#### **Aflatoxins: Risks and Options for Control**

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#### **Mycotoxins**

- Mycotoxins = toxins produced by fungi.
- Our interest/ concern is in mycotoxins that enter the food chain, particularly those that affect stored grains, staple foods and foods used for children.



#### **Critical Mycotoxins for Food Safety**

- Aspergillus flavus and A. parasiticus produce aflatoxins B1, B2, G1, and G2. "B" and "G"
- Fusarium graminearum/ Gibberella zeae, ear rot: produces mycotoxins: deoxynivalenol, zearalenone, zearalenol
- Fusarium moniliforme/ Gibberella fujikuroi): produces fumonisins
- Ocratoxins
- Deoxynivalenol (DON)
- Zearalenone
- **T-2/HT-2** toxins.
- Others toxins from fungi in food crops: ergot egometrine, patulin (apples), etc.

#### **Fumonisins**

- EU limit in maize flour for human consumption: 1,000 mg /kg.
- However high maize consumption in Africa suggested a maximum tolerated limit (MTL) of 122 mg/kg (rural), 202 mg/ kg (urban) areas.

#### Aflatoxins

- Aflatoxin B1 is one of the most potent naturally occurring chemical liver carcinogens known.
- Strong correlation with retarded growth (stunting) in West Africa (BMJ)
- Retarded mental development in children.
- Effects on child health are so sever that the limits are revised downwards as each research paper is published.
- Widespread: groundnuts, maize, coffee, wheat, barley.
- Key issues for NGOs:
  - Possible effects of aflatoxins on child growth and development, leading to stunting (chronic malnutrition).
  - Possible increased severity of AIDS-related infections.

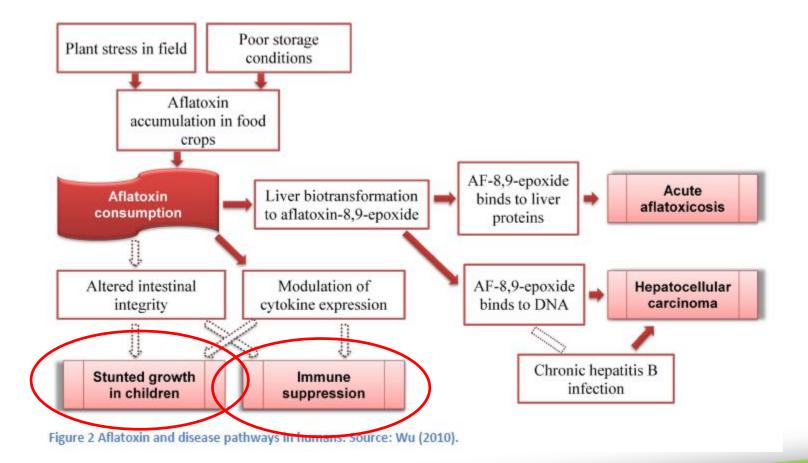
### Liver Cancer, Hepatocellular Carcinoma (HCC)\*

- Most common cancer in men in Africa.
- Hepatitis A is the main risk factor.
- Mozambique: 8% of meals have measurable aflatoxin levels, average consumption  $38.1\mu g/kg$ , highest in the world.
- Mechanism for carcinogenesis is not well known. Aflatoxin B is metabolize to aflatoxin B2,3 epoxide, the carcinogen.
- May suppress cell-mediated immunity and facilitate persistent HBV infections, and hence HCC.

#### **Field Data**

Country	Location	Aflatoxin B1 ingestion, ug/kg	HCC rate/ 100,000/ year
Kenya	High altitude	3.5	1.2
	Middle altitude	5.9	2.5
	Low altitude	10.0	4.0
Swaziland	High veldt	5.1	2.2
	Middle veldt	8.9	3.8
	Low Veldt	43.1	9.2
Mozambique	Inhambene	77.7	12.1
	Homoine-Maxixe	131.4	17.7
	Zavala	183.7	14

#### **Disease Pathways**



#### **Correlation or Causation?**

- Strong correlation between aflatoxins and high levels of stunting, but correlation ≠ causation.
- Could be due to confounding factors: stunting may be due to eating maize and little else rather than due to aflatoxins in the maize.
- Plausible biochemical/ physiological pathways to explain the affects of aflatoxins on child growth and development.
- Positive results from *in vitro* cells tests but results may not be applicable to the whole organism.
- Positive results from tests on animals but higher doses of aflatoxins used in tests than would occur in human diets.

#### Scale of the problem

- >5 billion people in developing countries worldwide are at risk of chronic exposure to aflatoxins through contaminated foods
- The primary disease associated with aflatoxin intake is hepatocellular carcinoma (HCC, or liver cancer).
- Liver cancer is the third-leading cause of cancer death globally according to WHO (2008), with about 550,000–600,000 new cases each year (83% in East Asia and sub-Saharan Africa).
- Question: does alcohol consumption increase the risk of aflatoxin related liver cancer?
- Hep B infection and aflatoxin synergize to produce ~30x higher liver cancer risk in HBV-positive, aflatoxin-exposed persons, as compared to HBV-negative persons

#### **Aflatoxin levels**

- Range of limits set for different foods and trading blocks for AFB1 & AFB2 (*Aspergillus flavus*) 2µg/kg → 20 µg/kg
- EU: 4 µg/kg = 4 ppb
- Codex Alimentarius 10 µg/kg = 10 ppb
- Zambia FRA: 10 ppb (Most African countries have adopted Codex standards)
- USA: 20 µg/kg = 20 ppb
- Cows that consume AFB1-contaminated feed can biochemically convert the toxin into 4-hydroxy derivative, aflatoxin M1 (AFM1), which is excreted in milk.
- AFM1 is about ten times less toxigenic than AFB1,
- International Agency for Research on Cancer: AFM1 group 1 carcinogen to humans.
- Milk: European Union (EU) No. 165/2010 and US Food and Drug Administration (USFDA) maximum level for AFM1 of 0.05 µg/L

 $1 \mu g/kg = 1 part per billion (ppb).$ 

#### **Aflatoxin levels**

- West Africa (Lancet):
- several west African countries show that more than 98% of children and adults have detectable aflatoxin B1 in their blood.

#### • Zambia:

- Fumonisins: 0.02 and 21.44 ppm
- Aflatoxins 0.7 and 108.39 ppb,
- Fumonisin was more pronounced in villages in forest areas
- Aflatoxin was highest in valley and forest areas in Zone II.

	Aflatoxin B1 (parts per billion)		
	Control (n=30)	Intervention (n-30)	P"
Survey 1	11 (9-17)	9 (5-11)	-
Survey 2	22 (17-33)	9 (8-11)	<0.0001
Survey 3	55 (39-72)	17 (11-22)	<0.0001

\*Compared with corresponding control group by non-parametric test of medians. Data are median (IQR).

Table 2: Aflatoxin B1 in groundnuts at main survey points

#### **Diagnostics and Sampling**

- Units: One part per billion is 1,000 times smaller than 1 ppm.
  One second in 32 years is 1 ppb.
- Neogen Europe: Reveal Q+ for Aflatoxin Green range 2-150 ppb, 25 samples, £150.00) + Accuscan Pro reader, £1,080.00



#### **Aflatoxin levels**

 Results of a post-harvest survey carried out by Concern Worldwide in Afghanistan

Location	Grain Type	Average ppb	Remark		
Takhar North	Wheat Lalmi	6.6	Considering that this sample was taken		
province	Pea Lalmi	5.7	from current season, ppb levels are		
	Almond	5.6	relatively high – meaning		
			contamination happened during		
			harvest.		
Last season results					
Takhar South	Pistachio	7.46	Apart from Corn, the rest had high		
Province	Walnut	13.06	levels of ppb due to prolonged		
	Corn/maize	4.43	duration in storage.		

#### Aflatoxins in Milk in Addis Ababa

- ILRI study of milk in Addis found:
- Only nine (8.2%) out of a total of 110 milk samples contained less than or equal to 0.05  $\mu$ g/L of AFM1 (EU/US upper limit).
- 29 (26.3%) milk samples exceeded 0.5 µg/L. upper limit
- All the feed samples were contaminated with AFB1 ranging between seven and 419  $\mu g/kg.$
- Only 16 feed samples out of 156 samples (10.2%) contained AFB1 at a level less than or equal to 10 μg/kg.
- 41 (26.2%) feed samples contained AFB1 at a level exceeding 100  $\mu g/kg.$
- less than one in five samples were above the limits set by the US/ EU.
- ILRI do not recommend that consumers stop consuming milk and dairy products in Addis Ababa because milk has very high nutritional value.
- Similar issues found in Iran.

#### **Source of Aflatoxin Contamination in Milk**

- All dairy farmers in the ILRI study used concentrate feed, mixture of wheat bran and *noug* (*Guizotia abyssinica*or) Niger seed cake.
- AFB1 in wheat bran 9 31  $\mu$ g/kg.
- AFB1 in Noug cake 290–397  $\mu$ g/kg

#### Control of Aflatoxin Contamination in Animal Feeds

- Danish study: Field-dried hay was heavily infected with *Aspergillus* and *Penicillium*, and in one case with *Rhizopus*, but hay dried on racks was almost completely free of fungal contamination.
- Detoxification: Lithuanian study. Contamination of composite fodder with zearalenone, deoxynivalenol, aflatoxins and T-2 toxin. *Saccharomyces cerevisiae* was found to detoxify T-2 toxin, and *Rhodotorula mulcilaginosa* detoxifies aflatoxins.
- Some evidence of detoxification in fermented / soured milk.
- Role for Effective Microorganisms (EM) treatment of hay/ straw? EM includes Lactic acid bacteria: *Lactobacillus casei*, Photosynthetic bacteria: *Rhodopseudomonas palustris*, Yeast: Saccharomyces cerevisiae

#### Control of Aflatoxin Contamination in Animal Feeds

- Absorption: use of absorbent carbon, bentonite, zeolite, ... in the diet. Only partly bind the mycotoxins and risk of absorbing/ sequestering valuable nutrients.
- Chemical detoxification: Ozone and Ammonia detoxifies AF1.
- Urea treatment of straw to improve palatability and N intake in diet produces ammonia.
- Could urea treatment also chemically detoxify straw?

#### **Agronomic Control Options**

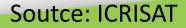
These are a selection of agronomic practices reported to reduce the risk of *Aspergillus f.* infection in the field:

- Seed treatment before planting (fungicide seed dressing).
- Adjust planting dates to avoid moisture stress.
- Resistant varieties (maize, groundnuts).
- Infect with non-pathogenic Aspergillus "<u>Aflasafe</u>" from IITA. 80-90% reduction in Nigeria. Available in Kenya, Nigeria, Zambia. <u>Afla-Guard</u> from Syngenta/ USDA.
- Fungal antagonists: Trichoderma viridis

#### **Agronomic Control Options**

- Lime application can reduce aflatoxin contamination by 72%
- Farm Yard Manure (FYM) by 47%
- Cereal crop residue by 28%
- Combination of FYM and lime by 84%,
- Combination of lime and residue by 82%,
- Combination FYM, and residue by 53%
- Combination of FYM, crop residues and lime by 85%

# Solution? Health, happy, unstressed plants that can fight-off *Aspergillus* infections?



#### **Post Harvest Control Measures**

- Aflatoxin production in storage, are favoured by high humidity (>85%), high temperature (>25 °C) and insect or rodent activity
- Crop drying off the ground (Humidity: 13-14% maize, 12% coffee, 7% groundnuts).
- Hand picking (up to 80% reduction in aflatoxins in groundnuts reported),
- Storage, store hygiene,
- Improved threshing and shelling.
- Avoid soaking groundnuts in water to soften the shells.
- Lye (potassium hydroxide) treatment of shell groundnuts.
- Natural fibre bags (jute, sisal & hemp rather than woven polypropylene).
- Insect and rodent control.
- Anaerobic storage (CIMMYT grain bins, double/triple wrapped "Purdue" Bags, <u>Grainpro</u> bags, ZeroFly bags)

### Control Measures in Small / Medium Scale Processing

- Hand sorting.
- Clean maize and rice mills daily.
- What happens to infected/ rejected crops? Risk to children? Ideally burry/ burn but is this realistic?
- Ozone treatment of contaminated grains (portable treatment units?)
- Aflatoxins can be filtered out of vegetable oil through bentonite filters.

#### Diet

- Some evidence that including montmorillonite clays in the diet prevents the update of aflatoxins from contaminated food – but probably inhibits the uptake of micro-nutrients as well.
- Solution?
- a diverse diet will reduce overdependence on a potentially contaminated crop



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