Aflatoxins

*an introduction*

Hans van Egmond, Jahorina, 4 April 2013
RIKILT – Institute of Food Safety

- Research institute within Wageningen University & Research
- History of > 100 years
- Located on the campus in Wageningen

- 200 staff, including PhD students and foreign guests
- Annual turnover 20 M€
- Main roles: statutory tasks and food safety research
Activities RIKILT (mostly for the government)

- Analysis
- Method development
- Research
- Advice & training

Themes
- Chemical contaminants
- Biological agents
- Veterinary drugs
- Feed
- Regulations
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52 years ago: “Turkey-X disease”
“We are fairly certain that this ‘toxic factor’ in groundnuts is not a new problem”

“We do not know the chemical composition of the ‘toxic factor’, but the source of toxicity is likely related to fungal contamination at a stage before processing”

“The ‘toxic factor’ is present in milk from cows fed with rations containing the toxic groundnut meal, and has shown to be a carcinogen”

“We think the whole problem is serious from a human and animal health point of view and from economic aspects”
Outline of presentation

- Introduction
- Risks and risk assessment
- Mycotoxin regulations
- European co-operation
- The combat of mycotoxins
- Summary
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Toxic effects of mycotoxins

- Carcinogenic
- Hepatotoxic
- Immunotoxic
- Nephrotoxic
- Neurotoxic
- Oestrogenic
- Teratogenic

Picture: courtesy Alain Pittet
Effects aflatoxin B₁

- Strong acute toxin in various animals
- Liver carcinogen in various experimental animals
- Possible causative role in human liver cancer in Asia and Africa
- Possibly associated with kwashiorkor
- Stunted growth in children, e.g. in West-Africa
- Kenya: recent outbreaks of aflatoxicosis
Human aflatoxicosis in Kenya, 2004

317 people in Kenyan villages got ill; 127 died, including many children

Photo: courtesy Henry Njapau and Masja Straetemans
Human aflatoxicosis in Kenya, 2004

- Village-grown maize suspected to play a causal role
- FDA emergency team confirmed hypothesis of human aflatoxicosis
- New outbreaks occurred in 2005, 2006 and in 2010

Photo: courtesy Henry Njapau and Masja Straetemans
Aflatoxins: pre- and post-harvest problems

- Produced primarily by some strains of *A. flavus* and almost all strains of *A. parasiticus*

- *A. flavus*: often in aerial parts of plants (leaves, flowers), dominant on maize, cotton seed, tree nuts (aflatoxins B$_1$, B$_2$)

- *A. parasiticus*: well adapted to a soil environment, prominent on peanuts (aflatoxins B$_1$, B$_2$, G$_1$ and G$_2$)
Chemical structures

aflatoxin B$_1$

aflatoxin M$_1$
Toxicity of aflatoxin M₁

- AFM₁ is hepatotoxic and carcinogenic
- IARC: aflatoxins (including AFM₁) are carcinogenic to humans (group I carcinogens)
- Carcinogenic potency one to two orders of magnitude less than AFB₁
- Uncertainties in risk characterization, due to limited studies and data
Aflatoxin M₁: the milk-aflatoxin

- Approx. 1-6 % of the aflatoxin B₁ in animal feedstuffs appears as aflatoxin M₁ in milk
- A seasonal trend is noted in many surveys, with lower levels occurring in summer months
- Dairy industry: common processes may lead to some AFM₁ redistribution, but no reduction
Risk Analysis Framework

Risk Assessment
- Hazard identification
- Hazard characterization
- Exposure assessment
- Risk characterization

Risk Management
- Assess policy alternatives
- Select and implement appropriate options

Risk Communication
- Interactive exchange of information and opinions

EFSA Journal

Official Journal of the European Union

(after WHO, 1998)
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Factors influencing mycotoxin regulations

- Hazard assessment
- Exposure assessment
- Availability of methods of sampling and analysis
- Trade contacts with other countries
- Sufficiency of food supply

\{ Risk assessment \}
Inquiries on mycotoxin regulations

- French, Spanish and Chinese translations available
Mycotoxin regulating countries in Europe

- 39 nations with known regulations (99 % of inhabitants of the region)
- EU harmonized limits exist for aflatoxins, ochratoxin A, patulin, DON, zearalenone, fumonisins
- EU indicative levels expected for T-2/HT-2 and ergot alkaloids
- EU feed limits exist for aflatoxin B₁
- EU feed guidance values exist for ochratoxin A and some *F.* toxins
Aflatoxin $M_1$ in milk

- 15 µg/kg: 1 country
- 5 µg/kg: 1 country
- 0.5 µg/kg: 19 countries
- 0.2 µg/kg: 1 country
- 0.05 µg/kg: 37 countries
- Not detectable: 1 country
Aflatoxin $B_1$ in feed for dairy cattle

- 50 µg/kg: 2 countries
- 25 µg/kg: 1 country
- 20 µg/kg: 3 countries
- 15 µg/kg: 1 country
- 10 µg/kg: 5 countries
- 5 µg/kg: 32 countries

Number of countries

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Number of countries
Examples of European collaboration

- Rapid Alert System for Food and Feed
- EFSA: risk assessments
- CEN: the European equivalent of ISO
- EU-RL for mycotoxins
- European research and networking projects
Rapid Alert System for Food and Feed

- Quick information-exchange in the EU on risks to human health
- Allows MS to identify potential problems and take measures
- In 2011: 631 mycotoxin issues
RASFF 2011: Border rejection notifications

Border rejection notifications 2011 (hazard category)

- Mycotoxins: 27%
- Pesticide residues: 12%
- Rest: 40%
- Poor or insufficient controls: 9%
- Foreign bodies: 6%
- Pathogenic microorganisms: 6%
EFSA and its mission

- EFSA: the EU’s scientific risk assessment body on food and feed safety, nutrition, animal health and welfare, and plant health and protection.
- EFSA panels, in cooperation with WGs, adopt scientific opinions on mycotoxins in food and feed.
- Opinions published on website and in EFSA journal.
CEN = Comité Européen de Normalisation

CEN standardizes mycotoxin methods

Performance criteria approach, usually based on collaborative studies

Most methods also approved by AOAC

22 CEN methods for aflatoxins, ochratoxin A, fumonisins, patulin and trichothecenes in foods
EU-RL and NRLs on mycotoxins

EC

EU-RL

27 NRLs

>> RFLs
Tasks of the EU-RL

- Provide NRLs with methods
- Coordinate comparative testing
- Organise training courses
- Technical assistance to the Commission
- Collaborate with labs in third countries
LCP Food, Agriculture and Fisheries, and Biotechnology, 2009-2013

“Reduction of mycotoxins in food and feed chains”

Focus on AFL, OTA, TCT, ZEA en FUM

Research and organisation of workshops and conferences (Europe, Asia, Africa, Latin America)

Research examples: screening hybrids of maize for *A. flavus* resistance and fungicides evaluation
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What to do against mycotoxins?

- Prevention: plant resistance, fungicides, insect control
- Irrigation under conditions of drought stress
- Biocontrol: biocompetetive exclusion
- Development of predictive models
- Post-harvest: rapid drying, cool storage
- Hygienic conditions at storage
- HACCP in production chains
- Development and application of decontamination systems
- Application of state-of-art methods to detect fungi
- State-of-art methods for sampling and analysis of toxins
- Enforcement of regulations and continuous monitoring
- Training, permanent education and networking
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Summary and conclusions

- Aflatoxins: problems of the past and the present
- Risk assessment: major ingredient for regulations
- More than 100 countries have aflatoxin limits
- EU: detailed and stringent regulations
- Strong European co-operation: get involved!
- Possibilities exist to combat the mycotoxin problem
Aflatoxins

an introduction

Hans van Egmond, Jahorina, 4 April 2013
Mark your calendars for upcoming events

35th MYCOTOXIN WORKSHOP
GHENT, BELGIUM, MAY 22-24, 2013
in cooperation with the Gesellschaft für Mykotoxinforschung
(Society for Mycotoxin Research)

27 - 31 May 2013
Martina Franca - Italy

2014

THE World Mycotoxin Forum®
CONFERENCE
NOVEMBER

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MEETS
IUPAC
2016

Singapore

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Research needs

- Better understanding of precise impact of climate change on insects and other pests
- Investigate consequences on soil characteristics: very relevant for fungal infestation
- Stimulate research from local to global scale to better understand the impact of climate change
- EMTOX project: one of the objectives: Quantify relationship between climate change and toxins
- Monitoring of mycotoxin occurrence, to observe possible changes in toxin profiles and levels
Dose response data for the critical effect

Response

Uncertainty factors

Threshold

TDI

NOAEL

Dose in mg/kg body weight

100

1000

(1) HAZARD IDENTIFICATION

(2) HAZARD CHARACTERISATION

(3) EXPOSURE ASSESSMENT

(4) RISK CHARACTERIZATION

(after M. Eskola, 2011)
Newer concept: Benchmark dose (BMD)

- Used for both thresholded and non-thresholded compounds
- Modelling benchmark dose (BMD) for 10% extra risk of the critical effect
- $\text{BMDL}_{10} = 95\%$ lower confidence limit of the BMD for 10% extra risk of the critical effect

(after M. Eskola, 2011)
Aflatoxin $M_1$: recent issues

- Recent problems in Europe: $AFB_1$ in maize leads to $AFM_1$ in milk
- Dairy farmers protest at Vojvodina Executive Council: milk dumped in the streets
- Aflatoxin affair spread to Germany and the Netherlands
- RIKILT involved in analyses of milk and maize for the Serbian authorities
- Problem of emerging aflatoxins in south-east Europe possibly due to climate change
Risk Analysis Framework

Risk Assessment

• Hazard identification
• Hazard characterization
• Exposure assessment
• Risk characterization
Risk Assessment Process

- **Hazard identification** – Establish that a substance has the apparent capacity to cause an adverse effect
- **Hazard characterization** – Assess the relationship between level of exposure, and severity of an effect
- **Exposure assessment** – Estimate the level, to which various individuals or populations are exposed
- **Risk characterization** – Assess human exposure in relation to the health-based guidance value
- For substances both genotoxic and carcinogenic: MOE approach can be used to assess risks
Safety evaluation of AFM$_1$ by JECFA, 2001
JECFA’s evaluation of AFM$_1$

- Requested by CODEX (CCFAC)
- TOR: “Examine exposure to AFM$_1$ and to conduct a quantitative risk assessment” to compare 2 standards for contamination of milk: 0.05 μg/kg and 0.5 μg/kg.
- With worst case assumptions, projected risks for liver cancer very small in both cases
- No significant health benefit, when a 0.5 μg/kg limit would be reduced to a 0.05 μg/kg limit
Factors influencing mycotoxin regulations

- Hazard assessment
- Exposure assessment
- Availability of methods of sampling and analysis

\[
\text{Risk assessment}
\]
Methodology for aflatoxin $M_1$ in milk

- TLC: CHCl$_3$ extractions, SiO$_2$ cleanup, 2dim. TLC
- HPLC: defatting, IA cleanup, C18 LC-FLD
- Immunoassays: ELISA, LFD
- LS-MS/MS
- Performance characteristics: CEN, EU
- Methods available by AOAC, CEN, IDF
- Milk powder CRMs for AQA: JRC-IRMM Geel
Aflatoxin M$_1$ in dairy products

- Milk: higher carry-over rate at lower AFB$_1$ concentration in feed, and at higher milk yield
- Dairy industry: common processes do not lead to AFM$_1$ losses:
  - no influence of pasteurization and sterilization
  - concentration in cheese (4x) and milk powder (8x)
- AFM$_1$ “well under control” in EU countries
The mycotoxin regulatory puzzle
Weighing the various factors: not trivial
Europe: mycotoxins regulated in food

- AFT
- AFB1
- AFB1/G1
- AFM1
- OTA
- PAT
- DON
- T-2
- ZEN
- FUMB1/2
- STE

number of countries

- 0
- 5
- 10
- 15
- 20
- 25
- 30
- 35
- 40
Europe: mycotoxins regulated in feed

- FUM
- ZEN
- DAS
- T-2
- DON
- OTA
- AFB1
- AFT

The diagram shows the number of countries regulating different mycotoxins in feed. The mycotoxins listed are FUM, ZEN, DAS, T-2, DON, OTA, AFB1, and AFT. The y-axis represents the mycotoxins, and the x-axis represents the number of countries.
Aflatoxin $B_1$ in food

- 20 µg/kg: 3 countries
- 15 µg/kg: 2 countries
- 10 µg/kg: 4 countries
- 5 µg/kg: 19 countries
- 2 µg/kg: 32 countries
- 1 µg/kg: 1 country
Total aflatoxins in food

- 35 µg/kg: 2 countries
- 30 µg/kg: 3 countries
- 20 µg/kg: 17 countries
- 15 µg/kg: 8 countries
- 10 µg/kg: 8 countries
- 5 µg/kg: 3 countries
- 4 µg/kg: 32 countries
- 3 µg/kg: 1 country
- 1 µg/kg: 3 countries
- 0 µg/kg: 1 country
RASFF trend analysis: mycotoxin reports

Specific mycotoxin hazards
RASFF, July 2003 - June 2009

Adapted from Kleter 2010

- Aflatoxins: 95%
- Ochratoxin A: 4%
- Fumonisins: 1%
- Deoxynivalenol: 0%
- Patulin: 0%
- Zearalenone: 0%