Toxic Mold and Mycotoxins

Food Toxicology
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University of Idaho

Learning Objectives

- Understand the relationship between mold growth, their potential mycotoxins, and disease.
- Explore the environmental conditions for mold growth.
- Understand the major species of toxic molds and their disease endpoints.
- Review the route of exposure of mycotoxins, general pharmacologic effects and clinical disease.
- Discuss some recent mycotoxin outbreaks.

Fungi and Mycotoxins

- > 100,000 species of fungi
- Mycotoxins: Substances produced by fungi that are harmful to animals and humans
- > 300 mycotoxins isolated
  - ~ 30 well-characterized and considered harmful to animals and humans (more?)

Corn Kernel with Mold

Mold Growth → Mycotoxin Production

- Substrate (plant) specific
- Environmental: (field and storage)
  - Temperature
  - Humidity
  - Moisture
  - Oxygen
- Crop damage:
  - Parasites
  - Drought
  - Pesticides

Mycotoxin Observations

- Not all moldy feeds/foods contain mycotoxins
- Not all feeds/foods containing mycotoxins are 'toxic'
- Feed/food does not have to look moldy to be contaminated
- May not be uniformly distributed

Toxic Mold Disease Endpoints

- Allergy
  - Sensitization to mold or mold products
- Mycosis
  - Direct infection by fungi
- Irritation
  - Mechanical effects of spores, mycelial debris, VOCs
- Mycotoxicosis
  - Response to toxin (mycotoxin)

Toxic Molds

Fungus	Allergy	Mycosis	Irritation	Mycotoxicosis
Stachybotrys	+	±	+	+
Coccidioides	±	+	±	-
Claviceps	+	±	+	+
Fusarium	+	±	+	+
Aspergillus	+	+	+	+

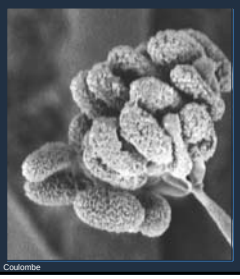
+ reported, ± possible, - not reported
Fung *et al.*, *Clinical Tox.* 36: 79-86 (1998)

Mycotoxins

- Resting stage secondary metabolites of fungus/mold
  - Low MW, not required for growth
  - Polyketide, amino acid, or terpene precursor
- Why? Ecological biochemistry?
  - Storage products? Competitive advantage?
- Beneficial uses
  - Antibiotics, other drugs
- Adverse effects
  - Toxic, carcinogenic

Stachybotrys

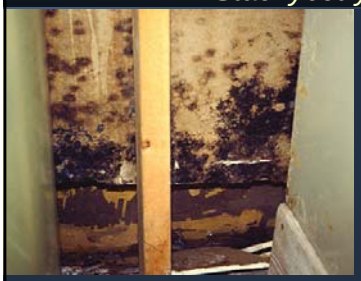
- *S. chartarum* (*aka. atra*)
- Hay and cellulose products common substrates
- Water-damaged buildings: 'toxic' house mold



Stachybotrys

Straw contaminated with *S. chartarum* (top); clean straw (bottom). Persons handling contaminated straw can develop stachybotryotoxicosis

Stachybotrys



Mold growth on water-damaged interior wall

Stachybotryotoxicosis

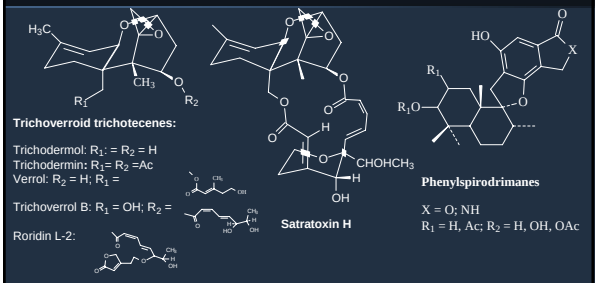
- Animals: clinical progression
  - Irritation of mouth, throat, and nose…
  - Shock…
  - Dermal necrosis…
  - Leukopenia...
  - Pulmonary (alveolar, bronchiolar, interstitial) inflammation and hemorrhage…
  - Nervous disorder; death

Stachybotryotoxicosis

- 19th Century Russia: numerous veterinary and human epidemics
  - ATA "Alimentary Toxic Aleukia"
- 1931 Ukraine
  - Inhalation of mold from hay and contaminated bedding
- Occupational cases
  - Cottonseed oil plants
  - Grain elevators; malting plants
  - Textile mills; twine factories

1996: Employees at a German horticultural facility developed painful, inflamed lesions on their fingertips followed by scaling off of the skin after handling decomposable paper pots infested with *S. chartartum*

Toxins from S. chartarum



Stachybotryotoxicosis

- Humans: inhalation, dermal exposure
- Clinical progression:
  - Dermatitis
  - Inflammation of mucous membrane
  - Upper respiratory symptom
  - Fever
  - Leukopenia
  - Headache, fatigue
  - Recovery(?)
- Cause of Infantile Pulmonary Hemorrhage (IPH)?
- CDC: Data insufficient to support association between *S. chartarum* and IPH

Coccidioides

- *Coccidioides immitis*

Coccidioidomycosis

- First described - 1894 (California)
- Inhalation of fungal hyphae
- Most cases asymptomatic, self resolving

Bilateral nodular infiltrates with progressive coccidioidomycosis

Coccidioidomycosis: The Americas

- San Joaquin Valley Fever
  - "Valley Fever"
- Endemic in Southwestern US
- CDC: "emerging disease"
  - Changing demographics
  - Immune compromised at risk

Coccidioidomycosis - Symptoms

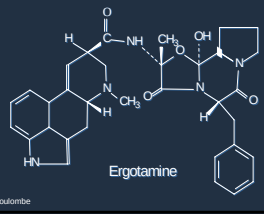
- Primary coccidiodomycosis:
  - Acute bronchitis: fever, cough, chills, sore throat
  - Pneumonia
  - Leukocytosis
- Clinical progression:
  - Low grade fever
  - Anorexia, weight loss
  - Skin ulcerations - face, abdomen
  - Abscesses
  - Progressive cyanosis
  - Renal, hepatic involvement

Claviceps

- *Claviceps purpurea, paspalli*
- Grows in wet and overwintered grains: rye, barley, wheat
- Sclerotia or "ergots"
  - Hard-packed mycelium
- "Ergotism"
  - Gangrene and/or convulsions and gastrointestinal symptoms
  - Livestock: decreased weight gains, milk production, reproductive efficiency

Ergotamine

- Ergotamine: analogue of lysergic acid dimethylamide (LSD)
- Vasoconstrictor
- Hallucinations, gangrene
- *St. Anthony's fire*



Coulombe

11-30-02
Herald Journal, Logan UT

A new spin on Salem witches

By Peter H. Gott, M.D.
Newspaper Enterprise Association

DEAR DR. GOTT: Could the witches of Salem have suffered from physical ailments?

DEAR READER: Historical documents indicate that 24 of the 30 victims suffered from "fits." Rather than convulsions, which in modern parlance involves loss of consciousness, the young women in Salem may well have exhibited spastic movements without fainting. They also complained of sensations of being bitten and pricked. In addition, they were reported to have experienced temporary blindness, deafness and speechlessness. They had hallucinations and out-of-body experiences, such as flying through the air. They were nauseated and weak. Some died, as did several cows in the communities. All these symptoms were blamed on witchcraft when, in fact, they were probably the results of epidemic ergot poisoning from tainted rye bread and contaminated rye grass.

Ergot is a natural alkaloid with effects similar to that of the hallucinogen LSD. Produced by a fungus in rye, ergot colors the flour cherry red. Baking, unlike boiling, does not diminish the toxicity.

In Salem (and similar communities), rye harvested in August usually lay without being threshed in barns until winter, an open invitation for fungal growth. Moreover, because of bad weather, there was a food scarcity in Salem during the summer of 1692. Thus, residents may have been forced unknowingly to rely on contaminated grain harvested more than a year before.

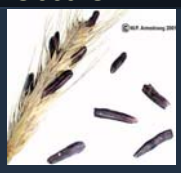
There are many other historical examples of societies that punished the socially unacceptable because of presumed religious reasons, most often heresy and consorting with the devil, when in fact the victims suffered from undiagnosed illnesses or poisonings of one kind or another.

This lesson should continue to give us pause.

How Ergot Exposure Occurs

- *Claviceps purpurea* – soil
- Spores released when grain flowers
- Land on stigma – germinate – hyphae extend into the ovary
- Replaces the ovary – hardens ergot body or sclerotium; recycle

How Ergot Exposure Occurs



- Seeds and sclerotia harvested together
- Screening techniques to remove the ergot based on size and weight
- Exposure occurs by ingesting grain / food

Ergot Toxins

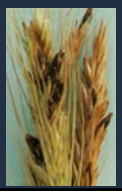
- Ergot alkaloids - sclerotia
  - Ergonovine
  - Ergovaline
  - Ergosine
  - Ergocristine
  - Ergotamine
    - Medicinally for vascular migraine, postpartum uterine hemorrhage in abortions
- Types and concentrations of alkaloids vary

Ergotamine

Ergot Pharmacological Effects

Coulombe

- Vasoconstriction
  - Gangrene
- Serotonin agonist
  - Neurological effects
- Dopaminergic agonist
  - Agalactia



Ergot Clinical Diseases

- Vasoconstriction → gangrene (toes, fingers, ears, worse) – 'frostbite'
  - Epidemics of gangrenous limbs / 40,000 people in France died (944 AD)
  - Pain, swelling, numbness
  - Necrotic tissue
  - Death
  - Abortion

Ergot Clinical Diseases

- Neurological / tremorgenic / convulsive form
  - Tingling (ants crawling under the skin)
  - Itching
  - Numbness
  - Twitching
  - Spasms
  - Seizures
- Action levels set for ergot in grains

Fusarium

Coulombe
Talcott

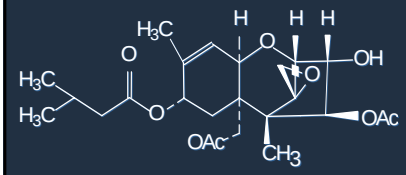
- *F. sporotrichioides* and *graminearum*
- Corn, wheat, barley
- Veterinary and public health concerns
- Major toxins:
  - Trichothecenes, zearalenone, fumonisin

Fusarium Mycotoxins

- Trichothecenes, Zearalenone, Fumonisin

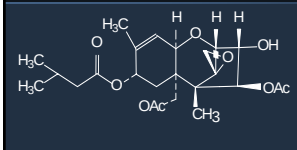
Tricothecenes

- Sesquiterpenoid tetracyclic compounds

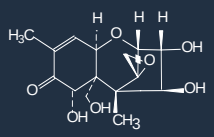


Have seen use as a biological warfare agent: "Yellow Rain"

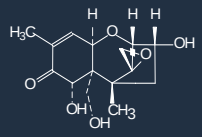
Tricothecenes



T-2 toxin

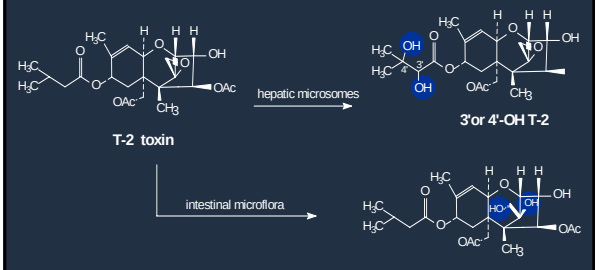


Nivalenol



Deoxynivalenol

Metabolism of T-2 Toxin



T-2 toxin

3'or 4'-OH T-2

deepoxy T-2 triol

- Metabolites are less toxic than T-2

T-2 Toxin: Animal Toxicity

- Digestive disorders:
  - Feed refusal, vomiting, bloody diarrhea, intestinal inflammation
- Hemorrhage
  - Stomach, heart, intestines, lung, bladder, kidney
- Edema
- Oral lesions
- Blood disorders
- Immunotoxic
- The oral LD_{50} of T-2 toxin in animals ranges from 3 to 5 mg/kg, and the DRC is very steep.

T-2 Dermal Toxicity

← Skin lesions on the back of a hairless guinea pig at 1 days after application of (bottom to top) 25, 50, 100, or 200 ng of T-2 toxin in 2 µL of methanol.

When human skin is exposed in vivo to small amounts of trichothecene mycotoxins, severe cutaneous irritations develop and can last 1 to 2 weeks after acute exposure.

T-2 Toxin: Human Toxicity

- Dermal exposure: local cutaneous necrosis and inflammation
- Oral exposure: lesions to the upper gastrointestinal tract (ATA)
  - Because of the lipophilic nature of trichothecenes, they are rapidly and completely absorbed from the GIT and quickly distributed to all major organs.
  - The mechanism by which T-2 toxin causes cell death is ribosomal binding and inhibition of protein synthesis.
- Ocular exposure: corneal injury.

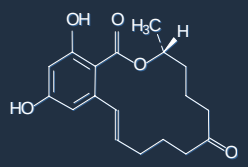
Alimentary Toxic Aleukia Toxicosis (ATA)

- **First stage:** immediately or several days after consumption of grain products contaminated with trichothecene mycotoxins.
  - Inflammation of the gastric and intestinal mucosa causes vomiting, diarrhea, and abdominal pain. In most cases, excessive salivation, headache, dizziness, weakness, fatigue, and tachycardia accompany this stage, and fever and sweating may also be present.
- **Second stage:** the leukopenic or latent stage—which is characterized by leukopenia, granulopenia, and progressive lymphocytosis.
  - When the ingestion of the toxin-contaminated food is not interrupted or if large doses are consumed, the next stage develops.
- **Third stage:** Characterized by the appearance of a bright red, or dark cherry-red, petechial rash on the skin of the chest and other areas of the body.
  - At first, the petechiae are localized in small areas, but they then spread and become more numerous. In the most severe cases, intensive ulceration and gangrenous processes develop in the larynx, leading to aphonia and death by strangulation. At the same time, affected individuals have severe hemorrhagic diathesis of the nasal, oral, gastric, and intestinal mucosa.
- **Fourth stage:** The necrotic lesions heal and the body temperature falls; the recovery stage begins.
  - During this period, exposed patients are susceptible to various secondary infections, including pneumonia. Convalescence is prolonged and can last for several weeks. Usually, 2 months or more are required for the blood-forming capacity of the bone marrow to return to normal.

37 Wannemacher and Weiner, 1997

Zearalenone (ZEN)

- *F. graminearium and F. sporotrichiodes*
- Corn, wheat, barley, oats, sorghum, hay
- High humidity, low temperature
  - Autumn harvest in upper Midwest US
- Often coincident with T-2



38 Coulombe

Zearalenone: Animal Toxicity

- Swine (>0.1 ppm): estrogenic effects
  - Vulvovaginitis, swollen mammae
- Swine (50-100 ppm): decreased reproduction
  - Cycling, conception, ovulation, implantation
- Boars (>0.1 ppm): feminization
  - Testicular atrophy, enlarged nipples
- Cows: decreased conception rates

39 Coulombe

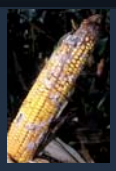
Zearalenone: Mechanism of Action

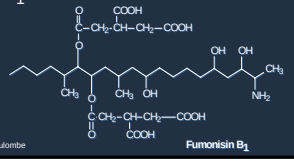
- Binds to estrogen receptor
- ZEN binding affinity
  - Equivalent with 17 β estradiol
  - Less than estradiol
- Estrogen receptor affinity
  - Swine > rat > chicken

40 Coulombe

Fumonisin

- *F. moniliforme* (universal in corn)
  - Corn, wheat, barley, oats, sorghum, hay
- High humidity, low temperature
  - Autumn harvest in upper Midwest US
- Often coincident with T-2
- Horses, pigs most susceptible
- FB_1 most toxic





41 Coulombe

Fumonisin B_1

Fumonisin B1: Animal Toxicity

- Neurotoxicity: Equine leukoencephalomalacia (ELEM)
  - "Moldy corn toxicosis"
  - Rapid onset (few hours)
  - Feed and water refusal, lameness, ataxia, paralysis
  - Severe cerebral edema, focal malacia (softening), liquefaction of white matter
- Pulmonary Edema: Porcine pulmonary edema syndrome (PPE)
  - Hydrothorax and lung edema
  - Usually fatal
- Liver cancer and liver toxicity

42 Coulombe, Talcott

Fumonisin B1: Animal Toxicity

- Horses (1-126 ppm):
  - Fatal ELEM
  - Liver toxicity at higher doses
- Swine (<1 – 5 ppm) :
  - Low dose: hepatic toxicity
  - High dose: acute pulmonary edema, hepatic toxicity
- Sheep:
  - Nephritis

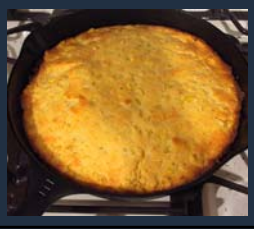
FDA-CVM: established action levels in animal feeds

Fumonisin Concern for Human Health

- Milk residues?
- Meat residues?
- 1996: 89% of corn grown in 3 areas of Costa Rica were contaminated with fumonisin

Fumonisin Carcinogenic Potential

- Carcinogen / promoter
- Esophageal cancer
  - South Africa, Italy, China, South Carolina
- Corn: staple, home brewed beer, moonshine, polenta

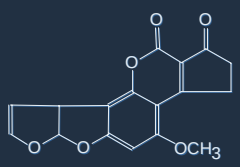


Aspergillus

- Dietary carcinogen

Aflatoxin B1

- From **A**spergillus **fla**vus
  - Universal food contaminant
  - Corn, peanuts, wheat, rice, etc.
- Animal carcinogen - 5 ppb
- FDA action level - 20 ppb

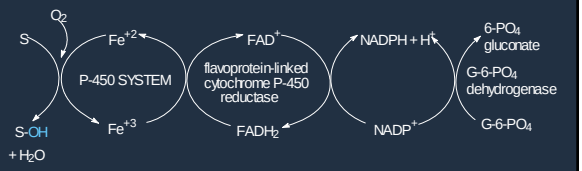


Aflatoxin B1

Coulombe

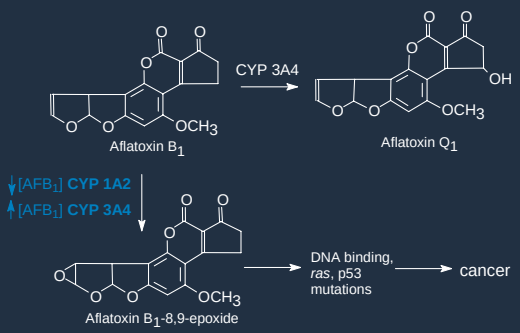
- Human liver carcinogen
  - Binds to N^7 Gua; DNA-adduct
  - G → T in p53 codon 249
- Lung cancer risk
  - Respirable grain dusts > 4000 ppb AFB1
- Problem in food industry and grain handling
  - Harvesting
  - Transport
  - Storage
  - Processing
- Requires bioactivation

Cytochrome P450 (CYP) Reaction Sequence

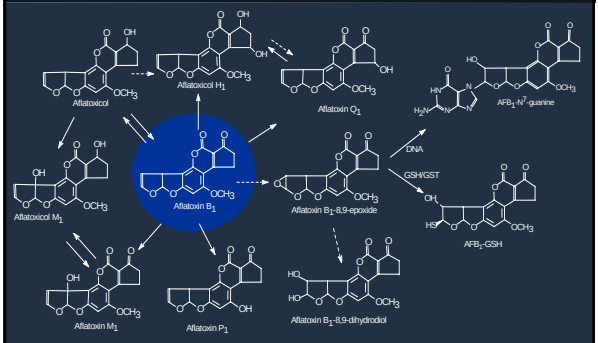


- Bioactivation

AFB1 Activation: Enzymology



Aflatoxin B1 Metabolic Pathways



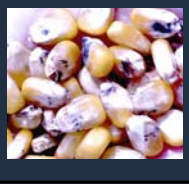
Aflatoxin: Etiological Role in Disease

- Indian childhood cirrhosis
- Hepatotoxicity
- Immunosuppression
- Kwashiorkor
- Carcinogenesis

Nigerian child with Kwashiorkor

Recent Aflatoxin Outbreaks

- Kenya: January to July, 2004
  - Outbreak of jaundice, liver failure
    - High fatality rate
  - 317 reported cases and 125 reported deaths
  - Consumption of maize – visibly discolored or moldy



Recent Aflatoxin Outbreaks

- Kenya: January to July, 2004
  - Range: 20 to 8,000 ppb
  - Widespread
  - Maize harvested when wet
  - Food shortage
  - Education needs
    - Harvesting, drying, storing