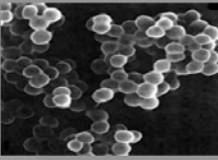

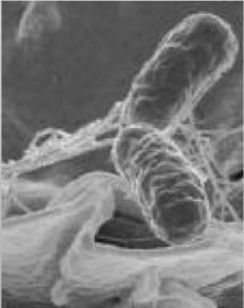




### Introduction



-  Ingesting food that has been contaminated by **bacteria**, viruses, parasites or chemicals causes food borne illness. Food-safety hazards can be introduced into food service operations in a number of ways, such as food, equipment, supplies and customers. The hazards may be biological (bacteria, viruses, parasites and fungi):
- 

### Major food borne illness include Salmonella Bacteria



- A large group of bacteria, salmonella can make people sick with a disease called salmonellosis. These bacteria are found in the natural environment, animal feed and animal intestines.

### Symptoms:



- Salmonellosis symptoms may include diarrhoea, abdominal cramps, vomiting and fever. These symptoms usually appear six to 48 hours after exposure and can persist for several days.

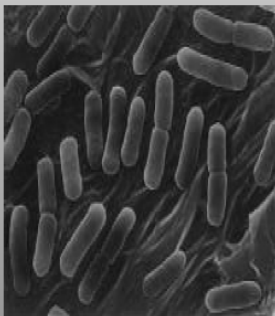
### Prevention

- Avoid cross-contamination, refrigerate food, thoroughly cook all meat to proper internal temperatures, rapidly cool cooked meats, practise good personal hygiene and proper hand washing.

### Sources

- Foods that are most likely to carry salmonella bacteria include raw and undercooked meats (especially poultry), raw milk, eggs and sprouts. Fruits and vegetables can become contaminated with salmonella bacteria if they have been exposed to contaminated soil, or have come in contact with a contaminated product or surface (such as a countertop or hands during food preparation).

### E.Coli0157:H7 Bacteria



This is found in the intestines of cattle, poultry and other animals. When an animal is butchered, the bacteria can be transferred to the meat's outer surface. E.coli 0157:H7 infection can be spread by hand-to-hand contact with an infected person or even from surfaces he/she may have touched.

© 2004 Dennis Kunkel Microscopy, Inc.

### Symptoms:



- A small number of people who become infected with E.coli do not get sick at all; some experience flu-like symptoms i.e. smallpox; others experience severe, even life-threatening symptoms. Symptoms include diarrhoea, severe abdominal pain, vomiting and low-grade fever.

### Symptoms:

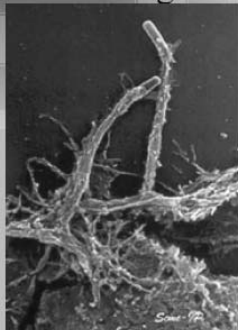
- It may also cause an unusual type of kidney failure and blood disorder called haemolytic uremic syndrome (HUS). HUS is commonly called hamburger disease, unpasteurized milk untreated water, vegetables and unpasteurized apple juice/cider contaminated with E.coli have made people ill.



### Prevention:

- Thoroughly cook ground beef to at least 70 C (158 F) for 15 seconds; avoid cross-contamination; avoid fecal contamination from food service employees by practising good personal hygiene and proper hand washing.

### Shigella Bacteria



- A gram(-) bacteria found in the intestinal tract of humans, shigella is rarely found in other animals. Its presence in foods is a sign of human contamination and lack of hygiene by food handlers. Another means of transmission is ingesting of contaminated water, such as water supplies contaminated by untreated sewage.

### Symptoms:



- Symptoms include diarrhoea, abdominal pain, fever, vomiting and dehydration. The most common foods to carry shigella are salads (potato, shrimp, tuna, chicken, macaroni, fruits, and lettuce). Chopped turkey, rice balls, beans, puddings, strawberries, spinach raw oysters, luncheon meats and milk may also carry shigella.

## Prevention

- Avoid cross-contamination; avoid fecal contamination from food service employees by practicing good personal hygiene and proper hand washing; use sanitary food and water sources.

## Cross contamination

- Diseases can also be spread by **CROSS-contamination**, which is the transfer of harmful substances or microorganisms to food by a variety of means. Utensils, washcloths, and human hands can contaminate ready-to-eat foods. Contamination can also occur via food-to-food, such as when thawing meats drip on ready-to-eat foods.

### What can I do to prevent cross contamination?

- **When shopping** ?- separate raw meat from other foods in your shopping trolley. Place these foods in plastic bags to prevent their juices from dripping onto other foods. The poultry industry now provides whole chickens in leak-proof packs and these should be selected wherever possible

### To prevent cross contamination:

- **In the refrigerator** - place raw meat in containers to prevent their juices dripping onto other foods. Juices will contain harmful bacteria if they are present on the meat
- **Defrosting** - *completely defrost meat before cooking so it will cook evenly. Ensure juices do not drip onto other foods*

### To prevent cross contamination:

- In the kitchen - harmful bacteria can spread throughout the kitchen and get onto chopping boards, utensils, and bench tops. To prevent this:
- Wash hands with soap and hot water before and after handling raw meat
- Wash chopping boards, dishes, utensils and benches with hot, soapy water after preparing each food item. Preferably, use one chopping board for fresh produce and a separate one for raw meat
- It is not necessary to rinse raw meat before cooking. Studies have shown that rinsing can spread bacteria around the kitchen.

### In kitchen

- Cook raw meat thoroughly until juices run clear. This is especially important for poultry meat
- When marinating, boil used marinade before brushing onto cooked meat
- When serving, never put cooked food back on the same plate or chopping board that previously held raw meat.

## ***Hazardous Effects of Lead & Benzene Present In Food***

***Swati S.Kadlag***  
***T.Y.BSc.-A***  
***R.No.36***

### **Introduction**

Due to the likely contamination of food and beverage products with pesticides, herbicides and many other materials that are considered a health risk, all such products on sale today must be carefully assayed. In addition, tests that identify the area or country in which the food was processed or grown may also be needed

The source of many plants (herbs and spices) can often be identified from the peak pattern of the chromatograms obtained directly from head space analysis. Similarly, unique qualitative and quantitative patterns from a GC analysis will often help identify the source of many alcoholic beverages.

### **LEAD**

- Lead is a naturally occurring bluish-gray metal found in small amounts in the earth. It has no special taste or smell and can be found in all parts of our environment . Human activities such as mining, manufacturing, and the burning of fossil fuels are the major sources of environmental lead.

### **LEAD**

- Because of health concerns, lead from gasoline, paints and ceramic products, caulking, and pipe solder has been dramatically reduced in recent years .

### **USES**

- It has many different uses, most importantly in the production of batteries, but also in ammunition, metal products (solder and pipes), roofing, and devices to shield x-rays . It has been used both for the bright colors on ceramic dishes and for the smooth, transparent glaze .

### **Sources of Lead in food & beverages**

- The major sources of [lead in drinking water](#) are lead plumbing, soil carried into water by rain and wind, and wastewater from industries that use lead . Food can contain lead if lead-containing dust gets onto crops while they are growing or during food processing . Lead can also get into food through food containers. pieces of china, pottery dishes,etc.

### Examples.

- acid foods and drinks will leach lead out of dishes much faster than non-acid foods. Spaghetti sauce, salsa, soy sauce, orange juice, applesauce, coffee, tea, cola drinks, and salad dressing are examples of acid foods. The major exposure of lead to the general population in food is through fruits and grains, according to the Agency for Toxic Substances and Disease Registry, part of the U.S. Public Health Service.

### Symptoms

- Some older pieces of china may contain lead which can leach out from the surface of the dish and get into foods and beverages. Then, when the food is eaten, the lead gets into the body. It can cause neurological disorders, reproductive problems, diminished intelligence and a host of other ills .

### Precautions

- Precautions that can be taken to reduce your exposure to lead in food include, avoiding the use of glazed pottery and pewter dishes to serve or store food, avoiding the storage of beverages in leaded glass decanters, keeping the home clean and as dust free as possible, eating a variety of foods, and eating foods rich in calcium, iron and Vitamin C so your body will absorb less lead from specific food sources that have been exposed to lead .

### Benzene

- Benzene is listed as a poisonous chemical shown to increase the risk of leukemia and other cancers(oral cancer).

### Benzene

- The problem is caused by two common ingredients – sodium benzoate and ascorbic acid (vitamin C) – which can react together to cause benzene formation. The US Department of Health and Human Services reported that in an internal memo: “*Benzene formation occurs at part per billion (ppb) levels in some food formulations containing sodium benzoate and ascorbic acid [vitamin C].*”

### Sodium Benzoate

- Use:
- Sodium benzoate, also known as E211, is used as a preservative by a range of food and drink producers. Its main advantage is its effectiveness at killing off bacteria under the acidic conditions of most beverages.

### Ascorbic Acid

- Use:
- Ascorbic acid, or vitamin C, is found naturally in fruit and vegetables but is also added as an antioxidant in food and drink production to help prevent spoilage and extend shelf-life.

### According to Lawrence:

- Glen Lawrence, another chemist who conducted benzene testing for the FDA, has also confirmed to **Beverage Daily.com** that sodium benzoate and ascorbic acid do react to form benzene in soft drinks .

### According to Lawrence,

- His study showed that ascorbic acid initially reacted with metals, such as iron or copper, found in the water to create 'free radical' particles known as hydroxyl radicals.
- Sodium benzoate, meanwhile, breaks down into benzoic acid when placed in acidic conditions, such as in a soft drink.

### According to Lawrence

- The hydroxyl radical attacks the benzoic acid, removing the carbon dioxide from it and leaving benzene in its wake. Lawrence's study said this reaction could take place "*under conditions prevalent in many foods and beverages*".

### According to Lawrence

- Lawrence said: "*There is no good reason to add ascorbic acid (vitamin C) to soft drinks, and those that may have ascorbic acid naturally in them (juices) should not use sodium benzoate as a preservative. So it is really very easy to avoid the problem.*"

*Thank You*

V.P.M''s

**B.N. Bandodkar College of Science, Thane**

Department of Chemistry

**Seminar on Contaminants in Food and Beverages**

2<sup>nd</sup> Preparatory Workshop : July 27, 2007 ( Time 10.00a.m. to 01.00 pm )

Venue:- Thorale Bajirao Peshwe Sabhagruha, Thane College Campus, Thane

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**Chief Guest Inaugural Lecture**

**Contaminants in Food and Beverages**

Dr. R.K. Shastri

**Presentation by College Teachers**

**A review of Mycotoxins as Contaminants in Food with special reference to Aflatoxins**

Dr. Moses J. Kolet

**Detection and determination of Chloramphenicol residue in Food and Agricultural products by liquid chromatography**

Dr. U.R. Pandit

**Component of Food and their contaminants**

Dr. R.P. Chavan  
(Dnyansadhana College, Thane)

**Presentation by College Students**

**Contamination of Food by Fungi**

Rakesh Kale  
(S.Y.B.Sc)

**Contamination in food oil on edible oil**

Pooja Dandekar  
(T.Y.B.Sc)

**Coca cola Case**

Raghunath Sakha  
(T.Y.B.Sc)



**Dr. Ranjan Shastri**

**ACADAMIC ACHEVEMENTS**

University of Poona  
GOLD MEDELS at B.Sc. And M.Sc

Ph.D.  
National Chemical Laboratory ,  
Poona

**POSITIONS HELD**

Sr. Scientist,  
R&D Manager  
Sr. R&D Manager  
From April 1976 to  
September 1993  
SANDOZ (INDIA) LIMITED,

- Synthesized more than 10000 New compound for Biological Evaluation
- Member of the invention team for the two successful products SAN 582 ( H ) and Cyproconazole [Azole fungicide]
- Designed and implemented Research Programs leading to potential crop protection.

Developed several analytical methods for the determination of Pesticides' Residue in variety of environmental substrates

Developed concepts and methodologies for treatment of effluent in Laboratory and implement them in plant.

Undertook programs to reduce the generation of waste by suitably altering manufacturing process

Special Training in the area of Environmental Sciences  
1988 to 1991  
SANDOZ Agrochemical Division,  
Switzerland

**Received Training in the area of**

GLP Good Laboratory Practices  
GFE Good Field Practices



**Research Papers**

Published Eight papers in a  
National and  
International Journal

**Research Guide**

Guided for 3 M.Sc. Students and  
1 Ph. D student

At present working as

Technical Director for Two companies  
And  
Advisor Technology  
to  
Various Chemical Companies

Visiting faculty to

Post Graduate Diploma in Analytical  
Chemistry : R.N. Ruia College,  
Mumbai

## **CONTAMINANTS IN FOOD & BEVEREGES**

Dr. R. K. Shastri  
July 27, 2007

## **Contamination**

- Contaminate (verb)
- Pollution
- Toxicants
- Impurities
- Residual unwanted materials

## **Contamination**

- Bulk drugs (pharmaceuticals)
- Pesticides
- Raw agricultural commodities
- Processed food
- Drinking water/ air
- Beverages (juice/soft drinks etc)

## **Bulk drugs**

- Impurities
- Harmful effects
- Heavy metal contamination
- Thalidomide episode

## **Pesticides**

- Impurities
- Phytotoxicity (injury to the host)
- Heavy metal contamination
- Loss of crop

## **Contaminants: Pesticide Residues**

- Raw agricultural commodities
- Processed food
- Drinking water/ air
- Beverages (juice/ soft drinks etc)

**Contaminants: Pesticide Residues**

**What are pesticides?**

- A chemical /biological substance which kills the insect(excluding honey bees) weeds and fungi selectively by not harming the host is called as pesticide
- The pesticide is not toxic to the mammals and is only toxic to the target pest

**Contaminants: Pesticide Residues**

**Major Types of Pesticides**

- Insecticides
- Herbicides
- Plant growth regulators
- Fungicides
- Rhodenticides
- House hold (Insect repellants)

**Types of Pesticides...**

**Insecticides**

- Organo-chlorine
- Organo-phosphates
- Carbamates
- Synthetic pyrethroids
- Benzoyl ureas
- Natural products

**Types of Pesticides...**

**Herbicides**

- Phenoxyacetic acids
- Chloroacetamides
- Phenoxy phenoxy propionic acids
- Phenyl ureas
- Nitro anilines
- Sulphonyl ureas

**Types of Pesticides...**

**Fungicides**

- Inorganic
- Products based on 2,6-dimethyl aniline
- Benzimidazoles
- Triazoles
- Strobilurim

**Types of Pesticides...**

**Rhodenticides**

Products based on 4-hydroxy coumarin

**Household products**

- Allethrin
- Prallethrin
- Trans-fluthrin
- Propoxur

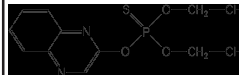
**Pesticide Residues...**

**Application of Pesticides**

- Number of products are used to combat with the pest
- Protection of a crop
- Agricultural practices

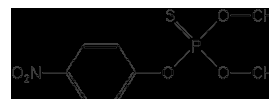
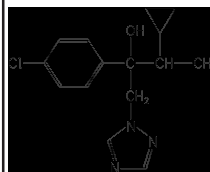
**Pesticide Residues...**

Metabolism in plants/animals

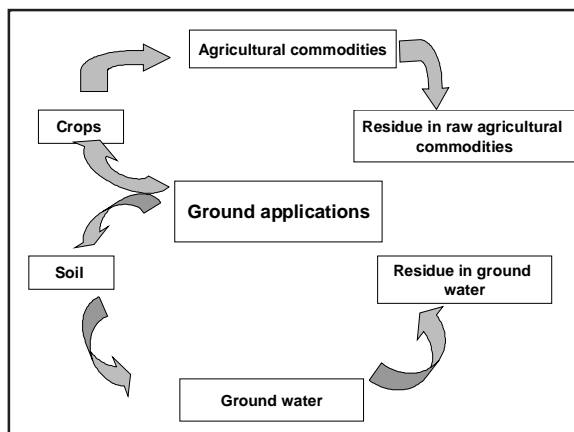


**Quinalphos**

**Methyl parathion**



**Cyproconazole**



**Pesticide Residues...**

**Developments**

- Earlier dosages – Very high [1.0ai -2.5ai kg/ha]
- Environmental concern: focus on inventing potent molecules
- Dosages reduced to 150-200 g ai/ha
- Further efforts gave more potent products  
Dosages now are 1.5g- 5g ai/ha
- Modern analytical techniques

**Pesticide Residues...**



Raw agricultural commodities	Processed food
Tea (green leaves)	Black tea
Spices	
Coffee seeds	Instant coffee
Oil seeds	Oil
Oil meal	Fodder
Raw food	Cooked food
Sugar cane	Sugar

**Pesticide Residues...**

**Estimation**

- LD<sub>50</sub> (value)
- NOEL(no observed effect level)
- ADI
- Residues

**Pesticide Residues...**

**LD<sub>50</sub> (oral)**

- It is a lethal dose (LD) of the pesticide expressed as mg/kg which kills 50% of TEST ANIMALS during the test.
- Each pesticide has a characteristic LD<sub>50</sub> value
- The other LD<sub>50</sub> values are for
  - Dermal toxicity
  - Inhalation toxicity

**Pesticide Residues...**

**NOEL**

NOEL = NOAEL (no observed adverse effect level)

NOEL is determined experimentally for

- Birth defect
- Cancer
- Reproduction changes
- Damage to the nervous system
- Effect on kidney or liver

**Pesticide Residues...**

**ADI [ Allowed Daily Intake]**

- Allowed daily intake for a given pesticide is
  - “A measure of the quality of a particular pesticide in food that can be consumed daily over a lifetime without any risk to health. It is expressed in relation to the body-weight”.

**Pesticide Residues...**

**Residues**

It is the amount of pesticide or toxic chemical remaining with the commodity (product) after the commodity is

- Harvested
- Processed

**Pesticide Residues...**

**Residues Determination**

Field trial part

- GFP
- 3 doses : normal, 1.5 N, 2N
- 3 replicates
- Sampling
- Sampling preservation until analysis (-20 deg Celsius Cold storage)

Purpose is to determine

- Waiting period
- Pre harvest interval

**Residues Determination...**

**Analytical part**

- Extraction
- Defatting
- Partitioning
- Purification by chromatography
- Concentration
- Dilution to a volume by a solvent
- Uses: Florosil, Silica gel, Neutral alumina, Activated carbon, C18 SiO<sub>2</sub> columns (solid phase extraction)

Residues Determination...

Quantification by GC or HPLC

- Reproducibility
- Linearity
- Limit of detection
- Limit of determination
- Quantification by external standard method
- GC/MS technique
- LC/MS technique

Residues Determination...

Residues are expressed as

- mg / kg = ppm
- mcg / kg = ppb
- pico gm / kg = ppt

Residues Determination...

Analytical methods

- Should be sensitive
- Should have modern detectors [ NPD, ECD]
- Capillary column up to 60-100 m length should be used
- Validation is must
- Multi-residue methods [ EPA]
- The entire analytical work should be done as per the guidelines of GOOD Laboratory Practices [GLP]

Aflatoxins

- Corn
- Soybeans
- Groundnuts
- *Aspergillus flavus*

Extremely carcinogenic

Aflatoxins ...

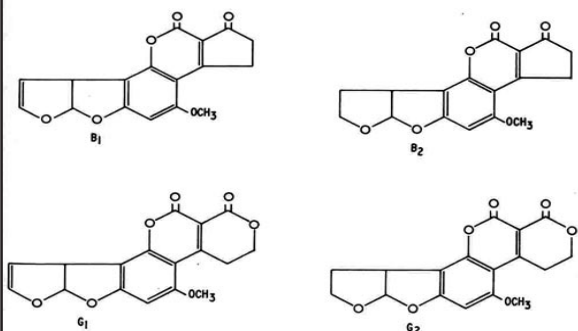


Fig. 1 Structures of aflatoxins B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub>, and G<sub>2</sub>.

Aflatoxins...

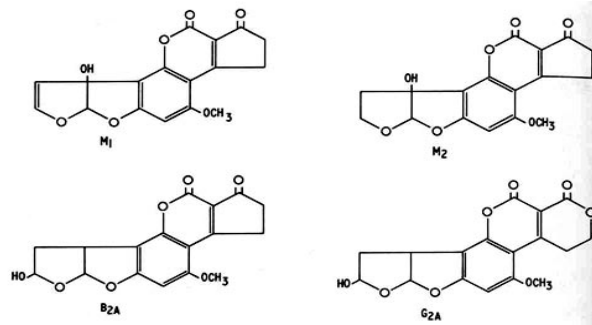


Fig. 2 Structures of aflatoxins M<sub>1</sub>, M<sub>2</sub>, B<sub>2A</sub>, and G<sub>2A</sub>.

### **Summary**

- Residual pesticide contaminants are always expected to be present in raw food materials.
- Processing of food decreases their levels.
- Modern residue determination methods are sensitive up to ppt level.

### **To conclude....**

**Contamination in food can be effectively controlled by discriminate and intelligent pest management by farmers and enforcing authorities.**

# **Thank You**



## A REVIEW OF MYCOTOXINS AS CONTAMINANTS IN FOOD, WITH SPECIAL REFERENCE TO AFLATOXINS

Dr. Moses J Kolet  
Reader in Botany  
B.N.Bandodkar College of Science  
Chendani, Thane (W) 400 601

- Mycotoxins :
- Mycotoxins are toxic substances produced by fungi (molds) growing on crops/grains in the field or in storage.
- Myco = of fungal origin
- These toxins are secondary metabolites.

## History

- Mycotoxins have caused epidemics in man and animals during historic times.
- St. Anthony's Fire-several epidemics in medieval Europe
- Alimentary toxic aleukia-killed over 1 lakh people
- Stachybotryotoxicosis-killed thousands of horses

## How was the concept of Mycotoxins developed ?

- An outbreak of an unknown disease killed poultry birds in 1960s
- This was named Turkey X disease
- After investigations it was finally traced to mycotoxins in groundnut meal feed imported from Brazil
- The feed was shown to contain a compound that could cause cancer
- Today we know this mycotoxin by the name of **AFLATOXIN** – A CLASS I CARCINOGEN

## Mycotoxins – Who are the culprits ?

- Evidence that fungi growing in food and feed produce mycotoxins has now emerged
- Thousands of fungi grow on stored agri-products but only a few produce mycotoxins
- Major mycotoxigenic fungi belong to only 3 genera:
  - 1 *Aspergillus*
  - 2 *Penicillium*
  - 3. *Fusarium*

## Entry of Mycotoxigenic Fungi

1. In the field.
2. After harvest and during storage

Fungi	Substrate	Mycotoxin
Aspergillus flavus	Maize, groundnut, oilseed, cotton seed	Aflatoxin
Aspergillus parasiticus	Maize, groundnut, oilseed, cotton seed	Aflatoxin
Aspergillus nomius	Maize, groundnut, oilseed, cotton seed	Aflatoxin
Aspergillus ochraceus	Barley wheat	Ochratoxin
Aspergillus carbonarius	Grapes wine coffee	Ochratoxin
Fusarium oxysporum	Wheat barley maize	Fumonisin
Fusarium sp.	Wheat barley maize	T-2 toxin
Penicillium verrucosum	Wheat barley maize	Ochratoxin
Claviceps purpurea	Rye	Ergot alkaloids
Stachybotrys	hay	satratoxins

## Mycotoxicoses

- Present major problems for clinicians.
- Diseases produced by mycotoxins are difficult to diagnose.

### Why so ?

- Very few mycotoxins produce overt signs of poisoning or other symptoms

### Why ?

- They are bizarre molecules with molecular weight 50 - >500.
- **Such small molecules induce no response in human immune system !**
- Major danger of mycotoxin in diet is our inability to detect them biologically.

## Toxicity of Mycotoxins

- ✓ Acute
- ✓ Chronic
- ✓ Mutagenic
- ✓ Teratogenic

## Acute toxicity

- Deterioration of liver and kidney functions, leading to death in extreme cases
- Interference with protein synthesis
- Skin sensitivity affected
- Necrosis of skin
- Immunodeficiency
- Neurotoxins cause trembling in small doses and brain damage or death in slightly higher doses

## Chronic toxicity

- Induction of cancer (Liver, oesophagus)
- Induction of tumors
- Induction is never detected at the time of ingestion and remains undetected till the disease is advanced

### Mutagenic & Teratogenic toxicity

- Replication of DNA is affected producing mutagenic and teratogenic effects

## Mycotoxin Toxicity: Effect on Humans

- Symptoms are as diverse as the chemical structures of the compounds
- Physiological and pathological changes
- Food poisoning
- Inhibition of protein synthesis
- Increase of tryptophan in blood and brain (affects appetite, muscular co-ordination and sleep)
- Anorexia
- Nausea
- Vomiting

- Headache
- Abdominal pain
- Chills
- Diarrhoea Giddiness
- Convulsions
- Alteration of capacity of cells to proliferate
- Reproductive and mammary changes
- Role in hormonal balance and breast cancer
- Precocious pubertal changes in children
- Breast enlargement in boys
- Role in cancer

### How many mycotoxins are there ?

- Today 300 - 400 mycotoxins are known
- Mycotoxins of human concern based on toxicity:
  - Aflatoxin
  - Deoxyniva-lenol (DON) or Vomitoxin
  - Zearalenone
  - Fumonisin
  - T-2 toxin
  - Ochratoxin A

### Some lesser known Mycotoxins

- |                      |                   |
|----------------------|-------------------|
| • Alternariol        | • Luteoskyrin     |
| • Citrinine          | • Neosolaniol     |
| • Cyclopiazonic acid | • Oosporein       |
| • Diacoumarol        | • Patulin         |
| • Ergotamine         | • Penicillic acid |
| • Ergotoxin          | • Penitrem        |
| • Fumitremorgen      | • Phomospin       |
| • Fusaric acid       | • Rubratoxin      |
| • Fusariocin         | • Sporidesmin     |
| • Islanditoxin       | • Tremorgens      |

### Aflatoxins

- Aflatoxins are a group of highly toxic carcinogenic secondary metabolites produced by fungi namely:-
  1. *Aspergillus flavus*
  2. *Aspergillus parasiticus*
  3. *Aspergillus nomius*

### Where do they come from ?

1. Moisture in crops – inadequate drying
2. Contamination during handling, storage and processing of foods

### Where are Aflatoxins found ?

- Aflatoxin producing fungi can grow on almost every raw as well as processed food
- Conditions to initiate Aflatoxin production are more specific
- Presence of lipids (oils) in foods favors Aflatoxin production
- Groundnuts, corn (maize), nuts and their products are susceptible for Aflatoxin accumulation
- Aflatoxin M<sub>1</sub> and M<sub>2</sub> are found in milk

## Types of Aflatoxins

- Naturally produced Aflatoxins – B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub>, G<sub>2</sub>
  - They undergo modifications during cellular metabolism and processing of foods to produce several derivatives such as M<sub>1</sub>, M<sub>2</sub>, P<sub>1</sub>, etc.
- International limit 15µg per kg food**

## Aflatoxin B<sub>1</sub>

- Best described Aflatoxin
- Molecular weight: 312
- Chemical Formula: C<sub>17</sub>H<sub>12</sub>O<sub>6</sub>
- Highly unsaturated structure
- Can cause damage even at extremely low doses of 0.3ppm

## Effects of Aflatoxins on Human Health

- Acutely toxic
- Carcinogenic
- Mutagenic
- Teratogenic
- Immunosuppressor
- Reports of outbreaks of hepatitis resulting from Aflatoxin ingestion
- Outbreaks of Aflatoxicoses-60% mortality
- Varied effects such as transient rash, nausea, headaches


## Aflatoxin B<sub>1</sub>

- It is the most potent aflatoxin
- **Classified as Class I Human carcinogen**
- It selectively targets human gene **p53** described as 'Guardian of the genome'

## Control Measures

- Control of insects in the field and during storage
- Detect early in the field
- Mycotoxin analysis
- Control moisture content of grains/feed
- Keep processing equipment clean
- Use fresh feeds
- Remove old stock
- Never mix old and new feed/grain stock
- Use mould inhibitors
- **Mycotoxins are not degradable. They tend to remain constant or increase in stored foods**

*Thank You !*



Detection and determination of sulfonamides residues in food products by LC –MSMS

By:Dr.U.R.Pandit

Analysis of Sulfonamides using LC-MSMS

	Molecular Weight
1. Sulfamerazine (SMR)	=264
2. Sulfadimidine (SDD)	=278
3. Sulfamonomethoxine (SMM)	=280
4. Sulfadimethoxine (SDM)	=310
5. Sulfaquinoxine (SQX)	=300

**Sources Of Sulphonamides**

- As these compounds are effective against gram positive and gram negative organisms, they are used in the agriculture industry.
- They are used in against bacterial species - paenibaccillus larvae and melissococcus pluton in honey bees.
- Used as feed additives for growth promotion

**Effects Of Sulphonamides residues**

- Development of antibiotic – resistant bacteria.
- Allergic reactions in humans.
- Some sulphonamides have been associated with carcinogenicity.

**Maximum residue limits allowed (in legislation)**  
(Total concentration of all sulphonamides)

100µg per kg  
(0.1 ppm)

**Why LC- MS-MS**

- > **Specific**
- > **Sensitive and accurate**
- > **Minimal sample preparation**  
Simple Methanol extraction
- > **Rapid**  
Total analysis time is Less
- > **Simultaneous analysis for many compounds in single chromatographic analysis.**
- > **Readily adaptable for the determination of new drug analogues**

**LC conditions**

Mobile Phase: 0.3% CH<sub>3</sub>COOH aq. (solvent A)  
ACN/H<sub>2</sub>O = 80/20 with 0.3% CH<sub>3</sub>COOH (solvent B)

Column: Symmetry Shield RP18, 2.1x150mm, 3.5µm

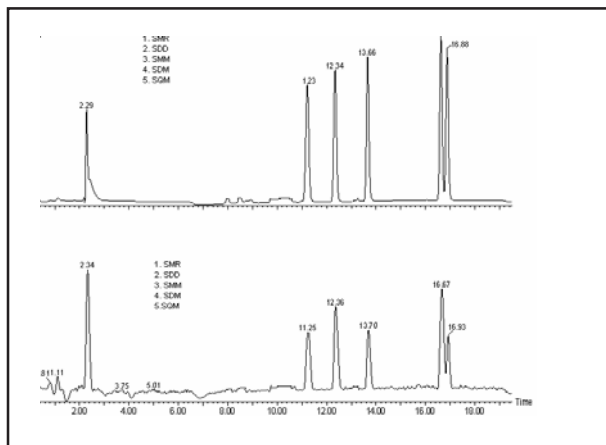
**MS conditions**

Capillary: 4.27kV  
Cone: 36V  
Scan: scan from 150 to 550

Sulfonamides:

- >system: Waters Alliance 2690
- >gradient program

Time (min)	% A	% B	Flow (ml/min)
0.00	100	0	4.00
2.00	100	0	4.00
3.00	0	100	4.00
3.50	0	100	4.00
3.60	100	0	4.00
6.00	100	0	4.00



Sample Preparation

### Extraction from Honey

Solid phase extraction

5 g honey  
+50ml of 30% Sodium chloride (dissolved)  
+60ml DCM (extraction)

↓

Dry the extract (DCM) with anhydrous Sodium sulfate

↓

Applied to Sep-Pak Florisil cartridge

↓

Wash with 10mL ACN/DCM (2:8)

↓

Elute with 10mL Methanol/DCM (2:8)

↓

Dry and reconstituted in HPLC mobile phase

Ref. J. AOAC Int., 75:786, (1992)

**Advantage of solid phase extraction**

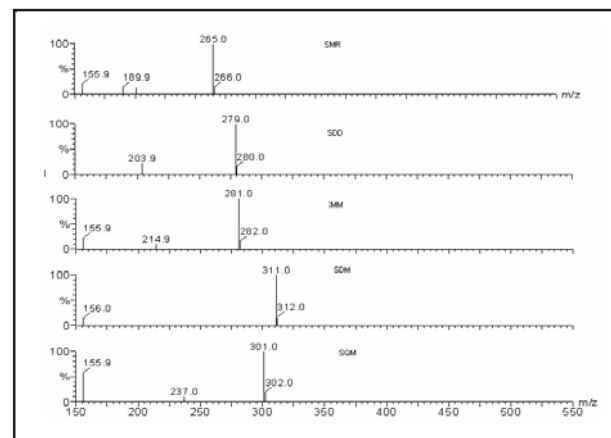
1. It removes impurities to greater extent.
2. Increases the column life.
3. Simple method to use.

### Extraction from Milks (Liquid – Liquid extraction)

3g Milk + 10 ml acidified methanol  
Mix well (3 min.)  
?

Centrifuge for 10 min ( 3500 RPM)  
Take supernat solution + 10 ml acidified methanol.  
Mix well (3 min.)

Centrifuge for 10 min. (3500 RPM)  
Evaporate the supernat solution in vacuum evaporator to dryness at 50°C.  
Add 5 ml acidified methanol. Mix well.  
Filter through 0.2µ nylon membrane filter.

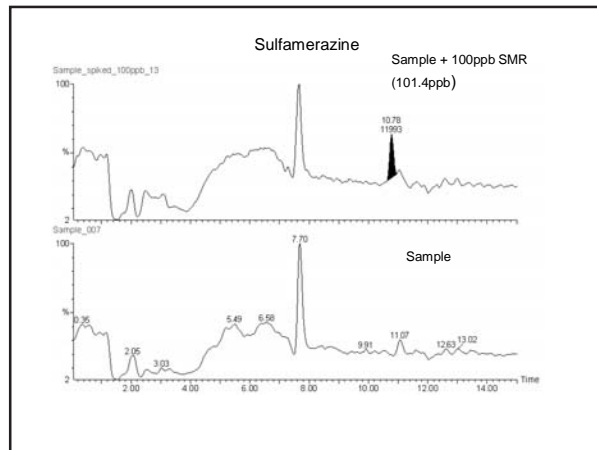


### Analysis of Sulfonamides using LC-MS/MS

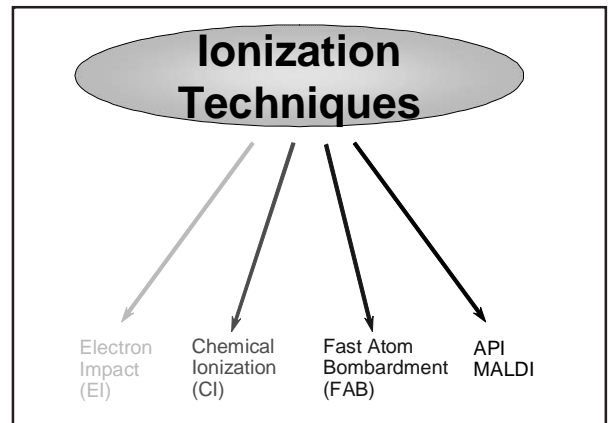
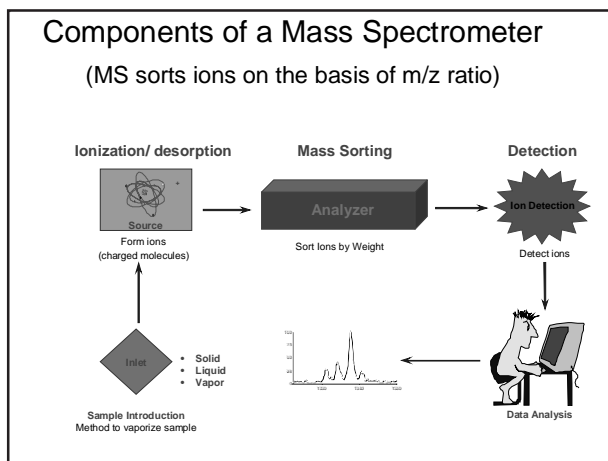
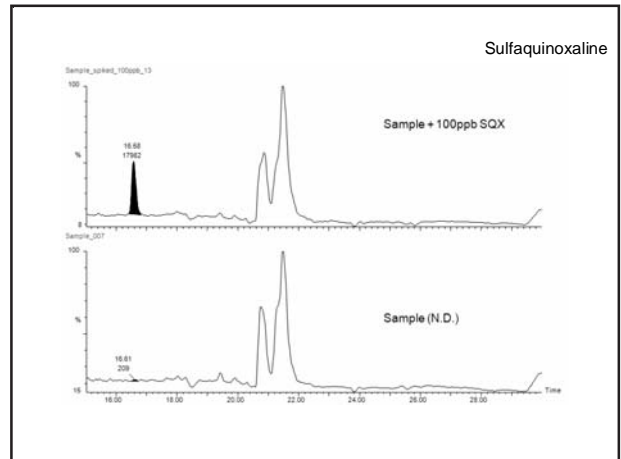
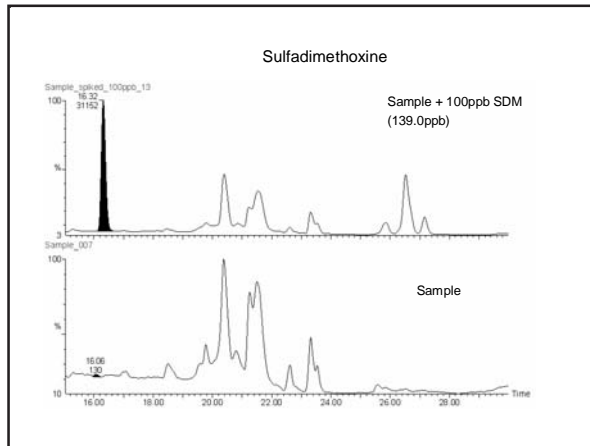
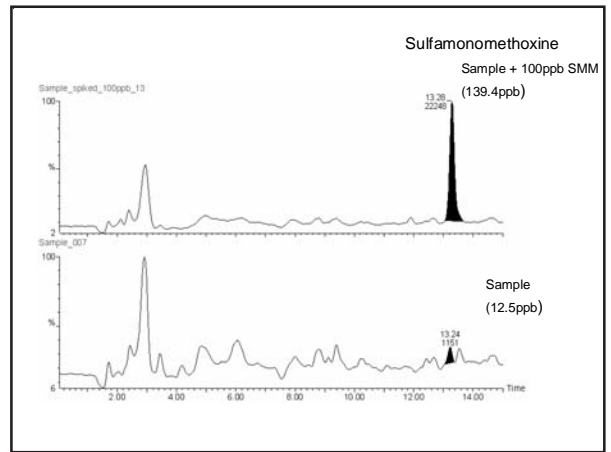
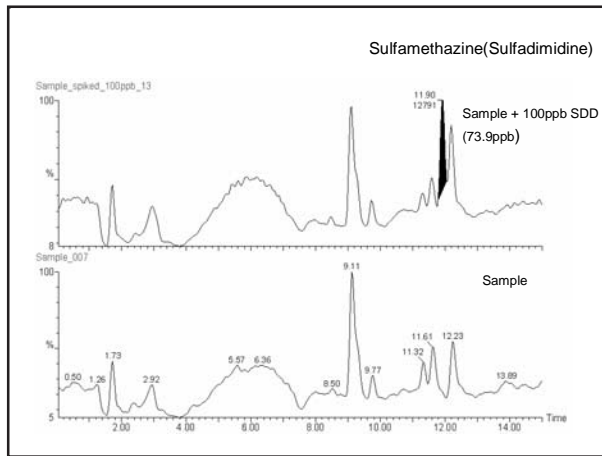
Precursor and fragment ions of sulfonamides obtained under optimal ESI (+) MS-MS conditions

	Precursor ion (m/z)	Fragment ions (m/z)	Cone voltage (V)	Collision energy (eV)
SMR	265	172, 156*, 108, 92	32	16

\* most abundant fragment ion

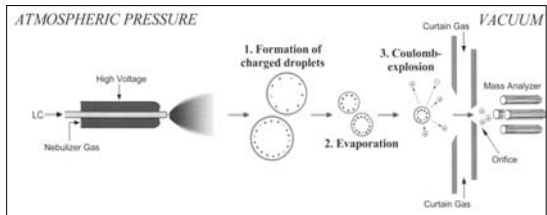


## Contaminants in Food and Beverages





## IonSpray - ESI



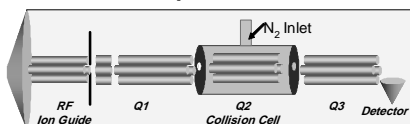
## Mass Analysers

1. Quadrupoles.
2. Time of flight (TOF) analysers.
3. Magnetic sectors.

## Detectors

1. Photomultiplier
2. Channel electro multiplier
3. Microchannel plate

## Schematics of a Triple Quadrupole MS



- RF Ion Guide: Focusses ion beam into Q1
- Q1: Analyzer Quadrupole 1
  - can be scanned or set to pass one specific m/z
- Q2: Collision Cell
  - can be pressurized with N<sub>2</sub> for fragmentation of ions
  - can be at low pressure for bandpassing all ions
- Q3: Analyzer Quadrupole 3
  - can be scanned or set to pass one specific m/z

## Information Expected From LC/MS/MS

- Isolation of the impurities.
- Molecular Weight.
- Accurate mass measurement.
- Mass determination with high resolution.
- Impurity Detection at low level.
- Search Capabilities based on suspected modifications.
- Structure elucidation and Confirmation of the structure.
- Quantitative analysis (Actual).

**THANK YOU**