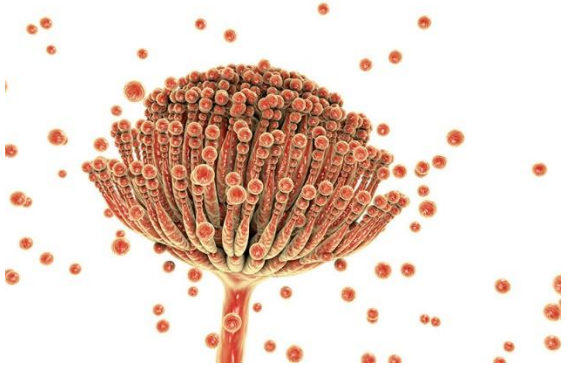
UNIVERSITA DEGLI STUDI DI TORINO

QUEEN'S UNIVERSITY BELFAST

# Mycotoxins & Plant Toxins

**Prof Katrina Campbell**
katrina.campbell@qub.ac.uk



UNIVERSITA DEGLI STUDI DI TORINO

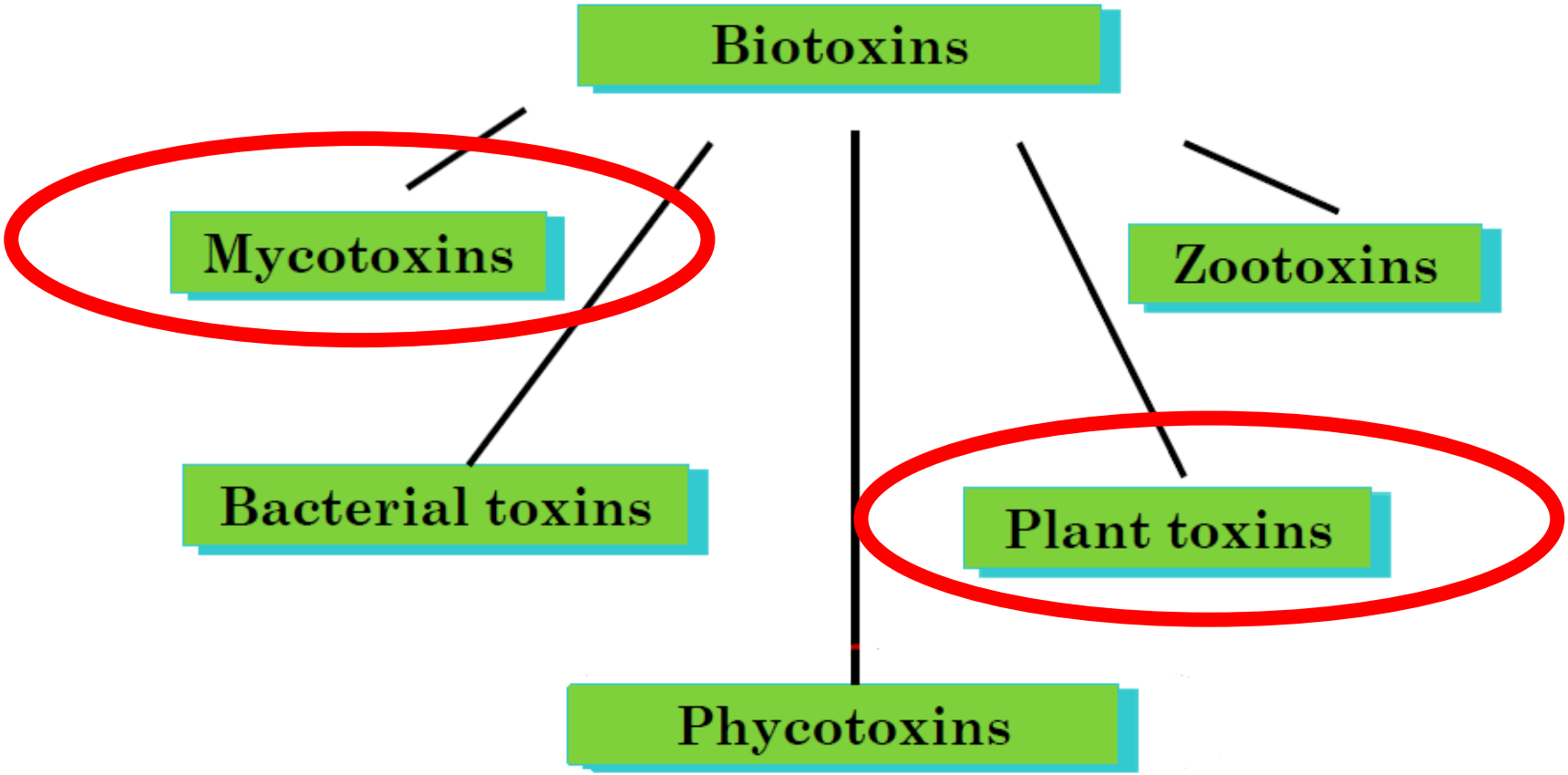
QUEEN'S UNIVERSITY BELFAST

UNIVERSITA DEGLI STUDI DI TORINO

QUEEN'S UNIVERSITY BELFAST

# Classification of Biotoxins

**Biotoxins/natural toxins can be divided into 5 main categories from their origin**



UNIVERSITA DEGLI STUDI DI TORINO

# Learning Outcomes

QUEEN'S UNIVERSITY BELFAST

- Mycotoxins:
  - Importance
  - Exposure & Infection
  - Food Contamination
  - Mycotoxicoses
- Major Mycotoxins
  - Formation
  - Occurrence
  - Adverse effects
- Regulations

UNIVERSITA DEGLI STUDI DI TORINO

# Mycotoxins

QUEEN'S UNIVERSITY BELFAST

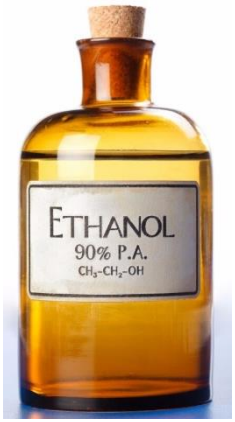
- Derived from the Greek 'Mykes' and Latin 'toxicum'
- Secondary metabolites of filamentous fungi
- No direct role in major metabolic pathways
- Structurally diverse group of low molecular weight organic compounds
- Associated with disease in humans and animals
- Number in the thousands
- >300 identified and characterized
- Major economic importance

UNIVERSITA DEGLI STUDI DI TORINO

# Mycotoxins

QUEEN'S UNIVERSITY BELFAST

- Other fungal-derived toxins which are not mycotoxins
  - Penicillin
  - Phytotoxins
  - Ethanol
  - Mushroom poisons


- Distinction
  - Produced by moulds
  - Toxic in low concentrations

# Classification

Clinicians - the organ affected.

Cell biologists - generic groups.

Organic chemists - chemical structure.

Biochemists - biosynthetic origins.

Physicians - illnesses caused.

Mycologists – genera of fungi.

UNIVERSITA DEGLI STUDI DI TORINO

# Importance

QUEEN'S UNIVERSITY BELFAST

- Implicated in disease in humans and animals
  - Acute and chronic toxicities at very low levels (ppb)
  - Carcinogenic, mutagenic
  - Teratogenic
  - Immunosuppressive
  - Reproductive and developmental toxicity
  - Inhibition of protein synthesis
- Most unfamiliar & least investigated

  food contaminants
- Not destroyed during normal food processing

A river in the US in which huge batches of milk were dumped because their mycotoxin content exceeded the regulatory level

UNIVERSITA DEGLI STUDI DI TORINO

# Importance

QUEEN'S UNIVERSITY BELFAST

- Great economic significance
  - loss of human and animal life,
  - increased health care
  - Increased veterinary care costs,
  - reduced livestock production,
  - disposal of contaminated foods
  - Disposal of contaminated feeds,
  - investment in research and
  - applications to reduce severity of the mycotoxin problem.


Impact of low mycotoxin contamination on animal health and performance


Moulds that can kill

When food grains are incorrectly handled or stored after harvesting, dangerous mycotoxins can develop. Aflatoxins in particular endanger human health and can prove fatal

by Ramesh V. Bhat

World Mycotoxin Journal

UNIVERSITA DEGLI STUDI DI TORINO

QUEEN'S UNIVERSITY BELFAST

# Exposure & infection

- Ingestion, absorption and inhalation
- Severity of infection:
  - Toxicity
  - Extent of exposure
  - Age
  - Health
  - Gender
  - Other chemicals

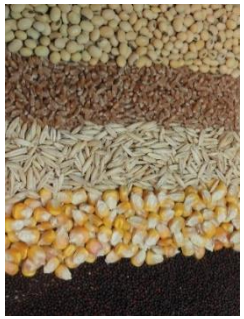

Paracelsus
(1493-1541)
Dosis facit venenum
"The dose makes the poison"

UNIVERSITA
DEGLI STUDI
DI TORINO

# Ingestion: pathways of exposure













UNIVERSITA DEGLI STUDI DI TORINO

# Contamination of food

QUEEN'S UNIVERSITY BELFAST

- Direct
  - ➢ Infection and mycotoxins formed
- Indirect
  - ➢ An ingredient has been contaminated with fungi, the fungi is destroyed but the mycotoxins remain.

All foods and feeds can be prone to fungal growth during production, processing, transport and storage.

UNIVERSITA DEGLI STUDI DI TORINO

# Contamination of food

QUEEN'S UNIVERSITY BELFAST

Mould damaged foodstuffs:

- Agricultural products:
  - Cereals
  - Oilseeds (groundnuts)
  - Fruits
  - Vegetables
- Consumer foods (secondary infections)
- Compounded animal feeds

UNIVERSITA DEGLI STUDI DI TORINO

# Contamination of food

QUEEN'S UNIVERSITY BELFAST

Residues in animal tissues and animal products:

- Milk
- Dairy produce
- Meat

Mould-ripened foods:

- Cheeses
- Fermented meat products
- Oriental fermentations

Fermented Products:

- Microbial proteins
- Enzymes
- Food additives such as vitamins

UNIVERSITA DEGLI STUDI DI TORINO

# Mycotoxin Pathway

QUEEN'S UNIVERSITY BELFAST

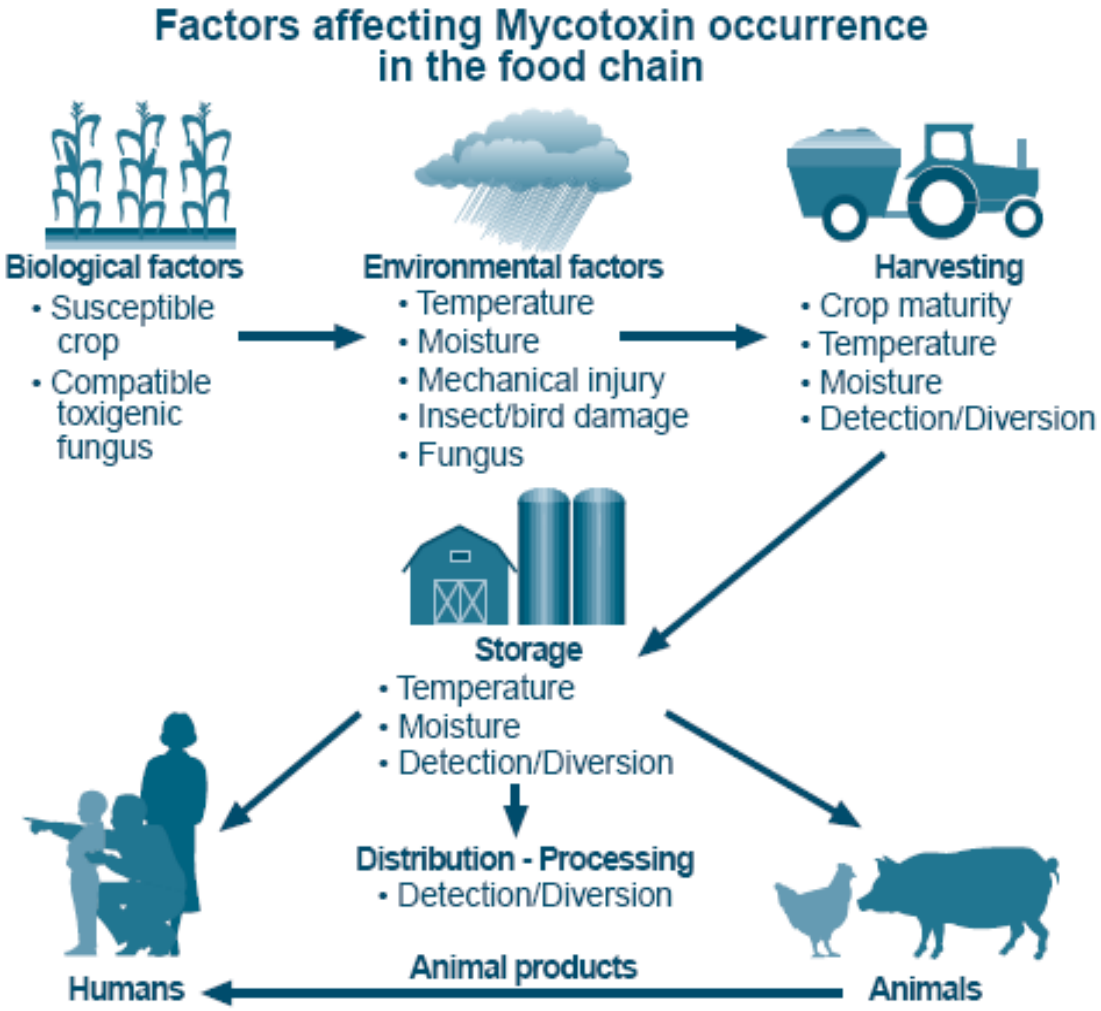


Photo courtesy of Dr. Fabio Mascher, Agroscope, Changins, Switzerland.

UNIVERSITA DEGLI STUDI DI TORINO

# Considerations

QUEEN'S UNIVERSITY BELFAST

- Do all feeds/foods with fungal growth contain mycotoxins?
- Are all feeds/foods containing mycotoxins 'toxic'?
- Does fungi have to be visible in feed/food for it to be contaminated?
- Are toxins distributed evenly in contaminated foods?

UNIVERSITA DEGLI STUDI DI TORINO

# Mycotoxicoses

QUEEN'S UNIVERSITY BELFAST

- Profound influence on health
- Difficult to diagnose
- Levels of infection depend on:
  - Genetic factors
  - Physiological factors
  - Environmental factors
  - Animal husbandry and management

UNIVERSITA DEGLI STUDI DI TORINO

# Mycotoxicoses

QUEEN'S UNIVERSITY BELFAST

- Acute primary mycotoxicoses
- Symptoms:
  - Hepatitis
  - Haemorrhage
  - Nephritis
  - Necrosis
- All systems affected e.g.
  - Vascular, digestive, respiratory, nervous, cutaneous, urinary and reproductive systems

# Mycotoxicoses

- Chronic primary mycotoxicoses
  - Reduced productivity (slower growth rates).
  - Reduced reproductive efficiency.
  - Inferior market quality.
  - Reduced feed conversion efficiency.
  - Reduced milk yields.
  - Reduced egg production


# Mycotoxicoses

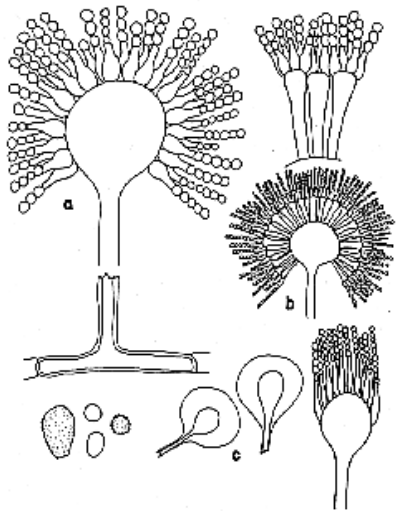

- Secondary mycotoxin diseases.
  - Less well defined.
  - Relates to effects on immune system.

- Animal mycotoxicoses
  - Contamination of feed.
  - Nutritional imbalances.
  - Toxic or synergistic molecules.
  - Micro-organisms.

UNIVERSITA DEGLI STUDI DI TORINO

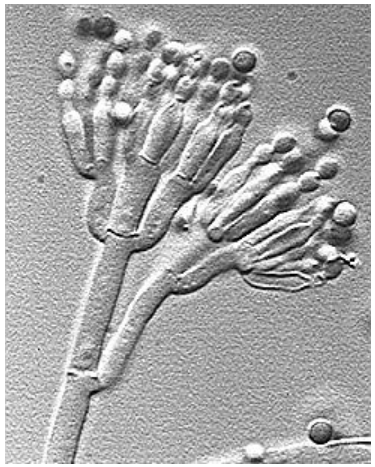
# Major Mycotoxin fungi

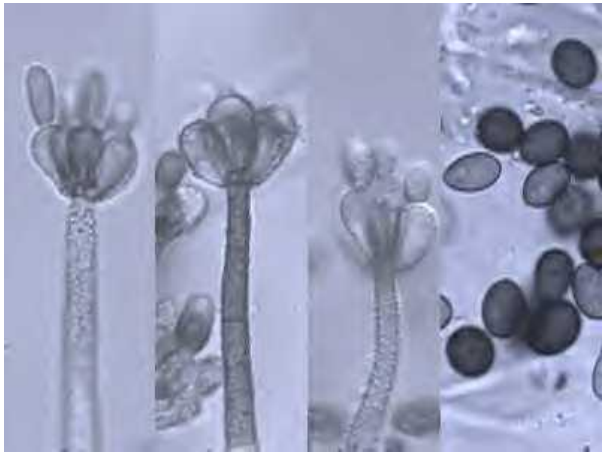
QUEEN'S UNIVERSITY BELFAST



Aspergillus

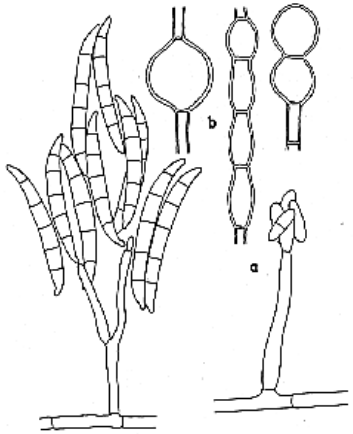
Penicillium





Stachybotrys

Fusarium



UNIVERSITA DEGLI STUDI DI TORINO

# Fungal Growth to Mycotoxin

QUEEN'S UNIVERSITY BELFAST

- Substrate specific (plant)
- Environmental Conditions (field and storage)
  - Temperature
  - Humidity
  - Moisture
  - Oxygen
- Crop damage
  - Parasites
  - Pesticides
  - Drought

UNIVERSITA DEGLI STUDI DI TORINO

# Major Mycotoxins

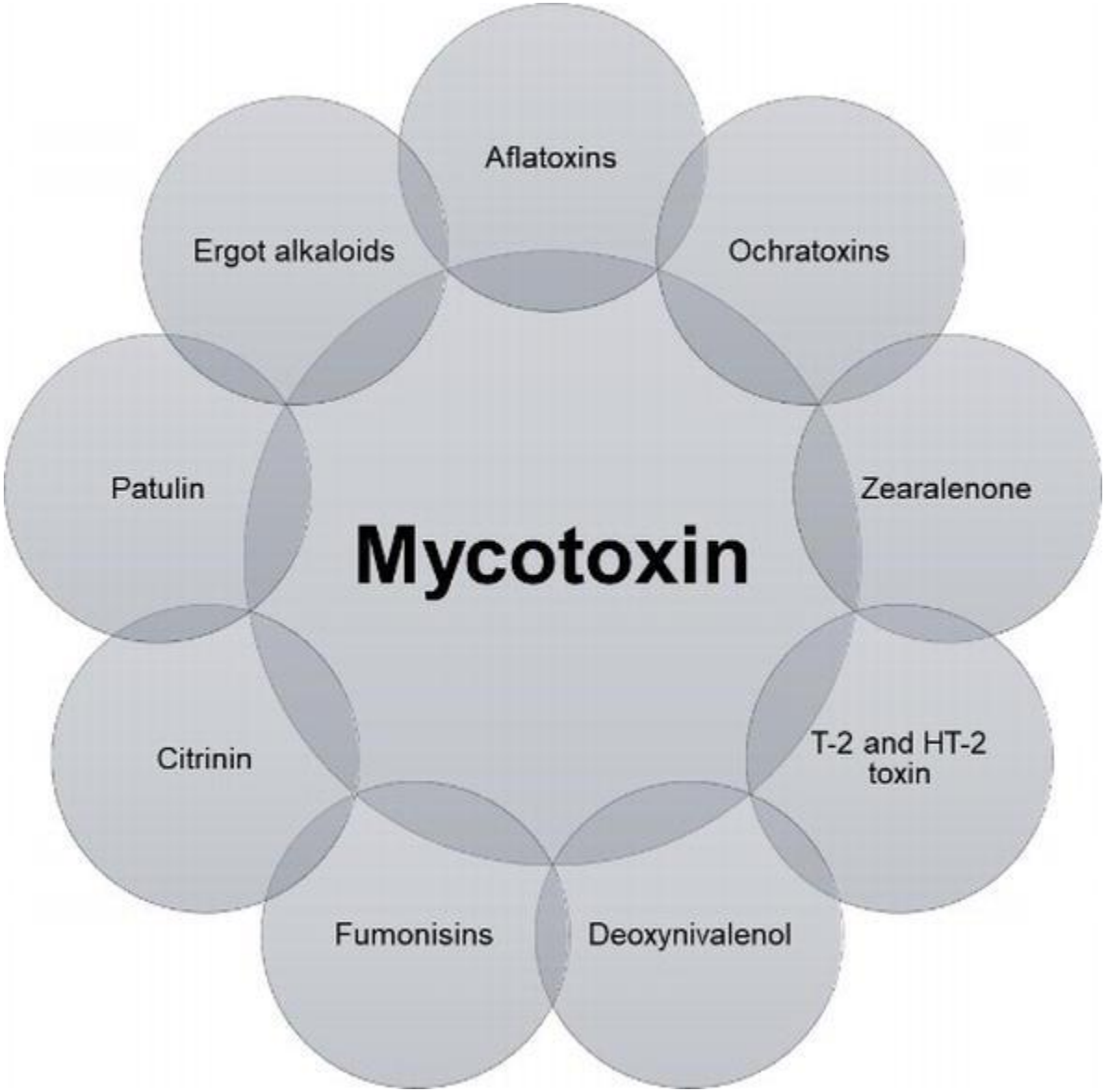
QUEEN'S UNIVERSITY BELFAST

- Responsible for production of:
  - Aflatoxins
  - Fumonisins
  - Trichothecenes
  - Ochratoxins
  - Zearalenone
  - Ergot alkaloids

- Pose the greatest potential risk to humans and animals.

UNIVERSITA
DEGLI STUDI
DI TORINO

QUEEN'S
UNIVERSITY
BELFAST



UNIVERSITA DEGLI STUDI DI TORINO

# Minor Mycotoxins

QUEEN'S UNIVERSITY BELFAST

- Cyclopiazonic acid
- Sterigmatocystin
- Gliotoxin
- Citrinin
- Penitrems
- Patulin
- Fusaric acid
- Penicillic acid
- Mycophenolic acid
- Roquefortine
- PR toxin
- Isofumigaclavines

UNIVERSITA DEGLI STUDI DI TORINO

QUEEN'S UNIVERSITY BELFAST

| MYCOTOXINS | FUNGAL SPECIES | FOODS |
|---|---|---|
| **Aflatoxins (B1, B2, G1, G2, M1)** | ***Aspergillus flavus; A. parasiticus;*** | **Nuts, maize, dried fruits, Oilseeds, milk and dairy** |
| **Ochratoxin A** | ***A. ochraceus; Penicillium verrucosum; P. viridicatum; P. cyclopium*** | **Cereal grains and products, pig products and raw coffee** |
| **Patulin** | ***P. expansum*** | **Apple & Silage** |
| **Zearalenone** | ***F. culmorum; F. graminearum; F. sporotrichioides*** | **Maize, Cereal grains** |
| **Type-A trichothecene T-2 toxin, HT-2 Diacetoxyscirpenol** | ***Fusarium sporotrichioides; F. poae*** | **Cereal grains (Wheat, oats, barley), maize, rice, beans and soya beans** |
| **Type-B trichothecene Deoxynivalenol Nivalenol** | ***F. culmorum; F. graminearum*** | |
| **Fumonisins; moniliformin; fusaric acid** | ***F. moniliforme*** | **Maize kernels** |
| **Ergot alkaloids** | ***Claviceps purpurea*** | **Cereal grains** |
| **Alternaria Toxins** | ***Alternaria sp*** | **Wheat, sorghum and barley, and also oilseeds and fruits and vegetables** |
| **Citrinin** | ***P. citrinum; P. expansum*** | **Cereal grains** |
| **Enniatins & Beavuericins** | ***Fusarium sp.*** | **Cereal grains** |

UNIVERSITA DEGLI STUDI DI TORINO

# *Aspergillus* Mycotoxins

QUEEN'S UNIVERSITY BELFAST

- Occupy subtropical and warm temperate climates.
- Grow under high temperature and low water conditions.
- Grow on inadequately dried products e.g.
  - Cereals, figs, oilseeds, nuts and tobacco.
- Contamination during storage.
- Serious threat to human and animal health e.g.
  - Aflatoxins*, Sterigmatocystin, Cyclopiazonic acid & Ochratoxin A*.

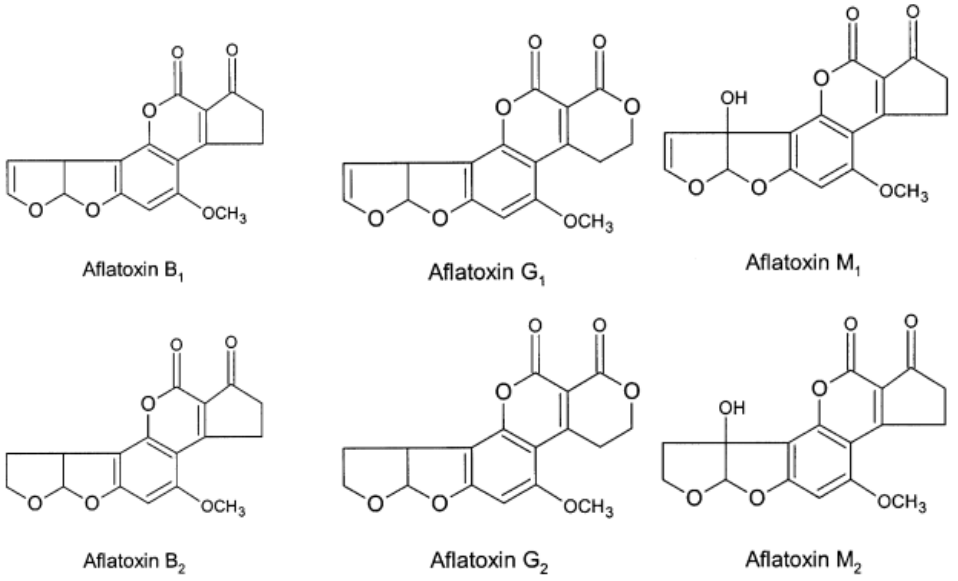
UNIVERSITA DEGLI STUDI DI TORINO

# Aflatoxins

QUEEN'S UNIVERSITY BELFAST

- Discovered in 1960s – Turkey X disease
- Produced by:
  - *Aspergillus flavus* (most common).
  - *Aspergillus parasiticus*.
  - *Aspergillus nomius*.
  - *Aspergillus pseudotamari*.

- Naturally occurring aflatoxins:
  - $B_1$, $B_2$, $G_1$, $G_2$
  - $M_1$, $M_2$



UNIVERSITA DEGLI STUDI DI TORINO

# Aflatoxins

QUEEN'S UNIVERSITY BELFAST

- Acutely toxic, carcinogenic, mutagenic, teratogenic.
- Disrupt protein synthesis.
- Primary target organ – liver - hepatotoxins
- Species susceptibility depends on:
  - Age
  - Weight
  - Diet
  - Exposure to infectious agents
  - Other mycotoxins
  - Pharmacologically active substances

UNIVERSITA DEGLI STUDI DI TORINO

# Aflatoxins

QUEEN'S UNIVERSITY BELFAST

- High level intake:
  - Necrosis of the hepatocytes
  - Derangement of clotting mechanisms and capillary fragility
  - Widespread haemorrhaging
  - Death
- Low level intake:
  - Reduced weight gain
  - Reduced milk yield
  - Reduced feed intake and feed conversion

UNIVERSITA DEGLI STUDI DI TORINO

# Human Aflatoxicoses

QUEEN'S UNIVERSITY BELFAST

- Reye's syndrome in children
- Cirrhosis
- Acute hepatitis
- Kwashiorkor syndrome: paediatric aflatoxicosis
- Burkitt's lymphoma
- Human breast milk contamination eg Ghana, Kenya and the Sudan
- Neonatal jaundice eg Nigeria
- Heroin addicts

UNIVERSITA DEGLI STUDI DI TORINO

# Human Aflatoxicoses

QUEEN'S UNIVERSITY BELFAST

- Acute human aflatoxicoses:
  - Jaundice, low grade fever, depression, anorexia, diarrhoea, fatty degenerative changes in the liver and tenderness near the liver.

- Chronic human aflatoxicoses:
  - Human cancer
    - Primary liver cancer.
    - Supported with epidemiological studies.

UNIVERSITA DEGLI STUDI DI TORINO

# *Penicillium* Mycotoxins

QUEEN'S UNIVERSITY BELFAST

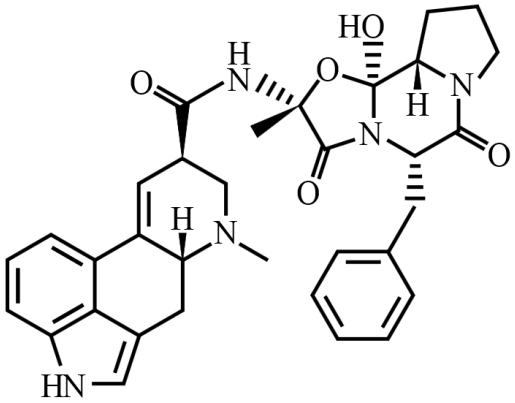
- Abundant in temperate climates
- Contamination of grain
- Main *penicillium* toxins:
  - Ochratoxin A
  - Cyclopiazonic acid
  - Citrinin
  - Citreoviridin
  - Patulin
  - Penicillic acid

UNIVERSITA DEGLI STUDI DI TORINO

# *Claviceps* Mycotoxins

QUEEN'S UNIVERSITY BELFAST

- Common pathogen of grasses
- Ergot alkaloids
  - Total 12 compounds considered for legislation
- Ergotism or St. Anthony's Fire
- Eating cereals infected with ergot sclerotia
  - ➢ Gangrenous form - blood supply.
  - ➢ Convulsive form – central nervous system
- Outbreaks:
  - ➢ 1951: Point Saint Esprit, France
  - ➢ 1975: India
  - ➢ 1977 and 2001: Ethiopia
  - ➢ 1999: Brazil
- Salem witchcraft affair




Ergotamine

Painting "The beggars" Pieter Brueghel, The Louvre, Paris, France

UNIVERSITA DEGLI STUDI DI TORINO

# Reported Cases of Ergot Alkaloids

QUEEN'S UNIVERSITY BELFAST

## Watch for ergot poisoning in cattle

University of Nebraska | Updated: 08/27/2013

ShareThis Resize text PRINT THIS

Cattle producers should be on the lookout a fungus in grasses that causes circulatory problems in livestock that consume them.

The fungus, ergot, has been confirmed in several surrounding states, including Missouri and Iowa, said Richard Randle, a University of Nebraska-Lincoln beef veterinarian. He and colleagues are aware of at least a couple of unconfirmed reports in Nebraska.

Climatic conditions are key to ergot's presence, and they were ideal in some parts of the state this year. Early moisture followed by heat causes certain grasses – especially rye, but also brome, wheatgrass and others – to grow quickly and develop seed heads faster than cattle can consume them. Ergot then can move in and infect the seed heads.

Producers should watch their animals for tips of ears and tails falling off as the fungus can shut off the blood flow to extremities, Randle said. As it progresses, it can affect cattle's feet, causing them to become lame, or cause swelling in the tops of hooves. Rear legs are typically most affected.

Ergot poisoning is unlikely to be fatal but because it can make cattle less tolerant of heat, they can be more susceptible to death from excessive heat. If they are removed from the source of the fungus, they are likely to recover.

Ergot shows up larger than typical seedheads, usually dark brown, purple to black.

Ergot poisoning does not affect meat from cattle.

UNIVERSITA DEGLI STUDI DI TORINO

# Reported Cases of Ergot Alkaloids

QUEEN'S UNIVERSITY BELFAST

**SCIENTIFIC REPORT submitted to EFSA**

**Survey on ergot alkaloids in cereals intended for human consumption and animal feeding** [1]

**Prepared by José Diana Di Mavungu, Daria A. Larionova, Svetlana V. Malysheva, Carlos Van Peteghem, Sarah De Saeger**

**Laboratory of Food Analysis, Department of Bio-analysis, Faculty of Pharmaceutical Sciences, Ghent University, Ghent, Belgium**

Study conducted from August 2010 and May 2011 in 13 European countries
Belgium, Czech Republic, Denmark, Finland, Germany, The Netherlands, Poland, Switzerland, Estonia, France, Sweden, Italy and the United Kingdom

In the analysis of Ergot alkaloids:
148 rye feed tested 52% presence of ergots
182 rye food tested, 95% presence of ergots,
137 wheat feed tested, 34% presence of ergots
127 wheat food tested, 86% presence of ergots,
27 triticale feed samples tested, 48 % presence of ergots
182 food products tested in Belgian shops,76% presence of ergots

Levels ranging from
1 to 12340 µg/kg

UNIVERSITA DEGLI STUDI DI TORINO

# Considerations

QUEEN'S UNIVERSITY BELFAST

What do think are the key concerns and implications for ergot alkaloids in relation to food safety ?

## A GUIDE TO MYCOTOXIN LEVELS IN FEED: MILD TO SEVERE DISEASE

| Fungus | Toxins | No clinical effect | Toxic level | Clinical signs |
|---|---|---|---|---|
| Aspergillus sp | Aflatoxins | < 100ppb | 300 - 2000ppb | Poor growth<br>Liver damage<br>Jaundice<br>Immunosuppression |
| Aspergillus sp and Penicillium sp | Ochratoxin & Citrinin | < 100ppb | 200 - 4000ppb | Reduced growth<br>Thirst<br>Kidney damage |
| Fusarium sp | T2<br>DAS<br>DON<br>(Vomitoxin) | < 2ppm | 4 - 20ppm | Reduced feed intake<br>Immuno-suppression<br>Vomiting |
| Fusarium sp | Zearalenone (F2 toxin) | < 0.05ppm | 1 - 30ppm | Infertility<br>Anoestrus<br>Rectal prolapse<br>Pseudo pregnancy |
| | | | < 30ppm | Early embryo mortality<br>Delayed repeat matings |
| Fusarium sp | Fumonisin | < 10ppm | 20 - 175 | Reduced feed intake<br>Respiratory symptoms<br>Fluid in lungs<br>Abortion |
| Ergot | Ergotoxin | < 0.05% | 0.1-1.0%<br>Ergot bodies by weight (sclerotium) | Reduced feed intake.<br>Gangrene of the extremities.<br>Agalactia due to mammary gland failure. |

**ppm** - parts per million **ppb** - parts per billion.

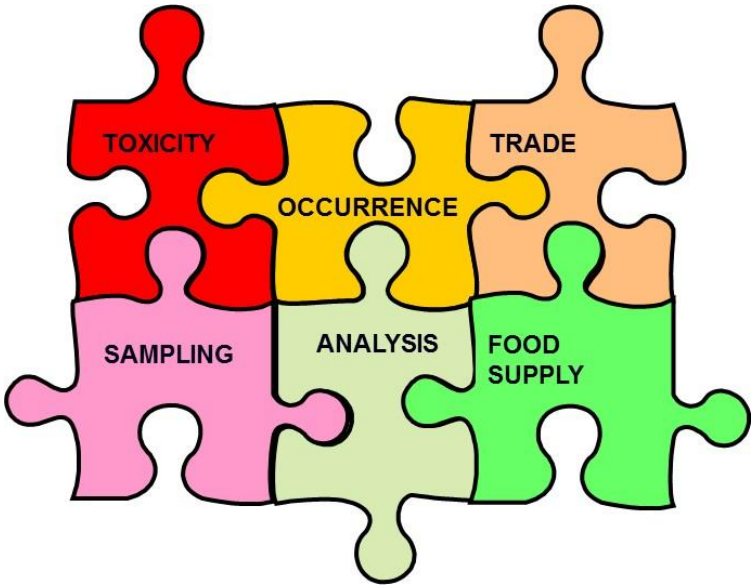
**sp** - species - each of these fungi have several species only some of which are toxic

UNIVERSITA DEGLI STUDI DI TORINO

# Regulatory Limits

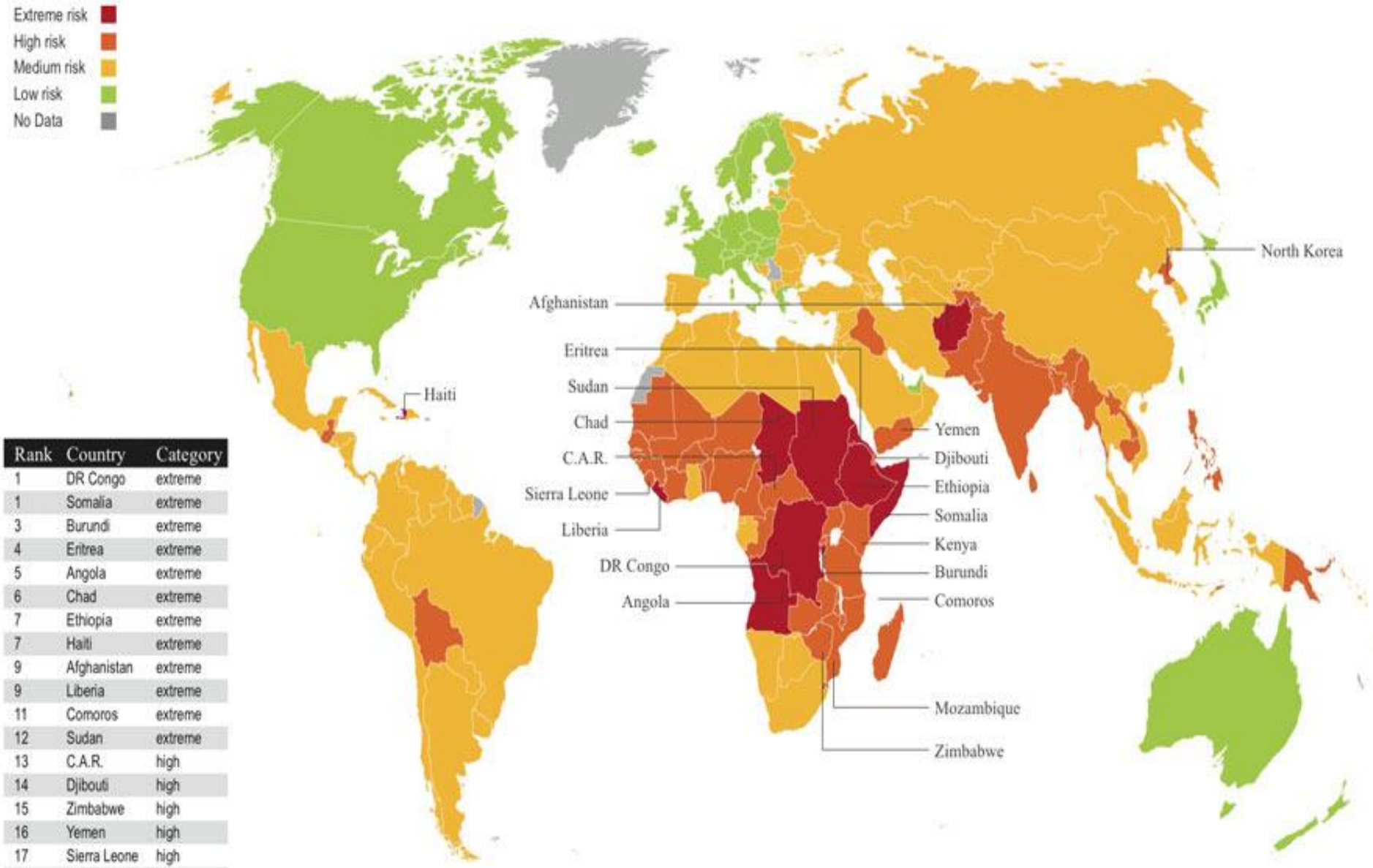
QUEEN'S UNIVERSITY BELFAST

- Toxicological and analytical survey data for risk assessments
  - identification
  - metabolic pathway
  - acute or chronic effects
  - occurrence
  - uptake and susceptibility of consumers
  - multiple commodities
  - global dietary patterns
- Occurrence and distribution of mycotoxins in various commodities.
- Sampling procedures and availability of analytical methods
- No worldwide harmonized mycotoxin regulations whereby trade agreements can be important in setting regulations
- Economic and political factors such as commercial interests and sufficiency of food supply
- Mycotoxins limits / guidelines have been set for certain mycotoxins in different countries
- Europe aims are to harmonise legislation but the Harmonisation of legislation between countries globally is difficult



# Food Security Risk Index 2011

maplecroft




| Rank | Country | Category |
|---|---|---|
| 1 | DR Congo | extreme |
| 1 | Somalia | extreme |
| 3 | Burundi | extreme |
| 4 | Eritrea | extreme |
| 5 | Angola | extreme |
| 6 | Chad | extreme |
| 7 | Ethiopia | extreme |
| 7 | Haiti | extreme |
| 9 | Afghanistan | extreme |
| 9 | Liberia | extreme |
| 11 | Comoros | extreme |
| 12 | Sudan | extreme |
| 13 | C.A.R. | high |
| 14 | Djibouti | high |
| 15 | Zimbabwe | high |
| 16 | Yemen | high |
| 17 | Sierra Leone | high |
| 18 | Mozambique | high |
| 19 | North Korea | high |
| 20 | Kenya | high |

UNIVERSITA DEGLI STUDI DI TORINO

# Regulatory Limits

QUEEN'S UNIVERSITY BELFAST

Harmonized regulations:

- Australia and New Zealand
- European Union
- Mercosur
- Association of Southeast Asian Nations (ASEAN).
- Codex Alimentarius Commission (CAC).

# EU Aflatoxin limits

Commission Regulation (EC) No 1881/2006
19 December 2006

| *aflatoxin $B_1$ / total aflatoxins* | (μg/kg) |
|---|---|
| - groundnuts, nuts & dried fruits (products) : | 2 / 4 |
| - groundnuts subject to sorting : | 8 / 15 |
| - nuts, maize & dried fruits subject to sorting : | 5 / 10 |
| - cereals & processed products : | 2 / 4 |
| - cereals to be sorted : | 2 / 4 |
| - Various spices : | 5 / 10 |

| *aflatoxin $M_1$* | |
|---|---|
| - milk: | 0.050 |
| - infant formulation | 0.025 |

UNIVERSITA DEGLI STUDI DI TORINO

# Global Aflatoxin limits

QUEEN'S UNIVERSITY BELFAST

| Country | Foodstuffs | Total aflatoxins (µg/kg) |
|---|---|---|
| Australia / New Zealand | Peanuts &Tree nuts | 15 |
| Canada | Nut and nut products | 15 |
| Codex<br>Saudi Arabia, United Arab Emirates (UAE), Kuwait, Bahrain, Oman, Yemen and Qatar<br>Nigeria | Peanuts, almonds, shelled Brazil nuts, hazelnuts pistachios intended for further processing | 15 |
| | Almonds, hazelnuts, pistachios, shelled Brazil nuts, "ready-to-eat" | 10 |
| India | Wheat, maize, jawar (sorghum) and bajra, rice, whole and split pulse (dal) masur (lentil), whole and split pulse urd (mung bean), whole and split pulse moong (green gram), whole and split pulse chana (gram), split pulse arhar (red gram), and other food grains | 30 |
| | Groundnut kernels (shelled) (peanuts); | 30 |
| USA | Brazil nuts, peanuts and peanut products, pistachio products | 20 |
| South Africa | Peanuts | 15 |

UNIVERSITA DEGLI STUDI DI TORINO

# Global Aflatoxin limits

QUEEN'S UNIVERSITY BELFAST

| Country | Foodstuffs | Aflatoxin $M_1$ (µg/kg) |
|---|---|---|
| China | Milk and milk products (for milk powder, calculated on a fresh milk basis) | 0.5 |
| | Formulated foods for infants (milk or milk protein based) | 0.5 (calculated on a dry powder basis) |
| | Formulated foods for older infants and young children (milk or milk protein based) | 0.5 (calculated on a dry powder basis) |
| | Formulated foods for special medical purposes intended for infants | 0.5 (calculated on a dry powder basis) |
| Codex, India, Kenya, USA | Milk | 0.5 |
| Argentina | Milk, liquid including milk used in the manufacture of milk and milk products and reconstituted milk | 0.5 / L |
| | Milk, powder | 5.0 |
| | Milk formula | ND |
| Mexico | Pasteurised, ultrapasteurised, sterilised and dehydrated milk, milk formula and combined milk products | 0.5 / L |
| South Africa | Milk | 0.05 |
| Europe | Milk | 0.05 |

UNIVERSITA DEGLI STUDI DI TORINO

# Mycotoxins for breakfast?

QUEEN'S UNIVERSITY BELFAST

Deoxynivalenol
Zearalenone
Ochratoxin A

Ochratoxin A

Aflatoxin $M_1$

Patulin

HT2 and T2 in oats
Ochratoxin A in dried fruit and cereals
Aflatoxins in nuts

Fumonisins

UNIVERSITA DEGLI STUDI DI TORINO

QUEEN'S UNIVERSITY BELFAST

# Synergistic and additive effects

Fungi and plants can produce multiple toxins
Synergistic and additive effects
Frequent co-occurrence - need to test for a number of toxins for food safety

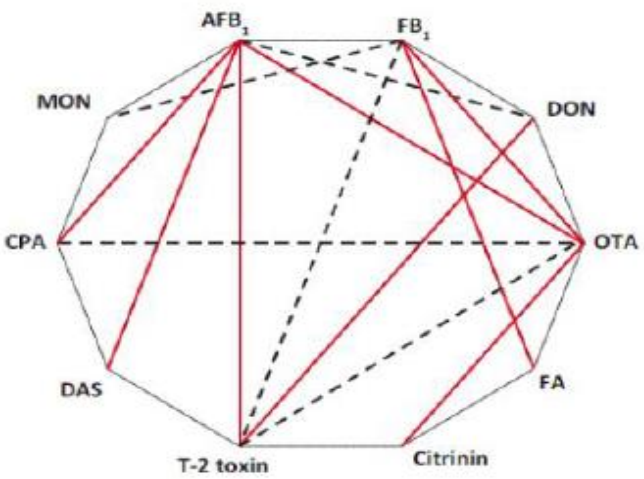



*Figure 1. Synergistic and additive effects of mycotoxins in poultry*

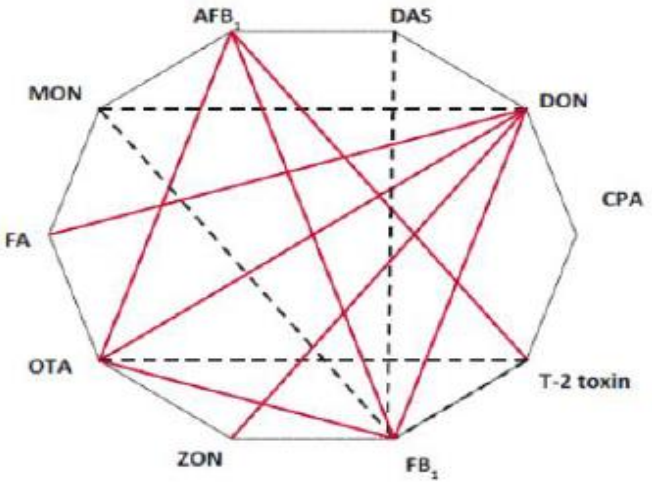



*Figure 2. Synergistic and additive effects of mycotoxins in pigs*

*synergistic effect*
*additive effect*

Source: Radka Borutova, Biomin 2010

UNIVERSITA DEGLI STUDI DI TORINO

QUEEN'S UNIVERSITY BELFAST

# Prevention and mitigation strategies

- Good Agricultural Practice
  - Early harvesting
  - Proper drying
  - Physical treatment
  - Sanitation
  - Proper storage
  - Insect management
  - Other methods
- Biological Control
- Chemical control
- Decontamination
- Breeding for resistance
- Legislation
- Surveillance and awareness creation

UNIVERSITA DEGLI STUDI DI TORINO

# Conclusions

QUEEN'S UNIVERSITY BELFAST

- Mycotoxins are an important health hazard
- Acute and chronic effects in humans and animals
- Many are potent carcinogens
- Difficult to prove mycotoxicoses
- Regulatory limits in foods and feeds need further consideration for harmonisation and cocktail effects
- Prevention and mitigation

UNIVERSITA
DEGLI STUDI
DI TORINO

QUEEN'S
UNIVERSITY
BELFAST

# Plant toxins

**Katrina Campbell**
**Prof of Food Security & Diagnsotics**
katrina.campbell@qub.ac.uk

UNIVERSITA DEGLI STUDI DI TORINO

# Plant Toxins

QUEEN'S UNIVERSITY BELFAST

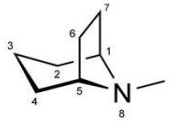
- Plant toxins are naturally occurring secondary metabolites that are toxic and/or have negative effects on the bioavailability of nutrients.
- Plant toxins may be present in fruits and vegetables which are common food sources.
- Products of secondary metabolism are species specific and give the plant its particular characteristics. They include plant pigments, flavours, and compounds that serve to protect the plants.
- Some of these secondary metabolic products may be growth inhibitors, neurotoxins, carcinogens, and teratogens.
- Common classes of plant toxins include alkaloids
  - pyrrolizidine alkaloids (eg herbs, tea, honey)
  - glycoalkaloids (eg potatoes)
  - cyanogenic glycosides (eg bitter apricot seed, cassava),
  - lectins (eg green beans and kidney beans)
  - saponins (eg root beers and soaps)

UNIVERSITA DEGLI STUDI DI TORINO

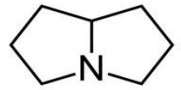
# Plant Alkaloids

QUEEN'S UNIVERSITY BELFAST

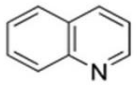
- Naturally occurring chemical compounds containing basic nitrogen atoms
- Physiologically active
- Insoluble or sparingly soluble in water
- Crystalline solids
- Form double-salts with compounds of Hg, Au, Pt, and other heavy metals
- Usually classified according to the ring structure of the main alkaloid group from which they are derived



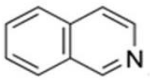
Tropane



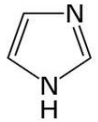
Pyrrolizidine



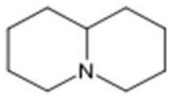
Quinoline



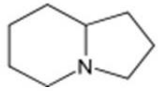
Isoquinoline



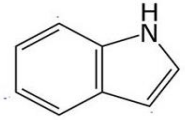
Imidazole



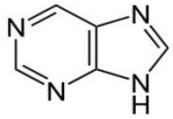
Quinolizidine



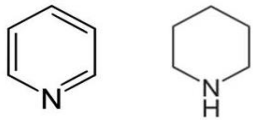
Indolizidine



Indole



Purine



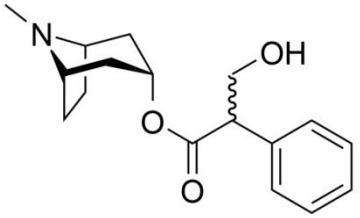
Pyridine-Piperidine

UNIVERSITA DEGLI STUDI DI TORINO

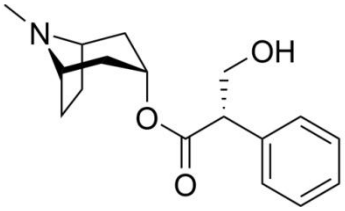
# Tropane alkaloids

QUEEN'S UNIVERSITY BELFAST

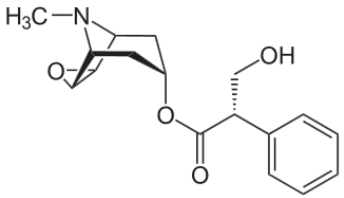
- Plant toxins, formed primarily by *Solanaceae;* seeds may contaminate plants, e.g. Soybean
- *>200 compounds, but most common tropane alkaloids are atropine, hyoscyamine and scopolamine*
- Humans: accidental exposure rare
- Animals: pigs very sensitive (*Datura* poisoning)
- Theses alkaloids used as a drug, poison or antidote act on acetylcholine receptors
- *Short term adverse effects eg dilated pupils, change of heart rate dryness of the mouth constipation, urinary retention and flushed skin*
- *Symptoms usually 30-60 minutes after consumption*
- *Excretion from the body from 12 to 48 hours with no long term health effects*




Atropine




Hyoscyamine




Scopolamine

# Tropane alkaloids

- Poisonings of humans by tropane alkaloids
  - unintended ingestions (contamination, mistaken identity, carry-over) and
  - intended ingestions (overdoses).

- Contamination can occur when toxic plant (parts) are accidently mixed into edible plants during harvest or processing.

  - France when Datura flower buds were mixed in with canned green beans.
  - mistaken identity has been reported where for example berries of deadly nightshade resemble edible berries like blueberry. 10 berries fatal.
  - Carry-over does not appear to be a real problem. There are only a few examples of animal products containing (low levels) of tropane alkaloids because the animals were fed with contaminated feed.

- Poisoning due to intended ingestions can be divided into consumption for recreational purposes (hallucinogenic effects) or medical properties (e.g. arthritis, anesthetic), homicides and suicides.

News 23.5.2013 14:56 | updated 23.5.2013 19:13

**Nine people now affected by datura poisoning**

Nine people have now been affected by datura contamination from frozen vegetables sold by a Finnish supermarket. Most symptoms are mild, with dry mouth, a quickening pulse and weakened vision the main effects, but some people have required hospital treatment.

Recommend 27 people recommend this. Sign Up to see what your friends recommend.

UNIVERSITA DEGLI STUDI DI TORINO

# Tropane alkaloids

QUEEN'S UNIVERSITY BELFAST

- In producing plants variable amounts of alkaloids are found in all parts of the plant
- Major concentration found in seeds
- Plants can produce 30,000 or more seeds.
- The seeds, when mature, are typically spread over an area of 1 to 4 sqM and may remain viable in the soil for more than a century

*Journal of Environmental Science and Health, Part B* (2013) **48**, 1034–1042

ISSN: 0360-1234 (Print); 1532-4109 (Online)
DOI: 10.1080/03601234.2013.824281

Taylor & Francis
Taylor & Francis Group

## Uptake of $^{14}$C-atropine and/or its transformation products from soil by wheat (*Triticum aestivum* var Kronjet) and their translocation to shoots

ZORA JANDRIĆ[1], MOHAMMAD N. RATHOR[1], SORIVAN CHHEM-KIETH[1], JOSEPH ADU-GYAMFI[2], LEOPOLD MAYR[2], CHRISTIAN RESCH[2], SOULEYMANE BADO[3], JAROSLAVA ŠVARC-GAJIĆ[4] and ANDREW CANNAVAN[1]

[1]*Food and Environmental Protection Laboratory, FAO/IAEA Agriculture and Biotechnology Laboratories, Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, International Atomic Energy Agency, Vienna, Austria*
[2]*Soil and Water Management and Crop Nutrition Laboratory, FAO/IAEA Agriculture and Biotechnology Laboratories, Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, International Atomic Energy Agency, Vienna, Austria*
[3]*Plant Breeding and Genetics Laboratory, FAO/IAEA Agriculture and Biotechnology Laboratories, Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, International Atomic Energy Agency, Vienna, Austria*
[4]*Department for Applied and Engineering Chemistry, Faculty of Technology, Novi Sad, Serbia*

UNIVERSITA DEGLI STUDI DI TORINO

# Tropane alkaloids

QUEEN'S UNIVERSITY BELFAST

www.bfr.bund.de

BfR
Bundesinstitut für Risikobewertung

**High tropane alkaloid levels in cereal products: Health impairments are possible in individuals with heart problems**

Opinion No 035/2014 of the BfR dated 13 November 2013

Tropane alkaloids (TA) are natural components contained in certain plants such as henbane, thorn-apple and deadly nightshade. To date, a total of more than 200 different TA have been identified in plants. Some of these alkaloids are also used in medicinal products, for example atropine (a mixture containing equal amounts of the isomers (-)-hyoscyamine and (+)-hyoscyamine), (-)-hyoscyamine and (-)-scopolamine. These alkaloids are known to affect the heart rate and the central nervous system even at low doses; drowsiness, headaches and nausea are typical symptoms.

Tropane alkaloids can also occur in cereal-based foods through the contamination of cereals

UNIVERSITA DEGLI STUDI DI TORINO

# Tropane alkaloids

QUEEN'S UNIVERSITY BELFAST

23.6.2015 EN Official Journal of the European Union L 157/97

RECOMMENDATIONS

**COMMISSION RECOMMENDATION (EU) 2015/976**

**of 19 June 2015**

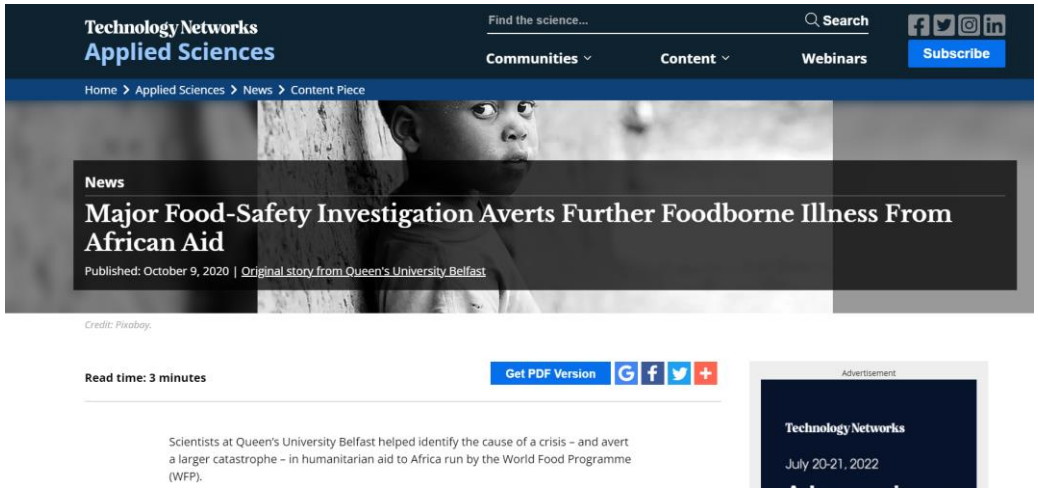
**on the monitoring of the presence of tropane alkaloids in food**

**(Text with EEA relevance)**

- The Limit of Quantification (LOQ) for atropine (racemic mixture of hyoscyamine enantiomers) and scopolamine should be:
  - <5 µg/kg and not >10 µg/kg for agricultural commodities, ingredients, food supplements and herbal teas
  - <2 µg/kg for finished foods (e.g. breakfast cereals)
  - 1 µg/kg for cereal-based foods for infants and young children.

UNIVERSITA DEGLI STUDI DI TORINO

QUEEN'S UNIVERSITY BELFAST




Researchers at the university's Institute for Global Food Security (IGFS) were called in to support an investigation after a major food-poisoning incident in Uganda in March 2019, which killed five people and saw hundreds hospitalised.

A second outbreak occurred in a refugee camp in a completely different area of Uganda in August 2019.

Contaminated Specialised Nutritious Foods / Super Cereals

UNIVERSITA DEGLI STUDI DI TORINO

QUEEN'S UNIVERSITY BELFAST

**Assessment of the Conclusions of the Joint FAO/WHO Expert Meeting on Tropane Alkaloids**

**Approved 9th March 2022**
**doi: 10.2903/j.efsa.2022.7229**

The main TAs considered in the assessments of EFSA and FAO/WHO were (-)-hyoscyamine and(-)-scopolamine, which exert their pharmacological and toxicological effects by acting as competitive antagonists of the muscarinic acetylcholine receptors.

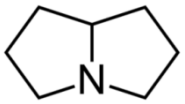
Both EFSA and FAO/WHO considered a study in human volunteers as the key study to assess the effects of TAs. The CONTAM Panel established a group acute reference dose (ARfD) of 0.016μg/kg body weight (bw) for the sum of (-)-hyoscyamineand (-)-scopolamine, based on decreased heart rate.

UNIVERSITA DEGLI STUDI DI TORINO

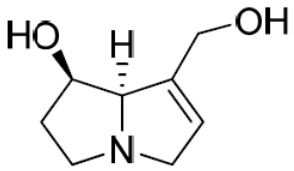
# Pyrrolizidine alkaloids

QUEEN'S UNIVERSITY BELFAST

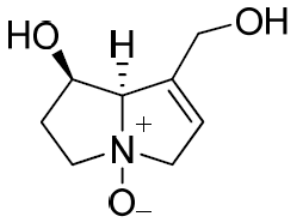
- Toxins formed in common plants that may contaminate food or feed especially flowering plants
- Plant secondary metabolites synthesized by 6000 species
- 600 different compounds with a common motif of pyrrolizidine
- Many pyrrolizidine alkaloids occur in two forms: as tertiary bases and as N-oxides
- Re-introduction of certain species in nature may increase intoxications in grazing animals
- Adverse effects in humans and livestock Eg HVOD, carcinogen




**common motif**




retronecine
*(7R)*




retronecine-*N*-oxide

UNIVERSITA DEGLI STUDI DI TORINO

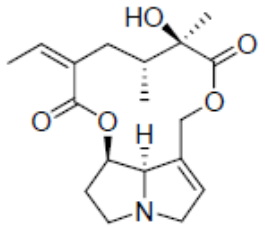
Scientific Opinion on Pyrrolizidine alkaloids in food and feed
EFSA Panel on Contaminants in the Food Chain (CONTAM )
EFSA Journal 2011: 9(11):2406

QUEEN'S UNIVERSITY BELFAST

4 groups of pyrrolizidine alkaloids (and their N-oxides) of particular importance for food and feed

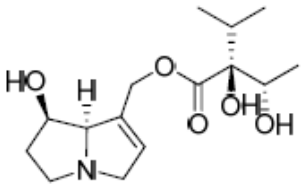
1. **Senecionine-type PAs**:
   - acetylerucifoline, erucifoline, integerrimine, jacobine, jacoline, jaconine, jacozine, retrorsine, senecionine, seneciphylline
   - Occur in the Senecioneae (Asteraceae family) and Crotalaria spp (Fabaceae family)

Senecionine
CAS: 130-01-8



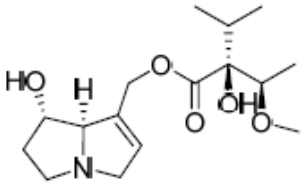
2. **Lycopsamine-type PAs**:
   - acetylechimidine and isomers, echimidine and isomers, echivulgarine, lycopsamine and isomers, vulgarine
   - Occur in the Boraginaceae family and Eupatorieae (Asteraceaae family)

Lycopsamine
CAS: 10285-07-1



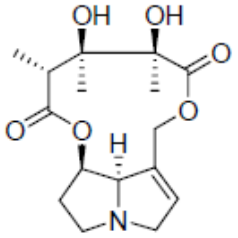
3. **Heliotrine-type PAs**:
   - europine, heliotrine, lasiocarpine.
   - Occur in the Heliotropium spp (Boraginaceae family)

Heliotrine
CAS: 303-33-3



4. **Monocrotaline-type PAs**:
   - fulvine, monocrotaline, retusamine, trichodesmine.
   - Occur in Crolalaria spp (Fabaceae family)

Monocrotaline
CAS: 315-22-0



UNIVERSITA DEGLI STUDI DI TORINO

# Reported Cases of Pyrrolizidine Alkaloids

QUEEN'S UNIVERSITY BELFAST

## Pyrrolizidine alkaloid poisoning of sheep in New South Wales

J T SEAMAN
New South Wales Department of Agriculture, Agricultural Research and Veterinary Centre, Forest Road, Orange, New South Wales 2800

**SUMMARY: Pyrrolizidine alkaloid poisoning of sheep in New South Wales was reviewed, based on the records of the New South Wales Department of Agriculture's Regional Veterinary Laboratories. The plant species causing significant mortalities were *Echium plantagineum* and *Heliotropium europaeum*. The syndrome of hepatogenous chronic copper poisoning was more frequently diagnosed than primary pyrrolizidine alkaloid poisoning, particularly when grazing *E. plantagineum*. The data indicated that adult crossbred ewes were the most commonly affected class of sheep.**
Aust Vet J 64: 164-167

Food Safety Watch
*The science of safe food*

NEWS | RESEARCH | BLOG | FEATURES | FACT SHEETS | E-BOOKS

Search Results for 'pyrrol'

### Plant toxins in teas and herbal teas could be health risk

Richard Lawley | July 20, 2013

The German Federal Institute for Risk Assessment (BfR) has announced that levels of pyrrolizidine alkaloids – naturally produced toxic substances in plants – found in some teas and herbal teas are "too high and should possibly be reduced." The finding has come out of a BfR research project to study the determination of these substances […]

*17-07-2013:* As the first results of the non-representative tests, a total concentrations of 0 to 3.4 milligrams of pyrrolizidine alkaloids per kilogram of analyzed sample were found.

UNIVERSITA DEGLI STUDI DI TORINO

# Reported Cases of Pyrrolizidine Alkaloids

QUEEN'S UNIVERSITY BELFAST

## An Outbreak of Hepatic Veno-Occlusive Disease in Western Afghanistan Associated with Exposure to Wheat Flour Contaminated with Pyrrolizidine Alkaloids

**Faizullah Kakar,[1] Zarif Akbarian,[1] Toby Leslie,[2] Mir Lais Mustafa,[1] John Watson,[3] Hans P. van Egmond,[4] Mohammad Fahim Omar,[1] and Jawad Mofleh[1]**

[1] *Ministry of Public Health, Kabul, Afganistan*
[2] *London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT, UK*
[3] *World Health Organisation, Geneva, Switzerland*
[4] *National Institute for Public Health & the Environment, RIVM, 3720 BA Bilthoven, The Netherlands*

Correspondence should be addressed to Toby Leslie, toby.leslie@lshtm.ac.uk

Received 16 November 2009; Revised 19 February 2010; Accepted 16 May 2010

Academic Editor: Peter John O'Brien



Pyrrolizidine alakloids (PAs) are known to cause hepatic veno-occlusive disease (VOD). Outbreaks have occurred in Western Afghanistan since 1974, the latest in February 2008. We conducted an outbreak investigation using a case-control design. Sixty-seven cases of VOD were compared with 199 community controls. Consumption of bread was strongly associated with disease (adjusted odds ratio: 35.8 [95%CI: 7.6–168.2]). Toxic doses of PA were found in plant extracts and in samples of wheat flour taken from the study area. Compared to wheat flour there was 1000 times less PA in milk and whey and in water samples the PA content was zero. Although direct analysis was not possible, contaminated wheat flour used to make bread was the likely source of PA causing the outbreak. Eating a more varied diet including meat and fruit may be protective. Prevention and control measures will rely on community awareness and agricultural interventions to ensure safety of the food supply.

## Concern over children's exposure to toxins in honey

By Jane Byrne, 08-Nov-2011

Related topics: Legislation

**The EU food safety assessor has concluded that there is a possible health concern for toddlers and children who are high consumers of honey arising out of the possible presence of pyrrolizidine alkaloids (PA).**

RELATED NEWS:

Young children at high risk from food toxins, warns study



The European Commission asked the European Food Safety Authority to deliver a scientific opinion on pyrrolizidine alkaloids (PA) in food and feed, taking into account new data since a previous assessment back in 2007.

PAs are toxins that are produced naturally by some plants, including ragwort (*Senecio* spp.) and borage (*Borago officinalis*). The UK's Food Safety Agency said there is a potential for these toxins to be transferred to honey by pollinating bees, but notes information regarding the transfer of PAs from ragwort and borage to honey is limited.

EFSA, meanwhile, said the results for 13,280 bulk honey and 1324 retail honey samples were provided to it by one member state only. And the agency cautions that as such they "*cannot be regarded as representative for the occurrence of PAs across Europe.*"

UNIVERSITA DEGLI STUDI DI TORINO

# Case study: Outbreak of liver disease in Hirat Province

QUEEN'S UNIVERSITY BELFAST

# Gulran district

- Approx. 150 km², approx.110, 000 inhabitants
- Remote villages, undulating hills, scanty vegetation, serving as pasture lands
- Inhabitants mostly wheat farmers who may keep sheep and goats
- Diet consists mainly of wheat bread, occasionally meat

# Outbreak of liver disease in Hirat district

- More than 270 people affected
- Approx. 50 people died
- WHO alarmed, and RIVM contracted to investigate the cause of the disease
- Hypothesis: PA poisoning
- Samples sent to RIVM, Public Health Laboratory Netherland
  - Weed animals were feeding on
  - Flour for the bread
  - Milk from the goats
  - Qurut a type of cheese
- Methodology: LC-MS/MS
  - High levels 200 -1000mg/kg in the charmac
  - Levels of 100-600 µg/kg in the Qurut

Charmac: Weed

Flour

Milk

Qurut

UNIVERSITA DEGLI STUDI DI TORINO

QUEEN'S UNIVERSITY BELFAST

- High concentrations of PA found in charmac
  - High levels 200 -1000mg/kg

- PA were found in wheat flour

- In dairy products PA were also found;
  - Qurut contained *trichodesmine*, not present in charmac
  - Levels in Qurut of 100-600 µg/kg

- Prolonged, regular exposure to contaminated wheat, in combination with low protein diet, likely to be the cause of the disease

UNIVERSITA DEGLI STUDI DI TORINO

QUEEN'S UNIVERSITY BELFAST

# Prevention and mitigation strategies

- Good Agricultural Practice
  - Early harvesting
  - Physical treatment
  - Sanitation
  - Proper storage

- Chemical control
- Decontamination
- Legislation
- Surveillance and awareness creation

# Methods of Analysis

**Tropane Alkaloids:**

Methods of analysis: HPLC, GC, RIA, CE-MS, LC-MS/MS,

**Pyrrolizidine alkaloids**

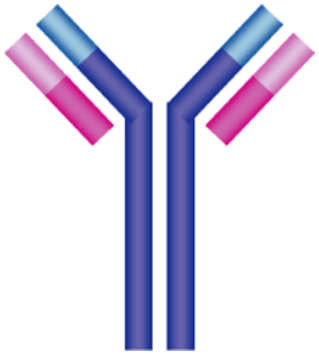
Methods of analysis: GC-MS, LC-MS/MS, ELISA

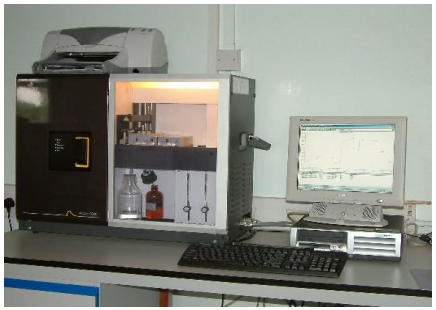
## Rapid field tests still in development

UNIVERSITA DEGLI STUDI DI TORINO

QUEEN'S UNIVERSITY BELFAST

**Task: Consider these rapid methods of analysis: Discuss what makes these methods of analysis rapid methods?**





UNIVERSITA DEGLI STUDI DI TORINO

# Summary

QUEEN'S UNIVERSITY BELFAST

- Plant alkaloids are potentially toxic compounds on human consumption especially to vulnerable groups
- More data on the occurrence of these compounds in food and animal feed is required
- Regulatory limits for alkaloids should be established particularly for pyrrolizidine alkaloids
- Need to monitor the risk of exposure of alkaloids
  - Herbal products
  - Teas
  - Honey
  - Seeds
  - Feed materials: soybean, linseed, rye
- Implement the use of rapid immunological
  - multi-analyte tests for analysis

**Field based sensor testing or HACCP Testing in Food supply chain**

UNIVERSITA DEGLI STUDI DI TORINO

QUEEN'S UNIVERSITY BELFAST

UNIVERSITA DEGLI STUDI DI TORINO

QUEEN'S UNIVERSITY BELFAST

# Mycotoxins & Plant Toxins

**Prof Katrina Campbell**
katrina.campbell@qub.ac.uk