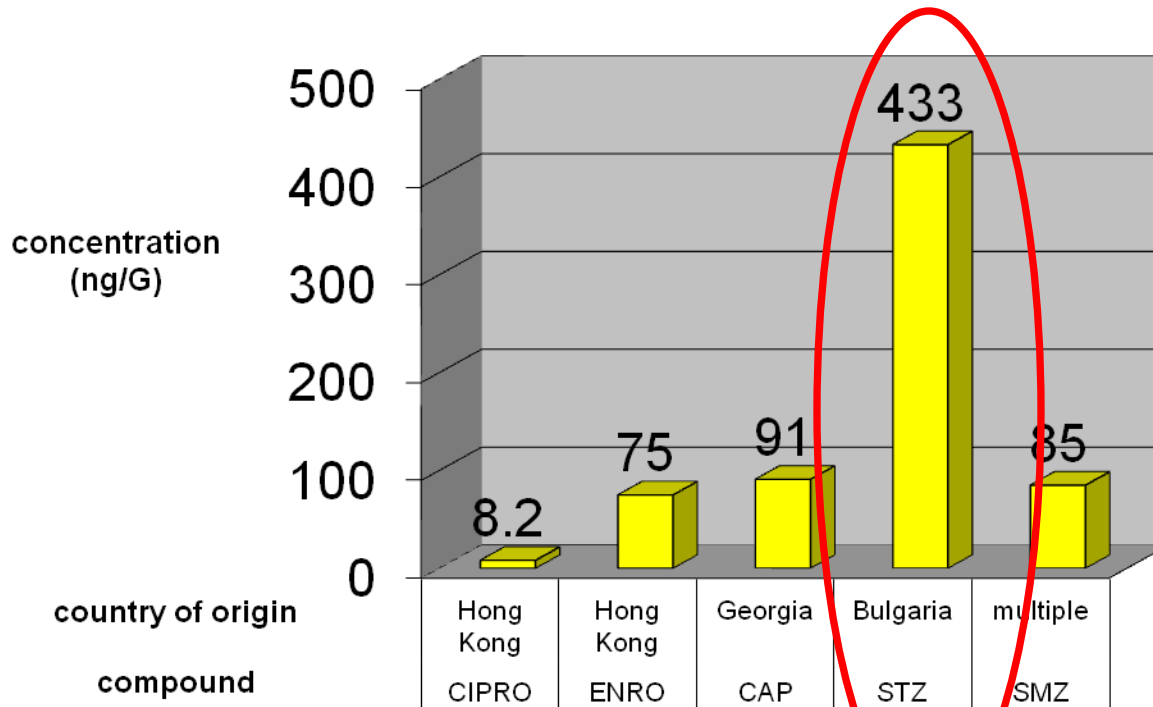
chloramphenicol
standard



chloramphenicol
in honey sample

Results since 2008

Samples of highest concentration

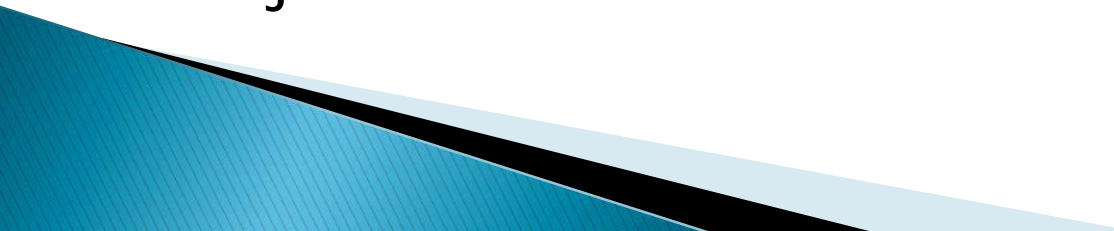


Sulfathiazole 433 ng/G (ppb)

Regulations – MRL's

- ▶ FDA recommends LOQ of Chloramphenicol 0.3 ppb. Sulfonamides not addressed
- ▶ EU sulfonamides 10 ppb
- ▶ New York action levels
 - Sulfonamides 10 ppb
 - Chloramphenicol 0.3 ppb
 - Fluoroquinolones 5.0 ppb
- ▶ International agreement would be helpful for trade

Arsenic in Juice

- ▶ 2008 FDA established a **level of concern** of 23ppb inorganic As for clear apple juice only
 - ▶ In 2008 Canada rejected pear juice from the US due to arsenic between 58 to 100 ppb
 - ▶ September 2008 Dr. Oz show reporting arsenic in juice > 10ppb (drinking water level)
 - ▶ NYS Dept. of Ag lab Validates method for total As in juice
- 



U.S. Food and Drug Administration
Protecting and Promoting *Your* Health

[A to Z Index](#) | [Follow FDA](#) | [FDA Voice Blog](#)

SEARCH

[Home](#)

[Food](#)

[Drugs](#)

[Medical Devices](#)

[Radiation-Emitting Products](#)

[Vaccines, Blood & Biologics](#)

[Animal & Veterinary](#)

[Cosmetics](#)

[Tobacco Products](#)

Food

[Home](#) [Food](#)

Foodborne Illness

Metals

[Arsenic](#)

[Lead](#)

[Mercury and Methylmercury](#)



Food and Drug Administration
College Park, MD 20740



Memorandum

Date: December 15, 2008
From: Chemical Hazard Assessment Team, Office of Food Safety (HFS-301)
Subject: Arsenic in Apple Juice
To: Henry Kim, Ph.D.
Office of Food Safety (HFS-300)

As requested, consumption of apple juice by the U.S. population has been estimated. This estimate is compared to the previous estimates of juice consumption used to assess the potential hazard from arsenic

Since consumption estimates from both surveys are similar, the **level of concern (LOC)** can be applied in the case of apple juice

apple juice (eaters only) and were calculated for two population groups: males and females (MF) from birth to 2 years of age and MF 2 years of age and older. The estimates included foods codes for products that were 100% apple juice, including infant apple juice; juice blends that included apple juice as an ingredient were not included. The consumption estimates are summarized in Table 1.

In the previous assessment, it was noted that, although the issue at hand was dietary exposure to arsenic from pear juice/concentrate, information on pear juice consumption was limited in both the CSFII and the NHANES. As an alternate approach, consumption estimates that took into account consumption of all fruit juices were used as a surrogate for consumption of pear juice. These estimates, which also represent 2-day average consumption by eaters only, are summarized in Table 2.

Results of both sets of consumption estimates are comparable. Relying on results of the more recent 2003-04 NHANES, consumption of apple juice by MF birth to 2 years was estimated to be 16.7 and 36.2 g/kg body weight/day at the mean and 90th percentile, respectively, compared with previous estimates of consumption

As in juice Method

LC-ICP-MS

Liquid chromatograph separates As compounds

ICP ionizes As

MS detects m/z of As as a chromatogram

Forms of Arsenic

DMAA

MMAA

Non toxic

As(III)

As(V)

Toxic



As in juice Method LC-ICP-MS

- ▶ Validation of method for inorganic As speciation
 - Low level chromatographic measurement
 - LOQ level (3 times each)
 - 5x LOQ level (3 times each)
 - 10x LOQ level (3 times each)
 - LOD level (twice)
 - Blank juice

Method Validation Requirements


➤ Quantitation

- spike recoveries must be 50% < > 150%
- typical for residue level methods

➤ Identification confirmation

- RT: $\pm 5\%$ from standard
- ICP/MS single quadrupole, measures element mass so no fragments and no ion ratio measurement is possible

Arsenic in juice

- ▶ Our lab has analyzed over 250 samples of various juices for total As. If result was > 23 ppb the sample was analyzed for inorganic As
 - ▶ Highest juice was 127 ppb total in blueberry juice. Method for speciating is not yet available.
 - ▶ FDA 23 ppb inorganic As level of concern only applies to apple juice
- 

Arsenic in Food

- ▶ Recently FDA proposed lowering level of concern for As in Apple juice to 10 ppb. This is the EPA standard for drinking water.
- ▶ Many other juices that may contain As
 - Blueberry and pomegranate are the highest
- ▶ Many other foods that contain As
 - Rice
 - Chicken– fed arsenical antibiotics to promote rapid growth
- ▶ State and Local regulatory labs do not have guidance

Known to be a problem since the mid 90s

By MELISSA MCNAMARA / CBS/AP / December 22, 2005, 12:36 PM

FDA: Get Lead Out Of Some Candy



candy, generic / AP

Comment / [f](#) Shares / [T](#)weets / [S](#)umble / [@](#) Email

More +

The U.S. government's **Food and Drug Administration** proposed on Thursday a stricter recommended limit on the amount of lead, a highly toxic metal, allowable in certain Mexican-style children's candies.

The list includes spicy lollipops widely sold in many Hispanic neighborhoods in the United States.

Lead in food

- ▶ The only food with an action level for lead is candy
 - High levels of lead were found in candy made with chili powder or salt from certain geographical locations
 - These candies have been linked to lead poisonings
 - Candies found to contain over 1.0 ppm lead



U.S. Food and Drug Administration

Protecting and Promoting *Your* Health

[A to Z Index](#) | [Follow FDA](#) | [FDA Voice Blog](#)

Most Popular Searches

SEARCH

[Home](#)

[Food](#)

[Drugs](#)

[Medical Devices](#)

[Vaccines, Blood & Biologics](#)

[Animal & Veterinary](#)

[Cosmetics](#)

[Radiation-Emitting Products](#)

[Tobacco Products](#)

Import Alert 33-10

[FDA Home](#) [Import Program](#) [Import Alerts](#) [Imports Alerts by Number](#) [Import Alert](#)



(Note:
issue

Import
Publis
Type:

Import Alert Name:

"Detention Without Physical Examination of Candy Due to Lead"

Reason for Alert:

NOTE:

The revision of this Import Alert dated 3/12/2010 lowers the level at which FDA will consider regulatory action for products found to contain lead to 0.1ppm, removes some of the consumption calculation as determined by CFSAN, and updates the guidance section. Changes are bracketed by asterisks.

det

Sources of excessive lead levels in candy that have been identified include ingredients such as mined salt with high levels of naturally occurring lead, chili prepared from unwashed peppers, and packaging/packing materials, such as wrappers printed with lead based ink and lead glazed ceramic bowls.

While incidents of lead contamination due to packing or packaging materials have not occurred since the mid-1990s, findings of elevated lead levels in candy products containing chili are still occurring.

In 1995, FDA initially provided guidance in this import alert that it would consider action against candy products that exceeded 0.5ppm lead. The

1995

with
imp
pro

Rec
ade
whi

FDA's Center for Food Safety and Applied Nutrition issued the document Guidance to Industry - Lead in Candy Likely to Be Consumed Frequently by Small Children: Recommended Maximum Level and Enforcement Policy in October, 2009. FDA is revising this Import Alert accordingly. ***

Guidance:

*** Districts may detain without physical examination, all products from the manufacturers and/or shippers identified on the Red List of this alert.

Surveillance sampling of other candy products is indicated. If the wrapper is considered to be a possible source of elevated lead levels, both the candy and wrapper should be analyzed for lead.

Since 1994 candy imported from Mexico have been found to contain levels of lead that may pose a health risk

In 1995 FDA would consider action against candy products that exceeded 0.5 ppm lead

Recent findings (2006) indicate adverse effects occur at lower blood levels than previously determined....FDA may consider action against products that contain lead in excess of 0.1 ppm



Lead in food Method

- ▶ **Inductively coupled plasma mass spectrometer (ICP-MS)**
 - Argon plasma ionizes elements, m/z measured
 - Linear dynamic range over several orders of magnitude
 - Highly selective
- ▶ **Standards**
 - 5 point curve to generate linear range
- ▶ **Closed vessel microwave digestion**
 - Nitric acid/hydrogen peroxide
- ▶ **QC**
 - Reagent blank
 - FAPAS check sample (lead in sugar)
 - FAPAS check sample (lead in chili sugar paste)
 - NIST SRM 1643e – elements in water (not digested)

Lead in Candy findings

Since 2004, 378 candy samples analyzed for lead

66 were found to contain lead $> 0.1 \text{ ug/g}$ (ppm)

2004–2013 17% violations

2011–2013 4% violations



Sampled in 2007

990 ppm lead in stick
350 ppm chromium

Lead Chromate
 PbCrO_4

<0.1 ppm lead in
candy



Sampled in 2010

990 ppm lead in stick

<0.1 ppm lead in candy



Sampled in 2007

Lead 0.93 ppm

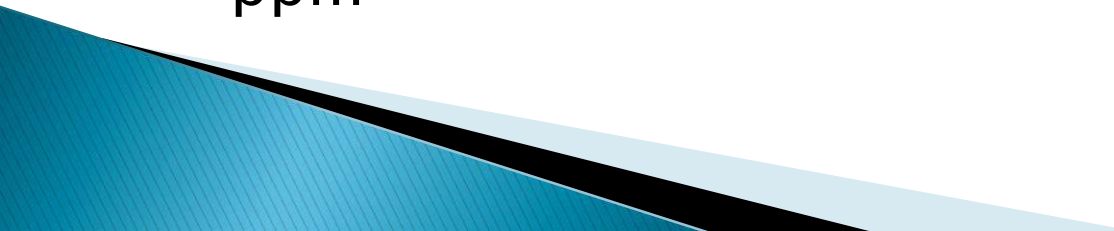


Sampled in 2011

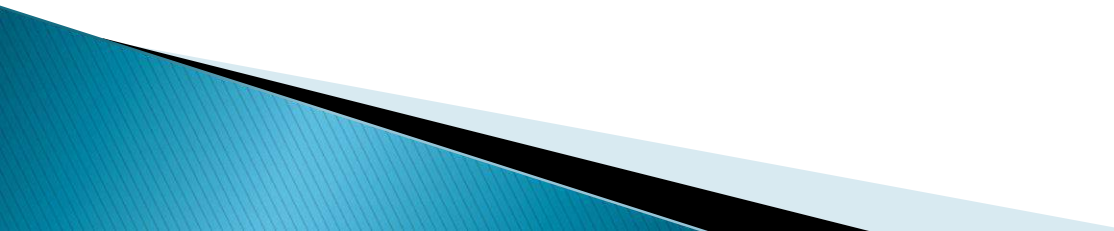
0.15 ppm Lead



Regulatory language

- ▶ FDA guidance on lead in candy refers to only candy marketed to children
 - Does not include foods containing chili powder high in lead
 - Does not include sweetened foods high in lead
 - No action taken on other foods with lead above 0.1 ppm
- 

Lead in other Foods

- ▶ Lead chromate and Lead sulfate are used as pigments in the paint, ink and textile industries
 - ▶ Unscrupulous food producers use these products particularly in spice production
- 

Indian Spices, Powders Linked to Lead Poisoning

March 15, 2010

By SUSAN DONALDSON JAMES



Several cases of children poisoned by lead in spices

Courtesy Diana Rohini LaVigne

Visi Tilak, a writer, musician and artist who emigrated from India, prepares her children's daily meals with fragrant spices that are a staple of Indian cooking, including tumeric, coriander and garam masala.

"They have been exposed to Indian spices since they were born," said the Ashland, Mass., blogger and mother of a 1-year-old and a 6-year-old.

Now, a study published today in the journal *Pediatrics* said young children who regularly ingest some imported Indian spices may be exposed to lead – a dangerous neurotoxin.

HOVER TO

Turmeric colored with lead chromate, a toxic chemical...

KANPUR: Do you know that food items like pulses and spices that are supposed to keep you healthy or add taste are making you sick instead?

Far-fetched as it may sound, this is the truth. The recent raids by the Kanpur Nagar Nigam (KNN) reveal that the local factories manufacturing food items are selling their products after adding dubious ingredients, which pose a threat to human health, in the longer run.

In a recent raid by the Food and Drug Authority (FDA) several anomalies were reported in a

turmeric manufacturing unit. It was found that 20 quintal of ash and yellow coloured rice bran was mixed with powdered turmeric to increase its quantity. Even 100-125 bags of raw turmeric coloured with lead chromate, a toxic chemical whose usage is prohibited under the Food and Adulteration Prevention Act, was found at the spot.

Also, in some of the raids, the food inspectors collected samples of pulses adulterated with 'khesari dal'. The dal is considered to be toxic and therefore, is banned by the government. It was earlier used to feed cattle but its side-effects were found to be dangerous. The head of department, Community Medicine, Ganesh Shankar Vidyarthi Memorial (GSVM) Medical College, Dr R P Sharma said that 'lathyrus sativus' commonly known as 'khesari dal' causes 'neurolathyrism' -- a crippling disease of the nervous system characterised by the paralysis of lower limbs.

Similarly, Argemone oil which is mixed with mustard oil is another most common toxic found

Ads by Google



Turmeric Powder

Origin: Bangladesh

Contains:

Lead Chromate

Lead 52.8 ppm

Chromium 10.3 ppm

Chromate Test = positive



Turmeric Powder

Origin: Bangladesh

Contains:

Lead Chromate

Lead 146.0 ppm

Chromium 30.0 ppm

Chromate Test = positive



**Excessive Levels** **Firm Press Release**

FDA posts press releases and other notices of recalls and market withdrawals from the firms involved as a service to consumers, the media, and other interested parties. FDA does not endorse either the product or the company.

OnTime Distribution Inc. Recalls PRAN brand Spice Powder TURMERIC Due To Excessive Levels of Lead**Contact:**

Consumer:
1-718-417-1100

FOR IMMEDIATE RELEASE - October 3, 2013 - OnTime Distribution Inc. of Brooklyn, NY, is voluntarily recalling PRAN brand Spice Powder TURMERIC because it was found to contain high levels of lead that could cause health problems to consumers, particularly infants, small children, and pregnant women if consumed. Recent analysis of the product found that it contained lead.

Le:
de:
Pe:

PR

Th
UP
BE
On

What is “Excessive Levels” ?**What if the distributor refuses to recall voluntarily?**

The recall was initiated after it was discovered that product contained high levels of lead based on sampling by New York

Elevated Levels

Recall -- Firm Press Release

FDA posts press releases and other notices of recalls and market withdrawals from the firms involved as a service to consumers, the media, and other interested parties. FDA does not endorse either the product or the company.

Fahman Enterprises Inc. - Recalls Pran Brand Turmeric Powder Due to Elevated Levels of Lead

Contact:

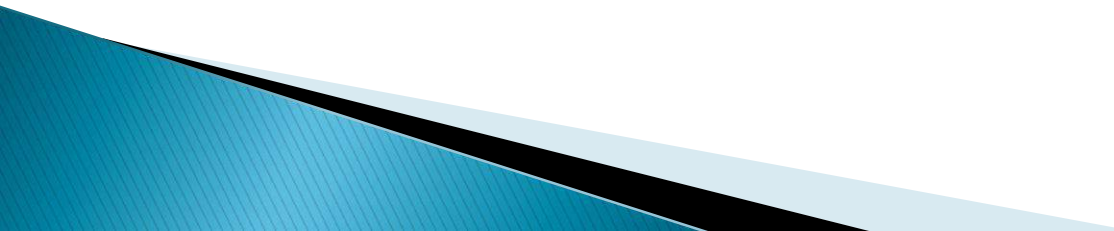
Consumer:
Najeeb Khan
214-597-5133

What is “Elevated Levels” ?

FOR IMMEDIATE RELEASE - October 17, 2013 - FAHMAN ENTERPRISES INC. of Dallas, TX is voluntarily recalling PRAN TURMERIC POWDER because it was found to contain high levels of lead that could cause health problems to consumers, particularly infants, small children, and pregnant women if consumed. The recall was initiated after it was discovered that product contained high levels of lead (48 ppm) based on sampling by The Food and Drug Administration.

Lead can be harmful to the body even in small amounts. Too much lead can cause health problems including

- ▶ Many foods do not have FDA tolerances for acutely toxic contaminants

 - ▶ Terminology needs clarification
 - Tolerance
 - Action level
 - Level of concern
 - Excessive
 - Elevated
- 

Thanks to:

- ▶ Virginia Greene
 - ▶ Thomas Tarantelli
 - ▶ Thomas King
- 

► Questions?