I am sure you all are familiar with poisons and toxins but did you ever stop to think what would be an exact definition. A **poison** is any substance that when ingested, inhaled, applied to, injected, or developed within the body; by virtue of its chemical or molecular action has the potential to cause damage to cellular structure and/or function. This certainly includes all manner of deleterious substances and is the most all-inclusive term. The term **toxin** is often used interchangeably with poison but it has a more specific meaning. A toxin is a protein produced by plants, certain animals, and bacteria that are dangerous for other living organisms. Importantly, toxins differ from simple chemical poisons (including plant alkaloids) by their high molecular weight and antigenicity. This introduces two additional terms that you should be familiar with. Do you know the difference between a **toxoid** and an **antitoxin**? A toxoid is a toxin that has been rendered less injurious but that is still immunogenic (antigenic). Toxoids are used to produce antitoxins; an example being tetanus vaccination. Antitoxins are antibodies directed against toxins that can be administered to a susceptible individual to provide passive immunity following exposure or suffering from the early stages of disease caused by toxins. **Venoms** are very similar to toxins. Venoms are typically antigenic (antivenoms are similar to antitoxins) and are injected into individuals by the stings or bites of insects, reptiles, etc. Venoms also often contain a mix of toxins having various effects. Poisons and toxins generally have a direct dose-response relationship. The higher the dose, the greater potential for structural or functional damage to cells, tissues, organs, or the body as a whole.

**Possible Mechanisms of Cellular Injury** (slides 3 – 8)

Four possible mechanisms that would account for tissue injury are given in the class presentation, most of you can probably think of others. Some poisons/toxins may cause injury by more than one mechanism. Additionally, for many poisons/toxins, the pathogenesis is unknown or incompletely understood.

- **Direct physical or chemical injury** to cells/tissues/organs through various mechanisms. The example given in the class presentation is the venom of the brown recluse spider (*Loxosceles reclusus*). The venom of this spider causes severe focal **necrotizing** lesions at the site of the bite. Additionally, severe systemic reactions (more common in children) can occur. Some of these symptoms include rash, fever/chills, nausea/vomiting, **arthralgia**, hemolysis/DIC, renal failure, seizures and coma. The venom of the brown recluse has multiple components including hyaluronidase, DNAase, RNAase, alkaline phosphatase, lipase, and sphingomyelinase. The sphingomyelinase is thought to account for much of the tissue destruction and **hemolysis** and can initiate a powerful host
inflammatory response that includes local and systemic release of cytokines and chemokines and activation of complement.

- **Interference with normal metabolic or biochemical pathways.** Several species in the legume family produce toxins (*Lathyrus sativa, odorata,* and other species) that can cause a variety of symptoms. The toxin produced by *L. sativa*, oxalylidiaminopropionic is a structural analog of the excitatory neurotransmitter glutamic acid and causes a neurological syndrome (neurolathyrism). *L. odorata* produces β-aminopropionitril that can lead to musculoskeletal abnormalities (slide 6). This toxin interferes with the function of lysyl oxidase (lectures 3 and 25).

- **Interference with gene expression and transcriptional pathways.** Later in this lecture, the effects of aflatoxin B1 on gene expression and transcriptional pathways will be discussed.

- **DNA damage.** The aflatoxin B1 mentioned above and later in this lecture also binds to DNA. Another example, this time of teratogenicity, is the development of cyclops lambs following ingestion of the toxic plant *Veratrum californicum* by the ewe during a specific time period in gestation (slide 7). Cycloamine, the poison in the plant is believed to alter normal gene signal transduction for formation of the neural tube.

One further example is for your interest only. This example demonstrates how a potential poison can cause severe defects in certain groups of individuals while in others, it can have beneficial effects as a therapeutic drug. This example is the drug thalidomide. Have you heard of it? Thalidomide was developed by a German pharmaceutical company and marketed between 1957 and 1961 as a drug to alleviate morning sickness in pregnant women. Teratogenic effects were found in approximately 10,000 babies worldwide. The most common malformation was phocomelia, very short or absent (agenesis) long bones, most common in the upper extremities with a flipper-like appearance of the hands. A pharmacologist and reviewer for the Food and Drug Administration, Francis Oldham Kelsey, rejected the application of an American company to market thalidomide in the United States; the result being that only 17 malformed babies were born in this country. The mechanism underlying the development of the malformations is poorly understood. It is suggested that thalidomide intercalates between guanine-cytosine rich regions of DNA. A known effect is the inhibition of angiogenesis which may be related to the teratogenic effects. Switch now to more modern times. Thalidomide received FDA approval for use in one manifestation of leprosy to alleviate pain in 1998. At least in part due to its anti-angiogenesis effect, the FDA approved use of the drug to treat multiple myeloma in 2006 and thalidomide or related analogs show promise in other forms of cancer.

### Outcomes of Poisoning (slide 9)

As with most other types of injuries, the outcomes of poisoning vary.

- **Detoxification and elimination after injury** – no or minimal structural or functional damage to the tissue with essentially full recovery
- **Tissue damage with repair/cellular adaptation** – restoration and recovery based on the type of tissue and extent of tissue damage
- **Chronic long-term injury** – persistent morbidity and potential carcinogenesis
- **Death** can occur at any of these stages depending on the agent
Biotransformation (slide 10)

As mentioned previously in lecture 3, the liver is the main organ responsible for **detoxification**, metabolism and elimination of toxins from the body, and the major subcellular organelle involved in the process is the smooth endoplasmic reticulum. Also mentioned in lecture 3 was the process of **biotransformation**. You should review this information if necessary. Some of the examples of poisons/toxins to be covered during the remainder of this lecture will involve biotransformation.

Examples of Poisoning/Intoxication

**Ethylene glycol** (slides 12 – 14)

Ethylene glycol is a major component (95%) of antifreeze. It purportedly has a sweet taste and is readily consumed by dogs and cats, the species most often affected in veterinary medicine. Some individuals have committed or attempted suicide by drinking antifreeze. It is ingested and rapidly absorbed from the gut and the majority is excreted in the urine. In the liver, ethylene glycol is converted by the enzyme alcohol dehydrogenase to glycoaldehyde which is then further oxidized to other toxic metabolites. Glycolaldehyde and glyoxylic acid deplete ATP stores, cause direct damage to cell membranes, and denature enzymes. Additionally, a metabolic acidosis can occur due to accumulation of lactic acid, hippuric acid, and CO$_2$. Oxalic acid in the blood can also bind to calcium resulting in hypocalcemia. Depending on dosage, death may occur during these acute stages. If the animal lives past the acute stage, calcium oxalate crystals are deposited in renal tubules and death can then occur from renal failure. There are other instances where calcium oxalates can be deposited in renal tubules. Certain plants can contain high levels of oxalates. Examples include *Halogeton glomerulatus* and *Sarcobatus vermiculatus* that can cause disease in herbivores grazing these plants. The fungus *Aspergillus niger* produces large quantities of oxalates on feedstuffs. There is also a hereditary condition called primary hyperoxaluria recognized in humans, dogs, cats, and one breed of cattle.

**Tryptophan, 3-methylindole, and lung disease** (slides 15 – 18)

Plants containing high levels of tryptophan can cause poisonings in animals, mainly cattle. In Wyoming, this most commonly occurs when cattle are moved from dry pastures to lush irrigated meadows in late summer or fall. In cattle, tryptophan is converted in the rumen by microbes to 3-methylindole. The indole is absorbed into the blood stream. In the lung, cells lining alveolar septae convert the indole to electrophilic intermediates initiating free radical-induced damage and necrosis of cells lining the alveolar septae. Enzymes involved in this conversion include cytochrome P450 and prostaglandin H synthase (see slide 20 for function of this enzyme). Since the 3-methylindole is delivered via the blood stream, the damage to the lung is diffuse. Initially, the damage results in an acute exudative phase with pouring of proteinaceous fluid and fibrin into alveoli forming hyaline membranes (slide 18). If the animal survives, within 3-5 days there is a proliferative phase with hyperplasia of cells lining the alveolar septae. With even longer survival, there can be fibrosis and scarring of alveolar septae and emphysema.
**Analgesic nephropathy** (slides 19 – 23)

Analgesics are drugs used to alleviate pain, fever, and other systemic signs of acute inflammation. Non-steroidal anti-inflammatory drugs (NSAIDs) typically act by inhibiting cyclooxygenase (COX) enzymes involved in the synthesis of prostaglandins and leukotrienes that mediate some of the vascular and cellular events of acute inflammation (slide 20). Analgesic nephropathy has been recognized in humans for many years and is associated with abuse of NSAIDs, typically combination drugs such as aspirin and phenacitin (acetaminophen). With this recognition, combination analgesic drugs have been withdrawn from the market place, and the incidence has decreased. Cases occur in animals; most cases have been observed in horses given the NSAID butazolidin and at WSVL we had one case involving a cat. From a morphological standpoint, one sees acute **coagulative necrosis** of the renal crest or renal pelvis (slides 21 and 22) implying ischemic injury to these portions of the kidney. The mechanism underlying the nephropathy is undetermined. Some NSAIDs or their metabolites may be directly toxic. Attention, however, has been focused on blockade of prostaglandin synthesis, particularly on PGE2 and its function as a vasodilator. Inability to control vascular tone in these vulnerable areas of the kidney may be at least partially responsible for the ischemic necrosis. Dehydration likely also plays a critical role in these events.

**Mycotoxins and the liver** (slides 24 – 34)

Mycotoxins are toxins produced by fungi. What other mycotoxin has been mentioned in previous lectures (see lecture 1)? In this final portion, another mycotoxin will be covered in some depth. **Aflatoxin B1** (AFB1) is one of the primary toxins produced by *Aspergillus flavus* that contaminates many feedstuffs around the world such as corn, rice, and peanuts. From this perspective, in humans AFB1 **aflatoxicosis** is of greatest concern in developing nations where grains (rice) are a dietary staple AND where there is inadequate screening for mycotoxins.

As the primary organ responsible for detoxification in the body, the liver is a target for aflatoxicosis. Aflatoxins can cause acute injury with necrosis of hepatocytes. With survival or low-grade persistent exposure, acute injury can become chronic with scarring and **cirrhosis** (slide 27). Exposure to AFB1 is also a major risk factor for the development of a form of liver cancer called **hepatocellular carcinoma** (slide 28). Mycotoxicosis related to AFB1 exposure is a example of **biotransformation** (what other examples have already been covered in this lecture?). AFB1 causes DNA damage (see slides 29 - 31) forming the basis for carcinogenesis. The point mutations caused by the guanine-cytosine:thymidine-adenosine transversion can involve many genes but the p53 tumor suppressor gene seems intimately involved with carcinogenesis (slides 30 and 31). In normal cells suffering from DNA damage, p53 upregulates target gene p21 to arrest cell division allowing the process of DNA repair to take place. DNA repair is associated with another target gene GADD53 to initiate repair. If repair fails, GADD53 interacts with another target gene BAX leading to apoptosis and removal of the damaged cell. Hypothetically, when p53 is defective, there is no cell cycle arrest, no DNA repair, and no apoptosis allowing the damaged cells to survive to eventually culminate in the formation of a malignancy. In this respect, would AFB1 be an initiator or a promoter in carcinogenesis? What then are the lines of evidence linking hepatocellular carcinoma to mutations in the p53 gene?
This specific mutation (GA:TC transversion) in the p53 gene is found in over 50% of hepatocellular carcinomas in areas of the world where there is a high incidence of aflatoxicosis compared to 1% in other parts of the world with a low incidence of aflatoxicosis.

Patients with positive urine AFB1-guanine excretion are 3 times more likely to develop hepatocellular carcinoma. Remember, the GA:TC transversion is due to binding of AFB1 to guanine.

Patients with hepatitis B virus (HBV) infection also provide further support for the role of the p53 gene in hepatocellular carcinoma. Infected individuals are 4-7 times more likely to develop hepatocellular carcinoma but when infection and aflatoxicosis occur together, individuals are 60 times more likely to develop the malignancy. There is then a synergism between HBV infection and AFB1 exposure. To wrap up the story of synergism and the link to the p53 gene:

- HBx protein, a gene product of HBV, inhibits nucleotide excision repair. This is the type of repair that would be needed in AFB1.
- One study indicates HBx inactivates the p53 gene
- Another study indicates HBx inhibits p53–induced apoptosis
- HBx promotes apoptosis following DNA damage when the p53 gene is functional but conversely, HBx gives a growth advantage to cells with the AFB1 mutation in the p53 gene.