Toxicology

BCH 436

COURSE SYNOPSIS

- Basic principles of toxicology
- Absorption and distribution of toxicants
- Metabolism and excretion of toxicants
- Toxicant-receptor interactions
- Mechanism of action of toxic substances in living organisms (hepatotoxicity & pulmonary toxicity)
- Metabolism, biological & pathological effects of some toxic substances in living organisms
- Carcinogenesis

- Toxicology is a branch of pharmacology that deals with the nature, effect, symptoms, detections, and treatment of poisons or poisoning.
- It is the study of poisonous materials & their effects on living organisms.
- Any exogenous substance that is chemically strange to the body, be it a drug, toxin or toxicant from any kind of origin, pesticide, or pollutant is known as XENOBIOTICS.
- They can be introduced into the body from the environment.
- Toxins are harmful substances that are produced naturally by living organisms like bacteria, fungi, and plants. E.g. Mycotoxins, ipomeol.
- Toxicants are harmful chemical substances or industrial wastes. E.g. pesticides, herbicides, monoethylglycol, etc.

Three (3) possibilities occur when any chemical, drug or toxic substance enters the body

(a) Some are excreted away unchanged: It depends on the volatility or polarity of the drug. E.g. some snake venoms, alcohol

(b) Some undergo spontaneous reaction by their catalyst/enzymes e.g. activated or deactivated in the gut.

(c)Some are metabolized and excreted: 98% of drugs fall under this category.

Classification of xenobiotic

Foreign chemicals (substances not native/natural to the body)

Major Categories of Xenobiotics

- Drugs
- Food constituents, cyanide in cassava, additives, preservatives, 4-ipomeanol, etc.
- Food additives
- Chemicals of abuse (alcohol, coffee, tobacco, marijuana...etc)
- Agrochemicals (fertilizers, insecticides, herbicides, pesticides, monoethylglycol etc.)
- Industrial chemicals (solvents, dyes, monomers,ploymers..etc)
- Pollutants: Air, waste water/sea, land

Definition

- Toxicity can be defined as “the capacity of a substance to cause adverse health effects on a living organism” (Altug, 2002).
- Paracelsus evidence-”All substances are poisons; there is none which is not a poison. The right dose differentiates a poison and a remedy.”

Food toxins

- Generally, food toxins are substances that cause foods to become toxic.

- Food is not only the elementary source of nutrients for humans but may also contain natural chemical substances with toxic properties.
- Some substances which are naturally occurring include cyanogenic glycosides, solanine, industrial pollutants, biogenic amines and mycotoxins.(Dabrowski & Sikorski, 2004).

General classification of food toxins

- **Physical**: glass, stone, metal, wood, etc.
- **Chemical-** natural toxins, residues, pesticides, metals, toxins formed during food processing.
- **Microbiological**: pathogenic microorganisms (bacteria, viruses, parasites, etc).

- Toxic substances such as dioxins, mycotoxins, heavy metals, pesticides, veterinary drugs and polycyclic aromatic hydrocarbons are almost ubiquitous in the environment. Thus, they are also present in ingredients for animal feed.
- Depending on their physico-chemical characteristics, some substances are metabolized into naturally occurring and generally harmless constituents. Most veterinary drugs and feed additives fall into this group.
(Kan & Meijer, 2007)

Further classification of food toxins

1) Natural toxins of plant origin
    1) Nitrogen containing e.g. alkaloids and glycosides.
    2) Nitrogen free e.g. organic acid and phenolic toxins.
2) Toxins of Animal origin
    1) Animal liver e.g. Bile acids and vitamin A
    2) Marine animals e.g. saxitoxin, tetrodoxin etc.
3) Fungal toxins e.g. mycotoxins of ergotism, alfatoxins.
4) Food toxins from industrial wastes
    1) Chlorinated hydrocarbons e.g. polychlorinated biphenyls
    2) Heavy metals e.g. Lead, mercury and cadmium

Further classification of food toxins cntd.

5) Bacterial toxins e.g. Cholera toxin and E. coli enterotoxin
6) Toxins from pesticide residues e.g. DDT and Chlorinated cyclodiene insecticide
7) Toxins from food additives and preservatives e.g. benzoic acid
8) Toxins formed during food processing e.g. polycyclic aromatic hydrocarbons, N-nitrosamines

Natural toxins of plant origin

- According to Dabrowski and Sikorski (2004) over 100,000 secondary compounds have been identified and categorized into two major classes:
  - Nitrogen containing compounds that include alkaloids, glycosides, proteins, polypeptides, amines and non protein amino acids.
  - Nitrogen free compounds that include some organic acids, alchohols, polyacetylenes, resinous toxins and mineral toxins.

Natural toxins of plant origin cntd.

- Cyanogenic glycosides are glycosides from which cyanide is formed by the activity of hydrolytic enzymes. They are widely spread in higher plants.
- Can be found in food plants like cassava, lima beans and the seed of some fruits (e.g. peaches) → cyanide content
- Fresh cassava cortex produces cyanide in quantities ranging from 1.0 to more than 60.0 mg per 100 g, depending on several conditions, including variety, source, time of harvest and field conditions.

MYCOTOXINS

Mycotoxins are secondary metabolites produced by micro-fungi that are capable of causing disease and death in humans and other animals

Types of Mycotoxins

Some of the different types of mycotoxins are:

- Aflatoxins: produced by Aspergillus) - includes Aflatoxin B1, B2, G1, G2, M1 and M2
- Ochratoxin: includes Ochratoxin A, B, and C
- Trichothecene: produced by Stachybotrys - includes Satratoxin-H, Vomitoxin and T-2 mycotoxins
- Fumonisins: includes Fumonisin B1 and B2
- Zearalenone

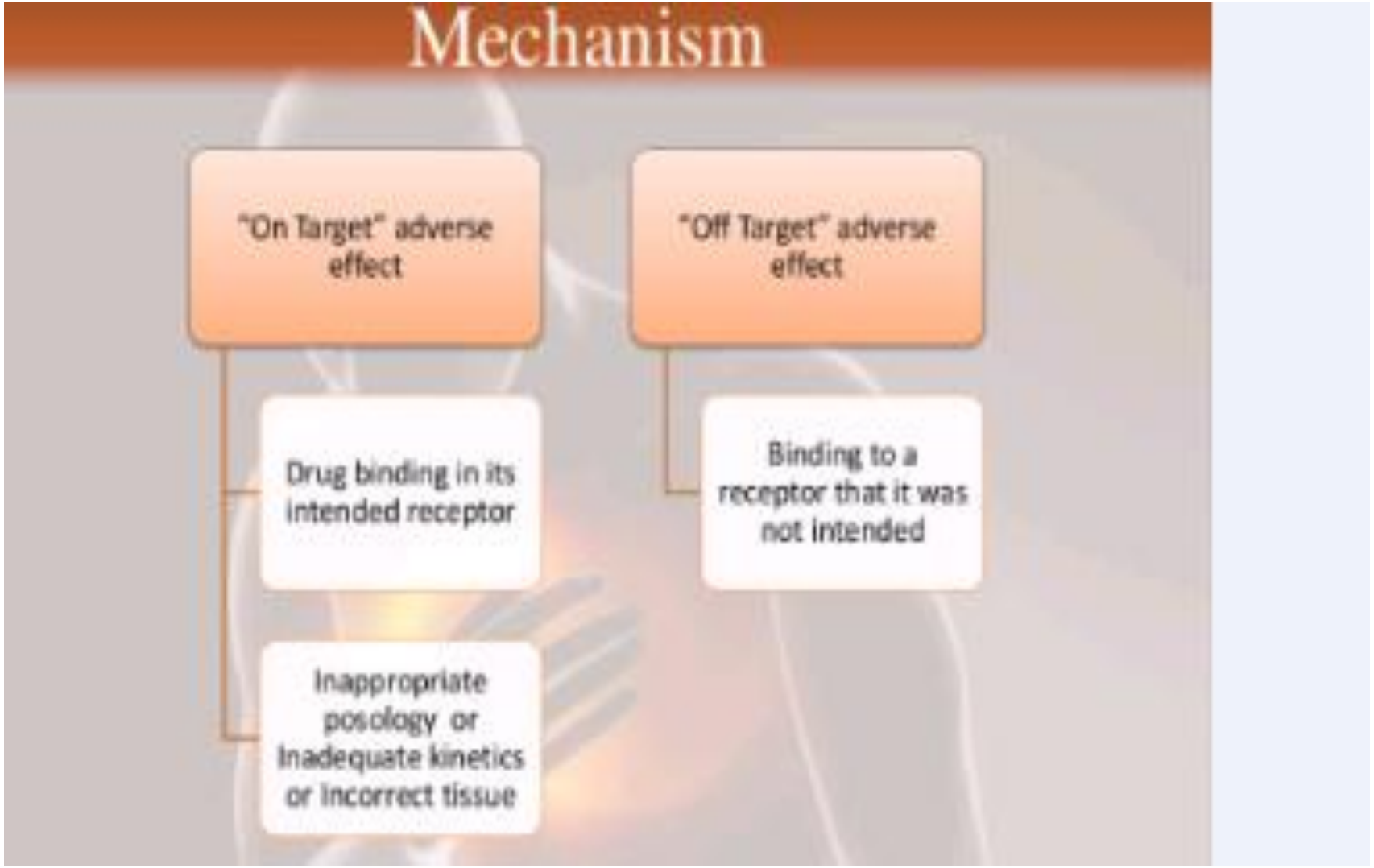
Fungal toxins contd.

- Aflatoxins are the most important mycotoxins, which is produced by certain species of *Aspergillus (A. flavus* and *A. parasiticus)*, which develop at high temperatures and humidity levels.
- Aflatoxins are carcinogenic substances and may be present in a large number of foods. This toxin can cause cancer, cirrhosis of the liver.
- For substances of this type there is no threshold below which no harmful effect is observed.

Fungal toxins contd.

- The most common commodities contaminated are tree nuts, peanuts, and corn and cottonseed oil.
- The major aflatoxins of concern are B1, B2, G1, and G2 → usually found together in various proportions. Aflatoxin B is usually predominant, and it is the most toxic and carcinogenic.

MECHANISM OF DRUG TOXICITY



Mechanisms of drug toxicity

- On target
- Off-target
- Production of toxic/harmful metabolites
- Harmful immune responses
- Idiosyncratic responses

1. **On-target (or mechanism-based) toxicity**: The toxicity is due to interaction of the drug with the same target that produces the desired pharmacological response.

Subclass and Important details of On-Target effects

- Drug action on the same receptor but on different tissue than the target one

May expose unknown functions of the biological target


On target Adverse Effect
Alteration of the
Dosing error
Exaggeration in
pharmacologic action

2. **Off-target toxicity**: the drug is not specific in its interactions and binding to an alternate target is the cause of toxicity.

- Example; antihistamine & terfenadine, which also inhibit the cardiac potassium channel.

3. **Production of toxic/harmful metabolites**: Many drugs are converted to reactive products (often termed (reactive) “metabolites”). These entities modify the proteins they react with and somehow cause toxicity.

- Example is acetaminophen a commonly used analgesic and antipyretic.

Harmful Metabolites of Acetaminophen

The high levels of acetaminophen will saturate the conjugation enzymes

First the glucuronate transferase and the sulfate transferase will be saturated

This will eventually saturate the glutathione transferase

When that happens high levels of NAPQI will accumulate inside the cell

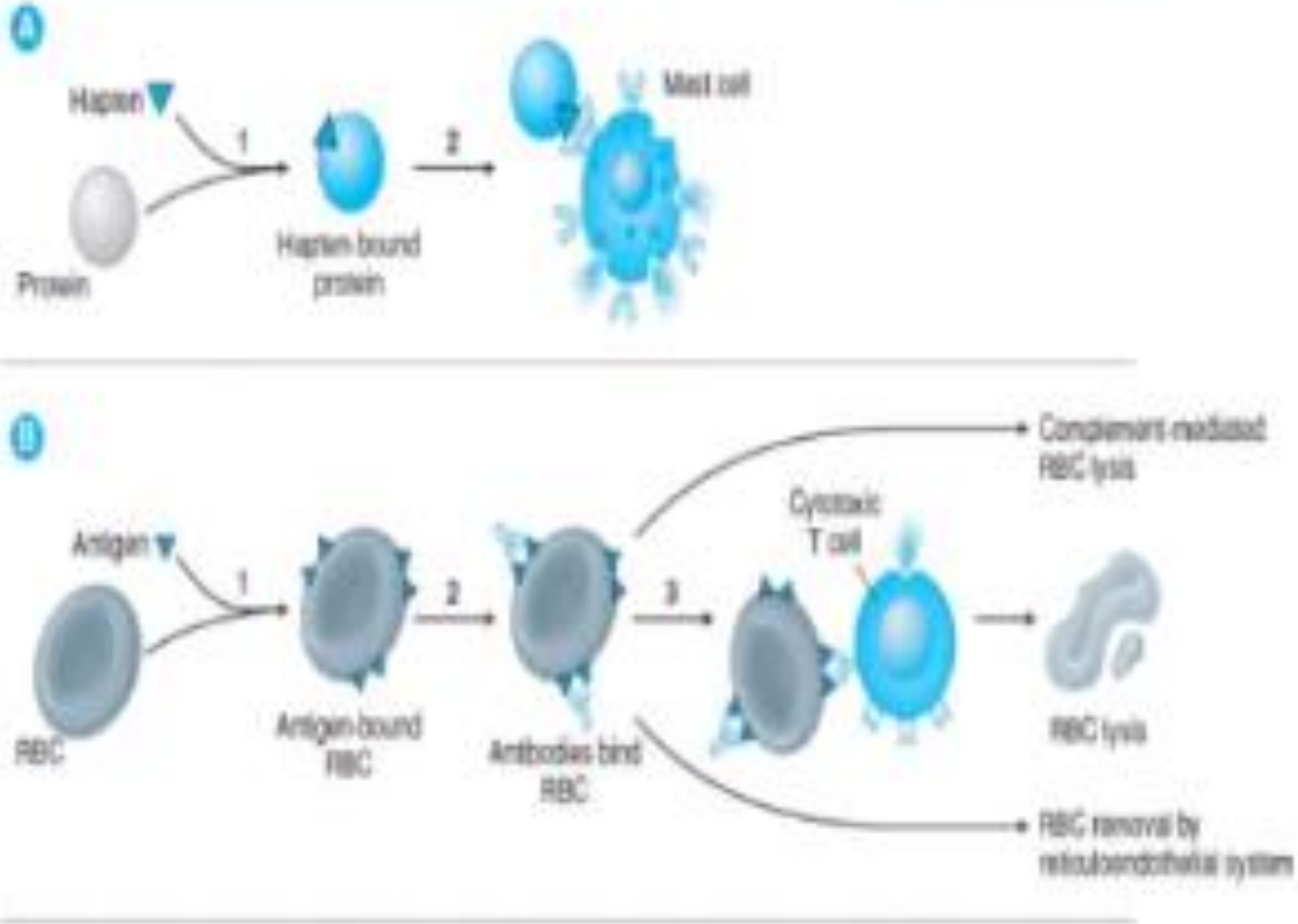
NAPQI will react with other hepatic proteins giving other toxic compounds

This will cause hepatotoxicity and will cause death

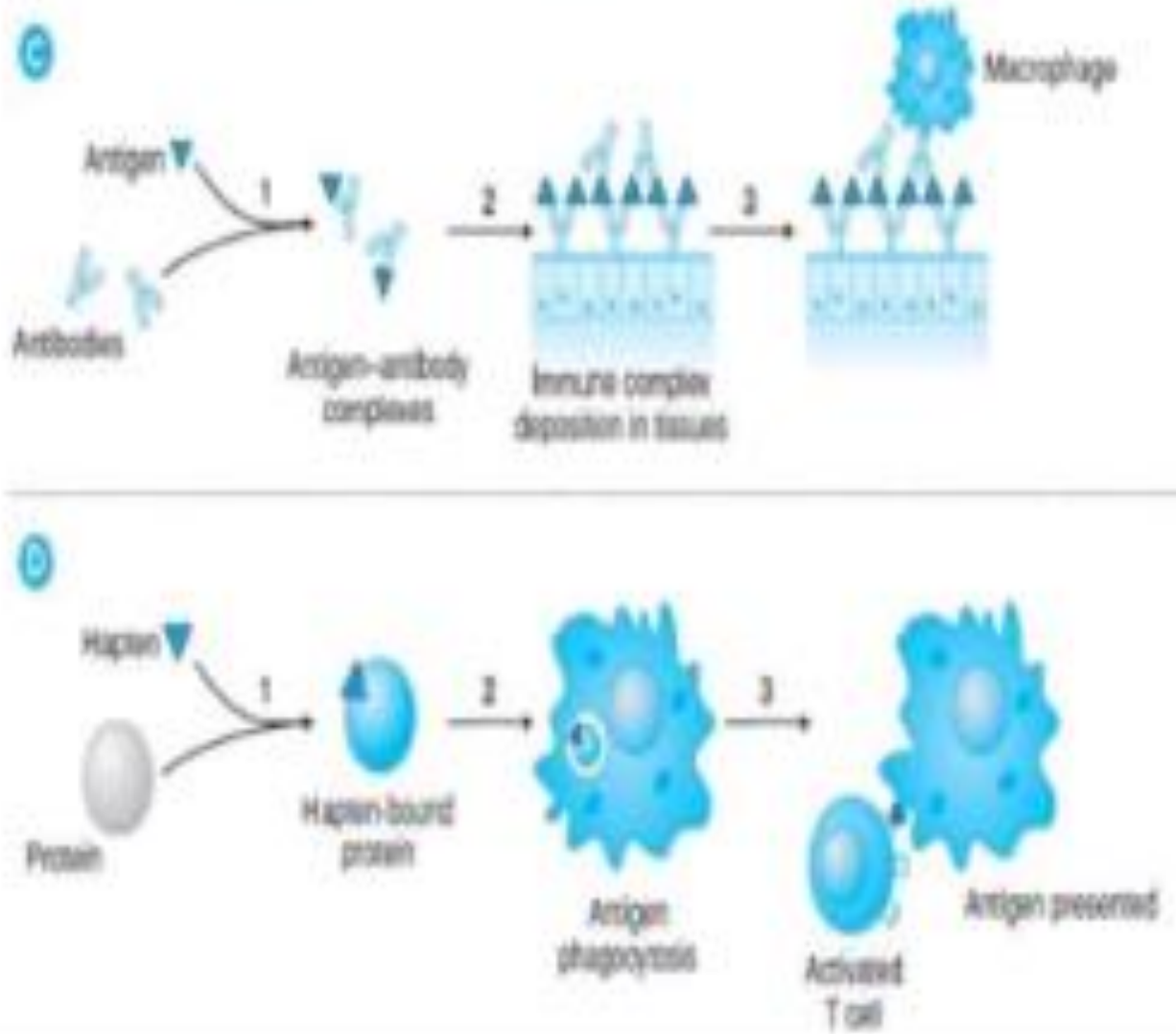
This is solved by giving the patient N-acetylcysteine

4. **Harmful immune response:** This concept is that drugs (or their metabolites) react with proteins in the body (as haptens) to induce antibodies and immune responses. In this case (penicillins for example), the chemical is not completely stable and has the potential to bind covalently to proteins and initiate antibody production.

Harmful Immune Responses



Harmful Immune Responses



Harmful immune responses

- type I hypersensitivity reaction occurs when hapten binds to a protein

(1) the antigen crosslinks the igE antibody on the surface of mast cell.

(2) Mast cell releases histamine and other inflammatory mediator

- type II hypersensitivity occurs when an antigen binds to the suface of a circulating blood cell, usually a red blood cell(RBC)

(1) antibodies to the antigen then bind surface of the RBC

(2) attracting cytotoxic T cells

(3) which release mediator that lyse the RBC binding of Ab to EBCs can also directly stimulate complement-mediated RBC-lysis & RBC removal by reticuloendothelial system.

Harmful immune responses cont

- type III hypersensitivity reactions occur when Ab binds to soluble toxins acting as an antigen

(1) The antigen-antibody complexes are then deposited in the tissues

(2) attracting macrophages

(3) and starting a complement-mediated sequence (not shown)

- type IV hypersensitivity reaction occurs when a hapten binds to a protein.

(1), and the hapten-bound protein is phagocytosed by a Langerhans cell

(2), the Langerhans cell migrates to a regional lymph node, where it presents the antigen to a T cell

AFLATOXINS

- They are a group of heterocyclic compound which was first discovered in England in 1960 after the out break of turkey disease resulted in deaths and development of cancer in rainbow trout fed with peanut and cotton seed meals

  Aflatoxins are produced predominantly by *Aspergillus parasiticus* (B_1,B_2, G_1, G_2) and *Aspergillus flavus* (B1, B_2)

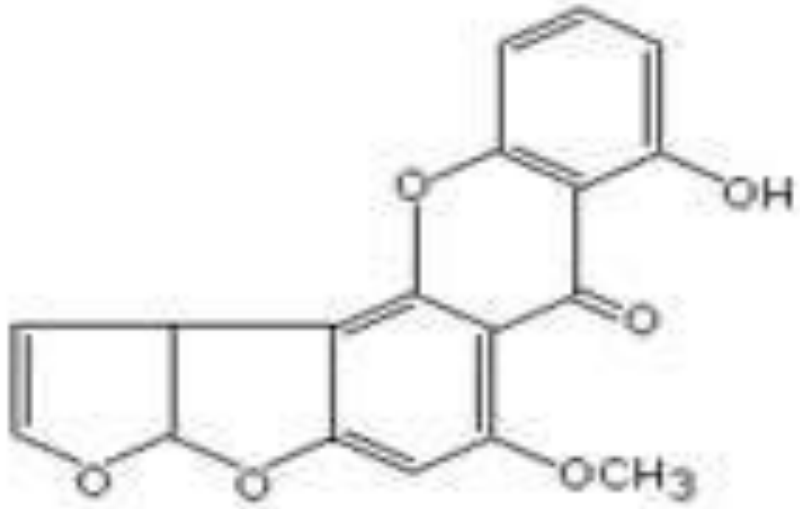
  AFB1 is the major component of these mixture.AFM1 is a hydroxylated metabolite of AFB1 found in milk.

  AFB1, AFG1 and AFM1 are the most biologically active aflatoxins by virtue of their activation to a reactive epoxide form, a reaction that does not occur with aflatoxins B2 and G2

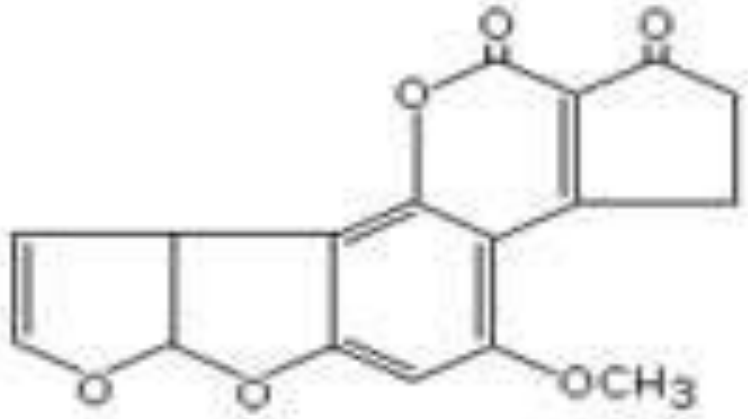
  Agricultural produce e.g. rice, corn, cassava, peanuts and spices are easily contaminated with aflatoxins due to lack of proper storage facilities.

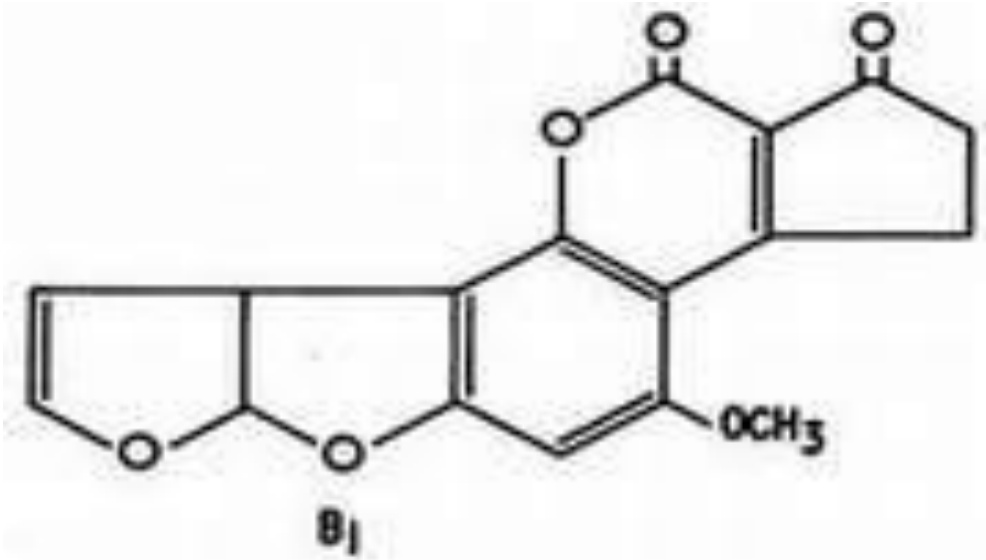
AFB_1

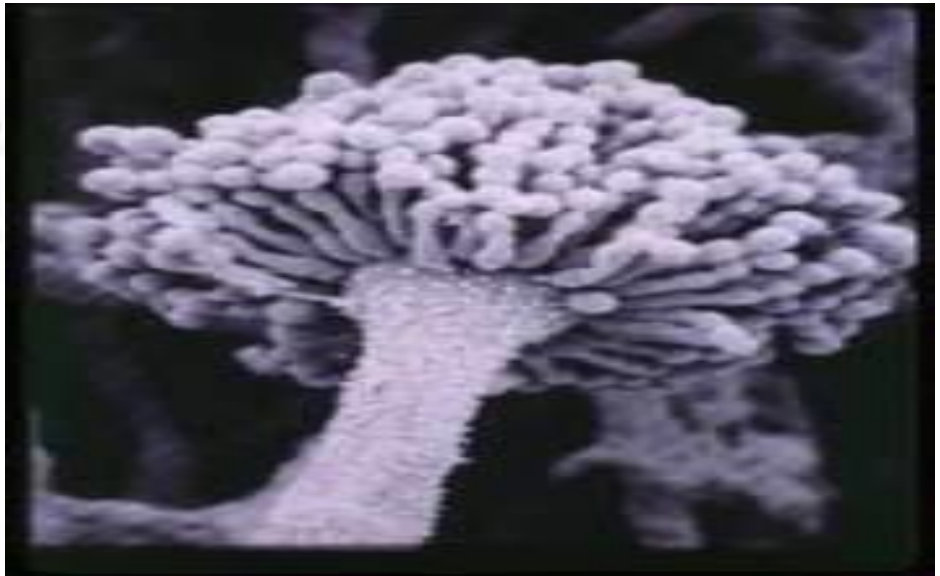
A



B







Mechanism of AFB_1

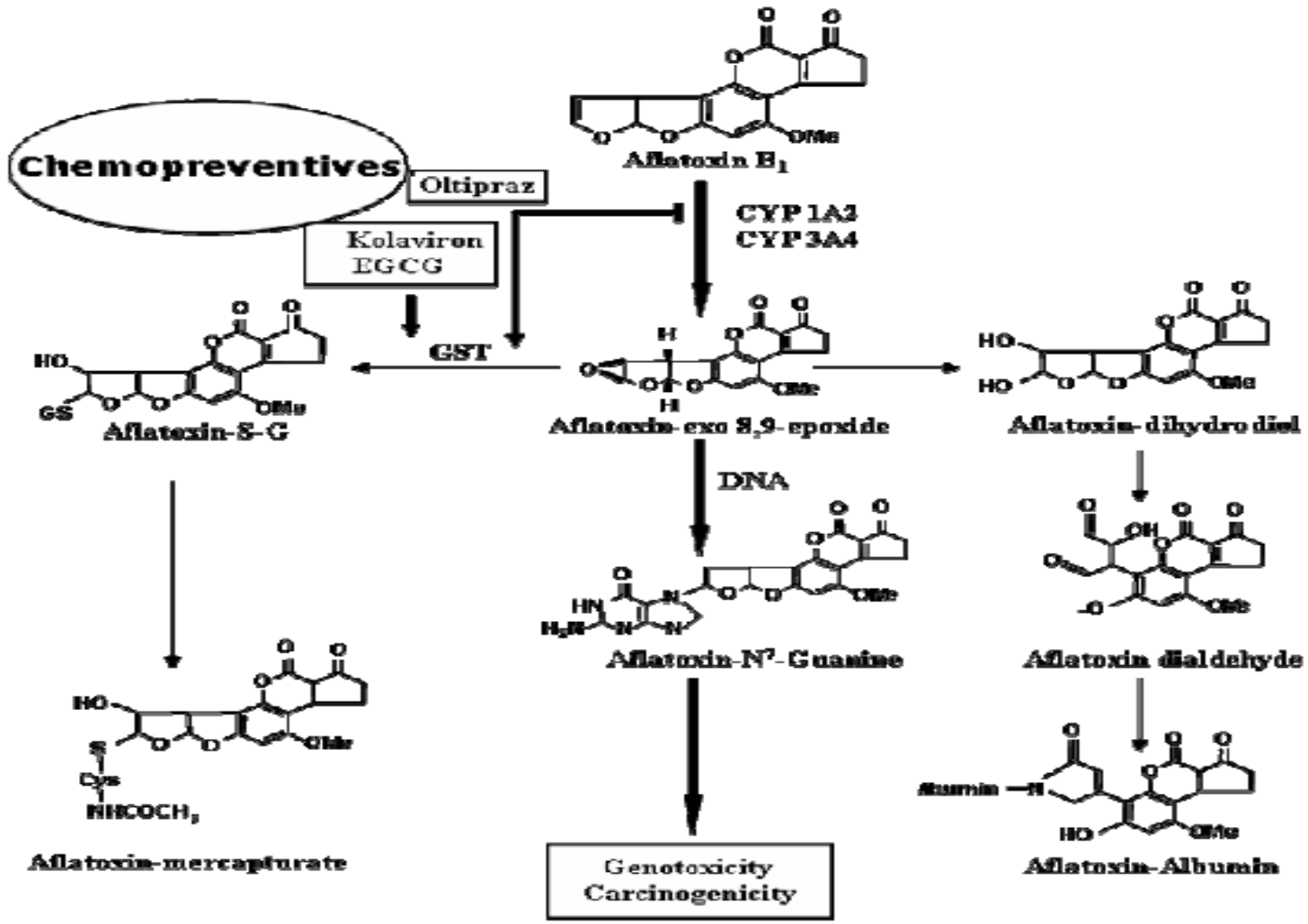


Figure 1. Major mechanism of biotransformation of aflatoxin B_1 (AFB_1) to AFB_1-exo 8, 9-epoxide leading to the formation of AFB_1-DNA adduct (AFB_1-N^7-guanine), detoxification product AFB_1-mercapturate catalysed by glutathione S-transferase (GST) and proposed site of action of selected chemopreventives.

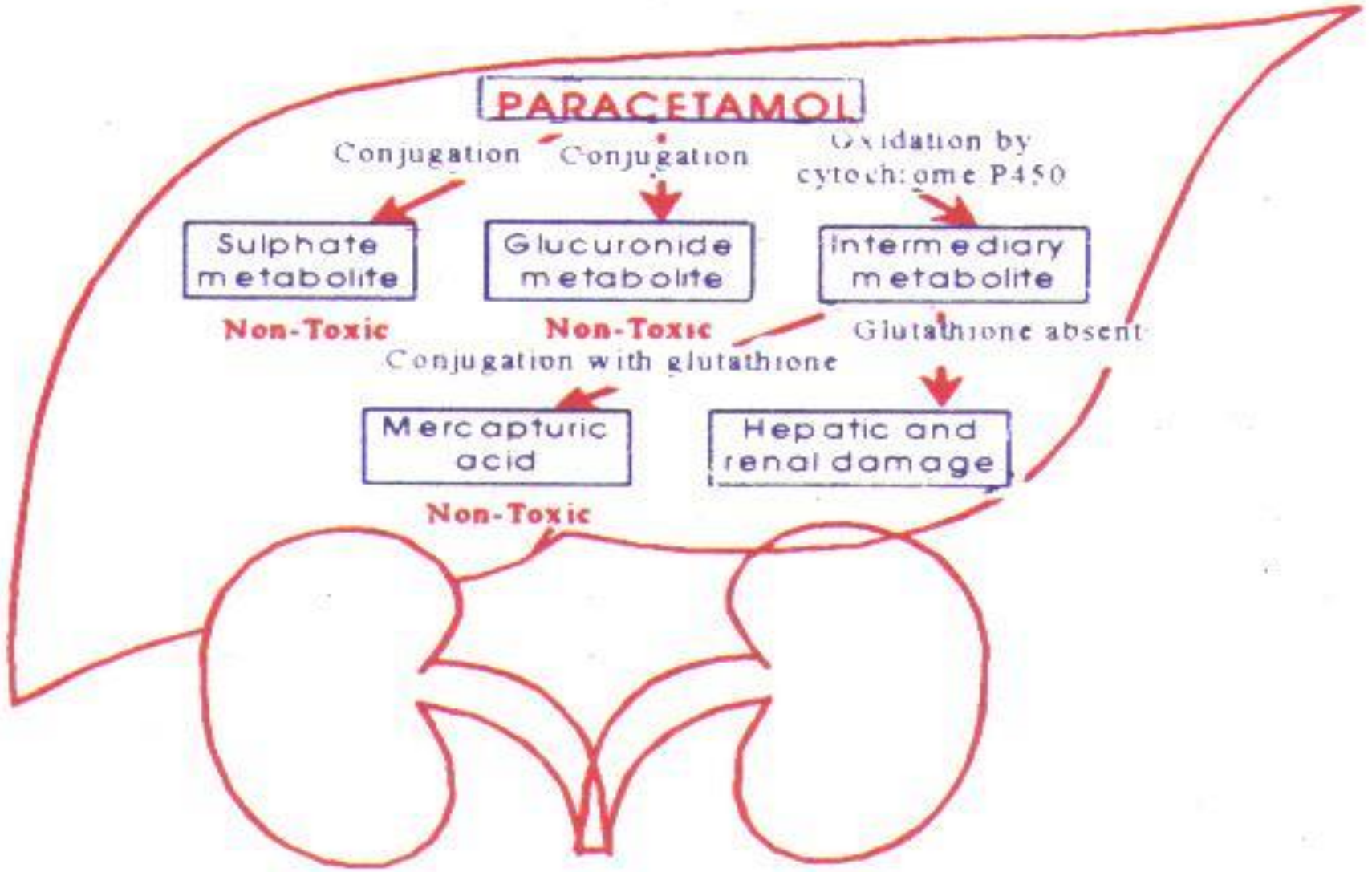
Food toxins from industrial wastes

- Other substances are persistent and remain in the animal and in animal products, like dioxins. Heavy metals are not metabolized at all. Some metals irreversibly are bound to body tissues, e.g. lead to bone or cadmium to kidneys.
- They result from either agricultural or industrial production or through accidental or deliberate misuse.
- These heavy metals include lead, cadmium, mercury and arsenic.

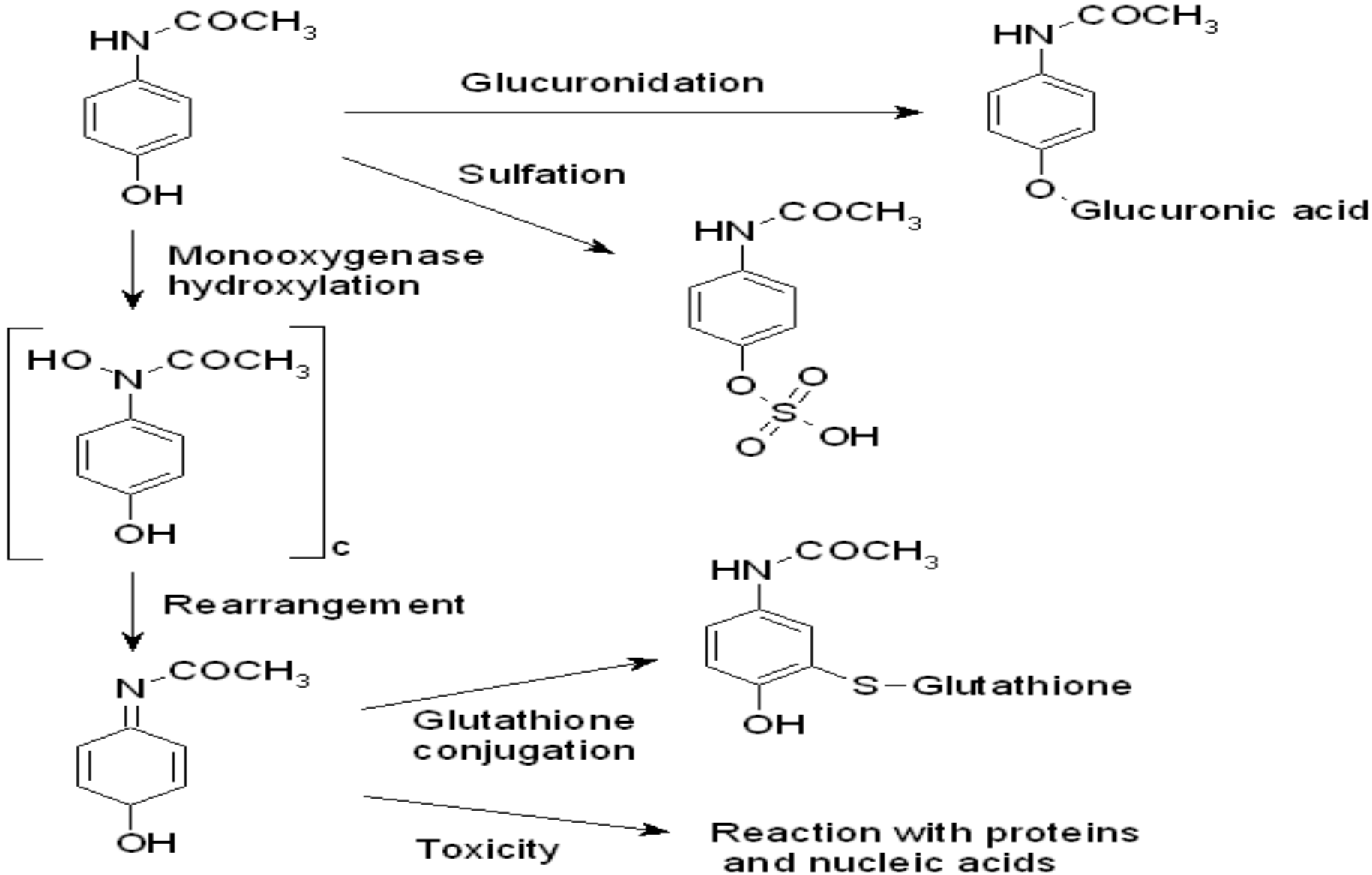
Toxins from pesticide residues

- For food toxicology the important categories of target organisms are insecticides, fungicides and herbicides.
- Glory years of nobel prize discovery, DDT.

Hepatotoxicity of Paracetamol (Mechanism of toxicity)



Mechanism of toxicity



PULMONARY TOXICITY

- The common drug that causes pulmonary toxicity is **4-Ipomeanol**-found on moldy sweet potatoes
- It causes necrosis of the non-ciliated bronchiolar epithelial or Clara cells
- Highly reactive metabolite covalently binds to the macromolecules of lung tissues causing necrosis of the cell type.
- **4-Ipomeanol is selectively toxic to the lung and relatively non-toxic to the liver**
- It may be because the liver lacks isoenzyme of CYP450 for its activation or well endowed with detoxifying phase II enzymes

TERATOGENS

- Drugs and chemicals that interfer with the process of embryo development are called Teratogens.
- Drugs given at the stage of organogenesis of cause the malformation of the embryo e.g. Cyclophosphamide–to treat cancers and immune disorders
- The study showed that the metabolites of cyclophosphamide are more potent teratogenic than the parent drug

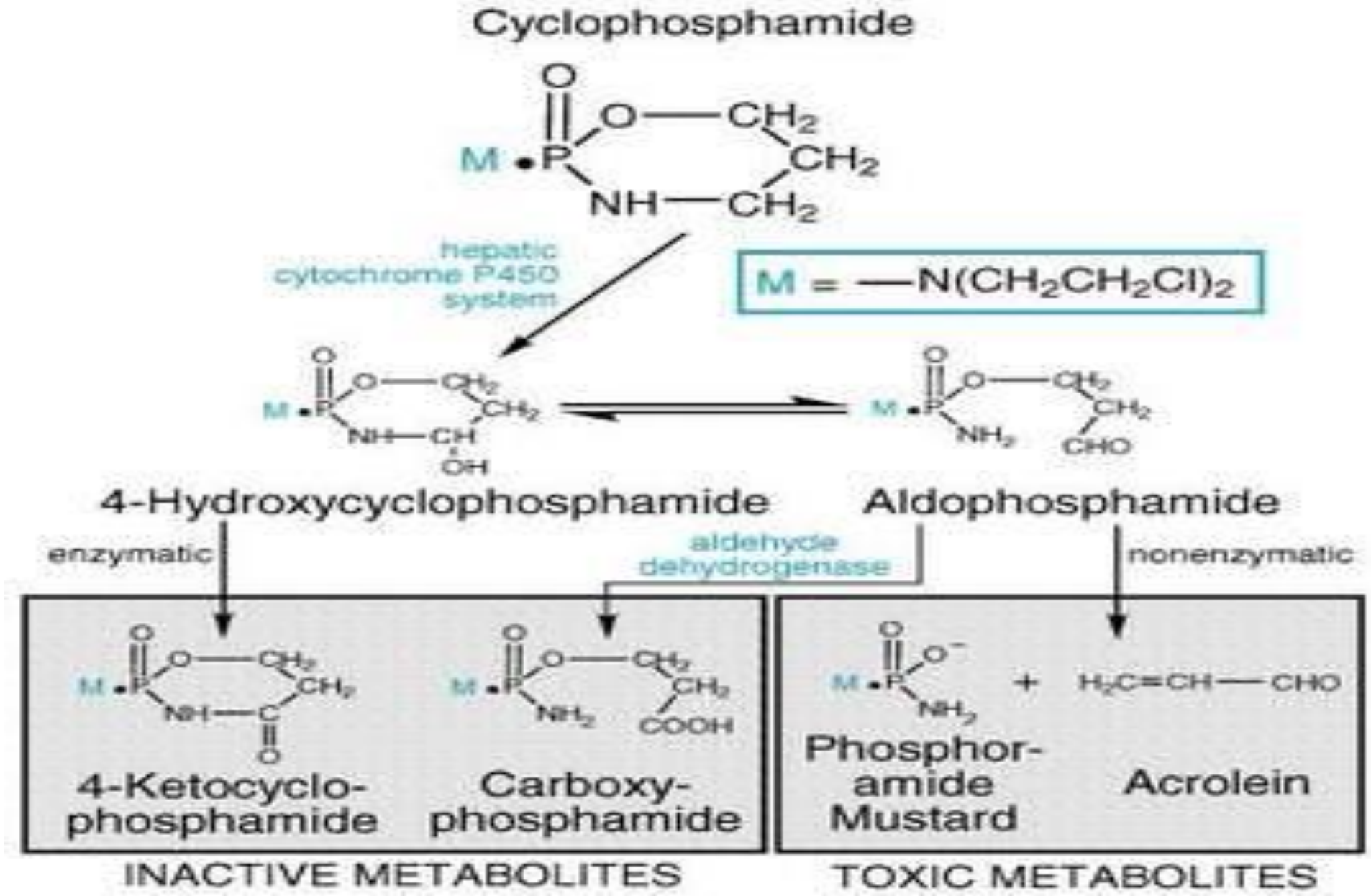
- CP undergoes CP450-dependent hydroxylation at 4-position and the hydroxylated metabolites serve as the precursor for the toxic metabolites e.g. **Acrolein** and **phosphamide mustard**
- Other example is **Thalidomide** which crosses the placenta by simple diffusion

CYCLOPHOSPHAMIDE

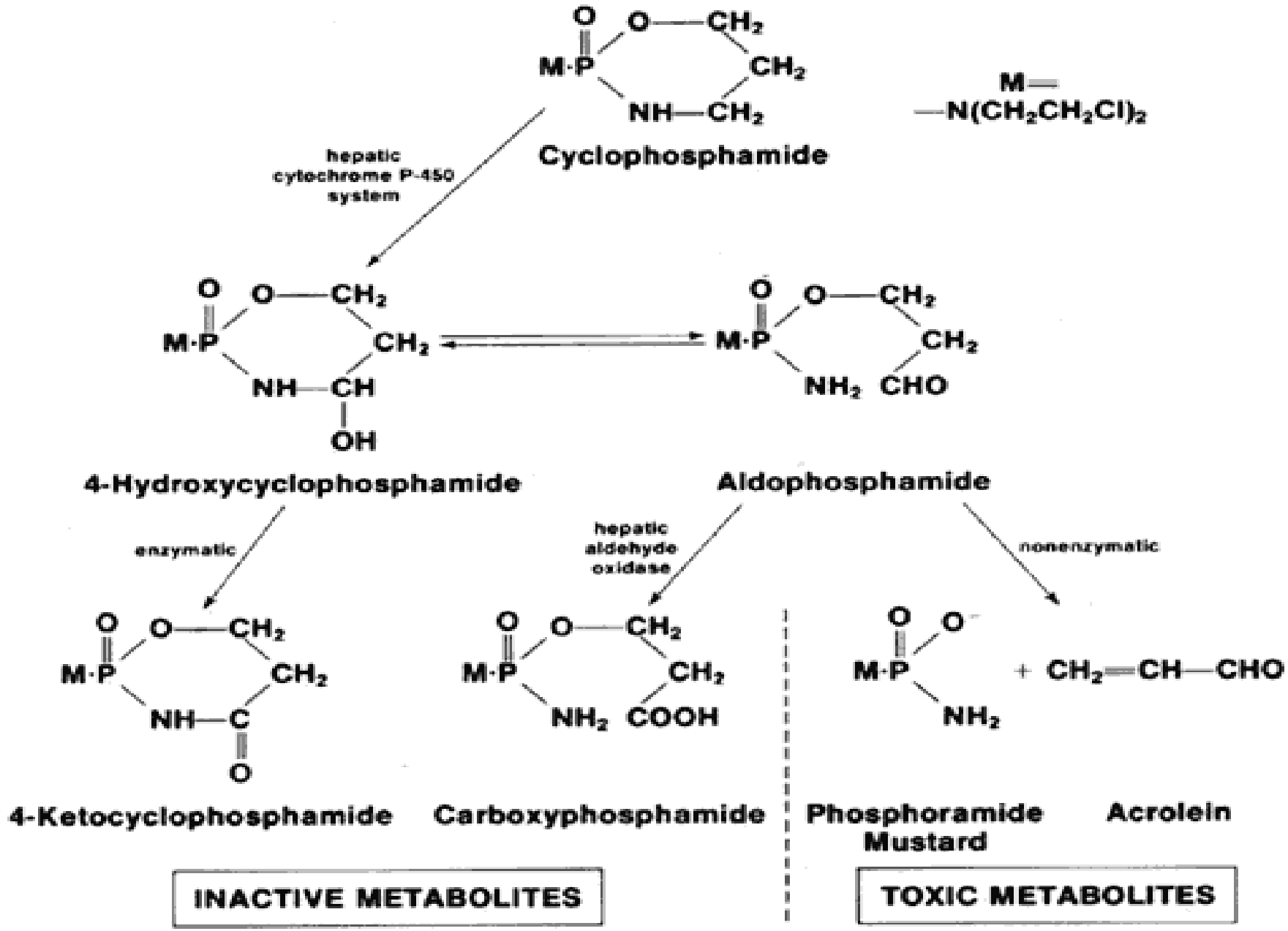
Cyclophosphamide, cont.

- **Effects on Dental Treatment**: Mucositis and stomatitis
- **Effects on Mental Status**: May cause dizziness
- **AAA's**
  - Antibiotics: NO Clarithromycin, NO Azole Antifungals, NO Doxycycline, NO Erythromycin, NO Ciprofloxacin
    - USE: Clindamycin, Azithromycin, Metronidazole
  - Analgesics: NO Nitrous Oxide
  - Anesthetics: No contraindications

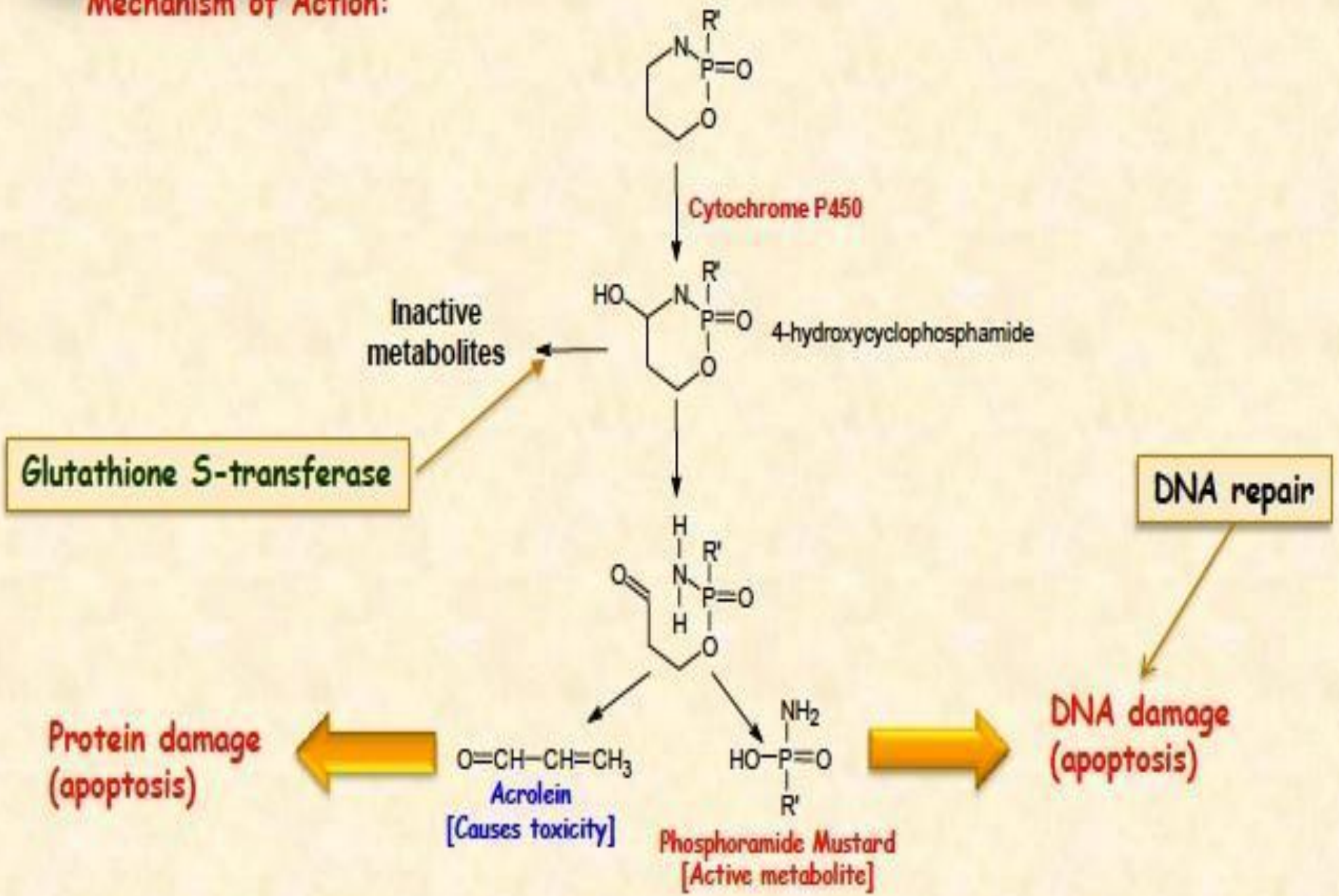
CYCLOPHOSPHAMIDE



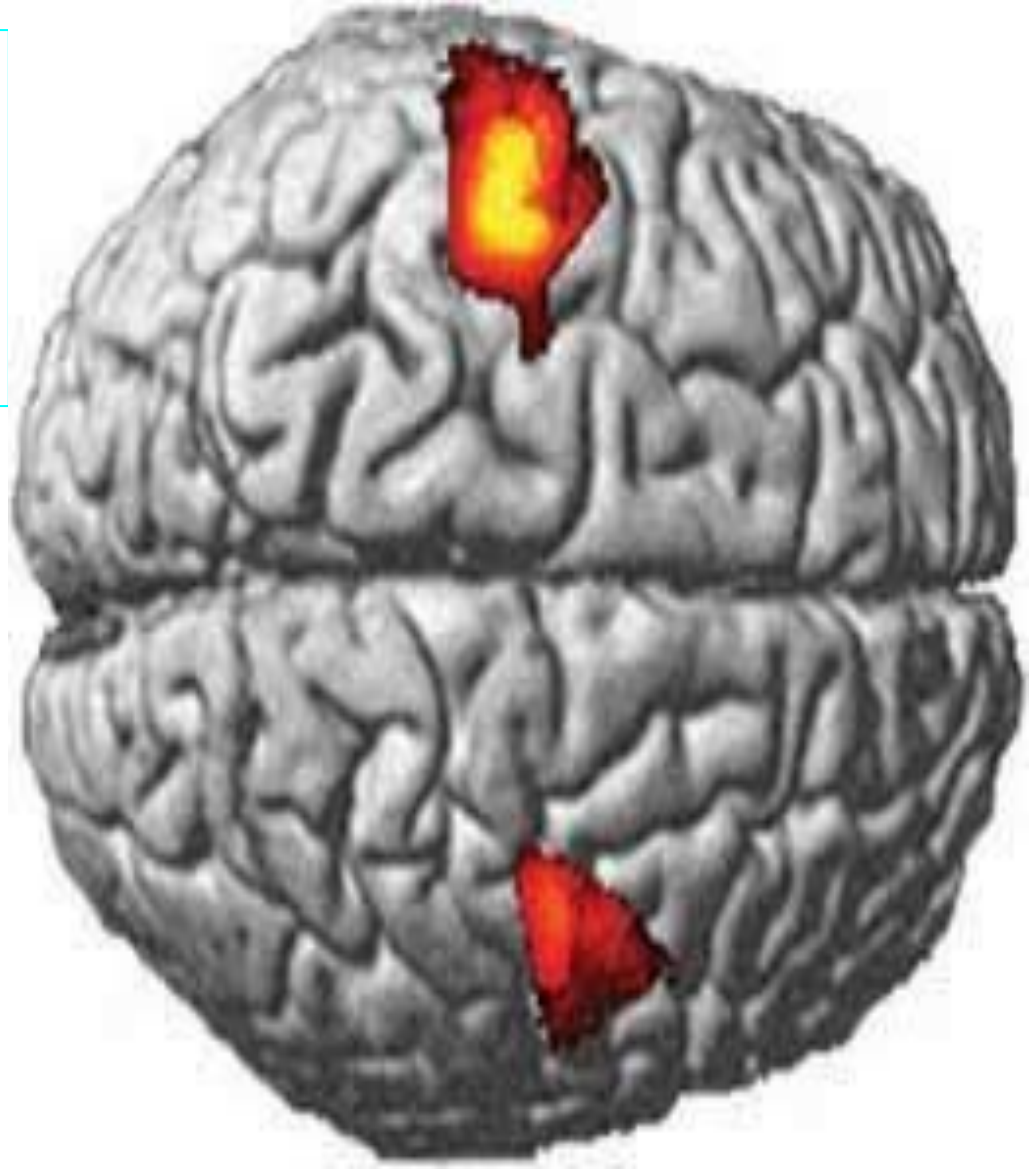
Metabolism of teratogenic agent-Cyclophosphamide



Mechanism of Action:



Haloperidol shrinks volunteers' striatums in two hours, but they bounced back within a day.



Tost, H et al., 2010

Metabolism & Toxicity

- Metabolism is of utmost importance in toxicity.
- There are several aspects or relationships between toxic substances and metabolism, as stated below:

i. Some substances are not themselves toxic but are metabolized to toxic substances. E.g. Most substances are often regarded as carcinogens, such as monosodium glutamate, maggi, smoke, cosmetics, etc. They are metabolically activated to produce species that are the utmost carcinogens.

ii. Toxic species are detoxified by metabolic processes. The metabolic processes act to counter their toxic effects.

Carcinogenesis

- Cancer is a group of diseases characterized by uncontrolled growth and spread of genetically damaged cells.

- Certain genes and proteins like tumor suppressor gene (p53), and oncogenes (mutated genes that have the potential to cause cancer, they transform normal cells into cancer cells; however, before they become mutated they are called proto-oncogenes) are damaged, so the abnormal cells no longer respond to regulatory signals, thus proliferate (multiply) autonomously (freely).

- Carcinogenesis is the formation of cancer, it is a process whereby normal cells are transformed into cells that no longer respond to regulatory signals due to mutations in the genes that control the cell's division.
- The study of cancer is known as oncology.

Note: Any agent with the ability to cause, influence, or promote cancer or carcinogenesis is called CARCINOGEN.

- **Note:** Any agent with the ability to cause, influence, or promote cancer or carcinogenesis is called CARCINOGEN. A number of them may include:
- i. Diet, e.g. High-fat consumption, fried food, burnt food, junk, artificial sugar, aspartame, saccharine
- ii. Chemicals, e.g. polycyclic aromatic hydrocarbons (PAHs, present in chimney soot, charcoal-charred/grilled meats, fish, corns, yam, auto exhaust, cigarette), aflatoxin, benzene, ethidium bromide cadmium chloride, benzo[a]pyrene, vinyl chloride, etc.
- iii. Radiations, e.g. UV-light, excessive exposure to X-ray
- iv. Viruses: Oncoviruses play an important role in specific human cancers, e.g. human papillomavirus in cervix cancer and certain skin cancers; hepatitis B virus in hepatocellular carcinoma; human T-cell leukemia virus in leukemia. The viruses are of two types: DNA viruses which incorporate into the cellular genome and retroviruses (RNA viruses) which cause the transformation of the cellular genome, leading to malignant changes in the infected cell.
- v. Environment, e.g. industrial areas, UV-light, air pollution

vi. Smoking, e.g. cigarette and tobacco

vii. Alcohol consumption

viii. Inherited condition, e.g. mutated genes

ix. Pro-carcinogens: a substance that is not itself a carcinogen but can sometimes be turned into a carcinogen.

- Pro-carcinogens are activation dependent. These compounds require cellular enzymatic metabolism into an ultimate carcinogen in order to exert their carcinogenic action.

- The resulting chemicals can be just as carcinogenic as direct carcinogens. E.g. Some therapeutic drugs, e.g. antibiotics, theobromine from cocoa powder; piperine from black pepper, mascara, cosmetics, etc.

Classification of carcinogens

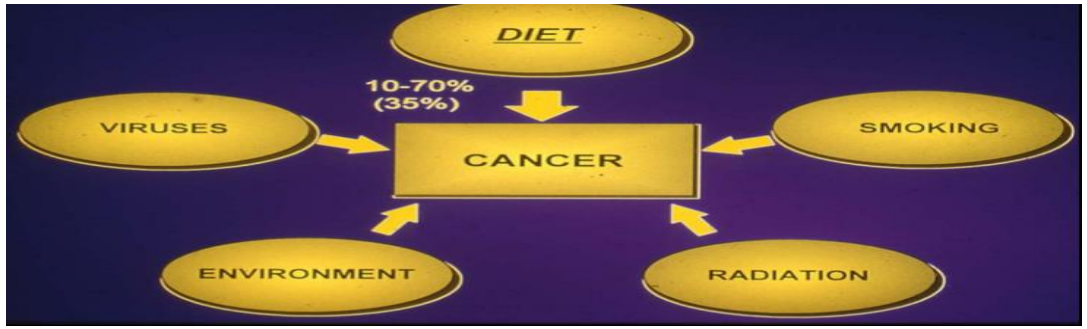
The carcinogens are classified as:

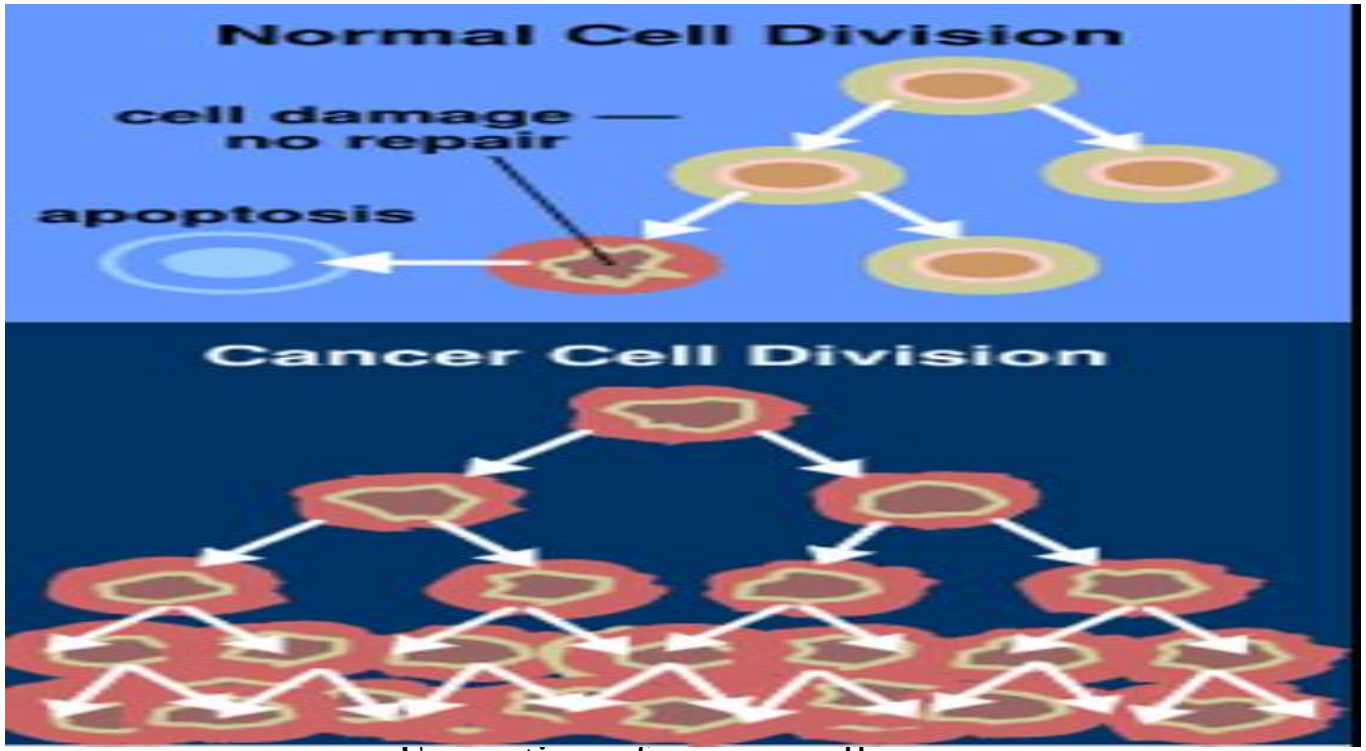
Genotoxic and Non-genotoxic / epigenetic carcinogens: Genotoxic carcinogens

- Possess the ability to damage DNA
- Cause gene mutation
- Majority of known human carcinogens are genotoxic

Non-genotoxic or Epigenetic carcinogens

- Neither damage DNA nor change DNA sequence but induce changes (methylation, acetylation, phosphorylation on DNA, RNA or histones) in cells, thereby altering transcription, translation and post-translational information.
- Alter the balance between cellular growth and death
- Lots of non-genotoxic chemicals are positive in animal studies





Formation of cancer cells

How do carcinogens enter into the body?

Through:

i. skin absorption: many chemical solvents (ethidium bromide, benzo[a]pyrene), bleaching creams, radiations and other chemicals go directly through the skin.

ii. Ingestion: swallowing of a carcinogen (aflatoxin on corns-infected with Aspergillus flavus, antibiotics, PAHs, alcohol, cigarette and tobacco).

iii. Inhalation: air pollution, breathing in gases, fumes and vapors are the most common forms of exposure.

What organs do carcinogens attack?

Skin, Brain, Lungs, Breast, Kidneys, Liver, Stomach, Reproductive system (cervix, prostate), Colon etc.

Four steps of carcinogenesis

1. Initiation: carcinogen binds with normal cells or DNA to form cell adducts. This stage is reversible.

2. Promotion: cells with adducts proliferate (multiply) and initiate formation of cancer cells.

3. Malignant conversion: cells tend to infiltrate (invade some parts of the body), metastasize (spread to other parts of the body) and terminate (burst)

4. Progression: a stage of genomic instability and uncontrolled growth.