Aflatoxins Control in Foods
Food Industry experience

P. Gallardo
Nestlé Quality Assurance Center
Singapore
Nestle Quality Policy

CONSUMER TRUST & PREFERENCE
Consumer confidence and satisfaction in all our Brands, products and services

ZERO-DEFECT, NO-WASTE ATTITUDE
We always strive for excellence and no-waste in everything we do

LEADING FOOD, NUTRITION, HEALTH AND WELLNESS COMPANY

FOOD SAFETY & FULL COMPLIANCE
We never compromise on food safety and always comply with all applicable regulatory requirements

EVERYBODY’S COMMITMENT
Quality is a Group-wide objective

Aflatoxins Control in Foods

NQAC Singapore
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

- Phytotoxines: 2%
- Dioxines/PCB: 8%
- Mycotoxines: 25%
- Heavy metals: 30%
- 3-MCPD: 1%
- Coumarine: 1%
- Vet drugs: 9%
- Pesticides: 14%
- PAH's: 10%
## EU Chemical Alerts 2007

<table>
<thead>
<tr>
<th></th>
<th>2007</th>
<th>2006</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Evolution</strong> 2006/2007</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mycotoxines</td>
<td>73</td>
<td>71</td>
</tr>
<tr>
<td>PAH's</td>
<td>30</td>
<td>27</td>
</tr>
<tr>
<td>Pesticides</td>
<td>40</td>
<td>21</td>
</tr>
<tr>
<td>Vet drugs</td>
<td>25</td>
<td>40</td>
</tr>
<tr>
<td>Coumarine</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>3-MCPD</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Heavy metals</td>
<td>88</td>
<td>56</td>
</tr>
<tr>
<td>tox.marines</td>
<td>6</td>
<td>20</td>
</tr>
<tr>
<td>Dioxines/PCB's</td>
<td>22</td>
<td>6</td>
</tr>
</tbody>
</table>
Outline

- E.U Alerts on Mycotoxins
  - Major Mycotoxins / Aflatoxins of concern
  - Management of the Upstream
  - Management at Factory level
  - Analytics on Mycotoxins
  - Regulations
In summary:
6 major chemical types of mycotoxins

<table>
<thead>
<tr>
<th>Mycotoxins</th>
<th>Main Producing Fungi</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aflatoxins $B_1, B_2, G_1, G_2$</td>
<td><em>Aspergillus flavus, A. parasiticus, A. nomius</em></td>
</tr>
<tr>
<td>Ochratoxin A</td>
<td><em>Penicillium verrucosum, A. alutaceus, A. carbonarius</em></td>
</tr>
<tr>
<td>Patulin</td>
<td><em>P. expansum, A. clavatus, Byssochlamys nivea</em></td>
</tr>
<tr>
<td>Fumonisins</td>
<td><em>Fusarium moniliforme, F. proliferatum</em></td>
</tr>
<tr>
<td>Deoxynivalenol (trichothecenes)</td>
<td><em>F. graminearum, F. culmorum, F. crookwellense</em></td>
</tr>
<tr>
<td>Zearalenone</td>
<td><em>F. graminearum, F. culmorum, F. crookwellense</em></td>
</tr>
</tbody>
</table>
Mycotoxins occurrence at a glance....

- Corn
- Wheat
- Barley
- Soybeans
- Milk
- Nuts
- Cocoa
- Coffee
- Fruits
- Spices

- AFL BG
- OTA
- Patulin
- Fumo
- DON
- ZEN
### Natural occurrence of aflatoxins

<table>
<thead>
<tr>
<th>High Risk</th>
<th>Moderate Risk</th>
<th>Low Risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn</td>
<td></td>
<td>Soybeans</td>
</tr>
<tr>
<td>Peanuts</td>
<td></td>
<td>Pulses</td>
</tr>
<tr>
<td>Cottonseed</td>
<td></td>
<td>Sorghum</td>
</tr>
<tr>
<td>Brazil nuts</td>
<td></td>
<td>Millet</td>
</tr>
<tr>
<td>Pistachio nuts</td>
<td></td>
<td>Wheat</td>
</tr>
<tr>
<td>Copra</td>
<td></td>
<td>Oats</td>
</tr>
<tr>
<td></td>
<td>Figs</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Almonds</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pecans</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Walnuts</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sultanas</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Spices</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Barley</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rice</td>
</tr>
</tbody>
</table>
E.U Alerts on Mycotoxins

Major Mycotoxins / Aflatoxins of concern

Management of the Upstream

Management at Factory level

Analytics on Mycotoxins

Regulations
 Routes of chemical contamination in plant-based foods

**Natural Toxins**
- Mycotoxins

**ENVIRONMENT**
- Pesticides
- Agrochemicals
- Veterinary drugs
- In situ formation due to heat, pH, etc.
  - e.g. chloropropanols
- Migration from packaging
  - e.g. BADGE
  - SEM
- Heat-induced carcinogens
  - e.g. heterocyclic aromatic amines, acrylamide

**Farming**

**Storage**
- Pesticides
- Mycotoxins

**Processing**

**Transport Distribution Retail**

**Consumer**
Routes for Contamination and Controls

CONTRIBUTING FACTORS

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
Aflatoxins Control in Foods

Routes for Contamination and Controls

Control factors

Pre-Harvest
- Pest Control
- Crop rotation
- Irrigation
- Plant Resistant varieties
- Control of diseases

Harvesting
- Timeliness
- Clean-up
- Drying

Storage
- Sorting
- Temperature
- Relative Humidity

Nestle Good Food, Good Life

NQAC Singapore
• 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

GOLD PRACTICES

• 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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Regulations
Aflatoxin contamination is very heterogeneous.

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

<table>
<thead>
<tr>
<th>Sample Size</th>
<th>Kernels (estimate)</th>
<th>Variability (μg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.54 kg (10 lbs)</td>
<td>30000</td>
<td>11.6-28.4</td>
</tr>
<tr>
<td>2.27 kg (5 lbs)</td>
<td>15000</td>
<td>8.1-31.9</td>
</tr>
<tr>
<td>1.13 kg (2.5 lbs)</td>
<td>7500</td>
<td>3.2-32.8</td>
</tr>
<tr>
<td>0.45 kg (1 lbs)</td>
<td>3000</td>
<td>1-40</td>
</tr>
</tbody>
</table>

Sampling procedure to obtain representative samples especially in nuts, dried fruits, grains are critical.
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.
The preparation of representative samples is key for Quality Assurance procedures.

Example of sampling in a non compartmented truck:

1. 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.

2. Proper grinding increases the chances of detecting contaminated particles.
### Number of incremental samples for lots of less than 15 tons

<table>
<thead>
<tr>
<th>Lot weight (Tons)</th>
<th>No. of incremental samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 0,1</td>
<td>10</td>
</tr>
<tr>
<td>0,1 - &lt; 0,2</td>
<td>15</td>
</tr>
<tr>
<td>0,2 - &lt; 0,5</td>
<td>20</td>
</tr>
<tr>
<td>0,5 - &lt; 1,0</td>
<td>30</td>
</tr>
<tr>
<td>1,0 - &lt; 2,0</td>
<td>40</td>
</tr>
<tr>
<td>2,0 - &lt; 5,0</td>
<td>60</td>
</tr>
<tr>
<td>5,0 - &lt; 10,0</td>
<td>80</td>
</tr>
<tr>
<td>10,0 - &lt; 15,0</td>
<td>100</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Lot Weight (Tons)</th>
<th>No. of incremental samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤ 1</td>
<td>10</td>
</tr>
<tr>
<td>&gt; 1 and ≤ 3</td>
<td>20</td>
</tr>
<tr>
<td>&gt; 3 and ≤ 10</td>
<td>40</td>
</tr>
<tr>
<td>&gt; 10 and ≤ 20</td>
<td>60</td>
</tr>
<tr>
<td>&gt; 20 and ≤ 50</td>
<td>100</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>High Risk</th>
<th>Securing Supply</th>
<th>Partnership Contract growing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Work with preferred suppliers</td>
<td>Leverage volume</td>
<td>Look for opportunities to standardise or substitute inputs</td>
</tr>
<tr>
<td>Process improvement – collaborative planning</td>
<td>Exchange of technical competence</td>
<td>Where possible broaden supplier market</td>
</tr>
<tr>
<td>Seasonal contract</td>
<td></td>
<td>Focus on demand management</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Develop suppliers</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Long term commitment</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>High Risk</th>
<th>Competition Supplier development</th>
<th>Tactical</th>
</tr>
</thead>
<tbody>
<tr>
<td>Best price analysis</td>
<td>Leverage price and volume in the (open) market</td>
<td>Best price analysis</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Transaction efficiency</td>
</tr>
<tr>
<td>Broaden supplier range</td>
<td>Exploit competition between suppliers where possible</td>
<td>Annual tender</td>
</tr>
</tbody>
</table>

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Low Risk

Low Supply risk (Need / availability)
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
### Aflatoxins Control in Foods

**Hazard**
- Aflatoxin Presence in pistachios above acceptable level

**Control Measure**
- Use of reliable, approved suppliers.
- Agreed specifications with supplier.

**Monitoring**
- Check on COA that aflatoxin is within specifications for each delivery.

**Verification**
- Testing for mycotoxins at reception (frequency based on risk and supplier confidence).

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**Example of HACCP**

**Management at Factory level**
Outline

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Analytics on Mycotoxins

Regulations
The two facets of the Analytics

- Sophisticated techniques to characterize ingredients

but also….

- Rapid tests for use in factories and routine laboratories
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**
Assay Procedure

1. Pipette 50µL of assay buffer into each conjugate well.
2. Add 50µL of sample extract into each well and mix.
3. Incubate for 5 minutes.
4. Put one strip into one well, start timing.

Interpret results:
- Negative results are confirmed as soon as two lines appear – within 1 minute (2ppb cutoff kit only).
- Positive results are confirmed after exactly 5 minutes.

Results Interpretation

- **Negative:** Aflatoxin less than cutoff level (two color lines)
- **Positive:** Aflatoxin distinct results below or above the cutoff level (one control line)
- **Invalid results:** no control line

* (10 g of sample extracted with 20 mL methanol/water (70:30))
EXAMPLE: LI-03.015 total aflatoxins BG (AgraStrip dipstick assay from Romer Labs)

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

Extract

- + control
- - anti S lgG
- + anti A
- - anti S lgG
- + anti S lgG
- - anti A

Lateral flow test (dipstick)
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 detected in a portable fluorometer
Promising rapid method: FLORIDA immunoassay

Fluorescence Labelled Optical-Read Immuno Dipstick Assay

1) If mycotoxin is absent or below LOD fluorescent labelled antibodies bind to immobilized mycotoxins at test-zone

2) Samples containing sufficient mycotoxin: Antibody-antigen complex passes test-line

3) Check: secondary antibodies have to capture labelled antibodies in any case
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.
**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

**Applications**
- aflatoxins, OTA, DON, fumonisins, ZEA

**Advantages**
- 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

- **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.

**Negative sample**
- Mycotoxin labelled with a fluorescent molecule (tracer)
- Slow rotation
- Light remains polarized
- Polarized light

**Positive sample**
- Mycotoxin-specific antibody
- Rapid rotation
- Light is depolarized
- Mycotoxin-antibody-komplex
Future trends in Mycotoxin analysis

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

- 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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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
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
• 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)
• 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