# Mycotoxin

**Secondary metabolite of fungi**
**Contaminating food and feeds**
**Environmental contamination**

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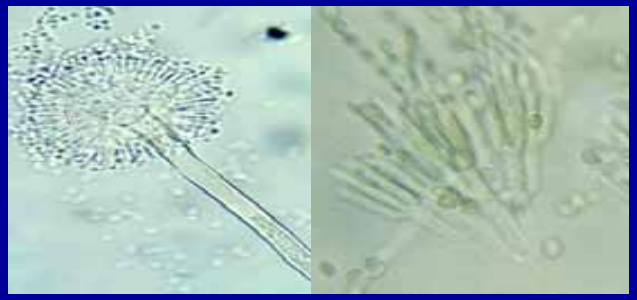


## Zearalenone

***Fusarium graminearum, F. Crookwellence*** **etc**

**Corn, wheat, barley, sorghum**

**Hyperestrogenism, hepatotoxic, hepatocarcinogenic**

**α-Zearalenol, β-Zearalenol**

**PMTDI=0.5 μg/kg bw (JECFA)**

## Trichothecenes

**A group of mycotoxin having a common structure, T-2 toxin, deoxynivalenol, nivalenol, etc.**

## Deoxynivalenol, Nivalenol

***Fusarium graminearum*** **etc**

**Wheat, barley, corn, processed foods derived from these**

**Vomit, feed refusal, diarrhea, decrease of leukocyte, depression of immune function**

**DON, PMTDI=1 μg/kg bw (JECFA)**

## Fumonisin

***Fusarium moniliforme, F.proliferatum* etc**

**Corn, processed food from corn**

**Human esophagal cancer, ELEM (equine leukoencephalomalacia), PPE (porcine pulmonary edema), human neural tube defect**

**PMTDI=2 μg/kg bw (JECFA)**

## Ochratoxin A

***Penicilium verrucosum, Aspergillus ochraceus etc***

**Wheat, dried grape, corn, wine, beer, coffee, pork kidney**

**Human endemic nephropathy & urinary tract tumor, porcine nephropathy, carcinogenic, nephrosis**

**PTWI = 100 ng/kg bw/week**

## Ergot alkaloids (ergotamine, ergotoxine, etc.)

***Claviceps purpurea* etc., *Neotyphodium coenophialum* etc.,**

**Rye, wheat, barley, rice, oats, corn, rye grass, tall fescue**

**Vasoconstriction, gangrene, neurotoxic symptoms, abortion in human and farm animals**

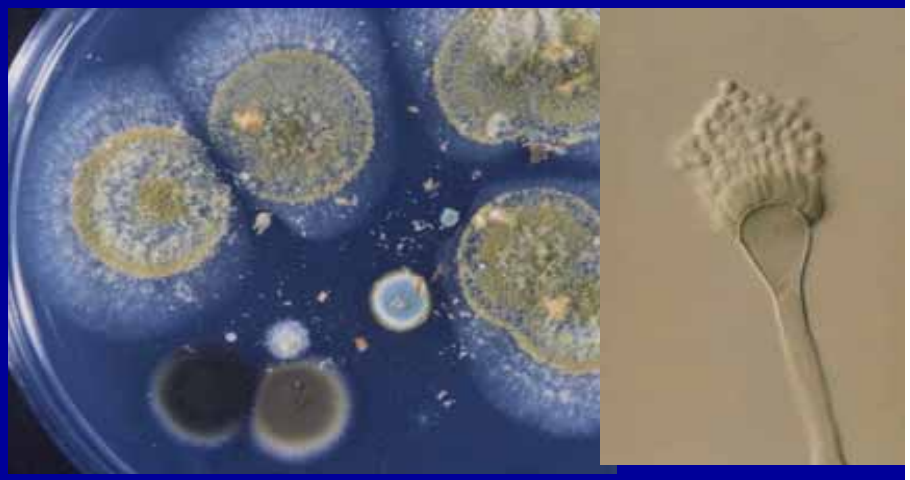
**Limit for feed contamination in some countries.**

# Aflatoxin

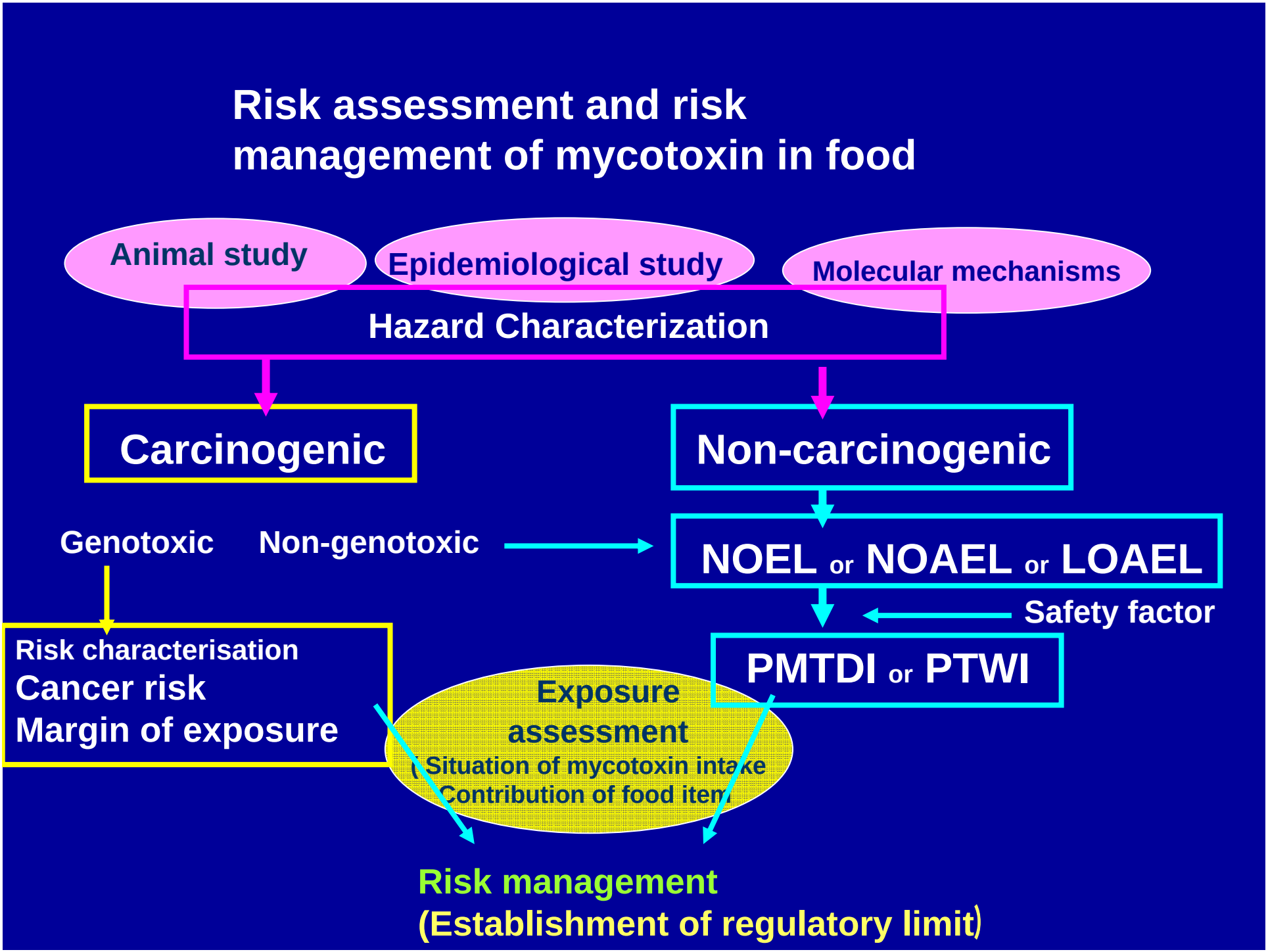
- In 1960, Spring-Summer：Turkey-X disease in England
- Causal feedstuff：peanut meal
- Food: nuts, spices, corn, cacao, coffee, rice, milk
- Producer：*Aspergillus flavus, A. parasiticus, other species*
- Toxicity：

  Acute—hepatotoxic

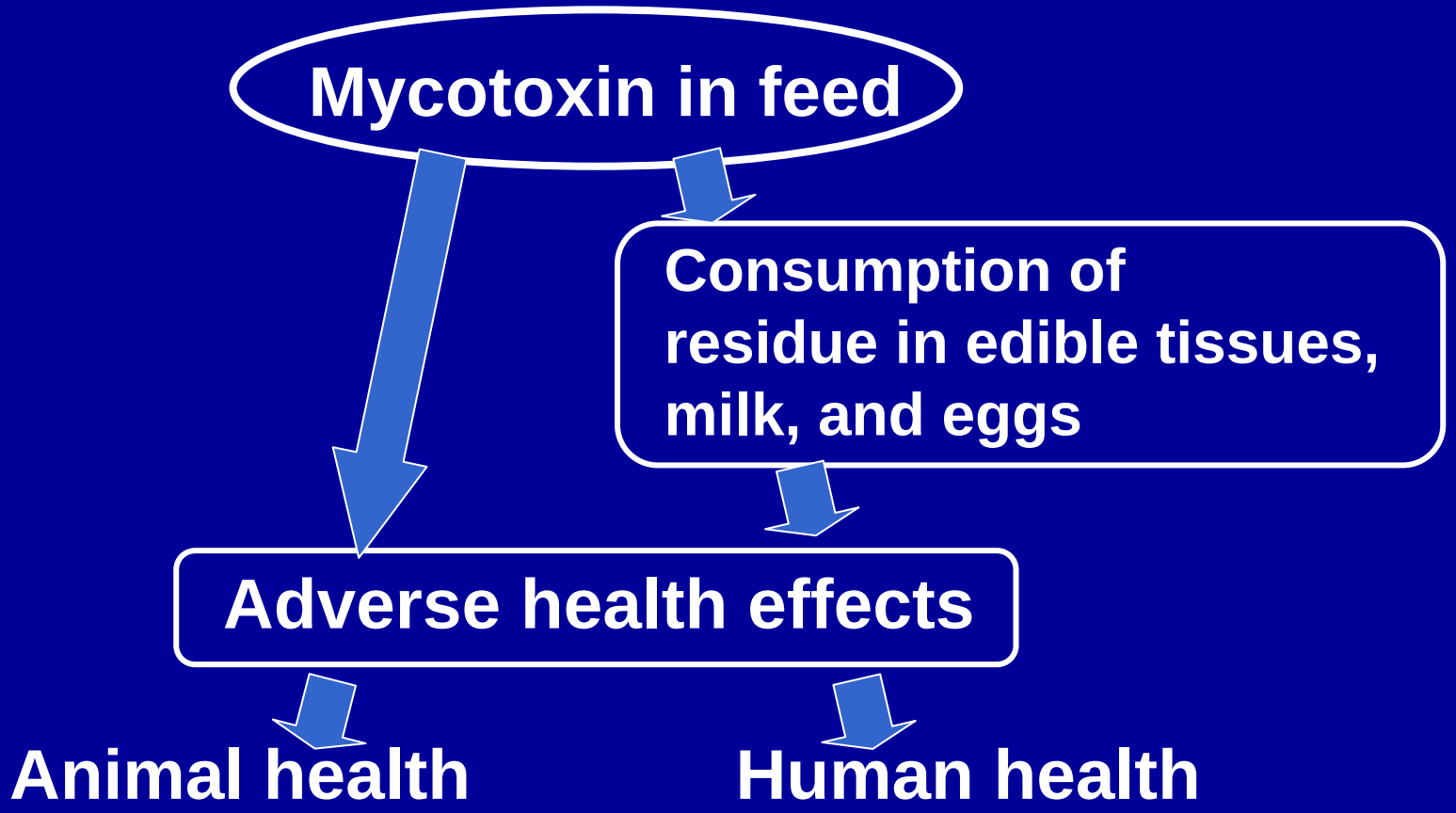
  Chronic—Liver cancer



# Risk assessment and risk management of mycotoxin in food



# The maximum level for mycotoxin in feed



The maximum level for feed should ensure both animal health and human health.

Effects of feed mycotoxin on animal health can be estimated based on data of animal experiments and natural intoxication cases of animals.

Effects of feed mycotoxin on human health can be estimated based on carry-over of mycotoxin from feed to animal derived food.

Mycotoxin concentration in food =
Mycotoxin concentration in feed / Carry over (ratio of mycotoxin concentration in feed to that in animal derived food, or, relationship between intake by animal and mycotoxin concentration in animal derived food)

Aflatoxin as an example

# Typical aflatoxins in Food

$AFB_1$ $AFB_2$ $AFG_1$ $AFG_2$

**Total aflatoxin** ⟶ **All foods**

**aflatoxin M1** ⟶ **Milk and products**

# Acute toxicity in animals

**Liver: Biliary proliferation, necrosis, jaundice**

**LD50 (mg kg bw) AFB1**

| Animal | po | ip |
|---|---|---|
| Duckling | 0.0335 | |
| Rabbits | 0.3 | |
| Cat | 0.55 | |
| Dog | 0.2 | |
| Guinea pig | 1.4 | |
| Rat, neonate | 0.56 | |
| Rat, adult | 2-30<br>Female>Male | 13 |
| Hamster | 10.2 | |

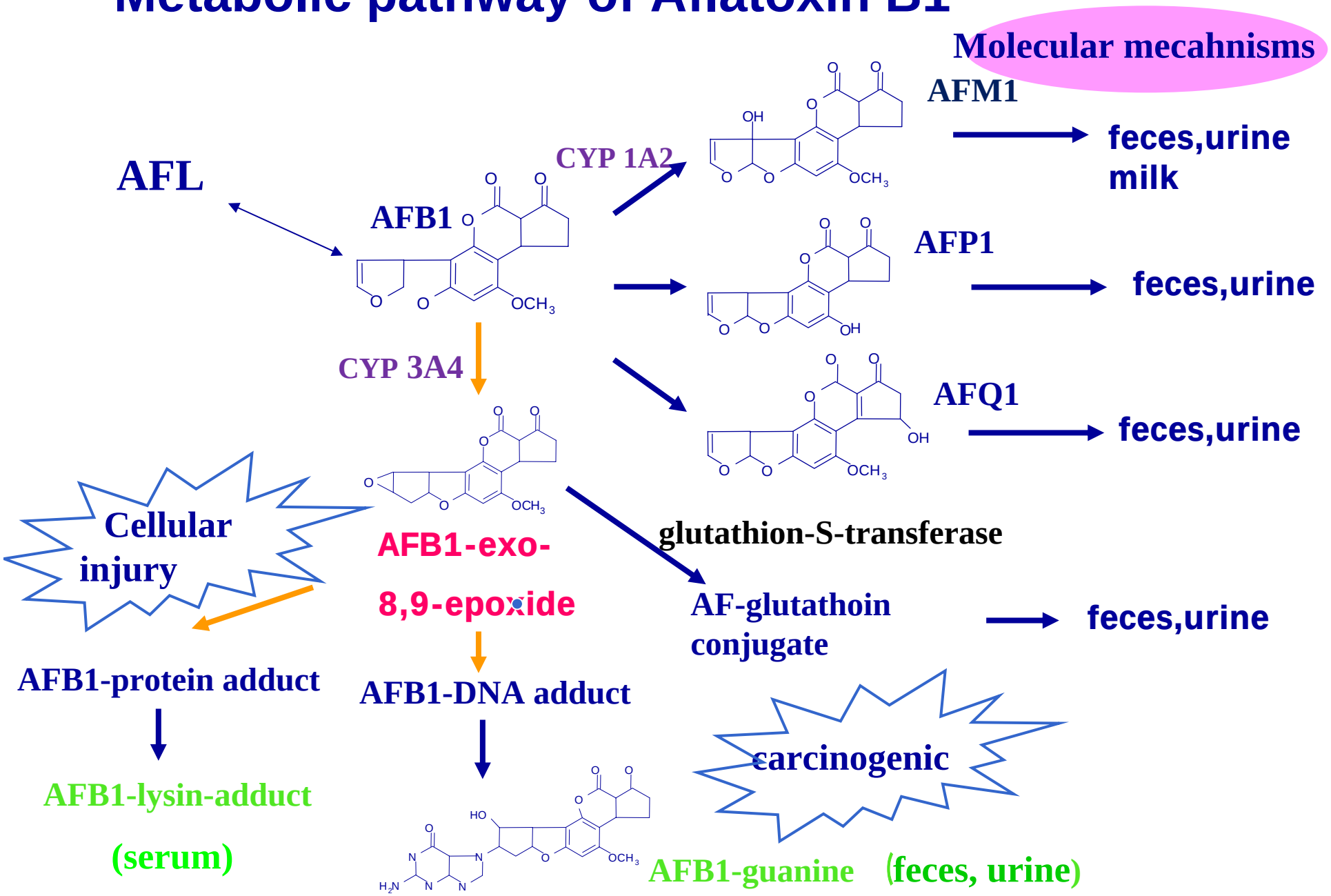
**AFB1 is metabolized in animal body to**

**AFM1 >>> Excreted into milk**
**AFP1**
**AFQ1**
**Aflatoxicol**

**AFB1-epoxide >>> AFB1-DNA >>> Cancer**
**AF-aldehyde >>> AFB1-protein >>> Acute toxicity**

**AFB1-glutathione conjugate**
**AF-diol etc.**

# Metabolic pathway of Aflatoxin B1



## Acute toxicity of AF metabolites

**AFB1=Aflatoxicol > G1, P1, M1, Q1 > B2, G2**

**Major Acutely Affected Human Cases**

**India 1974.11-1975**
397 Cases. 106 Deaths. Dogs also died.
Clinical symptoms: Ascites, jaundice, fever, legs edema, vomit.
Liver histopathology: Bile duct proliferation, gastrointestinal hemorrhage, periductal fibrosis.
Moldy corn: 6.25-15.6 ppm afl.
Ingested amount: 0.055 mg/kg/day< for several wks.

**Kenya 1982.4-5**
20 Cases. 12 Deaths. Dogs also died.
Clinical symptoms: Same as India.
Pathology: Liver necrosis
Stored crops: 3.2-12 ppm afB1 (corn), 1.6 ppm afB1 (bean)
Ingested amount: 0.038 mg/kg/day< for some days.

**Kenya 2004.1-6**
397 Cases. 125 Deaths.
Clinical symptoms: Jaundice.
Corn: mean 354.53 ppb, max 20ppm afl.

**Kenya 2005.4-6**
76 Cases. 32 Deaths. Max 33 ppm afl.

**Kenya 2006.4-6.**
51 Cases. 21 Deaths. Higher than 1 ppm.

Human cancer

Epidemiological studies

## The relationship between primary liver cancer and intake of aflatoxin

| Area | Occurrence of Primary liver cancer ($10^5$/year) | Intake of AF (ng/kg body weight／day) |
|---|---|---|
| Kenya highland | 0.7 | 3.5 |
| Thailand-Song khla | 2.0 | 5.0 |
| Swaziland | 2.2 | 5.1 |
| Kenya midland | 2.9 | 5.8 |
| Swaziland- midland | 4.0 | 8.9 |
| Thailand-Ratchaburi | 6.0 | 45.0 |
| Swaziland- lowland | 9.7 | 43.1 |
| Mozambique－Inhambane | 13.0 | 222.4 |

International risk assessment of Aflatoxins

# Carcinogenecity of AFB1 (Rat)

Strain dependent

Animal study

| Sex | AFB1 in feed (ug/g) | period (week) | Occurrence of liver cancer |
|---|---|---|---|
| ♂ | 1 | 41 | 18/22 |
| ♀ | 1 | 64 | 4/4 |
| ♂ | 0.3 | 52 | 6/20 |
| ♀ | 0.3 | 70 | 11/11 |
| ♂ | 0.015 | 68 | 12/12 |
| ♀ | 0.015 | 82 | 13/13 |

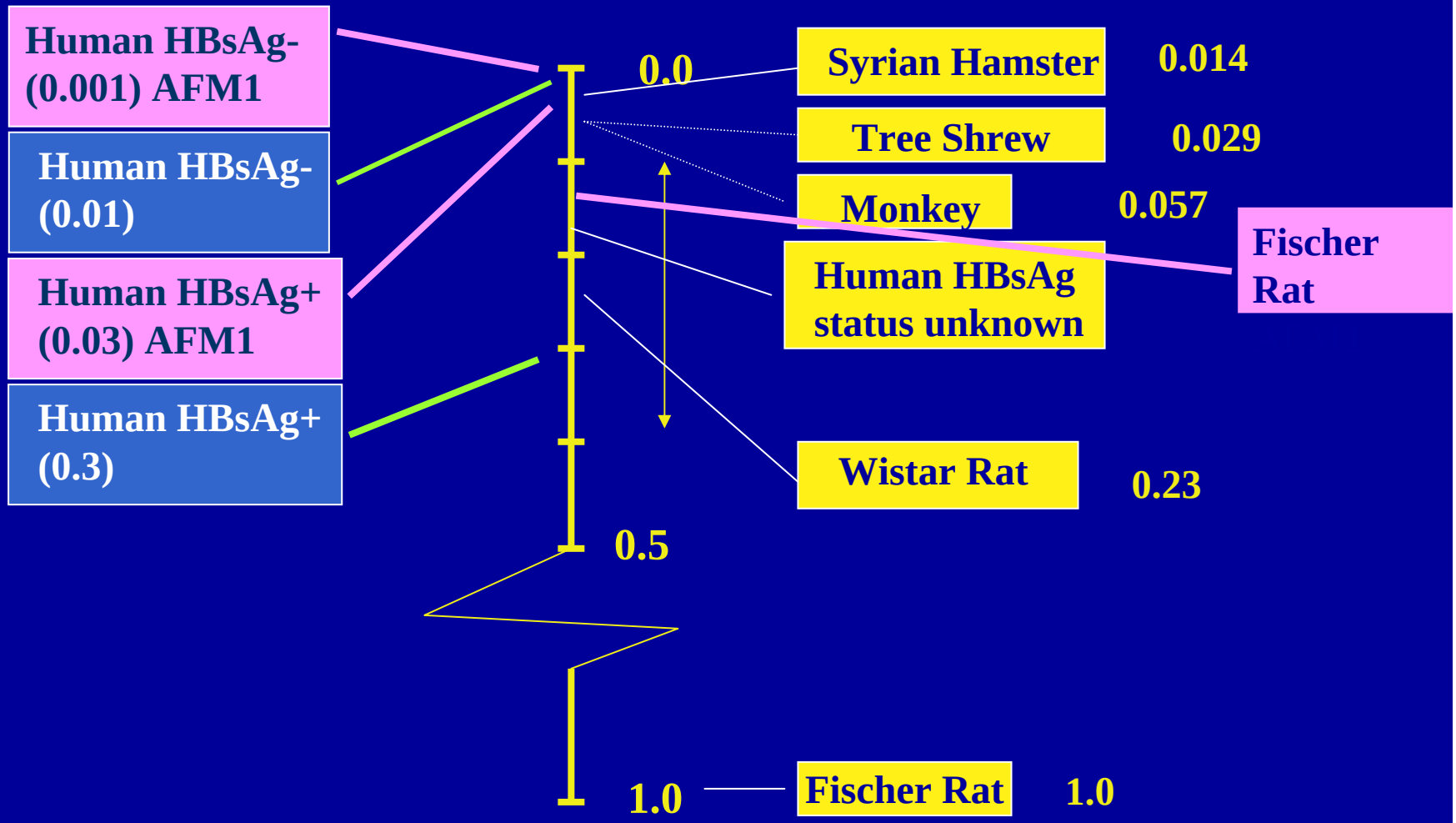
Wogan,G.N. and Newberne, P.M., Cancer Res.27, 2370-2376, 1967

Aflatoxin is a potent carcinogenic & genotoxic mycotoxin in human.

Tolerable intake has not been set for human for regulation.

Regulation for food is based on population risk.

# Risk chracterization




**Potency estimates (cancers/year per 100,000 people per ng/kg bw per day)**
**for human liver cancer resulting from exposure to aflatoxin B1 or M1 derived from epidemiological and toxicological studies. ( JECFA, 1997)**

# Aflatoxin M1

Metabolite of Aflatoxin B1

Excreted in Milk

Potency of AFM1 is approximately 1/10 times that of AFB1

(from the data of animal experiment)

# Worldwide limits for AFM1in milk


From "Worldwide regulations for mycotoxins in food and feed in 2003" by FAO Rome 2004

**How much AFB1 level gives the limit levels of AFM1 in milk?**

**Carry over from feed to cattle milk?**

## Carry-over of feed AFB1 to milk AFM1

Ratio of AFB1 level in feed to AFM1 level in milk
= 34:1 ~ 1600:1

From available data, 75:1 was proposed by Park & Pohland (1986) as most likely ratio.

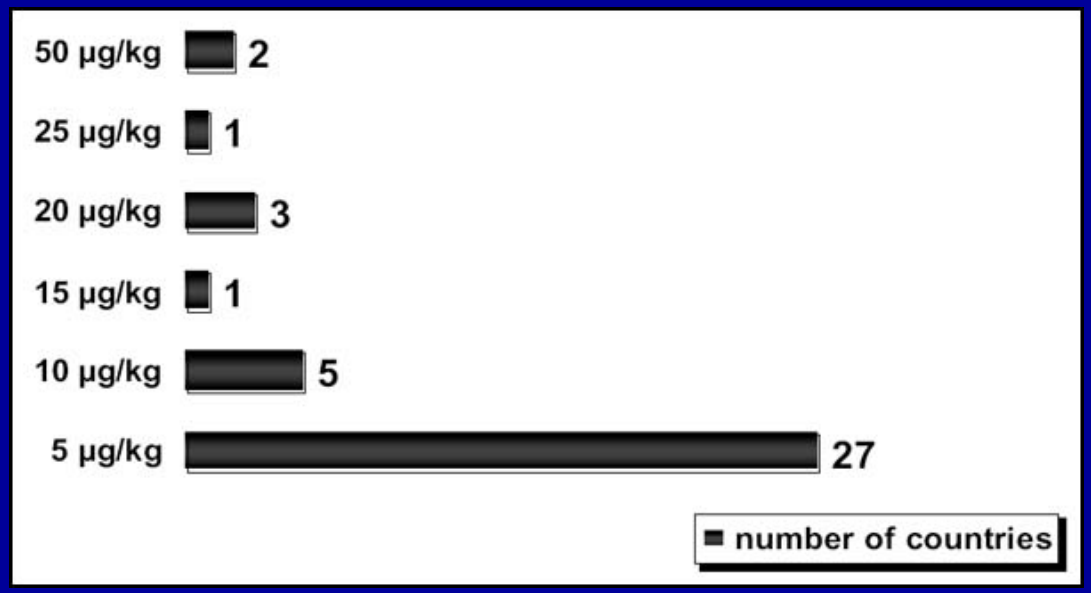
If this ratio is used,
3.75 µg AFB1 in 1 kg feed is converted to 0.05 µg AFM1 in 1 kg milk (EU regulation)
37.5 µg AFB1 in 1 kg feed is converted to 0.5 µg AFM1 in 1 kg milk (USA regulation)

AFM1(ng/kg of milk)=1.2 X AFB1 intake
（μg/ cow/day）＋1.9　（Veldman et al, 1992)

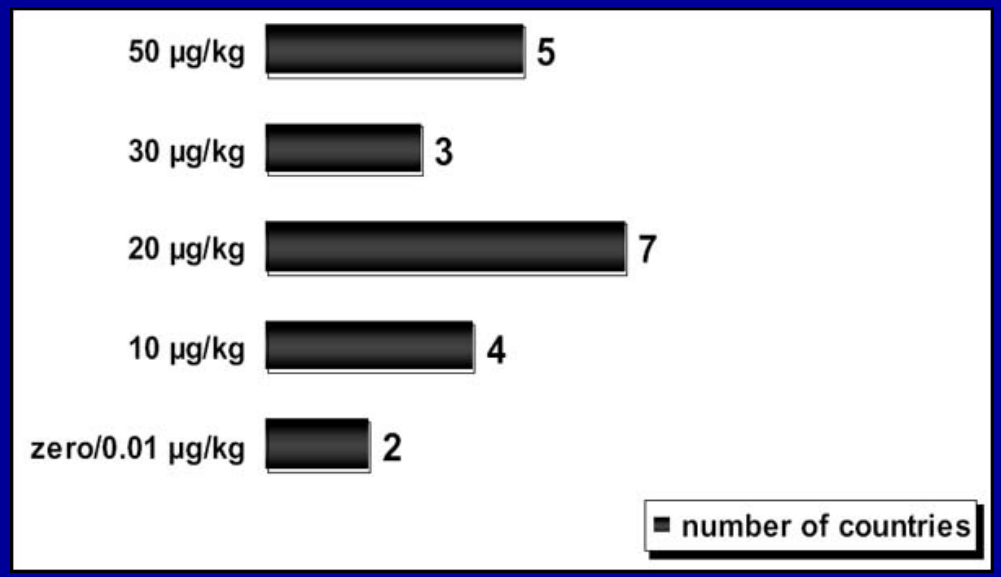
AFM1 (ng/kg milk) = 10.95 + 0.787 x AFB1 intake
(μg/cow/day)（Petterson 1998)

## Worldwide limits for AFB1 in feed for dairy cattle

5 ppb can ensure 0.5 ppb AFM1 in milk, but might not 0.05 ppb, under the assumption. Exposure assessment is required based on data of AFM1 concentration in milk.

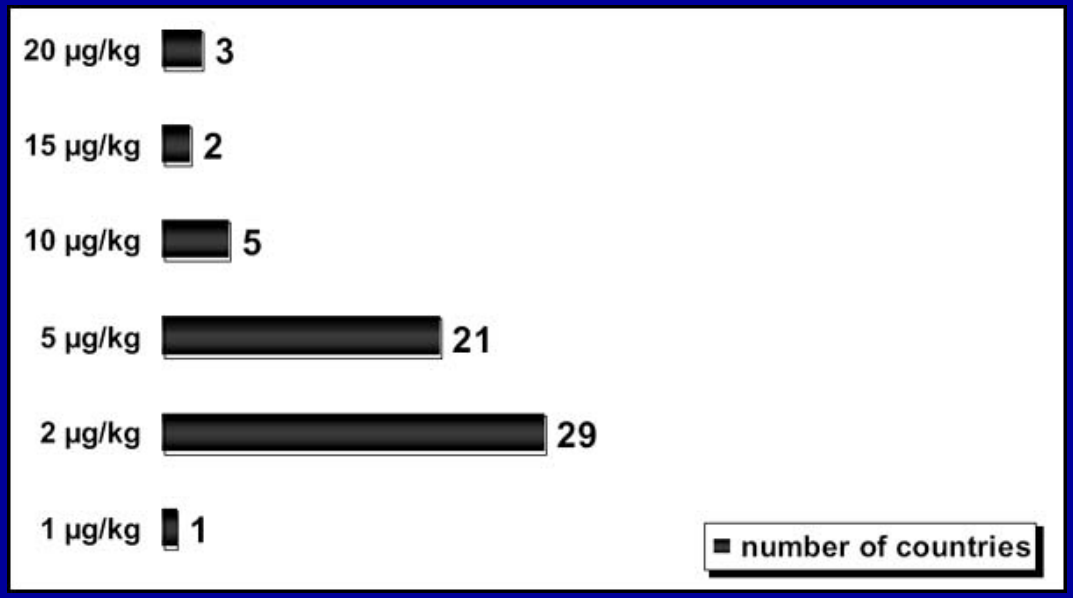



## Worldwide limits for Total AF in feed for dairy cattle

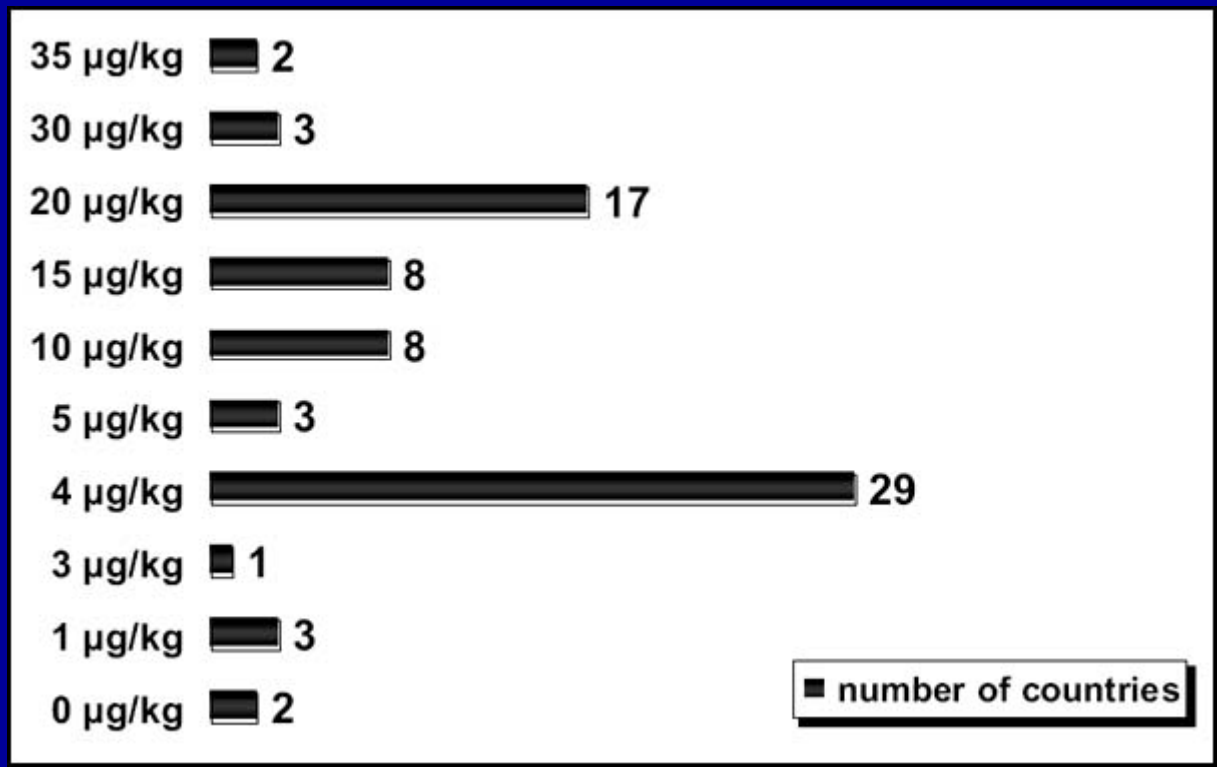



From “Worldwide regulations for mycotoxins in food and feed in 2003” by FAO Rome 2004

## Worldwide limits for AFB1in food




## Worldwide limits for Total AF in food

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The maximum levels for fumonisin, DON and zearalenone in feed are regarded to be set based on
their adverse effects on animal health, because their carry-over from feed to edible tissues, eggs and milk is too small to affect human health.

However,
data of carry-over are not enough for many other mycotoxins,  and more research works are required.


**Thank you**