

Mycotoxins that are Potential Environmental Exposure Risks for Minnesotans

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Chemophobia vs The Benevolence of Nature

- The assumption is widely held that all "chemicals" are bad and all "natural" things are good.

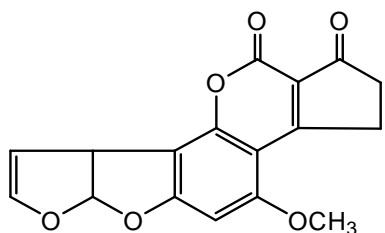
Causes of Chemophobia

- Tragic accidents at Bhopal and Seveso.
- Rachel Carson's book *Silent Spring*, which portrayed chemistry as a blind and brutal enemy of birds and other living creatures.
- General mistrust of all authority, including that of toxicologists, ever since Richard Nixon's 17 minutes of silence.
- Decline of science education, particularly among journalists.
- Confusion of toxicity with other real and imaginary dangers beyond current public understanding, such as radioactivity and genetically modified organisms.

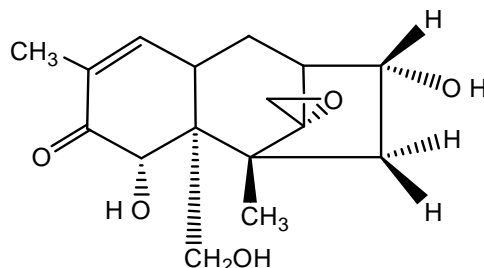
What is harder to understand is why anyone would think natural is always safe

- Novel man-made chemicals are no more likely to be toxic than new natural products.
- A wide variety of plants, animals and microorganisms are making strenuous and continuing efforts to produce, collect and disseminate toxic materials to the detriment of mankind.

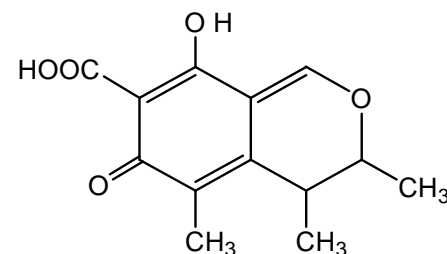
Mycotoxins of interest to Agriculture and Food Safety



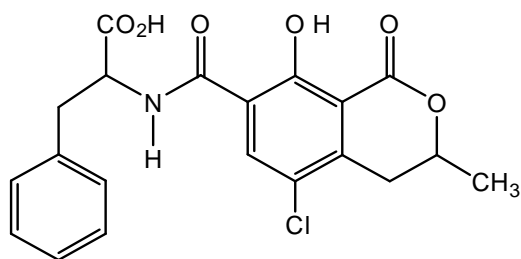
Aflatoxin B₁



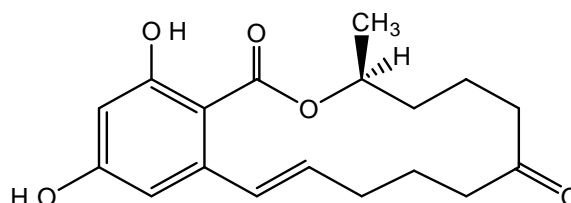
Deoxynivalenol



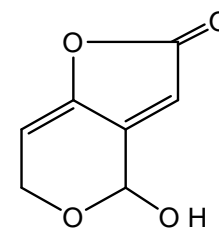
Citrinin



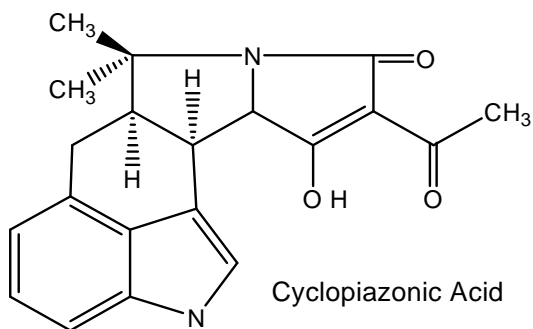
Ochratoxin A



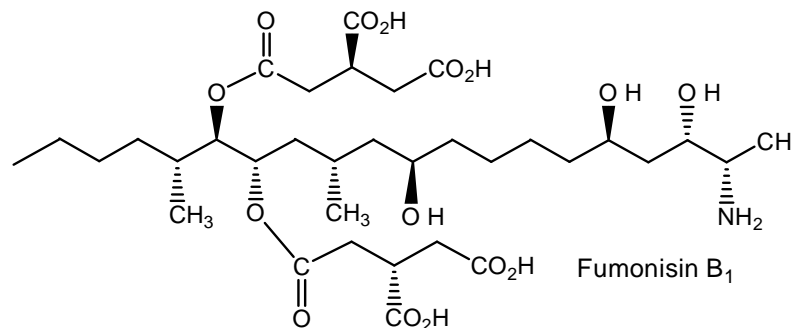
Zearalenone



Patulin



Cyclopiazonic Acid



Fumonisin B₁

Mycotoxin Nomenclature

Aflatoxin	<i><u>Aspergillus flavus</u> toxin</i>
Fumonisin	<i><u>Fusarium moniliforme</u> toxin</i>
Ochratoxin	<i><u>Aspergillus ochraceus</u> toxin</i>
Trichothecene	<i><u>Trichothecium roseum</u> toxin</i> the <u>alkene</u> form
Zearalenone	<i><u>Gibberella zea</u> resorcylic <u>acid lactone</u> in the <u>alkene</u> and <u>ketone</u> form</i>

Effect of Climate on Mycotoxin Production

Mycotoxin	Climate for Optimal Production
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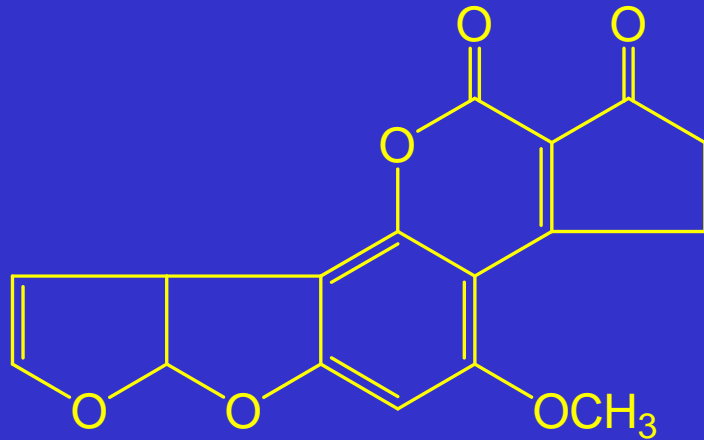
Aflatoxin	Hot, Dry
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Fumonisin	Hot, Humid
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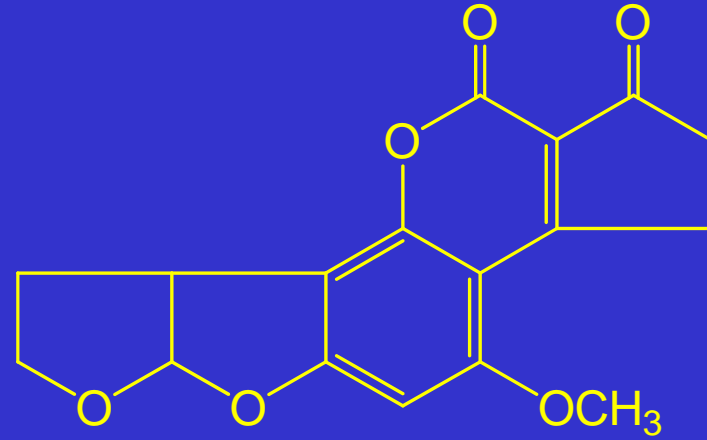
Vomitoxin (DON)	Cold, Damp
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Aflatoxins

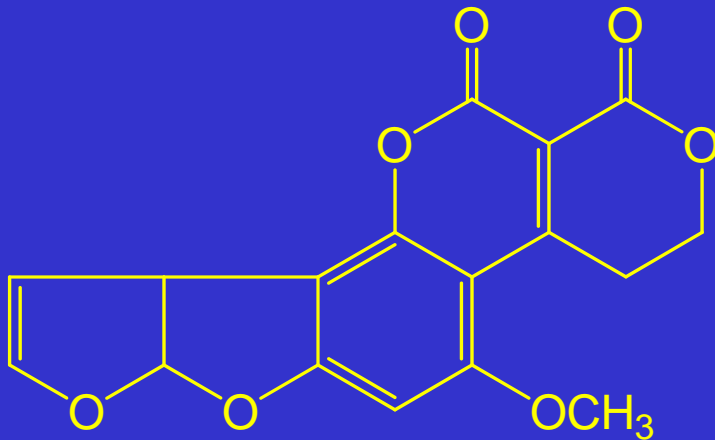
Structures of the Major Aflatoxins



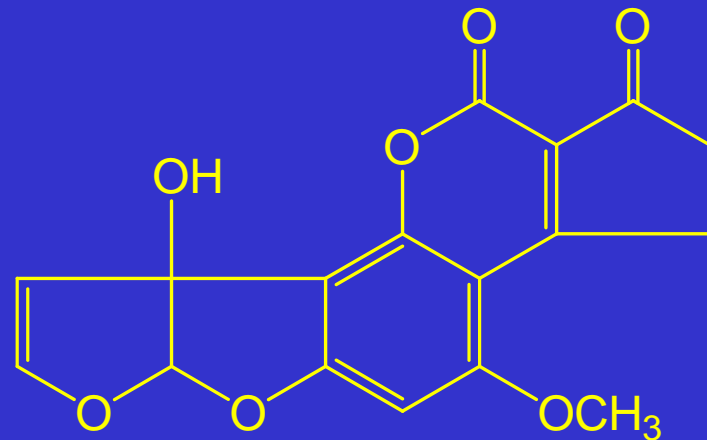
Aflatoxin B₁



Aflatoxin B₂



Aflatoxin G₁



Aflatoxin M₁

Aflatoxins

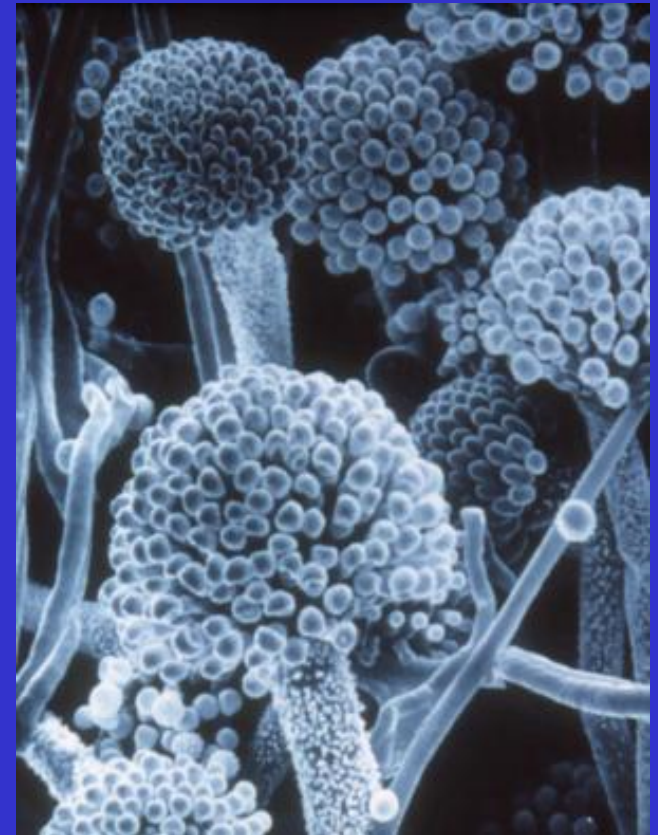
Produced by: Many *Aspergillus* species, most notably *A. flavus* and *A. parasiticus*.

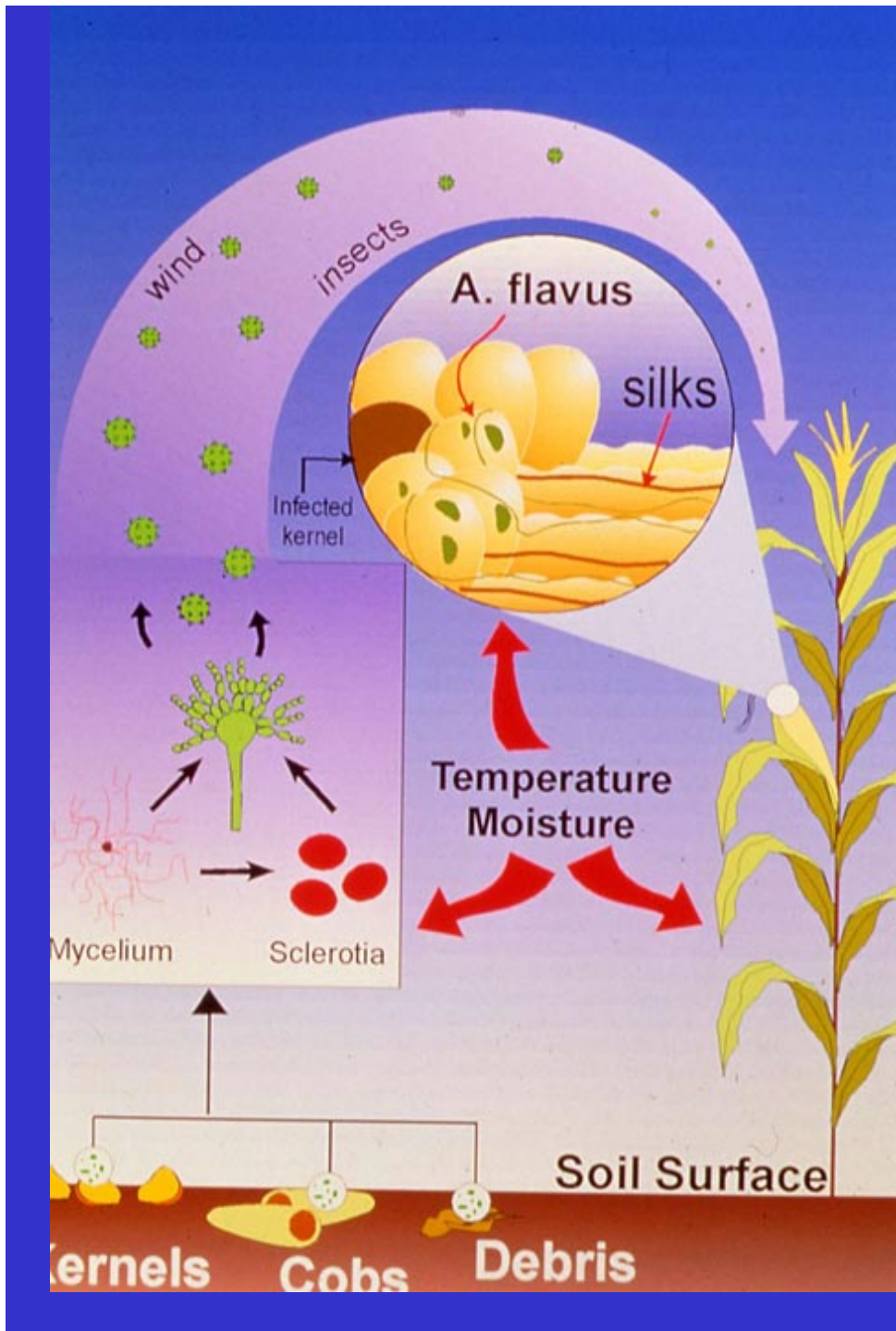
Analyses: Because aflatoxins are fluorescent without derivatization, very sensitive TLC and HPLC assays have long been available.

Issues: Because of the sensitivity of the assays, almost all lots of peanut butter produced and consumed in the US contain readily detectable levels of aflatoxins.

Aspergillus flavus Growing on Corn

Source: www.extension.umn.edu/cropenews/images/2007/07MNCN42-2.jpg



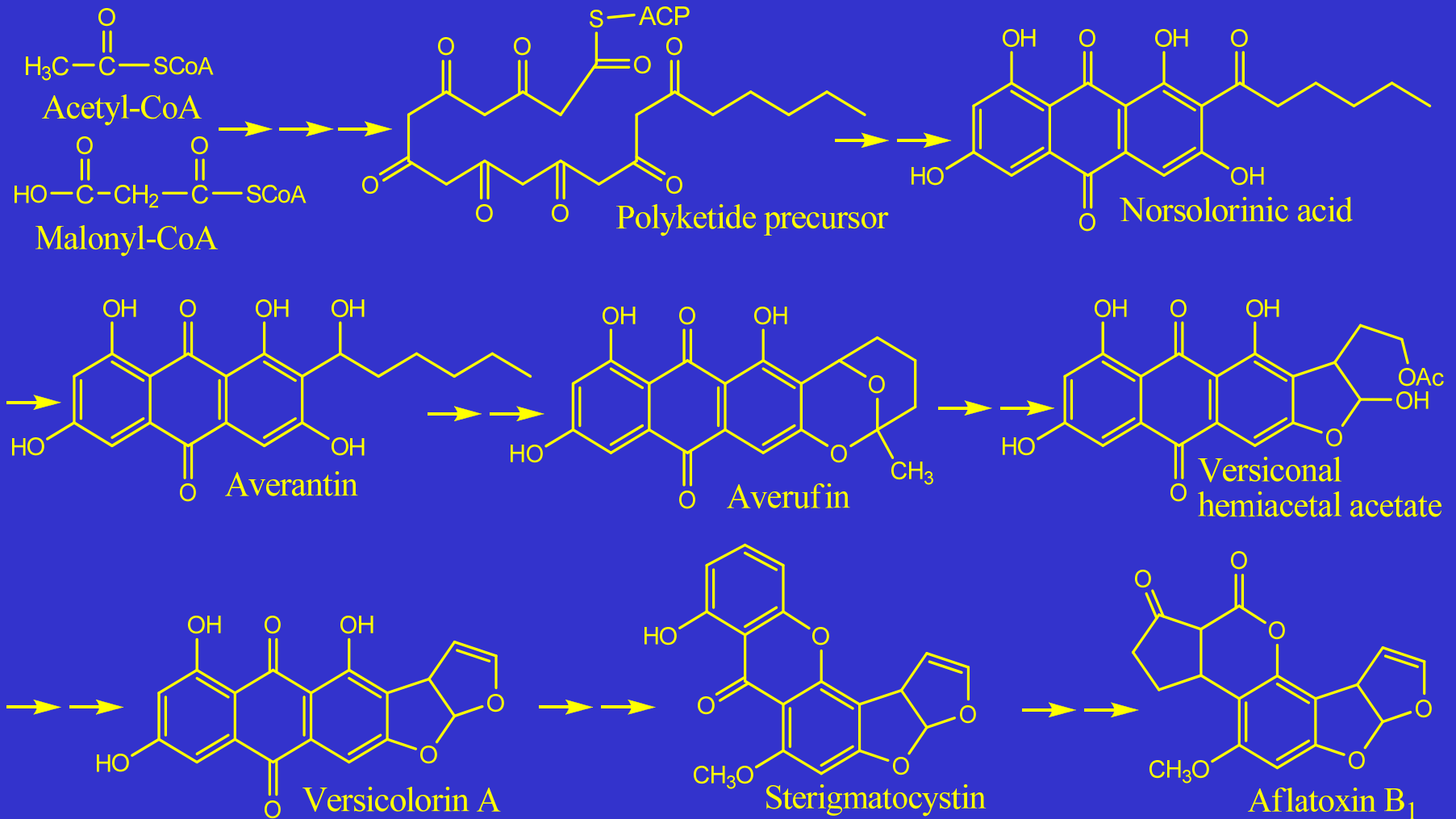


Aspergillus flavus
infects corn
via the silk from a
reservoir in soil
and surface debris
between silking
and blacklayer

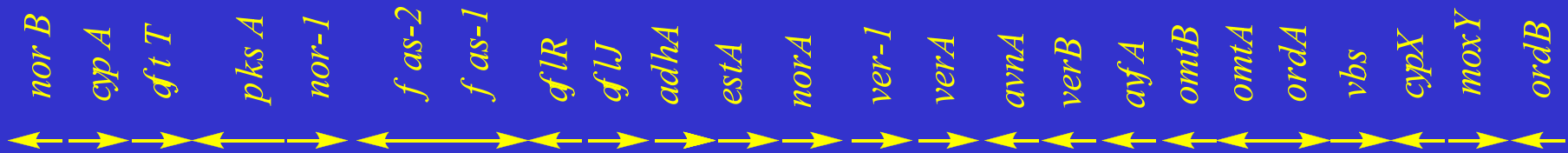
Source:

www.aspergillusflavus.org/aflavus

Key Intermediates in the Biosynthesis of Aflatoxin B₁



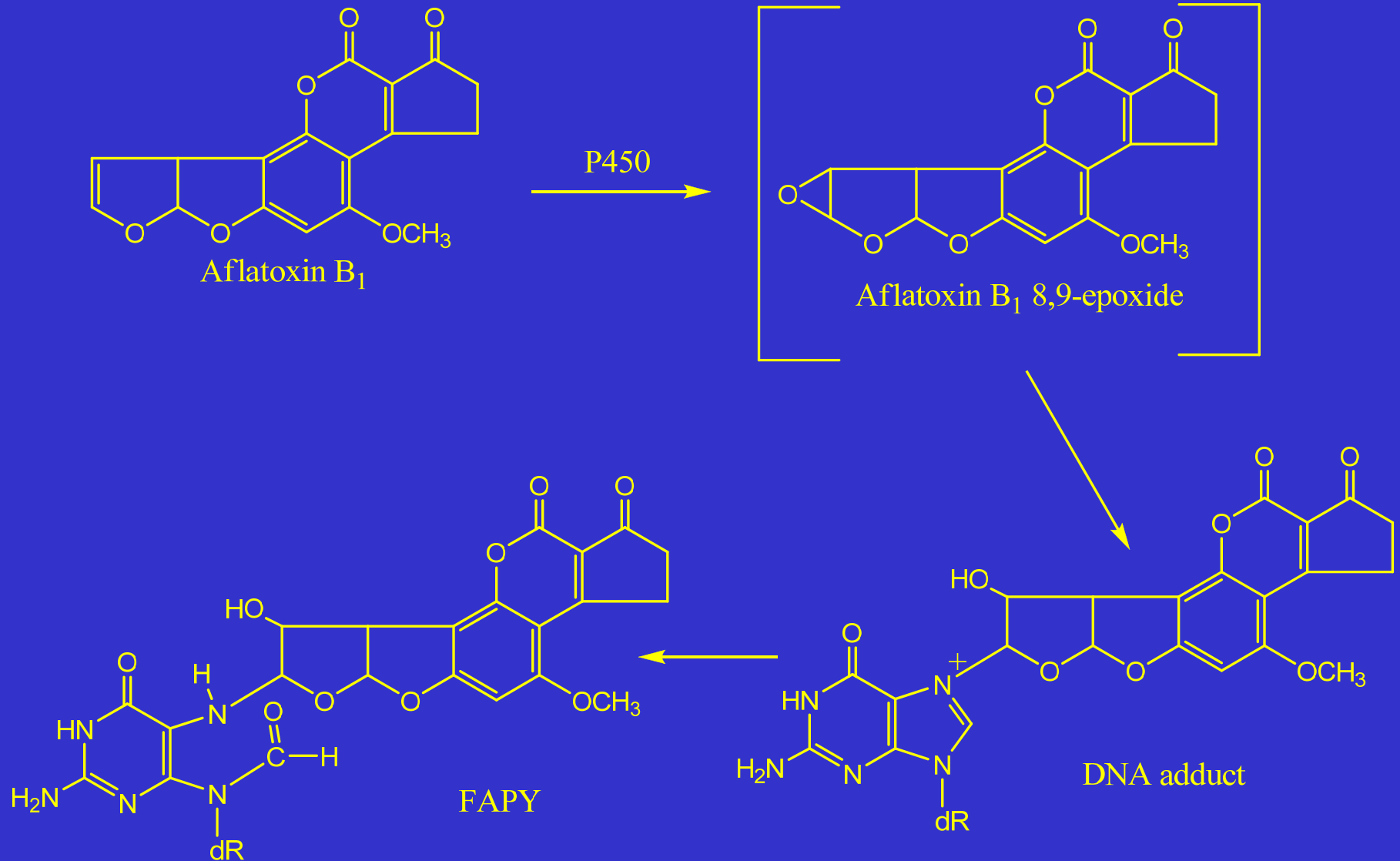
The Aflatoxin Biosynthetic Gene Cluster



Gene	Function
<i>NorB</i>	Dehydrogenase
<i>cypA</i>	Monooxygenase
<i>aflT</i>	Aflatoxin transporter
<i>pksA</i>	Polyketide synthase
<i>nor-1</i>	Reductase
<i>fas-2</i>	Fatty acid synthase-2
<i>fas-1</i>	Fatty acid synthase-1
<i>aflR</i>	Transcription regulator
<i>aflJ</i>	Co-Activator
<i>adhA</i>	Dehydrogenase
<i>estA</i>	Esterase
<i>norA</i>	Aryl alcohol dehydrogenase

Gene	Function
<i>ver-1</i>	Dehydrogenase
<i>verA</i>	Monooxygenase
<i>avnA</i>	Monooxygenase
<i>verB</i>	Desaturase
<i>avfA</i>	Dehydrogenase
<i>omtB</i>	Methyl transferase
<i>omtA</i>	Methyl transferase
<i>ordA</i>	Monooxygenase
<i>vbs</i>	Ver B synthase
<i>cypX</i>	Monooxygenase
<i>moy</i>	Monooxygenase
<i>ordB</i>	Monooxygenase

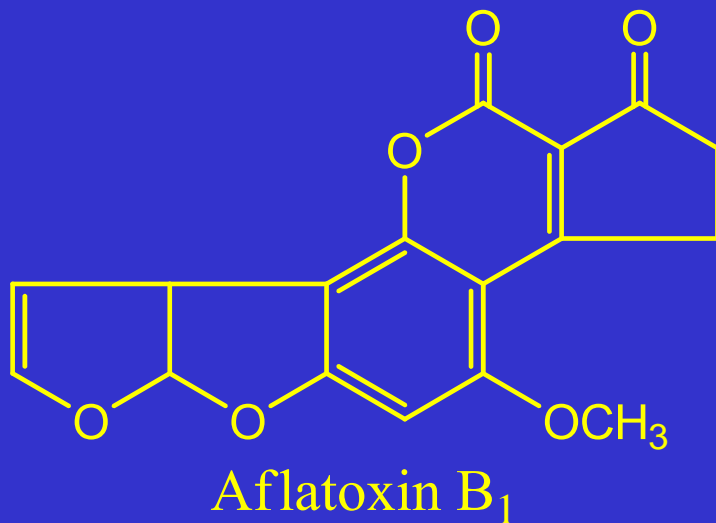
Metabolic Activation of Aflatoxin B₁ in Mutagenesis and Carcinogenesis



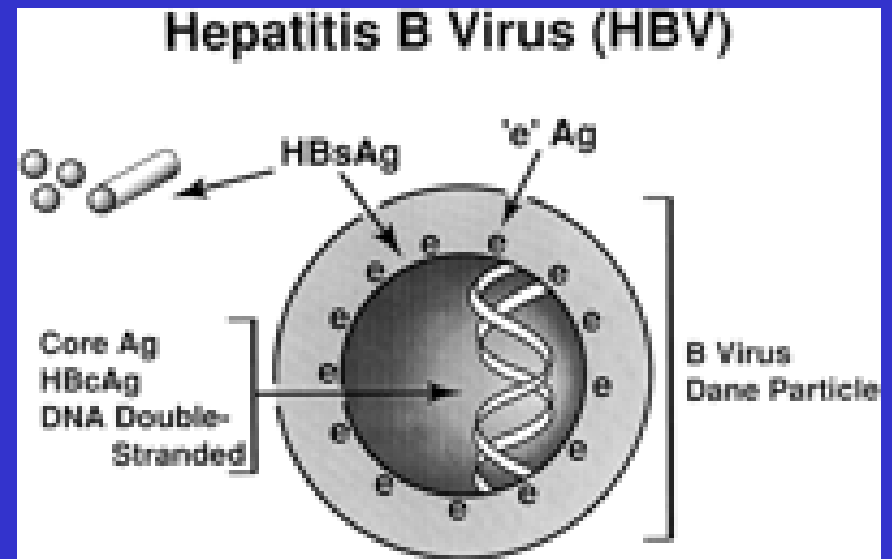
Chronic Toxicity of Aflatoxin B1: Carcinogenesis

- Aflatoxin B1 is the most potent natural carcinogen known. Only dioxins significantly exceed AFB1 in potency. It is 1000 times more potent as a carcinogen than benzo pyrene.
- The metabolite aflatoxin 8,9 epoxide forms an adduct with guanine moiety in the DNA for the tumor suppressor protein p53 in liver causing a G to T transversion at codon 249.

Is Primary Hepatocellular Carcinoma Caused by Aflatoxins or Hepatitis B Virus?

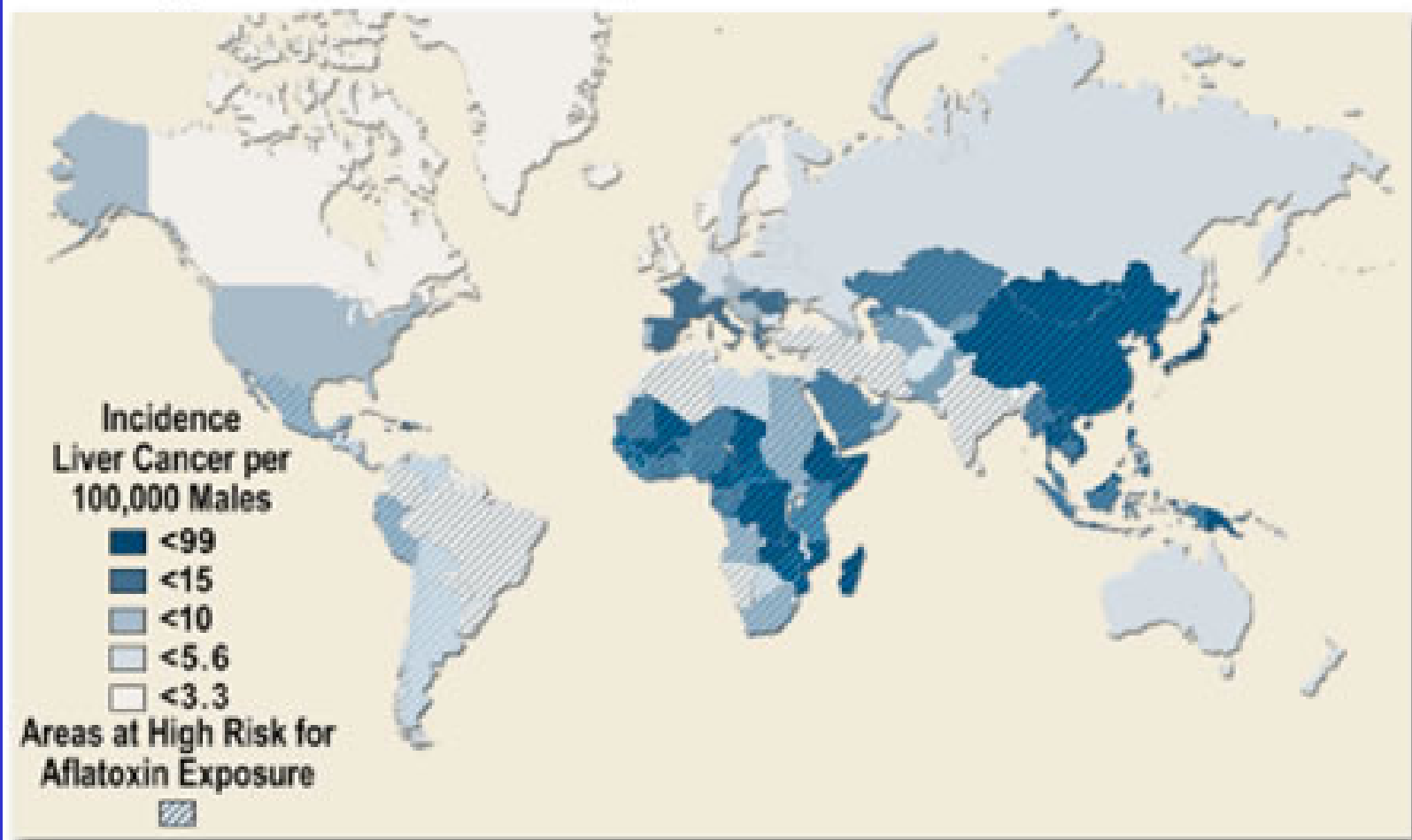


VS



Primary hepatocellular carcinoma is endemic in some Asian and African countries with high rates of hepatitis B infection resulting from vertical transmission from mother to child during birth.

Correlation Between Populations with High Liver Cancer Rates and High Risk of Chronic Exposure to Aflatoxin Contamination



*Liver cancer data from the GLOBOCAN 2002 database
(http://www-dep.iarc.fr/GLOBOCAN_frame.htm)*

Aflatoxin data from Williams et al., Human Aflatoxicosis in Developing Countries, Am J Clin Nutr 80:1106–22, 2004.

Q. Is Primary Hepatocellular Carcinoma Caused by Aflatoxins or Hepatitis B Virus?

A. Both

- When people who test positive for the hepatitis B virus are also exposed to aflatoxin in the diet, their risk for developing liver cancer is about 60 times that of unexposed individuals.
- This increase in cancer risk is much greater than that observed with either aflatoxin or hepatitis B virus alone.

Hepatitis B Vaccine

- an Anti-Cancer Vaccine

- In Taiwan implementation of a nationwide hepatitis B vaccination program in 1984 was associated with a decline in the incidence of childhood hepatocellular carcinoma.
- Thus, hepatitis B vaccine can be thought of as the first human anti-cancer vaccine.
- In practice it is cheaper to control primary hepatocellular carcinoma with hepatitis B vaccination than lowering aflatoxins in the diet, so that is what is done.

Acute Toxicity of Aflatoxin B1

Species	LD50 (mg/kg)
Rabbit	0.30
Duckling (11 day old)	0.43
Cat	0.55
Pig	0.60
Rainbow trout	0.80
Dog	0.50 - 1.00
Sheep	1.00 - 2.00
Guinea pig	1.40 - 2.00
Baboon	2.00
Chicken	6.30
Rat (male)	5.50 - 7.20
Rat (female)	17.90
Macaque (female)	7.80
Mouse	9.00
Hamster	10.20

Acute Toxicity of Aflatoxin B1

- Aflatoxins were first identified after acute intoxication in turkeys in 1968
- Acute aflatoxicosis occurs only in Africa only when it is an alternative to starvation.
- Aflatoxin in dry dog food manufactured by Diamond Pet Foods was responsible for at least 23 dog deaths due to liver failure between Dec 2005 and early 2006. There was another recall in 2007.

Acute Toxicity of Aflatoxin B1

- News Item: In 1995, the government of Saddam Hussein admitted to United Nations weapons inspectors that its scientists had weaponized a biological agent called aflatoxin.
- The joke among weapons inspectors was that aflatoxin would stop a lieutenant from making colonel, but it would not stop soldiers from advancing across a battlefield.

Comparison of Aflatoxin Problems in Developed and Developing Countries

Factor	Developed Countries	Developing Countries
Period of most Aflatoxin production	Pre-Harvest	Post-Harvest
Crops affected	Corn, peanuts, cotton, tree nuts	Corn, peanuts, copra, chickpeas, millet, yam flour, cassava flour
#1 Concern	Regulatory costs associated with monitoring aflatoxin levels	Chronic toxicity, including primary hepatocarcinoma, reduced immunity and rare acute toxicity
#2 Concern	Yield loss in animal feed	Yield loss in animal feed

Maximum Aflatoxin Allowed in US Corn

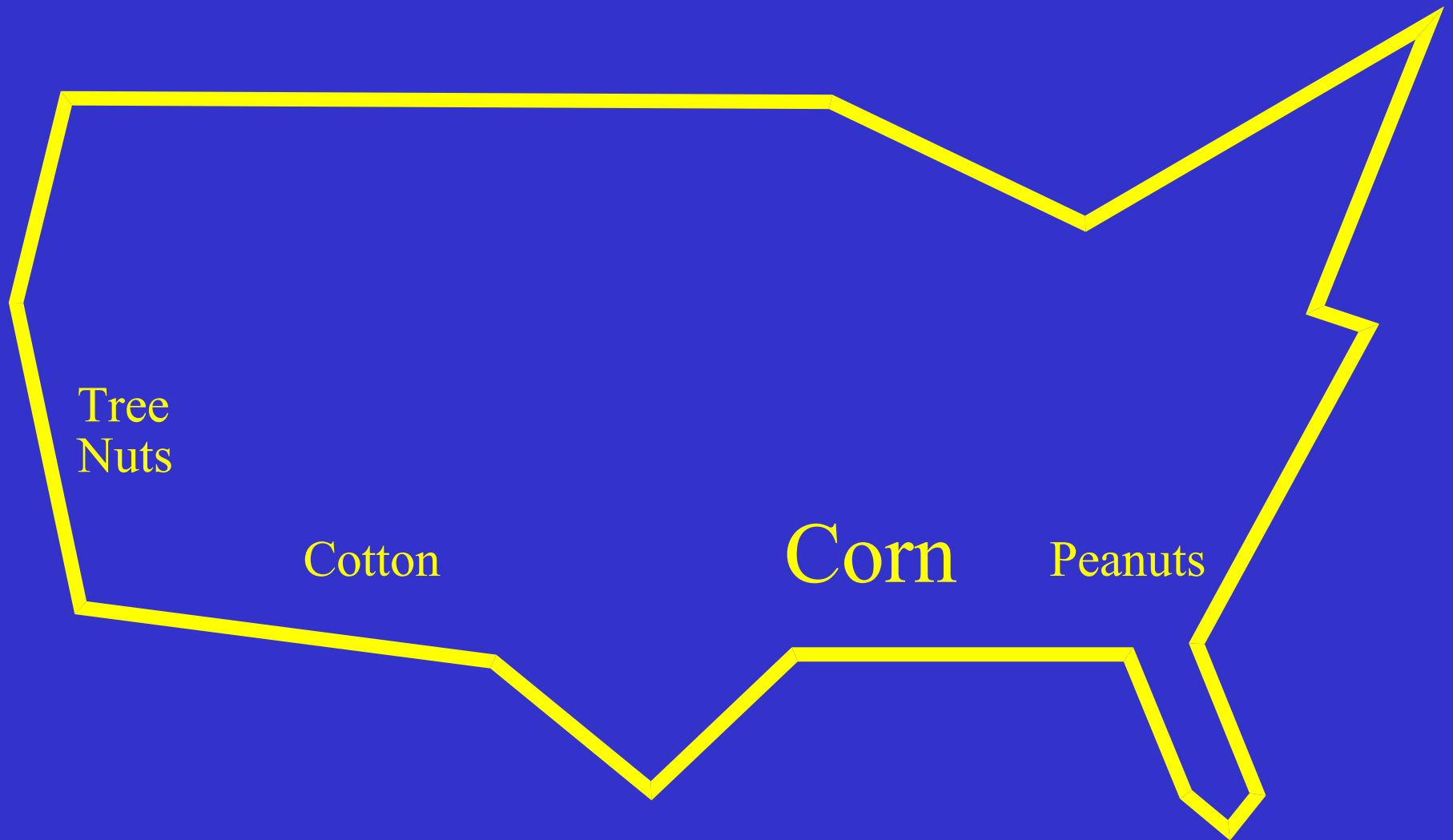
Corn Use	Aflatoxin Level (ppb)
Alcohol fermentation	none
Feed for finishing cattle	300
Feed for finishing swine	200
Feed for poultry and breeding livestock	100
Feed for immature livestock	20
Feed for dairy animals	20
Food for direct human consumption	20
Corn for export to Europe	5

Source: Adapted from US Food and Drug Administration, 2000

Aflatoxin Costs to US Agriculture

- Crop management (e.g., irrigation)
- Sampling
- Testing
- Crop value shift
- Remediation costs
- Research
- Insurance

Crops Affected by Aflatoxins in the United States



Factors which Affect Aflatoxin Production in US Corn

- Planting time
- Drought stress
- Heat stress – High temperature and Low temperature
- Nutrition stress – N, P, Ca, or K
- Population density
- Weeds
- Insects
- Other fungi
- Harvesting, handling and post-harvest storage

Effect of Source on Toxigenicity of *Aspergillus flavus* Isolates

Source of Isolates	Number of Isolates	Percent Toxigenic
Maize	451	62.1
Wheat	170	42.2
Rice	257	55.2
Barley	289	38.9
Green gram	167	36.5
Groundnut	230	57.1
Mustard	304	41.7
Coconut	230	51.0
Coriander	121	38.5
Cardamon	212	31.6
Soil	221	73.5

Source: Bilgrami & Choudhary, J. Stored Prod. Res., 29, 351 (1993)

The Lin and Dianese and Saito and Machida Cultural Tests for Aflatoxigenicity in *Aspergillus flavus*

Before Treatment

NH₄OH-treated

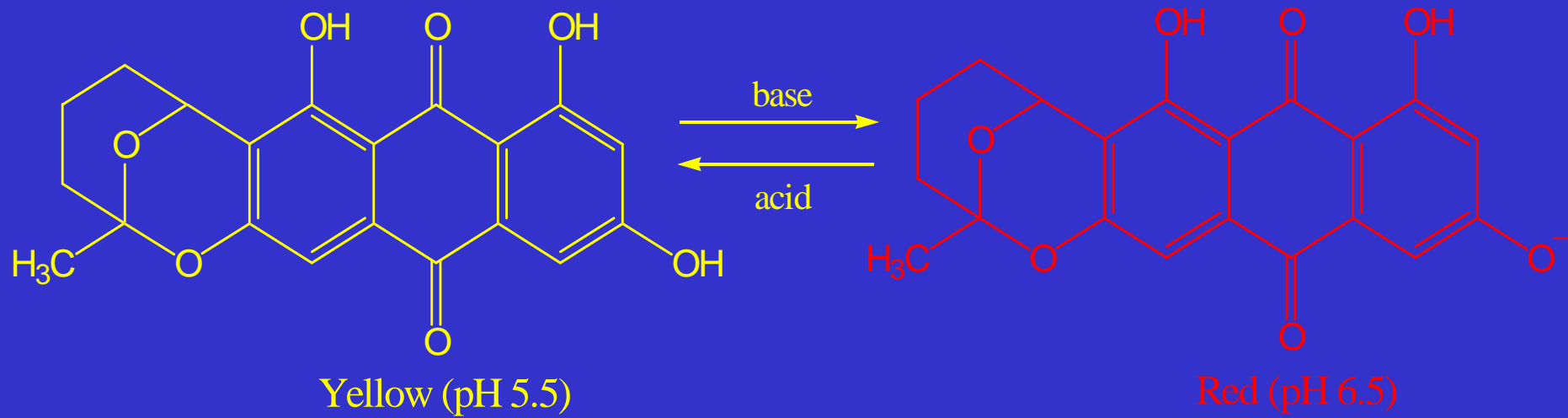
Toxigenic
A. flavus



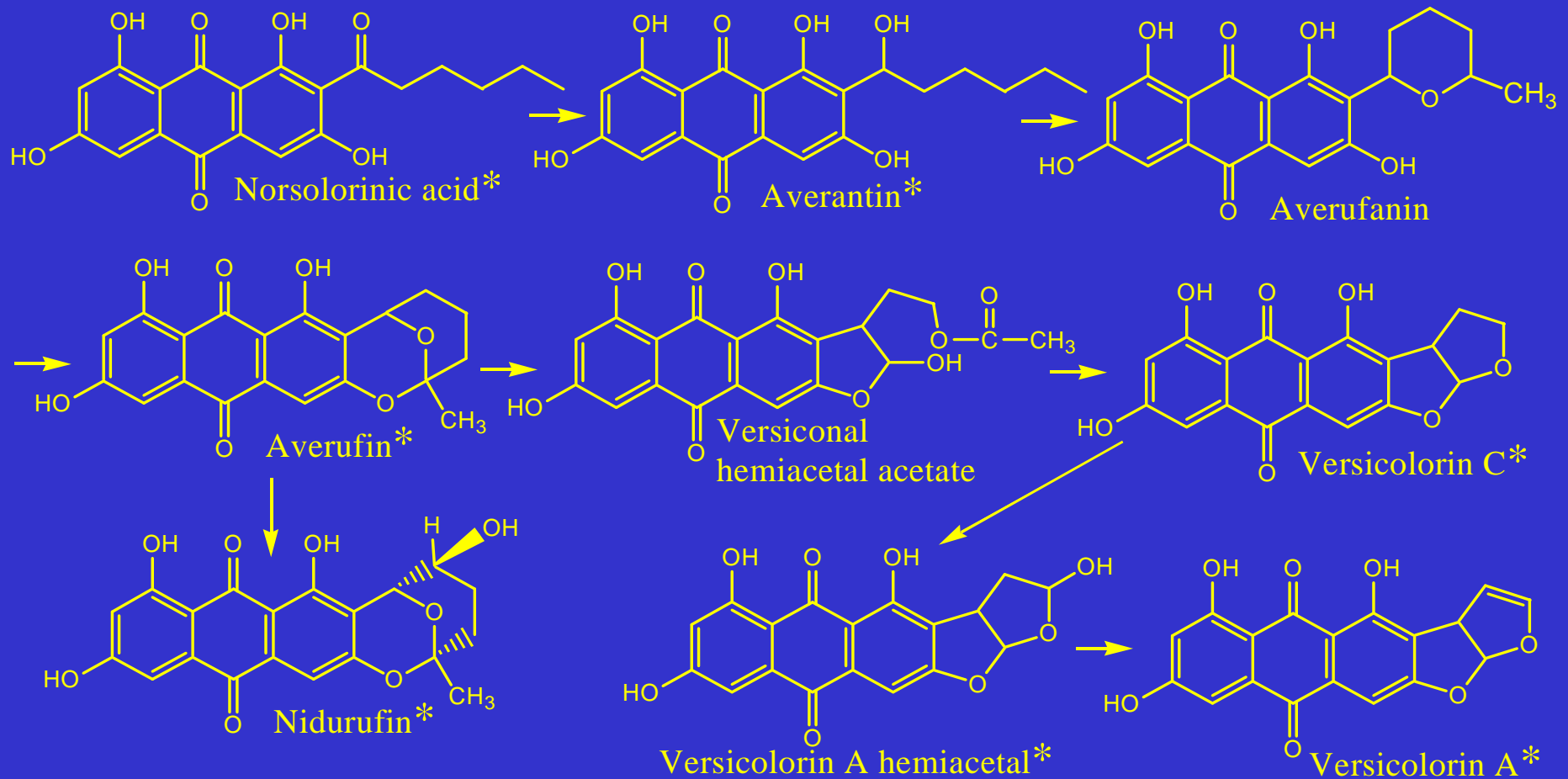
Non-toxigenic
A. flavus



Averufin as a pH Indicator Dye

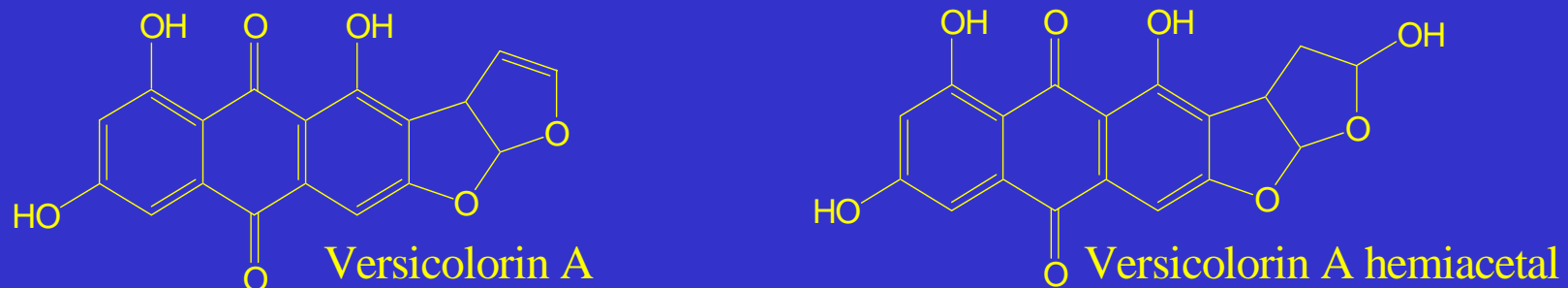
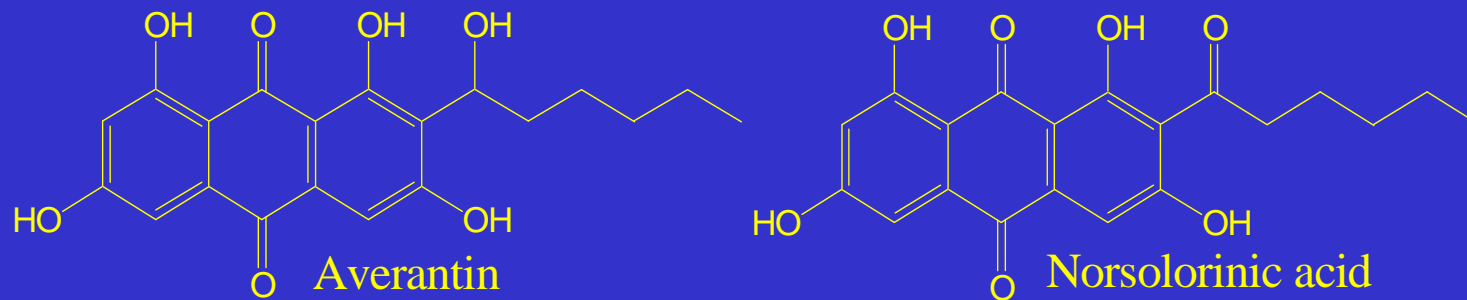
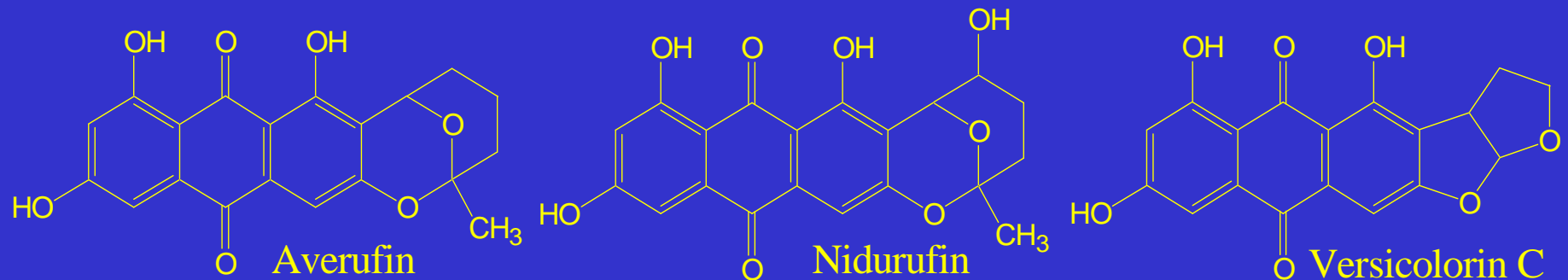


Anthrone Pigments on the Aflatoxin B₁ Biosynthetic Pathway



* Pigment identified in extracts of toxigenic *Aspergillus flavus*, but absent in non-toxigenic strains

Structures of Yellow Pigments Produced by Toxigenic Strains of *Aspergillus flavus*



Copra Inoculated with Aflatoxigenic *Aspergillus flavus* (F3w4)



Untreated

Ammonia treated

Fumonisin

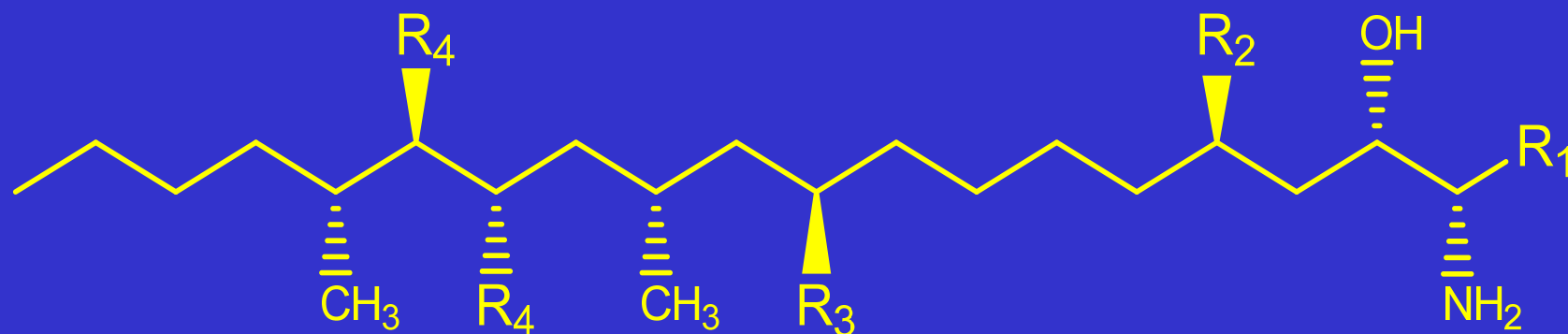
Fumonisin are produced by
Fusarium verticillioides and other
Fusarium spp.



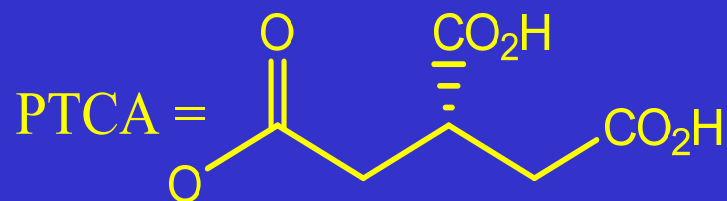
*Fusarium
verticillioides*
growing on
corn

Source: www.extension.umn.edu/cropenews/images/2007/07MNCN42-2.jpg

Structures of Fumonisin



Fumonisin	R ₁	R ₂	R ₃	R ₄
B ₁	H	OH	OH	PTCA
B ₂	H	OH	H	PTCA
B ₃	H	H	OH	PTCA
B ₄	H	H	H	PTCA
C ₄	CH ₃	H	H	PTCA

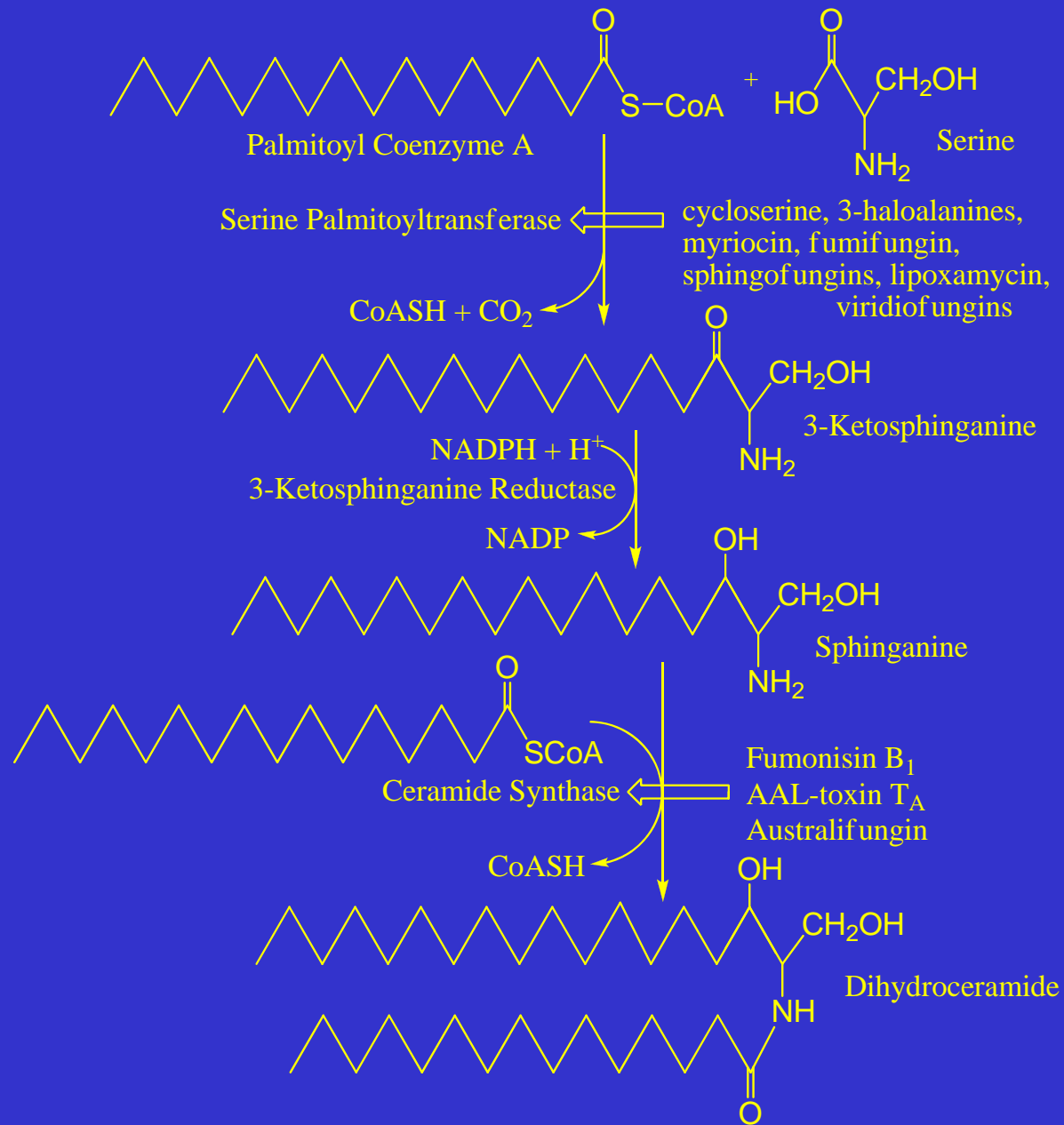


Maximum Fumonisin in US Corn

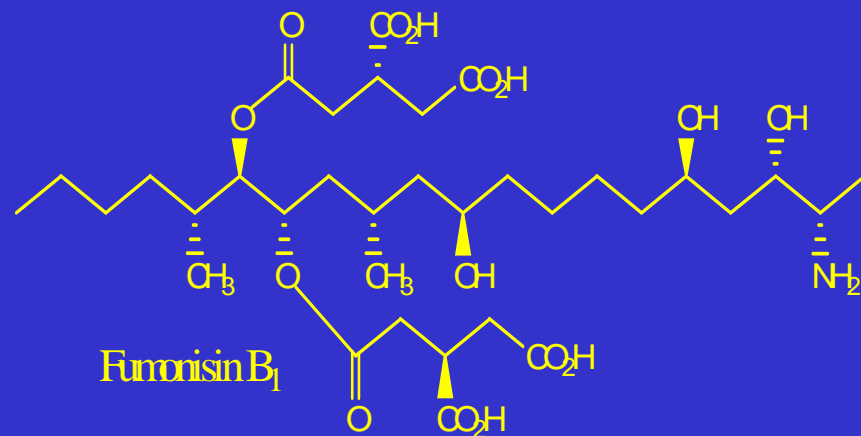
Corn Use	FDA Advisory Level
Humans - dry milled corn, fat < 2.25%	2 ppm
Humans - dry milled corn, fat > 2.25%	4 ppm
Humans – popcorn	3 ppm
Horses	5 ppm
Swine	20 ppm
Breeding ruminants, poultry	30 ppm
Ruminants for slaughter	60 ppm
Poultry for slaughter	100 ppm
Other species	10 ppm

Source: Adapted from US Food and Drug Administration, 2000

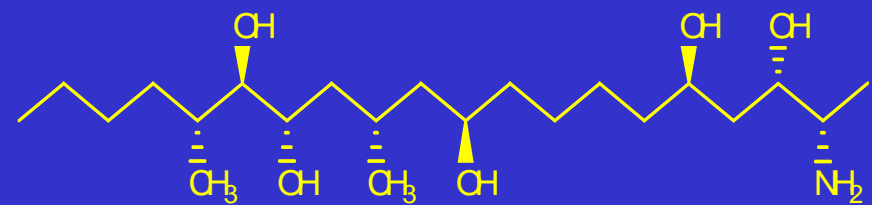
Fumonisin Inhibits Ceramide Synthase



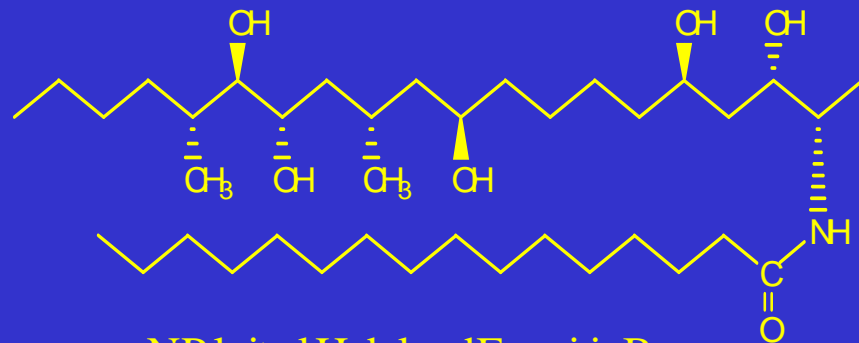
Structures of FB₁ and some abiogenic conversion products proposed to be formed during cooking of FB₁-contaminated foods



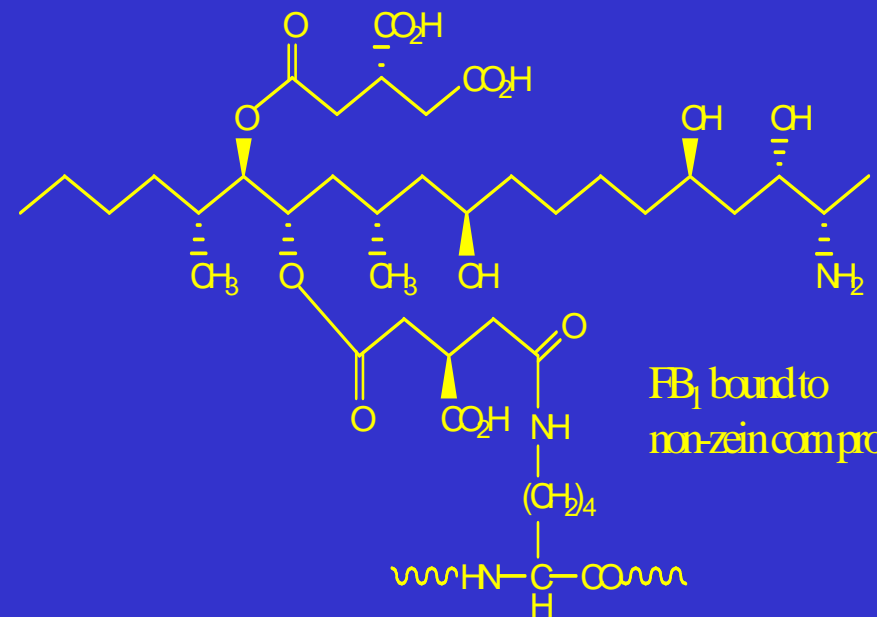
Fumonisin B₁



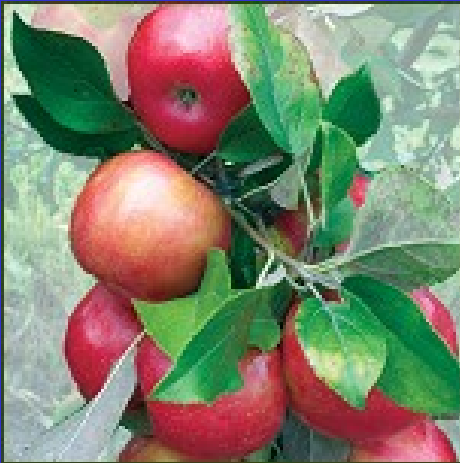
Hydrolyzed Fumonisin B₁



N-Palmitoyl Hydrolyzed Fumonisin B₁



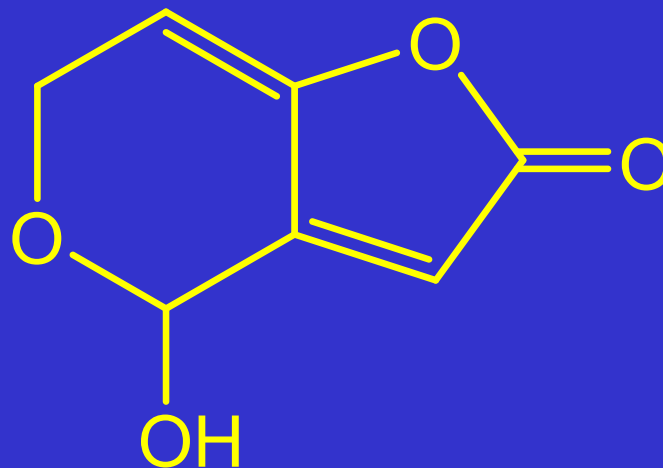
FB₁ bound to non-zein corn protein



Patulin

Produced by: Species of *Penicillium*, *Aspergillus*, and *Byssochylamys* which cause brown rot on fruit.

Structure:



Patulin

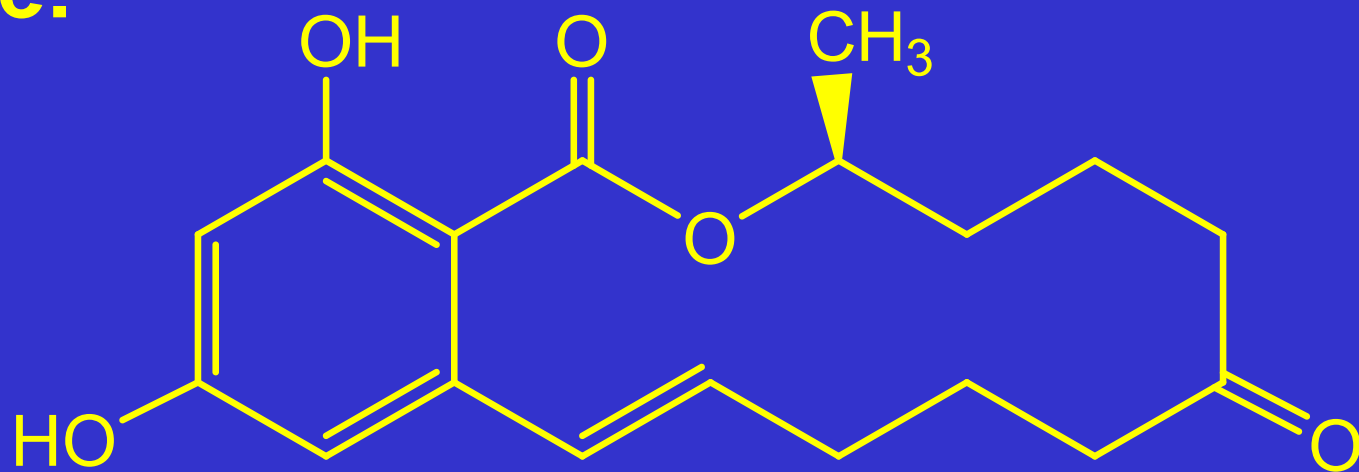
- **Mechanism of action:** Patulin is genotoxic in a number of tests.
- **Toxicity:** The FDA action level is 50 µg/L in apple juice.
- **Issues:** In fresh fruit patulin is discarded with contaminated tissue. Fermentation destroys patulin, but not heat. Apple juice is the main concern.

Zearalenone

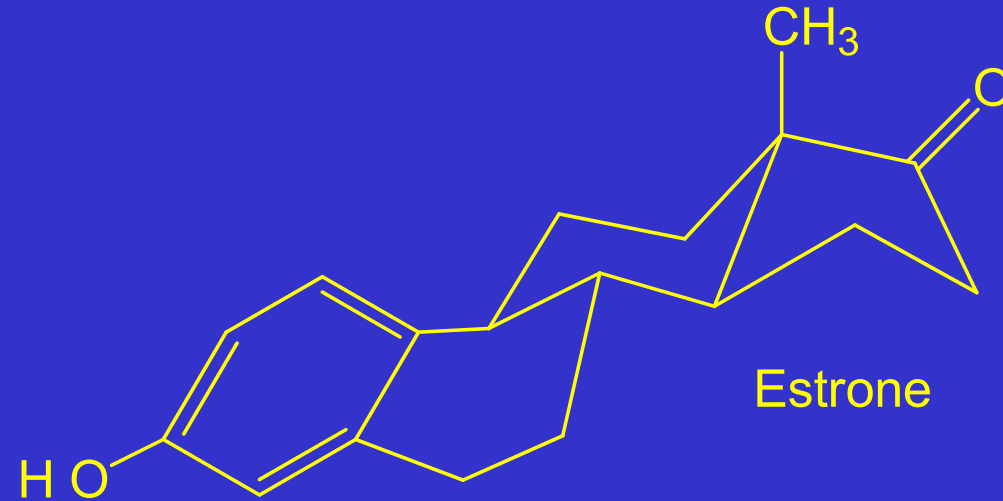
Produced by: *Fusarium graminearum*, *Fusarium culmorum*, *Fusarium equiseti*, and other *Fusarium* species.

Mechanism of action: Zearalenones are potent estrogens

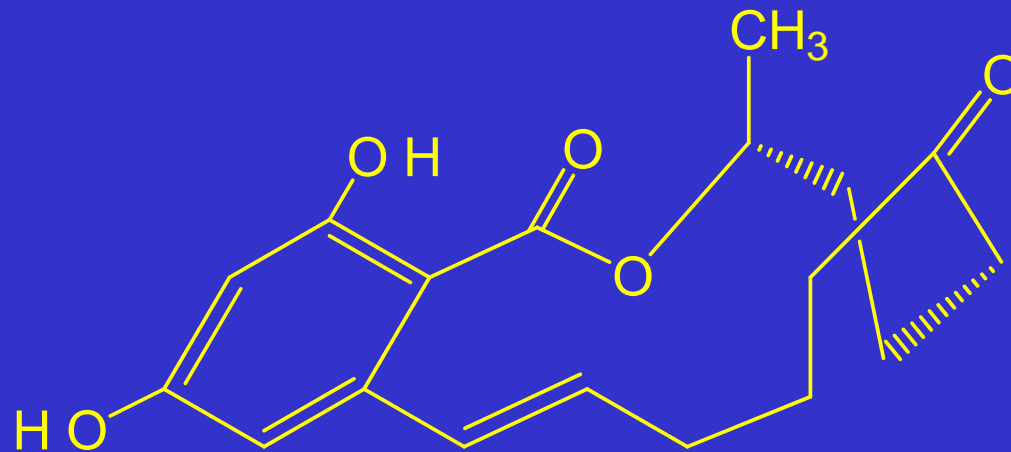
Structure:



Zearalenones can fold to fit
a receptor for estrogens . .

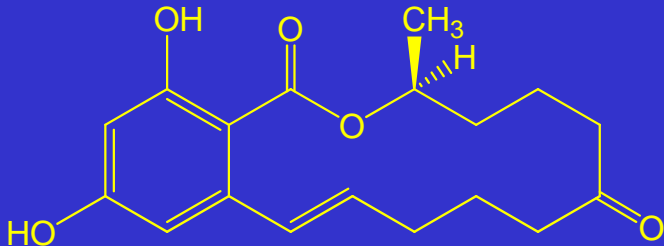
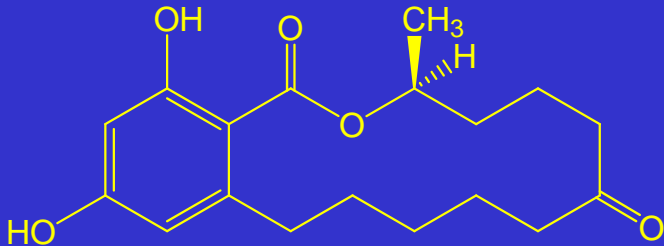
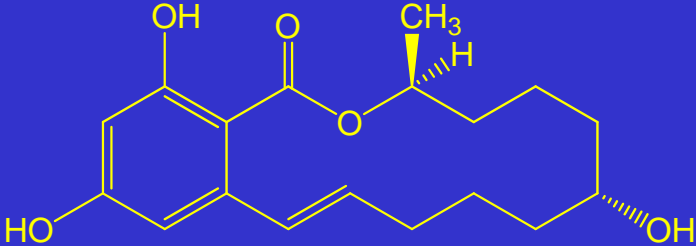
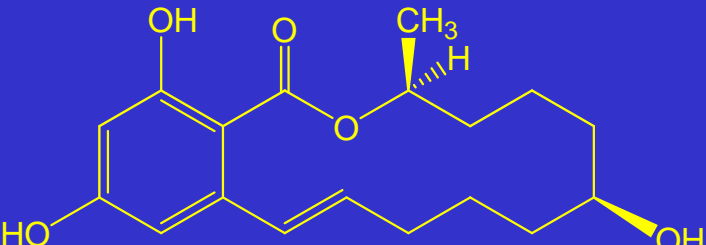


Estrone



Zearalenone

Structure-Activity Relationships of Zearalenones

Zearalenone Derivative	Structure	~EC ₅₀ for Stimulation of MCF-7 Proliferation	Relative Estrogenicity
Zearalenone		0.2 nM	1
Zearalanone		0.05 nM	4
a-Zearalenol		0.01 nM	20
b-Zearalenol		0.2 nM	1

Zearalenone

- **Toxicity:** Zearalenone causes infertility, abortion and other breeding problems, especially in swine and poultry.
- **Issues:** Zearalenone is heat-stable and is found worldwide in a number of cereal crops, such as corn, barley, oats, wheat, rice, and sorghum. It is also a problem in copra. It is second only to aflatoxins in causing economic loss to agriculture. It can often be detected in breakfast cereal and bread.

Ochratoxin A

Produced by: *Aspergillus ochraceus* and *Penicillium verrucosum*. Human exposure occurs mainly through consumption of improperly stored food products, particularly contaminated grain and pork products, as well as coffee, grapes and wine.

Structure:



Ochratoxin A

Toxicity: Ochratoxin A is listed as potentially carcinogenic to humans (Group 2B). It inhibits weight gain in chicks at 2.5 mg/kg feed.

Issues: It is a major concern in Europe, but is largely ignored in the US. It may be the cause of Balkan endemic nephropathy and epidemic nephropathy currently occurring in northern Africa.

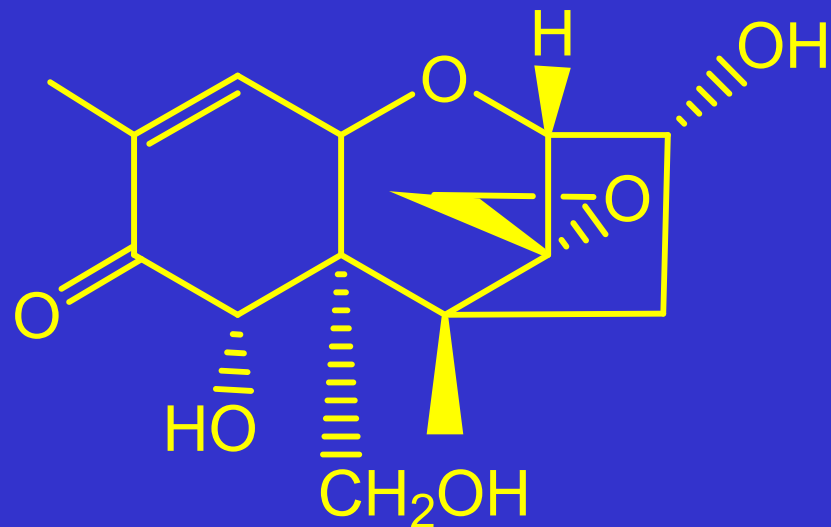
Simple Trichothecenes

Produced by: *Fusarium graminearum* and other *Fusarium* spp.

Plant Diseases: They cause head rot, stalk rot, root rot, ear rot in cold climates

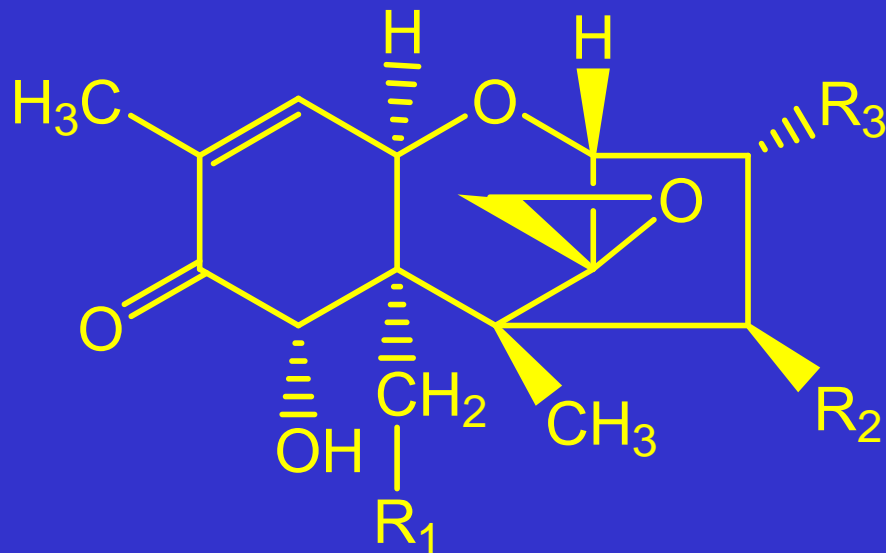
Major Concern: They cause feed refusal in livestock.

Structure:



Vomitoxin (DON)

Simple Trichothecenes



Trichothecene	R ₁	R ₂	R ₃
Deoxynivalenol	OH	H	OH
3-Acetyl-deoxynivalenol	OH	H	OAc
15-Acetyl-deoxynivalenol	OAc	H	OH
Nivalenol	OH	OH	OH
Fusarenone-X	OH	OAc	OH

FDA Regulation of Deoxynivalenol (Vomitoxin) Levels

Use	FDA Advisory Level
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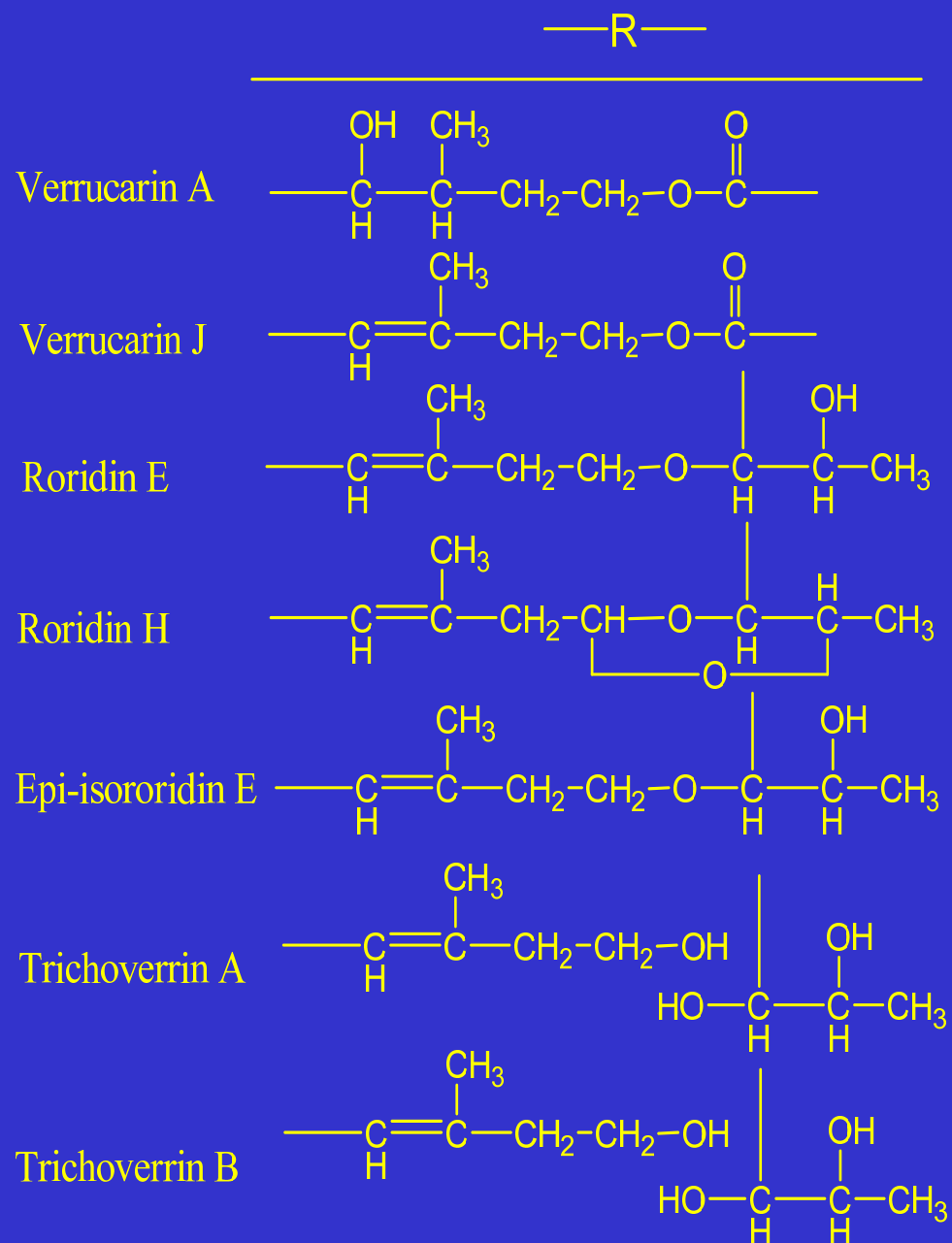
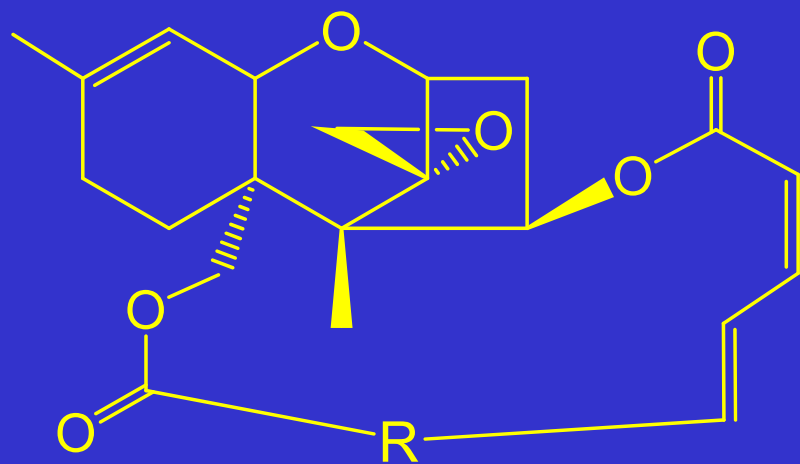
Humans - Wheat, Barley	1 ppm
Cattle and chickens (all grains)	10 ppm
Swine (all grains)	5 ppm

Source: Adapted from US Food and Drug Administration, 2000

Macrocyclic Trichothecenes

- **Produced by:** *Stachybotrys chartarum* and other *Stachybotrys* spp.
- **Toxicity:** 10 to 1000 times more toxic than simple trichothecenes.
- **Issue:** Believed to cause sick building syndrome. The spores contain high levels of toxin and are released from fungi growing in duct systems.

Macrocyclic Trichothecenes

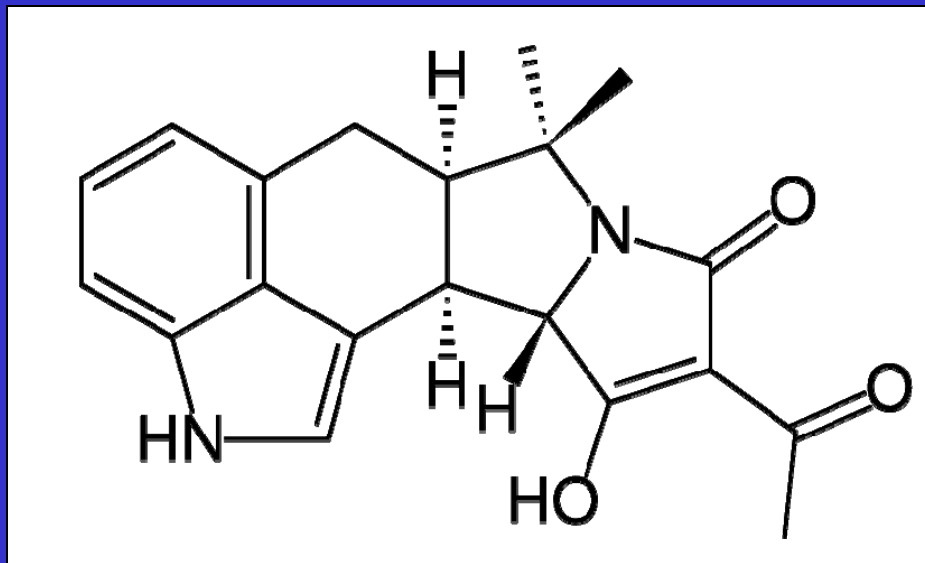


**Mycotoxins the USDA
doesn't want you to
know about**

Cyclopiazonic acid

Produced by: *Penicillium cyclopium*, *P. griseofulvum*, *Aspergillus flavus* and *A. versicolor*. It is co-produced with aflatoxin by *A. flavus*.

Structure:



Cyclopiazonic acid

Mechanism of action: Specific inhibitor of the calcium pump of calciosomes, subcellular organelles involved in the regulation of intracellular calcium. Thus, it depletes intracellular calcium stores.

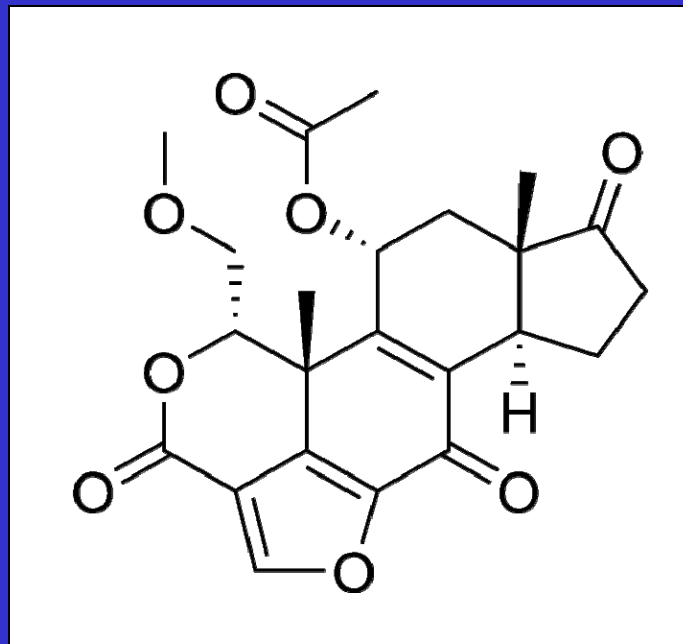
Toxicity: Inhibits weight gain in chicks at 34 mg/kg feed.

Issues: It is co-produced with aflatoxins

Wortmannin

Produced by: *Penicillium funiculosum* (fka *P. wortmannii*), *Fusarium oxysporum* and many other *Fusarium* species.

Structure:



Wortmannin

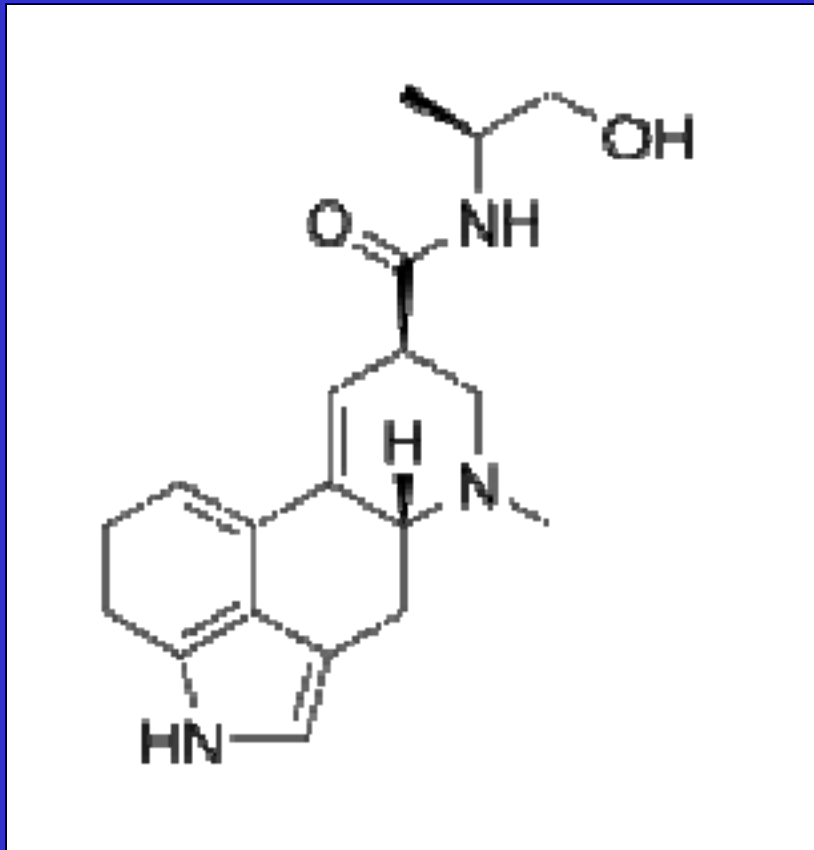
Mechanism of action: Inhibits phosphoinositide-3-kinase (PI-3-K) involved in an important cell survival signaling pathway. PI-3-K is constitutively activated in several types of cancer.

Toxicity: LD50 = 3 to 10 mg/kg oral in rats

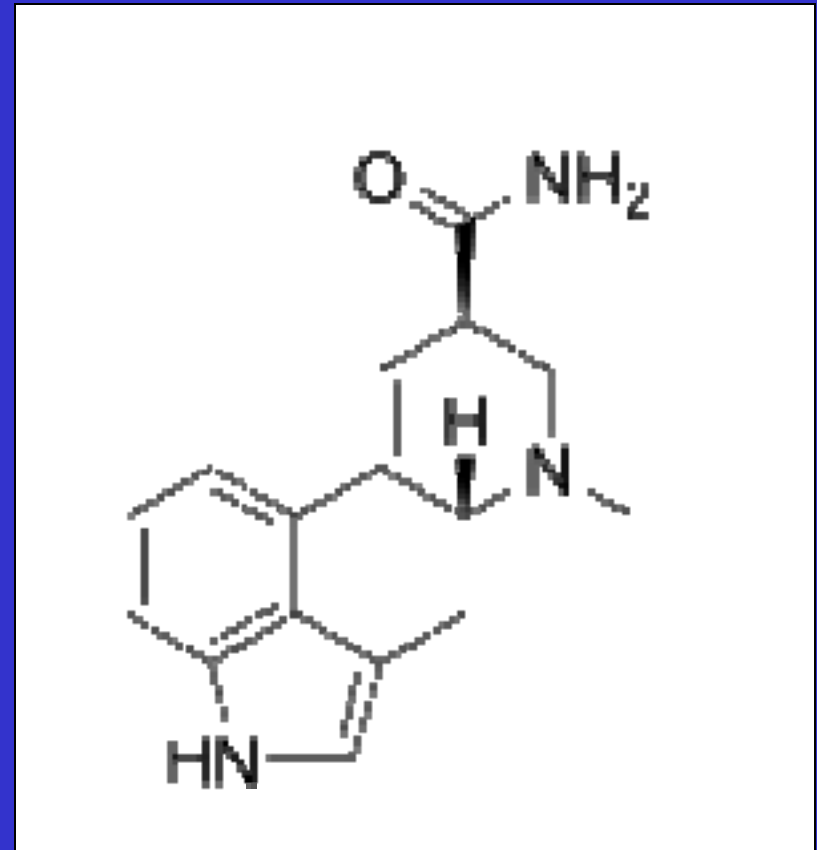
Issues: Hemorrhagic activity lead to its implication as a “yellow rain” toxin.

Other Important Mycotoxins

Ergot Alkaloids



Ergonovine



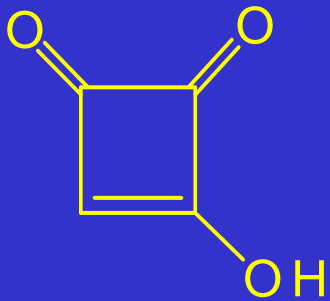
Ergine



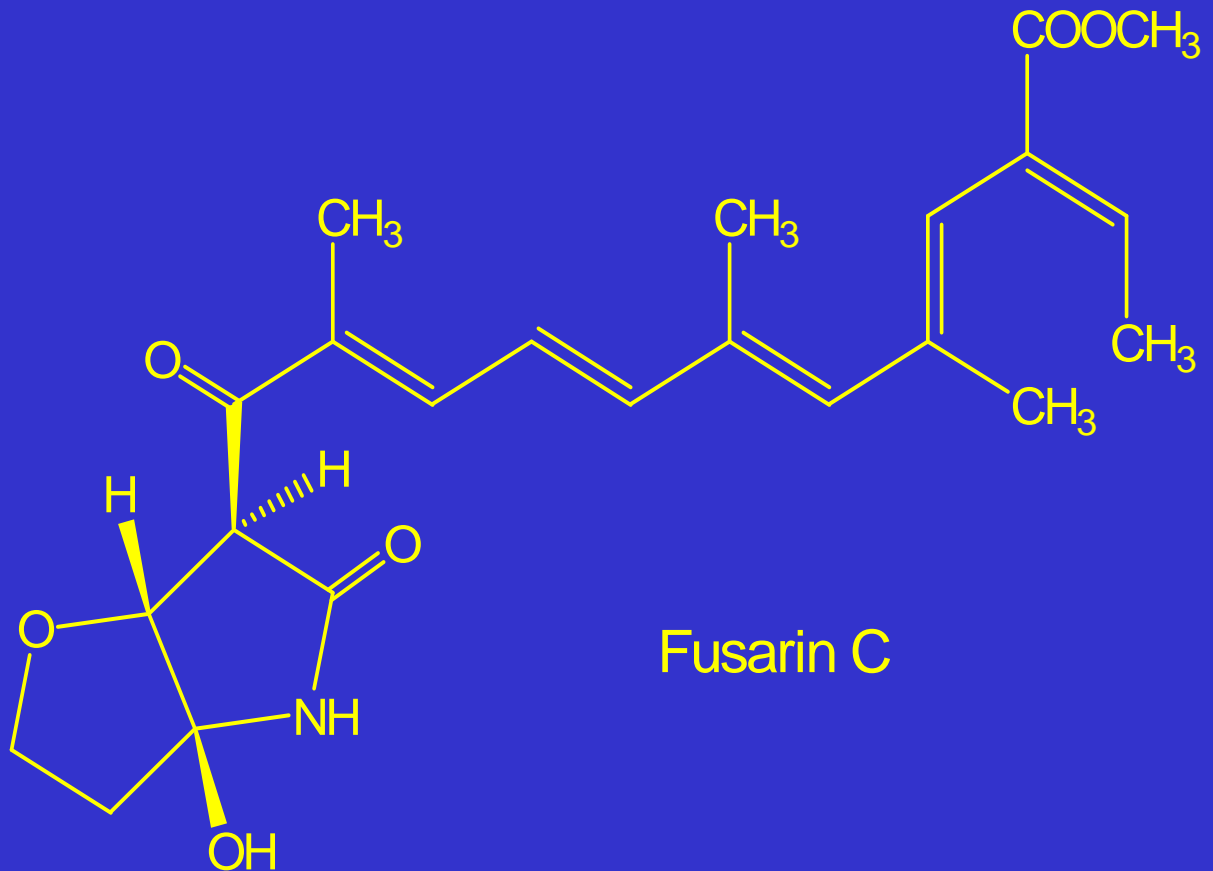
Ergot Alkaloids

- Produced by *Claviceps* spp, particularly on wheat, but are also the main alkaloids of morning glory.
- They are potent vasoconstrictors. This property underlies medical uses of ergonovine to facilitate delivery of the placenta and to prevent bleeding after childbirth, but is also the basis of pathological effects, known in the Middle Ages as St. Anthony's fire, including gangrene.

Other Mycotoxins from *Fusarium verticillioides*

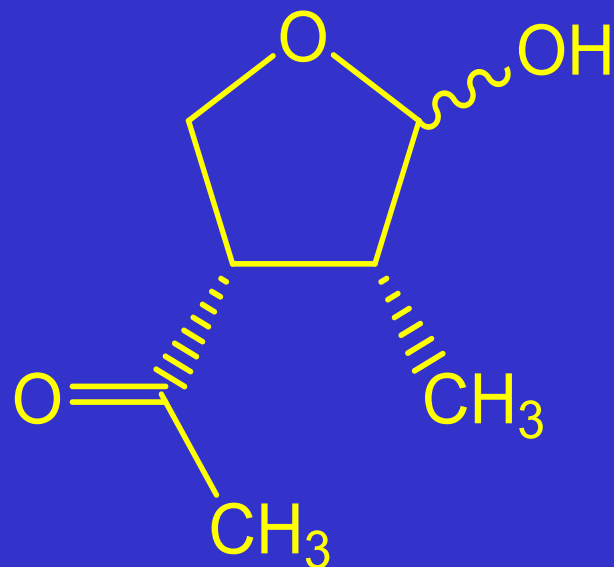


Moniliformin

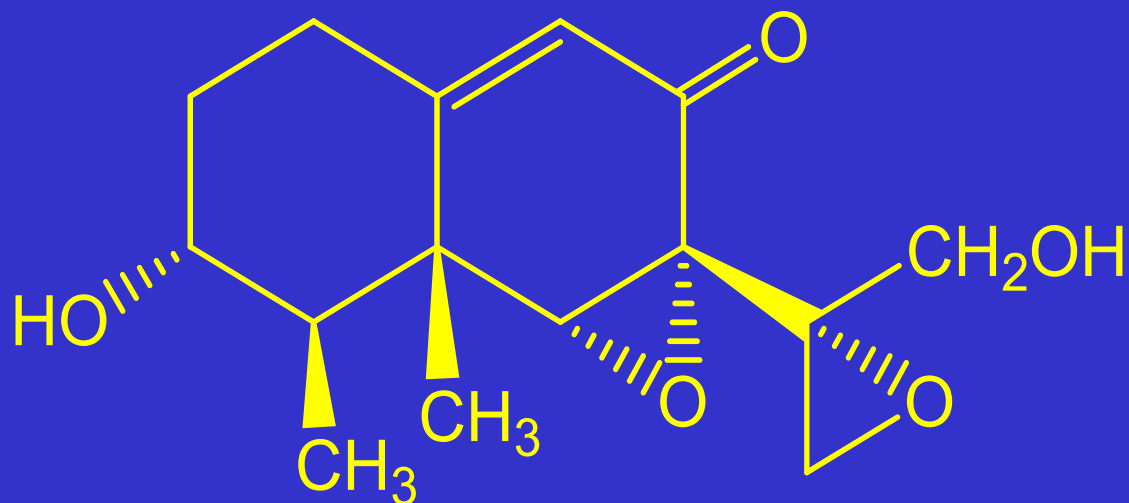


Fusarin C

A Mycotoxin from the Charcoal Rot Fungus *Macrophomina phaseolina*



(-)-Botryodiplodin



Phaseolinone

A Soybean Field with Charcoal Rot Disease



Tiny dark fungal structures (microsclerotia) on stem and root tissue look like charcoal dust



Questions?