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Aflatoxins Control in Foods

Food Industry experience

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Nestle Quality Policy

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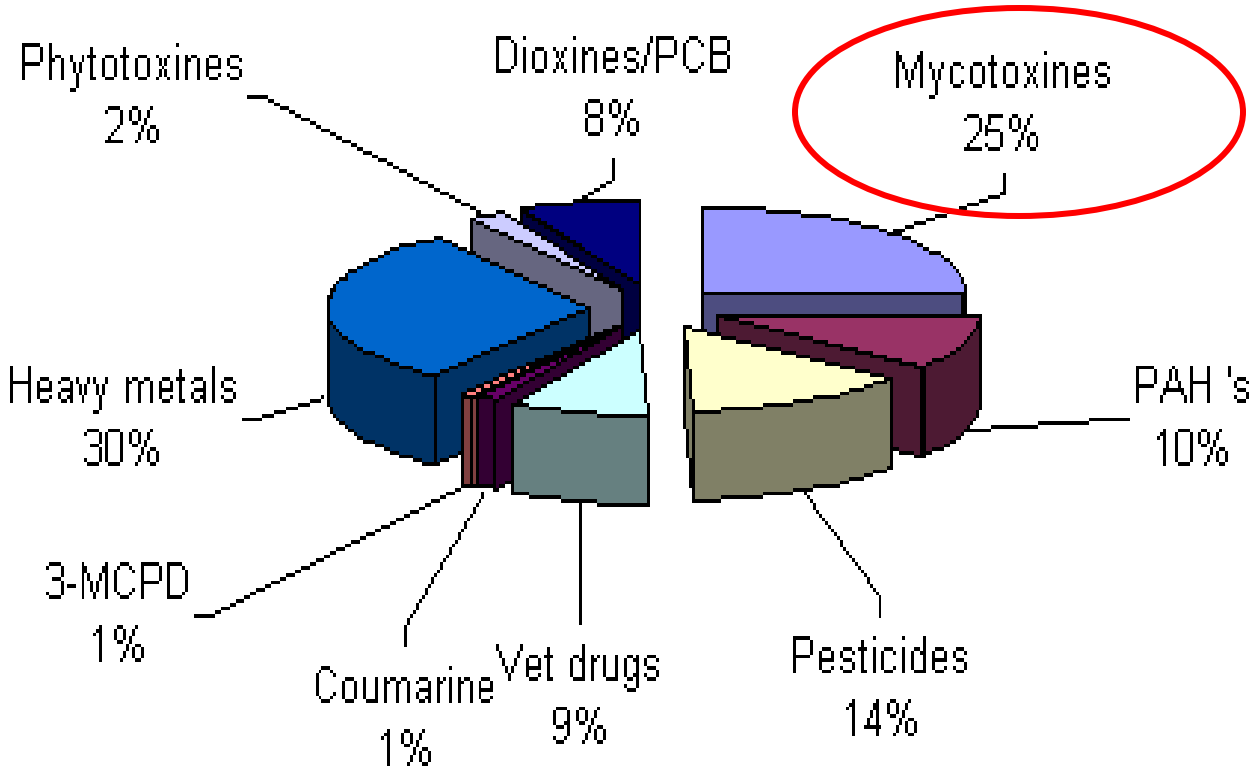
Outline

- E.U Alerts on Mycotoxins
- Major Mycotoxins / Aflatoxins of concern
- Management of the Upstream
- Management at Factory level
- Analytics on Mycotoxins
- Regulations

EU Chemical Alerts 2007



EU Chemical Alerts 2007

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Evolution 2006/2007	2007	2006
Mycotoxines	73	71
PAH's	30	27
Pesticides	40	21
Vet drugs	25	40
Coumarine	4	0
3-MCPD	2	2
Heavy metals	88	56
tox.marines	6	20
Dioxines/PCB's	22	6

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In summary:
6 major chemical types of mycotoxins

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Mycotoxins	Main Producing Fungi
Aflatoxins B_1, B_2, G_1, G_2	*Aspergillus flavus, A. parasiticus, A. nomius*
Ochratoxin A	*Penicillium verrucosum, A. alutaceus, A.carbonarius*
Patulin	*P. expansum, A. clavatus, Byssochlamys nivea*
Fumonisins	*Fusarium moniliforme, F. proliferatum*
Deoxynivalenol (trichothecenes)	*F. graminearum, F. culmorum, F. crookwellense*
Zearalenone	*F. graminearum, F. culmorum, F. crookwellense*

Mycotoxins occurrence at a glance….

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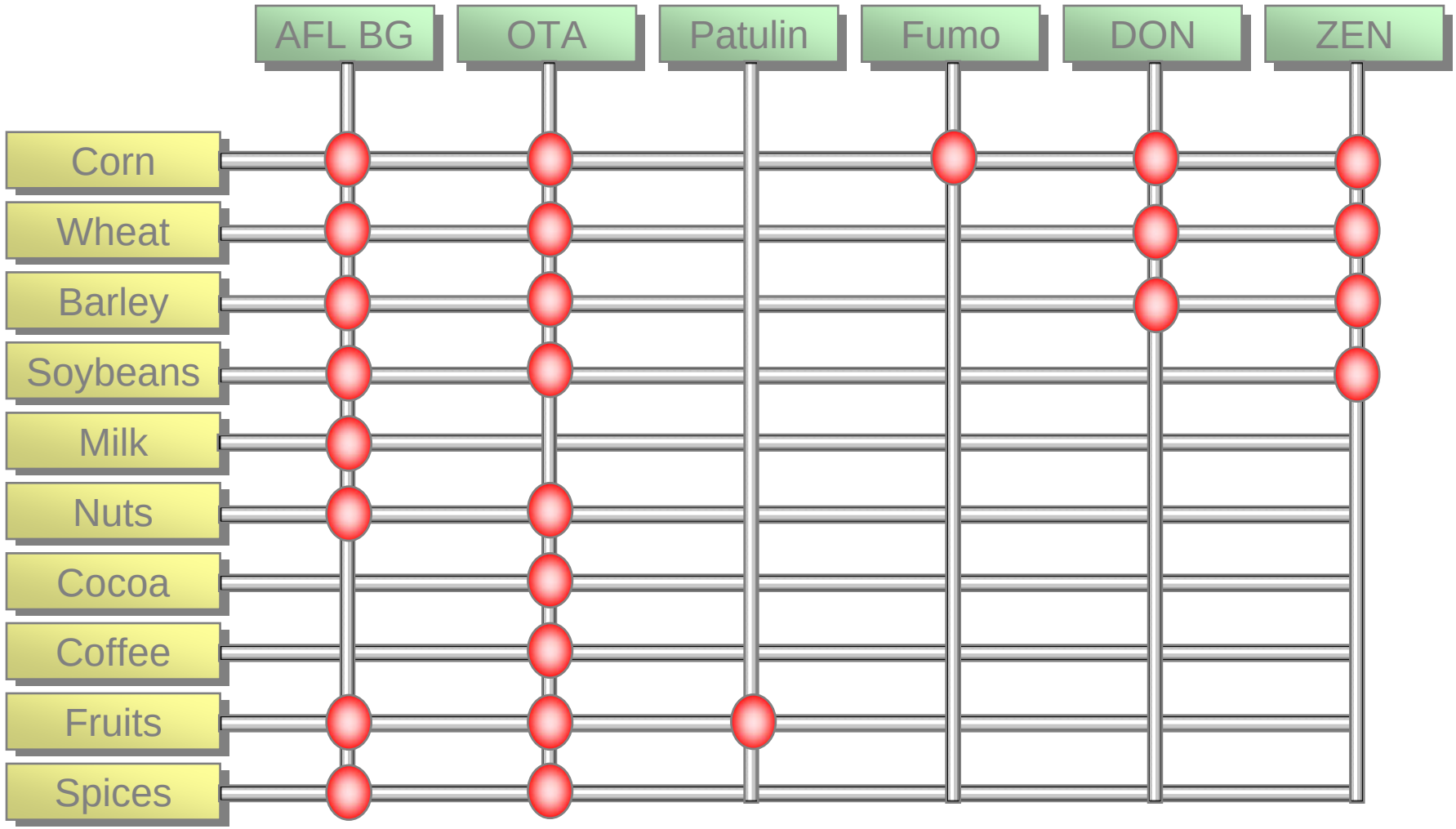

Natural occurrence of aflatoxins

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high risk	moderate risk	low risk
• Corn • Peanuts • Cottonseed • Brazil nuts • Pistachio nuts • Copra	• Figs • Almonds • Pecans • Walnuts • Sultanas • Spices	• Soybeans • Pulses • Sorghum • Millet • Wheat • Oats • Barley • Rice

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Routes of chemical contamination in plant-based foods

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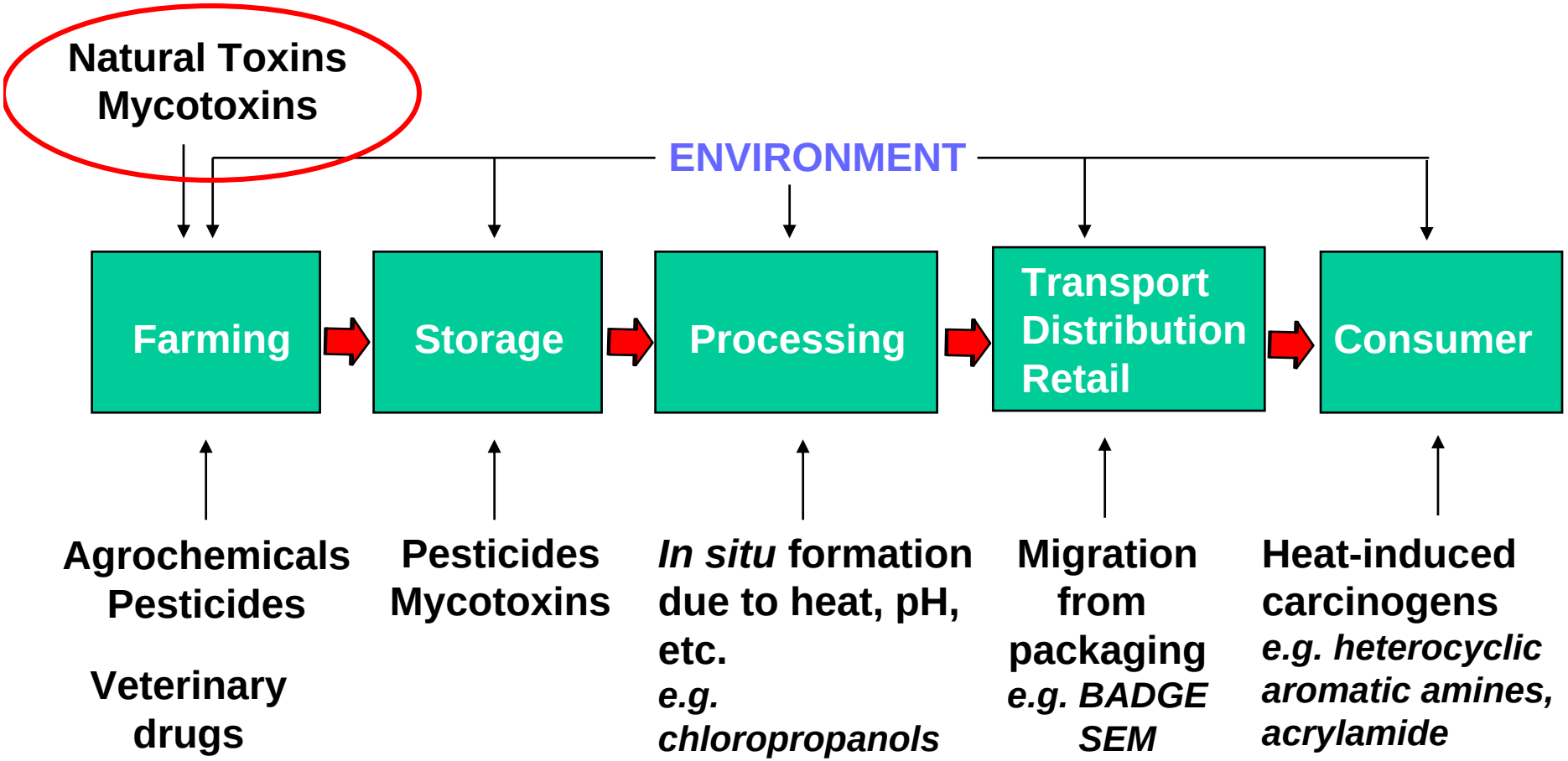

Routes for Contamination and Controls

CONTRIBUTING FACTORS

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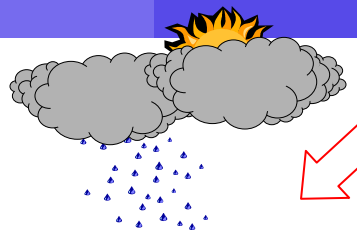
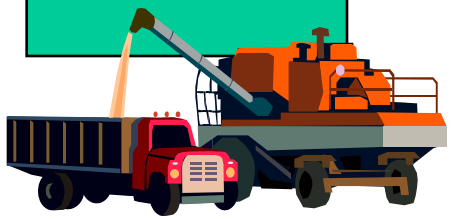


Pre- Harvest

- Weather
- Insect infestation
- Drought
- High Moisture
- Plant varieties

Harvesting



- Late Harvest
- Temperature
- Humidity
- Mechanical damage

Storage

- Temperature /Humidity
- Cleaning (previous stock)
- Roofing / Walls
- Ventilation
- Infestation

Routes for Contamination and Controls

Control factors

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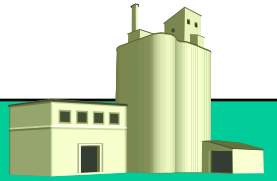
Pre- Harvest

Pest Control
Crop rotation
Irrigation
Plant Resistant varieties
Control of diseases

Harvesting

Timeliness
Clean-up
Drying

Storage



Sorting
Temperature
Relative Humidity

Routes for Contamination and Controls
GOOD PRACTICES

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- Pre- harvest:
  - Avoid stress to the crop during grain filling and maturation
  - Minimise insect, disease and mechanical damage to the grain
- Harvest
  - Harvest grain at full maturity and low moisture content
  - Avoid mechanical damage to the grain
- Drying
  - Dry the grain to 14% moisture as rapidly as possible

Routes for Contamination and Controls
GOOD PRACTICES

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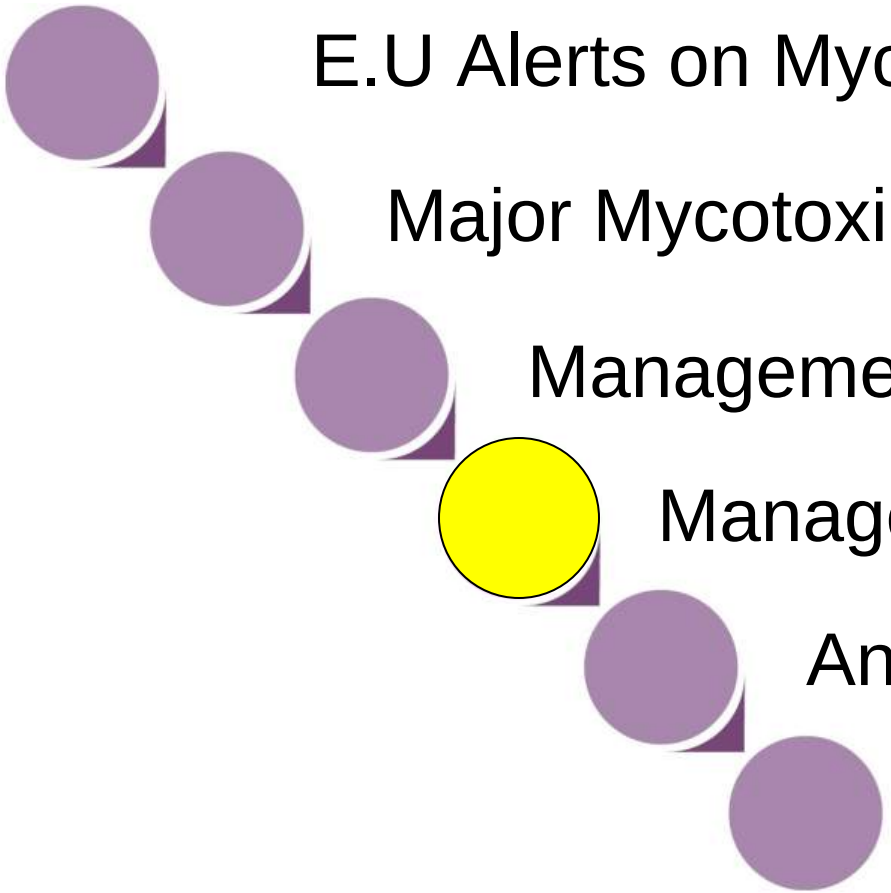
- Storage (Main area of risk)
  - Use only storage which are ventilated and are protected from rain and moisture.
  - Store at low temperature and monitor temperature continually in order to identify “hot spots”
- Transport
  - Ensure that transport machinery and containers are well cleaned
  - Avoid transporting over long distances (time) without temperature control systems

Outline



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Management at Factory level Issues

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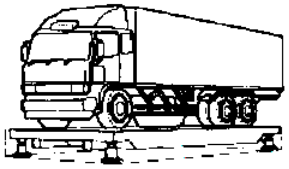
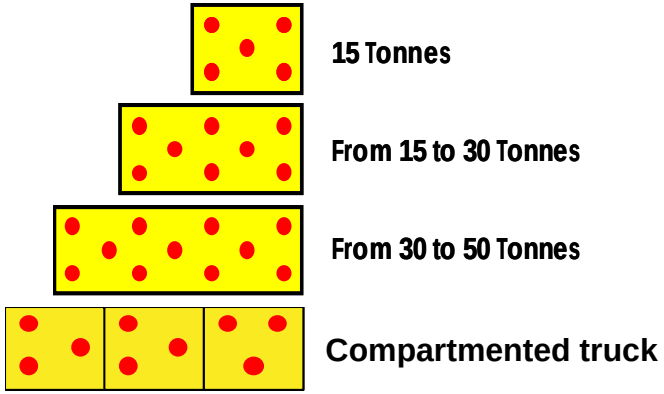
Aflatoxin contamination is very heterogeneous

Example: truck containing 20 µg/kg aflatoxin contaminated corn:

Sample Size	Kernels (estimate)	Variability (µg/kg)
4.54 kg (10 lbs)	30000	11.6-28.4
2.27 kg (5 lbs)	15000	8.1-31.9
1.13 kg (2.5 lbs)	7500	3.2-32.8
0.45 kg (1 lbs)	3000	1-40

Sampling procedure to obtain representative samples especially in nuts, dried fruits, grains are critical





Management at Factory level

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What does it mean?

Sampling is usually the largest source of variability associated with the mycotoxin test procedure.

Because of this variability, the true mycotoxin concentration in the lot cannot be determined with 100% certainty.

Correct decision (bulk lot) can only be made if the **mycotoxin concentration** in the lot can be determined with high degree of **accuracy and precision.**

Management at Factory level

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The preparation of representative samples is key for Quality Assurance procedures

Example of sampling in a non compartmented truck

For raw materials delivered in bulk, the sampling must be done from the top to the bottom of the truck with dedicated equipment such as a double tube probe

Proper grinding increases the chances of detecting contaminated particles.

Management at Factory level

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Number of incremental samples for lots of less than 15 tons

Lot weight (Tons)	No. of incremental samples
< 0,1	10
0,1 - < 0,2	15
0,2 - < 0,5	20
0,5 - < 1,0	30
1,0 - < 2,0	40
2,0 - < 5,0	60
5,0 - < 10,0	80
10,0 - < 15,0	100

Remark: For cereal lots under 15 tons, a sampling plan consisting of, depending on the lot weight, with incremental samples, resulting in an aggregate sample of 1 to 10 kg may be used.

Management at Factory level

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Number of incremental samples to be taken depending on the weight of the lot of cereals ≤ 50 Tons

Lot Weight (Tons)	No. of incremental samples
≤1	10
> 1 and ≤ 3	20
> 3 and ≤ 10	40
> 10 and ≤ 20	60
> 20 and ≤50	100

Remark: For cereal lots under 50 tons, a sampling plan consisting of, depending on the lot weight, 10 to 100 incremental samples each of 100 grams, resulting in an aggregate sample of 1 to 10 kg may be used.

Safety Risk Vs Supply Strategy assessment

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High

Raw Material Quality / Safety Risk

Low

Securing Supply

- Work with preferred suppliers
- Leverage volume
- Process improvement – collaborative planning
- Exchange of technical competence
- Seasonal contract

Partnership Contract growing

- Look for opportunities to standardise or substitute inputs
- Where possible broaden supplier market
- Focus on demand management
- Develop suppliers
- Long term commitment

Competition Supplier development

- Best price analysis
- Leverage price and volume in the (open) market

Tactical

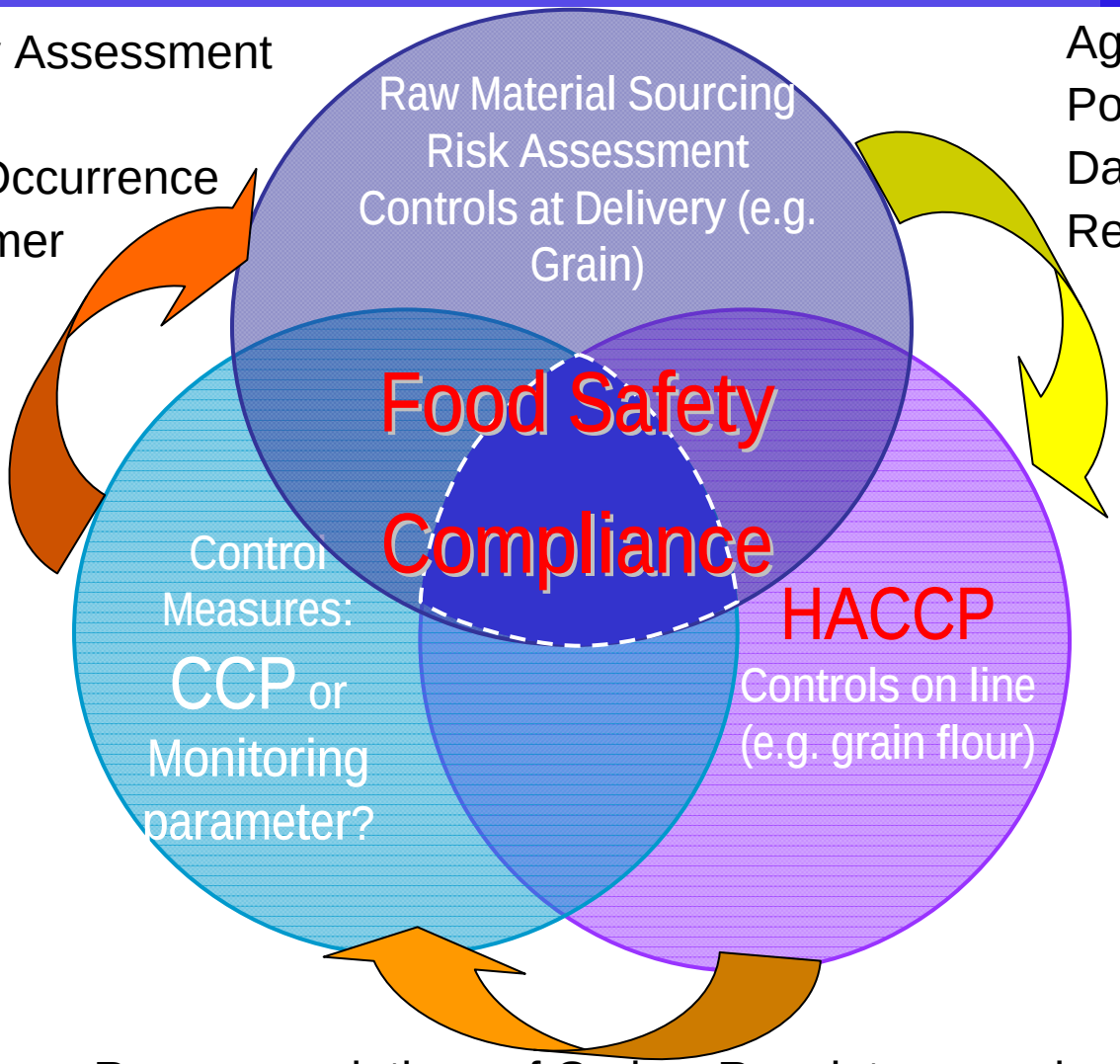
- Best price analysis
- Transaction efficiency
- Broaden supplier range
- Exploit competition between suppliers where possible
- Annual tender

Low — **Supply risk** (Need / availability) — High

Management at Factory level

Supply Strategy Assessment based on:
- Likelihood of Occurrence
- Target Consumer

Agricultural Practices
Potential Occurrence
Data from Surveys
Regulation



Recommendations of Codex, Regulatory requirements
Significance of Hazard, Due Diligence

Management at Factory level
Example of HACCP

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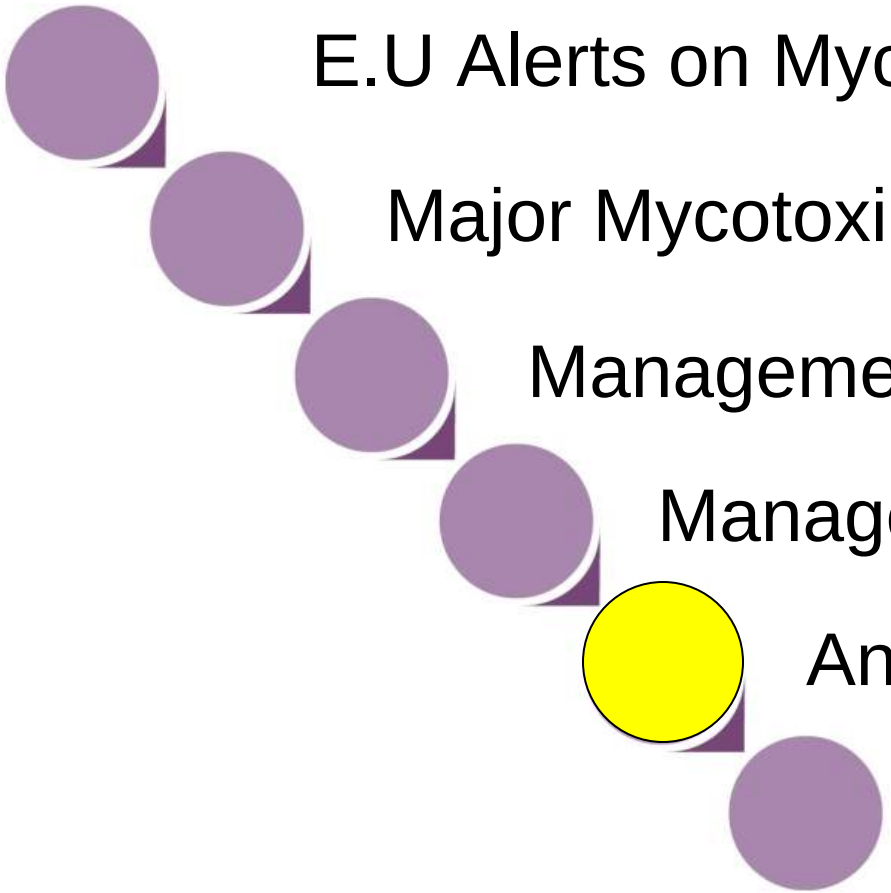
Hazard	Control Measure	Monitoring	Verification
Aflatoxin Presence in pistachios above acceptable level	Use of reliable, approved suppliers. Agreed specifications with supplier.	Check on COA that aflatoxin is within specifications for each delivery.	Testing for mycotoxins at reception (frequency based on risk and supplier confidence).

Outline



- E.U Alerts on Mycotoxins
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Analytics is fundamental to support the quality of the products

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The two facets of the Analytics

- Sophisticated techniques to characterize ingredients

but also….

- Rapid tests for use in factories and routine laboratories

Analytics is fundamental to support the quality of the products

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When establishing a release control or a surveillance plan, particular attention should be given to:

- **Analytical capabilities Vs regulatory requirement.**
- **Use of accredited reliable analytical methods**
- **Quick turn-around time from sample to result**
- **Broad scope of analytical groups**

Lateral flow test (dipstick)

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Assay Procedure

Pipette 50µL of assay buffer into each conjugate well

Add 50µL of sample extract into each well and mix *

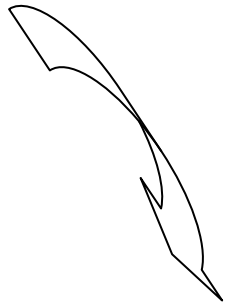
Put one strip into one well, start timing

Incubate for 5 minutes

Interpret results:

- Negative results are confirmed as soon as two lines appear – within 1 minute (20ppb cutoff kit only).
- Positive results are confirmed after exactly 5 minutes.

AgraStrip™ from Romer Labs, cutoff level at 4, 10 or 20 ppb total aflatoxins



Results Interpretation

* (10 g of sample extracted with 20 mL methanol/water (70:30))

Lateral flow test (dipstick)

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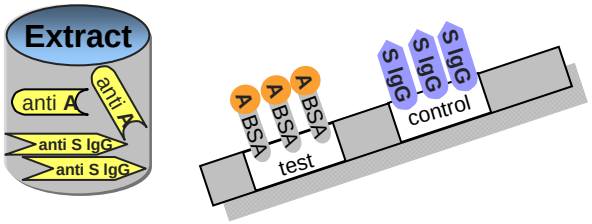
EXAMPLE: LI-03.015 total aflatoxins BG (AgraStrip dipstick assay from Romer Labs)



1) **Test Equipment:**

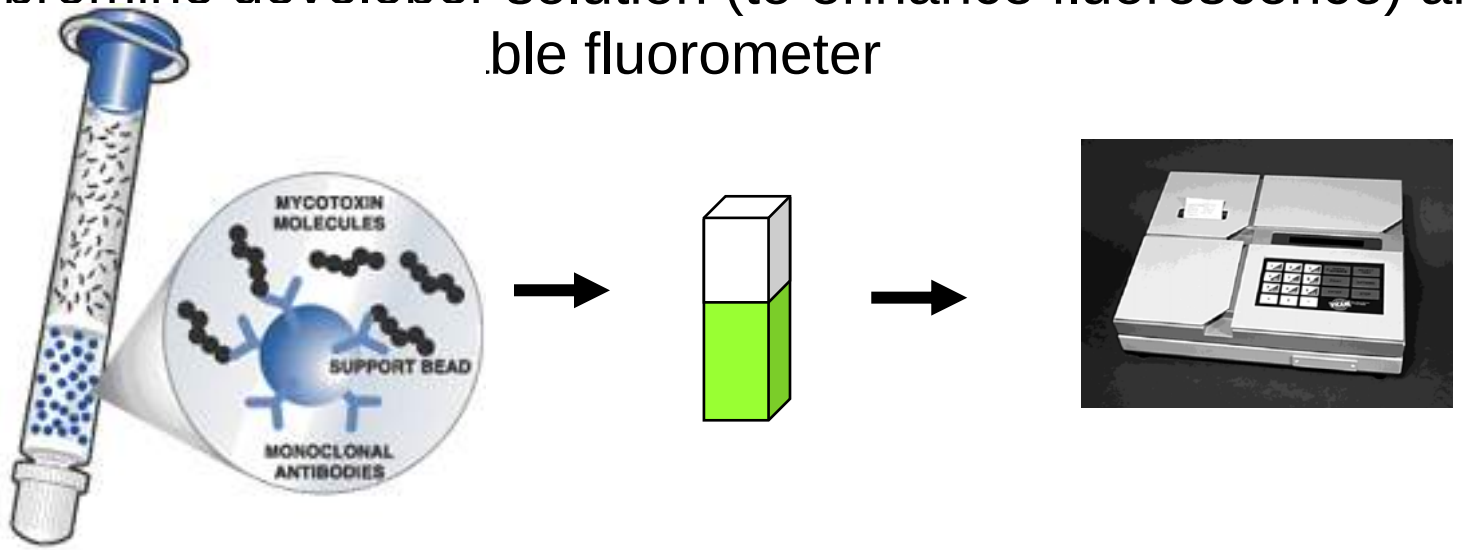
- Gold-labelled anti-aflatoxin antibodies anti A and gold-labelled anti-swine lgG antibodies anti S IgG
- Test zone with immobilized aflatoxin-BSA A BSA conjugate, control zone with immobilized swine lgG (S lgG). S IgG

Rapid Methods: Solution fluorometry

EXAMPLE: LI-00.123 total aflatoxins BG

- Clean-up of sample extract over immunoaffinity columns (mycotoxins are bound to antibodies)
- Mycotoxins are eluted in a cuvette, derivatized by adding a bromine developer solution (to enhance fluorescence) and

ble fluorometer



Promising rapid method: FLORIDA immunoassay

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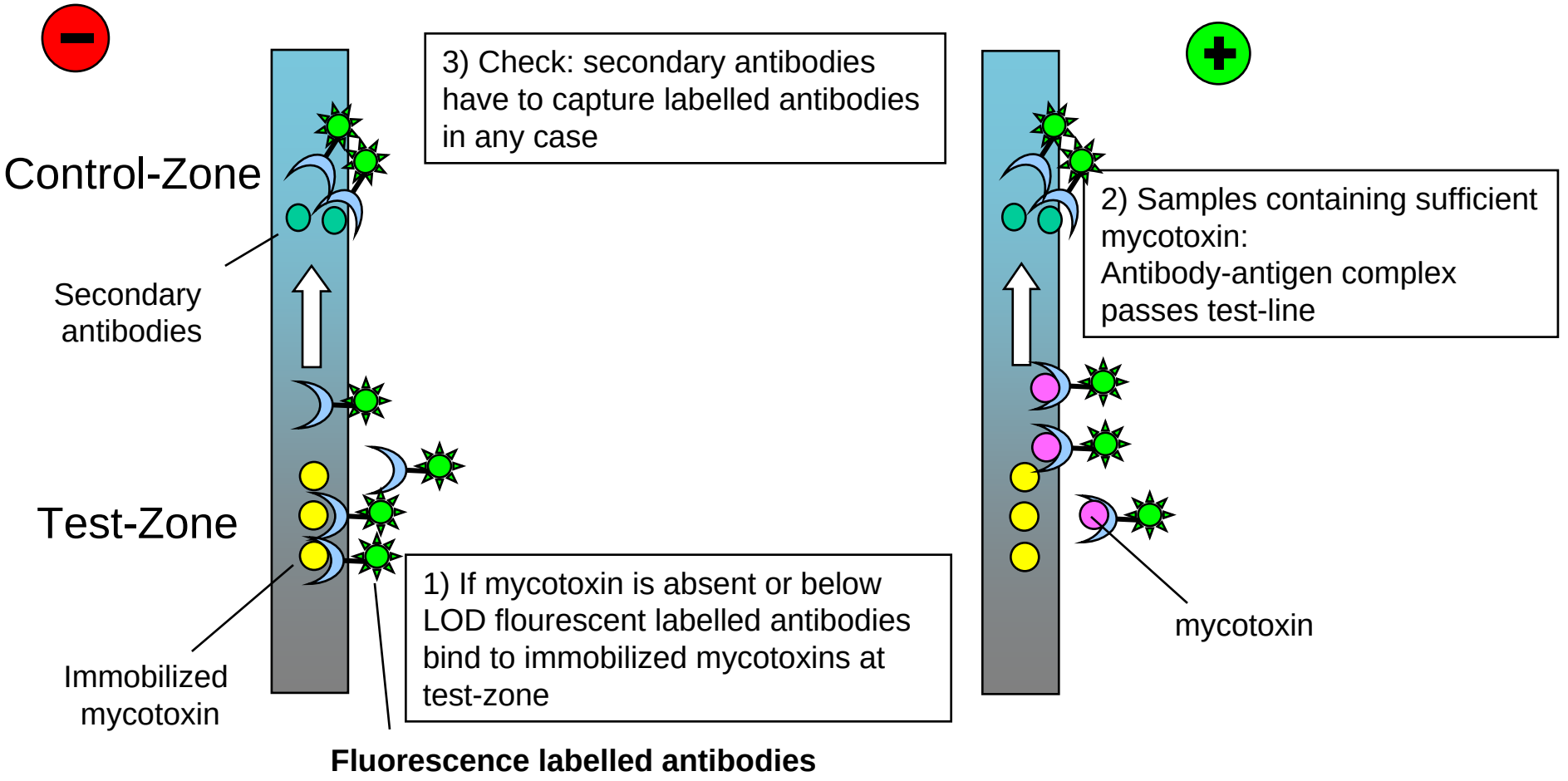
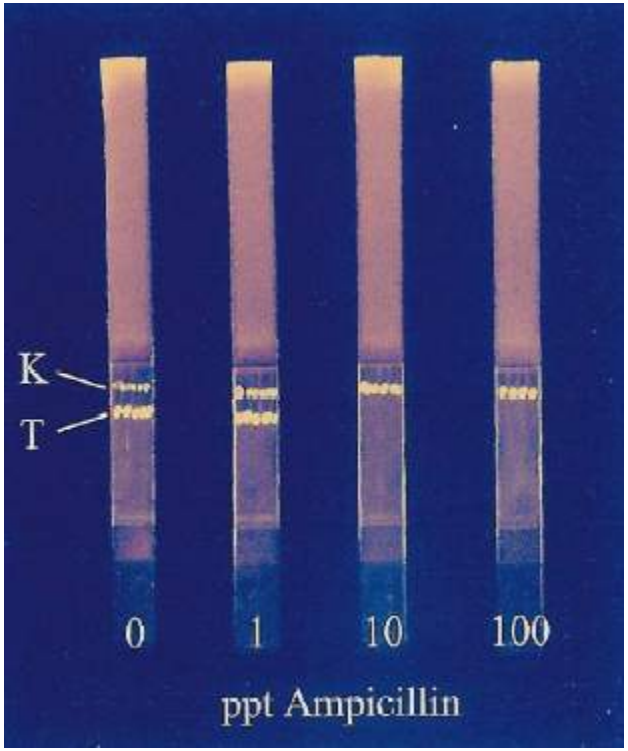
Fluorescence Labelled Optical-Read Immuno Dipstick Assay



Promising rapid method: FLORIDA immunoassay

Fluorescence Labelled Optical-Read Immuno Dipstick Assay



- **extremely sensitive**
  - cut-off values in the range of 10 ppt
  - increase of sensitivity up to 3 orders of magnitude
- **simple and rapid**
  - lateral flow dipstick assay
  - visually detectable signals
- **field method**
  - fluorophores excited with handheld lamp
  - bright signals even under subdued lighting

Bonenberger J., Hagenmaier S. and Polackova J.
Poster presented at "Rapid Methods Europe 2005", Noordwijk aan Zee, The Netherlands, 24-25 May 2005

Promising rapid method: Fluorescence polarization

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Applications

- aflatoxins, OTA, DON, fumonisins, ZEA

Advantages

- FP is a solution-phase assay:

  - no need to attach the antibody to a solid surface
  - no need to separate the “free” and “antibody-bound” toxin

Limitations

- Positive bias of 20 to 30% when compared with HPLC data
  - from matrix effects
  - from cross reactivities
- Not a high throughput method (samples need to be analyzed serially not as a batch)

Promising rapid method: Fluorescence polarization

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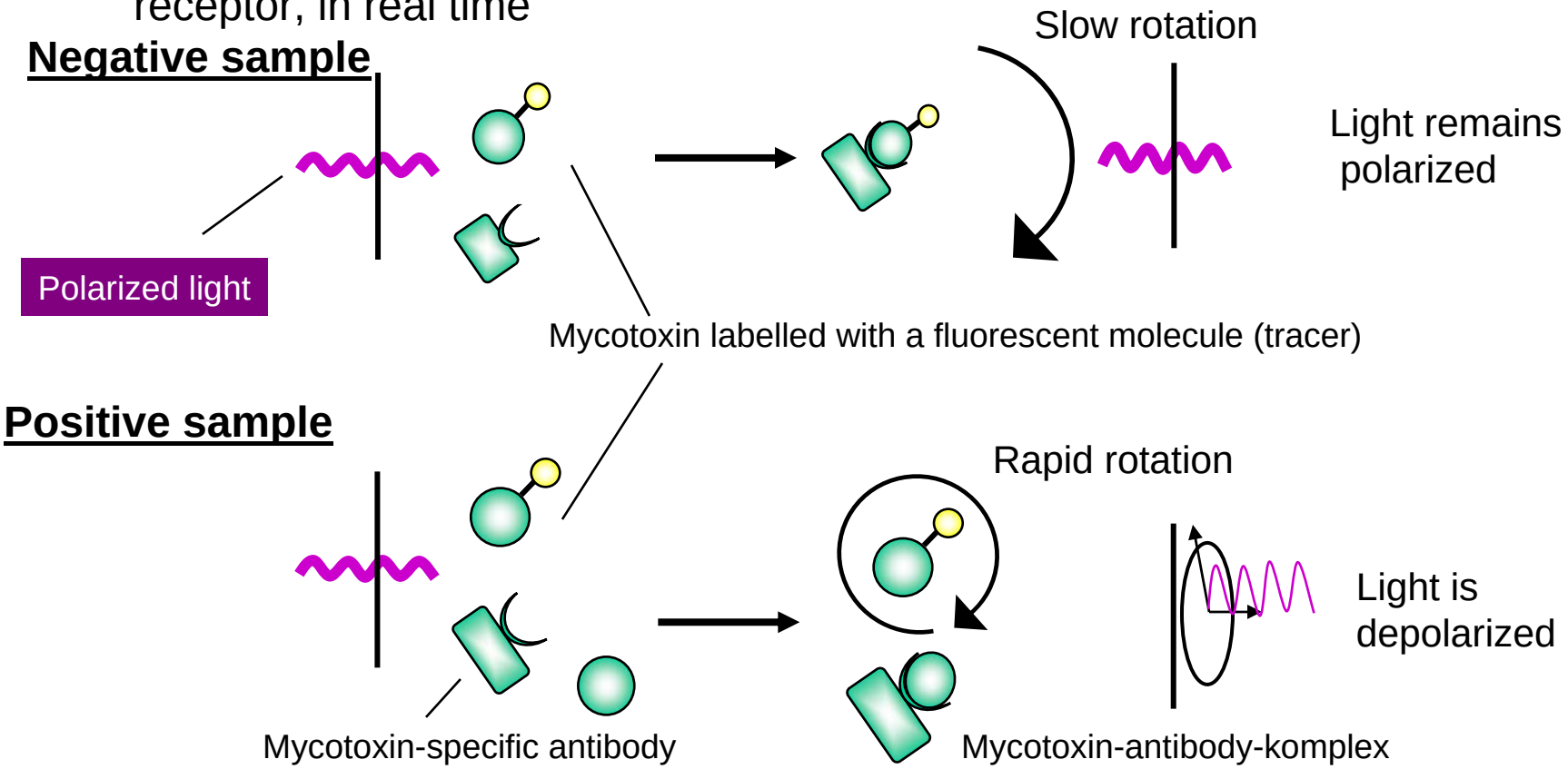
- **BACKGROUND**: All molecules in solution rotate; small molecules have higher rates of rotation and lower polarization than larger molecules
- **PRINCIPLE**: Monitor a change in the size of a mycotoxin-fluorophore conjugate (tracer) and hence detect its binding to a larger one, such as an antibody or receptor, in real time



Future trends in Mycotoxin analysis

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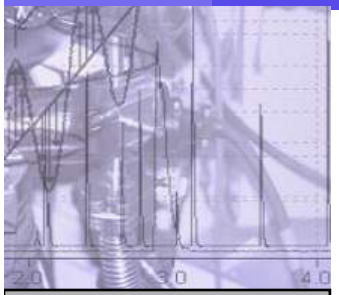

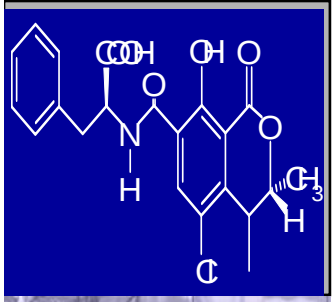

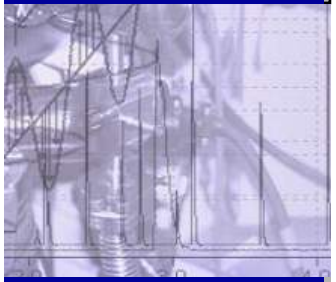

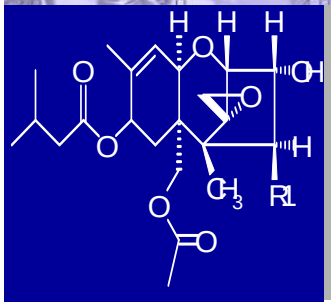

Rapid Screening Methods

Complemented by

Classical Confirmatory Methods (HPLC / UPLC)

Emerging approaches

LC-MS/MS
for high-throughput multi-toxin analysis

TIME

Multi residue method for mycotoxins based on LC-MS/MS

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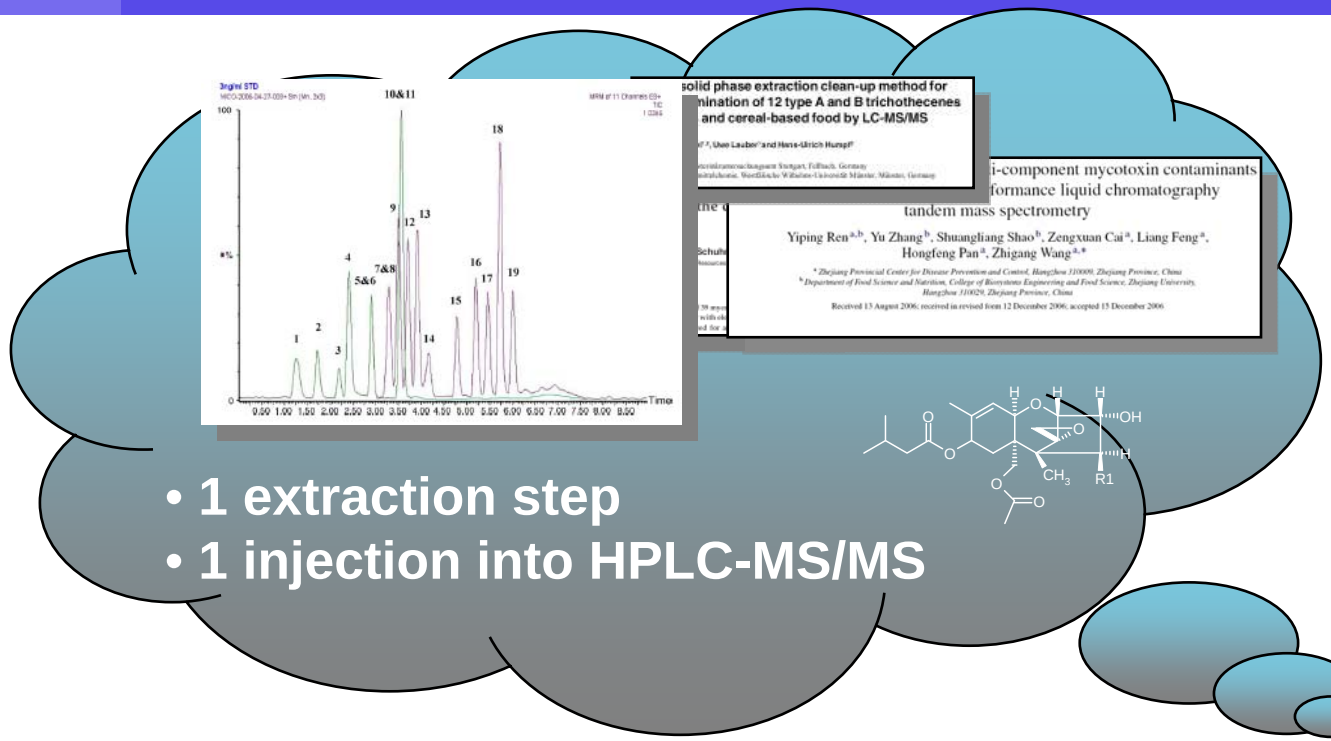

- 1 extraction step
- 1 injection into HPLC-MS/MS

Challenges:

- complexity of sample matrices
- diversity of analyte polarities
- different ionization capabilities

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REGULATION MYCOTOXINS

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To keep exposure to mycotoxins under control:

➔ Specific limits and regulations have been established in many countries

➔ Codex Alimentarius recommendations are referred to in the absence of national food legislation

➔ For raw materials at risk, Nestle has established internal limits which are based on Codex Alimentarius recommendations at the minimum for setting of critical limits in the context of HACCP plans or Raw materials specifications

REGULATION for MYCOTOXINS

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- EC Regulation 401/2006 (23/02/06), codifying EC sampling Directives for Afla., OTA, Patuline and Fusarium Toxins- Application 01/07/07

- EC Regulation 1126/2007, amending EC Regulation 1881/2006 :
  - limits for Fumonisins in maize products, including infant products (200 ppb)
  - ZEN, limits at 400 ppb for maize oil (instead of 200 ppb)
  - T2/HT2 not yet regulated
  - Application 01/07/2007

REGULATION for MYCOTOXINS

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- EC Decision 2007/563-aflatoxins- Amendment EC decision 2006/504, special conditions on the import into the EU of the almonds from the USA–Sampling according to Reg. 401/2006-Application 01/09/2007

- Meeting between European Commission and stakeholders concerning **T2/HT2**. **Limits to be fixed likely in 2009**.

- GI 31.019 – Guidelines on the management of mycotoxins in food (03/07)

- Code of good practice FSA for the reduction of mycotoxin contamination in cereals (06/07) + 2 GMP's (EU) for OTA and Fusarium toxins (02/07)

Harmonization of Food Safety Standards

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- 7th Asean Food Safety Standards Harmonization workshop (May 2008, Malaysia)

➔ Country members agreed to add contaminants in their harmonization targets for next meeting in 2009

- **Heavy Metals**: Tin, Lead, Mercury, Arsenic (Organic / Inorganic), Cadmium.
- **Mycotoxins**: Aflatoxins (B/G, M1), Ochratoxin
- **Process contaminants**: 3-MCPD

Thank you for your attention