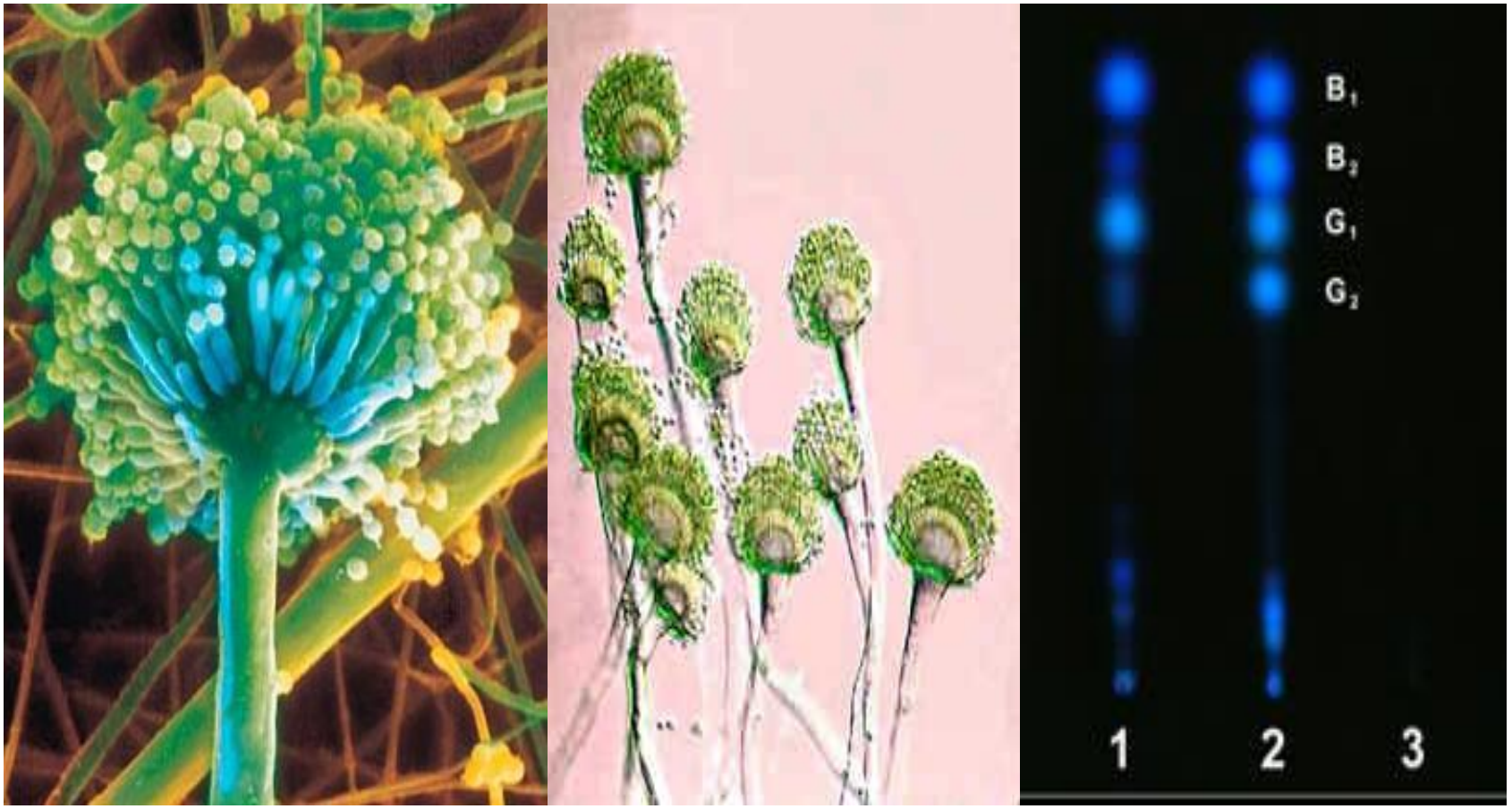
Aflatoxin in Milk
–Risk Assessment and Remediation

Arun Sharma

Consultant, FSSAI and RRF, DAE

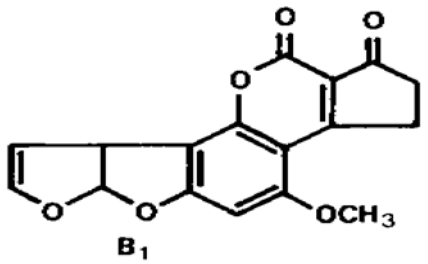
mail.ksarun@gmail.com

Aspergillus flavus toxin "Aflatoxin"

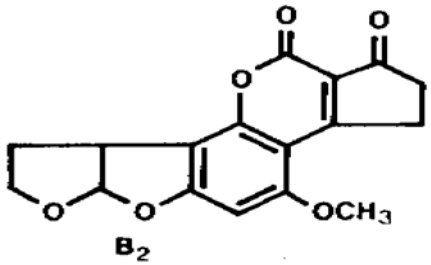


***A. flavus*: produces B Group toxins; *A. parasiticus*: produces both B and G Group toxins**

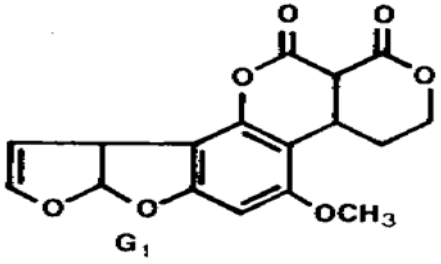
Major aflatoxins



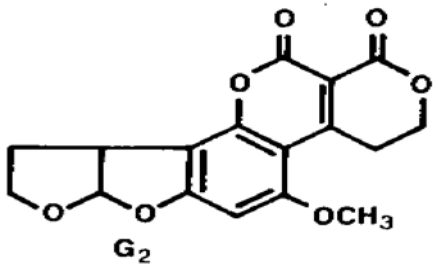
B_1



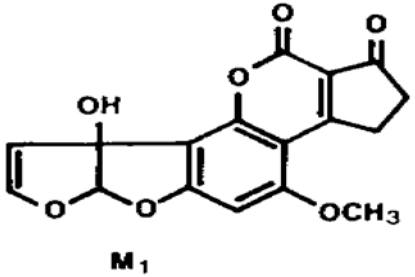
B_2



G_1



G_2



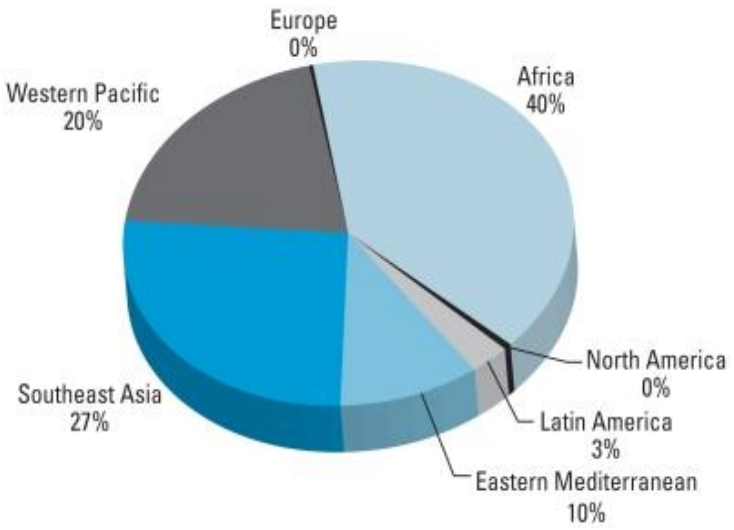
M_1

Aflatoxicosis in humans

- **Acute aflatoxicosis**
  - hemorrhage
  - acute liver damage
  - edema
  - altered digestion, absorption, and metabolism
  - death
- **Chronic aflatoxicosis**
  - impaired food conversion
  - slower growth
  - immunity problems
  - cirrhosis
  - liver cancer

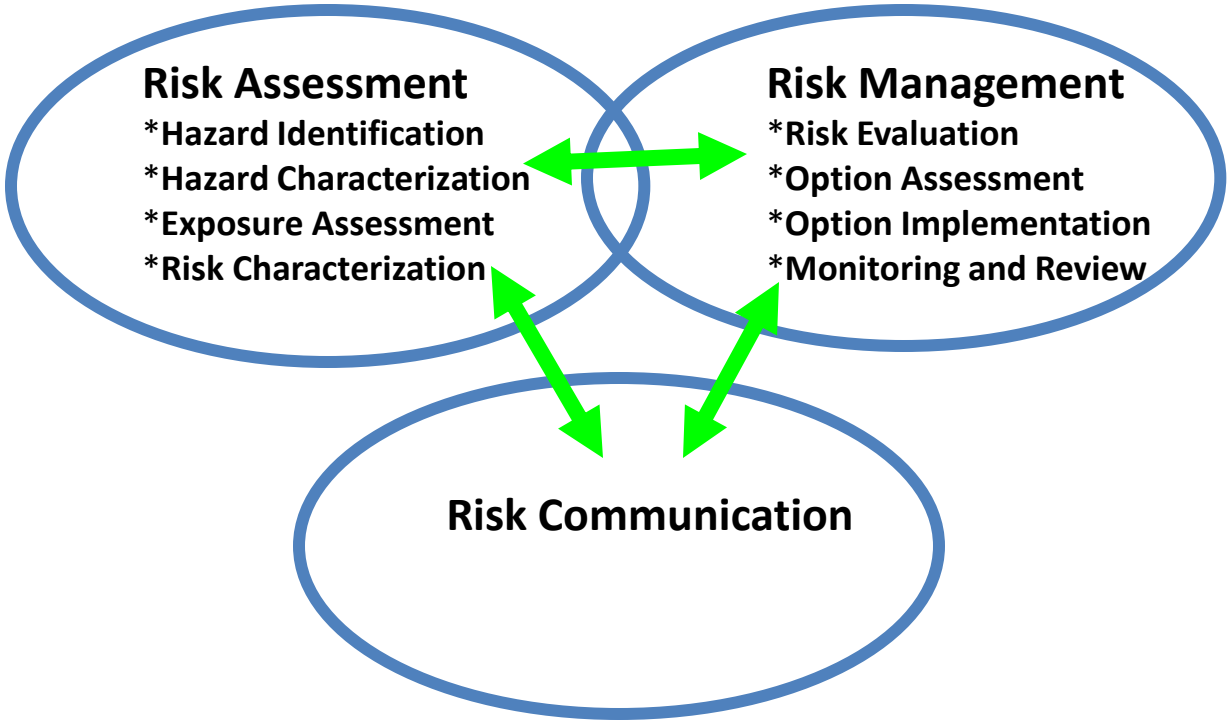
Aflatoxins- Hepatocarcinogens

- **AFB_1 & AFM_1 are most potent carcinogens known**
- **AFM_1 is 4-hydroxy derivative of AFB_1, the major toxin metabolite found in milk and urine in animals and humans exposed to dietary AFB_1**
- **AFM_1 is around 10 times less carcinogenic than AFB_1, based on animal data**
- **Group 1 carcinogens- have proven carcinogenicity to humans- International Agency for Research in Cancer**
- **Aflatoxin may play a causative role in 4.6–28.2% of all global HCC cases**



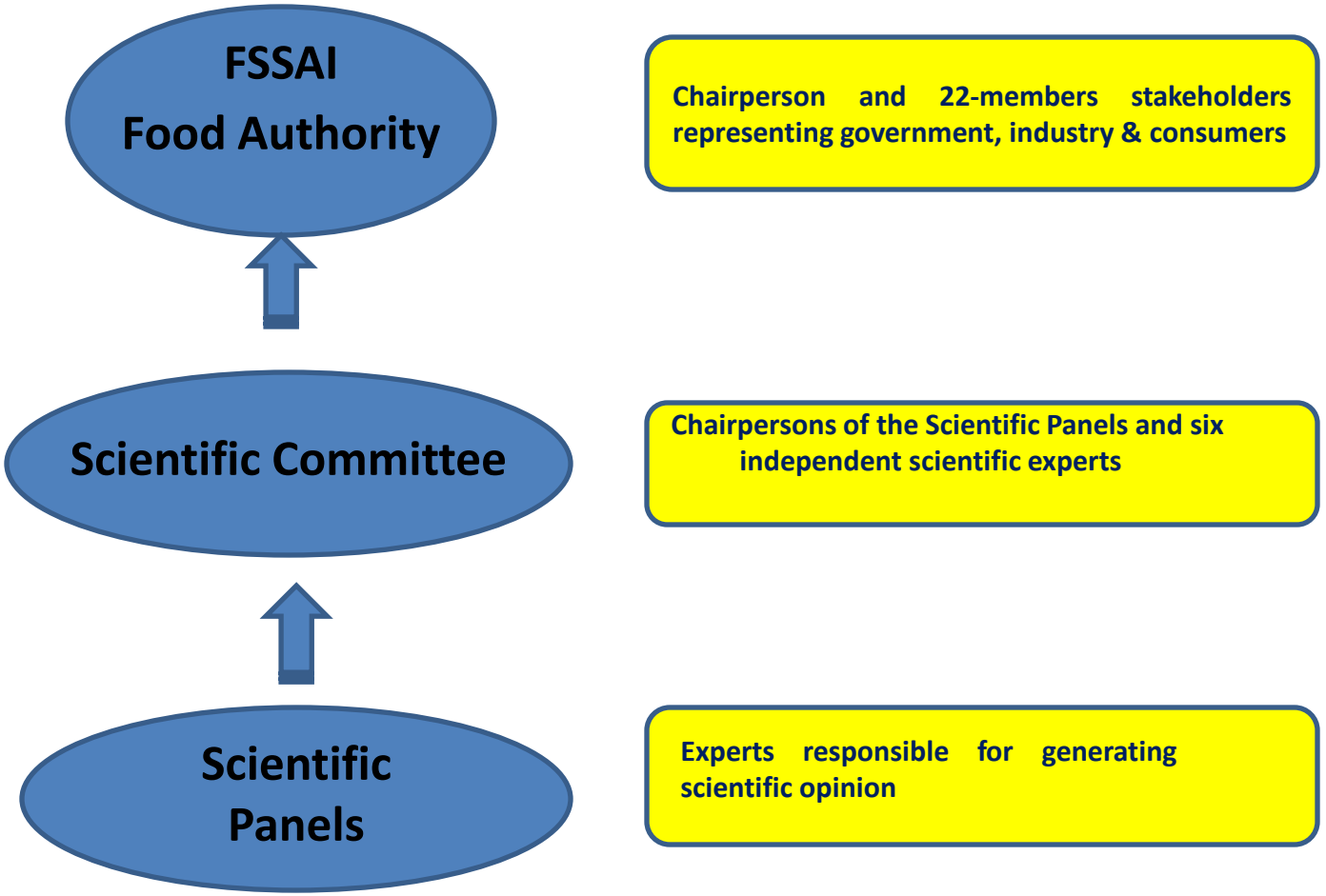
Food Safety Risk Management- Decision making
A Science-based approach

- Primary goal
  - Protection of human health
- Secondary considerations
  - Industry & economy
  - Socio-political



Food Safety & Standards Act, 2006

Laying down science based standards for articles of food and to regulate their manufacture, storage, distribution, sale and import, to ensure availability of safe and wholesome food for human consumption.

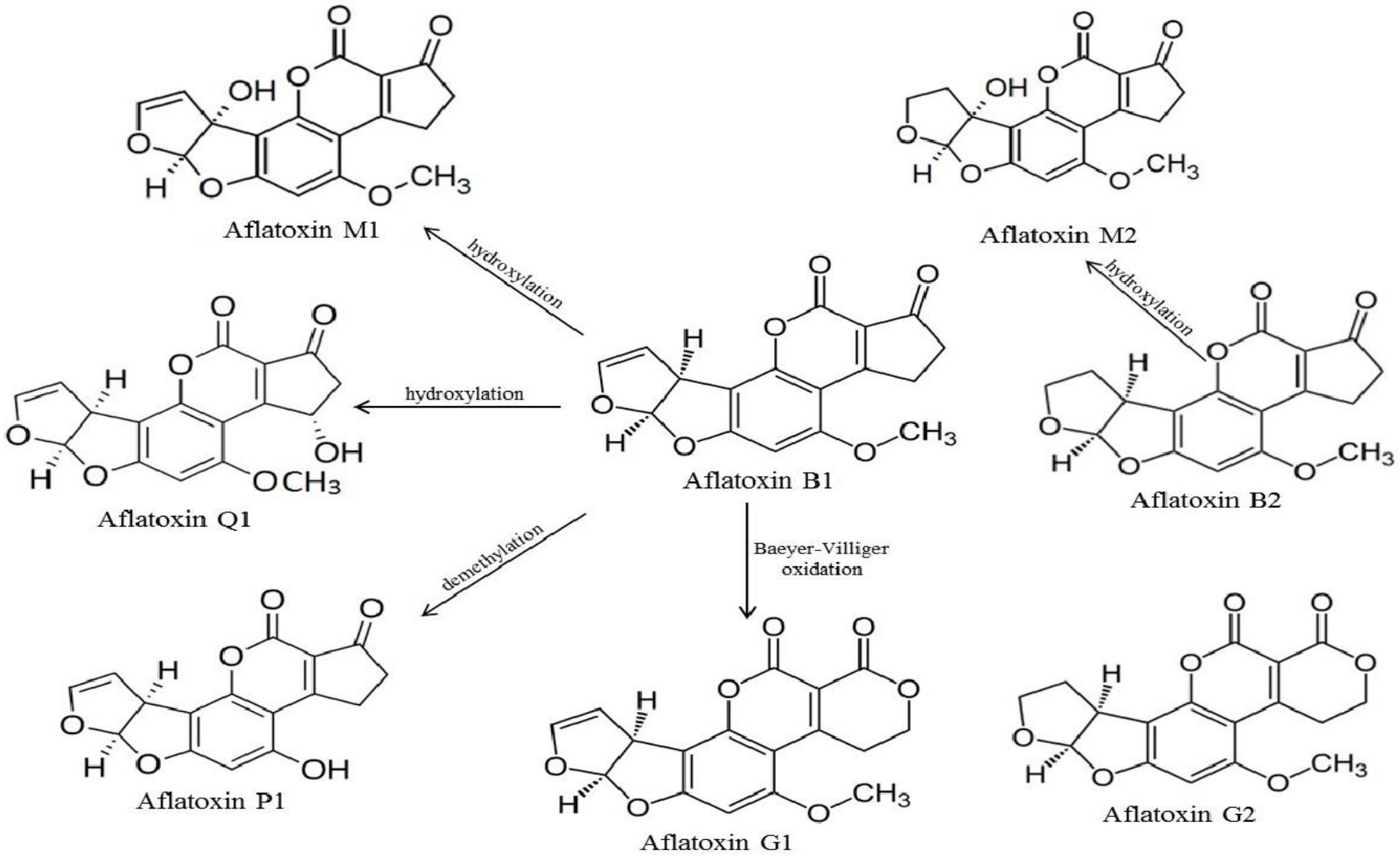


Food safety risks- Contaminants

Food Safety and Standards (Contaminants, Toxins and Residues) Regulations, 2011

- **Metal contaminants 10 (Pb, Cd, Cr, Mn, Ni, As, Sn, Cu, Hg, MeHg)**
- **Toxins: Mycotoxins (Aflatoxins (Total, B1, M1); Ochratoxin A; Patulin, DON)**
- **NOTS: Agaric acid, Hydrocyanic acid, Hypericine, Saffrole**
- **Chemicals: Polychlorinated biphenyls (Sum of PCB28, PCB52, PCB101, PCB138, PCB153 and PCB180); Polychlorinated biphenyls (Sum of PCB28, PCB52, PCB101, PCB138, PCB153 and PCB180); Benzo(a)pyrene; melamine**
- **Banned insecticides: 20 [The Extraneous MRL of the above mentioned banned insecticides shall be 0.01 mg/kg except for DDT for which it shall be 0.05 mg/kg.]**
- **Residues: Insecticides (150) with MRLs; Antibiotics and veterinary drug residues: 125**
- **Fish biotoxins and histamines**

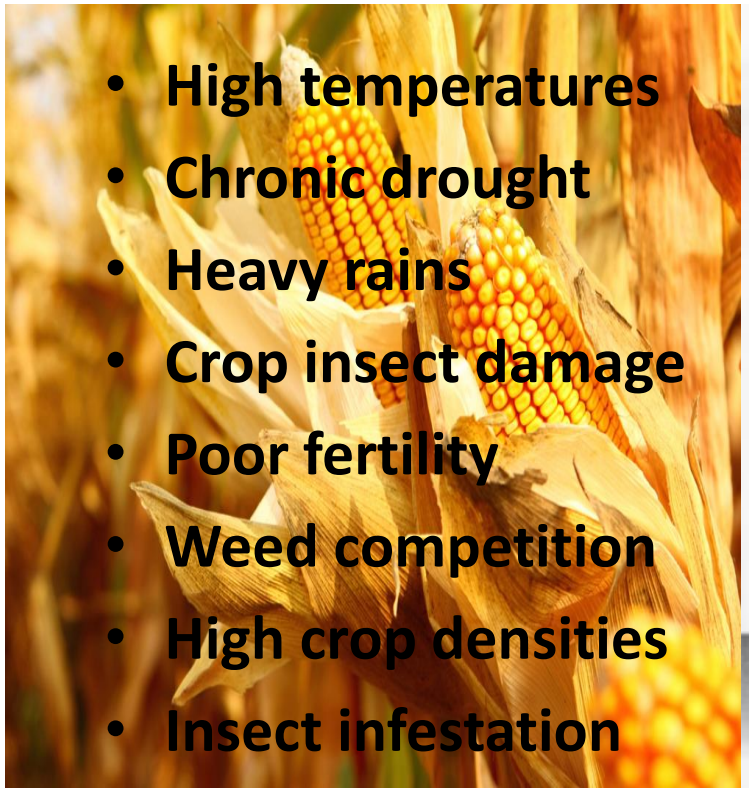
Transformation of aflatoxin B1



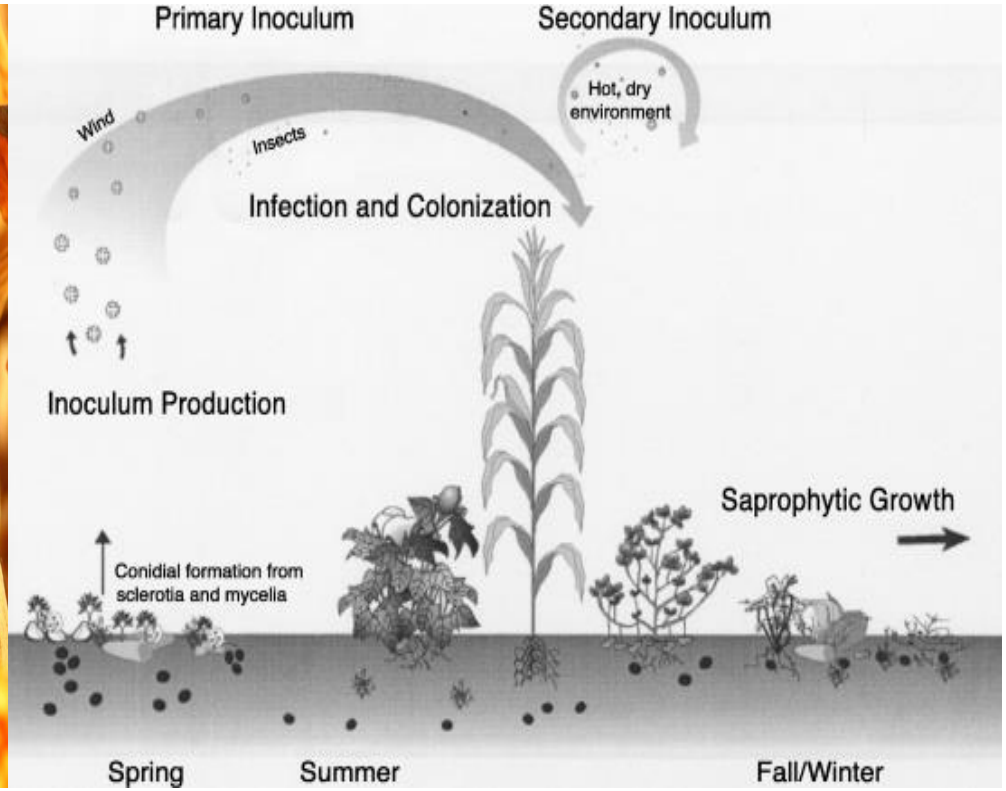
Sources of aflatoxin in milk

- **Feed ingredients**
  - molded grains
  - molded oilseed cakes
- **Stored feed**
  - post processing molding and mycotoxin production
- **Poor feed handling and feeding practices in dairy**

Pre-harvest factors causing mold infection in crops



- High temperatures
- Chronic drought
- Heavy rains
- Crop insect damage
- Poor fertility
- Weed competition
- High crop densities
- Insect infestation



Indian context

- **Regional Occurrence data**
  - **Cattle feed**
  - **In liquid milk**
    - **Primary vendor**
    - **Pooled sample**
  - **In milk products**
    - **Primary vendor**
    - **Pooled sample**
- **Processing factors**
- **Regional Consumption data/ Dietary survey**
  - **Fluid milk**
  - **Mil products**
- **Exposure assessment**
  - **Vulnerable groups**
  - **Hepatitis B infection**
- **Validating risk assessment**
- **Risk management**
  - **Regulating aflatoxin in feed**
  - **Reviewing current limits**

Prevalence: Aflatoxin in Groundnut

State	% Exceeded Limit
HR, MH	26-29
PB, UP, BR, OR, GJ, AS	20-24
KA, AP, WB	15-19
Median Levels	<5-20 ug/ kg
Max (AP)	125 ug/ kg
Total Samples	2062
% Exceeded Limit	21

Prevalence: Aflatoxin in Maiz

State	% Exceeded Limit
MH, MP, UP	32-52
GJ, AS, KA, HR	22-29
BR, OR, WB, AP	5-18
Median Levels	<5-35 ug/ kg
Max (UP)	160 ug/ kg
Total Samples	2074
% Exceeded PFA Limit	26

Prevalence: Aflatoxin in Rice

- **Samples of paddy (675) and milled rice (525) collected from 20 states across India.**
- **Assessed for *Aspergillus* spp. infection on selective medium and aflatoxin B_1 (AFB1) by indirect competitive ELISA.**
- ***Aspergillus flavus* contamination dominated in all the seed samples. The other major contaminants were *Aspergillus niger*, *Aspergillus ochraceus* and *Aspergillus parasiticus*.**
- **67.8% showed AFB1 ranging from 0.1 to 308.0 µg/kg**
- **All the paddy samples from Chattishgarh, Meghalaya and Tamil Nadu showed AFB1 contamination.**
- **Milled rice grains from different states showed below the permissible levels of AFB1 (average 0.5–3.5 µg/kg)**
- **Eighty-two percent of samples from open storage that were exposed to rain showed AFB1 contamination followed by one-year-old seed.**
- **Out of 1200 samples, 2% showed AFB1 contamination above the permissible limits (>30 µg/kg).**

K.R.N. Reddy, C.S. Reddy and K. Muralidharan Department of Plant Pathology, Directorate of Rice Research, Rajendranagar, Hyderabad – 500 030, Andhra Pradesh, India , Food Microbiology, 2008

Co-occurrence

Ingredients / feed	Number of positive samples in respective range of mycotoxin concentrations					
	Aflatoxin B_1, ppb		Ochratoxin A, ppb		T-2 toxin, ppb	
	0-50	50-100	0-50	50-100	0-50	50-100
Cereals	88	4	68	44	72	44
Cereal byproducts	48	8	4	16	32	8
Oilseed meals	152	8	64	40	60	24
Finished feed	328	76	200	80	268	96

Several recent surveys of the prevalence of mycotoxins indicate the seriousness of the mycotoxin problem in the Asia-Pacific Region. During the years 2004-2005, a survey was conducted to study the incidence of aflatoxin, ochratoxin and T-2 toxin in different feed ingredients and finished feeds collected from different states of the country. Out of 984 samples analyzed, 824 samples were found to be positive for the presence of aflatoxin, ochratoxin and T-2 toxin (Devegowda et al., 2005) (Table 2). 91, 94, 97 and 97% of cereals, cereal byproducts, oilseed meals and finished feeds, respectively, were tested positive for mycotoxins. These authors reiterated that aflatoxins are not the only problem in the region but also ochratoxins and T-2 toxin.

Aflatoxin in milk

- Factors affecting rate of carry over AFB1 from feed to milk (Duarte et al., 2013)
  - feeding regimens
  - rate of ingestion
  - rate of digestion
  - health of animal
  - hepatic biotransformation capacity,
  - actual milk production
- There is a linear relationship between AFB1 in feed consumed and AFM1 secreted of in milk (Kamkar, 2005)
- Appears in milk in 2-3 days following ingestion
- 2-3 days are required to reduce the AFM1 to zero level when a diet without aflatoxins is fed (Prandini et al., 2009)

National Milk Survey 2018

Test group Parameter	Samples, numbers	Processed, No. of samples	Processed, %	Raw, No. of samples	Raw, %	Overall, %
Total NC with safety issue	638	450	17.3	188	4.9	9.9
NC for Aflatoxin M1	368	227	8.6	141	3.7	5.7

AF M1 was detected in 368 (out of 6,432 samples), that is 5.7% of the samples had Aflatoxin at levels above the permissible limit. TN (24%), NCT (10%), Kerala (10%), Pb (8%), UP (8%), MH (5%), OR(5%), GJ (3%).

Risk assessment

Estimating magnitude and probability of a harmful effect to individuals or populations

- **Hazard identification**
  - **determining whether exposure can increase the incidence of a adverse health effect**
- **Dose–response assessment**
  - **Epidemiological studies on the relationship between exposure to a mycotoxin and particular harmful effects**
  - **Cohort studies**
  - **Case–control studies**
  - **Human studies**
  - **Animal studies**
  - **Cell and tissue culture studies**
  - **Toxic effects**
  - **Carcinogenic effects**
  - **Genotoxic effects**

Risk assessment

- **Exposure setting**
  - **Physical conditions**
  - **Populations exposed**
    - **Questionaires**
    - **Food dieries**
    - **Biomarkers**
  - **Exposure pathways**
  - **Quantify exposure**
    - **Average daily dose**
    - **Lifetime average daily dose**
- **Risk characterization of non-carcinogenic toxins**
- **Risk characterization of carcinogens**
  - **Risk = Life time average daily dose (LADD) x slope factor (slope factor of a carcinogen is derived by taking the slope of the linearized dose–response curve)**

AFB_1 risk assessment JECFA

- For non-carcinogenic toxins
  - ADI = NOAEL/ Safety Factor
  - Exposure < ADI or Exposure > ADI
- For carcinogenic toxins (Aflatoxin)
  - The average cancer potency (cancers/year/ 100,000/ ng kg^{-1} bw day^{-1})
    - $HBsAg^+$ = 0.3
    - $HBsAg^-$ =0.01
  - Exposure (ng/ kg)= Contamination levels (ug/ kg)x Amount consumed (g)/ body weight/kg
  - Population risk = Exposure × Average potency
  - Say the estimated AFB_1 intake
  - Maize is 0.10- 6 ng kg^{-1} bw day^{-1}
  - Peanuts is 0.8- 69 ng kg^{-1} bw day^{-1}
  - These data indicate that the risk from peanut was about 10 times or more

Current status of aflatoxins limits under FSSR

Name of the contaminant	Article of food	Limit ug/kg
Aflatoxin	Cereal and cereal products	15
	Pulses	15
	Nuts	15
	RTE Nuts	10
	Dried figs	10
	Oil or oilseeds	15
	RTE oilseeds	10
	Spices	30
	Arecanut/ betelnut	15
Aflatoxin M1	Milk	0.5
Ochratoxin A	Wheat, barley and rye	20
Patulin	Apple juice and Apple juice ingredients in other beverages	50
Deoxynivalenol	wheat	1000

Proposed aflatoxins limits under FSSR

Name of the contaminant	Article of food	Total Aflatoxin	Aflatoxin B1
		Limit ug/kg	
	Cereal and cereal products	15	10
	Pulses	15	10
	Nuts	15	10
	RTE Nuts	10	10
	Dried figs	10	10
	Oil or oilseeds	15	10
	RTE oilseeds	10	10
	Spices	30	15
	Arecanut/ betelnut	15	10
	Food product containing any of the above mentioned foods	20	10

Proposed mycotoxins limits under FSSR

Aflatoxin M1	Milk (Liquid)	0.5
	Skimmed milk powder	**6**
	Whole milk powder	**4**
Ochratoxin A	Wheat, rye, barley	20
Patulin	Apple juice	50
	Apple juice used as an ingredient in other beverages	50
Deoxynivalenol	Wheat	1000

Codex Alimentarius Comission recommendation is 0.5 ng/g

EC Legislative limits of mycotoxins in food and feedstuffs (EC 1881/2006)

- Oilseeds: Total 4 ppb, AFB_1 2 ppb (µg/kg); 8 & 15 ppb respectively for oilseeds for further processing
- Tree nuts: Total 10, AFB_1 5 ppb
- Baby milk/food: AFB1 $\leq$ 0.1 & AFM1 0.05
- OTA - 10 ppb in dried vine fruits & soluble coffee; 5 ppb for roasted coffee; 6-10 ppb for instant coffee; 2 ppb for wine and grape juice; 0.2 ppb for beer
- OTA - 15-30 ppb in spices applicable from 1 July 2010

U.S. Food and Drug Administration Guidelines for Aflatoxin Levels

Aflatoxin Level (ppb)	Commodities & Species
20 ppb	**Corn, peanut products, cottonseed meal and other animal feeds and feed ingredients intended for dairy animals; for animal species or uses not specified below, or when the intended use is not known**
20 ppb	**Corn, peanut products and other animal feeds and feed ingredients, but excluding cottonseed meal, intended for immature animals**
100 ppb	Corn and peanut products intended for breeding beef cattle, breeding swine or mature poultry (e.g. laying hens)
200 ppb	Corn and peanut products intended for finishing swine (100 pounds or more)
300 ppb	Cottonseed meal intended for beef cattle, swine or poultry (regardless of age or breeding status)
300 ppb	Corn and peanut products intended for finishing beef cattle (i.e., feedlot cattle)

Corn containing less than 20 parts per billion aflatoxin is considered safe by the U.S. Food and Drug Administration for use in all animal feed. Corn exceeding 20 ppb can be fed only for specific age, weight, and production stage. Corn exceeding 300 ppb must be blended with corn containing little or no aflatoxin before feeding is allowed to specific age, weight, and production stage of livestock. Before corn can be blended, the U.S. Food and Drug Administration must be notified and permission obtained from the Missouri Department of Agriculture for this to be allowed.

Action Levels (ng/ g) for Total Aflatoxins

Commodity	USFDA	Codex	EU	India
Animal Feed	300			
Poultry Feed	100			
Milch Animal Feed	20			
Foods	20	10	10/ 4	15
Milk	0.5	0.5	0.5	0.5

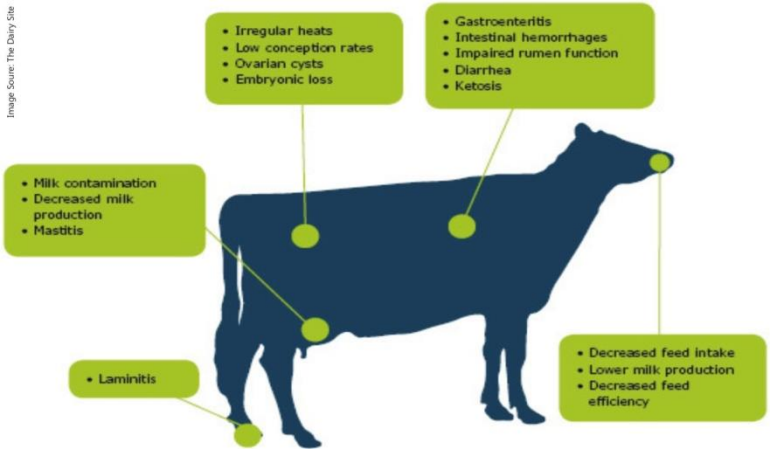
Detection limit 1 ng/ g in foods; 0.05 ng/ml for M1

AFM1: Risk management

- **Feed manufacturers**
  - Check raw materials for AFB1 at procurement
    - Cereal grains
    - Oil cakes
    - Other constituents
  - Check raw material before processing
  - Check AFB1 after processing
  - Check AFB1 during storage and shelf-life
  - Instruction for use
- **Regulating aflatoxin B1 in feed**
- **Incentivizing afla-free feed**
- **Awareness and education of farmers**
  - GAP for afla-free produce
  - Incentivising afla-free produce

AFM1: Risk management

- **Milk producers**
  - Mold / aflatoxin free feed
  - Monitoring animal health
  - Preventive measures
  - Ensuring aflatoxin free milk
- **Dairy**
  - Checking in-coming milk for AFM1
  - Tracing source of AFM1
  - Eliminating AFM1 contaminated milk
  - Incentivizing afla-free milk
- **Developing afla-free logo**
- **Awareness and education of farmers**



Food Safety- A shared responsibility

Thank You