Microbial Toxins in Food: Focus on Aflatoxin

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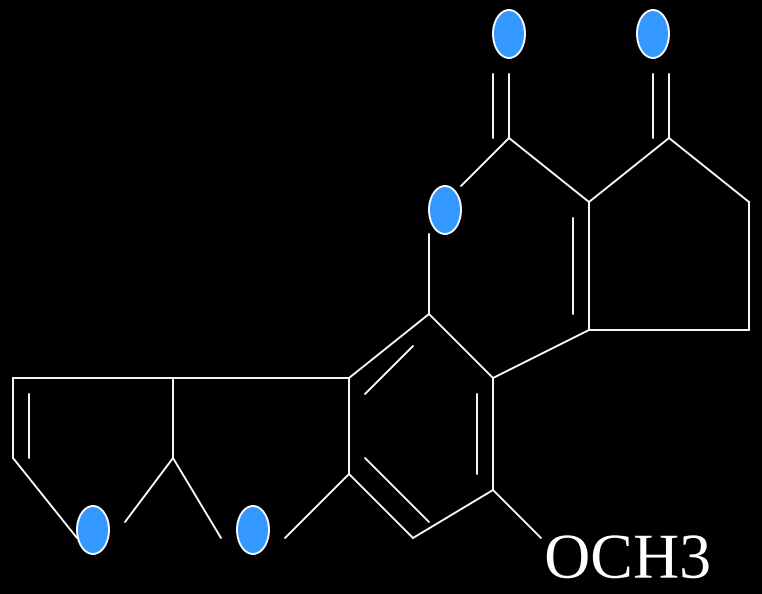
Why aflatoxin?

- Potent carcinogen
- Presence in foodstuff requires special techniques for detection
- Very heat-stable and not easily degraded
- Could be an unrecognized killer in the food that we eat

AFLATOXINS:

A group of polyketide-derived furanocoumarin toxins produced by some *Aspergillus* species, of which the most toxic and carcinogenic member is aflatoxin B1 (AFB1).



AFB1

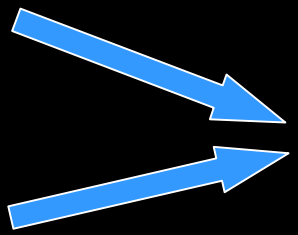
FOUR MAJOR TYPES OF AFLATOXINS:

B1 : $C_{17}H_{12}O_6$

B2 : $C_{17}H_{14}O_6$

G1 : $C_{17}H_{12}O_7$

G2: $C_{17}H_{14}O_7$



Dihydroxy derivatives

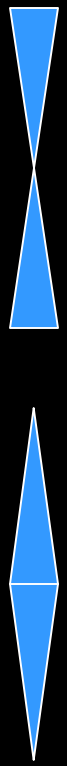
ADDITIONAL METABOLIC PRODUCTS:

M1 : 4-hydroxy AFB1

M2 : 4-dihydroxy AFB2

Known aflatoxin-producing species include:

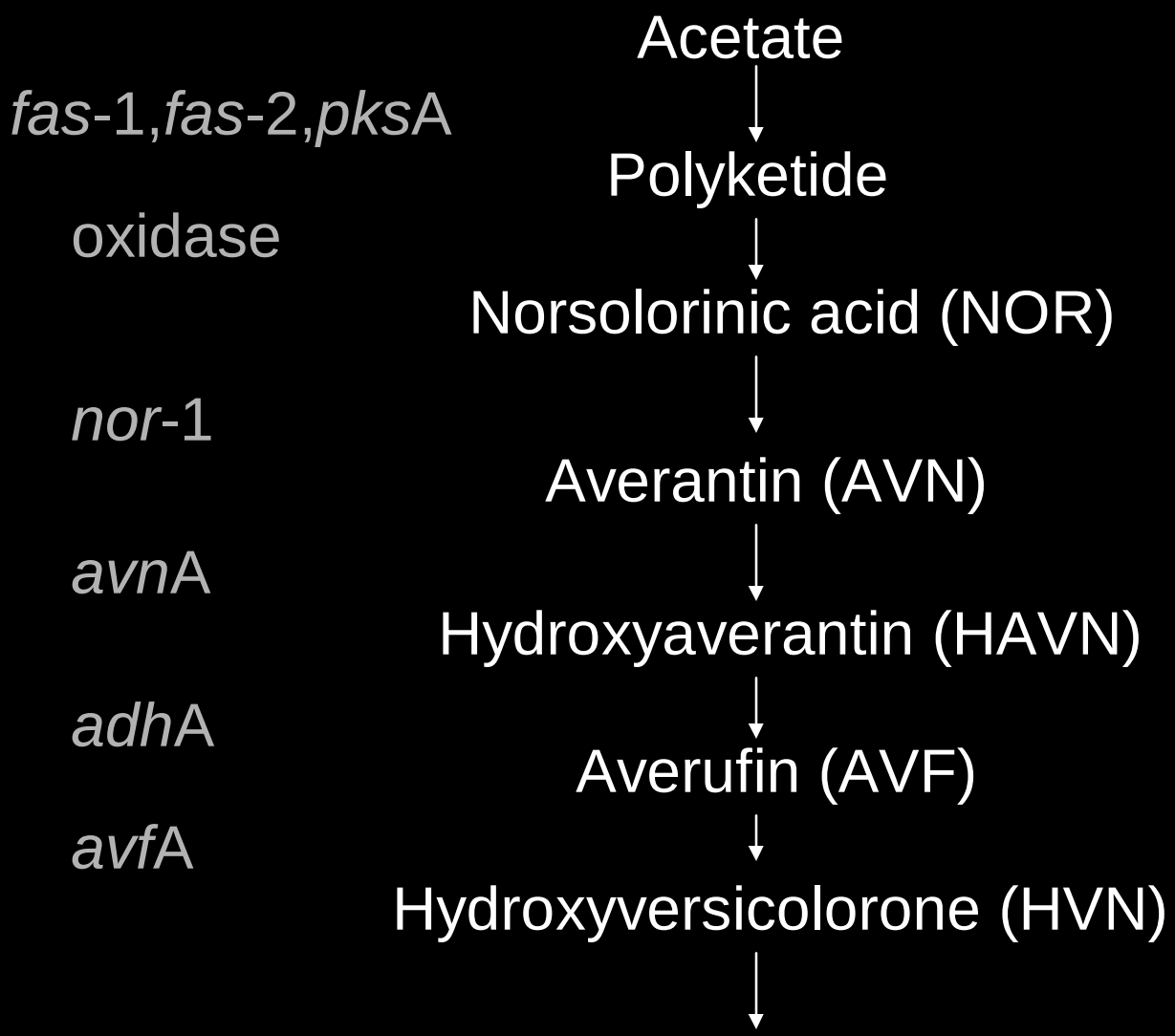
1. ***A. flavus***
2. ***A. pseudotamarii***
3. ***A. ochraceoroseus***
4. ***A. nomius***
5. ***A. bombycis***
6. ***A. parasiticus***
7. **West African unkown taxon**

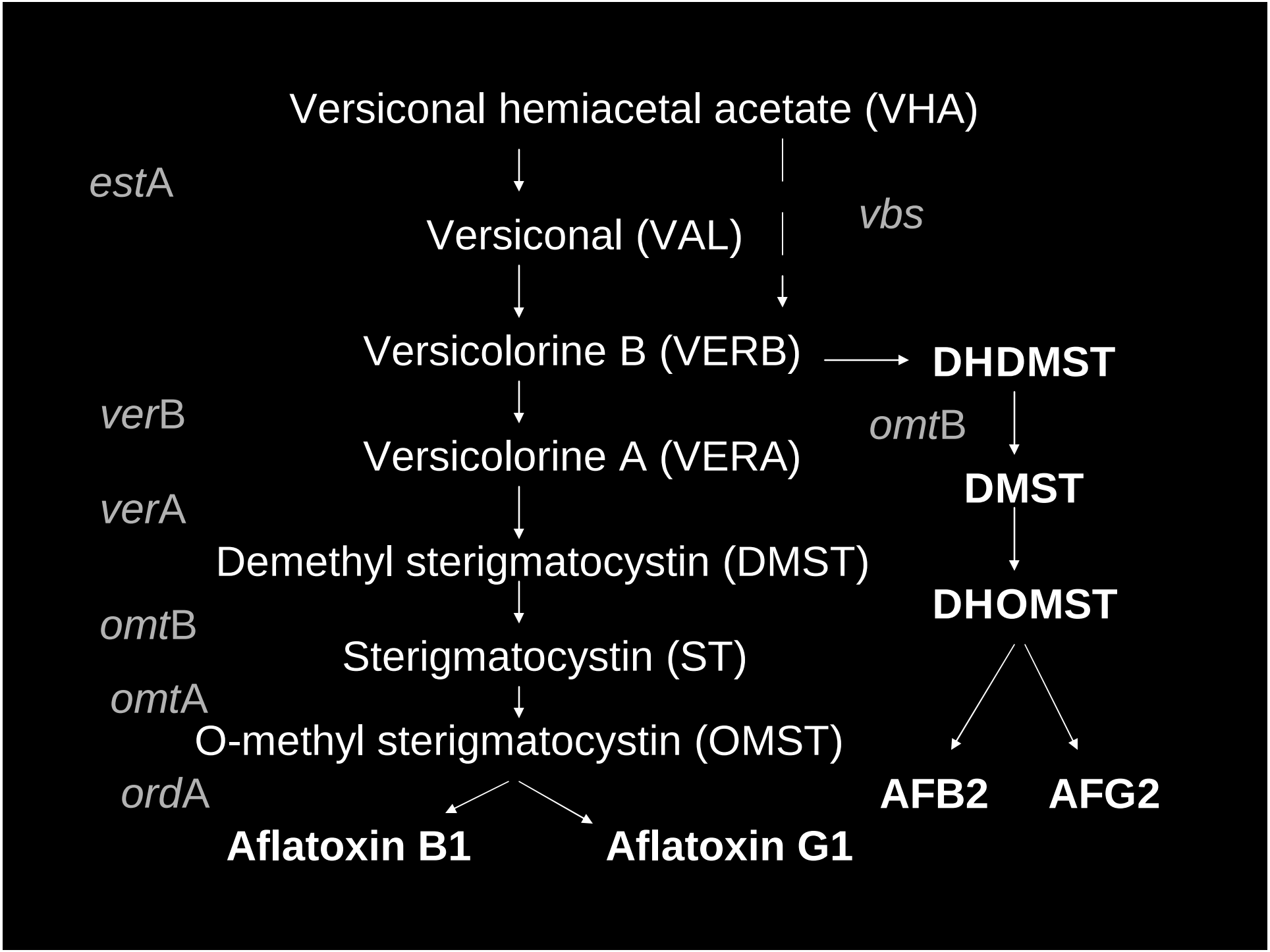


Produce only B aflatoxins

Produce both B and G aflatoxins

AFLATOXIN BIOSYNTHESIS




Versiconal hemiacetal acetate (VHA)
estA
Versiconal (VAL)
vbs
Versicolorine B (VERB)
DHDMST
verB
omtB
Versicolorine A (VERA)
DMST
verA
Demethyl sterigmatocystin (DMST)
DHOMST
omtB
Sterigmatocystin (ST)
omtA
O-methyl sterigmatocystin (OMST)
ordA
AFB2
AFG2
Aflatoxin B1
Aflatoxin G1

Important Mycotoxins (Sales, 2008)

HUMAN CARCINOGEN (Class 1 & 2B, IARC* 1993)

Aspergillus	*Aspergillus, Penicillium*	*Fusarium*		
AF/AFM1	OTA	Zea	NIV & DON	FM
(1961)/(1963)	(1965)	(1965)	(1968-1973)	(1988)
Peanut & products Corn & products Edible nuts, spices other cereals fruits, feeds Animal & diary Products	Cereals, coffee bean Edible tissue ham-bacon, sausage milk, dairy products	Cereal & products, corn, wheat, barley rice, oats, walnut		Corn & products
Liver cancer Reyes syndrome Acute hepatitis Indian childhood cirrhosis Fatty liver Changes Kwashiorkor Bile duct cell Profeleration Haemorrhages	Balkan Nephropathy Renal Tumors	Premature Telarche Cervical cancer Esophageal cancer	Red mould disease Esophageal cancer	Esophageal cancer

* International Agency for Research on Cancer

Mechanisms for aflatoxin exposure

(Sales, 2008)

Aflatoxins in foods and feeds

Processing

Dusts in occupational environments

Aflatoxins/ Aflatoxigenic fungi in dusts

Inhalation

Respiratory tract

CANCER

Digestive tract

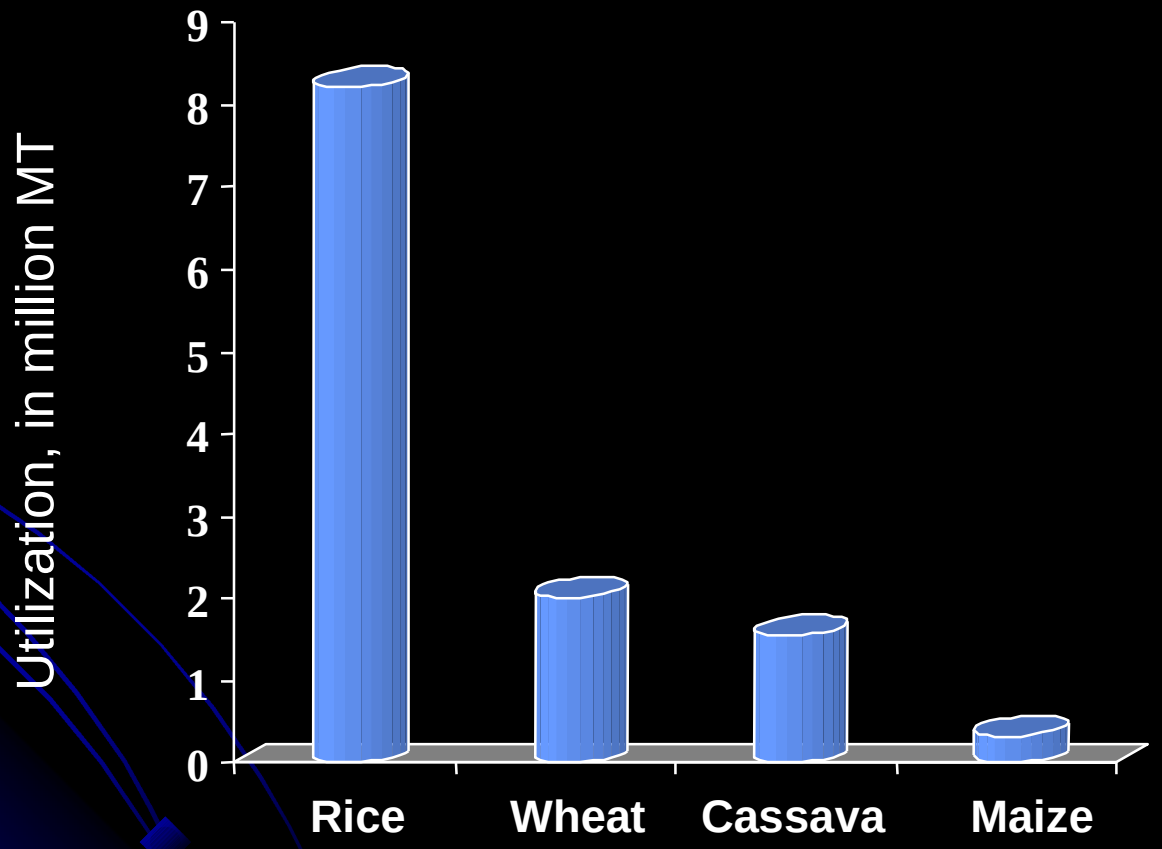
Ingestion by humans

Ingestion by animals

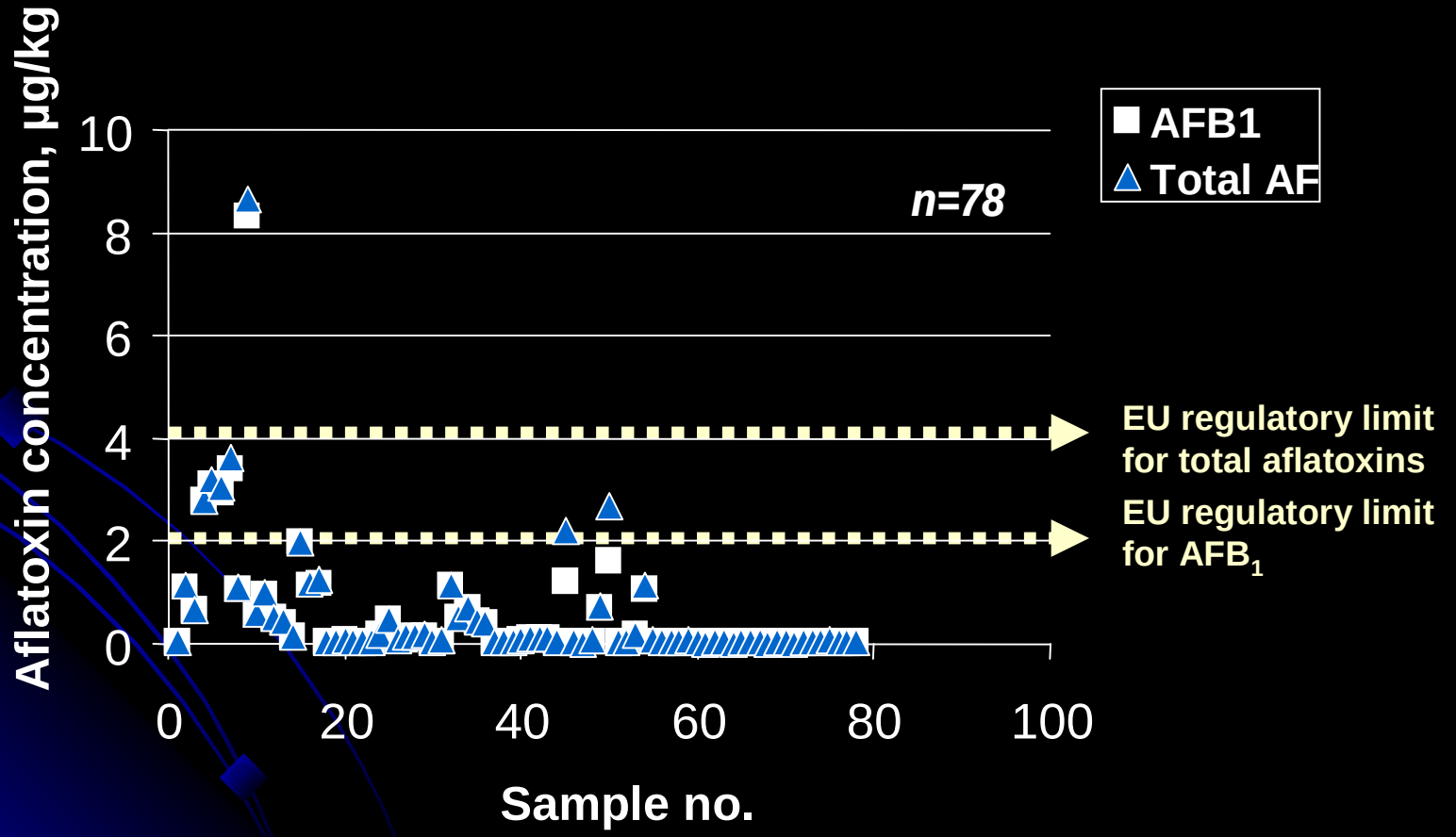
Aflatoxins in animal products

Utilization of major agricultural crops in the Philippines, 2005

(Sales, 2008)

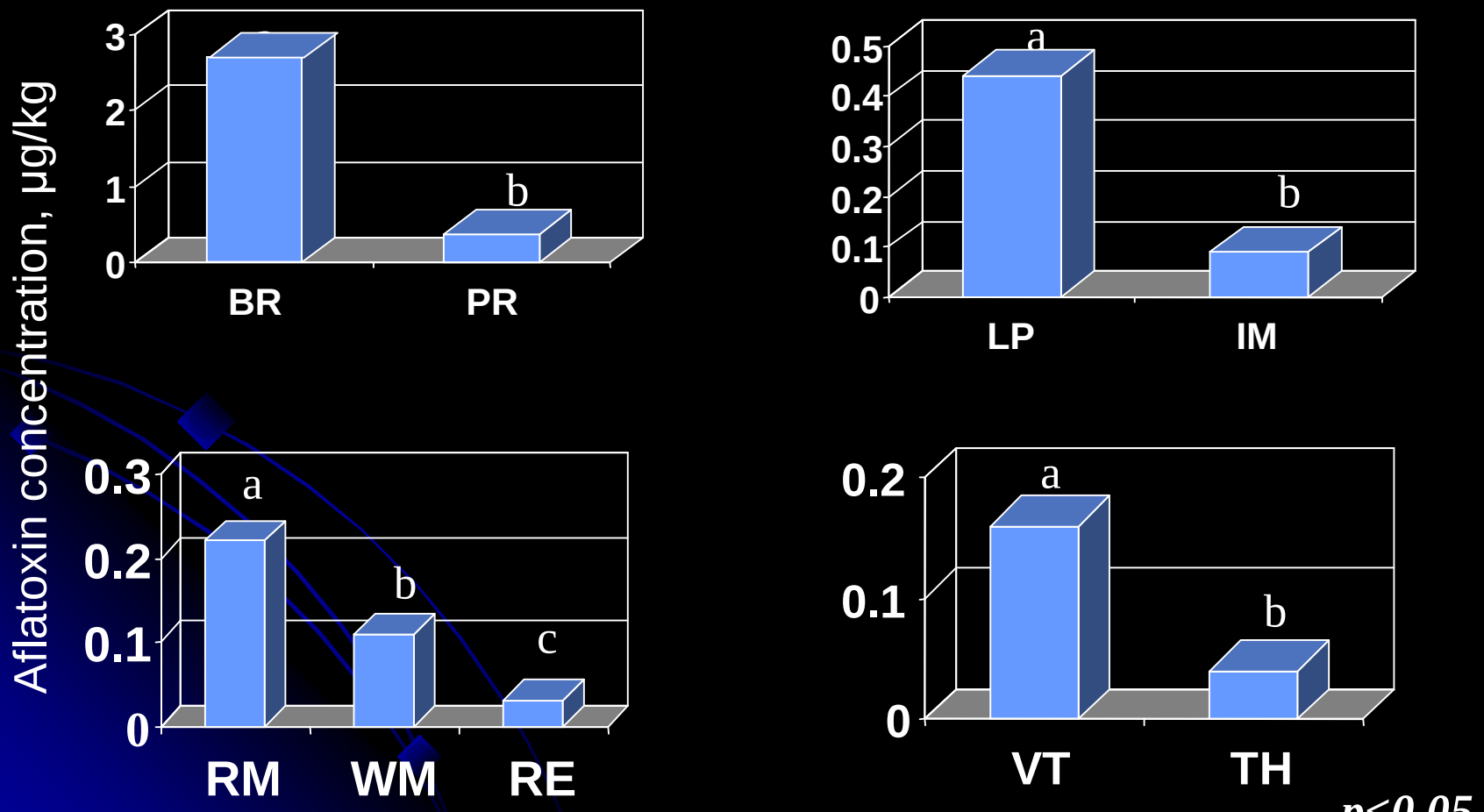


Levels of aflatoxins in polished and brown rice from the Philippines vis a vis EU regulatory limits (Sales, 2008)



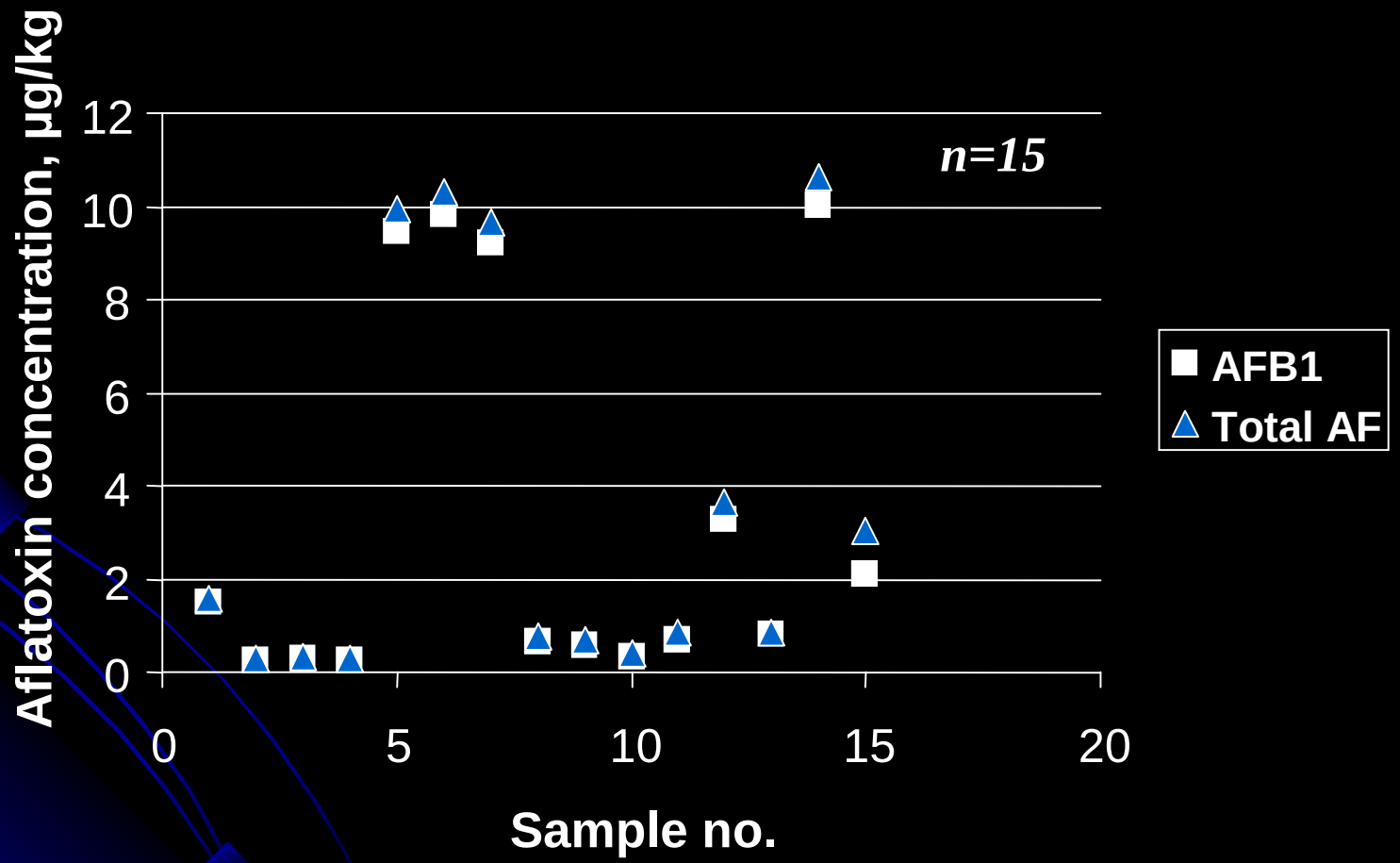
Aflatoxin profiles of brown and polished rice from the Philippines

(Sales, 2008)

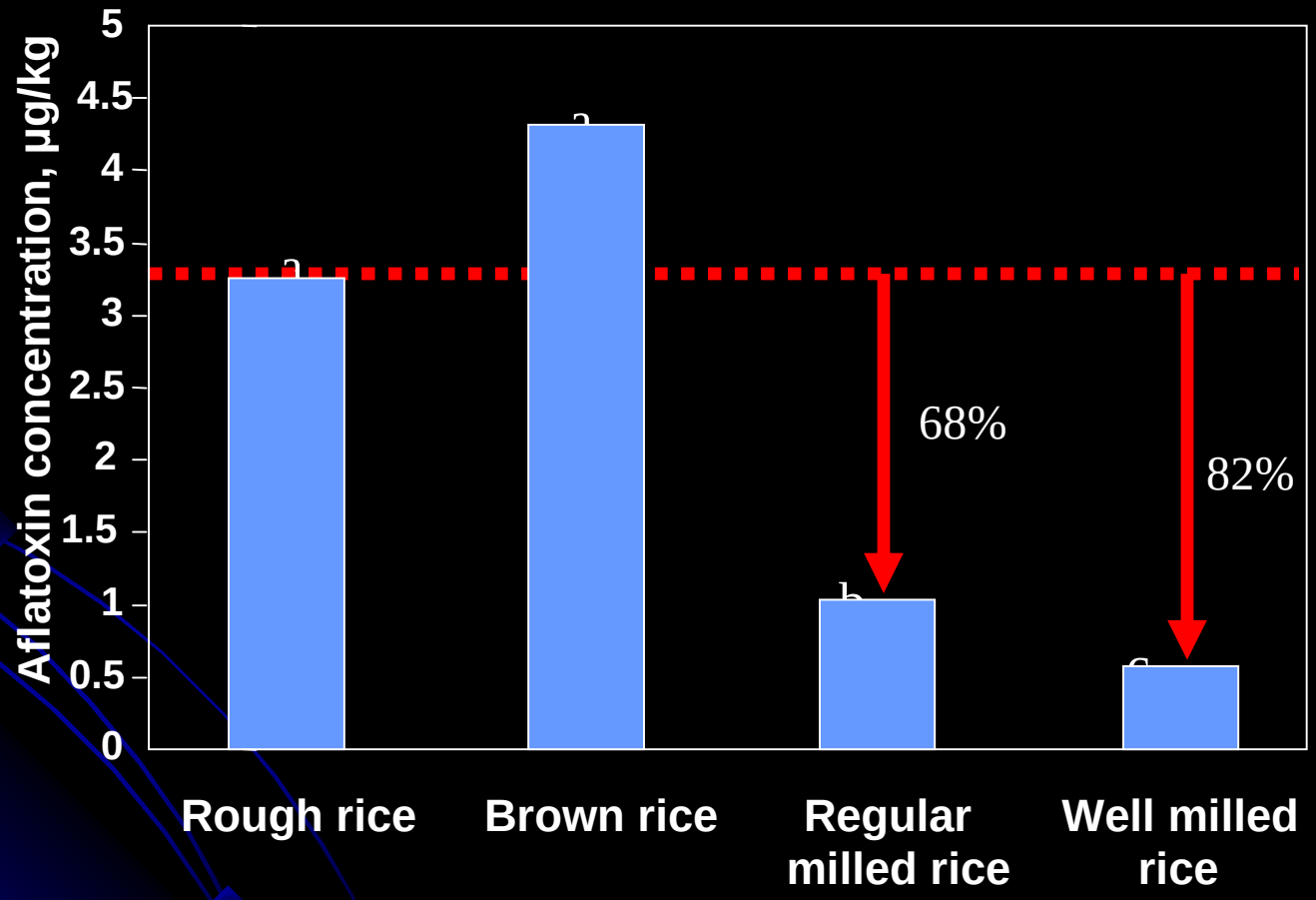


$p \leq 0.05$

Levels of aflatoxins in rice hulls, rice bran, and settled dust (Sales, 2008)



Reduction in aflatoxins during milling of rice (Sales, 2008)



n=3; p≤0.05

BIOLOGICAL EFFECTS:

Biologically, aflatoxins cause aflatoxicosis when taken in high concentrations characterized by vomiting, abdominal pain, pulmonary edema, convulsions, coma and death with cerebral edema and fatty conditions of the liver, kidneys and heart.

Chronic exposure to aflatoxins by ingestion can cause hepatocellular carcinoma or hepatoma.

Aflatoxins are also recognized as teratogenic, mutagenic, carcinogenic agents, immuno-suppressants, and potent inhibitors of protein synthesis.

Figure 1. Liver with hepatocellular carcinoma.

Corn, peanuts, cottonseed, tree nuts, including coconut, are the major agricultural crops affected by aflatoxins.

Screening of food for human consumption and animal feeds revealed the presence of aflatoxins worldwide but more commonly in Asian and African countries.

Regulatory guidelines have been established because of health concerns for foodstuff.

Allowable limit for aflatoxins in foodstuff is 20 ppb in the U.S. and <5 ppb in the E.U.

Table 1. Acceptable aflatoxin levels in food/feed (U.S.).

AFLATOXIN LEVELS (ppb)	COMMODITIES
20	CORN/OTHER GRAINS FOR IMMATURE ANIMALS AND DAIRY ANIMALS; OTHER KINDS OF ANIMAL FEEDS
100	CORN/OTHER GRAINS FOR BREEDING BEEF CATTLE, BREEDING SWINE, MATURE POULTRY
200	CORN/OTHER GRAINS FOR FINISHING SWINE OF 100 POUNDS OR GREATER
300	CORN/OTHER GRAINS FOR FINISHING BEEF CATTLE; COTTONSEED MEAL FOR BEEF CATTLE, SWINE OR POULTRY

Correlation between aflatoxin ingestion and hepatocellular carcinoma is well-established primarily because most studies on biological effects of aflatoxins are focused on their presence in foodstuff.

Potential exposure risk due to aflatoxins in Philippine rice (Sales, 2008)

Concentration of AFB_1 in polished rice, µg/kg	Toxin intake[a] µg/person/day	PDI[b], µg/kg of bw/day	Ratio of PDI/TDI[c]	Ratio of PDI/TD_{50}[d]
0.50 (mean)	0.05	0.0010	**9.10-5.30**	0.0017
1.23 (90th percentile)	0.35	0.0070	63.64-36.84	0.0061

[a] Calculated on the basis of daily average rice intake of Filipinos of 282 g.
[b]Probable daily intake (PDI), calculated on the basis of a body weight (bw) of 50 kg.
[c]Tolerable daily intake (TDI) of 0.11-0.19 ng/kg of bw/day in Asia, at a risk level of 10^{-5}.
[d]TD_{50} of 1.15 µg/kg of bw/day in rat, the dose at which 50% of the animals would have developed tumors

A prospective risk assessment of exposure to aflatoxins by inhalation in agricultural workers

	Aflatoxin concentration in dust, ng/g	Airborne dust concentration, mg/m^3	Airborne aflatoxin concentration, ng/m^3	Amount of aflatoxins inhaled, ng	
				In an 8-h Work-shift	In a 40-h workweek
Rice milling					
Minimum	**1.51**	**60.00**	**0.09**	**0.72**	**3.62**
Maximum	**91.61**	**80.71**	**7.39**	**59.15**	**295.75**
Corn milling					
Minimum	1.01	7.00	0.01	0.06	0.28
Maximum	34.16	417.00	14.22	113.80	568.99
Feed compounding					
Minimum	1.40	26.20	0.04	0.29	1.47
Maximum	37.90	350.00	13.26	106.12	530.60
Copra processing					
Minimum	5.08	7.90	0.04	0.32	1.60
Maximum	26.10	18.90	0.49	3.95	19.73

Talking about inhalation…..

Very few people realize that aflatoxin may also be present in tobacco.

Fungal contamination of tobacco leaves during storage is the most common cause for its occurrence.

Although first reported in the U.S. in the 1960s, its significance was seemingly downplayed perhaps because of the great economic implications of the findings.

Aflatoxins retain their potent carcinogenicity in primary and secondary smoke (=environmental tobacco smoke or ETS), primarily because of their extraordinary heat stability, which only begin to decompose at 269°C, way above the combustion temperature of a lighted cigarette.

How are aflatoxins related to other potential carcinogens in cigarette smoke, particularly benzopyrene?

- **Aflatoxin B1 is 200x more potent than benzopyrene as a carcinogen.**
- **Use of smokeless tobacco products often leads to oral cancers in a few years, therefore in theses cases, benzopyrene is not the culprit, which is mainly generated only through combustion.**
- **Uncombusted aflatoxin may be the causative agent or promoter of the early onset of oral malignancies.**

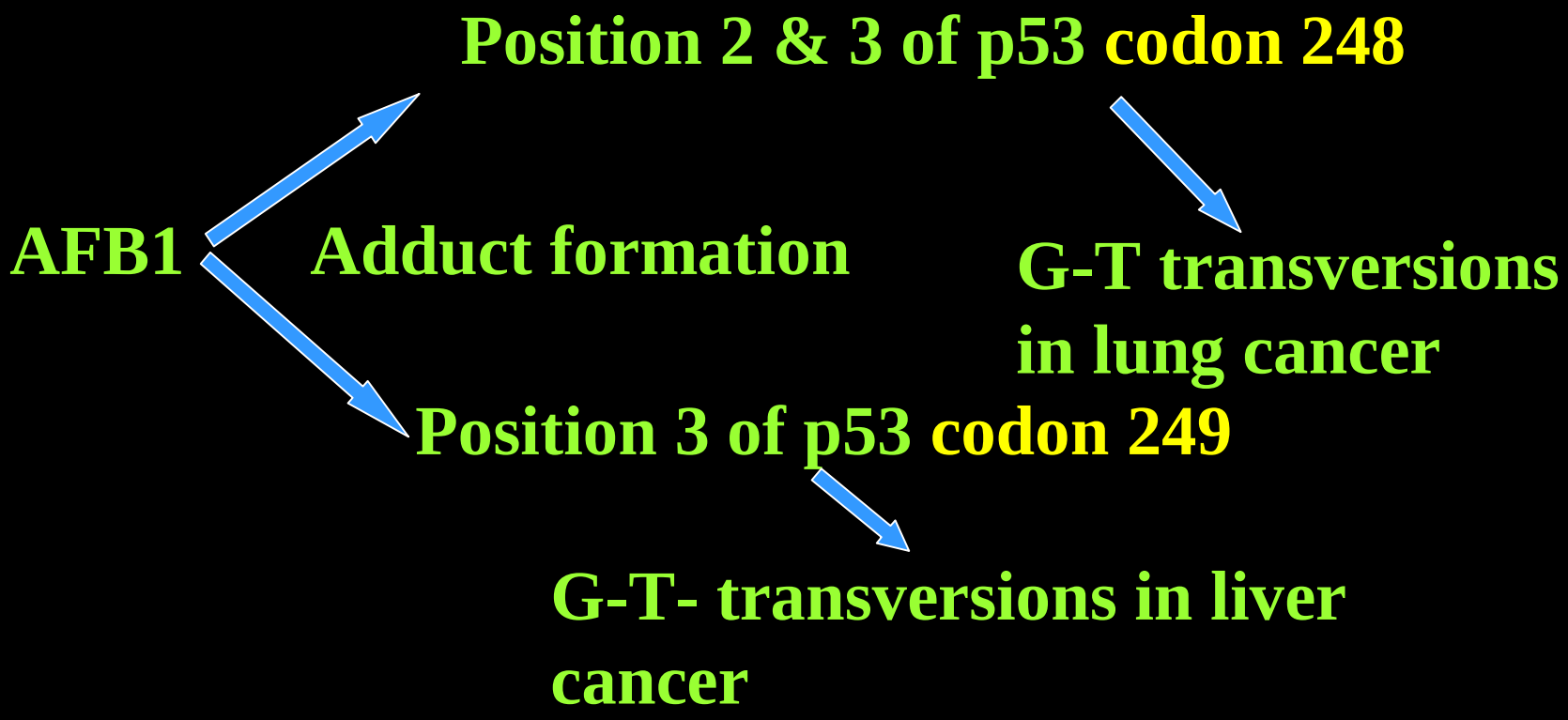
The Lung Cancer Connection

In animal model studies, AFB1 is a pulmonary carcinogen.

Epidemiological studies have shown an association between AFB1 exposure and lung cancer in humans.

A Common Theme at the Molecular Level

AFB1 causes p53 tumor-suppressor gene and *ras* gene mutations.



Approximately ½ of all human cancer cases exhibit p53 mutations, many are consistent with an aflatoxin etiology.

G:C to T:A transversions are the most common substitutions in cancers of the lung, breast, esophagus and liver.

p53 mutation studies

Environmental carcinogens are a cause of:

Breast cancer (44-50%)

Esophageal cancer (44-50%)

Lung cancer (56%)

Ovarian cancer (44-50%)

Prostate cancer (44-50%)

Skin cancer (44-50%)

p53 mutations have been found in lung cancer patients exposed to ETS who are either smokers or non-smokers. This may compound the problem with agricultural workers who are also smokers.

CONCLUSION

Mode of entry of aflatoxin into the human body could be through the diet and inhalation, the former being more established than the latter. However, effects of inhaled aflatoxin either from agricultural mills or from tobacco smoke need to be investigated seriously as this may have a more significant contribution to the high incidence of lung cancer on a global scale.

Possible link with prevalence of heart disease may be deduced from the fact that aflatoxin also mediates the uncoupling of control of cholesterol feedback regulation in hepatocellular carcinoma cases.

Thank you for your attention.

Daghang Salamat.