

Mycotoxins

Refer lecturer for course updated notes.

Students are obliged to follow the courses for evaluation process and presented notes are preliminary drafts for the whole evaluation process.

- Myco: Fungus
- Toxin:Naturally-produced poison
- Natural products produced by fungi that ewoke a toxic response when introduced in low concentrations to higher vertebrates by a natural route.
- 350-400 known mycotoxins

Definition

- Mycotoxins are naturally occurring toxins produced by certain moulds (fungi) and can be found in food.
- Mycotoxins are secondary metabolites produced by microfungi that are capable of causing disease and death in humans and other animals
- All mycotoxins are low-molecular-weight natural products (i.e., small molecules) produced as secondary metabolites by filamentous fungi.

- The term mycotoxin was coined in 1962 in the aftermath of an unusual veterinary crisis near London, England, during which approximately 100,000 turkey poults died- turkey X- A.flavus
- While all mycotoxins are of fungal origin, not all toxic compounds produced by fungi are called mycotoxins.
- The target and the concentration of the metabolite are both important.

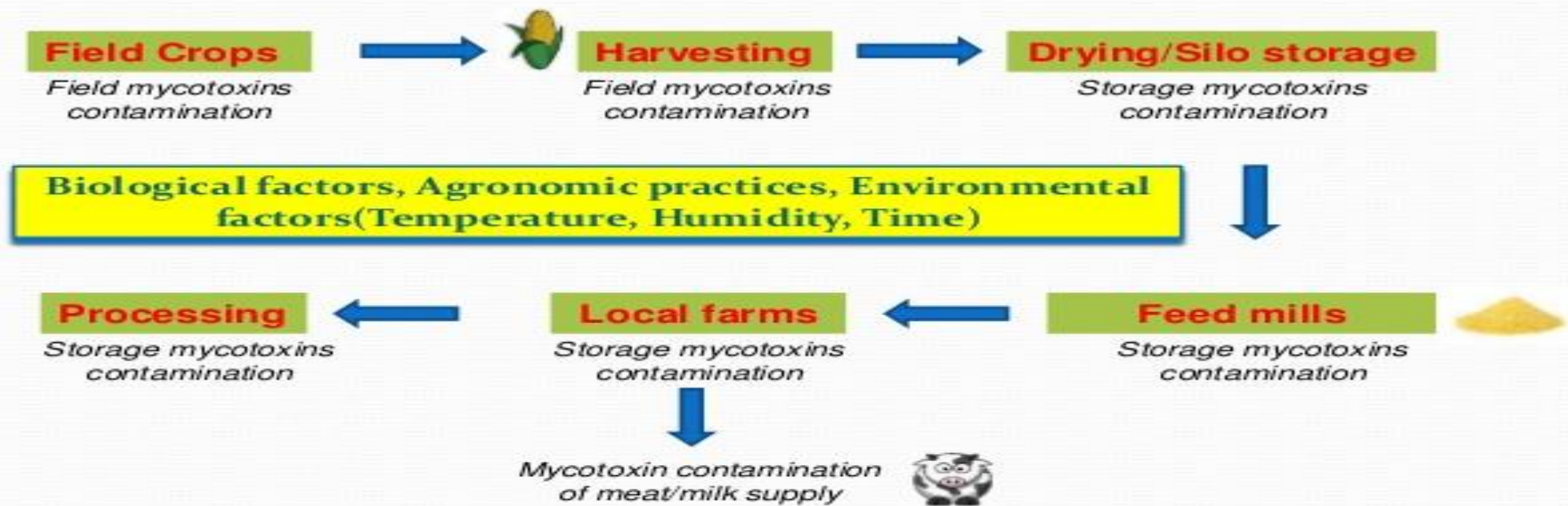
One Health-Mycotoxins approach

- The moulds grow on a variety of different crops and foodstuffs including cereals, nuts, spices, dried fruits, apples and coffee beans, often under warm and humid conditions.
- Mycotoxins can cause a variety of adverse health effects and pose a serious health threat to both humans and livestock.- ONE HEALTH principle – ATTENTION (!) RESIDUES
- The adverse health effects of mycotoxins range from acute poisoning to long-term effects such as immune deficiency and cancer.

Mycotoxicosis predisposing factors

- 1. Moisture:
 - Relative humidity 50% ↑ or higher
 - 9-10% of the moisture content in the growth medium ↑
- 2. Heat:
 - - The optimal temperature for the growth of fungi is 27 °C. However, even below 0 °C and above 55 °C, some fungal species can grow. (Produces very well at 20-30 °C. Ochratoxin cold climate)
- 3. Oxygen
 - Mushrooms are aerobic organisms. Therefore, if the CO₂ concentration in the environment rises above 10%, the fungal microflora is quickly suppressed.
- 4. Type of food
 - Especially agricultural products that are rich in available carbohydrates and fat content undergo rapid mold growth.
- Mechanically damaged during harvest and processing (parasite attack.vs)
- 5. They mostly grow between pH 2 and 7.5. Generally, foods with a slightly acid pH are more suitable for fungal activity than basic environments.
- 6. The presence of various metal ions, fungicidal substances and radiation in the living environment adversely affects the fungal life. They grow within 2-4 days when the conditions are appropriate.

Mycotoxin occurrence



Transport and storage: risks (Taşıma ve depolama: Riskler)

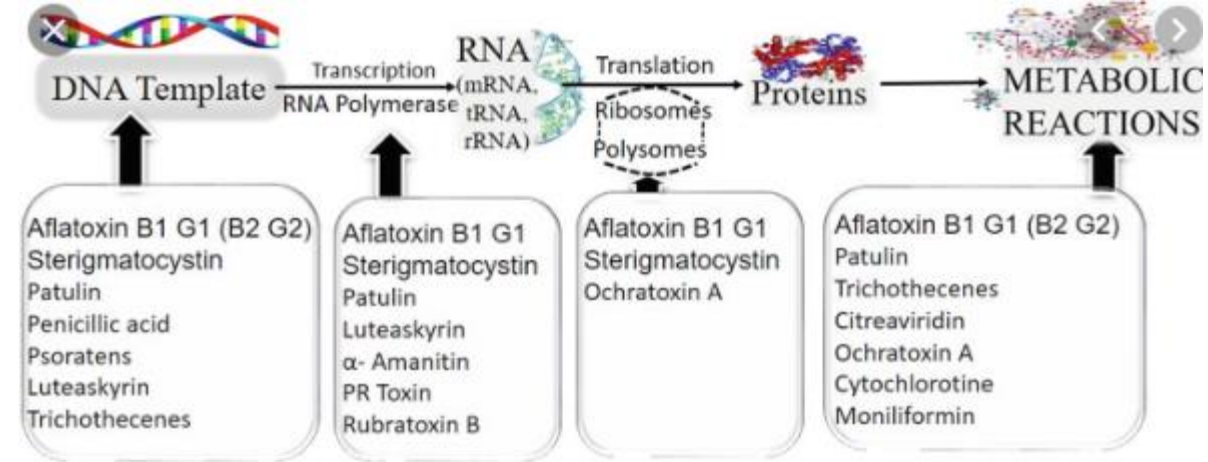
- Major risk: re-wetting

Temel risk : tekrar ıslatma

- leaky storage sızdıran depo
- insufficient ventilation yetersiz havalandırma
- bags against walls duvarlara karşı çuvallar
- loading container in rain kutuları yağmurda yüklemek
- leaky containers sızdıran kutular
- insufficient moisture control in containers kutularda eksik nem kontrolü
- keeping coffee in container after arrival ulaştıktan sonra kahveyi kutuda saklamak



Mechanism



- 1. Interaction with DNA Pattern → Aflatoxin, PRtoxin, luteoskirin, T-2 toxin
- 2. Prevention of mold release, spelling and translation → OA, Aflatoxin, Patulin, Trichothecene
- 3. Change in cell membrane permeability → Citrinin, rubratoxin, moliniformin, cytokalacin B
- 4. Inhibition of cell respiration → Aflatoxin, ochratoxin
- 5. Hormonal effect → Zearelenone

- Aspergillus toxins
 - Aflatoxins, aspergillic acid, gliotoxin
- Penicilium toxins
 - Rugulosin, luteoskirin, citrinin, ochratoxins, patulin
- Fusarium toxins
 - Fusarium, mirotesium, trichothecenes
- Others
 - Alternariol, cytochalcons

According to Structures

- Macrocyclic lactones
- Quinon and the like
- Amino acid-peptide compounds
- Oxygen bearing heterocyclic compounds
- Alicyclic compounds
- Aromatic compounds

Control of Mycotoxin

- Control of Mycotoxins is for the purpose of public health importance and economic improvement in the country. Hence, a number of strategies for reduction and control of mycotoxins have been considered in different areas of world.
- **The control of mycotoxins involves:**
 - 1.Prevention of mould or fungus growth in crops and other feedstuffs;
 - 2.Decontamination of mycotoxin contaminated feeds/foods as a secondary strategy;
 - 3.Continuous surveillance of mycotoxins in agricultural crops, animal feedstuffs and human food.

Control

- Control of Fungal Invasion in Field Conditions
 - Captain, Thiram, Zineb, Proprionic acid, Acetic acid (weed medicines)
- Harvesting, Drying
 - Ammonia, phosphine
 - (50 mg phosphine + 50 mg ammonia per liter) - Phosphine does not affect spores.

Decontamination of mycotoxin contaminated feeds/foods

- Includes **physical, chemical and biological approaches**.
- **Physical approaches** enlist as sorting, washing and crushing combined with dehulling of maize grains, were effective in removal of Aflatoxin and Fumonisin in Benin (Fandohan, Gnonlonfin, Hell, Marasas, & Wingfield, 2005).
- **Chemical approaches** are the activities incorporating application of fungicides such as prochloraz, propiconazole, epoxyconazole, tebuconazole, cyproconazole, Oltipraz, chlorophylin and azoxystrobin for reduction of Fumonisin and Aflatoxin contamination (Haidukowski et al., 2005; Hayes et al., 1998; Ni & Streett, 2005).
- **Biological approaches** depend on the development of atoxigenic fungi that compete with toxigenic fungi in the environment. Introduction of atoxigenic strains of *A. flavus* and *A. parasiticus* to soil of developing crops resulted in 74.3 to 99.9% reduction in the Aflatoxin contamination of peanuts in USA (Dorner, Cole, & Blankenship, 1998)

Control

- Physical
 - Selection of feed
 - Open for sun dry
 - UV- Gamma irradiation

Control

- Storage
 - The rate of foreign matter in storage should not exceed 10%.
- Combined Feed Protection
 - Propionic acid (2.5 kg eating sodium and calcium propionate-1 ton), lactic acid, sorbic acid, formic acid

Chemical Control

- Aerofungin, thiram, captan, orthophenylphenate, burgundy slurry
- +
- Organic acids (propionic acid, sorbic acid, acetic acid, benzoic acid)
- Copper sulphate
- Liquid ammonia (browning)
- Oxidizing agents (hydrogen peroxide, ozone, sodium bisulfite and sodium metabisulfite)

Competitive biocontrol agents to reduce infection (**enfeksiyonu azaltan rekabetçi biyokontrol temsilcisi**)

- *B.subtillis* – effective in lab conditions but difficult to deliver in field
(Kimura and Hirano, 1988)
B.Subtillis – Laboratuvar koşullarında etkili fakat alanda dağıtmak zor
- *Pseudomonas cepacia* – spray inoculated onto cottonseed
(Misaghi *et al* 1995)
Pseudomonas cepacia – pamuk tohumu üzerine sprey aşılama
- Saprophytic yeasts – applied to almonds and pistachio orchards
(Hua, 2002)
Saprophytic mayalar – badem ve fıstık meyve bahçelerine uygulanan
- Non-aflatoxigenic fungi – peanuts, cottonseed and maize
(Cole *et al.*, 1989; Cotty, 1990; Brown *et al.*, 1991)
Aflatoksijenik olmayan mantarlar – yer fıstığı, pamuk tohumu ve mısır



Binding agents

- HSCAS (hydrated sodium calcium aluminum silicate)
- 0.1-0.3% is added at most 0.5. If clay substances are added more than this rate, feed utilization (due to the binding of vitamins and minerals) is reduced.
- Bentonite 10%
- Coal
- cholestyramine

Mycotoxin Binder

Bio-Adsorbent



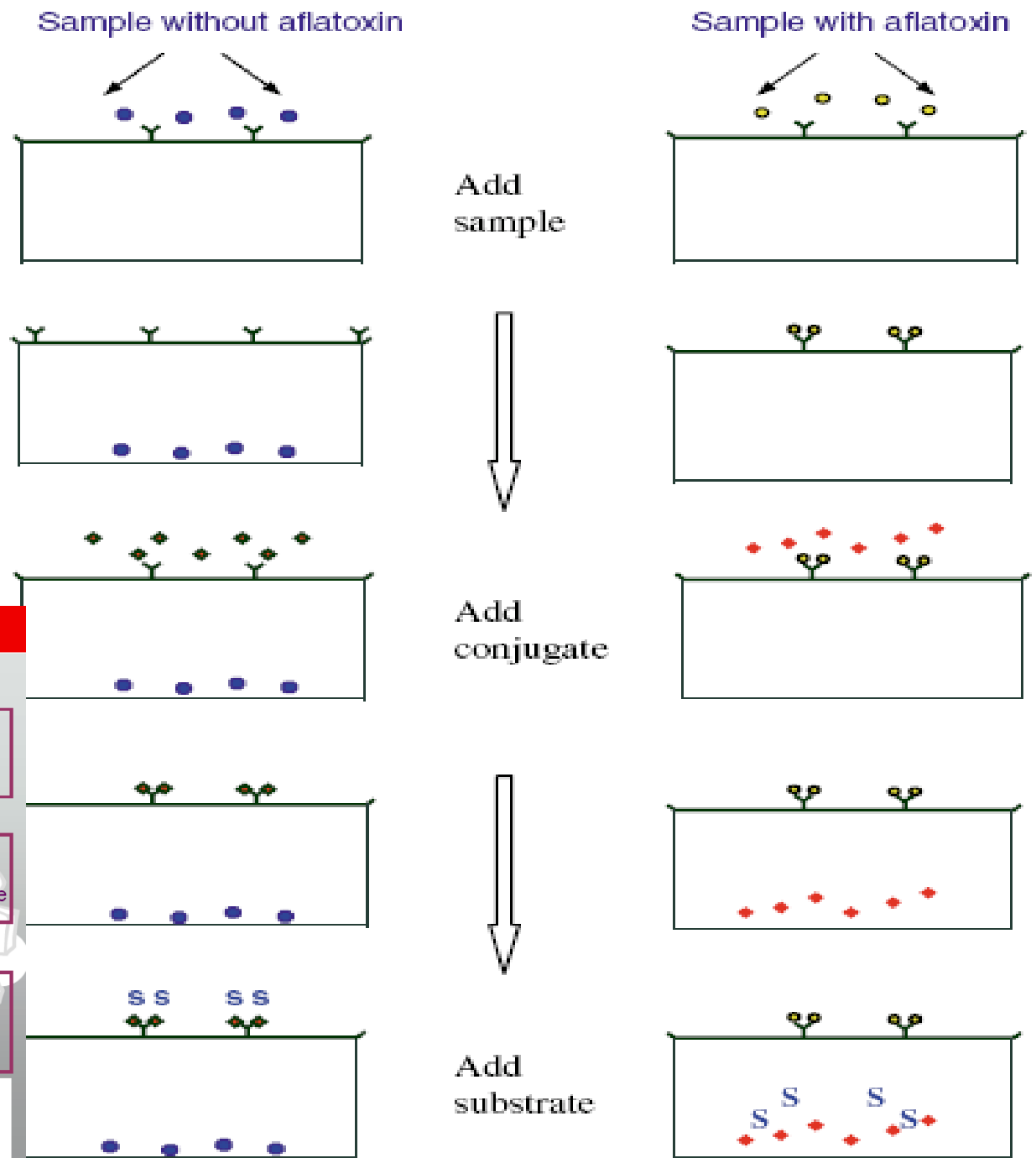
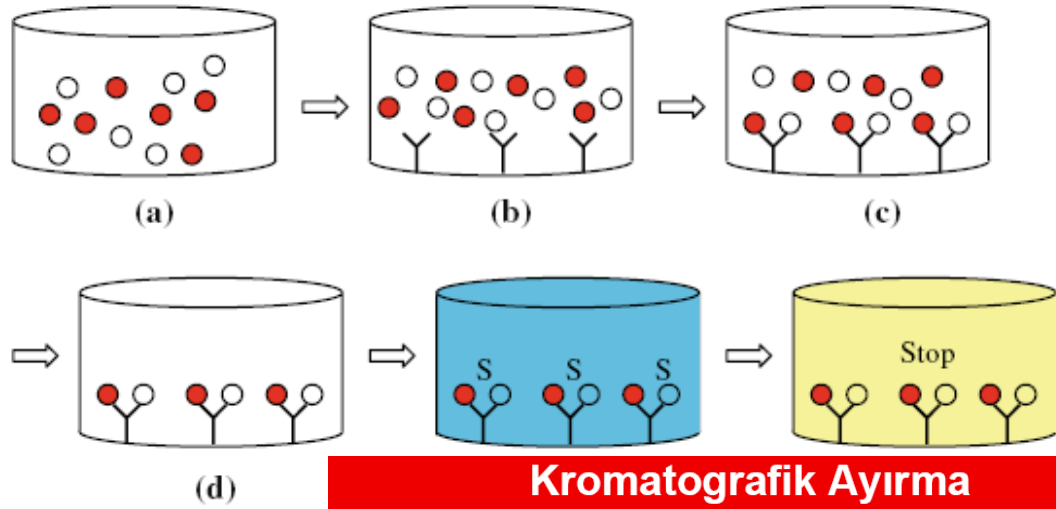
Comprehensive protection for animals
Selectively adsorbing mycotoxins only

Mycotoxin Binders

- Numerous products are marketed as anticaking agents to sequester or "bind" aflatoxins and reduce absorption from the GI tract.
- One effective binder for aflatoxins is **hydrated sodium calcium aluminosilicates (HSCAS)**, which reduce the effects of aflatoxin when fed to pigs or poultry at 10 lb/ton (5 kg/tonne).
- They also provide substantial protection against dietary aflatoxin. HSCAS reduce aflatoxin M₁ in milk by ~50% but do not eliminate residues of aflatoxin M₁ in milk from dairy cows fed aflatoxin B₁.
- Other adsorbents (**sodium bentonites, polymeric glucomannans**) have shown variable but partial efficacy in reducing low-level aflatoxin residues in poultry and dairy cattle. To date, the FDA has not licensed any product for use as a "mycotoxin binder" in animal feeds.

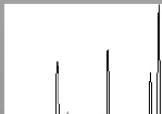
- Chlorinating agents → sodium hypochlorite, chlorine dioxide (gaseous chlorine)
- Oxidizing Agents → hydrogen peroxide, ozone (AFB1, AFB2) and sodium bisulfite
- Hydrolytic Substances → acids, alkalis

- Mycotoxin-enzyme conjugate
- Mycotoxin
- Y Anti-mycotoxin antibody
- S Substrate



Kromatografik Ayırma

TLC	→	<ul style="list-style-type: none"> 😊 Kalitatif analiz 🚫 Kantitatif analiz
GC / GC-MS	→	<ul style="list-style-type: none"> 😊 Hassas ve seçici 🚫 Pahalı, GC için türevlendirme
HPLC-FLD	→	<ul style="list-style-type: none"> 😊 Hassas ve güvenilir 🚫 Pahalı, temizleme türevlendirme,



Aflatoxicosis

- *A.flavus*, *A.parasiticus*- difuranocoumarin derivatives produced by a polyketide pathway by many strains of mentioned *Aspergillum* species
- Moldy peanuts, soybeans, cottonseeds, rice, sorghum, corn (maize), other cereals
- All poultry, pigs, cattle, sheep, dogs
- Major effects in all species are slow growth and hepatotoxicosis
- Major forms of aflatoxin in feedstuff-B1, B2, G1, G2, M1, M2- fluorescence under UV light (blue or green) and relative chromatographic mobility during thin-layer chromatography
- Mycotoxin-producing molds grow at temperatures of 24–35°C, moisture of 18–20%.

Aflatoxicosis

- toxic response and disease in mammals and poultry varies in relation to species, sex, age, nutritional status, and the duration of intake and level of aflatoxins in the ration
- Aflatoxicosis occurs in many parts of the world and affects growing poultry (especially ducklings and turkey poults), young pigs, pregnant sows, calves, and dogs

Aflatoxicosis

- metabolized in the liver (Cytochrome P450 enzymes) - epoxide - 8,9-epoxide form (also referred to as aflatoxin-2,3 epoxide in the older literature)- binds to macromolecules, especially nucleic acids and nucleoproteins.
- mutagenesis due to alkylation of nuclear DNA,
- Carcinogenesis - Inactivation of the p53 tumor suppressor gene
- teratogenesis,
- reduced protein synthesis,
- and immunosuppression.

Aflatoxin Metabolism

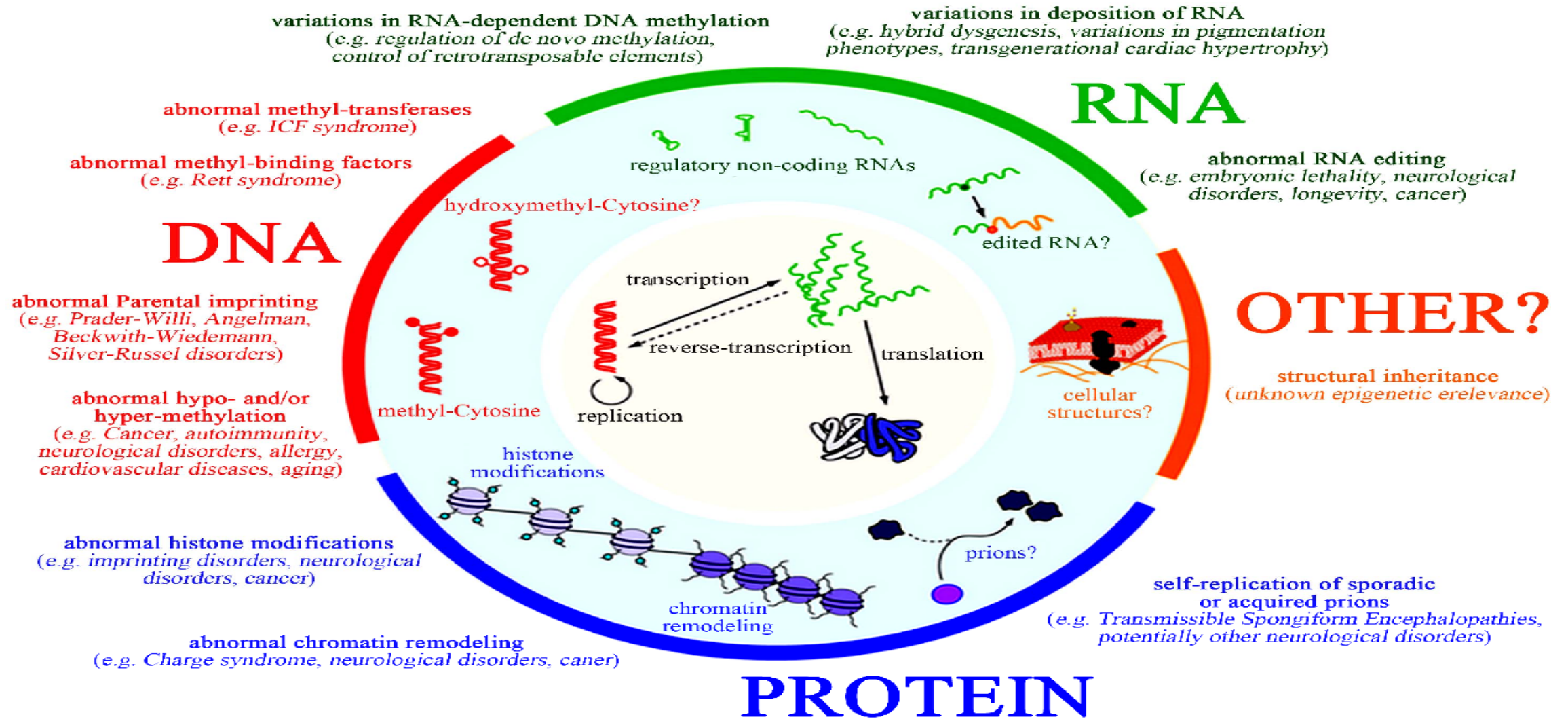


Figure 6. Targets of xenobiotics like aflatoxins on various sites of protein synthesis and their effects on genetic and epigenetic processes.

Aflatoxicosis

- Reduced protein synthesis - reduced production of essential metabolic enzymes and structural proteins for growth.
- LIVER (!!!) is the principal organ affected.
- High dosages of aflatoxins result in hepatocellular necrosis; prolonged low dosages result in
 - reduced growth rate,
 - immunosuppression, and liver enlargement

Aflatoxicosis-pathological findings

- Macroscopy
 - hepatic enlargement, congestion, yellow discoloration, and friability; petechiae or more generalized hemorrhage; and edema and ecchymotic or petechial hemorrhages of the gall bladder.
- Microscopy- depend upon the dose and duration of exposure
 - hepatocellular degeneration and necrosis in the centrilobular zone.
- Fibrosis and bile duct proliferation may be extensive and found together with fibrotic veno-occlusion of the central veins.

Susceptible Species/Tolerable Dietary Levels

- Aflatoxicosis occurs in many parts of the world and affects growing poultry (especially ducklings and turkey poults), young pigs, pregnant sows, calves, and dogs. Adult cattle, sheep, and goats are relatively resistant to the acute form of the disease but are susceptible if toxic diets are fed over long periods.
- Experimentally, all species of animals tested have shown some degree of susceptibility.
- **Dietary levels of aflatoxin (in ppb) generally tolerated are:**
- ≤ 50 in young poultry, ≤ 100 in adult poultry, ≤ 50 in weaner pigs, ≤ 200 in finishing pigs, < 50 in dogs, < 100 in calves, and < 300 in cattle.

Approximately two times the tolerable levels stated is likely to cause clinical disease, including some mortality.

Dietary levels as low as 10–20 ppb result in measurable metabolites of aflatoxin (aflatoxin M₁ and M₂) being excreted in milk; feedstuffs that contain aflatoxins should not be fed to dairy cows.

Acceptable regulatory values in milk may range from 0.05 ppb to 0.5 ppb in different countries; individual state or federal regulatory agencies should be consulted when contamination occurs.

Aflatoxin in Food

Commodity	Maximum Level		
	B1	Total	M1
Groundnuts (peanuts) and other oilseeds for sorting or processing	8 ppb	15 ppb	-
Groundnuts (peanuts) and other oilseeds intended for direct human consumption	2 ppb	4 ppb	-
Almonds, pistachios and apricot kernels for sorting or processing	12 ppb	15 ppb	-
Almonds, pistachios and apricot kernels intended for direct human consumption	8 ppb	10 ppb	-
Hazelnuts and Brazil nuts for sorting or processing	8 ppb	15 ppb	-
Hazelnuts and Brazil nuts intended for direct human consumption	5 ppb	10 ppb	-
Tree nuts, other than those listed for sorting and processing	5 ppb	10 ppb	-
Tree nuts, other than those listed intended for direct human consumption	2 ppb	4 ppb	-
Dried fruit for sorting and processing	5 ppb	10 ppb	-
Dried fruit intended for direct human consumption	2 ppb	4 ppb	-
Maize and rice (including brown rice) for sorting and processing	5 ppb	10 ppb	-
All cereals, including maize and rice, intended for direct human consumption	2 ppb	4 ppb	-
Spices (capsicum, pepper, nutmeg, ginger, turmeric)	5 ppb	10 ppb	-
Milk (raw milk, milk for manufacturing of milk based products and heat treated milk)	-	-	0.05 ppb
Baby foods and processed cereal based foods for infants and young children	0.1 ppb	-	-
Infant formulae and follow-on formulae, including infant milk and follow-on milk	-	-	0.025 ppb
Dietary foods for special medical purposes intended specifically for infants	0.1 ppb	-	0.025 ppb

Aflatoxin in Feed

Commodity	Maximum Level (B1)
All feed materials	20 ppb
Complementary and complete feed	10 ppb
Complementary and complete feed with the exception of: <ul style="list-style-type: none"> • compound feed for dairy cattle and calves, dairy sheep and lambs, dairy goats and kids, piglets and young poultry animals 	5 ppb

Citrinin

- *Penicillium citrinum*
- dozen species of *Penicillium* and several species of *Aspergillus* (e.g., *Aspergillus terreus* and *Aspergillus niveus*), including certain strains of *Penicillium camemberti* (used to produce cheese) and *Aspergillus oryzae* (used to produce sake, miso, and soy sauce), *Monascus ruber* and *Monascus purpureus*
- yellow rice disease in Japan
- porcine nephropathy
- Nephrotoxin
- LD50 for ducks is 57 mg/kg; for chickens it is 95 mg/kg; and for rabbits it is 134 mg/kg

Citrinin

- can act synergistically with ochratoxin A to depress RNA synthesis in murine kidneys
- Wheat, oats, rye, corn, barley, and rice have all been reported to contain citrinin

CITRININ

Citrinin in Food

Commodity	Maximum Level
Food supplements based on rice fermented with red yeast <i>Monascus purpureus</i>	2000 ppb

PATULIN

Patulin in Food

Commodity	Maximum Level
Fruit juices and drinks containing apple juice or derived from apples	50 ppb
Solid apple products, such as apple puree, intended for direct human consumption	25 ppb
Apple juice and solid apple products for infants and young children	10 ppb

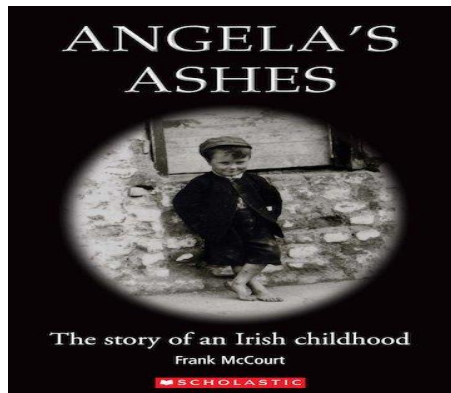
Ergot Alkaloids

- indole alkaloids- *Claviceps purpurea*
- Lysergic acid derivatives
- An Assyrian tablet dated to 600 B.C.E., referring to a “noxious pustule in the ear of grain
- Europe in the Middle Ages- Salem witchcraft

ERGOTISM



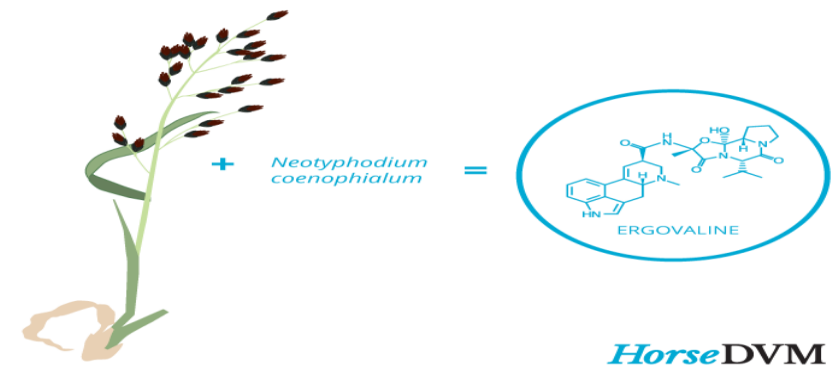
Matthias Grünewald's "The Temptation of St Anthony." Note the character in the bottom left corner, said to represent the symptoms of ergotism.



«Black Hands» Long term Ergotism effects



Clinical Findings and Lesions



- ergotized hay or grain or occasionally by grazing seeded pastures infested with ergot.
- Lameness, the first sign 2–6 wk or more after initial ingestion, depending on the concentration of alkaloids in the ergot and the quantity of ergot in the feed.
- Hindlimbs are affected before forelimbs, but the extent of involvement of a limb and the number of limbs affected depends on the daily intake of ergot.
- Body temperature and pulse and respiration rates are increased.
- **Epidemic hyperthermia** and hypersalivation may also occur in cattle poisoned with *C purpurea* (Also see [Fescue Poisoning](#)). Ergot alkaloids may interfere with embryonic development in pregnant females.

Diagnosis

- **causative fungus (ergot sclerotia)** in grains, hay, or pastures provided to livestock showing signs of ergotism.
- Ergot alkaloids may be extracted and detected in suspect ground grain meals. **At 200–600 ppb, ergot alkaloids may cause clinical signs and effects**; however, this is influenced by the relative amounts of various ergot alkaloids in the grain.
- Identical signs and lesions of **lameness**, and sloughing of the hooves and tips of ears and tail, are seen in fescue foot in cattle grazing in winter on tall fescue grass infected with an endophyte fungus, in which the ergot alkaloid ergovaline is considered a major toxic principle. In gilts and sows, lactation failure not associated with ergot alkaloids must be differentiated from prolactin inhibition due to ergot.

Treatment

- In horses, parenteral use of the dopamine D2 antagonist domperidone (1.1 mg/kg, PO, bid for 10–14 days) is effective in prevention ofagalactia from ergot alkaloids in fescue.
- Use against the same alkaloids produced by *C purpurea* could be medically logical .

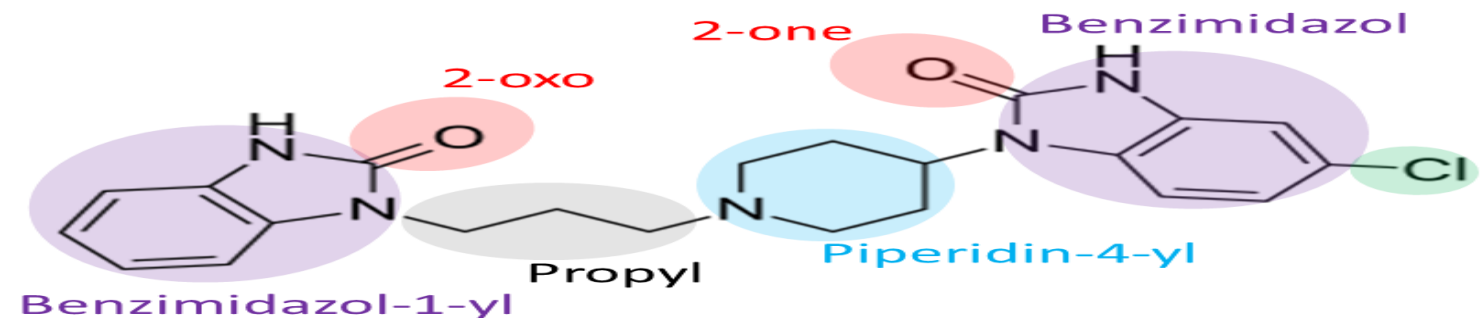


How to memorize drug structures

Blocker of dopamine receptors

Domperidone

6-chloro-3-[1-[3-(2-oxo-3H-benzimidazol-1-yl)propyl]piperidin-4-yl]-1H-benzimidazol-2-one



Control

- Intake of ergot bodies should be <0.1% of the total diet, and concentrations of ergot alkaloids should be <100 ppm in the total diet.
- Ergotism can be controlled by an immediate change to an ergot-free diet.
- In pregnant sows, however, removal of ergot in late gestation (<1 wk before parturition) may not correct the agalactia syndrome, and animals with clinical peripheral gangrene will not likely recover.
- Under pasture feeding conditions, frequent grazing or topping of pastures prone to ergot infestation during the summer months reduces flower-head production and helps control the disease. Grain that contains even small amounts of ergot should not be fed to pregnant or lactating sows.

Fescue Poisoning

Fescue foot	Ergovaline	<i>Neotyphodium coenophialum</i>	USA, Australia, New Zealand, Italy	Tall fescue grass (<i>Lolium arundinacea</i>)	Cattle, horses	Lameness, weight loss, hyperthermia, heat intolerance, dry gangrene of extremities, agalactia, thickened fetal membranes. Also see Fescue Poisoning .
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- Fescue lameness, which resembles ergot poisoning, is believed to be caused by ergot alkaloids, especially ergovaline, produced by the endophyte fungus *Neotyphodium coenophialum* in tall fescue grass (*Lolium arundinaceum*, formerly *Festuca arundinacea*).
- It begins with lameness in one or both hindfeet and may progress to necrosis of the distal part of the affected limb(s). The tail and ears also may be affected independently of the lameness.
- In addition to gangrene of these extremities, animals may show loss of body mass, an arched back, and a rough coat. Outbreaks have been confirmed in cattle, and similar lesions have been reported in sheep.

ERGOT ALKALOIDS

Ergotalkaloids/Rye Ergot (*Claviceps purpurea*) in Feed

Commodity	Maximum Level
Feed materials and compound feed containing unground cereals	1000 ppm

Fumonisin

- *Fusarium verticillioides* (previously *F moniliforme* Sheldon) and *Fusarium proliferatum*, and *Fusarium nygamai*, as well as *Alternaria alternata* f. sp. *lycopersici*
- Three toxins produced by the fungi have been classified as fumonisin B₁ (FB₁), B₂ (FB₂), and B₃ (FB₃).
- The most abundantly produced member of the family is fumonisin B₁

Fumonisin Toxicosis

- interfering with sphingolipid metabolism
- **Equine leukoencephalomalacia**-CNS that affects horses, mules, donkeys and rabbits
- pulmonary edema and hydrothorax - swine
- hepatotoxic and carcinogenic effects and apoptosis -liver of rats
- Humans-probable link with esophageal cancer
- Feeding of moldy corn (maize), usually over a period of several weeks.
- The toxins are concentrated primarily in molded, damaged, or light test weight corn.
- Major health effects are seen in Equidae and swine.

- Cattle, sheep, and poultry are considerably less susceptible to fumonisins than are horses or swine.
- Cattle and sheep tolerate fumonisin concentrations of 100 ppm with little effect.
- Dietary concentrations of 150–200 ppm cause inappetence, weight loss, and mild liver damage.
- Poultry are affected by concentrations of >200–400 ppm and may develop inappetence, weight loss, and skeletal abnormalities.
- Unlike most known mycotoxins, which are soluble in organic solvents, fumonisins are hydrophilic.

Fumonisin in Food

Commodity	Maximum Level (B1 & B2)
Unprocessed maize not intended for wet milling	4000 ppb
Maize and maize based foods intended for direct human consumption	1000 ppb
Maize based breakfast cereals and maize based snacks	800 ppb
Processed maize based foods and baby foods for infants and young children	200 ppb

Fumonisin in Feed

Commodity	Guidance Level (B1 & B2)
Maize and maize based products	60 ppm
Complementary and complete feeding stuffs for pigs, horses, rabbits and pet animals	5 ppm
Complementary and complete feeding stuffs for fish	10 ppm
Complementary and complete feeding stuffs for poultry, calves (<4 months), lambs and kids	20 ppm
Complementary and complete feeding stuffs for adult ruminants (>4 months) and mink	50 ppm

Ochratoxin

- *Aspergillus ochraceus*
- Members of the ochratoxin family have been found as metabolites of many different species of *Aspergillus*, including *Aspergillus alliaceus*, *Aspergillus auricomus*, *Aspergillus carbonarius*, *Aspergillus glaucus*, *Aspergillus melleus*, and *Aspergillus niger*
- *Penicillium* species, it is now thought that *Penicillium verrucosum*,

Ochratoxin

- kidney is the primary target organ.
- human disease called endemic Balkan nephropathy
- Ochratoxin A is a nephrotoxin to all animal species studied to date and is most likely toxic to humans
- Ochratoxin has been detected in blood and other animal tissues and in milk, including human milk

Ochratoxin in Food

Commodity	Maximum Level
Unprocessed cereals	5 ppb
All products derived from unprocessed cereals (including cereal products and cereal grains intended for direct human consumption)	3 ppb
Dried wine fruit (currants, raisins and sultanas)	10 ppb
Roasted coffee beans and ground roasted coffee	5 ppb
Soluble coffee (instant coffee)	10 ppb
Wine (including sparkling wine, excluding liqueur wine and wine with an alcoholic strength of not less than 15 % volume) and fruit wine	2 ppb
Flavoured wine, flavoured wine based drinks and flavoured wine product cocktails	2 ppb
Grape juice, concentrated grape juice as reconstituted, grape nectar, grape must and concentrated must as reconstituted, intended for direct human consumption	2 ppb
Species of spices (including white and black pepper, nutmeg, ginger and turmeric, excluding <i>Capsicum</i> spp.)	15 ppb
<i>Capsicum</i> spp. spices (including chillies, chilli powder, cayenne and paprika)	20 ppb
Mixtures of spices	15 ppb
Liquorice root	20 ppb
Liquorice extract	80 ppb
Baby foods and processed cereal based foods for infants and young children	0.5 ppb
Dietary foods for special medical purposes intended specifically for infants	0.5 ppb
Wheat gluten not sold directly to the consumer	8 ppb

Ochratoxin in Feed

Commodity	Guidance Level
Cereals and cereal products	0.25 ppm
Complementary and complete feeding stuffs for pigs	0.05 ppm
Complementary and complete feeding stuffs for poultry	0.1 ppm
Complementary and complete feeding stuffs for cats and dogs	0.01 ppm

Patulin

- 4-hydroxy-4H-furo[3,2c]pyran-2(6H)-one
- *Penicillium patulum* (later called *Penicillium urticae*, now *Penicillium griseofulvum*)
- *Penicillium expansum*, the blue mold that causes soft rot of apples, pears, cherries, and other fruits, is recognized as one of the most common offenders in patulin contamination.
- unfermented apple juice, although it does not survive the fermentation into cider products
- World Health Organization Expert Committee on Food Additives has established a provisional maximum tolerable daily intake for patulin of 0.4 mg/kg of body weight per day

Trichothecenes

- *Fusarium, Myrothecium, Phomopsis, Stachybotrys, Trichoderma, Trichothecium*
- macrocyclic or nonmacrocyclic, depending on the presence of a macrocyclic ester or an ester-ether bridge between C-4 and C-15
- Nonmacrocyclic trichothecenes
 - type A, which have a hydrogen or ester type side chain at the C-8 position, T-2 toxin, neosolaniol, and diacetoxyscirpenol,
 - type B group contain a ketone and include fusarenon-x, nivalenol, and deoxynivalenol
- *Fusarium* is the major genus implicated in producing the nonmacrocyclic trichothecenes.

Trichotecenes

Deoxynivalenol

- common mycotoxins found in grains
- causes nausea, vomiting, and diarrhea- VOMITOXIN
- at lower doses, pigs and other farm animals exhibit weight loss and food refusal- FOOD REFUSAL FACTOR
- barley, corn, rye, safflower seeds, wheat, and mixed feeds

Trichotecenes

T-2 and diacetoxyscirpenol

- cytotoxic
- immunosuppressive effect -decreased resistance to infections
- gastrointestinal, dermatological, and neurologic symptoms
- human disease called alimentary toxic aleukia- inflammation of the skin, vomiting, and damage to hematopoietic tissues. The acute phase is accompanied by necrosis in the oral cavity, bleeding from the nose, mouth, and vagina, and central nervous system disorders

T-2 & HT-2 in Food

Commodity	Guidance Level (T-2 & HT-2)
Unprocessed cereals	
<ul style="list-style-type: none"> • Barley (including malting barley) in maize 	200 ppb
<ul style="list-style-type: none"> • Oats (with husk) 	1000 ppb
<ul style="list-style-type: none"> • Wheat, rye and other cereals 	100 ppb
Cereal grains for direct human consumption	
<ul style="list-style-type: none"> • Oats 	200 ppb
<ul style="list-style-type: none"> • Maize 	100 ppb
<ul style="list-style-type: none"> • Other cereals 	50 ppb
Cereal products for human consumption	
<ul style="list-style-type: none"> • Oat bran and flaked oats 	200 ppb
<ul style="list-style-type: none"> • Cereal bran except oat bran, oat milling products other than oat bran and flaked oats and maize milling products 	100 ppb
<ul style="list-style-type: none"> • Other cereal milling products 	50 ppb
<ul style="list-style-type: none"> • Breakfast cereals including formed cereal flakes 	75 ppb
<ul style="list-style-type: none"> • Bread (including small bakery wares), pastries, biscuits, cereal snacks, pasta 	25 ppb
<ul style="list-style-type: none"> • Cereal-based foods for infants and young children 	15 ppb

T-2 & HT-2 in Feed

Commodity	Guidance Level
Cereal products for feed and compound feed:	
<ul style="list-style-type: none"> • Oat milling products (husks) 	2000 ppb
<ul style="list-style-type: none"> • Other cereal products 	500 ppb
<ul style="list-style-type: none"> • Compound feed, with the exception of feed for cats 	250 ppb
<ul style="list-style-type: none"> • Compound feed for cats 	50 ppb

Trichotecenes

- *Stachybotrys atra* (*Stachybotrys chartarum*) trichotecenes
- satratoxins
- roridins
- Verrucarins
- atranones

Trichotecenes

- **Stachybotryotoxicosis**
- Stachybotryotoxicosis was first described as an equine disease of high mortality associated with moldy straw and hay.
- Cutaneous and mucocutaneous lesions, panleukopenia, nervous signs, and abortions have been seen. Death may occur in 2–12 days.
- stachybotryotoxicosis was considered a rare occupational disease limited largely to farm workers who handle moldy hay

Zearalenone

- (6-[10-hydroxy-6-oxo-*trans*-1-undecenyl]-B-resorcylic acid lactone)- formerly called F₂ toxin)
- secondary metabolite from *Fusarium graminearum* (teleomorph *Gibberella zeae*)- *Fusarium culmorum*, *Fusarium equiseti*, and *Fusarium crookwellense*
- nonsteroidal estrogen
- mycotoxin with primarily estrogenic effects
- produced concurrently with deoxynivalenol.

Zearalenone

- reduced feed intake or reproductive dysfunction
 - presence of deoxynivalenol may limit exposure to zearalenone, thus reducing its practical effect.
- binds to receptors for 17β -estradiol- bind estradiol sites on DNA.
- Specific RNA synthesis leads to signs of estrogenism.
- Zearalenone is a weak estrogen with potency 2–4 times less than estradiol.
- vulvovaginitis in prepubertal gilts fed moldy corn (maize),
- sporadic outbreaks in dairy cattle, sheep, chickens, and turkeys.
- High dietary concentrations (>20–30 ppm) are required to produce infertility in cattle and sheep, and extremely high dosages are required to affect poultry.

Zearalenone

- Physical and behavioral signs of estrus -1 ppm dietary zearalenone.
- In -prepubertal gilts, causing hyperemia and enlargement of the vulva (known as vulvovaginitis).
- hypertrophy of the mammary glands and uterus, and abdominal straining results in prolapse of the uterus in severe cases. Removal of affected grain results in return to normal in ~1 wk.
- Sexually mature sows-inhibiting secretion and release of follicle-stimulating hormone (FSH), resulting in arrest of preovulatory ovarian follicle maturation.
- Zearalenone fed at 3–10 ppm on days 12–14 of the estrous cycle in open gilts results in retention of corpora lutea and prolonged anestrus (pseudopregnancy) for up to 40–60 days. Zearalenone fed at ≥ 30 ppm in early gestation (7–10 days after breeding) may prevent implantation and cause early embryonic death. Zearalenone metabolites can be excreted in milk of exposed sows, resulting in hyperestrogenic effects in their nursing piglets.

Zearalenone

- In cattle, dietary concentrations >10 ppm may cause reproductive dysfunction in dairy heifers, although mature cows may tolerate up to 20 ppm.
- Young males, both swine and cattle, may become infertile, with atrophy of the testes. However, mature boars appear unaffected by as much as 200 ppm dietary zearalenone.
- Ewes may show reduced reproductive performance (reduced ovulation rates and numbers of fertilized ova, and markedly increased duration of estrus) and abortion or premature live births.

Zearalenone in Food

Commodity	Maximum Level
Unprocessed cereals other than maize	100 ppb
Unprocessed maize	350 ppb
Cereals intended for direct human consumption, cereal flour, bran as end product for direct human consumption	75 ppb
Maize intended for direct human consumption, maize based snacks and maize based breakfast cereals	100 ppb
Refined maize oil	400 ppb
Bread, pastries, biscuits, cereal snacks and breakfast cereals	50 ppb
Processed cereal and maize based foods and baby foods for infants and young children	20 ppb

Zearalenone in Feed

Commodity	Guidance Level
Cereals and cereal products with the exception of maize by-products	2 ppm
Maize by-products	3 ppm
Complementary and complete feeding stuffs for piglets, gilts (young sows), puppies, kittens, dogs and cats for reproduction	0.1 ppm
Complementary and complete feeding stuffs for adult dogs and cats other than for reproduction	0.2 ppm
Complementary and complete feeding stuffs for sows and fattening pigs	0.25 ppm
Complementary and complete feeding stuffs for calves, dairy cattle, sheep (including lamb) and goats (including kids)	0.5 ppm

Facial Eczema



Facial eczema
(Pithomycototoxicosis)

Sporidesmins

Pithomyces chartarum

Widespread

Toxic spores on
pasture litter

Sheep,
cattle,
farmed
deer

Also see [Facial Eczema](#).

Facial Eczema

- of grazing livestock, the toxic liver injury commonly results in photodynamic dermatitis.
- In sheep, the face is the only site of the body readily exposed to ultraviolet light, hence the common name.
- Sheep, cattle, and farmed deer of all ages can contract the disease, but it is most severe in young animals.

Etiology and Pathogenesis

- Sporidesmins are secondary metabolites of the saprophytic fungus *Pithomyces chartarum*, which grows on dead pasture litter.
- this fungus restrict disease occurrence to hot summer and autumn periods shortly after warm rains.
- excreted via the biliary system-produce severe cholangitis and pericholangitis as a result of tissue necrosis.
- Biliary obstruction - restricts excretion of bile pigments and results in jaundice. Similarly, failure to excrete phylloerythrin in bile leads to photosensitization.
- Previous ingestion of toxic spores causes potentiation; thus, a succession of small intakes of the spores can lead to subsequent severe outbreaks.

Clinical Findings, Lesions, and Diagnosis:



- photosensitization and jaundice appear ~10–14 days after intake of the toxins. Animals frantically seek shade.
- Even short exposure to the sun rapidly produces the typical erythema and edema of photodermatitis in nonpigmented skin.
- The animals suffer considerably, and deaths occur from one to several weeks after photodermatitis appears.
- Characteristic liver and bile duct lesions are seen in all affected animals whether photosensitized or not. In acute cases showing photodermatitis, livers are initially enlarged, icteric, and have a marked lobular pattern.
- atrophy and marked fibrosis.
- The clinical signs together with characteristic liver lesions are pathognomonic. In live animals, high levels of hepatic enzymes may reflect the extensive injury to the liver.

Mycotoxin
Regulation
Food and Feed
MRL

Bölüm 2- Mikotoksinler

İstenmeyen maddeler	Hayvan yemi olarak kullanılan ürünler	Kabul edilebilir en çok miktar mg/kg (ppm) (% 12 rutubet içeren yeme göre)
(1)	(2)	(3)
1. Aflatoksin B1	Yem maddeleri	0,02
	Tamamlayıcı ve tam yemler; aşağıdakiler dışında:	0,01
	-Süt sığırları ve buzağılar, süt koyunları ve kuzular, süt keçileri ve oğlaklar, domuz yavruları ve genç kanatlı hayvan karma yemleri	0,005
	-Sığır (süt sığırları ve buzağılar hariç), koyun (süt koyunları ve kuzular hariç), keçi (süt keçileri ve oğlaklar hariç), domuz (domuz yavruları hariç), kanatlı (genç kanatlılar hariç) karma yemleri	0,02
2. <u>Cavdar mahmuzu (Rye Ergot)</u>	<u>Yem maddeleri ve öğütülmemiş tahıl içeren karma yemler</u>	1000
3. <u>Deoxynivalenol</u>	Yem maddeleri:	
	-Mısır yan ürünleri hariç tahıllar ve tahıl yan ürünleri	8
	<u>-Mısır yan ürünleri</u>	12
	<u>Aşağıdakiler dışında tam ve tamamlayıcı yemler:</u>	5
<u>-Domuz tam ve tamamlayıcı yemleri</u>	0,9	
<u>-Kuzu, oğlak ve 4 aydan küçük buzağı tam ve tamamlayıcı yemleri</u>	2	

4. Zearalenone	Yem maddeleri:	
	-Mısır yan ürünleri haric tahıllar ve tahıl yan ürünleri	2
	-Mısır yan ürünleri	3
	Tam ve tamamlayıcı yemler:	
-Domuz yavruları ve genç anaç domuz tam ve tamamlayıcı yemleri	0,1	
-Anaç domuz ve besi domuzu tam ve tamamlayıcı yemleri	0,25	
-Buzağı, süt ineği, kuzu dahil koyun ve oğlak dahil keçi tam ve tamamlayıcı yemleri	0,5	
5. Ochratoxin A	Yem maddeleri:	
	-Tahıllar ve tahıl ürünleri	0,25
	Tam ve tamamlayıcı yemler:	
-Domuz tam ve tamamlayıcı yemleri	0,05	
-Kanatlı tam ve tamamlayıcı yemleri	0,1	
6. Fumonisin (B1+B2)	Yem maddeleri:	
	-Mısır ve mısır ürünleri	60
	Tam ve tamamlayıcı yemler:	
	-Domuzlar, tektırnaklılar, tavşanlar ve ev ve süs hayvanları	5
	-Balık	10
-Kanatlılar, kuzular, oğlaklar ve 4 aydan küçük buzağılar	20	
-4 aydan büyük yetiskin gevişgetiren hayvanlar ve vizon	50	

Further regulations

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TROPANE ALKALOIDS

Tropane Alkaloids (Atropine and Scopolamine) in Food

Commodity	Maximum Level
Processed cereal-based foods and baby foods for infants and young children, containing millet, sorghum, buckwheat or their derived products	1 ppb

Disease	Toxins (When Known)	Fungi or Molds	Regions Where Reported	Contaminated Toxic Foodstuff	Animals Affected	Signs and Lesions
Aflatoxicosis	Aflatoxins	<i>Aspergillus flavus</i> , <i>A parasiticus</i>	Widespread (warmer climatic zones)	Moldy peanuts, soybeans, cottonseeds, rice, sorghum, corn (maize), other cereals	All poultry, pigs, cattle, sheep, dogs	Major effects in all species are slow growth and hepatotoxicosis. Also see Aflatoxicosis and see Mycotoxicoeses .
Diplodiosis	Unknown	<i>Diplodia zeae</i>	South Africa	Moldy corn (maize)	Cattle, sheep	Nervous system disorders, cold and insensitive limbs. Recovery usual on removal of source.
Ergotism	Ergot alkaloids	<i>Claviceps purpurea</i>	Widespread	Seed heads of many grasses, grains	Cattle, horses, pigs, poultry	Peripheral gangrene, late gestation suppression of lactation initiation. See Ergotism .
	Paspalinine and paspalitrems, tremorgens	<i>C paspali</i> , <i>C cinerea</i>	Widespread	Seed heads of paspalum grasses	Cattle, horses, sheep	Acute tremors and ataxia. See Paspalum Staggers .

Disease	Toxins (When Known)	Fungi or Molds	Regions Where Reported	Contaminated Toxic Foodstuff	Animals Affected	Signs and Lesions
Estrogenism and vulvovaginitis	Zearalenone	<i>Fusarium graminearum</i> Perfect state: <i>Gibberella zeae</i>	Widespread	Moldy corn (maize) and pelleted cereal feeds, standing corn, corn silage, other grains	Pigs, cattle, sheep, poultry	Vulvovaginitis in pigs, anestrus or pseudopregnancy in mature sows, early embryonic death of swine embryos, estrogenism in cattle and sheep, reduced egg production in poultry. Also see Estrogenism and Vulvovaginitis .
Facial eczema (Pithomyctotoxicosis)	Sporidesmins	<i>Pithomyces chartarum</i>	Widespread	Toxic spores on pasture litter	Sheep, cattle, farmed deer	Also see Facial Eczema .
Fescue foot	Ergovaline	<i>Neotyphodium coenophialum</i>	USA, Australia, New Zealand, Italy	Tall fescue grass (<i>Lolium arundinacea</i>)	Cattle, horses	Lameness, weight loss, hyperthermia, heat intolerance, dry gangrene of extremities, agalactia, thickened fetal membranes. Also see Fescue Poisoning .

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