Mycotoxins in cereals in a changing world

EuroCereal, December 6, 2011

Monique de Nijs

RIKILT WAGENINGEN UR

Outline of presentation

- RIKILT – Institute of Food Safety;
- Introduction & history;
- Regulations/RASFF results;
- Challenges:
  - Methods of analysis;
  - Emerging risks (new legislation, masked mycotoxins, climate, changing environment);
  - Decontamination / binders;
  - Bioethanol;
- Summary

RIKILT WAGENINGEN UR

RIKILT - Institute of Food Safety

www.rikilt.wur.nl

- Part of Wageningen UR since 1998
- About 200 employees

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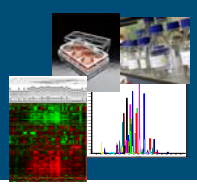
RIKILT –Institute of Food Safety



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- Environment & process contaminants:
  - *Dioxins in eggs in Germany;*
- Radioactivity:
  - *Imports from Japan;*
- Pesticides;
- Natural toxins (*mycotoxins, plant toxins, phycotoxins*);
- Animal treatment medicines and residues;
- GMO's;
- Allergens;
- Nutrients / Quality:
  - *Identify organically produced eggs;*
  - *Authenticity identification (is this the fruit juice I selected?).*

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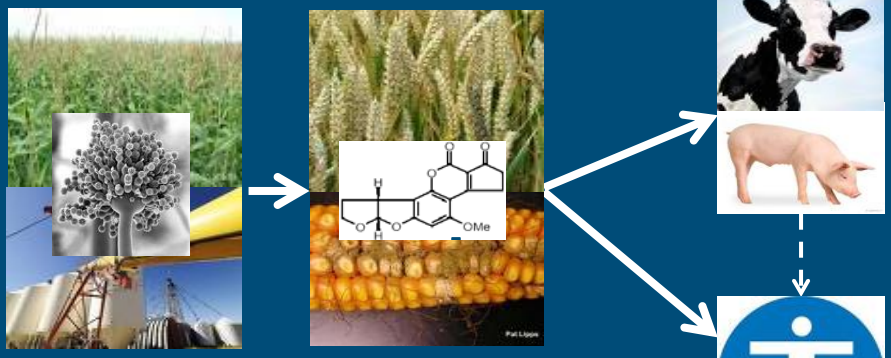
Introduction – *Contamination and exposure route*



Fungi contaminate (harvested) grain

Fungi excrete mycotoxins in (harvested) cereals

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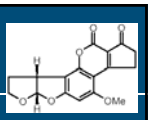
Introduction – *Incidents and issues*



Year	*Toxicosis*	*Toxin*	*Fungus*
994	Holy fire	lysergic acid deriv.	*C. purpurea*
1890	Cardiac beriberi	citreoviridin	*P. citreo-viride*
1913	Alimentary toxic aleukia	trichothecenes	*F. sporotrichioides*
1952	Balkan endemic nephropathy	ochratoxin	*P. verrucosum*
1960	Turkey X disease	aflatoxins	*A. flavus*
1988	Hole in the head syndr.	fumonisins	*Fusarium*
2004& '05&'10	Hum. aflatoxicosis	aflatoxins	Not determined *317 people ill, 127 fatalities (Kenya)*

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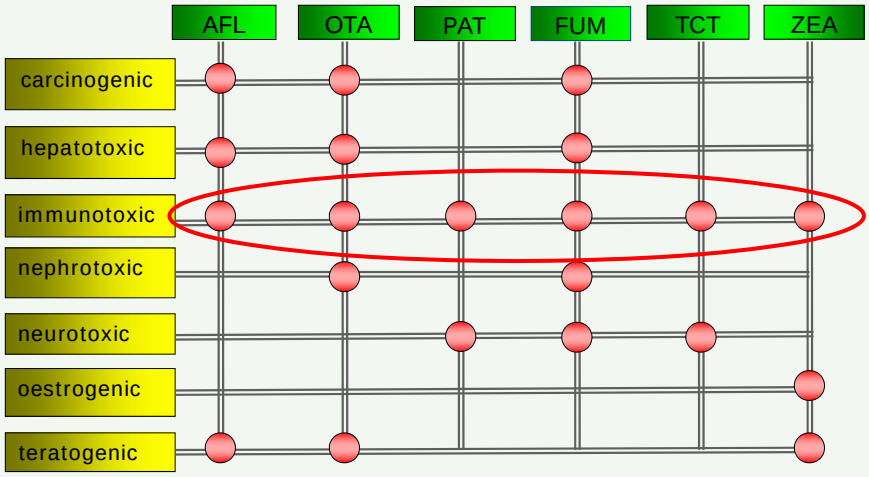
Introduction - *Toxic effects of mycotoxins*



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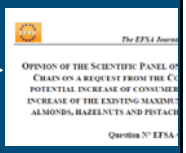
Regulations – *Procedures*

Toxicity

MS: Consumption

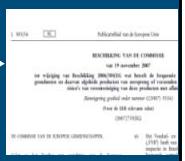
MS: Occurrence

Risk assessment:
EFSA (EU) / JECFA (ww)



Risk management :
EU & MS / CODEX (ww)

- Regulatory limits (EU) / Standards (CODEX)
- Methods & sampling (CEN)



Risk communication:
Government to public
EFSA / JECFA / CODEX

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MS=member state
ww = world wide

Regulations – *EU Food (1/2)*

- Commission *Regulation* (EC) No 1881/2006 (and its amendments): Setting maximum levels for certain contaminants in food
- Commission *Decision* (EC) No 401/2006 (and its amendments): Laying down the methods of sampling and analysis for the official control of the levels of mycotoxins in food

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Regulations – *Member state (the Netherlands) Food (2/2)*

- The Netherlands: Warenwetbesluit bereiding en behandeling van levensmiddelen Artikel 12

  Fungal and bacterial toxins in quantities that can be harmful to the public health must be absent in food, drinks and raw materials

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Regulations - *EU Feed (1/2)*

- *Directive* (EC) of the European Parliament and the Council No 2002/32 (and its amendments): On undesirable substances in animal feed
- Commission *Recommendation* 2006/576/EC: On the presence of deoxynivalenol, zearalenon, ochratoxin A, T-2 and HT-2 and fumonisins in products intended for animal feeding

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Regulations - *EU Feed (2/2)*

- Commission *Regulation* (EC) No 152/2009: Laying down the methods of sampling and analysis for the official control of feed

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Regulations - *EU import controls*

- Commission *Regulation* (EC) No 669/2009 (and its amendments): Implementing Regulation (EC) No 882/2004 of the European Parliament and of the Council as regards the increased level of official controls on imports of certain feed and food of non-animal origin and amending Decision 2006/504/EC
- Commission *Regulation* (EC) No 1152/2009: imposing special conditions governing the import of certain foodstuffs from certain third countries due to contamination risk by aflatoxins and repealing Decision 2006/504/EC

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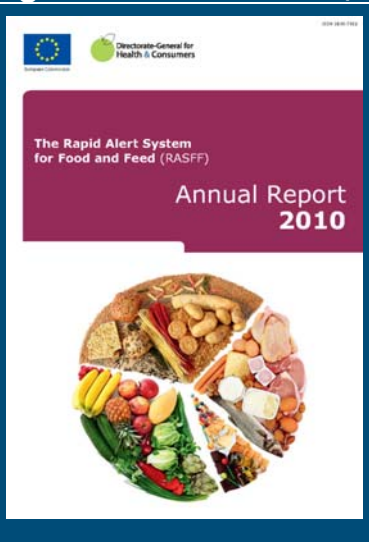
Regulations - *EU Rapid alert system for food & feed*



- Quick information-exchange in the EU on risks to human health;
- Allows MS to identify potential problems and take measures;
- In 2010: 679 mycotoxin issues incl 586 border rejections

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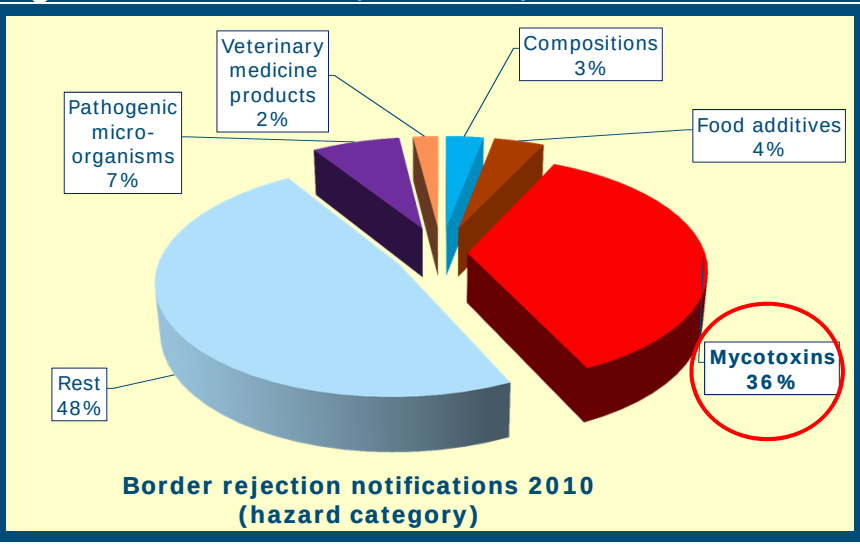
Regulations - *EU Rapid alert system for food & feed*



Border rejection notifications 2010 (hazard category)

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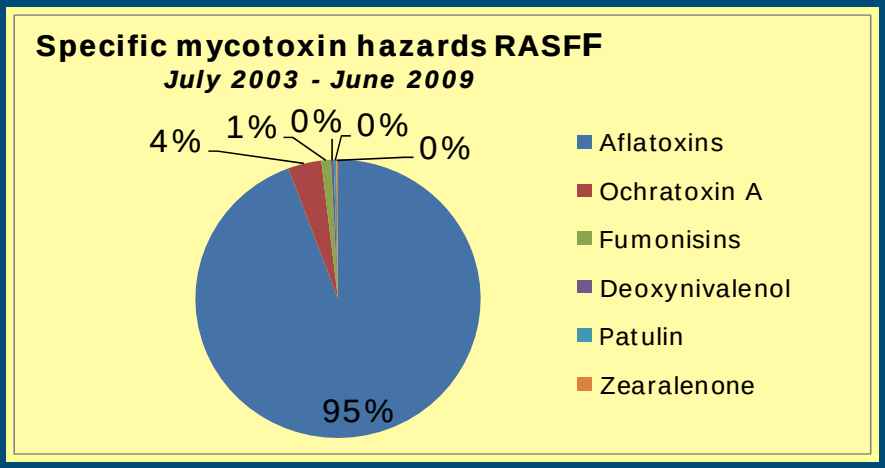
Regulations - *EU Rapid alert system for food & feed*



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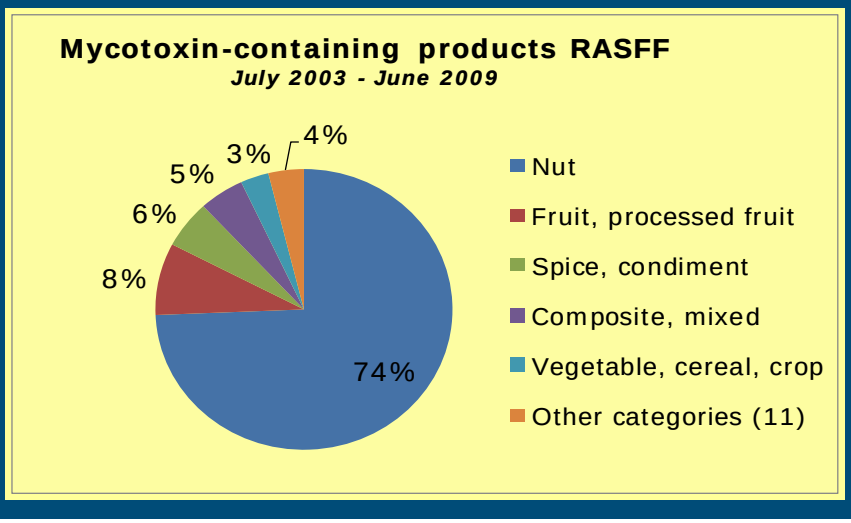
Adapted from Kleter 2010

Regulations - *EU Rapid alert system for food & feed*



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Challenges – *Analytical methods*

Current legislation: single compound chemical analysis

AOAC INTERNATIONAL

- US based, international involvement;
- Development and validation of methods of analysis and improvement of AQA;
- Approx. 45 mycotoxin methods in "OMA".

- European equivalent of ISO;
- Performance criteria approach, usually based on interlaboratory studies;
- 22 mycotoxin methods standardized;
- EU interlaboratory studied methods → CEN

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Challenges – *Analytical methods*

Single compound chemical analysis

Multiple compound chemical analysis

Single / multiple compound effect analysis

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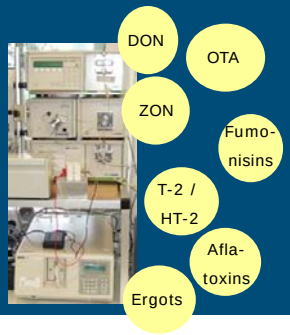
Challenges – *Analytical methods*

Single compound methods:

- Optimized extraction;
- SPE and/or IAC clean-up (derivatisation);
- LC-UV, LC-Flu, GC-ECD



Multi-compound methods:

- Generic extraction;
- No/limited clean up;
- Dilute and shoot;
- LC-MS/MS *(RIKILT 37 mycotoxins + 10 plant toxins)*

Aflatoxins
OTA DON
Fumonisins
ZON T-2/HT-2
Ergot alkaloids
Many other mycotoxins

Spanjer et al, Food Additives and Contaminants (2008), 25, 472;
Mol et al, Anal. Chem. (2008), 80, 9450;
Mol et al. Food Additives and Contaminants (2011) accepted

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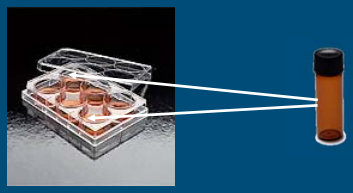
Challenges – *Effect analysis (1/2)*

In vitro testing: effects of mycotoxins on gene expression



1. Culture human Caco-2 cells
2. Seed cells; expose to compounds or sample extracts of interest
3. Isolate RNA from Caco-2 cells;
4. Conversion in cDNA, amplify cDNA, labelling;
5. Hybridisation, detection and data acquisition;
6. Data analysis.

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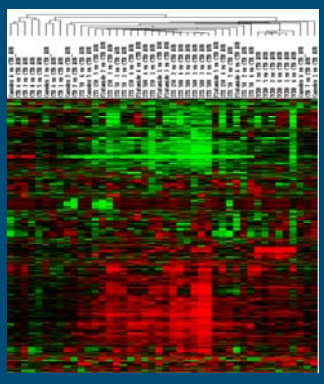
Challenges – *Effect analysis (2/2)*

Affected genes whole array
(44000 spots from 25000 genes)
- Red: up-regulated
- Green: down-regulated



Further method development, e.g.:

- Caco-2 cell line clones with luciferase expression when exposed to mycotoxins;
- Multiplex qRT-PCR on several specific up- and down-regulated genes;

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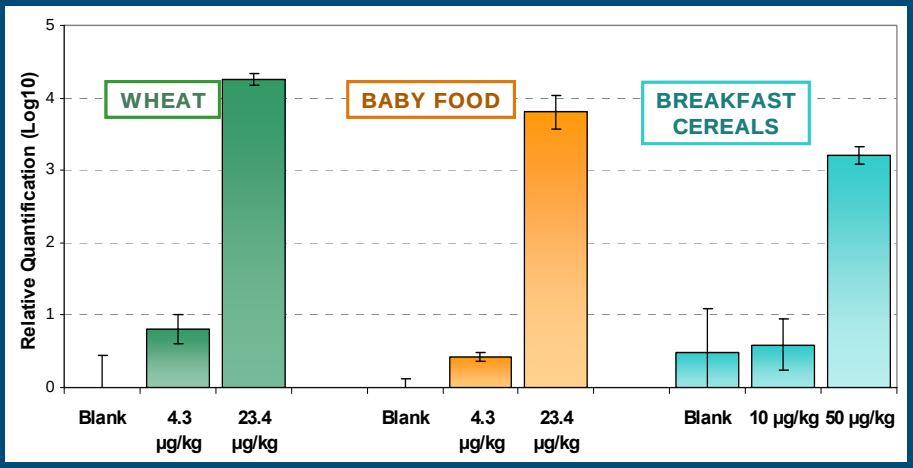
Challenges – *effect analysis – example transcriptomics assay for T-2*



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Lancova *et al* (2009). *World Mycotoxin Journal* 2, 247-257

BioCop

Challenges - *Emerging risks – New legislation*

Mycotoxin	Target date EFSA opinion
T-2 and HT-2 toxin	31 Dec 2011
Alternaria toxins	Oct 2011
Ergot alkaloids	2012
Beauvericin & enniatins	2012
Moniliformin	
Diacetoxyscirpenol	
Nivalenol	2012
Citrinin	2013
Sterigmatocystin	2013
Phomopsins	2013

http://www.efsa.europa.eu/en/topics/topic/mycotoxins.htm

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Challenges - *Emerging risks – Masked mycotoxins*

- First report 1985 by Miller JD & Young JC;
  (Deoxynivalenol in an experimental Fusarium graminearum *infection of wheat, Canadian Journal Plant Pathology, (1985), 7, 132-134);*
- Plant metabolises the mycotoxin into, compound that is harmless to the plant;
- Most known: deoxynivalenol-3-*β*-D-glucoside (D3G);
- Relevance:
  - Occurrence: D3G analyzed by LC MS/MS;
  - Toxicity:
    - Increased exposure by release in intestinal tract?
    - Absorbed by intestinal cells?

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Challenges - *Emerging risks – Climate changes*

- Expected: changes in range of latitudes where certain fungi are able to compete;
  - *Example:* F. graminearum *growth: NIV increase*
- Drought, flooding and other consequences of climate change may result in more mycotoxins and changed toxin profiles;
  - *Example: aflatoxins found in Italian cereals since 2003, and in other parts of Central Europe*
- Response of insects and plant diseases to climate change poorly understood, but increases expected.

Ine van der Fels-Klerx

10:00 hours

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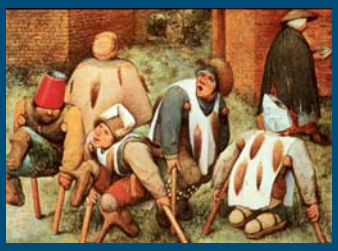
Challenges - *Emerging risks – Changing environment*



994: Holy fire lysergic acid deriv. *C. purpurea*

1568: Pieter Brueghel *The Cripples*
'Holy fire' by ergot alkaloids in **rye**
(gangrene followed by *necrosis / hallucinations*)

2011: Ergots in **cereals**; EFSA opinion expected in 2012
Regulatory limits on groups of toxins (in addition to ergot sclerotia)

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Challenges - *Emerging risks – Changing environment*

- Handicraft production / locally sourced food (locatarians):
  - Insufficient knowlegde on contamination routes:
    - New cultivars, susceptible cultivars;
    - Storage of harvested product;
  - Patulin in handicraft produced apple juice (2009): 18 of 42 samples > regulatory limit 50 µg/l *(adult)*
    (Gillard et al (2009) WMJ (1) 95-104)

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Challenges - *Emerging risks – Changing environment*

- (Re-)introduction of crops:
- Introduction of lupin as GMO-soy replacer: risk of phomopsin contamination (Australia & NZ 5 µg/kg)

- Increase area oats as healthy grain: no recent reports on occurrence of mycotoxins in oats in the Netherlands.

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Challenges - *Decontamination – Binders*

- In EU decontamination is not allowed;
- Mycotoxin binders are considered as feed additives *regulation (EC) No 1831/2003 of the European parliament and of the council on additives for use in animal nutrition);*
- Admission of the binder can only take place when efficacy is proven and no toxicity is detected. *regulation (EC) No No 429/2008 on detailed rules for the implementation of Regulation (EC) No 1831/2003 of the European parliament and of the Council as regards the preparation and the presentation of applications and the assessment and the authorisation of feed additives;*
- AND.... only when feed meets the EU limits on mycotoxins;

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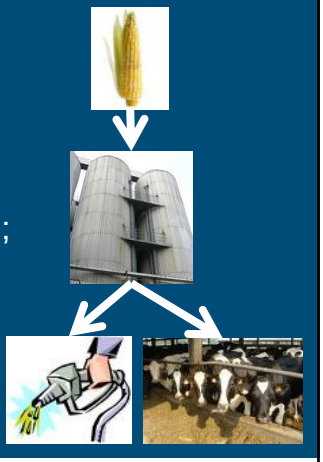
Challenges - *Bioethanol*

- Animal feed is by-product of bioethanol production
- Raw material quality defines feed quality:
  - Use of chemical plant protection;
  - Cultivars, monoculture in area;
  - GMO;
  - Storage of raw materials;
  - Drying of feed;



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Summary

- Legislation for more mycotoxins;
- Challenges due to climate and lifestyle changes;
- Further work on the relevance of masked mycotoxins;

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Mycotoxins

Past performance is no guarantee of future results!

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Thank you for your attention

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Thank you!

Questions

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