European Commission

Better Training for Safer Food

Initiative

Aflatoxins

BTSF

Consumers, Health, Agriculture and Food Executive Agency

Addis Ababa, Ethiopia, 29 August – 2 September 2016

Mycotoxins:

Definition and general properties

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Mycotoxins

Secondary metabolites produced by fungi

on a **wide range of plant agricultural products**

(cereals, peanuts, nuts, coffee, cocoa, grapes, spices ...)

both in the field and the post-harvest, especially during storage,

depending on the environmental conditions,

with potential toxicity for both humans and animals

Natural food contaminants

According to FAO, at least 25% of the world's food crops are contaminated with mycotoxins

Mycotoxins

Can also contaminate:

Food products of plant origin (beer, wine, animal feed...) due to their thermal and chemical stability

By transfer, **food products of animal origin** (milk, eggs, meat and offal from animals consuming contaminated food)

Mycotoxin contamination along the food supply chain

PRE-HARVEST

Plant

FIELD

POST-HARVEST

STORAGE

Food products of plant origin
Direct consumption or after processing

Feed

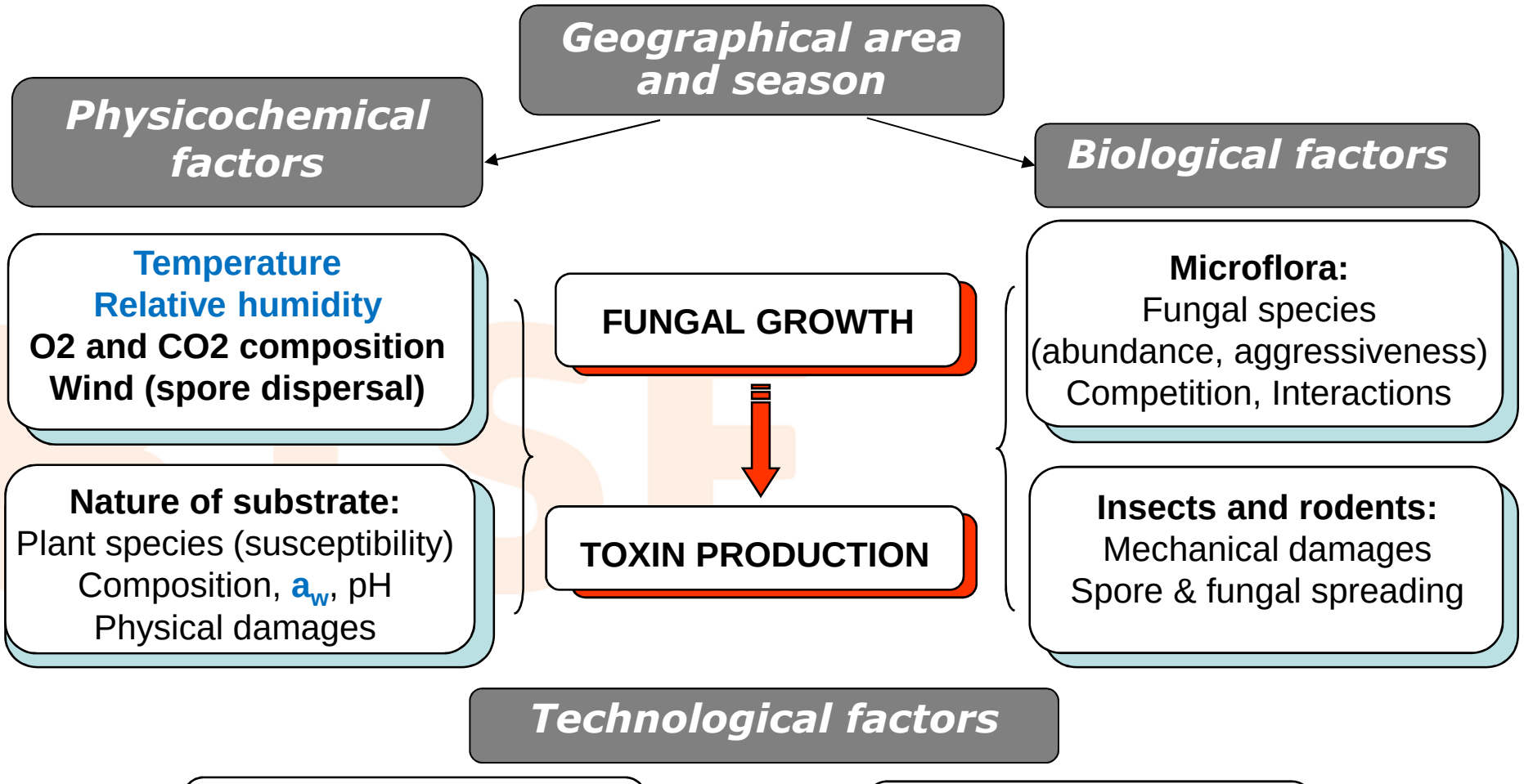
Animal

Food products of animal origin
milk, eggs, meat, offal

Human food

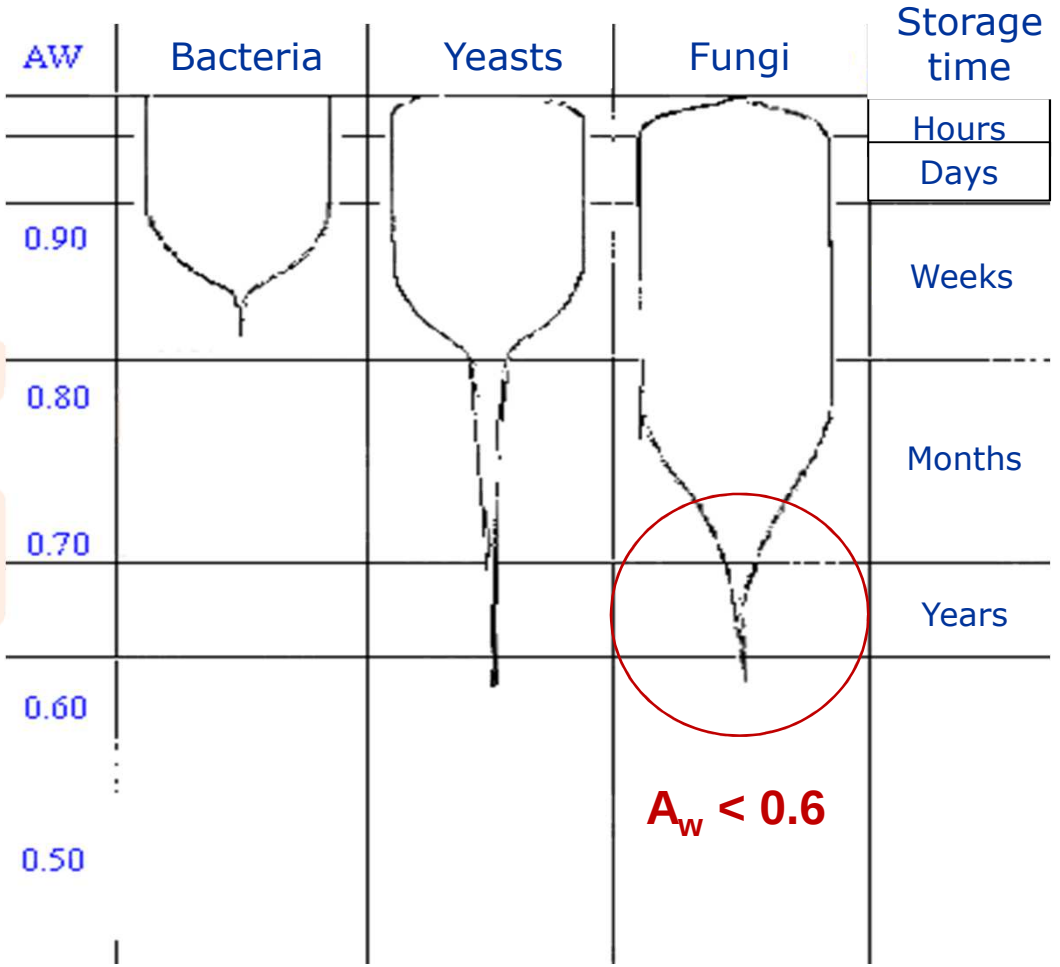
Mycotoxins

Factors affecting fungal growth and toxin production



Water activity - a_w

- "**Available water**" for biological reactions and microorganism growth
- **Varies** between **0** (water retained by salts, sugars, proteins) and **1** (pure water)



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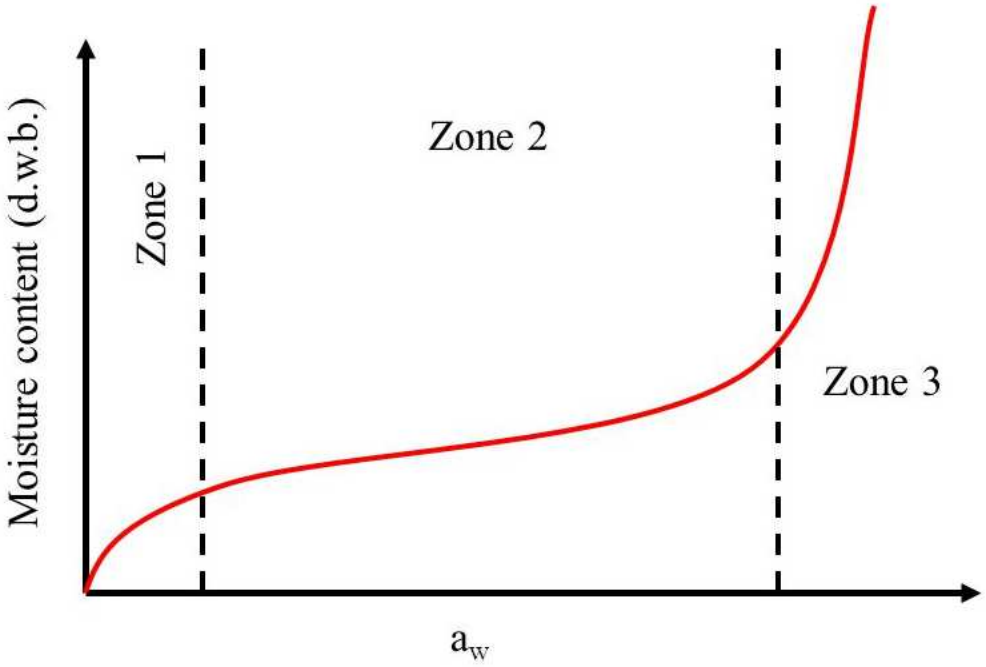
Water activity - a_w

- **The relationship between water activity and moisture content of a food, at a given temperature,** can be studied by the **determination of the sorption isotherms**



Zone 1 (aw <0.3): strongly bound water
Zone 2 (0.3 <aw <0.7): weakly bound water
Zone 3: (aw> 0.7): Free Water

Mycotoxins: major concern for human and animal health

Various toxic effects antagonistic, additive or synergistic

(carcinogenic, hepatotoxic, nephrotoxic, neurotoxic, genotoxic, immunotoxic...)

Presence in food and feed can cause **acute or chronic intoxications in both humans and animals,** which are sometimes **fatal**

Regulation of mycotoxins

Numerous countries, particularly in Europe, have set maximum acceptable levels for mycotoxins in order to protect the consumer health

Mycotoxins: significant economic losses for the chain actors

Impact on trade:

Foods with mycotoxin levels higher than the regulatory limits are:

- **Rejected** or even **destroyed** by food control authorities,
- **Reprocessed** for market acceptance by the chain actors, or
- **Shipped to less demanding markets** that may constitute a new risk to human health

Ingestion of contaminated food can **lower livestock performance and even cause their death**

Mycotoxins considered most significant from a food and health perspective, and regulated

More than 300 mycotoxins identified,
Although only around 30 with toxic properties of concern

Aflatoxins (AFB1, AFB2, AFG1, AFG2)

Ochratoxin A (OTA)

Fumonisins (FUM)

Trichothecenes (Deoxynivalenol - DON)

Zearalenone (ZEA)

Patulin

Mycotoxins considered most significant from a food and health perspective, and regulated

From the African perspective, 2 classes of mycotoxins estimated to be widespread in major dietary staples:

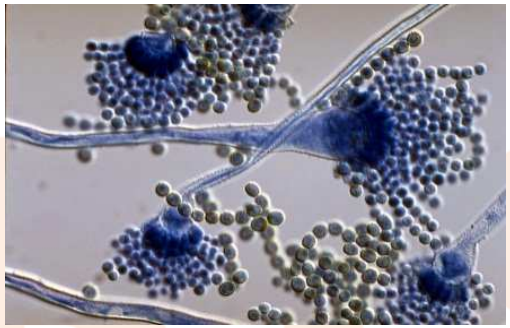
- **Aflatoxins, mostly in maize and peanuts**
- **Fumonisins, prevalence on maize from different parts of Africa**

(Wagacha and Muthomi, 2008 - http://www.ncbi.nlm.nih.gov/pubmed/18258326)

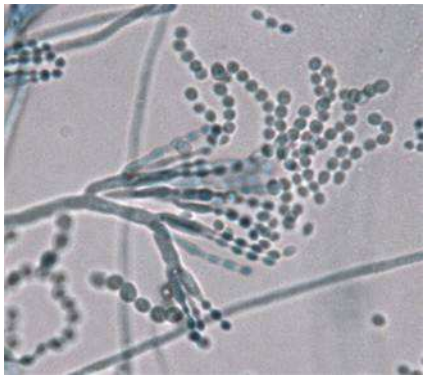
Mycotoxin-producing fungi

Belong in particular to genera:

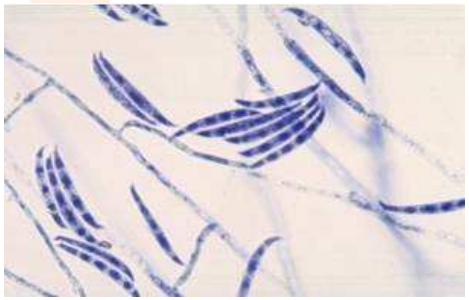
Aspergillus



Penicillium



Fusarium



Mycotoxin-producing fungi

Two groups of mycotoxin-producing fungi can be distinguished:

Group 1:
Invade their substrate and produce mycotoxins on plants in the field (**field toxins**)

Group 2:
Produce toxins after harvesting (**storage toxins**).
Fungi from the ground or plant debris may disseminate their spores onto the plant or seeds and then proliferate during storage if conditions allow

(AFSSA, Summary report, December 2006 - https://www.anses.fr/fr/system/files/RCCP-Ra-MycotoxinesEN.pdf)

Mycotoxins and fungi

One fungus	→	**Several mycotoxins**
≠ fungi	→	**Same toxin**
Presence of fungi	↛	**Presence of mycotoxins**
Absence of fungi	↛	**Absence of toxins** **Toxin can persist**

Mycotoxins:

Regulations worldwide, in Europe and Africa
Codex standards

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Regulation of mycotoxins on a worldwide basis

In 2003:

- **At least 99 countries had mycotoxin regulations for food and/or feed**
- **All countries have at least limits for AFB1 or AF B1+B2+G1+G2**
- **For several other mycotoxins, specific regulations exist as well**
- **Regulations harmonized between countries belonging to economic communities (EU, Australia/New Zealand, Mercosur)**

(FAO FNP 81, 2004 - http://www.fao.org/docrep/007/y5499e/y5499e00.htm)

Regulation of mycotoxins in Europe

- **In 2003, 39 countries with mycotoxin regulations (99% of the continent's population)** (FAO FNP 81, 2004)

- **Europe has the most extensive and detailed mycotoxin regulations in food**
  EU harmonized limits for AF, OTA, FUM, DON, ZEA, Patulin, Citrinin in foodstuffs (Regulation 1881/2006/EC as amended by regulations 1126/2007/EC, 105/2010/EU, 165/2010/EU, 594/2012/EU, 1058/2012/EU and 212/2014/EU)

- **EU guidance values for T-2 / HT-2 in food and feed** (Recommendations 2013/165/EU and 2016/1319/EU)

- **EU limits for ergot sclerotia in food** (Regulation 2015/1940/EU) and **EU recommendation** (2012/154/EU) **for monitoring ergot alkaloids in food and feed**

- **EU feed limits for AFB1** (Directive 2002/32/EC)
- **EU feed guidance values for OTA, DON, ZEA, FUM** (Recommendation 2006/576/EC as amended by recommendation 2016/1319/EU)

Regulation of mycotoxins in Africa

In 2003:

- **15 countries with specific mycotoxin regulations (59% of the continent's population)**
- **Majority of the countries: regulations (probably) do not exist**
- **Several countries recognize that they have problems due to mycotoxins and that regulations should be developed**
- **Regulations mainly for aflatoxins**
- **Most detailed: Morocco**

(FAO FNP 81, 2004)

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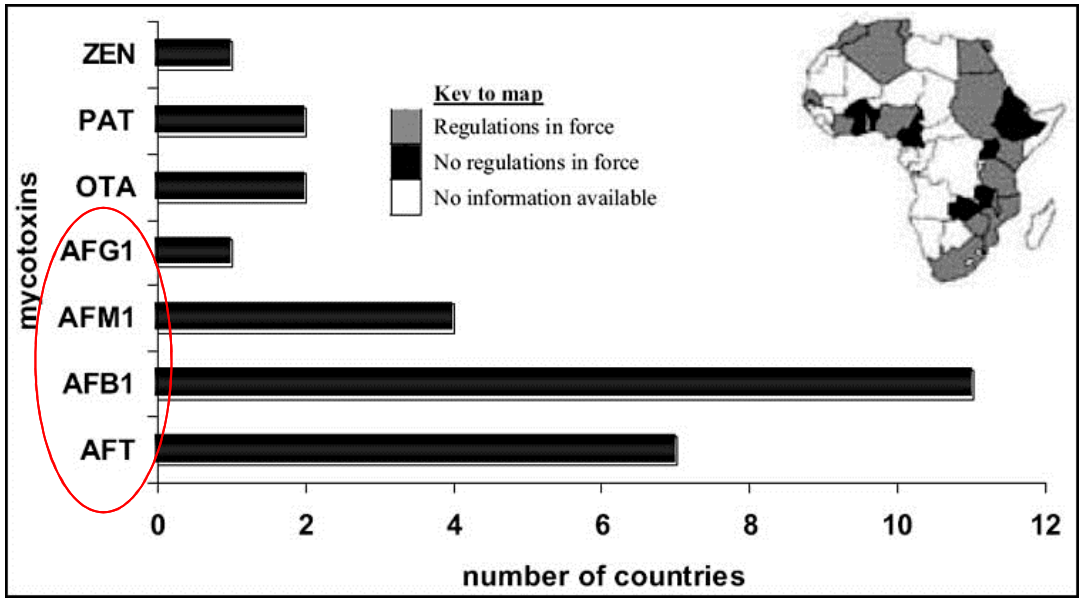
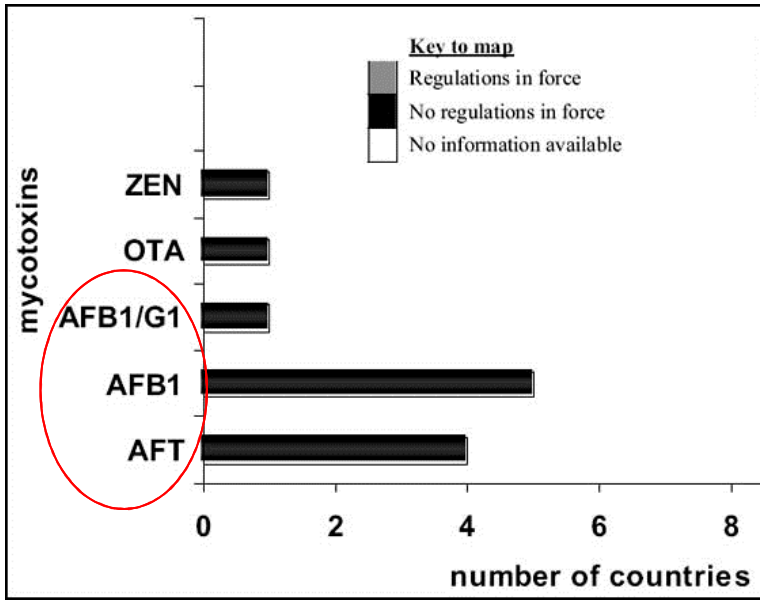
Regulation of mycotoxins in Africa

In food, 2003



In feed, 2003



(FAO FNP 81, 2004)

Codex standards for mycotoxins
- Codex STAN 193-1995 -

- **Total Aflatoxins in almonds, Brazil nuts, hazelnuts, peanuts, pistachios and dried figs**
- **AFM1 in milks**
- **DON in cereals and derived products**
- **FUM B1 + B2 in maize grain, flour and meal**
- **OTA in wheat, barley and rye**
- **Patulin in apple juice**

CODEX ALIMENTARIUS
International Food Standards
World Health Organization
Food and Agriculture Organization of the United Nations

http://www.fao.org/fao-who-codexalimentarius/standards/list-of-standards/en/

Aflatoxins:

Nature and structure

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The main aflatoxins (AF)

AF = the most studied and regulated group of mycotoxins

Isolated and identified in 1960 in animal feed (peanut meal) responsible for the deaths of 100 000 turkeys in the UK

AF B1, B2, G1, G2

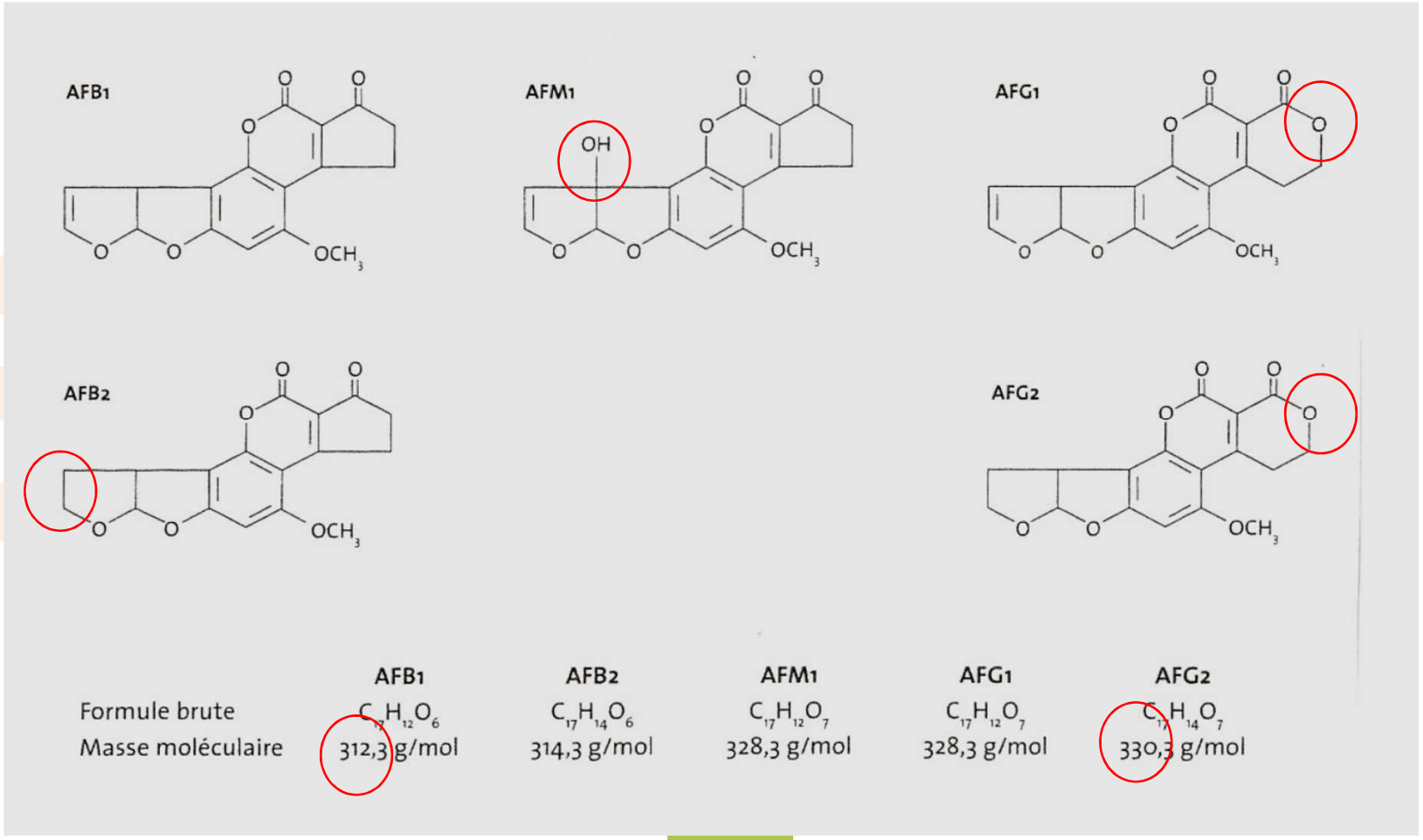
AFB1: major representative both in terms of content and prevalence in at risk foods and of its toxic effects

AF M1 : hydroxylated metabolite of AFB1

AFB1 absorbed by dairy cows is metabolized in the liver and excreted in milk as AFM1

Chemical structure of aflatoxins

Polyacetates of low molecular weight



	AFB1	AFB2	AFM1	AFG1	AFG2
Formule brute	$C_{17}H_{12}O_6$	$C_{17}H_{14}O_6$	$C_{17}H_{12}O_7$	$C_{17}H_{12}O_7$	$C_{17}H_{14}O_7$
Masse moléculaire	312,3 g/mol	314,3 g/mol	328,3 g/mol	328,3 g/mol	330,3 g/mol

Aflatoxins:

Aflatoxin-producing fungi

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Aflatoxin-producing fungi: *Aspergillus* species

Most common *Aspergillus* species associated with AF contamination of food crops

A. flavus (maize, peanuts and cottonseed)
Especially abundant in the Tropics with hot, humid climates
Toxigenic and atoxigenic strains
It has been estimated that only about 30–40 % of known isolates produce AF
Produces AF B1, B2

A. parasiticus (peanuts)
Also a tropical species, more restricted geographically and less commonly found
Most produce AF B1, B2, G1, G2

(Perrone *et al.*, 2014; Varga *et al*, 2011; IARC, 2012
http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4104701/
http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3161756/pdf/simycol_69_1_005.pdf
http://monographs.iarc.fr/ENG/Monographs/vol100F/mono100F.pdf)

Aflatoxin-producing fungi: *Aspergillus* species

Other *Aspergillus* species of Section *Flavi* responsible of AF contamination

A. nomius in corn, nuts, and brazil nuts, especially in certain geographical area

A. arachidicola in peanuts

A. mottae, ***A. sergii*** and ***A. transmontanensis*** in maize and almonds in Portugal

Production of AF B1, B2, G1, G2

(Perrone *et al.*, 2014)

Aflatoxin-producing fungi: *Aspergillus* species

- **Infection in field, but essentially during storage**
- **Tropical and temperate zones (especially during hot and humid seasons)**
- ***A. flavus:*** **a_w: 0.84-0.86** **Temperatures: 25-40°C**

(AFSSA, 2009 - https://www.anses.fr/en/system/files/RCCP-Ra-Mycotoxines2009.pdf)

Aflatoxins:

Contaminated foods

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Foods contaminated by aflatoxins

AF B et G

Cereals (corn, wheat, rice ...) and derivatives

Peanuts, treenuts (pistachios, almonds, Brazil nuts ...)

Spices

Dried fruits (figs ...)

AF M1: Milk and dairy products

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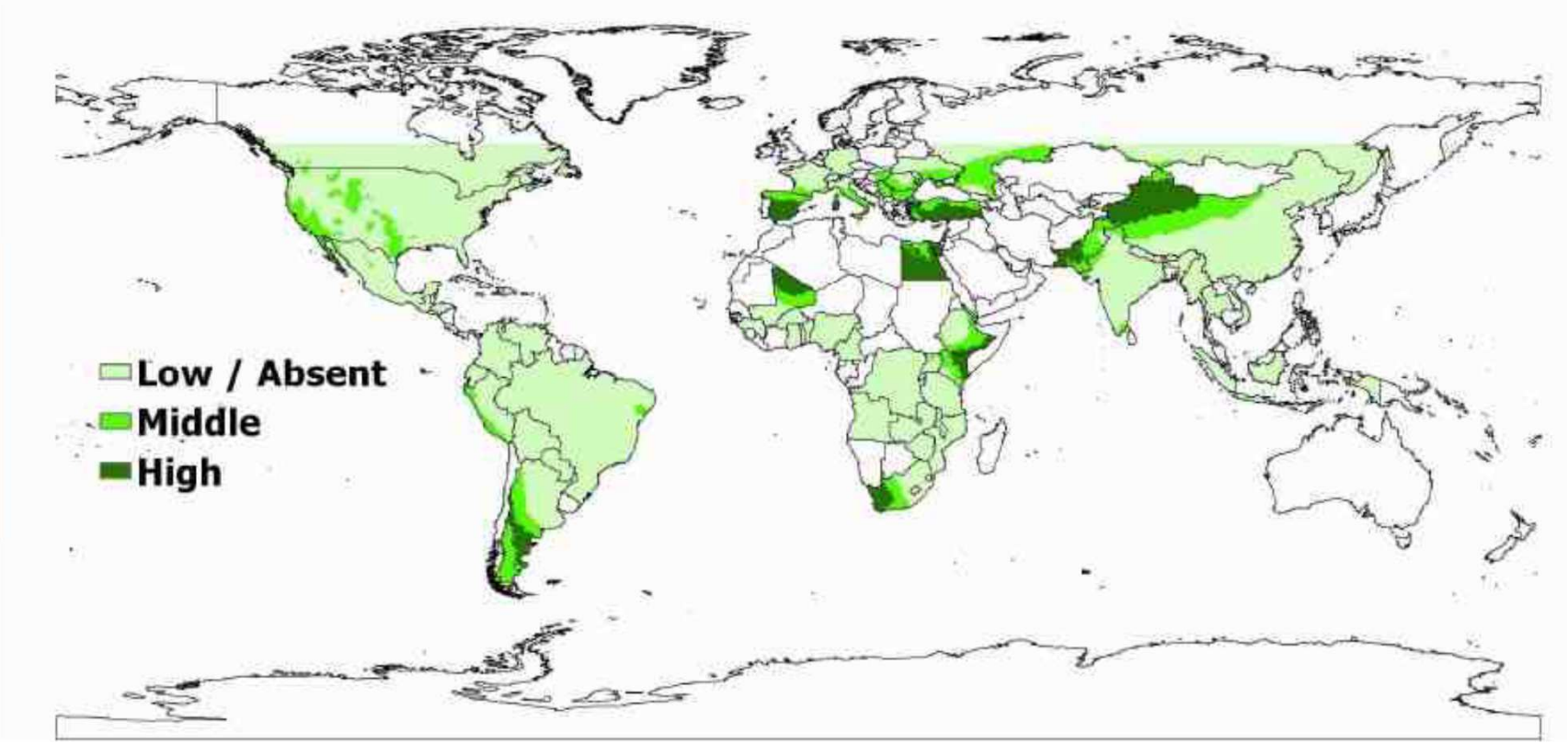
Foods contaminated by aflatoxins

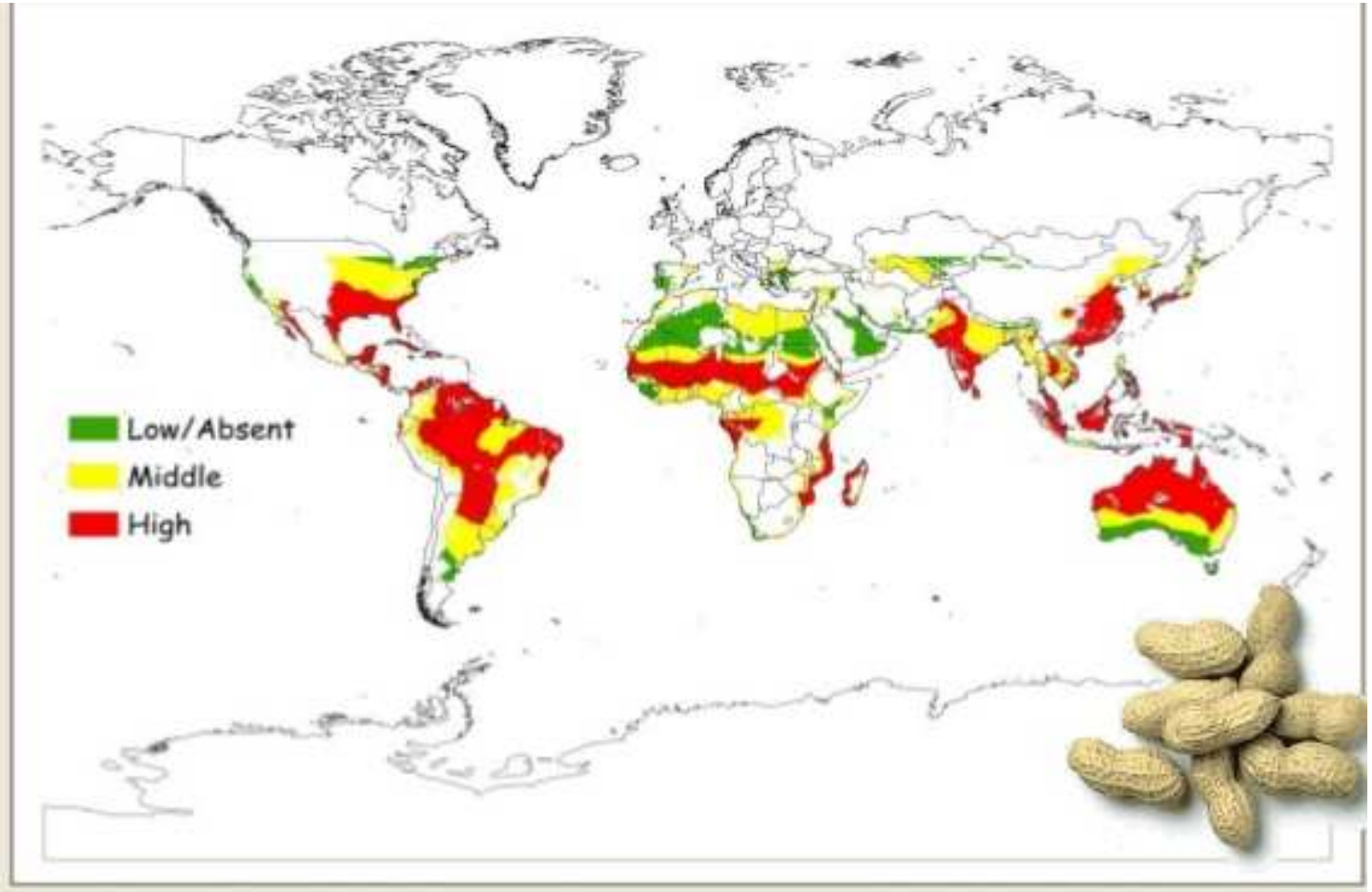
Map of aflatoxin risk in maize



(Battilani and Logrieco, 2013)

Foods contaminated by aflatoxins

Prediction map of AFB1 risk in peanut growing areas



(Battilani and Logrieco, 2013)

Foods contaminated by aflatoxins

Examples of AF occurrence data in Africa (range)

Due to consumption patterns, **maize and peanuts dominate** in terms of level of **AF exposure in Africa**:

- Peanut cake from Nigeria (20–455 µg/kg)
- Raw peanut from Kenya (ND to 7525 µg/kg) and Botswana (12-329 µg/kg)
- Maize from Benin (2–2500 µg/kg), Ghana (20–355 µg/kg), and Zambia (1–109 µg/kg)

Other AF-contaminated food sources reported in various African countries include **cassava, tiger nuts, cowpeas, sorghum, okra, and hot peppers**

(IARC, 2015 - http://www.iarc.fr/en/publications/pdfs-online/wrk/wrk9/IARC_publicationWGR9_full.pdf)

Aflatoxins:

Toxicity

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Toxicity of aflatoxins

Carcinogenic
Hepatotoxic
Genotoxic
Immunotoxic

AFB1 > AFG1 > AFB2 > AFG2

(AFSSA, 2009)

According to IARC – International Agency for Research on Cancer: **(**http://monographs.iarc.fr/ENG/Classification/latest_classif.ph**)**

- **Aflatoxins classified as Group 1 :** carcinogenic to humans
- **AF M1 classified as Group 2B:** possibly carcinogenic to humans

AF B1: the most toxic and potent liver carcinogen aflatoxin

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Toxicity of aflatoxins

→ Mycotoxin risk assessment

Main factors affecting mycotoxin toxicity:

- Bioavailability
- Combined effects of several mycotoxins
- Mycotoxin amount consumed
- Continuous or intermittent ingestion of contaminated food
- Weight, age, health status, etc. of the exposed population

Toxicity of aflatoxins

→ Mycotoxin risk assessment

Risk assessment

Scientific phase/ Independent Scientific Experts

-> scientific advices

EU : **EFSA** (http://www.efsa.europa.eu/fr)

FAO/WHO: **JECFA** - Joint Expert Committee on Food Additives (http://www.codexalimentarius.org/scientific-basis/jecfa/fr/)

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Toxicity of aflatoxins

→ Mycotoxin risk assessment

Risk assessment is a probability

Zero risk does not exist

Method developed to define the health effects of exposure of individuals or populations to hazards (chemical or biological) using the scientific facts

Include 4 steps:

1) Hazard Identification: mycotoxins

2) Hazard characterization: dose-response relationship

3) Hazard exposure: level of food contamination and food consumption data

4) Risk Characterization

☞ From hazard concept to risk concept

Toxicity of aflatoxins

→ **Mycotoxin risk assessment**

Toxicological Studies
Acute toxicity
Chronic toxicity

No Observed Adverse Effect Level (NOAEL)

Uncertainty factor of 100 applied

Tolerable daily intake (TDI ng / d / Kg body weight)

Eating habits

Acceptable Daily Intake (ADI)
Maximum amount that can be ingested throughout life without health effect

LD 50 (mg/kg): Lethal dose 50 : dose that kills 50% of animals

Uncertainty factor: the lowest NOAEL in animal studies is divided by 100, 10 for extrapolation from animals to humans and 10 for variation between individuals, to arrive at a ADI

Toxicity of aflatoxins

No acceptable daily intake (ADI):

Genotoxic carcinogenic effects with no threshold (i.e. no-effect concentration limit cannot be established, toxic at all tested concentrations), hence application of the **ALARA** (As Low As Reasonably Achievable) **principle**

According to epidemiological data in Europe:

Ingestion of 1 ng of aflatoxins / kg b.w./d would increase the incidence of liver cancer of 0,013 cancer per year per 100 000 people (JECFA)

Oral LD 50 (Average Lethal Dose) in mouse (mg / kg b.w.)
9 (AFB1)

(AFSSA, 2009)

Aflatoxins:

Regulations in Europe and Africa

Codex standards

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Maximum levels of aflatoxins (µg/kg)

Commission regulations (EC) 1881/2006 as amended by (EU) 165/2010 & 1058/2012

Foodstuffs	B1	B1+B2+G1+G2
Intended for direct human consumption or use as an ingredient in foodstuffs		
Almonds, pistachios and apricot kernels	8	10
Hazelnuts and Brazil nuts	5	10
Peanuts, other tree nuts, dried fruit, **cereals and derived products**	2	4
To be subjected to sorting, or other physical treatment, before human consumption or use as an ingredient in foodstuffs		
Peanuts, hazelnuts and Brazil nuts	8	15
Almonds, pistachios and apricot kernels	12	15
Other tree nuts, dried fruit, **maize** and rice	5	10
Dried figs	6	10
Spices	5	10
Processed cereal-based foods and baby foods for infants and young children	0,10	-

Maximum levels of aflatoxins (µg/kg)

Commission regulations (EC) 1881/2006 as amended by (EU) 165/2010

Foodstuffs	B1	B1+B2+G1+G2	M1
Raw milk, heat-treated milk and milk for the manufacture of milk-based products	-	-	0.05
Infant formulae and follow-on formulae, including infant milk and follow-on milk	-	-	0.025
Dietary foods for special medical purposes intended specifically for infants	0.10	-	0.025

Maximum levels of aflatoxins (µg/kg)

General standard for contaminants and toxins in food and feed
- CODEX STAN 193-1995 -

Foodstuffs	B1+B2+G1+G2	M1
Almonds, Brazil nuts, hazelnuts, pistachios (after removal of shell) • "Ready to eat" • Intended for further processing	 10 15	
Peanut seeds or kernels intended for further processing	15	
Dried figs "ready to eat"	10	
Milks		0,5

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Maximum levels of aflatoxins (µg/kg)

In Africa

Country	Food	B1	B1+B2+G1+G2	M1
Kenya	Peanut (product)s, vegetable oils		20	
Malawi	Peanuts (export)	5		
Mauritius	All foods Peanuts	5 5	10 15 (+M1M2)	
Mozambique	Peanut, peanut milk		10	
South Africa	All foodstuffs Milk	5	10	 0,05
Tanzania	Cereals, oil seeds	5	10	
Zimbabwe	Foods, peanuts, maize, sorghum	5		

(FAO FNP 81, 2004)

Aflatoxins:

Health and economic impacts

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Aflatoxin impacts on human health

Chronic toxicity

Over 5 billion people worldwide are **at risk of chronic exposure to AF** in food, mainly in developing countries

Effects of chronic low-level exposure to AF:
Hepatocellular carcinoma (HCC), liver cirrhosis
Immuno-suppression
Increased susceptibility to some infectious diseases (HIV-AIDS), and maternal and child health problems such as anemia, malnutrition, stunting, wasting

Concomitant exposure to AF and HBV (hepatitis B virus) **greatly increases HCC risk**

In sub-Saharan Africa, about **26 000 people die each year** of liver cancer associated with AF exposure

(IARC, 2015; Wu and Guclu, 2012; Strosnider *et al*., 2006
http://journals.plos.org/plosone/article/asset?id=10.1371%2Fjournal.pone.0045151.PDF
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1764136/pdf/ehp0114-001898.pdf **)**

Aflatoxin impacts on human health

Acute toxicity

Acute high-level exposure to AF, which is less common, can result in **aflatoxicosis,** which manifests as severe, acute hepatotoxicity **with a case fatality rate of about 25%.**

Early symptons: anorexia, malaise and low-grade fever
Later symptoms: vomiting, abdominal pain, jaundice, fulminant hepatic failure and death

Acute aflatoxicosis outbreaks → recurring public health problem **in developing countries:**

In Kenya, several acute aflatoxicosis outbreaks over the past 25 years with hundreds dead. In 2004, 317 people became ill and 125 died as a result of consuming highly AF contaminated maize (with **AFB1 levels as high as 4400 µg/kg**)

In Nigeria, 2005: more than 100 deaths

In Western India, 1974: 397 cases and 106 deaths

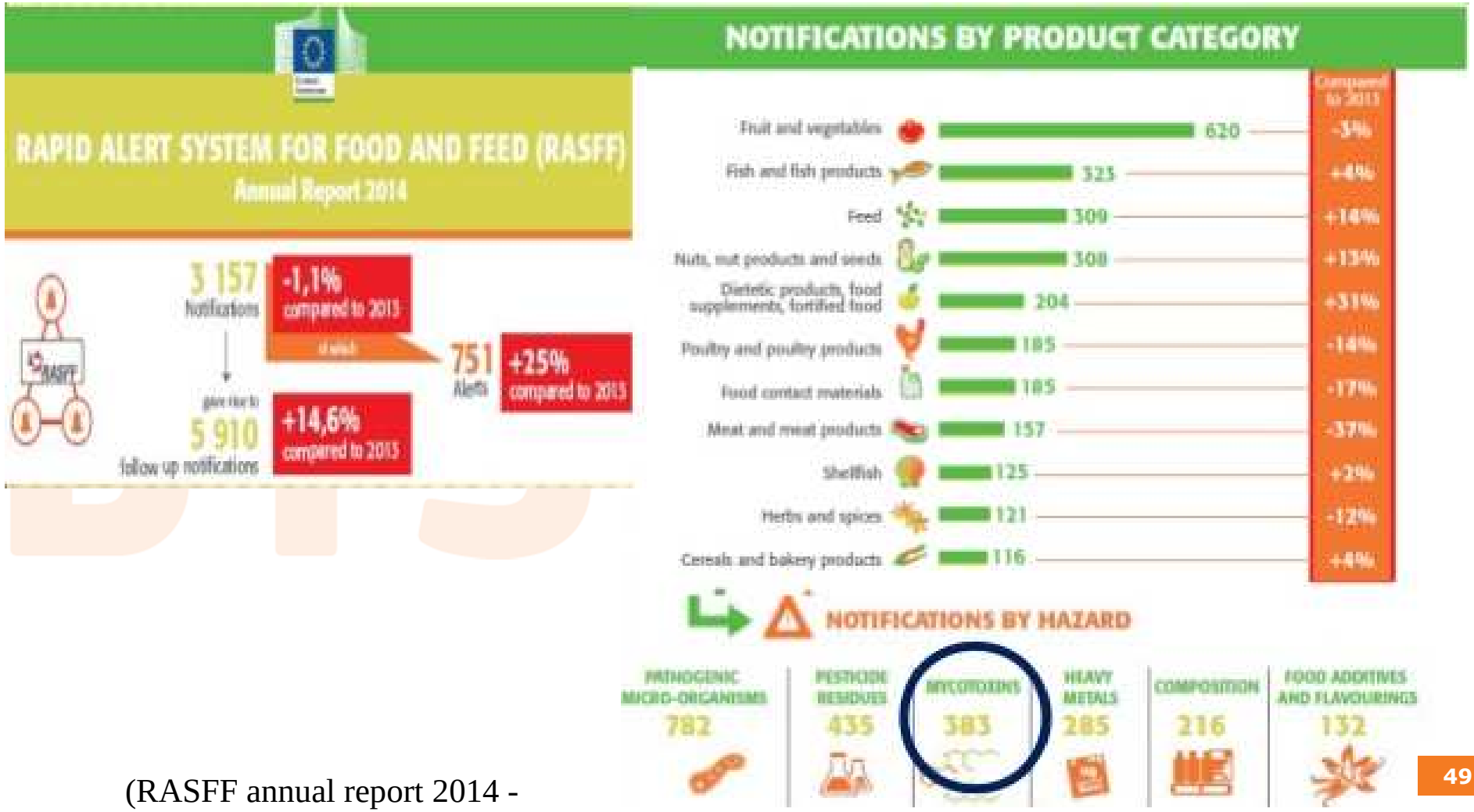
(Wagacha and Muthomi, 2008
Strosnider *et al*, 2006**)**

Aflatoxin impacts on animal health

- **Liver damage**
- **Gastrointestinal dysfunction**
- **Immune system suppression**
- **Lower feed conversion ratios**
- **Reduced productivity**
- **Decreased milk and egg yield**
- **Decreased reproductive performance**
- **Embryonic death**
- **Death (cattle, turkey, poultry, swine..)**

(Iheshiulor *et al.*, 2011
http://scialert.net/qredirect.php?doi=ajas.2011.19.33&linkid=pdf)

RASFF notifications in 2014



(RASFF annual report 2014 - http://ec.europa.eu/food/safety/rasff/index_en.htm)

RASFF 2014 mycotoxin notifications in food and feed

Notification Type	Food	Feed	Food & Feed
Alert	46	7	53
Border rejections	270	10	280
Information for attention	37	7	44
Information for follow up	4	2	6
TOTAL	**357**	**26**	**383**

About 73%

(RASFF annual report 2014)

RASFF 2014 aflatoxin notifications in food and feed

Mostly from nuts, nut products & seeds (218: about **64%**)
of which a significant part was for **peanuts** (89: about **41%**)

About 88 %

Hazard	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014
Aflatoxins	839	946	801	705	902	638	649	585	484	341	338
Deoxynivalenol (DON)				10	4	3	2	11	4	8	6
Fumonisins	14	2	15	9	2	1	3	4	4	7	2
Ochratoxin A	27	42	54	30	20	27	34	35	32	54	37
Patulin		6	7		3						
Zearalenone			1	6	2				4		
Total mycotoxins	880	996	878	760	933	669	688	635	528	410	383

(RASFF annual report 2013 and 2014
http://ec.europa.eu/food/safety/rasff/docs/rasff_annual_report_2013.pdf)

Aflatoxins:

Prevention and control

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Aflatoxins: prevention and control

Understand fungal growth & AF production along the production chain

Identification of critical points & factors

Develop & validate preventive and controlling measures in pre- et post-harvest

Sampling and analytical methods for AF surveillance & monitoring

Key Point: preparation of representative samples

Adoption of a food safety management system (FSMS), applied to aflatoxin control, by the food chain actors

Promote the implementation of good production practices (GAP, GSP, GMP, HACCP)

Aflatoxins: prevention and control

- **Integrated approach, from farm to fork**

  Limit the risks of AF contamination along the food chain

- **Concerted effort of all actors along the food production chain**

  Private sector (farmers, industries…), R&D institutes, public and regulatory authorities, NGO, Civil Society Organizations…

  ☞ Need for adequate social organization & coordination between chain actors

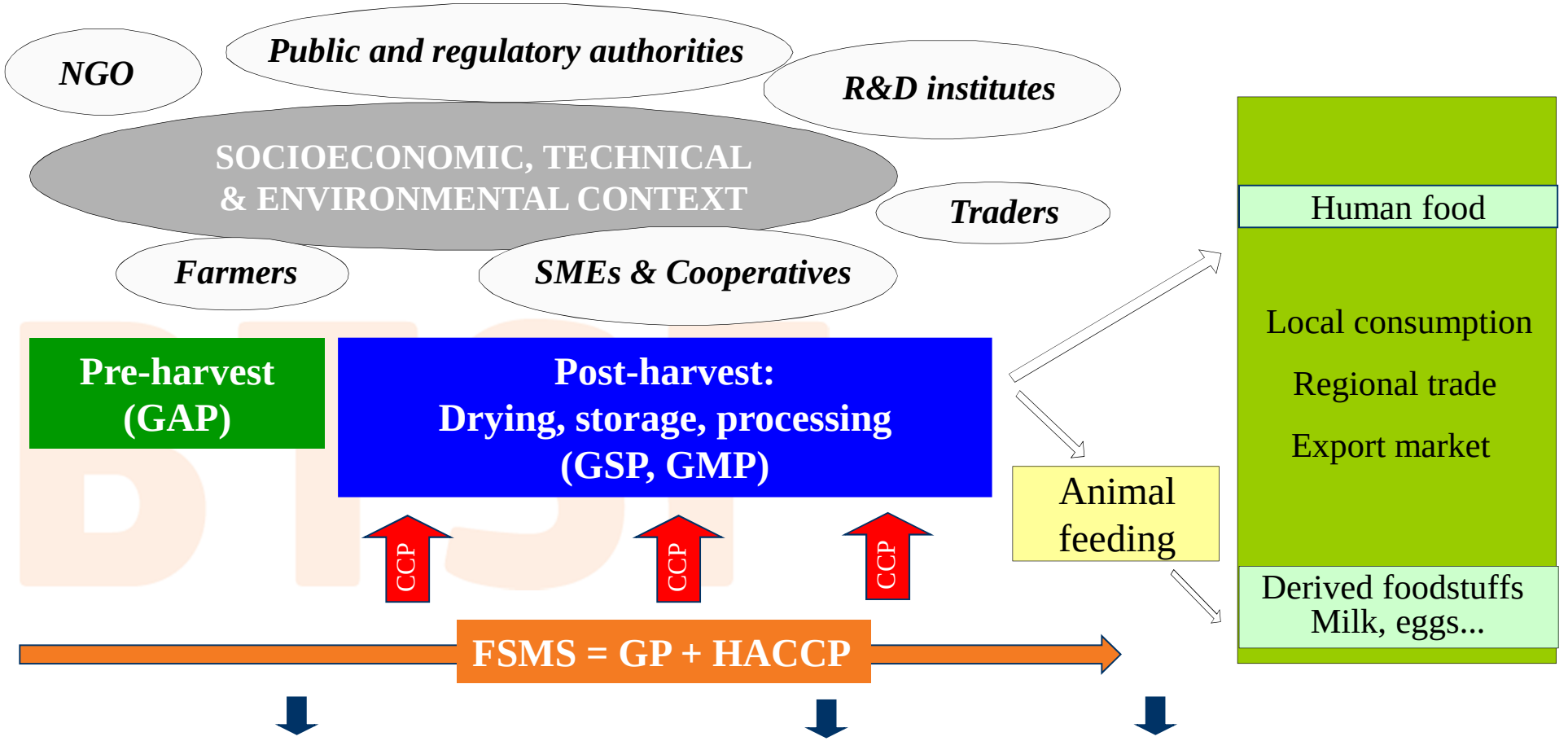
- **Multidisciplinary approach**

  By integrating technical, socio-economical and environmental aspects to develop a sustainable AF management system

- **Prioritize preventive measures to curative measures**

- **Promote incentives for the adoption of the FSMS**

Aflatoxins: prevention and control



Aflatoxins: prevention and control

Codes of Practice of Codex alimentarius (CAC/RCP)

http://www.codexalimentarius.org/standards/list-standards/en/?no_cache=1

On food hygiene:

General Principles of Food Hygiene
Code of Hygienic Practice for specific food

On aflatoxin prevention and reduction:

Codes of Practice for the Prevention and Reduction of :

- **Mycotoxin** Contamination in Cereals
- **Aflatoxin** Contamination in Peanuts
- **Aflatoxin** Contamination in Tree Nuts
- **Aflatoxin** Contamination in Dried Figs

Code of Practice for the Reduction of **Aflatoxin B1** in Raw Materials and Supplemental Feedingstuffs for Milk-Producing Animals

Aflatoxins: prevention and control

Referential harmonizing food hygiene management in Africa

elaborated as part of the program
"Better Training for Safer Food - BTSF- Africa"
funded by the European Union

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Aflatoxins: prevention and control

Aflatoxigenic fungi
(Aspergillus)

Mycotoxins

Field

Storage

Human food and animal feed

Safe for humans and animals

In accordance with established regulations

Varietal selection
Farming practices
Bio-control

Control of temperature & humidity

Physical
Chemical
Biological
Treatments

Prevention

Decontamination

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Aflatoxins: prevention at pre-harvest

Varietal selection: resistant varieties

- Example: early peanut varieties resistant to aflatoxins
  The resistance lies in the maturing capacity of seeds under high water stress (drought)

  Seed maturity promotes the production of substances recognized for their protective and anti-fungal properties: phytoalexins, tannins and phenolics

- Plant breeding:
  Elimination of genes contributing to their susceptibility to mycotoxins
  Adding anti-mould and anti-toxin genes

Aflatoxins: prevention at pre-harvest

Farming practices

- Crop rotation and tillage
- Remove debris that can serve as a substrate for fungi
- Planting and irrigation date adapted to prevent water stress
- Keep the recommended spaces between plants
- Appropriate use of fertilizers, insecticides / fungicides, herbicides
- Avoid mechanical damages of plants
- Harvest maturity

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Aflatoxins: prevention at pre-harvest

Biological control

Using competitor microorganisms: native atoxigenic fungi

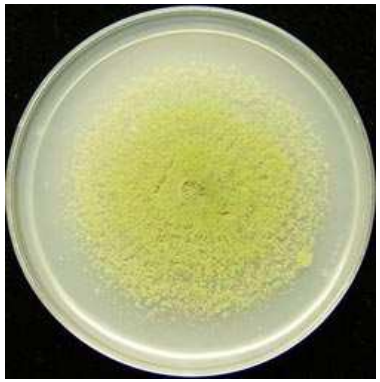
- Aflasafe for maize and peanut in Nigeria
- AF36 for maize and cotton, and afla-guard® for maize and peanut in USA

NB: Biocontrol methods: check that other mycotoxins are not produced

Aflatoxins: prevention at pre-harvest

Biological control

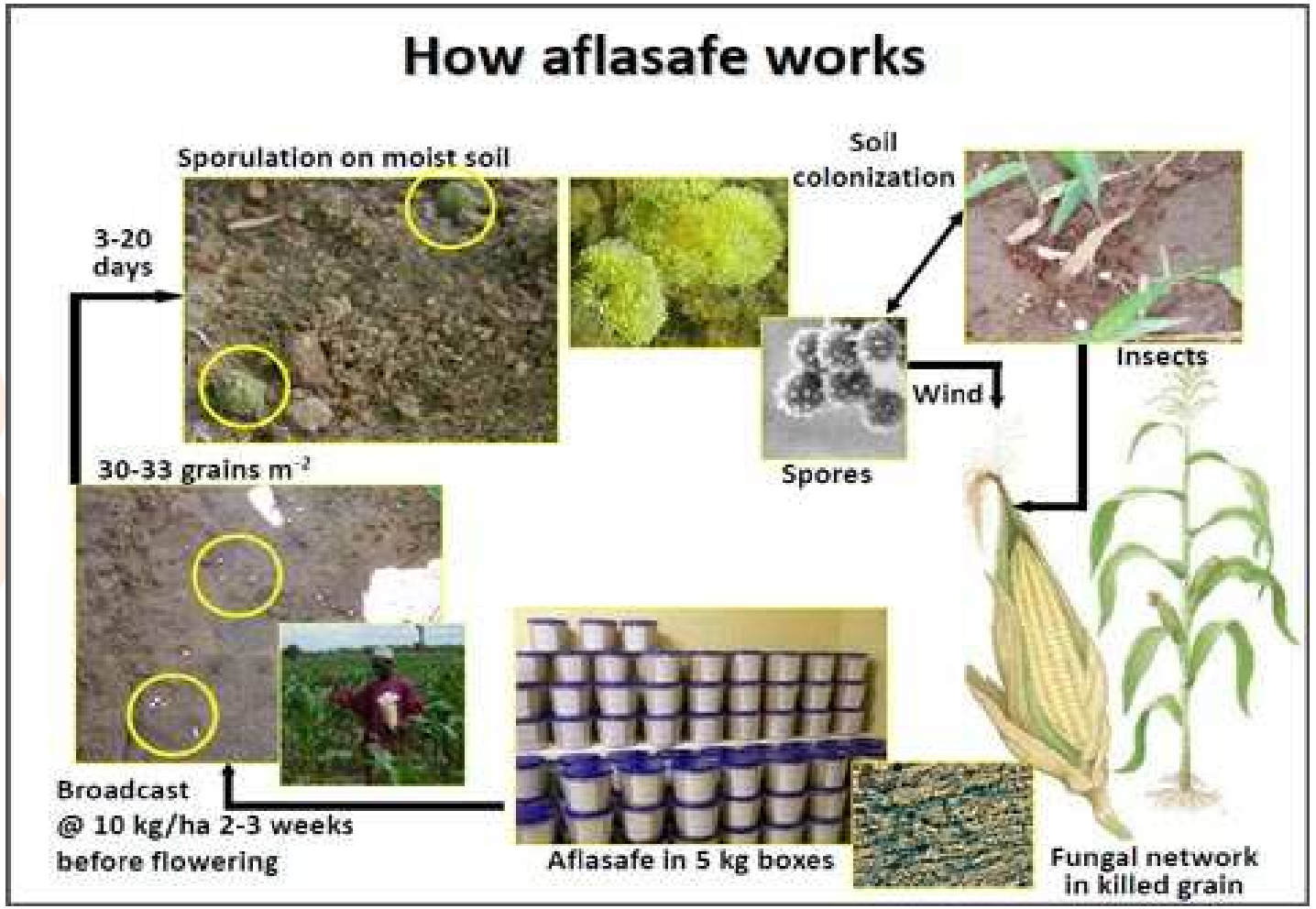
- Aflasafe for peanut, maize : http://www.aflasafe.com/



Contains a mixture of 4 atoxigenic strains of A. flavus of Nigerian origin
They have inherent defects in one or more of the 26 genes in the aflatoxin biosynthetic pathway
The selected atoxigenic strains belong to genetic groups that posses only atoxigenic strains

Reduction of 80 to 90% of AF contamination in maize and peanuts in Nigeria

Aflatoxins: prevention at pre-harvest



Aflatoxins: prevention at post-harvest

Critical factors	Proper drying as quickly as possible
Low moisture content and water activity (a_w)	**Appropriate temperature & time** Products should be dried to a safe moisture content (a_w <0.7) Grains: MC < 14% Peanuts: MC < 8% Cotton seeds: MC < 10% Process adjustments where operating limits are violated
Avoid cross-contamination	Cleaning of dryers

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Aflatoxins: prevention at post-harvest

Critical factors	Proper storage
Low moisture content and a_w to be kept after drying (avoid re-wetting) Temperature	**Control of humidity, temperature, ventilation** Appropriate storage facility & packaging Process adjustments where operating limits are violated
Avoid immature, mouldy & damaged products	**Manual or mechanical sorting/segregation**, based on product density, colour, damages, greenish-yellow fluorescence under UV light… Use of antifungal treatments
Avoid pest physical damages	Appropriate packaging General hygiene Pest control
Avoid cross-contamination	Cleaning of stores & packaging

Intact

Insect attack

Mouldy

Consumers, Health, Agriculture and Food Executive Agency

Aflatoxins: prevention at post-harvest

Critical factors	Proper transportation
Low moisture content and a_w to be kept	Control of humidity, temperature, ventilation Appropriate packaging
Avoid mouldy & damaged products	Appropriate food state
Avoid pest physical damages	Appropriate packaging General hygiene Pest control
Avoid cross-contamination	Cleaning of containers… & packaging Containers should be clean, dry and free of insects & fungal growth

Consumers, Health, Agriculture and Food Executive Agency

Aflatoxins: post-harvest treatments

Physical treatments	**Heating** (autoclaving, groundnut roasting; maize extrusion…) Decreases AF levels, but AF not completely destroyed **Controlled atmosphere:** AF production greatly restricted if O2<1% and CO2 increased **Milling:** Separation of grains into fractions and elimination of the toxic portions (bran and germ in dry milling) **Pulsed light** (UVC-near IR), during 300 µs, up to 5 times/s
Chemical treatments	**Ozonation,** but nutritional value affected **Application in feedstuff industry:** **AF adsorption/binders:** calcium alumino-silicates **AF decomposition** (95-98%): Ammoniation
Biological treatments	**Microbial detoxification** **Fermentation, silage**

Aflatoxins:

Analytical methods

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Aflatoxins: analytical methods

Aflatoxin analysis in food includes 3 main steps:

1. **Sampling**
2. **Sample preparation and sub-sampling**
3. **Analysis**

The approach followed is:

- Get a relatively **large primary sample representing a lot**,
- Grind it to **reduce the particle size and homogenize it**,
- **On a small manageable and representative portion**, perform aflatoxin extraction and detection/quantification

Aflatoxins: analytical methods

Appropriate handling of the sample until AF analysis

Sample integrity must be guaranteed, i.e. stability characteristics

Avoid conditions that damage the sample and degrade AF content (moisture, UV light, cross contamination ...)

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Aflatoxins: analytical methods

Variability associated with each step of the aflatoxin test procedure

Sampling is the main source of error

And therefore plays a crucial part in the precision of the determination of aflatoxin levels

Problem «Hot spots of contamination»

Aflatoxin distribution in a lot is **very heterogeneous**, especially for **food products having large particle size** such as dried figs or peanuts

Aflatoxin distribution in **processed products** is generally **less heterogeneous** than in the unprocessed products (such as grains)

Aflatoxins: analytical methods

Example:
Aflatoxin distribution in a lot of peanut kernels

- **A small percentage of peanut kernels is contaminated**
  - **Only 0.03 %** for an average aflatoxin concentration in the lot of 5 µg/kg
  - **Less than 1 kernel in 1000 (0.1 %)** is contaminated in a lot of shelled peanuts

- **A single kernel can contain very high aflatoxin levels :** as much as 1100 µg/kg

Aflatoxins: analytical methods

Example:
Error distribution in peanut lots tested for aflatoxins

Individual contributions to the total variability

Aflatoxins in farmers' lots of peanuts

Sample of 2.27 kg, sub-sample of 100 g, AF concentration analysed by HPLC: 100 µg/kg

- **Sampling** **92.7%**
- Sample preparation 7.2%
- Sample analysis 0.1%

(Whitacker et al., 1994: J AOAC Int, 77,107)

Aflatoxins: analytical methods

The highly unequal distribution of aflatoxins in a lot represent a great challenge to measure the actual level of contamination in the lot

Risks of misclassification of lots based on the limits of acceptance / rejection

Aflatoxin analysis requirements

- **Protect the consumers** (by not accepting contaminated batches)
- **Protect the sellers** (producers ...) (by not rejecting healthy lots)
- **Be defensible before a court of law** in case of dispute

Sampling method for aflatoxin analysis

Sampling considerations

A sample taken from a lot must be representative of this lot

Appropriate sampling method must be applied:

- **Random sampling**: sample has to be taken in such a way that every kernel in the lot has an equal chance of being chosen
- Sample has to be the accumulation of many small **incremental samples taken at many different locations** throughout the lot
- **Large sample size** for reducing sampling error
  But too large samples cannot be handled and are also expensive

Sampling plans with a balance between rigorous statistics and laboratory practices

Sampling method for aflatoxin analysis

In addition to the maximum levels of AF permitted in food, **provisions for sampling and analytical methods for official control purposes have been implemented** by:

- The countries
- The Codex Alimentarius Commission
- The European Commission

Sampling method for aflatoxin analysis

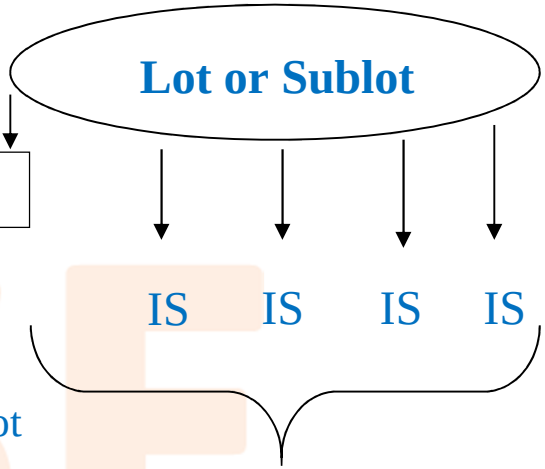
Commission regulation (EC) No 401/2006

Lays down the methods of sampling and analysis for the official control of the levels of mycotoxins in foodstuffs

- Cereals and cereal products
- Dried fruit, including dried vine fruit and derived products, except dried figs
- Dried figs, groundnuts and nuts
- Spices
- Milk and milk products; Infant formulae and follow-on formulae, including infant milk and follow-on milk
- Coffee and coffee products
- Fruit juices including grape juice, grape must, cider and wine
- Solid apple products and apple juice and solid apple products for infants and young children
- Baby foods and processed cereal based foods for infants and young children

Sampling method for aflatoxin analysis

Commission regulation (EC) No 401/2006



Incremental sample (IS)

Quantity of material taken from a single place in the lot.
As far as possible IS shall be taken at various places distributed throughout the lot

Lot: quantity of a food commodity having common characteristics (such as origin, variety, treatments…)

Aggregate sample
The combined total of all the incremental samples taken from the lot or sublot

Division, as necessary

Laboratory sample

Sampling method for aflatoxin analysis

Other EU regulations

- **Commission Regulation (EU) No 519/2014** amending Regulation (EC) No 401/2006 as regards methods of sampling of large lots, spices and food supplements, performance criteria for T-2, HT-2 toxin and citrinin and screening methods of analysis
- **Commission Regulation (EU) No 178/2010** amending Regulation (EC) No 401/2006 as regards groundnuts (peanuts), other oilseeds, tree nuts, apricot kernels, liquorice and vegetable oil

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Sample preparation for aflatoxin analysis

In order to improve sample representativeness and reduce sample preparation errors:

- **Sample dry grinding** to reduce the particle size **followed by an homogenization**

  Sample can also be slurried with water (1: 1-1,5) to produce small size particles with a high-shear mixer

  The sample particle size plays a major role in the variance of the subsampling: more the particles are small, the more ground sample is homogeneous and the variance is small

- **A division** to obtain a representative and manageable analytical sample

Aflatoxin analysis

Include 3 steps:

- **Aflatoxin extraction** with a suitable aqueous organic solvent mixture
- **Extract purification** to remove impurities, and **toxin concentration,** where necessary
- Aflatoxin **detection and quantification**

AF	Very slightly soluble in water Insoluble in nonpolar solvents Very soluble in slightly polar organic solvents (e.g. chloroform and methanol)
	Fluorescent under UV light AFB1, AFB2: blue fluorescence AFG1, AFG2: green fluorescence AFM1: blue-purple fluorescence

Aflatoxin analysis

Reference methods (confirmation)

Chromatographic methods such as HPLC / fluorimeter
Quantitative method, highly sensitive, accurate and precise
However they are demanding in equipment, expertise and time

Rapid screening methods

- Immuno-enzymatic kits (ELISA - Enzyme linked immunosorbent assay)
- Immuno-chromatographic tests (LFD – Lateral Flow Device)
- ToxiMet system

Aflatoxin analysis

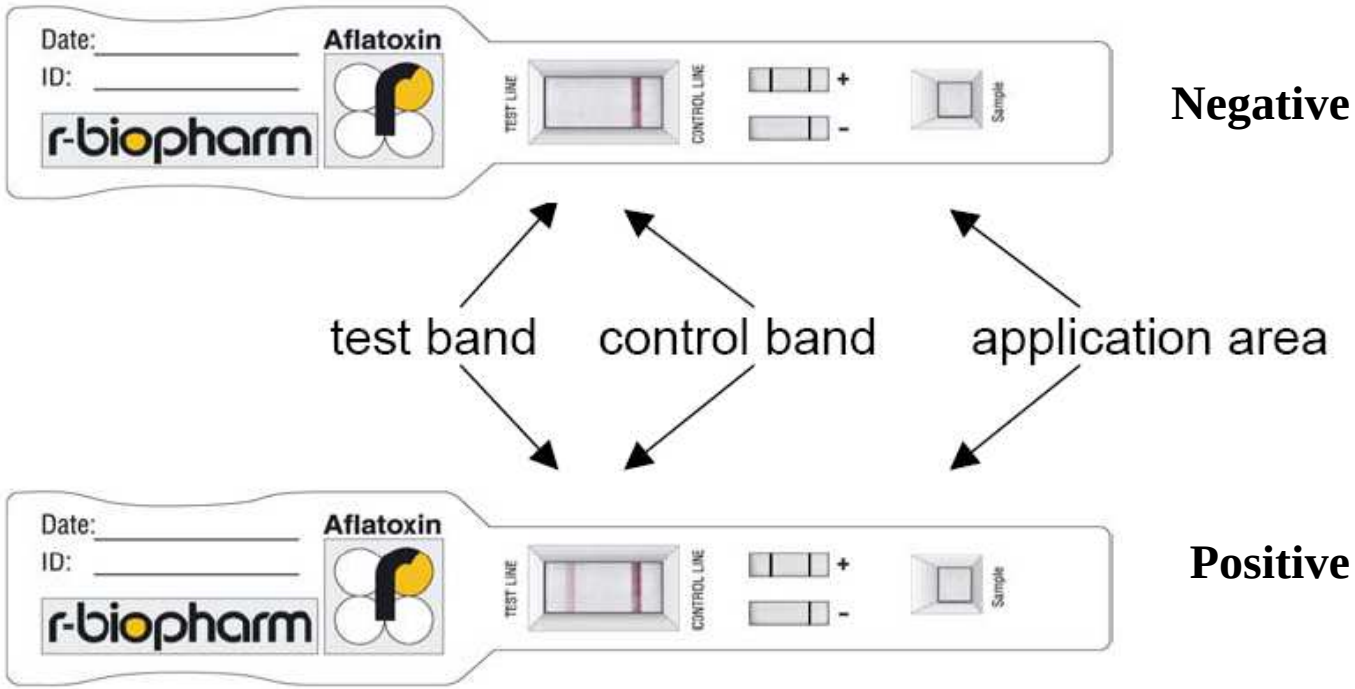
ELISA= Enzyme Linked ImmunoSorbent Assay

- **Quantitative analysis of aflatoxins**
- **A microtiter plate spectrophotometer is required for quantification**
- **Sensitivity: µg/kg level**
- **Selectivity – specific antibody-antigen interaction**

Aflatoxin analysis

Lateral Flow Device - LFD

- Semi-quantitative (visual assessment by observing the development of colored bands) or quantitative (using a spectrophotometer) analysis



Aflatoxin analysis

ToxiMet system: http://www.toximet.com/

Analysis includes the following steps:

1. **Take a representative sample** of the matrix to be tested
2. **Extract toxin** with an aqueous organic solvent and filter
3. **Clean-up the filtrate** with toxin **purification cartridge (ToxiSep)**
4. Load the purified extract on toxin **immobilisation and detection cartridge (ToxiTrace)**
5. Insert ToxiTrace into **ToxiQuant – aflatoxin fluorescence and detection**
6. Read results of aflatoxin concentrations

Aflatoxin analysis

Reference Methods: CEN and AOAC

CEN: European Committee for Standardization
AOAC: Association of Analytical Communities

CEN and AOAC establish performance criteria for mycotoxin analytical methods

Usually based on collaborative studies and expert opinions

Performance criteria fulfil criteria laid down by Regulation (EC) 401/2006

Not mandatory but can be used, e.g. in case of official control and surveillance and case of dispute

9 CEN (http://www.cen.eu/Pages/default.aspx) and about 40 AOAC (http://www.aoacofficialmethod.org/) methods for aflatoxin analysis

Aflatoxin analysis

The analytical methods should be evaluated for their performance (such as recovery, repeatability, reproducibility ...) and regularly checked to ensure the precision and accuracy of the results:

- **Use of internal standards or certified reference materials** with known concentrations of aflatoxins

- **Participation in inter-laboratory tests**

  Each laboratory receives a certified reference material to be tested, and its performance are evaluated relative to the concentration and other laboratories

Regulation (EC) 401/2006, CEN and AOAC set performance criteria for methods of aflatoxin analysis

THANK YOU FOR YOUR ATTENTION

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Better Training for Safer Food

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