PHYSIOLOGY HONOURS (CBCS) SEMESTER-IV
(MODULE-SEC-B)

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Food additives: (Definition and Example)

Substances that are added to food to maintain or improve the safety, freshness, taste, texture, or appearance of food are known as food additives. Some food additives have been in use for centuries for preservation – such as salt (in meats such as bacon or dried fish), sugar (in marmalade), or sulfur dioxide (in wine. They are added intentionally.
WHO, together with FAO, groups food additives into **3 broad categories** based on their function.

1. **Flavouring agents:** which are added to food to improve aroma or taste –. There are hundreds of varieties of flavourings used in a wide variety of foods, from confectionery and soft drinks to cereal, cake, and yoghurt. Natural flavouring agents include nut, fruit ETC.
2. **Enzyme preparations:** Enzyme preparations are a type of additive that may or may not end up in the final food product. They can be obtained by extraction from plants or animal products or from micro-organisms.. They are mainly used in baking (to improve the dough), for manufacturing fruit juices (to increase yields), in wine making and brewing (to improve fermentation), as well as in cheese manufacturing (to improve curd formation).
3. **Other additives:** Other food additives are used for a variety of reasons, such as **preservation, colouring, and sweetening.** They are added when food is prepared, packaged, transported, or stored, and they eventually become a component of the food.

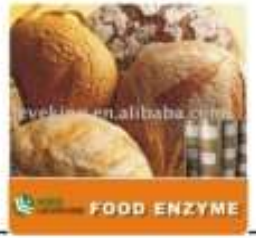
Over 3000 additives are approved.

Additive Class	Function	Chemical substance	Foods in which used
Colours and adjuncts*  	Provide, preserve or enhance the colour of a food	Annatto, carotene, cochineal, chlorophyll, nitrates Red – Ponceau 4 R, carmoisine erythrosine Yellow - Tartrazine, Sunset yellow FCF Blue- indigo carmine Brilliant Blue FCF Green – Fast green FCF	Ice- cream, biscuits, cakes confectionery sweets, savouries fruit syrup, fruit squash, fruit drink and beverage, soft drink, jam, 200 mg/kg. permitted

Additive Class	Function	Chemical substance	Foods in which used
Flavour Enhancers* ** 	Supplement, enhance or modify the original flavor or aroma of a food with out contributing flavours of their own.	Monosodium glutamate (up to 1%) and yeast.	Masalas/spices used in noodles/Chinese cookery.
Flavouring agents and adjuvant 	Impart or help impart a taste or an aroma in food	Natural extracts and synthetic flavor chemicals are used, Basically they are aldehydes, ketones, hydroxyl and ester or etheric in nature.	Soft drinks, confectionery, chewing gum.

Additive Class	Function	Chemical substance	Foods in which used
Antimicrobial! Agents*	Prevent the growth of bacteria, moulds, fungi and yeast	Benzoic acid, esters of p-hydroxy – benzoic acid, o – chlorobenzoic acid or salicylic acid sulbhites sodium benzoate and sorbic acid.	Squashes, crushes 
Curing and pickling agents	Impart unique flavor or colour to a food, often increase shelf life and stability.	Sodium nitrite gets converted to nitric oxide which combines with myoglobin to form nitric oxide myoglobin, inhibits growth of clostridium and streptococcus.	Meat 
Maintain appearance palatability and wholesomeness	To prevent spoilage caused by moulds, bacteria, yeast	Propionic acid Calcium and sodium salts or Propionic acid ascorbic acid, Butylated hydroxyanisole (BHA) Butylated hydroxyl toluene (BHT) propylene glycol	Bread, pie filling, cakes mixes potato chips, crackers, cheese, syrup, fruit juices, frozen and dried fruits margarine, shortening.

Additive Class	Function	Chemical substance	Foods in which used
Sweeteners* non - nutritive**	Provide less than 2% of the caloric value of sucrose per equivalent unit of sweetening capacity when used to sweeten the food.	Aspartame (fruit flavour) Acesulfame - k 	Soft drinks, chewing gum, instant coffee and tea.
Sweeteners nutritive* **	Provide greater than 2% of the caloric value of sucrose per equi valent unit of sweetening capacity when used to sweeten the food.	Sorbitol mannitol – other polyols. 	Chocolates, ice – cream, pastries jams, drinks.

Additive Class	Function	Chemical substance	Foods in which used
Enzymes 	Digestion and clarification 	Pepsin, rennet, papain, amylase, pectinase	Cheese making, tenderizing meat, beverage clarifying
Neutralizing agents	To remove excess acidity	Alkaline Salts of Na, K, Mg and Ca	Wine, ice – cream
Stabilizing agents	Help in ensuring a stable emulsion emulsifier/ thickener	Caragean gums, gum arabi	Chocolate in milk, ice- cream, foam stabilizer in beer.
Firming Agents	To retain texture of canned fruit and vegetables	CaCO3, Sodium aluminium sulphate, Calcium citrate.	Canned tomatoes

Additive Class	Function	Chemical substance	Foods in which used
Propellants, aerating agents and gases	Supply force to expel a product	CO_2 gas and Na_2CO_3 or $CaCO_3$ with an acid to generate gas.	Soft drinks, alcoholic beverages.
Solvents and vehicles	Extract or dissolve another substance	Ethyl alcohol, propylene glycol, glycerin, oils and fatty acids	Vanilla extract
Leaving agents	To increase in volume of dough or batter resulting in light fluffy, spongy, produce or stimulate CO_2 production in baked goods.	Baking powder Sodium bicarbonate with another acid component like tartaric acid or calcium hydrogen phosphate.	Bread, biscuits, cakes

FOOD ADULTERANTS (Definition and Example):

Food Adulteration can be defined as the **practice of adulterating food or contamination of food materials by adding a few substances,** which are collectively called adulterants. Adulterants are the **substance or poor quality products** added to food items for economic and technical benefits. Addition of these adulterants reduces the value of nutrients in food and also contaminates the food, which is not fit for consumption.

For example- **high fructose corn syrup** or cane sugar, used to adulterate honey, roasted **chicory roots** used as an adulterant for coffee, **water**, for diluting milk and alcoholic beverages, **water or brine** injected into chicken, pork, or other meats to increase their weight etc.

Listed here are the **main reasons for adulterating food products**:-

- To increase the quantity of food production and sales,
- To meet increased food demand for a rapidly growing population,
- To make maximum profit from food items by fewer investment.
- for imitation of some other food substance, practiced as a part of the business strategy
- lack of knowledge of proper food consumption, ,

METANIL YELLOW

1. Metanil yellow commonly known as acid yellow 36, is a **water-soluble yellow azo dye**. It is basically 3-[[4-(Phenylamino) phenyl] azo] benzene sulfonic acid.

2. It is commonly used as a **yellow coloring factor** in food stuffs, textiles, cosmetics and turmeric due to its cheap price and availability.

3. Metanil Yellow is known to be an **illegal food dye worldwide**. The acceptable daily intake (ADI) of metanil yellow was maintained by the Food and Agriculture Organization (FAO) at 0-0.3 mg/ 1 kg body weight. In India, it was deemed by the Government of India as illegal coloring according to the **Food Adulteration Act 1954.**

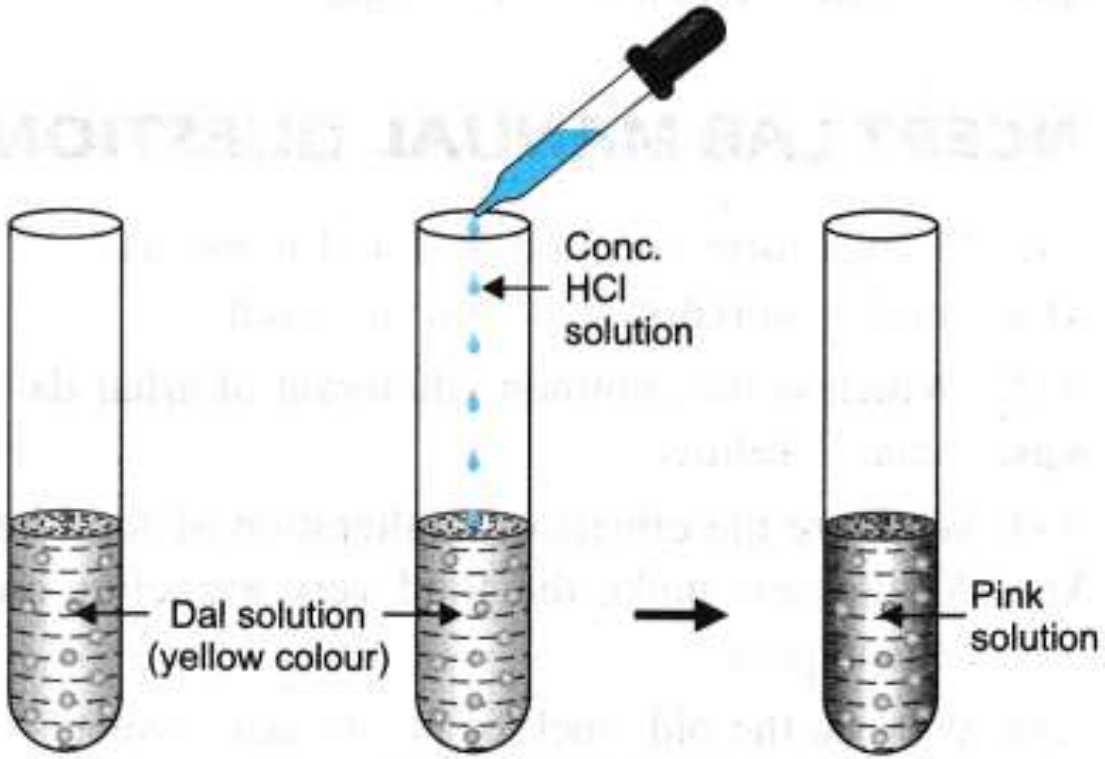
4. Metanil yellow being an unauthorized food colorant is still used in many kinds of food items and has been found to cause damage in different key internal tissues.

Sweets

- Adulterants : Metanil yellow used to brighten the colour of pulses, turmeric powder and sweetmeats, is colours not permitted.
- Health effect :tumor and cancer

Test for identification in food sample:

Take one gram turmeric powder in a test tube. Add 5 ml of water. Add a few drops of **concentrated HCl.** Observe any change in color Inference. Appearance of pink/violet color which disappears on dilution with water shows the presence of unadulterated turmeric. If the color persists, indicates the presence of metanil yellow.



Pathophysiological effects:

1. **On Nervous System**:

- Damages adult as well as developing brain in Wistar rats.
- It was found that amine levels (neurotransmitters) in certain areas of brain such as the stratum, hypothalamus and brain stem were markedly .
- A delayed but persistent decrease in the level of acetylcholine esterase level was observed in the hippocampus.
- Learning was adversely effected in rats with metanil yellow administration
- It damages both the granular and Purkinje cell layer of brain.

2. Effects on Digestive System:

- It causes gatrotoxicity, and damages the intestine.
- it causes disruption and disarrangement of gastric folds, destroys the epithelial cells, caused loss of microridges from the apical plasma membrane and fragmentation.
- Metanil yellow also caused erosion and degeneration of gastric glands.
- it loosened the structural configuration of absorptive columnar epithelial cells in intestine.
- Intestinal microvilli were also observed to be disrupted heavily. All those caused loss of absorption capacity of nutrients.
- The lamina propria was also severely necrosed.
- Studies in fish model shows hepatotoxicity that extensive degeneration of cytoplasm, pyknosis of nuclei and damage occurred in central vein region of liver tissue on metanil yellow exposure.

3. **Effects on Cardiovascular System**:

• Metanil yellow induces damage in heart tissue and causes cardiotoxicity.

• It raises the level of lipid peroxidation and also altered the level of the endogenous antioxidant enzyme, catalase in goat heart in vitro.

4. Effects on Excretory System :

• It caused histopathological lesions in kidneys.

• Necrosis of tubular epithelium, cloudy swelling of epithelial cells of renal tubules and disruption in Bowman's capsule.

•Several deteriorative changes were observed in the distal convoluted tubule and the collecting tubules in kidneys.

5. Effects on Reproductive System:

• it is toxic to both male and female reproductive system.

• It can disrupt the normal estrous cycle in female rats.

• It impaired folliculogenesis in female rats and also has been found to inhibit the secretion of FSH and estradiol from the ovary,

• It induces oxidative stress in hypothalamic–pituitary–gonadal axis.

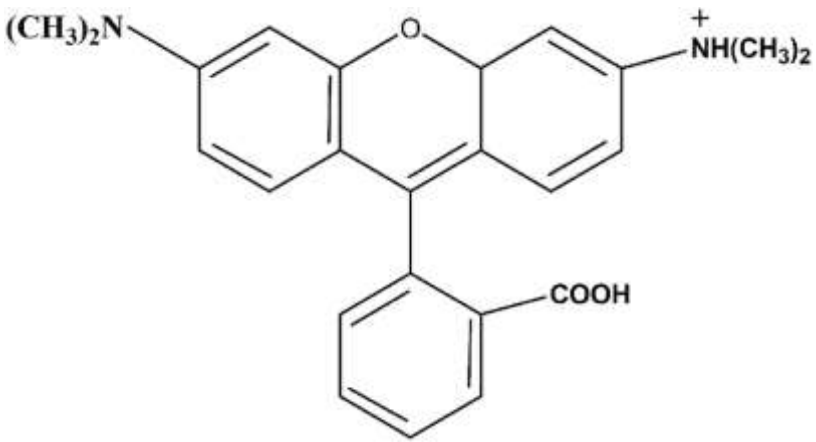
• It causes damages to testicular tissues. It was found that it induced degenerations in the seminiferous tubules and spermatocytes.

RHODAMINE B

Rhodamin B is a **synthetic food colour.** It appears green in powder form but when added to water turns into a vivid fluorescent pink.

It is not a permitted food colour. It is an organic chloride salt and a dye. It is illegally used to impart a red colour to **chili powder and pickles**. Rhodamine is a banned dye as per PFA act (1954), by Government of India.

Identification test: Take 2 grams sample (chili powder) in a test tube. Add 5 ml of acetone. Observe color of acetone layer. Immediate appearance of red color indicates presence of Rhodamine-B.




Ministry of Health
& Family Welfare
fssai
mygov
DETECTING
RHODAMINE B
ADULTERATION
IN SWEET POTATO

XCWY

Pathophysiological effects:

Acute effects:

- Direct Contact produces irritation, burning of skin
- Burning of eyes with possible eye damage.
- Inhalation can irritate nose and throat causing coughing, wheezing and chest tightness.
- It can also cause headache, nausea and vomiting.

Chronic effects:

- Long term effects may last for months to years after exposure. Adverse reproductive effects have been reported in many animals.
- Rhodamine B is suspected to be carcinogenic.
- It is harmful to humans because of its reproductive, developmental toxicity and neurotoxicity.

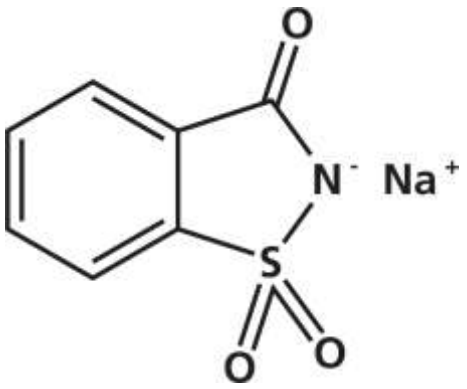
SACCHARIN

Saccharin is an **artificial and non-nutritive sweetener** used for the production of various foods like jams, jellies, baked goods, chewing gum, low calorie candies, toothpaste, diet drinks, tinned fruit etc.

is an artificial sweetener with effectively no food energy. It is about **300–400 times as sweet as sucrose** but has a bitter or metallic aftertaste, especially at high concentrations.

People uses saccharine to stay in better health and control weight. It is the oldest artificial sweetener, and was discovered in 1879.

Identification test: High pressure liquid chromatography with UV detection (HPLC-UV) is the most commonly used method to identify and quantify saccharin in non-alcoholic drinks. It can be done by FTIR spectroscopy also. Both methods are unsuitable for small laboratories.


O
N⁻
Na⁺
S
O
O

Pathophysiological effects:

1. Saccharin and toxicity are arguable.. Most publications reference that saccharin increases the rate of **urinary bladder cancer** in rats when fed with large doses. Saccharin was banned in 1981 because of fear of *possible* carcinogenesis. Experimentally, no harmful effects on humans were observed with consumption of 5 g saccharin daily over 5 months.
   As for saccharin, humans would need to drink the equivalent of 800 twelve-ounce diet sodas with saccharin daily to reach the carcinogenic doses.

2. Saccharin may induce **oxidative stress** on the liver cells through lowering catalase activity and the total antioxidant concentration (TAC) in plasma. It was demonstrated that saccharin harmfully affects both **hepatic and renal tissues.**
3. Saccharin is a sulfonamide compound which can cause **allergic reactions** in people that can't tolerate sulfa drugs. Common allergic reactions include breathing difficulties, headaches, skin irritation, and diarrhea.
4. Saccharin can also be found in some brands of children's vitamins and infant formulas and has been linked to **irritability, insomnia** and short-term problems with muscle tone.

MONO-SODIUM GLUTAMTE (MSG)

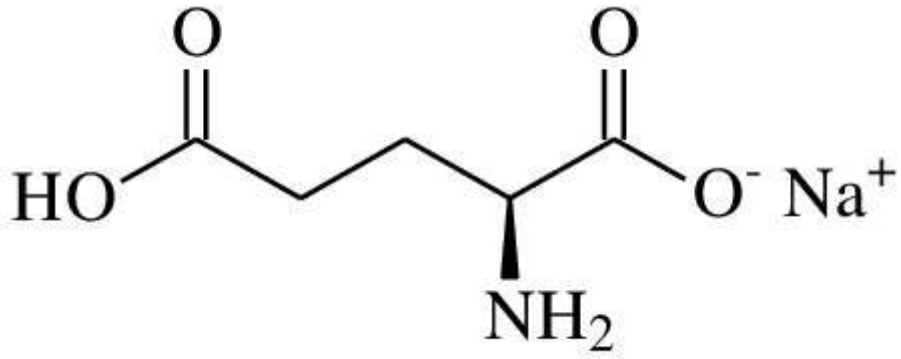
1. Monosodium glutamate is the **sodium salt of glutamic acid.** It is a **flavour enhancer** commonly added to Chinese food, canned vegetables, soups and processes meats. It is found naturally in foods including cheese and tomatoes. MSG enhances the savory, meaty umami flavour of foods. The Food and Drug Administration (FDA) has classified MSG as a food ingredient that's **"generally recognized as safe**," but its use remains controversial.

(Unlike common belief, **Ajinomoto** is not an ingredient itself, but is a Food and Chemical corporation based in Japan that uses its name as a trademark for its original product Monosodium Glutamate).

Identification test:

MSG can be tested in foodstuffs by analytical methods- **High Performance Liquid Chromatography (HPLC)** and **High Performance Thin Layer Chromatography (HPTLC)**.

MSG can also be analyzed through **chemical Analysis (Potentiometric Titration Method)**- Dissolve about 200 mg of the sample, previously dried and weighed accurately, in 6 ml of formic acid, and add 100 ml of glacial acetic acid. Titrate with 0.1 N perchloric acid determining the end-point potentiometrically. Run a blank determination in the same manner and correct for the blank. Each ml of 0.1 N perchloric acid is equivalent to 9.356 mg of $C_5H_8NNaO_4 \cdot H_2O$.)


O
O
HO
O⁻ Na⁺
NH2


AJI-NO-MOTO
UMAMI SEASONING
味の素
SINCE 1909
PURITY GREATER THAN 99%
MONOSODIUM GLUTAMATE
MADE IN FRANCE
AJINOMOTO FOODS EUROPE SAS
153 RUE DE COURCELLES, 75017 PARIS, FRANCE
UNDER LICENSE OF AJINOMOTO CO., INC-TOKYO, JAPAN
NET WEIGHT 454 g/1lb


Maggi
2-Minute noodles
"Mummy Im hungry"
Maggi
2-Minute noodles
"Mummy Im hungry"
Enjoy GRAIN SHAKTI

Pathophysiological effects:

1. **Chinese restaurant syndrome**: The term 'Chinese restaurant syndrome' (CRS) was first used more than four decades ago. At the onset of symptoms patients experience complaints such as a **burning sensation at the back of the neck, blistering on both arms and occasionally on the anterior thorax, general weakness, fatigue and palpitations.** These symptoms occur 20 minutes after consumption of a meal rich in MSG. Other symptoms that may appear later include flushing, dizziness, syncope and facial pressure.

2. **MSG symptom complex:** FDA in US has reported many anecdotal reports of adverse reactions to foods containing MSG. These reactions are collectively known as 'MSG symptom complex'. The symptoms include: **Headache, Flushing, Sweating, Facial pressure or tightness, Numbness, tingling or burning in the face, neck and other areas, Rapid, fluttering heartbeats (heart palpitations), Chest pain, Nausea, Weakness.**

3. Excitotoxin: Scientists claim that MSG leads to excessive glutamate in brain and excessive stimulation of nerve cells. For this reason, MSG has been labeled an excitotoxin. The average intake of 0.3-1.0 g MSG per day potentially **disrupts neurons** and might have adverse effects on behavior.

4. **Obesity:** In China, increased MSG intake has been linked to **weight gain** — with average intake ranging from 0.33–2.2 grams per day. MSG intake causes a disrupted energy balance by increasing the palatability of food and disturbing the leptin-mediated hypothalamus signalling cascade, potentially leading to obesity.

5. **Liver damage:** MSG induces a significant decrease in **liver transaminases indicating hepatic damage.** This damage was likely the result of non-alcoholic steatohepatitis which is associated with long lasting inflammation. MSG triggers micro-RNA (mRNA) expression of interleukin-6 (IL-6) and tumour necrosis factor-alpha (TNF-α).

6. **Reproductive damage:** Both animal models and human studies have shown toxic effect of MSG on the reproductive system. Administration of MSG at a dose of 2 mg/g during various perinatal periods of life leads to disruption primary **spermatocytes**. MSG also causes disruption of stroma cell vacuolations and basement membrane and cellular hypertrophy of the theca **folliculi in the ovaries**.

ALUMINIUM FOIL

Aluminum foil is a common household product that's often used in cooking. **Aluminum foil, or tin foil, is a paper-thin, shiny sheet of aluminum metal. It's made by rolling large slabs of aluminum until they are less than 0.2 mm thick.**

It's used industrially for a variety of purposes, including **packing, insulation and transportation**. At home, people use aluminum foil for **food storage, to cover baking surfaces and to wrap foods,** such as meats, to prevent them from losing moisture while cooking.

These food preparation and practice can expose consumers to different aluminum amounts in dependence to food types and composition. **This interaction between aluminum foil and food wrapped in it represents a potential hazardous source of aluminum in the human diet**. For example, one study found that cooking red meat in aluminum foil could increase its aluminum content by between 89% and 378%. Leaching of aluminum compounds was highest when acidic foods such as lemon juice or tomatoes came into contact with aluminum foil.

Pathophysiological effects:

1. Dietary aluminum has been suggested as a potential factor in the development of Alzheimer's disease**. Alzheimer's disease** is a neurological condition caused by a loss of brain cells. People with the condition experience memory loss and a reduction in brain function. Aluminum is diversely affecting the growth rate of human brain cells. Aluminum is also considered to be a **neurotoxin.**

2. In addition to its potential role in brain disease, a handful of studies have suggested that dietary aluminum could be an environmental risk factor **for inflammatory bowel disease (IBD).**

3. Aluminum salts can be accumulated by the gut and different human tissues (bones, parathyroid, and brain). Aluminum either directly or indirectly impacts osteoblast production which leads to **bone wasting**. It predisposes us to **osteoporosis.**

4. Aluminum causes **skin pigmentation** and eczema if used for long.
5. People who have kidney disease may be more vulnerable to aluminium toxicity.

CHICORY

1. Chicory is widely used in India and Africa to **substitute and adulterate coffee.** Chicory root is derived from the root of the ***Cichorium intybus*** plant which has been cultivated since ancient Egypt.
2. It is a biennial crop native to Europe (Mediterranean). It was used as a western herbal medicine and discontinued due its side effects. Chicory became popular in 18th century France when coffee was scarce during the Napoleonic wars. It was used as an additive or adulterant to overcome the shortage of coffee.
3. Chicory is not coffee. Nor does it come from a coffee bean. According to Word Web it is "the root of the chicory plant, roasted and ground to substitute for or adulterate coffee". Chicory costs only one fourth the price of Arabica coffee and does not contain any caffeine. Chicory only mimics a bitter coffee taste. Roasted chicory contains none of the volatile oils and aromatics that are contained in roasted coffee.

Tests for identification:

Tumbler test: Take a transparent glass. Fill it with water. Drop a pinch of coffee powder on it gently. If the powder floats for some time before sinking, it is coffee. If the powder sinks quickly, it is chicory or some other seed. If it readily diffuses brownish or yellowish colour, it contains Caramel or Chicory.

Pathophysiological effects:

1. **On CNS:** The two components of chicory that cause the bitter taste are lactucin and lactacoprin. These two components affect the central nervous system by relaxing it.

2. On female reproductive test: Chicory may stimulate the uterus and in turn result in menstruation, which could risk abortion in a pregnant woman. It is advised to avoid using chicory root during pregnancy and breastfeeding since it has stimulatory effects on menstruation.

3. On skin: According to the Ohio State University, there have been reports of contact dermatitis when handling Chicory. Contact dermatitis can involve a wide spectrum of side effects like inflammation of the skin. Handling or consuming Chicory root extract may result in hives, intensive itching, swelling, wheezing, dizziness, pale skin or loss of consciousness.

4. On GI system: Chicory root extract and chicory seed are possibly safe for most adults when taken by mouth in medicinal amounts and short-term. Taking chicory by mouth might cause minor GI side effects including gas, bloating, abdominal pain, and belching. The Coffee Board of India has wisely persuaded the Food Safety Standards Authority of India (FSSAI) to reduce the permitted chicory mix content in coffee from 50% to 30

MARGARINE

1. Margarine is a processed food that is designed to **taste and look similar to butter**. It is often recommended as a heart-healthy replacement.

2. Modern types of margarine are made from **vegetable oils,** which contain polyunsaturated fats that can lower the 'bad' cholesterol (LDL) when used instead of saturated fat. Since vegetable oils are liquid at room temperature, their chemical structure are modified to make them **solid like butter**. For the past few decades, a process known as **hydrogenation** has been used to **harden the vegetable oils in margarine**. Hydrogenation increases the oil's saturated fat content, but unhealthy **'trans' fats** are formed as a side product.

3. However, a more recent process called **interesterification** achieves similar results **without forming any 'trans fats'**. In addition to hydrogenated or interesterified vegetable oils, modern margarine may contain **several food additives**, including emulsifiers and colorants. Put simply, modern margarine is a **highly processed food** product made from vegetable oils, while butter is basically concentrated dairy fat.


Or
Butter
Margarine

BUTTER

VERSUS

MARGARINE

BUTTER	MARGARINE
Butter is a dairy fat made by churning the cream of milk from cows	Margarine is a substitute for butter made from vegetable oil
Made of dairy fat	Made of vegetable oil
Involves churning and separation	Involves hydrogenation
More natural	Highly processed
Rich in saturated fats	Rich in trans fat
Has a soft, creamy and rich taste	Does not have the rich taste of butter

Tests for identification:

Take about one teaspoon full of melted sample of **butter** with equal quantity of concentrated **Hydrochloric acid** in a stoppered test tube and add to it a **pinch of sugar**. Shake for one minute and let it stand for five minutes. Appearance of **crimson colour** in **lower layer (acid) indicates Vanaspati or Margarine.**

(Note: The test is specific for sesame oil which is compulsorily added to Vanaspati and Margarine. Some coal tar colours also give a positive test.

- If the test is positive i.e. red colour develops only by adding strong Hydrochloric acid (without adding crystals of sugar) then the sample is adulterated with **coal tar dye.**
- If the crimson or red colour develops after adding and shaking with sugar, then alone Vanaspati or **Margarine** is present).

Pathophysiological effects:

1. **High level of trans fat**: Although margarine may contain some good nutrients, it often contains **'trans' fat**, which has been associated with an **increased risk of heart disease and other chronic health issues** Hydrogenation involves exposing the oils to high heat, high pressure, hydrogen gas and a metal catalyst. Hydrogenation changes some of the unsaturated fat into saturated fat, which is solid at room temperature, and also increases the product's shelf life. Unfortunately, 'trans' fat is formed as a side product. A high intake of industrial 'trans' fat has been linked to an increased risk of chronic disease.

2. **High level of omega-6 fatty acid**. Omega-3 fats are considered anti-inflammatory, meaning they act against inflammation. Conversely, eating too much omega-6 fat may promote chronic inflammation. Based on ancestral diets, **the optimal ratio of omega-6 to omega-3 is estimated to be around 1:1**. **People who eat margarine are eating far too much omega-6 fat today. In fact, the ratio is estimated to be as high as 20:1 in developed countries.** Observational studies have linked a high intake of omega-6 fat to an increased risk of obesity and chronic diseases, such as heart disease and inflammatory bowel disease.

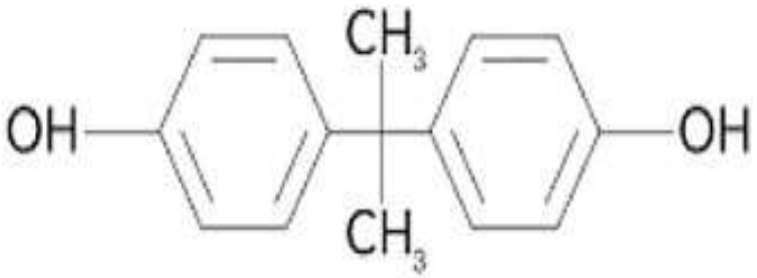
BISPHENOL A

1. Bisphenol A (BPA) is a chemical produced in large quantities for use primarily in the production of **polycarbonate plastics and epoxy resins**. Polycarbonate plastics have many applications including use in some food and drink packaging, e.g., **water and infant bottles, compact discs, impact-resistant safety equipment, and medical devices.** Epoxy resins are used as lacquers to coat metal products such as **food cans, bottle tops, and water supply pipes.** It is one of the highest volume chemicals produced worldwide, with over 6 billion pounds produced each year.

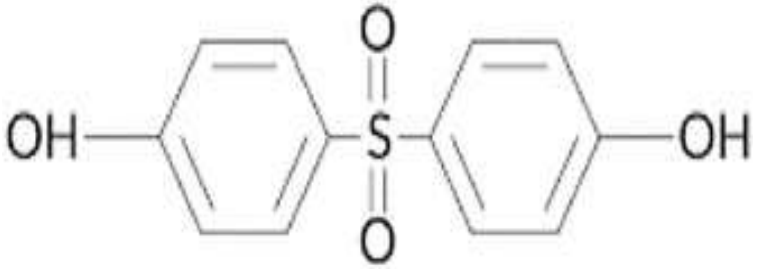
2. The primary source of exposure to BPA for most people is through the **diet**. While air, dust, and water are other possible sources of exposure, **BPA in food and beverages accounts for the majority of daily human exposure**. Bisphenol A can leach into food from the protective internal epoxy resin coatings of canned foods and from consumer products such as polycarbonate tableware, food storage containers, water bottles, and baby bottles.

3. The degree to which BPA leaches from polycarbonate bottles into liquid may depend more on the temperature of the liquid or bottle, than the age of the container.

Bisphenol A



Bisphenol S



BPA May Be Lurking In:

Canned foods

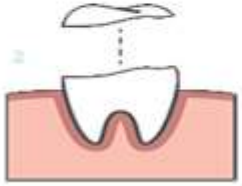
Toiletries

Feminine hygiene products

Items packaged in plastic containers

Household electronics

Thermal printer receipts



Dental filling sealants

Sports equipment

PATHOPHYSIOLOGICAL EFFECTS:

1. **Endocrine disruptor**: BPA is an endocrine disruptor. The Environmental Protection Agency (EPA) notes that BPA can **imitate the body's hormones** and interfere with the production of, response to, or action of natural hormones. For example, it can behave in a similar way to estrogen and other hormones in the human body. BPA is well known to have **estrogenic effects**. Specifically, it is known to bind to both types of nuclear estrogen receptors (ER), ERα and ERβ.

2. **On reproductive system**: In 2013, scientists published study showing that BPA exposure can affect egg maturation in humans. BPA can affect puberty and ovulation and may lead to infertility. They add that the impact may be **"lifelong and transgenerational."** In males, the endocrine-disrupting activity of BPA has been found to have several significant consequences, including changes in sex steroid concentrations, sexual function, and spermatogenesis.

3. **On cardiovascular system:** Research in humans has linked even low dose BPA exposure to cardiovascular problems, including coronary artery heart disease, angina, heart attack, hypertension, and peripheral artery disease. It can trigger arrhythmias, atherosclerosis, and blood pressure changes.

4. On diabetes: There is evidence that human exposure to BPA may contribute to type 2 diabetes by impacting insulin resistance, weight gain and metabolic syndrome. BPA may aggravate or raise the risk of glucose intolerance.

5. On nervous system: Environmental exposure to BPA has the potential to affect the **developing brain during gestation**, according to research. This could have effects on social behavior and anxiety after birth. It is in the evolving brain that variations in the estrogenic environment affect several features of cellular reproduction, together with neuritis flexibility and branching, synaptic development, expression of neurotransmitters, cell survival, and death.

6. On breast cancer: BPA, with its estrogen-like behavior, could increase the risk of breast, prostate, and other cancers in people who experienced exposure to the chemical in the womb. In 2015, a group of researchers concluded that exposure to BPA before birth could have long-term effects on carcinogenesis in certain organs. This in turn could lead to the development of hormone-related cancers. In 2009, scientists reported that BPA could interfere with the effectiveness of chemotherapy in breast cancer treatment.

BISPHENOL S

1. **Bisphenol S** (BPS) is an industrial chemical which is recently used to **replace the potentially toxic Bisphenol A (BPA)** in making polycarbonate **plastics, epoxy resins and thermal receipt papers.**

2. These BPA-free plastics are made using **bisphenol analogs** with very similar structural and chemical properties. **BPS is the most common bisphenol analog marketed as a BPA-free product.**

3. Bisphenol S has been the most studied of the bisphenol analogues and is the most common substitute for BPA. The reasoning behind the substitution of BPS for BPA was that **BPS was less likely to leach monomers into food and drink since BPS is generally more tolerant to heat and is more photo-resistant than BPA.**

4. Mainly, exposure to BPA and analogues such as BPS comes from **microwaving food in plastic containers made from these materials, from using plastic bowls and cups that are worn out** and may be leaching monomers, or even from tap water in areas where bisphenols were used to coat the **inside of water pipes.**

PATHOPHYSIOLOGICAL EFFECTS

1. **On reproductive system:** Research has documented a PCOS-like condition after exposure to BPS. Polycystic Ovary Syndrome **(PCOS)** has been known to cause infertility in humans. Other showed that BPS, BPA, and BPE all caused problems with the follicular development of mice. There was a significant decrease in the rate of pregnancy, a decrease in the number of live births and more problems associated with giving birth to offspring. Finally, there was another study that reported decreased sperm counts, sperm motility, and spermatogenesis. BPS was tied with BPA for being the **second most spermatotoxic bisphenol analogue**.

2. **Obesity:** Most articles have reported a correlation between all bisphenols and increased body weight. The study went on to propose that BPS is causing lipid accumulation and promoting the **differentiation of pre-adipose cells** via the peroxisome proliferator-activated receptor-γ (PPARG) pathway.

3. **Breast cancer:** Bisphenol compounds promote hormone-dependent breast cancer in varying degrees. All of the phenolic compounds, including BPS and BPA, not only increased the expression of breast carcinogen, but that they also increased the proliferation of ERα positive breast cancer cells.

4. DNA damage: All bisphenols caused **oxidative damage to both purines and pyrimidines**, but purines were most strongly affected. BPS caused the least damage to DNA bases. In addition, BPS was more likely than BPA to cause abnormalities in the formation of spindle assemblies and to cause **chromosomal misalignments** in bovine oocytes.

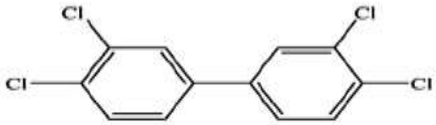
5. On blood: BPS **lowers the number of circulating RBCs and depressing the synthesis of hemoglobin.** This may be due to the inhibition of RBC formation (erythropoiesis) from the committed stem cells of the bone marrow and/or promotion of haemolysis**.** Which might be further due to the inhibition on the secretion of erythropoietin, promotion of the inactivation of erythropoietin at the liver and/or inhibition of the erythropoietin signaling in the committed stem cells by the BPS. Scientists reported that **BPA** which has been previously used in the industries before the substitution by BPS induces **oxidative damage of the bone marrow**. BPS also **decreases total WBC count** in the BPS exposed groups of rats comparing to the control.


BPA
FREE

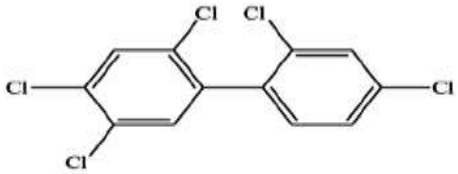
POLYCHLORINATED BIPHENYLS (PCB)

Polychlorinated biphenyls are a group of 209 different chemicals which share a common structure but vary in the number of attached chlorine atoms. PCBs are **synthetic chlorinated hydrocarbon compounds** that consist of two benzene rings linked by a single carbon-carbon bond, with from 1 to all 10 of the hydrogen atoms replaced with chlorines. PCBs have been produced commercially since **1929.**

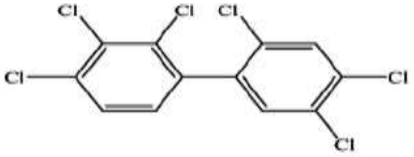
PCB77



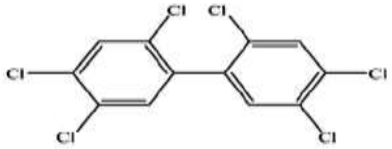
PCB99



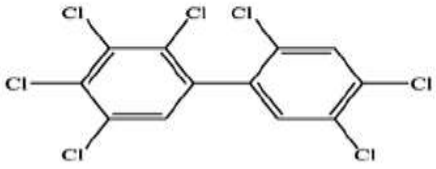
PCB138



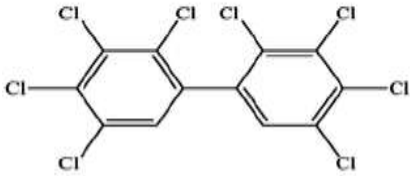
PCB153



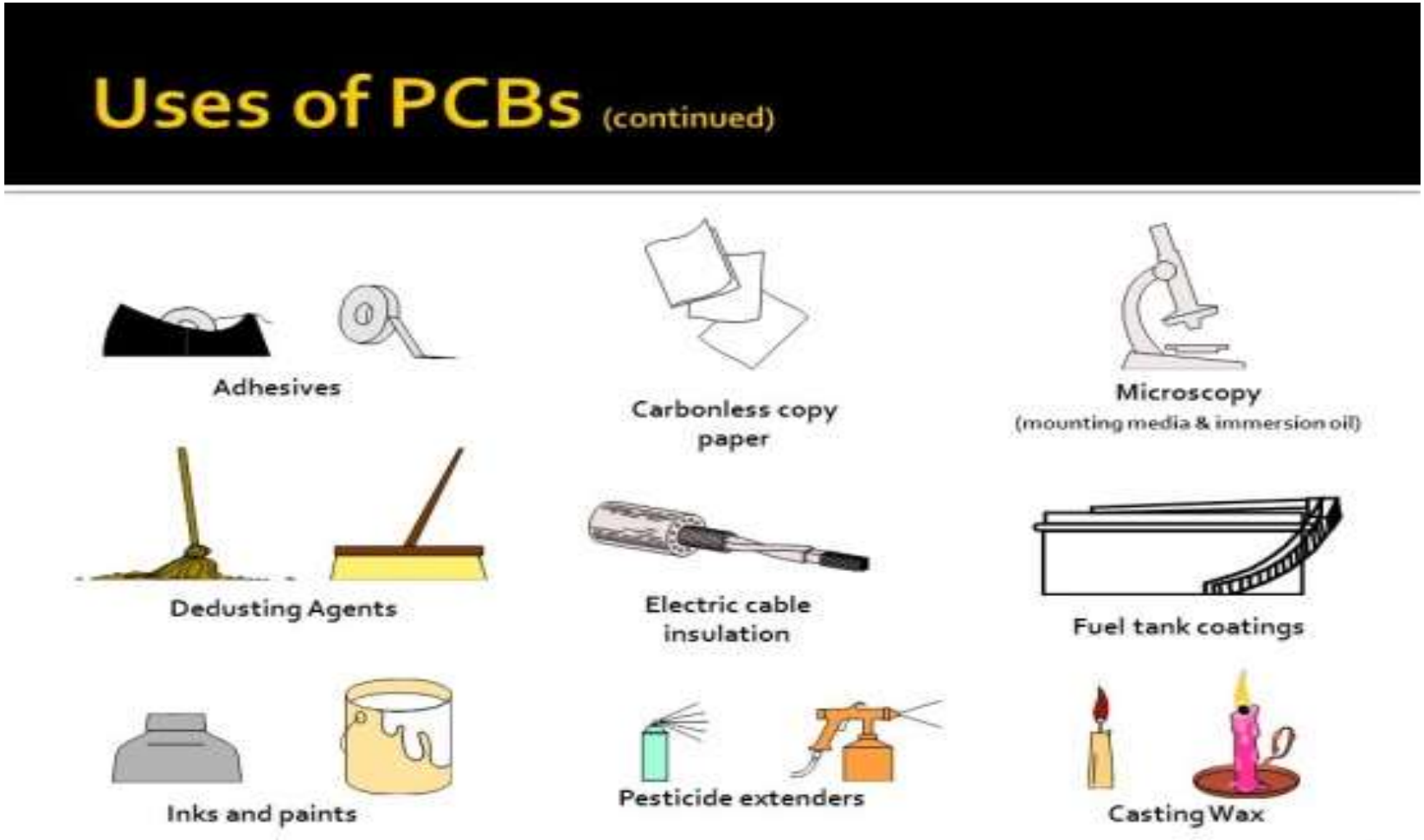
PCB180



PCB194



Uses: They have been used in **plasticizers, surface coatings, inks, adhesives, flame retardants, pesticide extenders, paints**, and microencapsulation of dyes for carbonless duplicating paper. Because PCBs resist both acids and alkalis and are relatively heat-stable, they have been used in dielectric fluids in **transformers and capacitors**. Further environmental contamination may occur from the disposal of **old electrical equipment** containing PCBs.



Applications of PCBs

Electrical

- Capacitors & Transformers 
- Voltage regulators 
- Bushings & Electromagnet 
- Cable Insulations 
- Fluorescent Bulbs 

Construction

- Plasticizer 
- Window glazing 
- Sealants & finishing on floor 
- Caulking and grout in floor and joints 

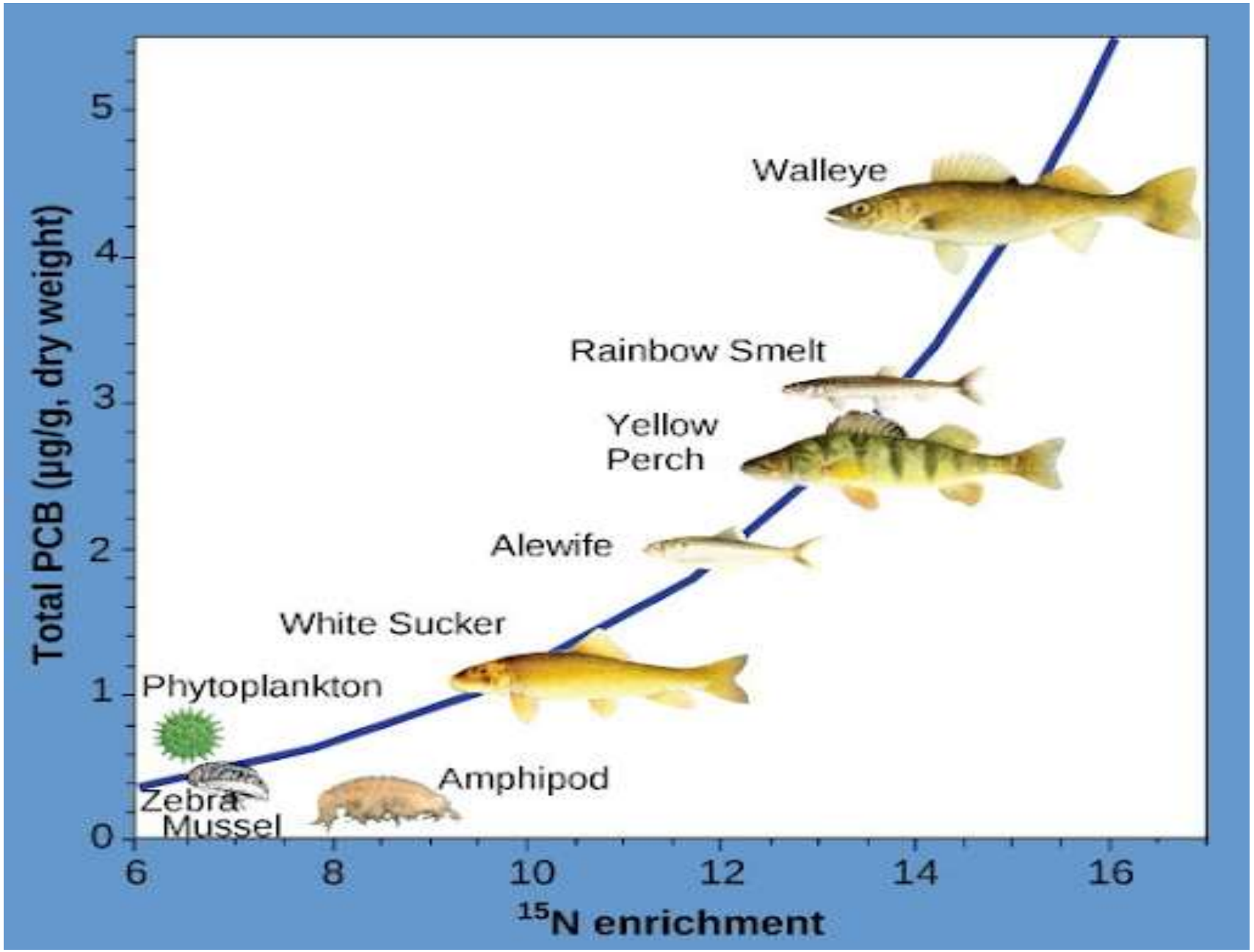
Industrial

- Paints 
- Printing Inks and Carbon less paper 
- Clothing pigments & dyes 
- Pesticides 
- Plastic water and baby bottles 

Exposure: Overall, humans are mainly exposed through consumption of **contaminated foods, particularly meat, fish, and poultry**. PCBs can enter human cells and tissues when **contaminated air is breathed in**, when **contaminated food** enters the digestive system, or through **contact with the skin**. Once in the gastrointestinal tract, ingested PCBs diffuse across cell membranes and enter blood vessels and the lymphatic system. PCBs, especially those that contain a greater number of chlorine atoms, are **readily soluble in fats** and thus tend to **accumulate in fat-rich tissues** such as the liver, brain and skin.

Bioaccumulation: **PCBs accumulate in the food-chain**. Once PCBs enter a person´s (or animal´s) body, they tend to be absorbed into fat tissue and remain there. Unlike water-soluble chemicals, they are not excreted, so the **body accumulates PCBs over years**. A small fish may absorb PCBs in water or by eating plankton, and these PCBs are stored in its body fat. When a larger fish eats the small fish, it also eats and absorbs all the PCBs that have built up in the small fish. **In this way, larger fish and animals can build up a highly concentrated store of PCBs**. Some types of PCBs may degrade into nontoxic form while they are stored in the body, but this process can take many years.

In the same way, **PCBs accumulate in women** and **pass on to their infants through breast milk.** This accumulation means that nursing infants may ingest PCB levels much higher than the levels in fish and other foods consumed by their mothers.


Total PCB (µg/g, dry weight)
0
1
2
3
4
5
6
8
10
12
14
16
15N enrichment
Walleye
Rainbow Smelt
Yellow Perch
Alewife
White Sucker
Phytoplankton
Amphipod
Zebra Mussel

Pathophysiological effects:.

1. Carcinogenicity: The International Agency for Research on Cancer and the Environmental Protection Agency classify PCBs as a **probable human carcinogen.** Epidemiological studies suggest exposure-related increases in cancers of the digestive system, especially **liver cancer, and malignant melanoma**.

2. Skin problems: Irritation of the nose and lungs, skin irritations such as severe acne (chloracne) and rashes, and eye problems. Characteristic skin changes included marked **enlargement, elevation, and keratotic plugging of follicular orifices, comedo formation, acneiform eruptions, hyperpigmentation, hyperkeratosis, and deformed nails**. Dark-coloured pigmentation frequently occurred in the gingival and buccal mucosa, lips, and nails and improved only gradually in most patients.

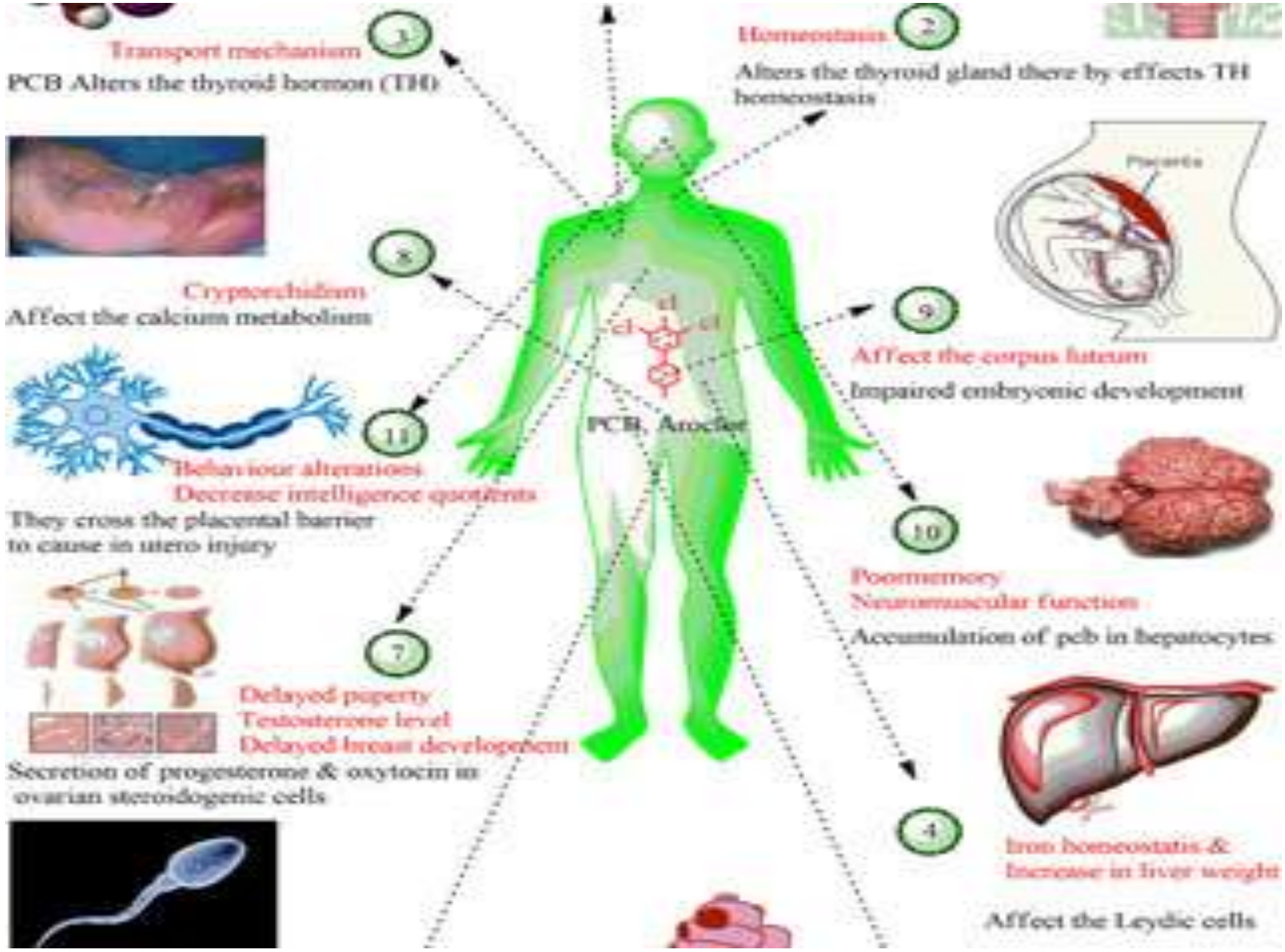
3. Developmental effects: Women exposed to PCBs before or during pregnancy can give birth to children with significant **neurological and motor control problems, including lowered IQ and poor short-term memory, decreased birth weight and head size,** lowered performance on standardized **memory**, psychomotor and behavioral tests. These effects lasted through at least 7 years.

4. Neurological effects: Symptoms such as **headache, dizziness, depression, fatigue, and a tingling sensation in the hands.** Persons regularly consuming fish from waters contaminated with PCBs performed poorly on tests that required **cognitive abilit**y, word naming, auditory recall, and complex motor tasks. Adult victims of the Yusho and Yu-Cheng incidents showed weakness, and neuralgia of limbs, hypoesthesia. Exposure also caused a reduction in sensory nerve and motor nerve conduction velocities.

5.Endocrine disruptor: PCBs with only a few chlorine atoms can mimic the body´s natural hormones, especially **estrogen**. Women who consumed PCB-contaminated fish from Lake Ontario were found to have **shortened menstrual cycles. PCBs are also thought to play a role in reduced sperm counts, altered sex organs, premature puberty,** and changed sex ratios of children. High PCB levels in adults have been shown to result in reduced levels of the thyroid hormone triiodothyronine,

6.Yusho disease: In **Japan in 1968**, 280 kg of PCB-contaminated rice bran oil was used as chicken feed, resulting in a **mass poisoning**, known as Yushō disease, in over 1800 people. Common symptoms included dermal and ocular lesions, irregular menstrual cycles and lowered immune responses.

7.Immunological deficits: Changes in different circulating lymphocyte types, decreased numbers of natural killer cells have been observed prevalence of recurrent middle-ear infections and chicken pox was related to plasma PCB concentrations in 3.5-year-old children.


Transport mechanism
PCB Alters the thyroid hormon (TH)
Homeostasis
Alters the thyroid gland there by effects TH homeostasis
Placenta
Cryptorchidism
Affect the calcium metabolism
Affect the corpus luteum
Impaired embryonic development
PCB, Anochor
Behaviour alterations
Decrease intelligence quotients
They cross the placental barrier
to cause in utero injury
Poormemory
Neuromuscular function
Accumulation of pcb in hepatocytes
Delayed puperty
Testosterone level
Delayed breast developement
Secretion of progesterone & oxytocin in
ovarian steroidogenic cells
Iron homeostatis &
Increase in liver weight
Affect the Leydic cells

DIOXIN

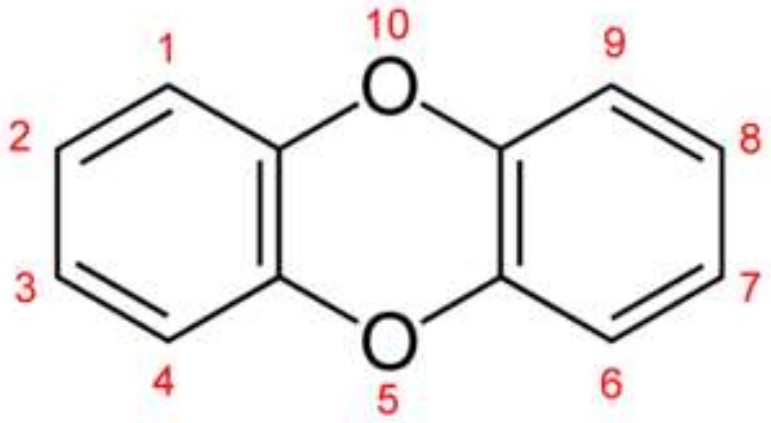
Dioxins and furans are some of the **most toxic chemicals known to science**. A draft report released for public comment in September 1994 by the US Environmental Protection Agency clearly describes dioxin as a **serious public health threat**. The public health impact of dioxin may rival the impact that DDT had on public health in the 1960‘s

Dioxin is a general term that describes a group of hundreds of chemicals that are **highly persistent in the environment**. Several hundred of these chemicals exist and are members of three closely related families-

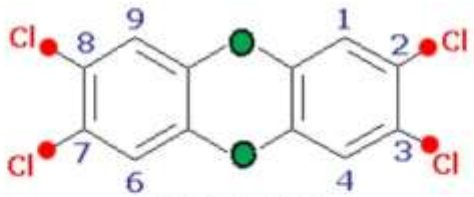
Polychlorinated dibenzo-p-dioxins (PCDDs),
Polychlorinated dibenzofurans (PCDFs) and
Certain polychlorinated biphenyls (PCBs).

Although hundreds of PCDDs, PCDFs, and PCBs exist, **only some are toxic.** Counting around the carbon rings, **those with chlorines at positions 2, 3, 7, and 8 are toxic**.

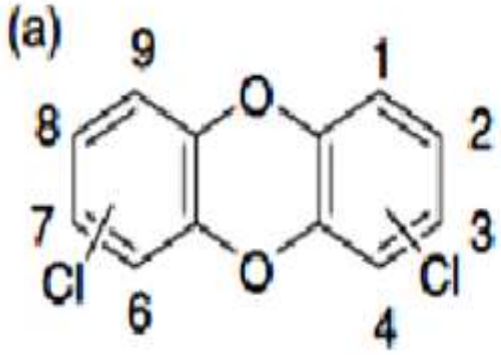
The **most toxic compound** is 2, 3, 7, 8-tetrachloro dibenzo-p-dioxin or **TCDD**. The toxicity of other dioxins and chemicals like PCBs that act like dioxin are measured in relation to TCDD.



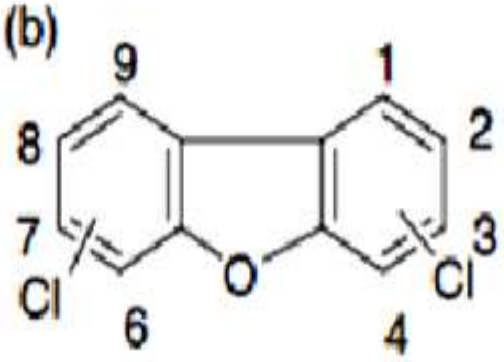
Molecular structure of dioxin



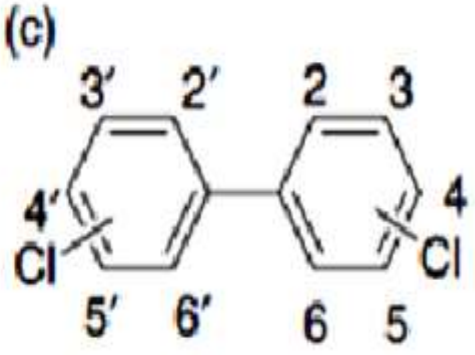
2,3,7,8-TCDD



PCDDs



PCDFs

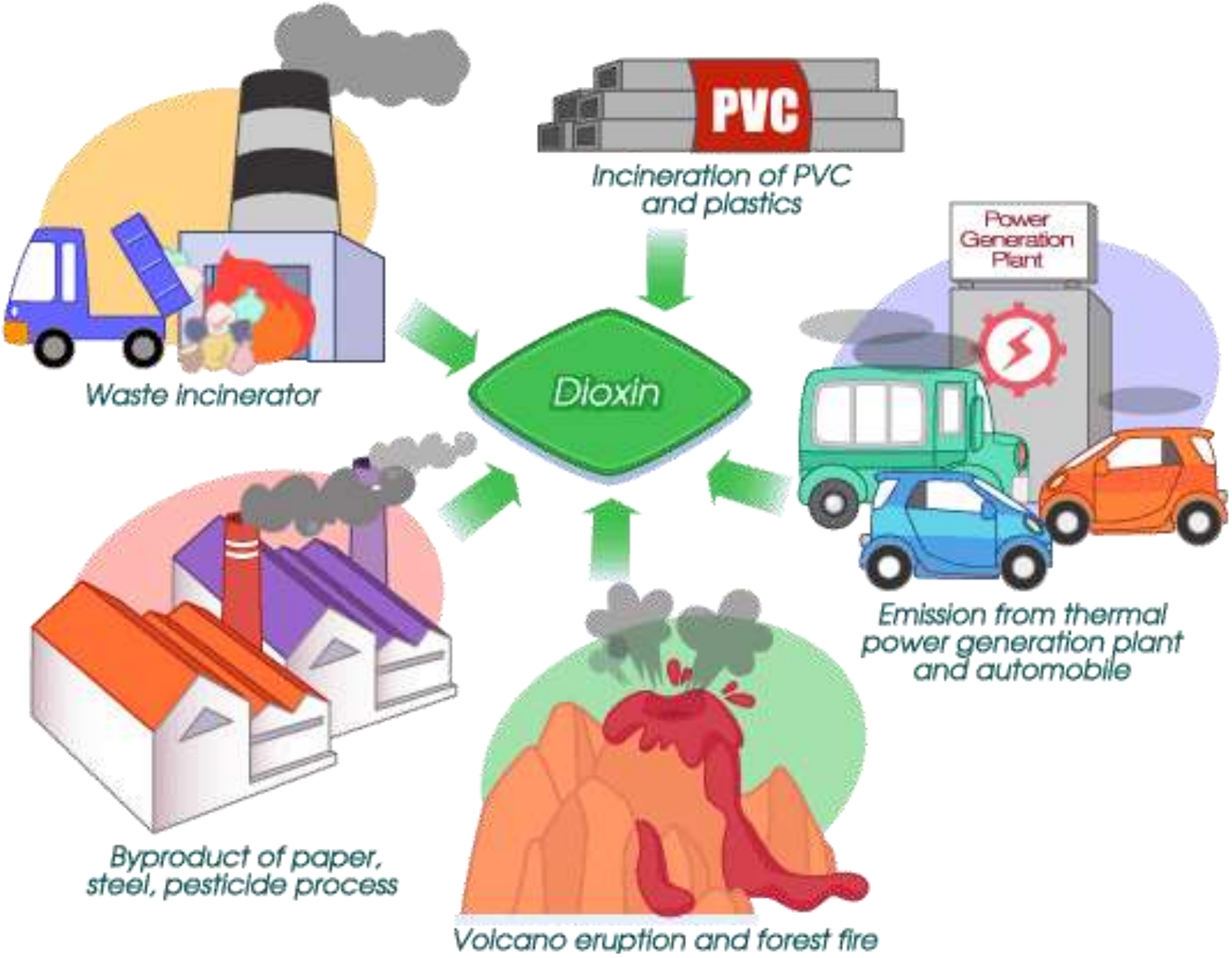


PCBs

SOURCE:

1. Industrial activities: It is a contaminant formed during the production of some chlorinated organic compounds, including a few **herbicides** such as Silvex. Dioxins are extremely persistent compounds and break down very slowly.
2. Burning: Combustion processes such as **waste incineration** (commercial or municipal) or burning fuels (like wood, coal or oil) form dioxins.
3. Bleaching: Chlorine bleaching of **pulp and paper** and other industrial processes can create small quantities of dioxins in the environment.
4. Smoking: Cigarette smoke also contains small amounts of dioxins.
5. Drinking Water: Dioxin can get into drinking water from: Air emissions from waste incineration and other combustion, with subsequent deposition to lakes and reservoirs.

6. Diet: The major sources of dioxin are in our diet. Since dioxin is **fat-soluble**, **it bioaccumulates, climbing up the food chain**. A North American eating a typical North American diet will receive 93% of their dioxin exposure from **meat and dairy products** (23% is from milk and dairy alone; the other large sources of exposure are beef, fish, pork, poultry and eggs). In fish, these toxins bioaccumulate up the food chain so that **dioxin levels in fish are 100,000 times** that of the surrounding environment. (The best way to avoid dioxin exposure is to **reduce or eliminate your consumption of meat and dairy products by adopting a vegan diet**.)
7. Natural processes: Such as volcanic eruptions and forest fires.


PVC
Incineration of PVC and plastics
Waste incinerator
Dioxin
Power Generation Plant
Emission from thermal power generation plant and automobile
Byproduct of paper, steel, pesticide process
Volcano eruption and forest fire

HISTORY OF CONTAMINATION:

1. In **2008**, contaminated animal feed led to pork products from **Ireland** containing over 200 times the permitted levels of dioxins.

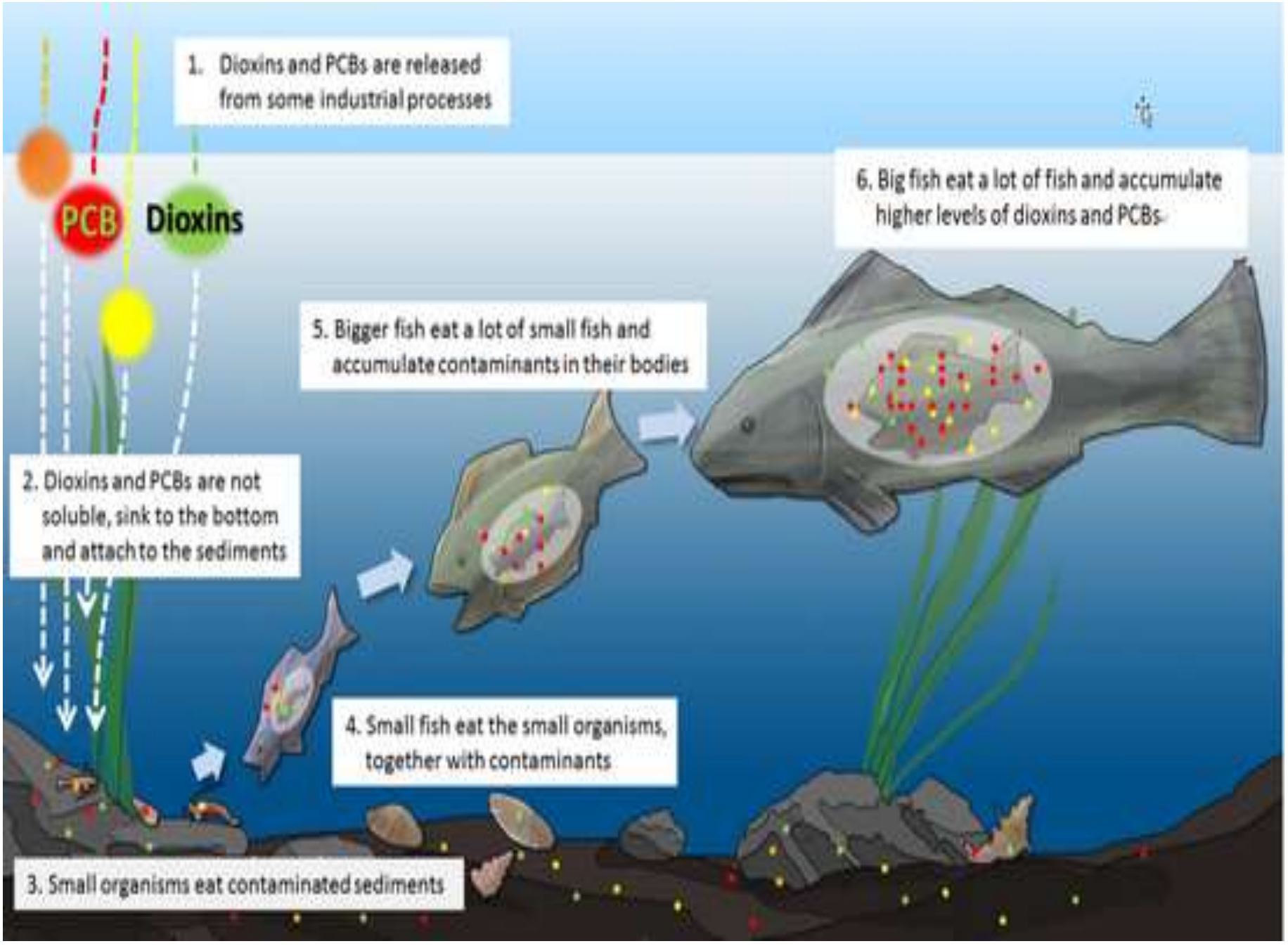
2. In **1999**, illegal disposal of an industrial oil caused animal feed and animal-based food products from **Belgium** and some other countries to be contaminated.

3. In **1976**, an industrial accident led to a cloud of toxic chemicals, including dioxins, affecting thousands of people in **Italy**.

4. In **2004**, Viktor Yushchenko, President of the **Ukraine**, was intentionally poisoned with dioxins.

BIOACCUMULATION:

Dioxins are called **persistent organic pollutants (POPs)**, meaning **they take a long time to break down once they are in the environment.** Dioxins are found throughout the world in the environment, and they **accumulate in food chains**, concentrating mainly in the fatty tissue of animals. It crosses the placenta into the growing infant and It is present in the fatty breast milk, which is also a route of exposure.


1. Dioxins and PCBs are released from some industrial processes
PCB
Dioxins
6. Big fish eat a lot of fish and accumulate higher levels of dioxins and PCBs
5. Bigger fish eat a lot of small fish and accumulate contaminants in their bodies
2. Dioxins and PCBs are not soluble, sink to the bottom and attach to the sediments
4. Small fish eat the small organisms, together with contaminants
3. Small organisms eat contaminated sediments

Spreading of Dioxin

- Biomagnification: The concentration of dioxin increases as you go up the food chain.
- Bioaccumulation: Dioxin accumulates in the animal's body and milk supply.
- Dioxin can then be spread to an animal's offspring through their milk or even the placenta.

PATHOPHYSIOLOGICAL EFFECTS :

1. **Carcinogenicity:** Dioxins are **well established carcinogens** in animal studies, although the precise mechanism is not clear. Dioxins are not mutagenic or genotoxic. The International Agency for Research on Cancer has classified **TCDD as a human carcinogen (class 1)** on the basis of clear animal carcinogenicity and subsequently also 2,3,4,7,8-PCDF and PCB 126 as class 1 carcinogens. The mechanism is thought to be mainly promotion, i.e. **dioxins can accelerate the formation of tumours caused by other factors, and adversely affect the normal mechanisms for inhibiting tumour growth**. Some researchers have also proposed that dioxin **induces cancer progression** through a very different mitochondrial pathway.

2. **Developmental problems:** Dioxin causes disturbances of tooth development and of sexual development in infant.
3. **Skin Problems:** Short-term exposure of humans to high levels of dioxins may result in skin lesions, such as chloracne and patchy darkening of the skin.
4. **Immune dysfunction:** Long-term exposure is linked to impairment of the immune system.
5. **Endocrine disruption:** It causes disruption of thyroid and pancreatic function.
6. **Reproductive damage:** Dioxin causes inability to maintain pregnancy, decreased fertility, reduced sperm count, endometriosis and lowered testosterone levels.

Effects of dioxins on human health

18

Short-term exposure

- Skin lesions, such as **chloracne**
- Patchy darkening of the skin
- Altered liver function
- Dermatitis
- Gastrointestinal problems.

19

- **Long term exposure** - effects on
  - Immune system
  - Endocrine system
  - Reproductive system
  - Nervous system
  - Cardiovascular system

20

Long-term exposure – effects on

- **Immune system**
  - Dioxin directly reduces the number of B-cells(immune cells that develop in bone marrow, then circulate throughout the blood and lymph, fighting off invaders) and it reduces number of T-cells(immune cells that develop in thymus)
  - EPA concludes that the even low doses dioxin attack the immune system.

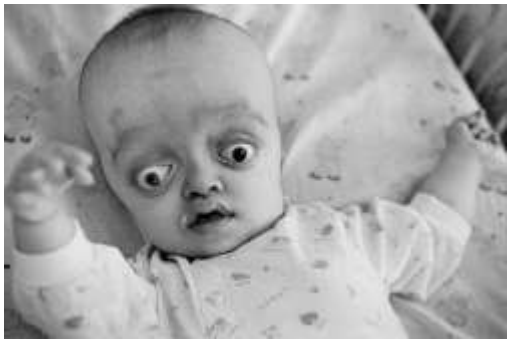
22

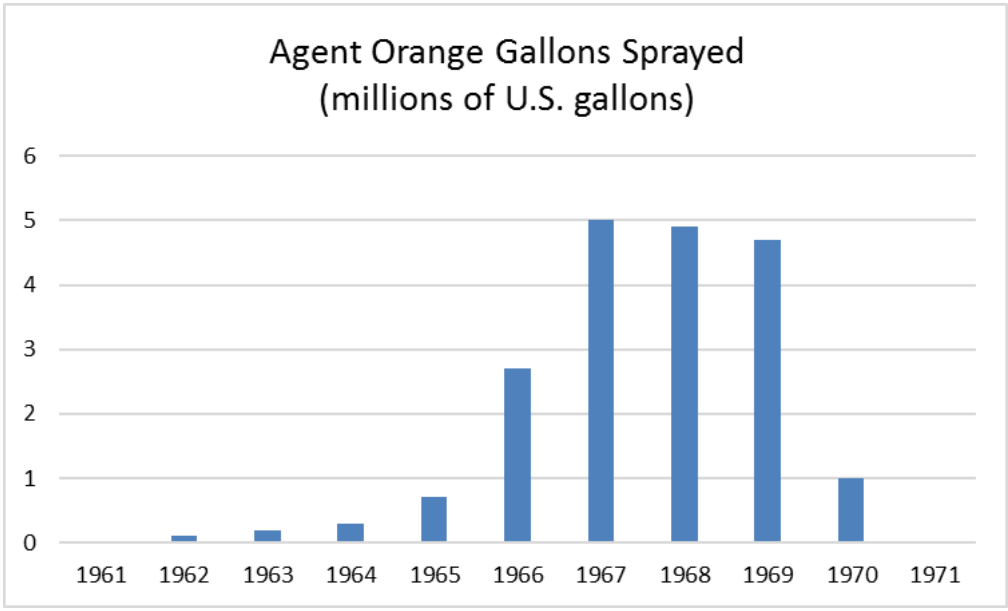
- **Reproductive system** –
  - Decrease in sperm production by almost 50%
  - Lower Infertility among young men
  - Increased risk of Cryptochidism
  - Decrease in testosterone (a male sex hormone that is important for sexual and reproductive development)
- **Nervous system** –
  - Causes nerve damage, birth defects
  - Delay in nervous system development

In **2004**, Viktor Yushchenko, President of the **Ukraine**, was intentionally poisoned with dioxins.

Major incidents

1. Agent Orange in Vietnam: TCDD, 1962 - 1971
2. Times Beach, USA: TCDD, 1970-1972
3. Binghamton, USA: Transformer fire. PCB, PCDF/D 1981
4. Seveso, Italy, TCDD: 1976
5. Yusho rice oil poisoning, Japan: PCB, PCDF, 1968
6. Yucheng rice oil poisoning, Taiwan: PCB, PCDF, 1976.
7. Belgium “dioxin” food poisoning: PCB/PCDF. 1990s.





AGENT ORANGE (TCDD) IN VIETNAM WAR

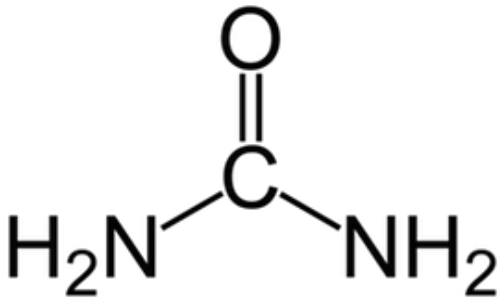
UREA

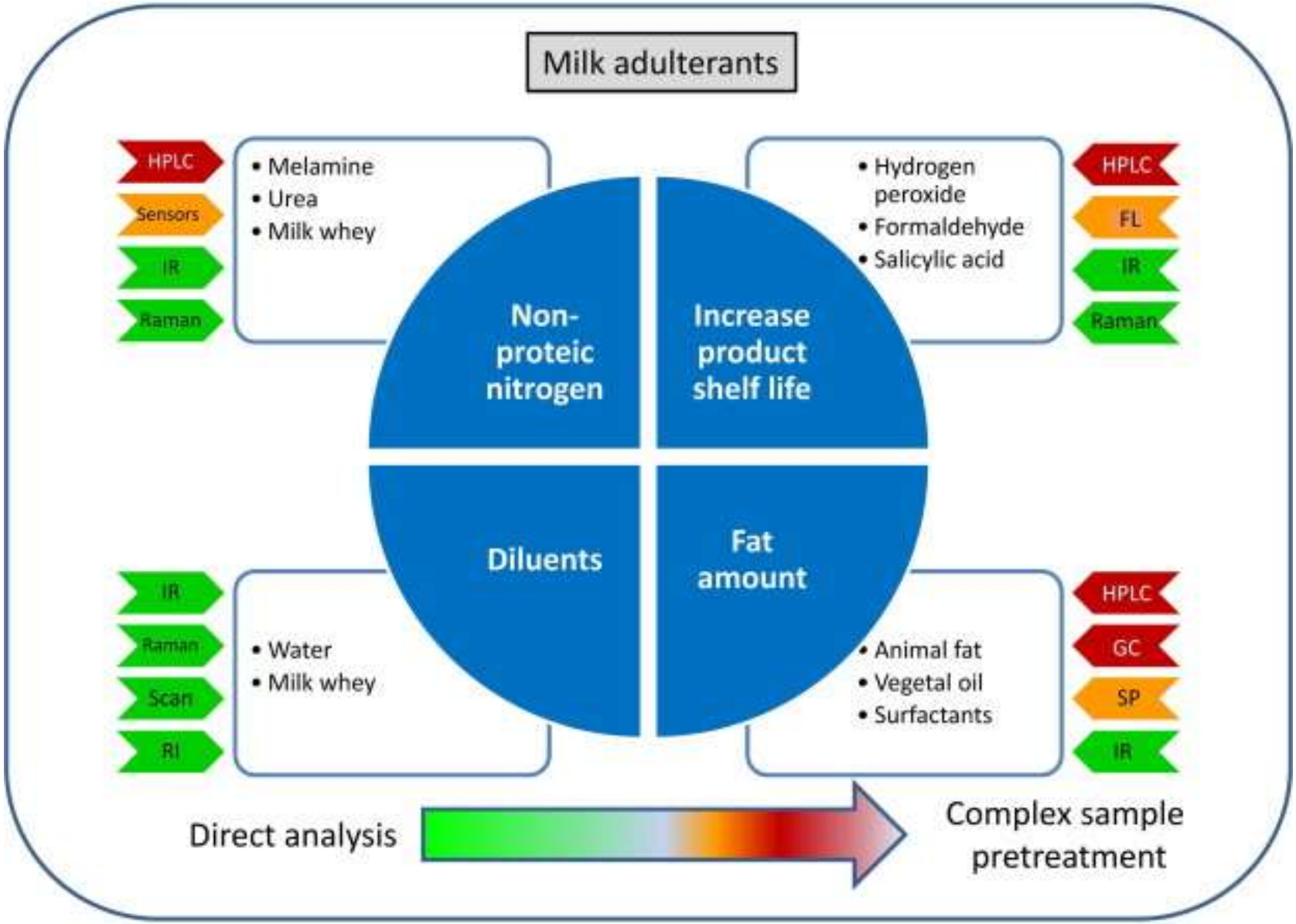
Urea, also called carbamide, the **diamide of carbonic acid**. Its formula is H_2NCONH_2. Urea has important uses as a **fertilizer and feed supplement**, as well as a starting material for the manufacture of **plastics and drugs**. It is a colourless, crystalline substance that melts at 132.7° C (271° F) and decomposes before boiling.

Use of urea as food additive:

Adulterants are added in **milk** to increase the milk quality in dishonest way. For example, cane sugar, starch, sulfate salts, urea and common salts are added to **increase solid-not-fat (SNF) in milk. Commercial urea is added to milk to increase non-protein nitrogen content and increase the shelf life of the milk**. Urea, being a natural constituent of milk, constitutes the major portion of non-protein nitrogen in milk.

According to FSSAI act 2006 and PFA rules 1955, **maximum allowable limit for urea in milk is 70 mg/100 mL.** Milk can be adulterated with urea in two ways – by intentional addition of urea and by addition of unspecified synthetic milk to natural milk.




Milk adulterants
HPLC
Sensors
IR
Raman
• Melamine
• Urea
• Milk whey
Non-proteic nitrogen
• Hydrogen peroxide
• Formaldehyde
• Salicylic acid
HPLC
FL
IR
Raman
Increase product shelf life
IR
Raman
Scan
RI
• Water
• Milk whey
Diluents
Fat amount
• Animal fat
• Vegetal oil
• Surfactants
HPLC
GC
SP
IR
Direct analysis
Complex sample pretreatment

Tests for Identification:

1. **Test 1:** Take 5 mL milk sample in a test tube. Add equal volume of 24% **TCA** to precipitate fat and proteins of milk. Take 1 mL filtrate and add 0.5 mL 2% **sodium hypochlorite**, 0.5 mL 2% **sodium hydroxide** and add 0.5 mL 5% **phenol** solution, then mix. A characteristic blue or bluish green colour develops in presence of added urea whereas pure milk remains colourless.

2. **Test 2:** Take 5 ml milk in a test tube, add 0.2 ml **urease** (20 mg/ml) Shake well at room temperature and then add 0.1 ml **Bromothymol Blue** (BTB) solution (0.5%). Appearance of blue colour after 10-15 min. indicates the presence of urea in milk. Normal milk shows faint blue colour due to natural urea present in milk.

3. **Test 3:** Take 5 mL milk sample in a test tube. Add 5 mL p-Dimethyl Amino Benzaldehyde **(DAB)** reagent. Appearance of distinct yellow color indicates presence of added urea whereas formation of slight yellow color indicates natural urea in milk.

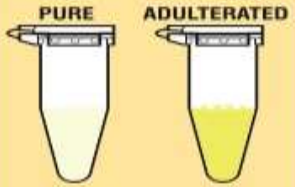
MILK TEST

MILK ADULTERATION TEST KIT

TEST FOR UREA

How to Use:

- Take 1ml of Milk in given tube
- Add 1ml of Urea Reagent
- Mix for a minute
- Refer chart for results



यूरिया के लिए परिक्षा कैसे इस्तेमाल करे:

- दी गई ट्यूब में 1 मि. ली. दूध लें।
- उसमें 1 मि.ली. यूरिया परिक्षण रासायन जोड़ें।
- एक मिनट के लिए मिलाएं।
- परिणामों के लिए चार्ट देखें।

UREA

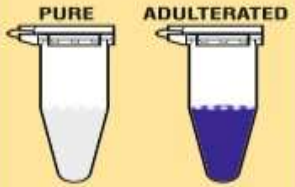
Pure Milk

Adulterated Milk

TEST FOR PRESERVATIVE (H_2O_2)

How to Use:

- Take 1ml of Milk in given tube
- Add 0.5ml of Preservative Reagent
- Mix for a minute
- Refer chart for results



प्रेजरवेटीव के लिए परिक्षा कैसे इस्तेमाल करे:

- दी गई ट्यूब में 1 मि.ली. दूध लें।
- उसमें 0.5 मि.ली. यूरिया परिक्षण रासायन जोड़ें।
- एक मिनट के लिए मिलाएं।
- परिणामों के लिए चार्ट देखें।

PRESERVATIVE H_2O_2

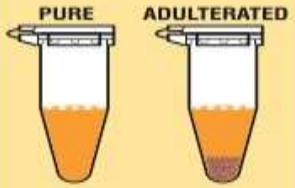
Pure Milk

Adulterated Milk

TEST FOR STARCH

How to Use:

- Take 1ml of Milk in given tube
- Add 0.5ml of Starch Reagent
- Mix for a minute
- Refer chart for results



स्टार्च् के लिए परिक्षा कैसे इस्तेमाल करे:

- दी गई ट्यूब में 1 मि.ली. दूध लें।
- उसमें 0.5 मि.ली. यूरिया परिक्षण रासायन जोड़ें।
- एक मिनट के लिए मिलाएं।
- परिणामों के लिए चार्ट देखें।

STARCH

Pure Milk

Adulterated Milk (At the bottom)

TEST FOR NEUTRALISER

How to Use:

- Take 1ml of Milk in given tube
- Add 1ml of Neutraliser Reagent
- Mix for a minute
- Refer chart for results

न्यूट्रलाईजर् के लिए परिक्षा कैसे इस्तेमाल करे:

- दी गई ट्यूब में 1 मि.ली. दूध लें।
- उसमें 1 मि.ली. यूरिया परिक्षण रासायन जोड़ें।
- एक मिनट के लिए मिलाएं।
- परिणामों के लिए चार्ट देखें।

NEUTRALISER

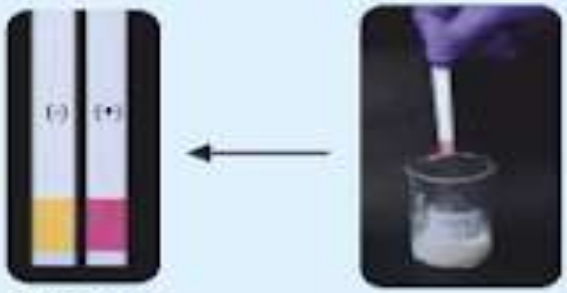
Pure Milk

Adulterated Milk

Strip Based Test for Detection of

Urea in milk

Steps for detection



Observe the colour change of strip after 3 min.

Colour Chart

Pathophysiological effects of urea in milk:

1.Urea in milk **overburdens the kidneys** as they have to filter out more urea content from the body.

2.Depending upon the level of its toxicity it can lead to **gastrointestinal and digestive disorders.**

3. Other symptoms are burning sensation in throat and chest; cough, dyspnea, exercise-induced asthma, **lung damage**-fibrosis and inflammation.

4. Redness in eyes and skin.

5. Headache; nausea, vomiting.

Mercury (Hg) Toxicity

1. Mercury poisoning refers to toxicity from **mercury consumption**.
2. Mercury is characterized as a highly malleable **liquid at normal temperature** and pressure. Its name is derived from the Latin word hydrargyrum, meaning metal that resembles liquid silver.
3. Mercury is classified into three main groups: **elemental mercury, inorganic mercury, and organic mercury.** Mercury is a type of **toxic metal** that comes in different forms within the environment. Small amounts of mercury are present in everyday foods and products, which may not affect our health. Too much mercury, however, can be poisonous.
4. More than 2500 A.C., the **prehistoric man used the cinabrio** (mercury sulfide), due to its red-gold color, to **draw on cave walls** and perform **face painting**. Subsequently, mercury has been used in the **amalgamation** (direct burning of metallic mercury on the gravel, promoting the separation of gold), in **photography** and as an **antiseptic** in the treatment of syphilis**.**
5. Mercury itself is naturally occurring, but the amounts in the environment have been on the rise from **industrialization**. The metal can make its way into **soil and water**, and eventually to animals like fish.

Source:

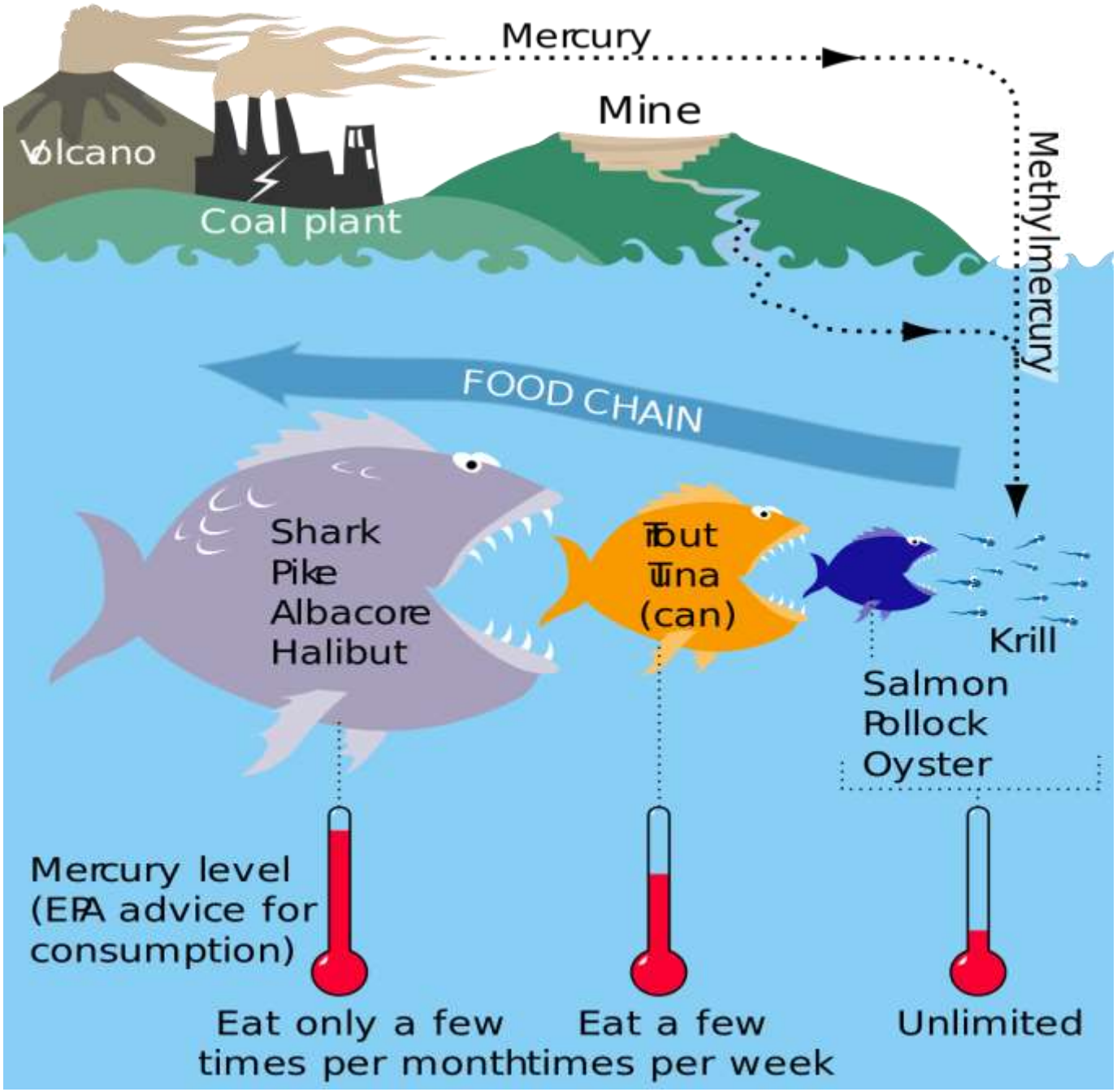
1. Mercury occurs naturally in the **earth's crust**. It is released into the environment from volcanic activity, weathering of rocks and as a result of human activity. Human activity is the main cause of mercury releases, particularly **coal-fired power stations, and residential coal burning for heating and cooking, industrial processes, waste incinerators** and as a result of mining for mercury, gold and other metals.

2. **Once in the environment, mercury can be transformed by bacteria into methyl mercury. Methyl mercury then bioaccumulates** (bioaccumulation occurs when an organism contains higher concentrations of the substance than do the surroundings) in fish and shellfish. Methylmercury also **biomagnifies**.

3. People may be exposed to mercury in any of its forms under different circumstances. However, exposure mainly occurs through **consumption of fish and shellfish contaminated with methylmercury** .Cooking does not eliminate mercury.

4. Fish get mercury from the water they live in. All types of fish contain some amount of mercury. Larger types of fish can have higher amounts of mercury because they prey on other fish that have mercury too. Sharks and swordfish are among the most common of these.

5. Other causes of mercury poisoning can be environmental or from exposure to other forms of the metal. These include, **broken fever thermometers, "silver" dental fillings, certain types of jewelry, mining for gold, and household gold extraction, skin care products**, exposure to toxic air in industrialized communities, CFL bulb breakage etc.


Mercury
Volcano
Coal plant
Mine
Methylmercury
FOOD CHAIN
Shark
Pike
Albacore
Halibut
Trout
Tuna
(can)
Krill
Salmon
Pollock
Oyster
Mercury level
(EPA advice for
consumption)
Eat only a few
times per month
Eat a few
times per week
Unlimited

Pathophysiological effects: Mercury toxicity for man varies depending on the form of mercury, dose, and rate of exposure.

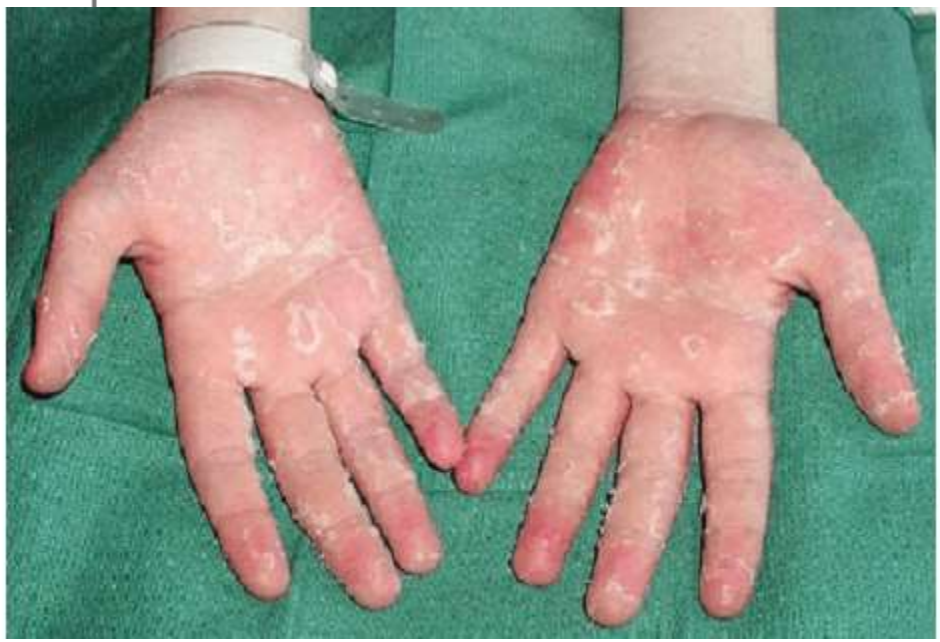
1: Neurological effects: Mercury is most notable for its neurological effects. Too much mercury can cause **anxiety, depression, irritability, memory problems, numbness, pathologic shyness, tremors.** Mercury poisoning symptoms in adults include **hearing and speech difficulties, lack of coordination, muscle weakness, nerve loss in hands and face, trouble walking, vision changes.**

2. Mercury poisoning can also disrupt **fetal and early childhood development**. Infants and young children may have delays in cognition, fine motor skills, speech and language development, visual-spatial awareness, learning disabilities.

3. Reproductive effects: Mercury poisoning also poses a risk to the reproductive system. It may cause **reduced sperm count or decreased fertility** and may also cause problems with the fetus. Possible effects of mercury poisoning include **deformity and a decreased survival rate of the fetus, and reduced growth and size of the newborn at birth.**

4. Cardiovascular risks: Mercury helps promote the accumulation of free radicals in the body, which puts the cells at risk for damage. This may lead to an **increased risk of heart problems, including heart attack and coronary heart disease.** it has been demonstrated that exposure to mercury by frequent consumption of fish has a strong positive correlation with **increased arterial blood pressure**. Other studies also correlate mercury exposure with increased risk of hypertension, **myocardial infarction, coronary dysfunction, and atherosclerosis**. The mechanism by which mercury produces toxic effects on the cardiovascular system is not fully elucidated, but this mechanism is believed to involve an increase in oxidative stress.

Neurological	Non-Neurological
Ataxia	Alopecia totalis
Chorea	Autoimmunity
Blindness	Fatigue
Depression	Hypersalivation
Drowsiness	Keratosis
Excitability	Melanosis
Fearfulness/anxiety	Recurrent infections
Insomnia	Ulcers
Irritability	
Low Intelligence Quc	
Memory loss	
Mental retardation	
Parasthesias	
Quarreling	
Restlessness	
Temper outbursts	
Tremors	



Mechanism of mercury toxicity:

1. The primary mechanism of mercury toxicity involves **its irreversible inhibition of seleno-enzymes, such as thioredoxin reductase**. **Although it has many functions, thioredoxin reductase restores vitamins C and E back into their reduced forms, enabling them to counteract oxidative damage.** Since the rate of oxygen consumption is particularly high in brain tissues, production of reactive oxygen species (ROS) is accentuated in these vital cells, making them particularly vulnerable to oxidative damage and especially dependent upon the **antioxidant protection provided by seleno-enzymes.** High mercury exposures **deplete the amount of cellular selenium** available for the biosynthesis of thioredoxin reductase and other selenoenzymes that prevent and reverse oxidative damage.

2. Exposure to methyl mercury causes increased levels of antibodies sent to myelin basic protein (MBP), which is involved in the myelination of neurons, and glial fibrillary acidic protein (GFAP), which is essential to many central nervous system (CNS). This causes an **autoimmmune response against MBP and GFAP** and results in the **degradation of neural myelin** and general decline in function of the CNS.

3. In addition, because of its high affinity for sulfhydryl groups in tubulin, methylmercury **inhibits the organization of microtubules** that are important in CNS development. The binding to SH groups also interferes with **the intracellular signaling of multiple receptors** (e.g., muscarinic, nicotinic, and dopaminergic) and promotes the blockade of Ca^{++} channels in neurons

Minamata disease

1. Minamata disease, sometimes referred to as **Chisso-Minamata disease**, is a **neurological disease** caused by severe mercury poisoning.

2. Signs and symptoms include **ataxia, numbness in the hands and feet, general muscle weakness, loss of peripheral vision, and damage to hearing and speech. In extreme cases, insanity, paralysis, coma, and death** follow within weeks of the onset of symptoms. A congenital form of the disease can also affect fetuses in the womb.

3. Minamata disease was first discovered in the **city of Minamata, Kumamoto Prefecture, Japan, in 1956.** It was caused by the release of methyl mercury in the industrial wastewater from a chemical factory owned by the **Chisso Corporation**, which continued from 1932 to 1968. It has also been suggested that some of the **mercury sulfate in the wastewater was also metabolized to methyl mercury by bacteria** in the sediment. This highly toxic chemical bioaccumulated and biomagnified in shellfish and fish in Minamata Bay and the Shiranui Sea, which, when eaten by the local population, resulted in mercury poisoning.

4. As of March 2001, **2,265 victims** had been officially recognised as having Minamata disease (**1,784 of whom had died**) and over 10,000 had received financial compensation from Chisso.


SHIRANUI SEA
Chisso Factory
Hyakken Harbour
Minamata Bay
Minamata City

LEAD TOXICITY

1. Lead is a naturally occurring **toxic metal** found in the Earth's crust. There is almost no function in the human body which is not affected by lead toxicity.

2. Lead is the **most important toxic heavy element** in the environment. Due to its important physico-chemical properties, its use can be retraced to historical times. Of all the organs, the **nervous system is the mostly affected target** in lead toxicity, both in children and adults.

3. The **toxicity in children** is however of a greater impact than in adults. **This is because their tissues, internal as well as external, are softer than in adults**. As of 2012, the Centers for Disease Control and Prevention (USA) have set the standard elevated blood lead level for adults to be 10 µg/dL and for children 5 µg/dL of the whole blood

4. Lead poisoning is believed to be primarily responsible for the collapse of the **Roman Empire**, in which lead acetate was used as a sweetener of wine. Its prolonged use was considered to have caused dementia to many Roman emperors. **Beethoven's death** has been treated in various reports. Many of them have concluded that he died because of the toxic doses of lead-based treatment administered by his doctor. Analysis of his hair was found to contain elevated levels of lead. **Organic lead is perhaps more toxic than inorganic lead because of its lipid soluble nature**, which results in rapid consequences.

SOURCE:

1. **Turmeric** as a source of lead (Pb) exposure due to the addition of **lead chromate ($PbCrO_4$)**, a yellow pigment used to enhance brightness. It is poisonous, acting as a neurotoxin when humans ingest or inhale. It is an essential spice that many people consume daily in South Asia.

2. Poisoning due to lead occurs mainly by **ingestion of food or water** contaminated with lead. Lead toxicity may be caused through fruits and vegetables contaminated with high lead levels from the soils where they were grown.

3. The soil accumulates lead levels generally from **pipes, lead paint and residual emissions from leaded gasoline**. Lead from water pipes, from plumbing and fixtures that are either made of lead or have lead solder are one of the major sources of lead poisoning.
4. Occasional lead poisoning was found to be caused by lead salts used **in pottery glazes leached by acidic fruit juices.** It is also assumed that in the eighteenth and early nineteenth century lead was illegally added to wine.

5. Lead is present in products, such as herbal and **traditional medicines, folk remedies, cosmetics and toys.** Some folk medicines (greta, azarcon, and pay-loo-ah) may contain lead. These folk medications may contain high amount (approximately 90%) of lead. Various cosmetic products such as surma, kohl, kajal, tiro, and tozali also contain a high amount of lead..


Image credit: istockphoto.com/margouillatphotos


paint
water from pipes
ceramics
toys

- Please Turn Over For More Information -

Sources of Lead Poisoning

Paint in Homes Built Before 1978

Lead Contaminated Soil

Lead Contaminated Dust

Traditional Remedies & Cosmetics

Imported Pottery

Imported Toys

Imported Jewelry

Imported Candy

Tests for identification:

1. To detect the presence of lead chromate, mix a teaspoon of turmeric powder with water. If adulterated, it will immediately leak streaks of water-soluble colour.

2. Detection of Lead Chromate in Turmeric Powder: Ash about 2 grams the sample. Dissolve it in 4-5 ml of 1:7 **sulphuric acid** (H_2SO_4) and filter. Add 1ml of 0.2% **diphenylcarbazide**. Observe any change in color Inference. A pink color indicates presence of lead chromate.

PATHOPHYSIOLOGICAL EFFECTS:

On Brain:

❑Blood lead levels from 25 and 60 µg/dL give rise to **neuropsychiatric effects** such as delayed reaction times, irritability, and difficulty in concentrating, as well as slowed down motor nerve conduction and headache In adults.

❑High blood lead levels which exceed 100 µg/dL cause very severe manifestations, like signs of **encephalopathy** (condition characterized by brain swelling) accompanied by increased pressure within the skull, delirium, coma, seizures, and headache.

❑**Synapse formation** is greatly affected in the cerebral cortex by lead.

Lead is able to pass through the endothelial cells at the blood brain barrier because it can substitute for calcium ions and be taken up by calcium-ATPase pumps, thereby interfering with synapse formation.

❑Lead usually interferes with the neurotransmitter glutamate which is important for many functions, like learning. It operates by **blocking NMDA receptors**. In addition to inhibition of the NMDA receptor, lead exposure also decreased the amount of gene for this receptor in part of the brain. Lead was also found to be involved in apoptosis of brain cells in animal studies.

❑An increase in Pb^{2+} concentration will effectively inhibit ongoing **long-term potentiation (LTP),** and lead to an abnormal increase in long-term depression (LTD) on neurons in the affected parts of the nervous system.

On blood:

❑Anaemia may appear at blood lead levels higher than 50 µg/dL. As lead disrupts the maintenance of the cell membrane, red blood cells with a damaged membrane become more fragile, resulting in **anaemia.**

❑Lead is also speculated to **alter the permeability** of blood vessels and collagen synthesis.

❑Lead interferes with the activity of an essential enzyme called delta-aminolevulinic acid dehydratase, or ALAD, which is important in the **biosynthesis of heme**. Heme precursors, such as aminolevulinic acid, have been found to build up due to their interference with lead, which may be directly or indirectly harmful to neurons

❑Pb can also inhibit the enzyme **ferrochelatase**, reducing iron (Fe) incorporation into heme

On CVS:

❑Prolonged exposure to lead is associated with **increase in blood pressure** and some studies showed a connection between lead exposure and coronary heart disease, heart rate variability, and death from stroke.

❑People exposed to very high doses of Pb (blood lead concentrations between 500–870 µg/L blood) can experience **sinus node dysfunction**, atrioventricular conduction disturbances and atrioventricular block.

❑Pb exposure can also lead to **morphological changes in the heart**, e.g., visible changes in the electrocardiographic picture, impaired systolic and diastolic function, changes in repolarization dispersion and increased blood pressure.

On Reproductive system:

❑ In males **sperm count is reduced** and other changes occur in the volume of sperm when blood lead levels exceed 40 µg/dL. Activities like motility and the general morphology of sperm are also affected at this level.

❑ Toxic levels of lead can lead to **miscarriages**, prematurity, low birth weight, and problems with development during childhood.

❑Long-term low-dose lead exposure was shown to **alter the signalling** system between the hypothalamus and pituitary gland of male rats. This signalling is disrupted by long-term exposure, altering thereby the gonadotropin-releasing hormone system in the male rat.

❑ Investigation of HCG indicated that lead acetate changed the secondary structure of HCG which resulted in **decreased bioactivities of HCG.**

On children:

❑ Pb is **especially harmful to children under the age of six**, most likely because of the rapid brain growth and development with associated periods of heightened vulnerability, and because of high demand for nutrients. Moreover, the absorption of lead occurs more quickly in children than in adults.

❑ In a child's developing brain, **synapse formation is greatly affected in the cerebral cortex by lead.** Lead also interferes with the development of neurochemicals, including neurotransmitters, and organisation of ion channels.

❑ Lead poisoning also causes **loss of neuron myelin sheath,** reduction in the number of neurons. Children with blood lead concentration greater than 10µg/dL are at higher risk for developmental disabilities.

❑Increased blood lead levels are also associated with a **decrease in cognitive performance** and with other psychiatric conditions like depression, anxiety, attention deficit hyperactivity disorder and antisocial behavior. Prenatal and early childhood lead exposure was reported in correlation with violent crimes in adulthood.

❑ The hippocampus is a part of the brain involved in learning and memory. The main reasons for lead interfering with learning particularly in children is that it **damages the cells within the hippocampus.**

On kidney: Chronic lead **nephropathy** occurred due to years of lead exposure with loss of proximal tubules and interstitial fibrosis.. Lead accumulation in the proximal tubule leads to hyperuricaemia and gout – presumably by inhibiting uric acid secretion – and also to diminished renal clearance, tubular reabsorption and glomerular filtration rate.

Metabolic and Genetic Effects:

❑Lead impairs multiple biochemical processes, including inhibiting calcium and reacting with proteins. Upon entering the body, **Pb takes the place of calcium** and then interacts with biological molecules, interfering with their normal function.

❑ Lead **reduces the activity of various enzymes,** causing changes in their structure, and inhibits their activity by competing with the necessary cations for binding sites.

❑**Oxidative stress** caused by lead is another main mechanism responsible for its toxicity, causing changes in the composition of fatty acids in membranes (affecting processes such as exocytosis and endocytosis, and signal transduction processes). Pb can also cause gene expression alterations.

Bone toxicity:

❑A significant reduction in the bone calcium content upon lead intoxication has been observed. This decrease in calcium content may be because of the increased bone resorption.

❑ Lead is one of the risk factors for the development of **osteoporosis** by altering bone mineral metabolism.

❑Osteopenia, osteoporosis, and osteomalacia with increased bone fragility in humans and experimental animals were observed because of lead exposure.

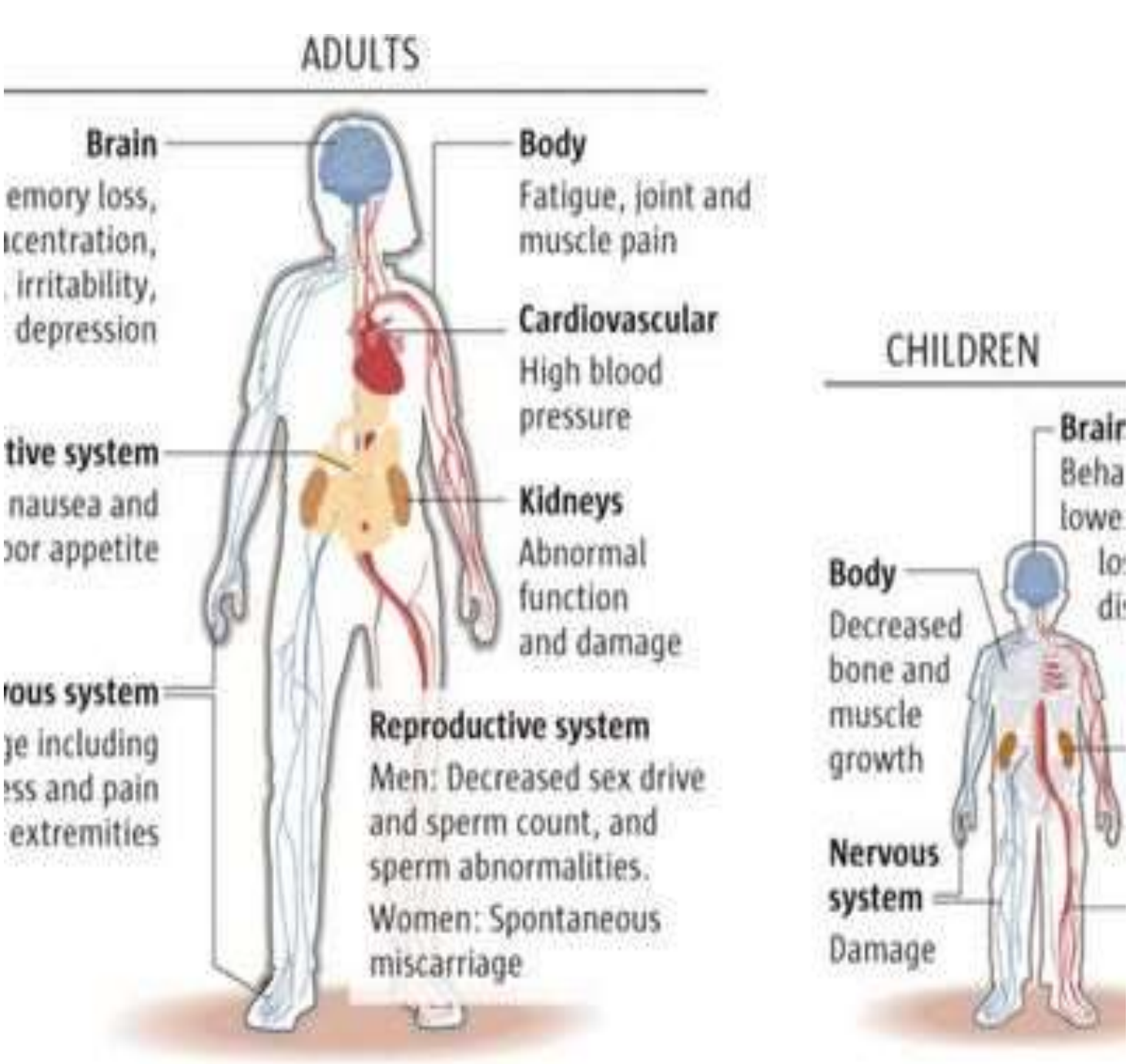
Hepatotoxicity of lead:

- Ingestion of Pb is one of the primary causes of its hepatotoxic effects. Hepatocarcinogenic effects of Pb is reported in animal toxicology studies.

- The present work revealed that lead accumulation is highly toxic to liver. Lead toxicity increased the expressions of cytochrome P450 1 A (CYP1A) and cytochrome.

- The lead toxicity also decreases the level of liver marker enzymes alanine transaminase, aspartate transaminase in serum.

- Chronic exposure to lead imposes a potent toxic effect on liver cells manifested as glycogen depletion, cellular infiltration and liver architecture in the form of initiation of periportal fibrosis that may progress to liver cirrhosis.


ADULTS
Brain
emory loss,
ıcentration,
irritability,
depression
tive system
nausea and
ɔor appetite
ous system
ge including
ess and pain
extremities
Body
Fatigue, joint and
muscle pain
Cardiovascular
High blood
pressure
Kidneys
Abnormal
function
and damage
Reproductive system
Men: Decreased sex drive
and sperm count, and
sperm abnormalities.
Women: Spontaneous
miscarriage
CHILDREN
Brair
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Decreased
bone and
muscle
growth
Nervous
system
Damage

Mechanism:

❑One of the mechanisms underlying the **neurotoxicity of lead lies in its ability to substitute for other polyvalent cations (particularly divalent cations, such as calcium (Ca2+) and zinc (Zn2+))** in the molecular machinery of living organisms. In most instances, the characteristics of lead allow it to bind with greater affinity than calcium and zinc ions to protein binding sites.

❑These interactions allow lead to affect different biologically significant processes, including metal transport, energy metabolism, apoptosis, ionic conduction, cell adhesion, intercellular and intracellular signalling, diverse enzymatic processes, protein maturation, and genetic regulation. Membrane ionic channels and signalling molecules seem to be one of the most relevant molecular targets that contribute to lead's neurotoxicity.

ARSENIC TOXICITY

1. The World Health Organization (WHO) lists arsenic as one of the 10 chemicals of **major public health concern.** Long-term consumption of drinking water contaminated with naturally occurring soluble inorganic arsenic leads to chronic arsenic poisoning, also called **arsenicosis.**

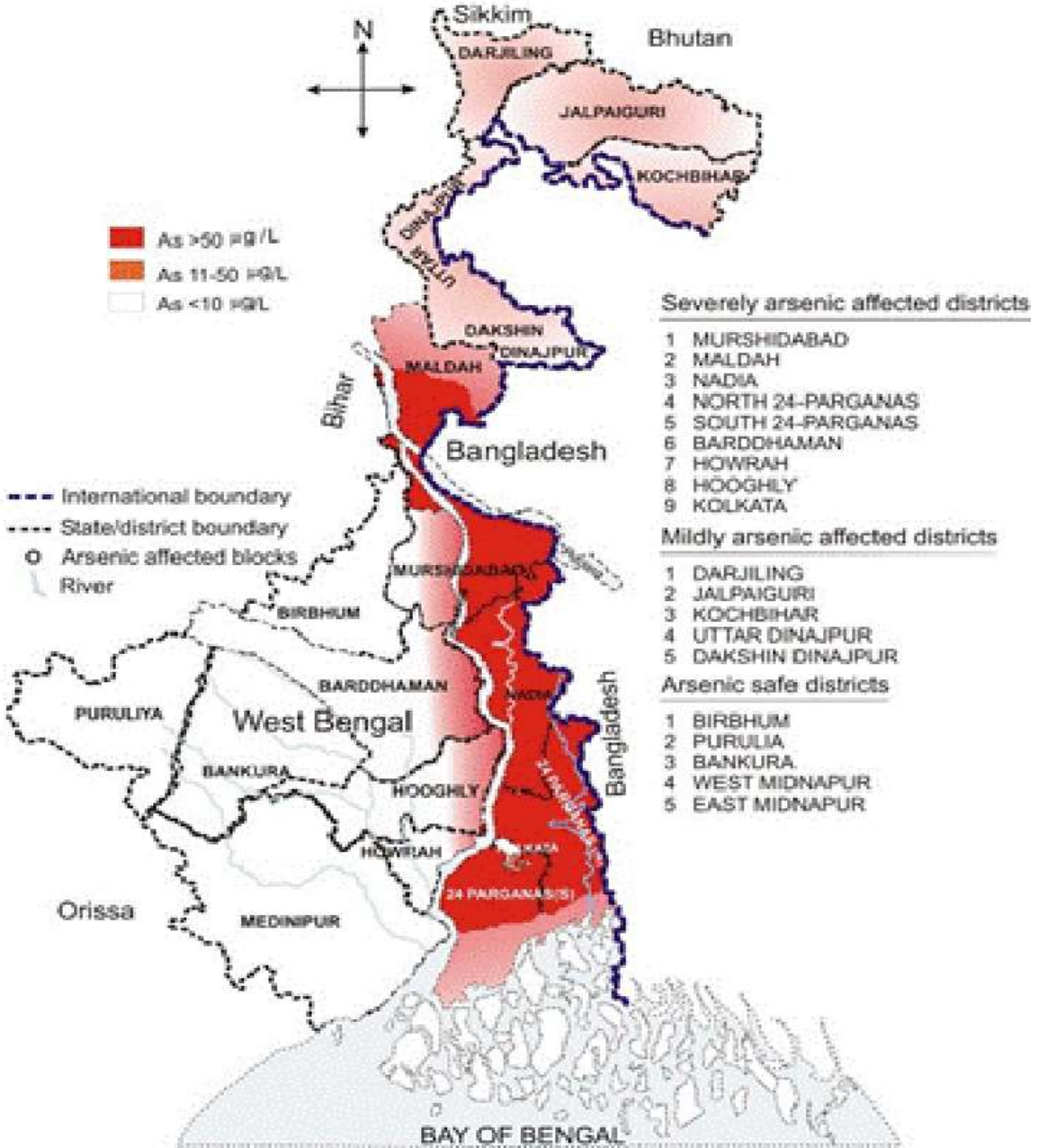
2. West Bengal (WB) is an **arsenic endemic state** in India, with at least **9 out of 18 districts exposed to groundwater contaminated with arsenic** (of geological origin) above the WHO's maximum permissible limit of 10 mcg/L. The State Government currently estimates at least 79 blocks (administrative units) across the state to be severely affected, involving 26 million individuals.

3. In fact, the Ganga-Brahmaputra plains in India (7 states) and the Padma-Meghna plains in Bangladesh together constitute the **most widespread arsenic-affected area in the world.** One of the worst incidents of arsenic poisoning via well water occurred in Bangladesh, which the World Health Organization called the **"largest mass poisoning of a population in history**" recognized as a major public health concern.

4. Arsenic is a steel-grey **semi-metallic element** and present in Group 15 in the periodic Table. All arsenic compounds are poisonous. Arsenate (pentavalent) and arsenite (trivalent) are the most common toxic inorganic forms; each has a different proposed mechanism based on valence state.

GROUNDWATER ARSENIC CONTAMINATION STATUS IN WEST BENGAL-INDIA

[Total number of arsenic affected districts 9 and blocks 111]



Severely arsenic affected districts

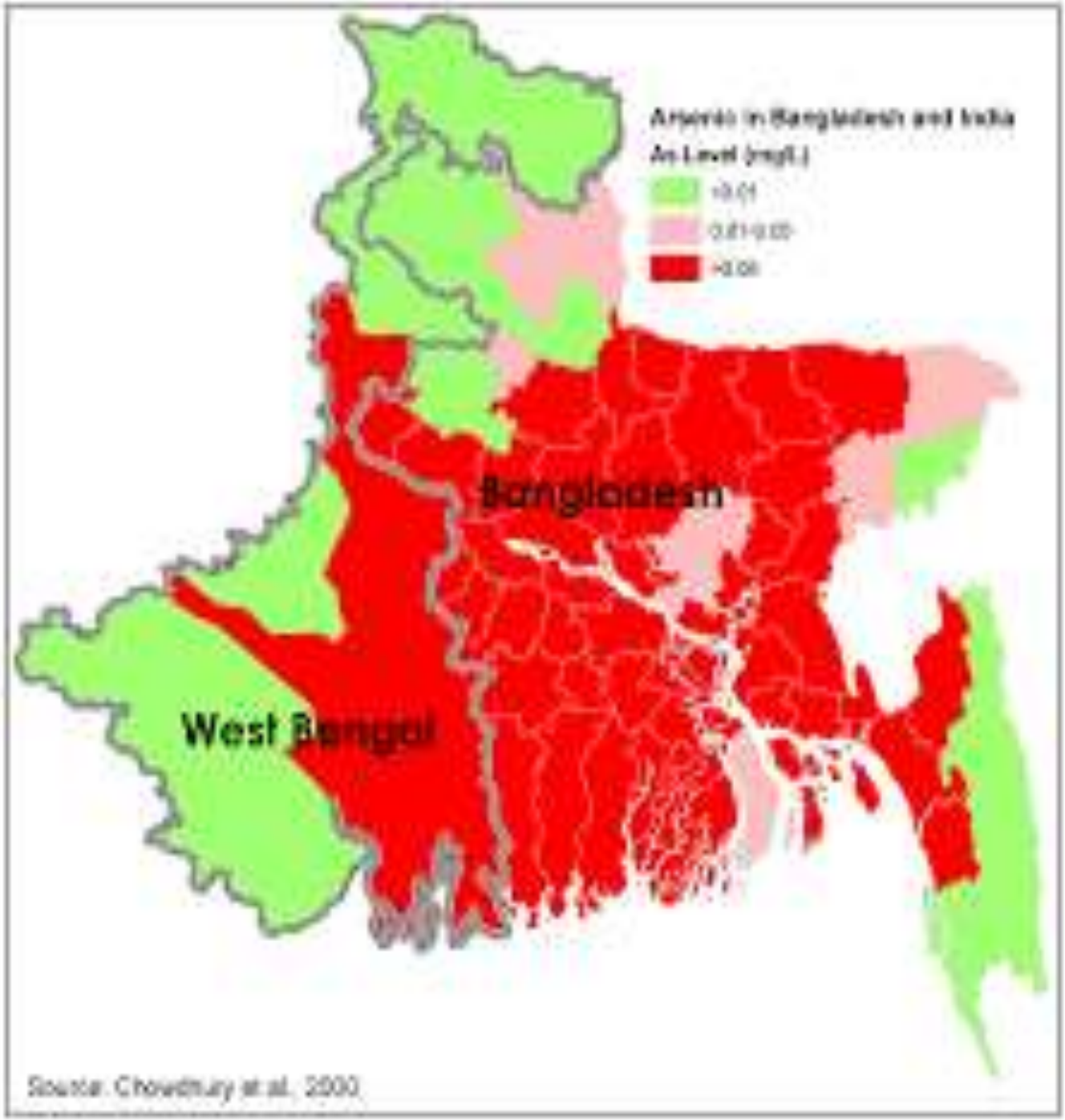
1 MURSHIDABAD
2 MALDAH
3 NADIA
4 NORTH 24-PARGANAS
5 SOUTH 24-PARGANAS
6 BARDDHAMAN
7 HOWRAH
8 HOOGHLY
9 KOLKATA

Mildly arsenic affected districts

1 DARJILING
2 JALPAIGURI
3 KOCHBIHAR
4 UTTAR DINAJPUR
5 DAKSHIN DINAJPUR

Arsenic safe districts

1 BIRBHUM
2 PURULIA
3 BANKURA
4 WEST MIDNAPUR
5 EAST MIDNAPUR


Bangladesh
West Bengal
Source: Chowdhury et al. 2000

Source:

1. Historically, **groundwater** has been utilized as the most affordable source of drinking water supply in rural WB. Exposure primarily occurs through **drinking groundwater contaminated with inorganic arsenic salts, and from food prepared or crops irrigated using high-arsenic water sources**

2. Bio-accumulation of arsenic is occurring from the **food grains** as irrigated with arsenic contaminated water. Inorganic arsenic has been found in raw (93.8%) as well as cooked rice (88.1%), the staple diet in the state.

3. **Fish, shellfish, meat, poultry, dairy products and cereals** can also be dietary sources of arsenic, although exposure from these foods is generally much lower compared to exposure through contaminated groundwater

4. Arsenic is introduced into soil and groundwater during weathering of rocks and minerals followed by subsequent leaching and runoff. It can also be introduced into soil and groundwater from anthropogenic sources. Arsenic is emitted into the atmosphere by high-temperature processes such as coal-fired power plants, burning vegetation and volcanism. **The source of arsenic in groundwater of lower gangetic delta is considered to be the arsenic-rich sediments which has transported from the Chotonagpur-Rajmahal highlands**. Some research workers believe that the leaching of arsenic in ground water is due to maximum use of ground waters for irrigation.

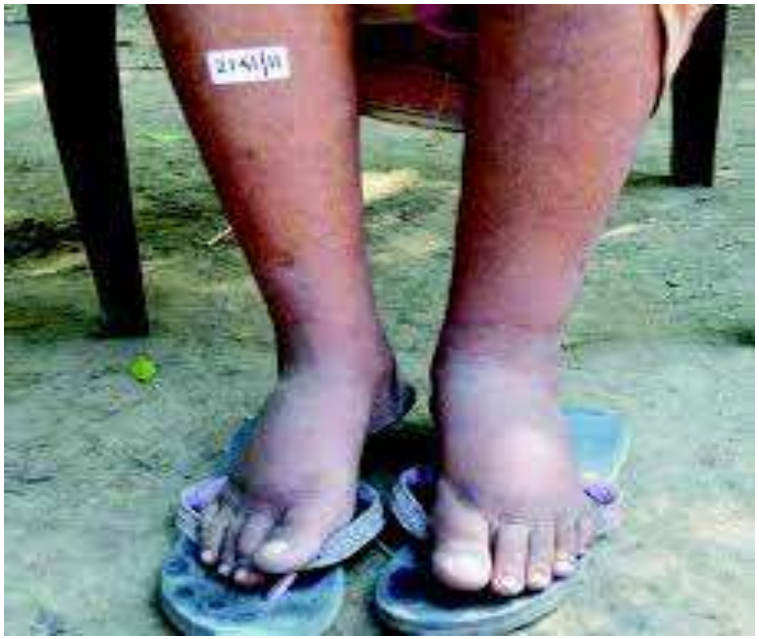
Pathophysiological effects:

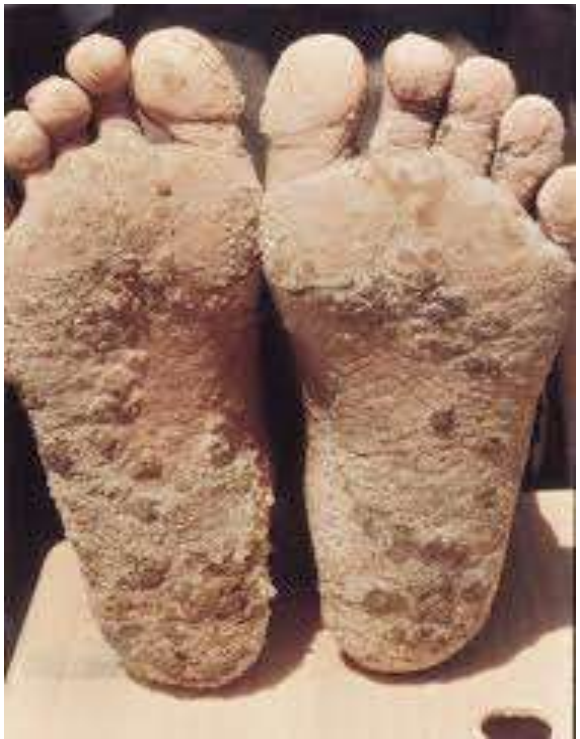
On skin: Classic skin lesions such as **"rain drop pigmentation"** and keratosis are more commonly described in arsenicosis. Major dermatological signs are diffuse or spotted melanosis, leucomelanosis, and keratosis.

On Lungs Symptoms of **chronic lung disease, chronic cough**, were present in 57% people with chronic arsenic toxicity. Lung function tests carried out on patients showed features of restrictive lung disease and combined obstructive and restrictive lung disease.

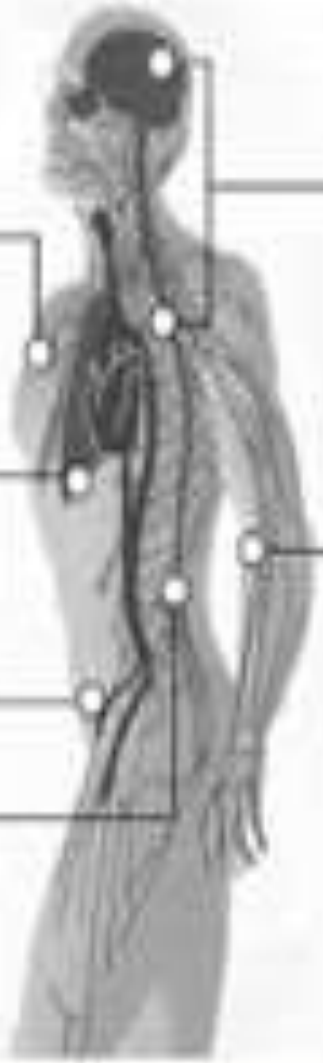
Gastrointestinal system: **Dyspepsia, gastroenteritis, nausea, diarrhea, anorexia and abdominal pain** was observed in patients with chronic arsenic toxicity studied in West Bengal. Patients developed features of portal hypertension with signs of liver fibrosis. There have also been case reports of liver cirrhosis after medication with inorganic arsenic compounds. Hepatomegaly was found.

On cardiovascular system: **Blackfoot disease (BFD)**, a form of peripheral vascular disease, has been reported to be one of the important complications of chronic arsenic toxicity. Comparable peripheral vascular disorders with varying degrees of severity, including Raynaud's syndrome and acrocyanosis, have also been reported among people drinking arsenic-contaminated water. Increased prevalence of hypertension among residents in the endemic area and a dose–response relationship between ingested inorganic arsenic and prevalence of hypertension has been found. Mortality rate from ischemic heart disease was found to be increased with endemic arsenicosis.





Arsenic poisoning



Nerve damage

Skin damage:
- Hyperkeratosis (scaling skin)
- Pigment changes

Circulatory problems in skin

Increased cancer risk:
- Lung
- Bladder
- Kidney and liver cancers

On nervous system: There are many reports of occurrence of peripheral neuropathy because of chronic exposure of arsenic through drinking water. Peripheral neuritis characterized by paresthesia (tingling, numbness, limb weakness, and others) was present in patients of chronic arsenicosis. They showed abnormal electromyography and altered nerve conduction velocity. Irritability, lack of concentration, depression, sleep disorders, headache, and vertigo were reported in arsenicosis people showing features of neuropathy in West Bengal.

On reproductive system High concentrations of arsenic (≥200 mg/L) during pregnancy were found to be associated with a six-fold increased risk for stillbirth.

Hematological effects A characteristic pattern of anemia, leucopenia, and thrombocytopenia was found in people exposed to arsenic in drinking water.

Diabetes A dose–response relationship between cumulative arsenic exposure and prevalence of diabetes mellitus was observed in Taiwan and Bangladesh.

Other effects: High incidences of weakness and fatigue have been reported in chronically arsenic-exposed people in West Bengal and in many other countries. Conjunctival congestion and non-pitting edema of the legs and hands have also been reported in patients.

Arsenicosis and cancer: Arsenic was potentially responsible for skin, urinary bladder, and lung cancers as result of chronic exposure to arsenic. In West Bengal, higher incidences of skin cancer and internal cancers were detected among patients of arsenicosis studied in arsenic-affected villages.

Genotoxicity: Several studies have investigated the genotoxic effects of arsenic after long-term exposure in drinking water. **Increased incidence of chromosomal aberration** and frequency of micronuclei in buccal and urothelial cells was observed by many workers after drinking of arsenic-contaminated water. Higher level of **cytogenetic damage** was found in symptomatic participants. Deficiency in DNA repair capacity and alteration in the methylation status of DNA may be involved in arsenic-induced carcinogenesis.

Mechanism:

1. Arsenate (pentavalent arsenic) may replace phosphate in several reactions. **Arsenate has a similar structure and similar properties to phosphate.** In vitro studies show arsenate reacts with glucose to form **glucose-6-arsenate**, which resembles glucose-6-phosphate. Glucose-6-arsenate is a substrate for glucose-6-phosphate dehydrogenase and can inhibit hexokinase. Depletion of ATP has been observed secondary to diminished ATP formation.

2. Arsenite (trivalent arsenic) **reacts with thiol and sulfhydryl groups, of many proteins and enzymes** found throughout the body. These reactions cause dysregulation and inhibition of these proteins and enzymes for eg., -**pyruvate dehydrogenase**. Pyruvate dehydrogenase (PDH) is a vital enzyme in the citric acid cycle; an altercation of PDH can lead to impairment of cellular respiration and ATP formation.

3. Definitive mechanism of action for the carcinogenic effects of arsenic remain unverified. Proposed mechanisms include **alteration of DNA repair, DNA methylated oxidative stress and genotoxicity.**

1. Why trivalent arsenic is more toxic than pentavalent arsenic? (2/2021)
2. Name two persistent organic pollutants. (2/2021)
3. Is it safe to use aluminium for wrapping food? (2/2021)
4. Does margarine clog your arteries? Justify your answer. (2/2021)
5. What are the major sources of dioxin? (2/2021)
6. Name one simple method of chiccory differentiation from coffee powder. (2/2021)
7. According to FSSAI guidelines MSG is not permitted in which food stuff? (2/2021)
8. Give examples of two flavouring food additives. (2/2021)
9. Mention two sources of bisphenol-a exposure on human body. (2/2021)
10. What is incidental adulteration? (2/2021)
11. State two uses of rhodamine B. (2/2021)
12. Mention two sources of PCBs. (2/2021)
13. Write short note on mercury toxicity on human health. (5/2021)
14. Write short note on PFA. (5/2021)
15. What are the toxic effects of metanil yellow on human body? (5/2021)
16. Write short notes on physiological effects of chicory in food. (5/2021)
17. Give an account of health effects of dioxin. (5/2021)
18. Briefly describe the neurotoxic and hepatotoxic effects of lead. (5/2021)
19. What are the long-term side effects of PCBs on human health? (6/2021)
20. State two pathological side effects of urea. (2/2021)
21. Describe the oxidative stress of arsenic on human. (4/2021)
22. Arsenic is toxic, then why it is used in medicine? (2/2021)
23. State the harmful effects of consumption of MSG. (4/2021)
24. How do you detect rhodamine-B? (3/2021)
25. Give an account of acute health effect of rhodamine- B. (4/2021)
26. Is rhodamine B a xanthene dye? Explain your answer. (3/2021)
27. **How can the presence of adulterants be detected in the following items? Ghee, milk, oil, honey and turmeric powder**. (2X5/2021)

1. Why trivalent arsenic is more toxic than pentavalent arsenic? (2/2021)

Arsenites (As^{3+} or trivalent, arsenic trioxide) are 5–10 times more toxic than arsenates (As^{5+} or pentavalent) due to their higher solubility. Arsenite the (trivalent form) has a slower excretion rate compared to arsenate the (pentavalent form) and organic arsenic, which may contribute to arsenite's increased toxicity compared to arsenate and organic arsenic.

2. Why arsenic tends to accumulate in keratin-rich tissues such as hair and nails?

Arsenic has high affinity for sulfhydryl groups (–SH). Since hair and nails contain –SH-rich keratin, arsenic accumulates in them.

3. Why arsenic can cause abortions but not nervous symptoms?

Arsenic can cross Placental barrier, hence can cause abortions. It cannot cross BBB, hence is unable to cause nervous symptoms.

4. **What is the mechanism of action of Arsenite (+3) toxicity?**

Arsenite (+3) reacts with sulfhydryl groups (–SH) of proteins and inhibits the enzymes by blocking the active groups. The arsenite inhibits alpha-keto oxidases which contain dithiol groups and are involved in oxidation of pyruvate. One crucial enzyme effected is pyruvate dehydrogenase, which is a protein complex that requires lipoic acid, a dithiol, for activation. Pyruvate dehydrogenase (PDH) is a vital enzyme in the citric acid cycle; an altercation of PDH can lead to impairment of cellular respiration and ATP formation.

5, What is the mechanism of action of Arsenate (+5) toxicity?

Arsenates (+5) are a little different. They are uncouplers of oxidative phosphorylation. The inorganic penta-valents may substitute phosphate in many reactions. The result is an increase in body temperature.

6. **Arsenic is toxic, then why it is used in medicine?**

For over 2,400 years, arsenic — from the Greek word arsenikon, meaning “potent” — has been used as both a **therapeutic agent** and a poison. Arsenic is a substance that has been well known to both the ‘healer’ and the ‘poisoner’ throughout history. Arsenic has been called the “King of Poisons”, because it had been used to poison royalty and thus alter who would ascend to the throne. In the past years As and its compounds were used as a medicine for the treatment of such diseases as diabetes, psoriasis, syphilis, skin ulcers, trypanosomisis and joint diseases. Nowadays As is also used especially in the treatment of patients with acute promyelocytic leukemia. Arsenic is a natural substance and a traditional poison. The toxicity of arsenic is a double-edged sword. In fact, it has also been used as a drug with appropriate application for over 2,400 years in both traditional Chinese medicine and the Western world.

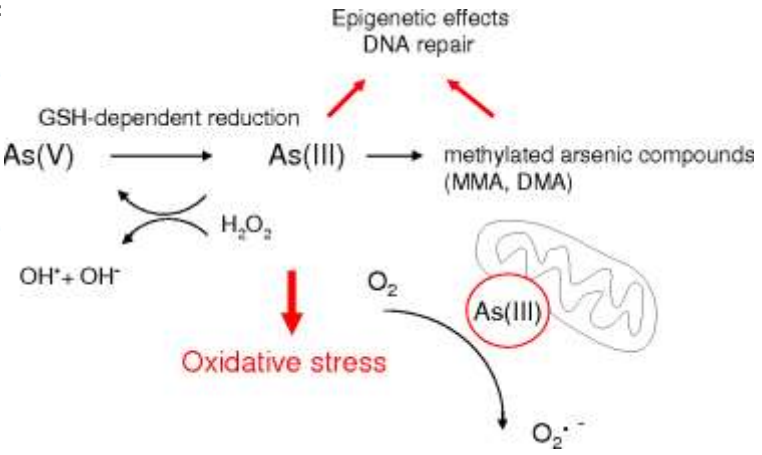
7. **What is Fowler’s solution?**

In the 1700s, English inventor Thomas Fowler developed a solution of arsenic trioxide in potassium bicarbonate (1% w/v) that was used to treat asthma, chorea, eczema, pemphigus, psoriasis, anemia, Hodgkin’s lymphoma and leukemia. In 1878, the compound, aptly named “Fowler’s solution,” was discovered to lower white blood cell counts in normal individuals, with a more significant decrease occurring in those with chronic myelogenous leukemia.

Qs. Describe the oxidative stress of arsenic on human.

- Reactive oxygen species (ROS)-mediated oxidative damage is a common denominator in arsenic pathogenesis. In addition, arsenic induces morphological changes in the integrity of mitochondria.

- When both humans and animals are exposed to arsenic, they experience an increased formation of **ROS/RNS, including peroxyl radicals (ROO•), the superoxide radical, singlet oxygen, hydroxyl radical (OH•) via the Fenton reaction, hydrogen peroxide, the dimethylarsenic radical, the dimethylarsenic peroxyl radical** and/or oxidant-induced DNA damage.



- Arsenic induces the formation of oxidized lipids which in turn generate several bioactive molecules (ROS, peroxides and isoprostanes), of which aldehydes [malondialdehyde (MDA) and 4-hydroxy-nonenal (HNE)] are the major end products.

- Arsenic alters cellular glutathione levels either by utilizing this electron donor for the conversion of pentavalent to trivalent arsenicals or directly binding with it or by oxidizing glutathione via arsenic-induced

Q. what is incidental and intentional adulteration?

- Incidental adulteration occurs when foreign substances are added to a food as a result of ignorance, negligence, or improper facilities. Eg. pesticides, droppings of rodents, larvae in food.

- Intentional adulteration involves the deliberate addition of inferior materials to a food to heighten appearance qualities and to gain greater profits. Eg. sand, marble chips, stones, chalk powder, etc

Q. What is metallic adulteration?

When the metallic substances are added intentionally or accidentally. Eg: arsenic, pesticides, lead from water, mercury from effluents, tins from cans, etc.

Q. According to FSSAI guidelines MSG is not permitted in which food stuff?

MSG is not permitted in more than 50 food products including pasta and noodles (dried products)

Q. What is PFA act?

- PFA (the prevention of food adulteration act) 1954 (Amended in 1964, 1976, 1986) provides the protection from adulteration / contamination of food that may lead to the health risk of consumers.
- The Act deals with the frauds also that can be perpetrated by the dealers by supplying cheaper or adulterated foods.
- The Act regulates the use of chemicals, pesticides, flavours and other additives in food preparation.
- Enrichment of flour, bread, or other cereals with vitamins or minerals, iodization of salt, vitaminisation of vansapati oil, addition of vitamin "C" in certain foods can be done under the provision made in this Act.
- There is a provision of penalty if anybody break the law for a maximum imprisonment of 1 year or a minimum fine or Rs. 2000 in the first instance and for imprisonment of 6 months which may extend to 6 years and cancellation of license on the second or subsequent offense

Q. Identification of adulteration in Ghee, milk, oil, honey and turmeric powder. (2X5/2021)

GHEE:

Xenobiotic Metabolism

DEFINITION:

1. Human beings are continuously exposed to several foreign compounds such as drugs, pollutants, food additives, cosmetics, pesticides, etc., From a metabolism viewpoint, they can be defined as *chemicals that are extrinsic to the normal metabolism of a living organism*. ('Xenos' means stranger- it is a Greek word.)

2. They are mostly regarded as synthetic substances, but they include naturally occurring chemicals and endobiotics, when present in higher concentrations than their normal levels, or produced by certain organisms as a defense mechanism, such as the toxins produced by some fungi, bacteria, or even herbs.

3. It has been estimated that humans are exposed to 1-3 million xenobiotics in their lifetimes. Most of these chemicals that gain access to the body via the diet, air, drinking water, drug administration, and lifestyle choices, undergo a broad range of processes of detoxication that in general render them less toxic, more polar, and readily excretable.

(Soucek, 2011). From a metabolism viewpoint, they can be defined as *chemicals that are extrinsic to the normal metabolism of a living organism*

TYPES OF XENOBIOTICS

1. Endogenous: Pigments, hormone etc.
2. Non-endogenous: Such as drugs, food additives, pollutants, toxin, etc.
3. Drugs . Food constituents devoid of physiological roles . Food additives (preservatives, coloring and flavoring agents, antioxidants, etc.) . Chemicals of leisure, pleasure, or abuse (ethanol, coffee and tobacco constituents, hallucinogens, doping agents, etc.) . Agrochemicals (fertilizers, insecticides, herbicides, etc.) . Industrial and technical chemicals (solvents, dyes, monomers, polymers, etc.) . Pollutants of natural origin (radon, sulfur dioxide, hydrocarbons, etc.) . Pollutants produced by microbial contamination (e.g., aflatoxins) . Pollutants produced by physical or chemical transformation of natural compounds (polycyclic aromatic hydrocarbons by burning, Maillard reaction products by heating, etc.)

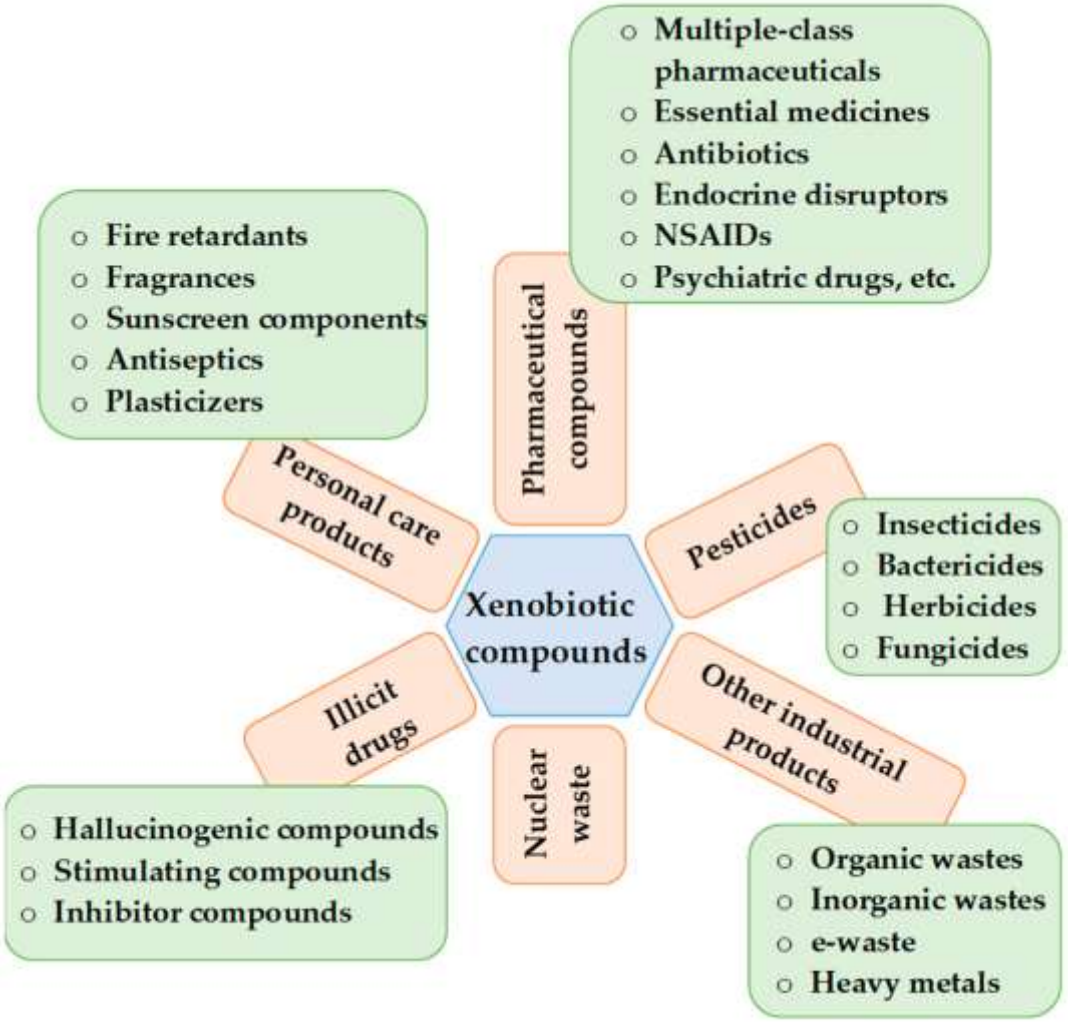
TYPES OF XENOBIOTICS

Exogenous:

- Drugs
- Food additives
- Pollutants
- Insecticides
- Carcinogens

Endogenous:

- Bilirubin,
- Bile acids
- Steroids



SOURCES OF XENOBIOTICS

the term ‘source’ is taken to mean a potential xenobiotic emission source. As such, the term covers a myriad of different commodities, facilities, and processes (activities) which have the potential to release xenobiotics into the wider environment, and which may therefore have implications for the urban water cycle. For the purposes of monitoring and management, these sources are often divided into subcategories such as point and diffuse sources, mobile and transient sources, natural and anthropogenic sources and so on.

1.Not all sources of a particular substance are associated with the deliberate use of
that substance and

2. Not all uses of a potential pollutant will necessarily result in its release

As evidence for the first of these statements, consider the combustion of wood for household heating. This is a common source of air pollution, emitting a range of pollutants including polyaromatic hydrocarbons (PAHs), volatile organic compounds (e.g. aldehydes), and metals (see, for example, Bonvalot et al. 2000; Hedberg et al. 2002) and yet it is very clear that the process of burning wood has nothing to do with the deliberate use of these substances.

Continuous sources vs. intermittent sources ·
This category is based on the pollutant release pattern from a particular source type.
Traffic in a city centre or effluent release from a municipal sewage treatment plant are examples of relatively continuous sources, whereas a forest fire (whether deliberatel it or accidental) is only an occasional source. In
fact, release patterns may actually show a characteristic daily, weekly, monthly or annual pattern,
Intentional vs. unintentional ·
This distinction is not intended to indicate that any release of pollution to the environment should be intentional, but rather that the use/release of a substance in a process/article is deliberate (e.g. biocide application) rather than accidental (e.g. due to spilling, or unintentional leaching), without purpose (e.g. by-products released from incomplete combustion), or even unrecognised (e.g. formation of a hazardous degradation product). In this case, the substance use is applied as an indicator of the type of source
Restricted vs. unrestricted (regulated vs. unregulated) ·
Some sources are more suited to regulatory control than others. For example, large industrial sources may require emission permits and/or have emission limits imposed, whereas household sources are much harder to control in this manner. Nevertheless, it is important to recognise that the cumulative effect of many small unregulated sources together may actually be greater than that of larger more restricted sources

Mobile vs. stationary sources ·
A mobile source is used to refer to a moving source of pollution such as an aeroplane, car, or ship, whereas a stationary source is easily demonstrated by an industrial facility. Although these categories may seem to overlap somewhat with point and diffuse sources they are not always the same. For example, many small stationary sources (e.g. households) in the same source area would be considered as a diffuse source, even though they are stationary
Natural vs. anthropogenic ·
Anthropogenic emission sources are clearly exemplified by any type of industrial activity releasing pollutants, whereas a natural source could be a forest fire, a volcano, or even an area of soil with naturally high metal concentrations
Receiving compartment (e.g. soil, water, air) ·
A single source can release pollutants to one or more different environmental compartments (see Fig. 2.1). The compartments most commonly used in emissions inventories are air and water. However, a range of more specific compartments may also be used to facilitate modeling applications for investigating substance fate
For example, major environmental compartments could be represented as air, surface water, groundwater, permeable surface, and impermeable surface. The distinc-

Indoor vs. outdoor source ·
The indoor vs. outdoor categories can be used simply to divide sources into those releasing substances within a confined space (i.e. indoors) and those releasing sub-
stances directly into the outdoor environment. Both indoor and outdoor sources may release to a variety of environmental com-
partments (i.e. water, air, etc.)

Fast vs. slow release ·
The time scale refers to the speed with which the substance is released from the source. Examples are the fast dispersal of anti-freeze substances due to the applica-
tion of de-icers, as opposed to the slow release of plasticisers from shoes due to wear and tear throughout the service life. The release time scale can be established
by considering the time from start up of the process/article use, until the time when
the substance can be detected in the ambient environment
Point sources vs. nonpoint sources (i.e. diffuse pollution) ·
Point source pollution comes from a single specific site and is generally used to refer to relatively large sources such as a factory smokestack or industrial wastewa-
ter outlet. The European Environment Agency (EEA) defines a point source as “a stationary location or fixed facility from which pollutants are discharged; any single

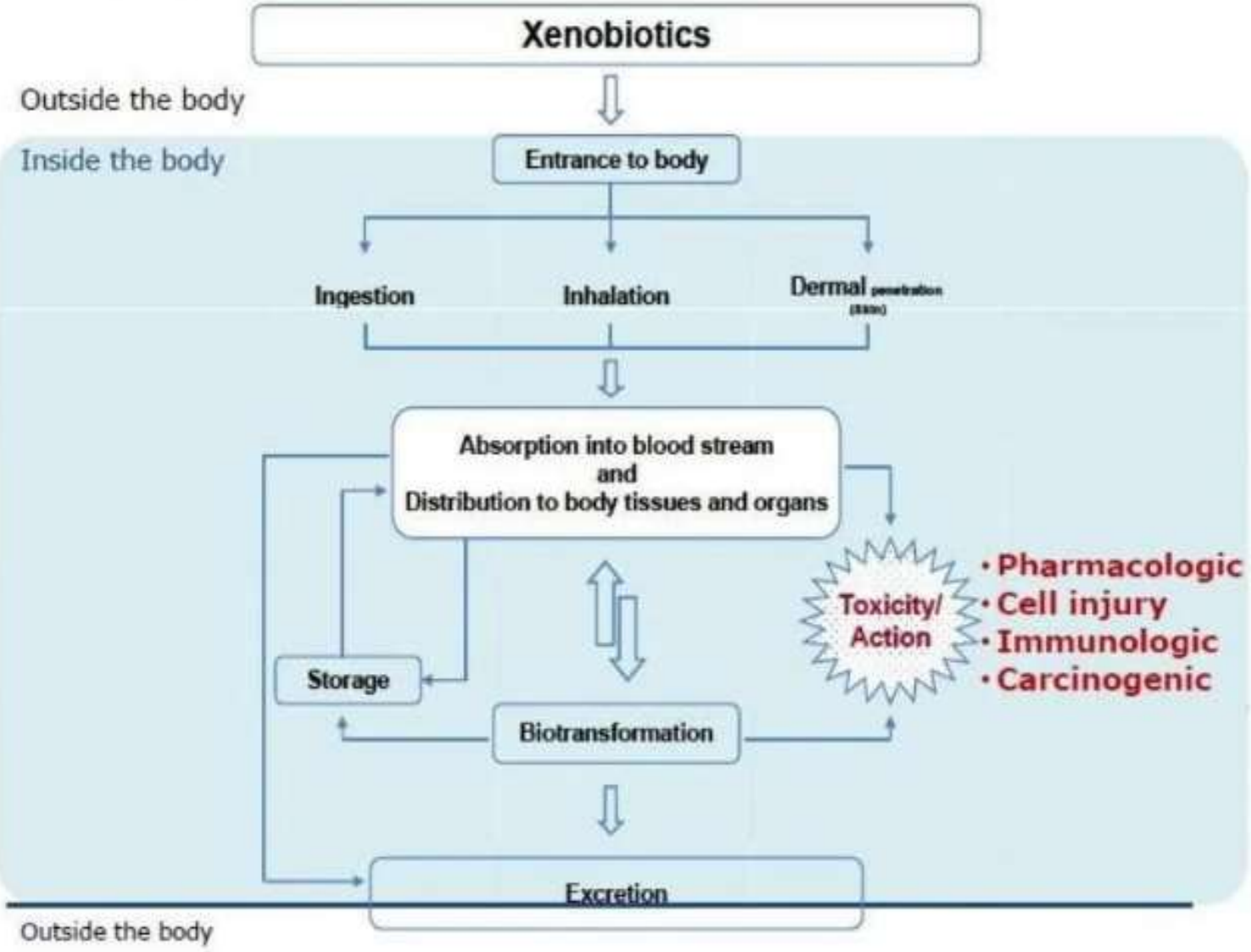
FATE OF XENOBIOTICS (XENOBIOTIC METABOLISM)

A knowledge of the fate of a drug, its disposition (absorption, distribution, metabolism, and excretion, known by the acronym ADME) and pharmacokinetics (the mathematical description of the rates of these processes and of concentration-time relationships), plays a central role throughout pharmaceutical research and development.

Pharmacokinetics is the study of drug absorption, distribution, metabolism, and excretion (Figure 46-1). A fundamental concept in pharmacokinetics is drug clearance, that is, elimination of drugs from the body,

The main difference between pharmacokinetics and pharmacodynamics is that **pharmacokinetics (PK) is defined as the movement of drugs through the body, whereas pharmacodynamics (PD) is defined as the body's biological response to drugs**.

Fate of Xenobiotics



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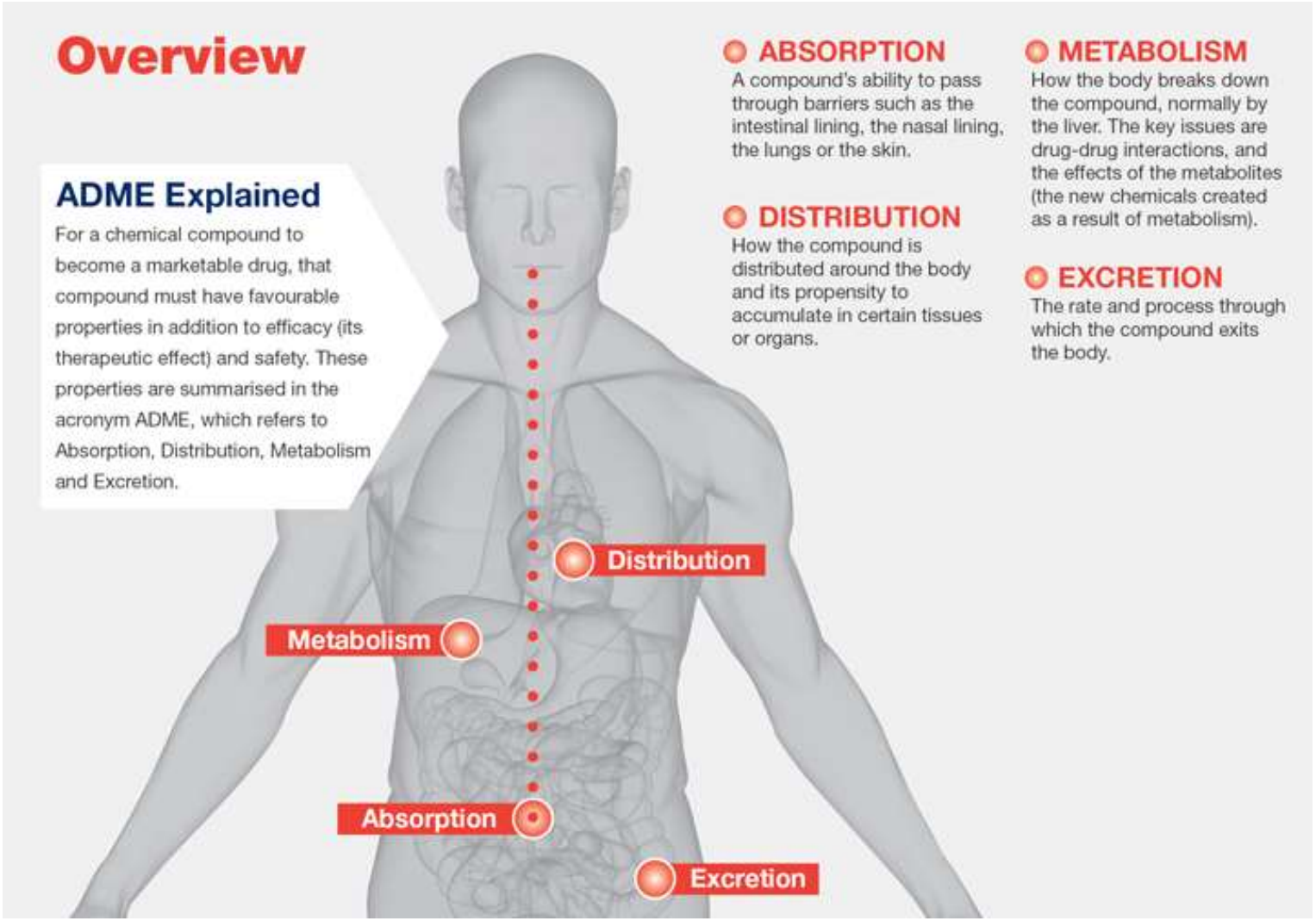
A knowledge of the fate of a drug, its disposition (absorption, distribution, metabolism, and excretion) is known as ADME.



Absorption

Absorption is the process that brings a drug from the administration, e.g., tablet, capsule, into the systemic circulation. Absorption affects the speed and concentration at which a drug may arrive at its desired location of effect, e.g., plasma. There are many possible methods of drug administration, including but not limited to oral, intravenous, intramuscular, intrathecal, subcutaneous, buccal, rectal, vaginal, ocular, otic, inhaled, nebulized, and transdermal. Each of these methods has its own absorption characteristics, advantages, and disadvantages.

Bioavailability

Bioavailability is the fraction of the originally administered drug that arrives in systemic circulation and depends on the properties of the substance and the mode of administration. It can be a direct reflection of medication absorption. For example, when administering medication intravenously, 100% of the drug arrives in circulation virtually instantly, giving this method a bioavailability of 100%. When oral medication is administered, it is often processed in large quantities by the liver, gut wall, or digestive enzymes, subsequently lowering the amount of medication that arrives in circulation; therefore, having a lower bioavailability

First-Pass Metabolism

First-pass metabolism (or **first-pass effect**) is the phenomenon in which the **concentration of the drug is reduced after absorption** but before it reaches the systemic circulation, thereby **lowering bioavailability.**

After absorption, the portal blood carries the drug to the liver. The **drug may undergo metabolism in the liver** (or even in the portal blood or gut wall itself); in addition, the drug can be excreted into the bile.

Transportation of drugs

A drug is absorbed from the site of administration (e.g., the gastrointestinal tract) by **passive diffusion** or **active transportation**, depending on the physicochemical properties of the drug.

For example, small lipid- or water-soluble drugs can move across the plasma membrane (through the membrane bilayers or aqueous channels/pores, respectively) from areas of high concentration to the low concentration. This is called **simple passive diffusion**. Some drugs require specialized transmembrane carrier proteins to aid their passage across the cell membrane. This is called **facilitated diffusion**. This process does not need energy and the drug molecule is not transported against the concentration gradient.

Some drugs need to be actively transported across the cell membrane by a carrier protein, a process that uses energy (usually in the form of ATP) and can be transported against a concentration gradient mostly in specific areas in the small intestine. This is called **active transport**. This usually occurs for drugs that are structurally similar to endogenous molecules, such as vitamins, sugars, and amino acids.

Drugs of very large sizes mainly protein drugs are transported by engulfment of the drug molecule by the cell membrane, which is transported inside the cell; this process is called **endocytosis**. This vesicle can either be used in the cell (e.g., iron), pushed across the cell (e.g., vitamin B12), or stored for later use (e.g., neurotransmitters).

All pharmacokinetic processes involve transport of the drug across the cell membrane.

Distribution

Distribution describes how a substance is spread throughout the body. This varies based on the biochemical properties of the drug as well as the physiology of the individual taking that medication. In its simplest sense, the distribution may be influenced by two main factors: diffusion and convection.[3] These factors may be influenced by the polarity, size, or binding abilities of the drug, the fluid status of the patient (hydration and protein concentrations), or the body habitus of the individual.[4] The goal of the distribution is to achieve what is known as the effective drug concentration. This is the concentration of the drug at its designed receptor site

Volume of Distribution (Vd)

This metric is a common method of describing the dissemination of a drug. It is defined as the amount of drug in the body divided by the plasma drug concentration. a small lipophilic molecule, such as chloroquine (Vd = 140 L/kg), would have a very large Vd as it can distribute throughout cells and into adipose tissues. When a molecule is very large, charged, or primarily protein-bound in circulation, such as the GnRH antagonist cetrorelix (Vd = 0.39 L/kg), it stays intravascular, unable to diffuse, reflected by a low Vd. Knowledge of the volume of distribution is an important factor for a practitioner to understand dosing schemes.

After entering the systemic circulating, a drug is distributed and eliminated, often at the same time. The body has a multi-fluid compartment structure, and the drug can be distributed in any or all of those compartments:

•**Central compartment:** Organs with good blood flow (e.g., brain, heart, kidney)

•**Peripheral compartment:** Tissues with lesser blood flow (e.g., fat and muscle tissues); drugs that are stored in fat, for example, take a long time to equilibrate and the tissues can act as a reservoir, e.g., thiopental sodium.

Drugs can be bound to plasma proteins. Only the **free (unbound) drug** is available to act on the cell membranes of the target tissues. Protein binding must be taken into account when interpreting the concentration of a drug in the blood.

Key points of protein binding

•Protein-bound drug is pharmacodynamically inert, neither metabolized nor excreted.

•Drugs bound to proteins through weak chemical bonds → reversible process.

•Irreversible binding of drugs can result in toxicity (e.g., irreversible binding of paracetamol metabolites to the hepatocytes).

•Most drugs bind to human serum albumin (HSA); it's also the most abundant plasma protein.

Barriers to the distribution of drugs

•CSF barrier

•Blood-brain barrier

•Placental barrier

•Blood-testis barrier

Metabolism

Metabolism is the processing of the drug by the body into subsequent compounds. This is often used to convert the drug into more water-soluble substances that will progress to renal clearance. Different strategies of metabolism may occur in multiple areas throughout the body, such as the gastrointestinal tract, skin, plasma, kidneys, or lungs, but the majority of metabolism is through phase I (CYP450) and phase II (UGT) reactions in the liver. Phase I reactions generally transform substances into polar metabolites by oxidation allowing conjugation reactions of Phase II to take place.[2] Most commonly, these processes inactivate the drug, convert it into a more hydrophilic metabolite, and allow it to be excreted in the urine or bile.

Excretion is the process by which the drug is eliminated from the body. The kidneys most commonly conduct excretion, but for certain drugs, it may be via the lungs, skin, or gastrointestinal tract. In the kidneys, drugs may be cleared by passive filtration in the glomerulus or secretion in the tubules.

Clearance

Clearance is an essential term when examining excretion. It is defined as the ratio of the elimination rate of a drug to the plasma drug concentration. This is influenced by the drug, blood flow, and organ status (usually kidneys) of the patient.

Half-life (t)

The half-life is the amount of time for serum drug concentrations to decrease by 50%. Defined by the equation

t=(0.693 X Vd)/Clearance,

it is directly proportional to the volume of distribution and inversely to clearance. Half-life (*t*1/2) is the **time required for the plasma concentration to reduce the amount of drug in the body by 50%.** Half-life is expressed in minutes or hours**.** Thus, after two half-lifes, 25% of the drug is left; after three, 12.5%; and after 4 half-lives, 6.25%.

The half-life determines the length of the drug's effect

Routes of drug excretion

- Renal excretion – drugs are filtered, secreted and reabsorbed by the kidneys (Nonsteroidal Anti-inflammatory Drugs)
- Biliary excretion (cardiac glycosides, rifampicin, chlorpromazine)
- Pulmonary excretion (halothane)
- Salivary (caffeine, theophylline, phenytoin, carbamazepine)
- Dermal (salicylic acid)
- Swat (heavy metals)
- Mammary (diazepam, nicotine, tetracycline, morphine, barbiturates)
- Gastrointestinal (quinine)
- Genital (ciprofloxacin)

PHASES OF XENOBIOTIC METABOLISM (BIOTRANSFORMATION OR DETOXIFICATION)

All the biochemical reactions involved in the conversion of foreign, toxic, and water insoluble molecules to non-toxic, water soluble and excretable forms are called as Detoxification or Biotransformation reactions. The purpose of which is to increase their water solubility (polarity) and thus excretion from the body.

In some cases, these reactions may instead increase the toxicity of a foreign compound, then these reactions are called as Entoxification reactions.

Conversion of lipophilic xenobiotics to water-soluble chemicals by a process catalyzed by enzymes in the liver and other tissues. In most cases, biotransformation lessens the toxicity of xenobiotics, but many must undergo the process to exert their toxic effects.

Purpose of biotransformation

1. facilitates excretion: To Converts lipophilic to hydrophilic compounds. 2. Detoxification/inactivation: To converts chemicals to less toxic forms 3. Metabolic activation: To converts chemicals to more toxic active forms

General Metabolic Pathways: Liver is the main site for biotransformation. Approximately 30 different enzymes catalyze reactions involved in xenobiotic metabolism; however, this note will only cover a selected group of them.

It is convenient to consider the metabolism of xenobiotics in two phases.

phase Ⅰ and phase II. Phase I: The reactions of Phase I are oxidation, reduction and hydrolysis. Reactions of functionalization comprise oxidations (electron removal, dehydrogenation, and oxygenation), reductions (electron addition, hydrogenation, and removal of oxygen), and hydrations/dehydrations (hydrolysis and addition or removal of water). hese reactions serve to convert lipophilic drugs into more polar molecules by adding or exposing a polar functional group such as -NH2 or -OH. These reactions also often create active metabolites and which is beneficial in activating prodrugs into their active and therapeutic state.

Phase II: These are the conjugation reactions, involving compounds such as glucuronic acid, amino acids (glycine), glutathione, sulfate, acetate and methyl group. Generally, detoxification of a compound involves phase I as well as phase II reactions. For instance, oxidation followed by conjugation is the most frequent process in the metabolism of xenobiotics. In phase I reactions, the functional groups (such as -OH, -SH, NH2, -COOH) of xenobiotics are either added or exposed. These include simple reactions such as oxidation, reduction and hydrolysis. Phase-II consist of relatively complex conjugation and synthetic reactions.

P450 cycle

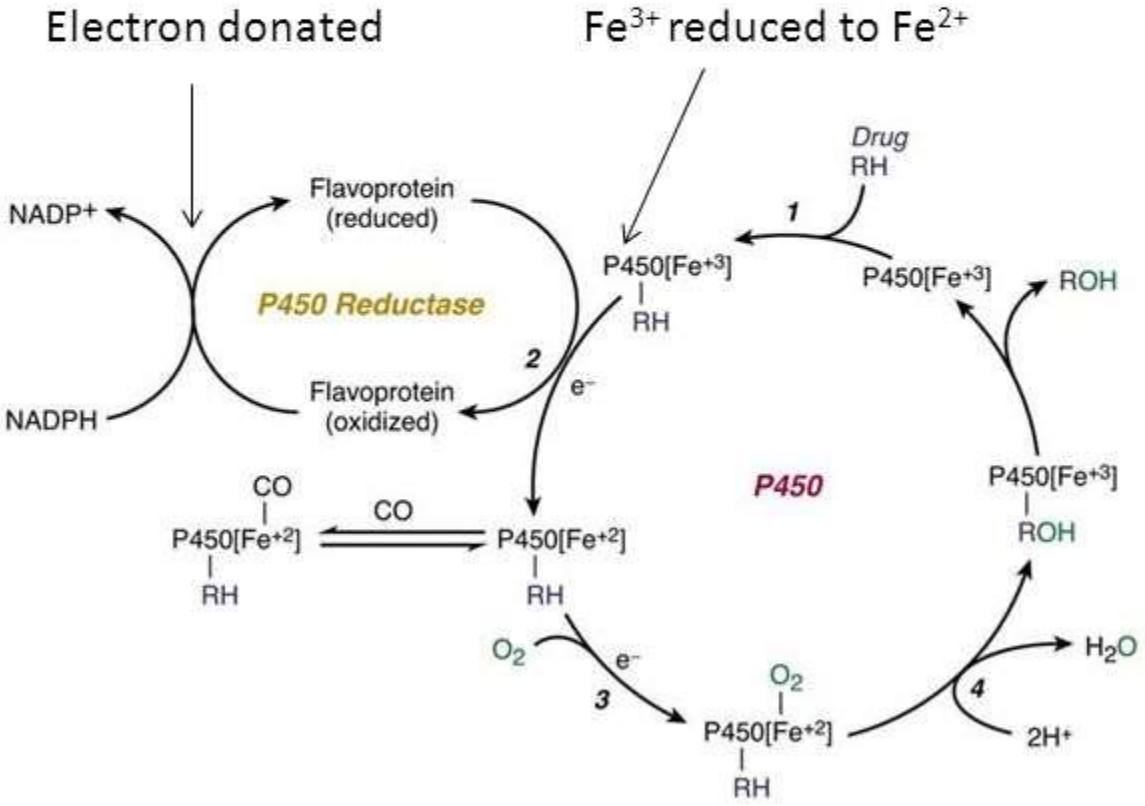
Drug + O_2 + NADPH + H^+ ⟶ Drug-OH + H_2O + $NADP^+$

Microsomal drug oxidations require:

1. P450
2. P450 reductase
3. NADPH
4. Molecular oxygen

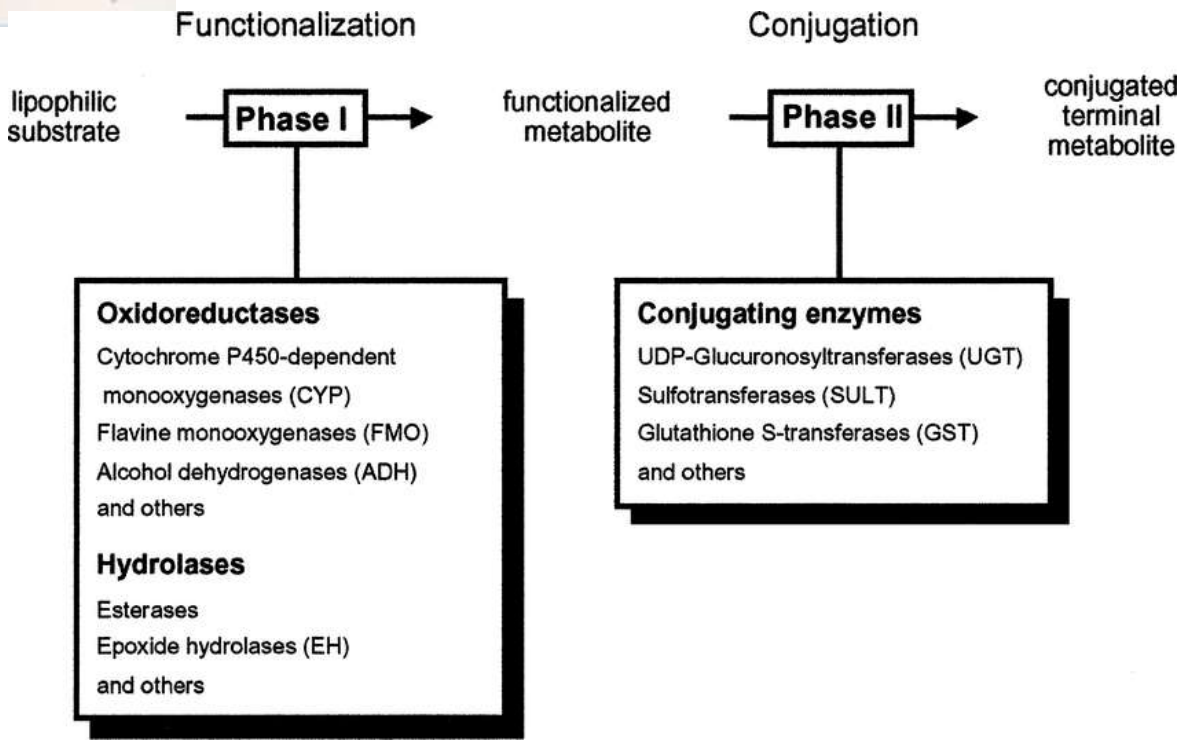
Steps of P450 mediated oxidation:

1. Oxidized P450 binds with drug to form a complex
2. P450 reductase reduces the P450/drug complex
3. P450 reductase reduces molecular oxygen to form an "activated oxygen"-P450/drug complex
4. Activated oxygen is transferred to drug to form oxidized product
5. One molecule of water is produced



Phases of Metabolism

- **Phase I**
  - Functionalization reactions
  - Converts the parent drug to a more polar metabolite by introducing or unmasking a functional group (-OH, -NH2, -SH).
- **Phase II**
  - Conjugation reactions
  - Subsequent reaction in which a covalent linkage is formed between a functional group on the parent compound or Phase I metabolite and an endogenous substrate such as glucuronic acid, sulfate, acetate, or an amino acid



Phase I reactions are very important and predominant reactions in the biotransformation of toxicants and are catalyzed by enzyme systems, such as cytochrome P-450 and cytochrome P-450 reductase and these enzyme systems are termed as mixed function oxidase (MFO) system. It is also termed as monooxygenases because they catalyze the incorporation of one atom of molecular oxygen into the substrate. But, the term mixed function oxidase is preferred. Some other hydrolytic enzymes, such as esterases and amidases, also expose the functional groups of xenobiotics. Hydrolysis, reduction, and oxidation reactions expose or introduce a functional group (—OH, —NH_2, —SH, or —COOH), and usually result in only a small increase in hydrophilicity.

A number of research workers have suggested the mixed function oxidation of xenobiotics by the system consisted of cytochrome P-450, NADPH-cytochrome P-450 reductase and phosphatidylcholine. This system oxidizes xenobiotics in the presence of NADPH and oxygen,

(i) Cytochrome P-450 system The most important enzyme system of microsomal mixed function oxidase is cytochrome P-450. It is a coupled enzyme system consisted of: (a) a haeme containing enzyme, cytochrome P-450, and (b) NADPH-cytochrome P-450 reductase, which prefers NADPH as its cofactor. Cytochrome P-450 is a haemoprotein of cytochrome b5 type with unique redox potential and is named from the unique wave-length of absorption maximum (at 450 nm) o At least 10 different forms of cytochrome P-450 have been isolated from rat liver microsomes

(ii) (ii) Cytochrome b5 system Associated with cytochrome P-450 system, there is another cytochrome system consisted of cytochrome b5 and cytochrome b5 reductase,

(iii) (iii) Mixed function amine oxidase Another important oxidative enzyme is referred to as mixed function amine oxidase. It is also present in endoplasmic reticulum and oxidizes nucleophilic nitrogen and sulphur atoms

Phase I / Non Synthetic Reactions

Oxidation

- Addition of oxygen/ negatively charged radical or removal of hydrogen/ positvely charged radical.
- Reactions are carried out by group of mono-oxygenases in the liver.
- Fianl step: Involves cytochrome P-450 haemoprotein, NADPH, cytochrome P-450 reductase and O2

Non-CYP Drug Oxidations

- Monoamine Oxidase (MAO), Diamine Oxidase (DAO)
  - MAO (mitochondrial) oxidatively deaminates endogenous substrates including neurotransmitters
  - Dopamine, serotonin, norepinephrine, epinephrine
- Alcohol & Aldehyde Dehydrogenase
  - Non-specific enzymes found in soluble fraction of liver
  - Ethanol metabolism
- Flavin Monooxygenases
  - Require molecular oxygen, NADPH, flavin adenosine dinucleotide (FAD)

oxidation:

The cytochrome P450 (CYP) system ranks first in terms of catalytic versatility and the sheer number of xenobiotics it detoxifies or activates. Cytochromes p450 (CYPs) are a superfamily of enzymes containing heme as a cofactor that function as monooxygenases. In mammals, these proteins oxidize steroids, fatty acids and xenobiotics and are important for the clearance of various compounds, as well as for hormone synthesis and breakdown. Cytochrome P450 enzymes are primarily found in liver cells but are also located in cells throughout the body. Within cells, cytochrome P450 enzymes are located in a structure involved in protein processing and transport (endoplasmic reticulum) and the energyproducing centers of cells (mitochondria).

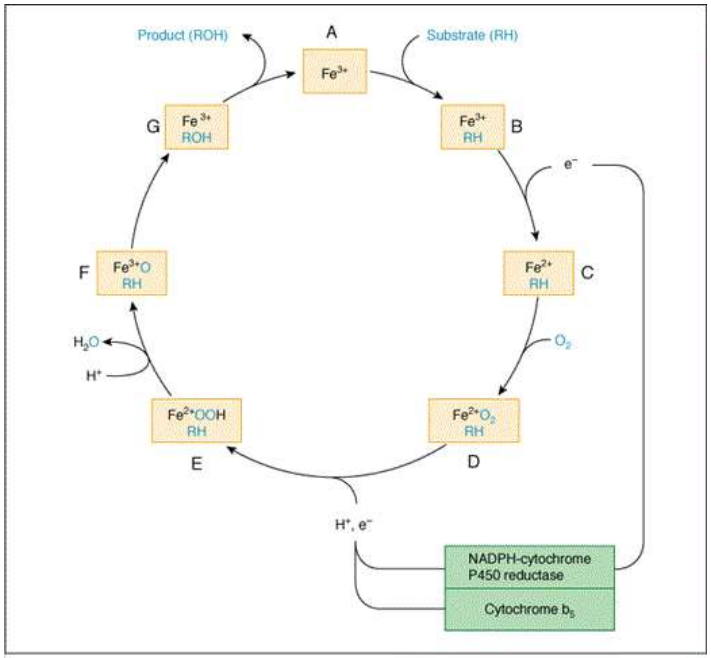
All CYP enzymes are heme-containing proteins that catalyze the monooxygenation of one atom of oxygen into a substrate, and the other oxygen atom is reduced to water with reducing equivalents derived from NADPH. During catalysis, CYP does not interact directly with NADPH or NADH. In the endoplasmic reticulum, electrons are relayed from NADPH to cytochrome P450 via a flavoprotein called NADPH–cytochrome P450 reductase. In mitochondria, electrons are transferred from NADPH to CYP via ferredoxin and ferredoxin reductase.

Salient features of cytochrome P450 :

Multiple forms of cytochrome P450 are believed to exist, ranging from 20 to 200. Atleast 6 species have been isolated and worked in detail. They are all hemoproteins, containing heme as the prosthetic group. Cytochrome P 450 species are found in the highest concentration in the microsomes of liver. In the adrenal gland, they occur in mitochondria. The mechanism of action of Cytochrome p450 is complex and is dependent on NADPH. The Cytochrome p450 is an inducible enzyme. Molecular mass is about 55 kDa. Exihibit broad substrate specificity. Cause introduction of one atom of oxygen into the substrate and one intowater. The hydroxylated products are more water soluble

NADPH-cytochrome P450 reductase is a flavin-containing enzyme, consisting of one mole of FAD (flavin adenine dinucleotide) and one mole of FMN (flavin mononucleotide) per mole of apoprotein; this is quite unusual as most other flavoproteins contain only FAD or FMN as their prosthetic group. The enzyme exists in close association with cytochrome P450 i

As shown in Figure 6–4, the first part of the catalytic cycle involves the activation of oxygen, and the final part involves substrate oxidation, which entails the abstraction of a hydrogen atom or an electron from the substrate followed by oxygen rebound (radical recombination). Following the binding of substrate to the CYP enzyme, the heme iron is reduced from the ferric (Fe^{3+}) to the ferrous (Fe^{2+}) state by the addition of a single electron from NADPH–cytochrome P450 reductase. Release of the oxidized substrate returns cytochrome P450 to its initial state. If the catalytic cycle is interrupted, oxygen is released as superoxide anion (O_2^-) or hydrogen peroxide (H_2O_2).



Other reactions

One-electron reduction	**C** (Fe^{2+} RH)	⟶	**A** (Fe^{3+}) + $RH^{\cdot}$
Superoxide anion production	**D** (Fe^{2+} O_2RH)	⟶	**B** (Fe^{3+} RH) + $O_2^{\cdot}$
Hydrogen peroxide production	**E** (Fe^{2+} OOH RH) + H^+	⟶	**B** (Fe^{3+} RH) + H_2O_2
Peroxide shunt	**B** (Fe^{3+} RH) + XOOH	⟶	**F** $(FeO)^{3+}$ RH + XOH

Cytochrome P450 catalyzes the following types of oxidation reactions:

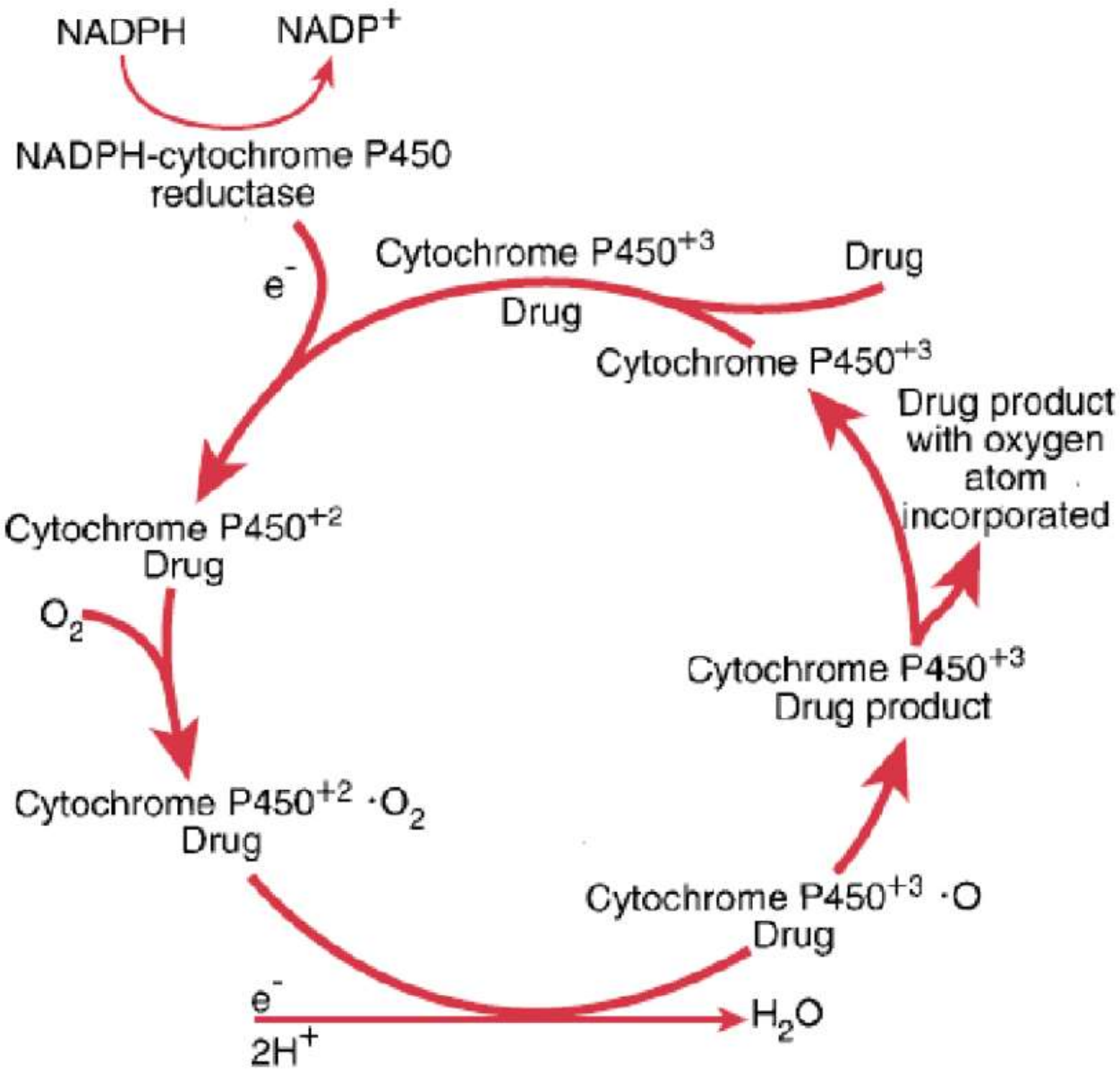
1. hydroxylation of an aliphatic or aromatic carbon;
2. epoxidation of a double bond;
3. heteroatom (*S*-, *N*-, and *I*-) oxygenation and *N*-hydroxylation;
4. heteroatom (*O*-, *S*-, *N*-, and *Si*-) dealkylation;
5. oxidative group transfer;
6. cleavage of esters;
7. dehydrogenation.

The general cytochrome P450-catalysed reaction is:

cytochrome P450 NADPH + H+ + O2 + RH NADP+ + H2O + ROH

where RH represents an oxidisable drug substance and ROH, the hydroxylated metabolite. As can be seen from the above reaction, reducing equivalents (derived from NADPH + H+) are consumed and only one atom of the molecular oxygen is incorporated into the substrate (generating the hydroxylated metabolite), whereas the other oxygen atom is reduced to water (the reaction is actually a hydroxylation rather than a genuine oxidation).

+


NADPH
NADP+
NADPH-cytochrome P450 reductase
e-
Cytochrome P450+2
Drug
O2
Cytochrome P450+2 ·O2
Drug
e-
2H+
H2O
Cytochrome P450+3 ·O
Drug
Cytochrome P450+3
Drug product
Drug product with oxygen atom incorporated
Cytochrome P450+3
Drug
Cytochrome P450+3
Drug

Oxidation:
Alcohol dehydrogenase: ADH is a cytosolic enzyme present in several tissues including the liver, which has the highest levels, the kidney, the lung, and the gastric mucosa. There are five major classes of ADH. The class I ADH isozymes (α-ADH, β-ADH, and γ-ADH) are responsible for the oxidation of ethanol and other small aliphatic alcohols. Class II ADH (π-ADH) is primarily expressed in liver where it preferentially oxidizes larger aliphatic and aromatic alcohols. Long-chain alcohols (pentanol and larger) and aromatic alcohols are preferred substrates for class III ADH (χ-ADH). Class IV ADH (σ- or μ-ADH), which is not expressed in liver, is the most active of the medium-chain ADHs in oxidizing retinol. Class V ADH has no subunit designation.

Aldehyde dehydrogenase (ALDH) oxidizes aldehydes to carboxylic acids with NAD^+ as the cofactor.

Monoamine oxidases (MAO) are involved in the oxidative deamination of primary, secondary, and tertiary amines, including serotonin and a number of xenobiotics. Oxidative deamination of a primary amine produces ammonia and an aldehyde, whereas oxidative deamination of a secondary amine produces a primary amine and an aldehyde. The aldehydes formed by MAO are usually oxidized further by other enzymes to the corresponding carboxylic acids. MAO is located throughout the brain and in the outer membrane of mitochondria of the liver, kidney, intestine, and blood platelets.
The substrate is oxidized by MAO, which itself is reduced using FAD. The oxygen incorporated into the substrate is derived from water, not molecular oxygen.

Oxidative bio-transformation of xenobiotics by peroxidases couples the reduction of hydrogen peroxide and lipid hydroperoxides to the oxidation of other substrates via a process known as *cooxidation.* An important peroxidase is prostaglandin H synthetase (PHS), which possesses two catalytic activities: a *cyclooxygenase* that converts arachidonic acid to prostaglandins and a *peroxidase* that converts the hydroperoxide to the corresponding alcohol PGH_2
Cytosolic oxidoreductases are the molybdenum hydroxylases, namely aldehyde oxidase and xanthine oxidase. The large and important ensemble of dehydrogenases/reductases include alcohol dehydrogenases (ADH) that are zinc enzymes found in the cytosol of the mammalian liver and in various extrahepatic tissues. Mammalian liver alcohol dehydrogenases (LADHs) are dimeric enzymes.

Liver, kidney, intestine, brain, and lung contain one or more FAD-containing monooxygenases (FMO) that oxidize the nucleophilic nitrogen, sulfur, and phosphorus heteroatom of various xenobiotics. The mammalian FMO gene family comprises five microsomal enzymes that require NADPH and O_2, and many of the reactions catalyzed by FMO can also be catalyzed by cytochrome P450.

The mechanism of catalysis by FMO is depicted in Figure 6–3. After the FAD moiety is reduced to $FADH_2$ by NADPH, the oxidized cofactor $NADP^+$ remains bound to the enzyme. $FADH_2$ then binds oxygen to produce a relatively stable peroxide. During the oxygenation of xenobiotics, the flavin peroxide oxygen is transferred to the substrate (depicted as $X \rightarrow XO$ in Figure 6–3). The final step in the catalytic cycle involves restoration of FAD to its oxidized state and release of $NADP^+$. This final step is rate-limiting, and it occurs after substrate oxygenation.

reduction
Certain metals and xenobiotics containing an aldehyde, ketone, disulfide, sulfoxide, quinone, *N*-oxide, alkene, azo, or nitro group are often reduced in vivo. The reaction may proceed enzymatically or nonenzymatically by interaction with reducing agents, such as the reduced forms of glutathione, FAD, FMN, and NADP. Likewise, enzymes, such as alcohol dehydrogenase (ADH), aldehyde oxidase, and cytochrome P450, can catalyze both reductive and oxidative reactions depending on the substrate and conditions.
Azo- and nitro-reduction are catalyzed by intestinal microflora and under certain conditions (i.e., low oxygen tension), by two liver enzymes: cytochrome P450 and NADPH-quinone oxidoreductase (also known as DT-diaphorase). The reactions require NADPH and are inhibited by oxygen. The anaerobic environment of the lower gastrointestinal tract is well suited for azo- and nitro-reduction.
The reduction of certain aldehydes to primary alcohols and of ketones to secondary alcohols is catalyzed by NAD(P)H-dependent reductases belonging to one of the two superfamilies, the aldo-keto reductases (AKRs) and the short-chain dehydrogenases/reductases (SDRs).
Thioredoxin-dependent enzymes in liver and kidney cytosol can reduce sulfoxides, which were formed by cytochrome P450. Under reduced oxygen tension, the NADPH-dependent reduction of *N*-oxides in liver microsomes may be catalyzed by cytochrome P450 or NADPH–cytochrome P450 reductase.
Quinones can be reduced to hydroquinones by two cytosolic flavoproteins, NQO1 and NQO2, without oxygen consumption. NADPH-quinone oxidoreductase-1 (DT-diaphorase) and NADPH-quinone oxidoreductase-2 have different substrate specificities.
here are three major mechanisms for removing halogens (F, Cl, Br, and I) from aliphatic xenobiotics: (1) *reductive dehalogenation* involves replacement of a halogen with hydrogen; (2) *oxidative dehalogenation* replaces a halogen and hydrogen on the same carbon atom with oxygen; and (3) *double dehalogenation* involves the elimination of two halogens on adjacent carbon atoms to form a carbon–carbon double bond

Reduction

- Converse of oxidation
- Drugs primarily reduced are chloralhydrate, chloramphenicol, halothane.

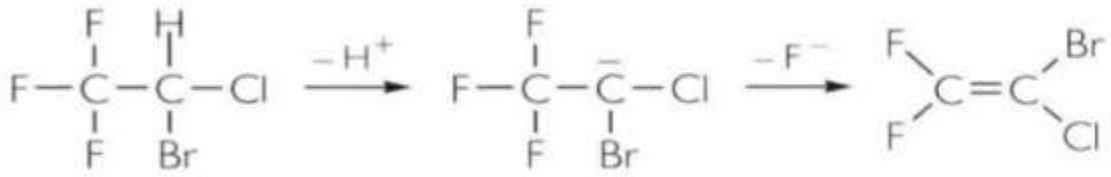


Figure 1.25 Reductive defluorination of halothane.

Actually, it represents the major route of metabolism for aromatic nitro- and nitroso-groups (as in chloramphenicol, nitroglycerine and organic nitrites), for the azo- group (as in prontosil) as well as for a wide variety of aliphatic and aromatic N-oxides. Generally, reductive processes involve two separate enzyme systems: NADPH-cytochrome P450 reductase, a ferrihemoprotein oxidoreductase, also known as NADPH- cytochrome c reductase, is considered to be the major oxidoreductase transferring electrons to microsomal cytochrome P450. The system contains one molecule each of FAD and FMN per polypeptide chain, and the NADPH (resulting from the pentose phosphate pathway), represents the preferred source of reducing equivalents.

(II) Microsomal hydrolytic enzymes

Hydrolysis occurs especially with esters and amides in reactions catalysed by various enzymes located in hepatic microsomes, kidneys and other tissues. Other compounds susceptible to such a biotransformation pathway are carbamates and hydrazides

(i) Epoxide hydrolase Epoxide hydrolase is an important hydrolytic enzyme and is believed to be located in the microsomal fractions, i.e. in close proximity of microsomal cytochrome P-450 systems. Epoxide hydrolase catalyses the hydration reactions of aliphatic and aromatic epoxides of xenobiotics; and thus inactivates a number of highly reactive epoxides. Hence, epoxide hydrolase is considered as a detoxication enzyme. Epoxide hydrolase catalyzes the *trans*-addition of water to alkene epoxides and arene oxides, and is present in virtually all tissues. It plays an important role in detoxifying electrophilic epoxides that might otherwise bind to proteins and nucleic acids and cause cellular toxicity and genetic mutations. There are five distinct forms of epoxide hydrolase in mammals: microsomal epoxide hydrolase (mEH), soluble epoxide hydrolase (sEH), cholesterol epoxide hydrolase, LTA4 hydrolase, and hepoxilin hydrolase. Epoxide hydrolase is one of the several inducible enzymes in liver microsomes.

(ii) Esterases and amidases A large number of nonspecific esterase and amidase enzymes occur in various mammalian tissues. Both of these hydrolyze ester and amide linkages of xenobiotics. The hydrolysis of carboxylic acid esters, amides, and thioesters is largely catalyzed by carboxylesterases and by two cholinesterases: true acetylcholinesterase in erythrocyte membranes and pseudocholinesterase, which is also known as butyrylcholinesterase and is located in serum. Phosphoric acid esters are hydrolyzed by paraoxonase, a serum enzyme also known as aryldialkylphosphatase. Phosphoric acid anhydrides are hydrolyzed by a related organophosphatase. The hydrolysis of xenobiotic esters and amides in humans is largely catalyzed by just two carboxylesterases called hCE1 and hCE2.

Carboxylesterases are glycoproteins that are present in serum and most tissues. Carboxylesterases hydrolyze numerous endogenous lipid compounds

Hydrolysis

- Cleavage of drug molecule by taking up a molecule of water.

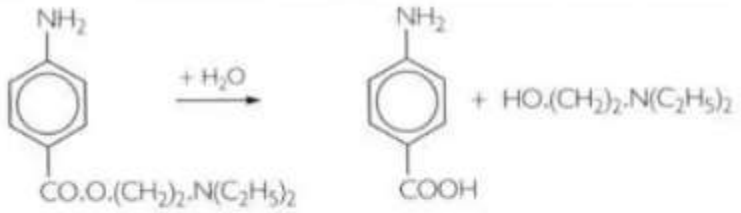


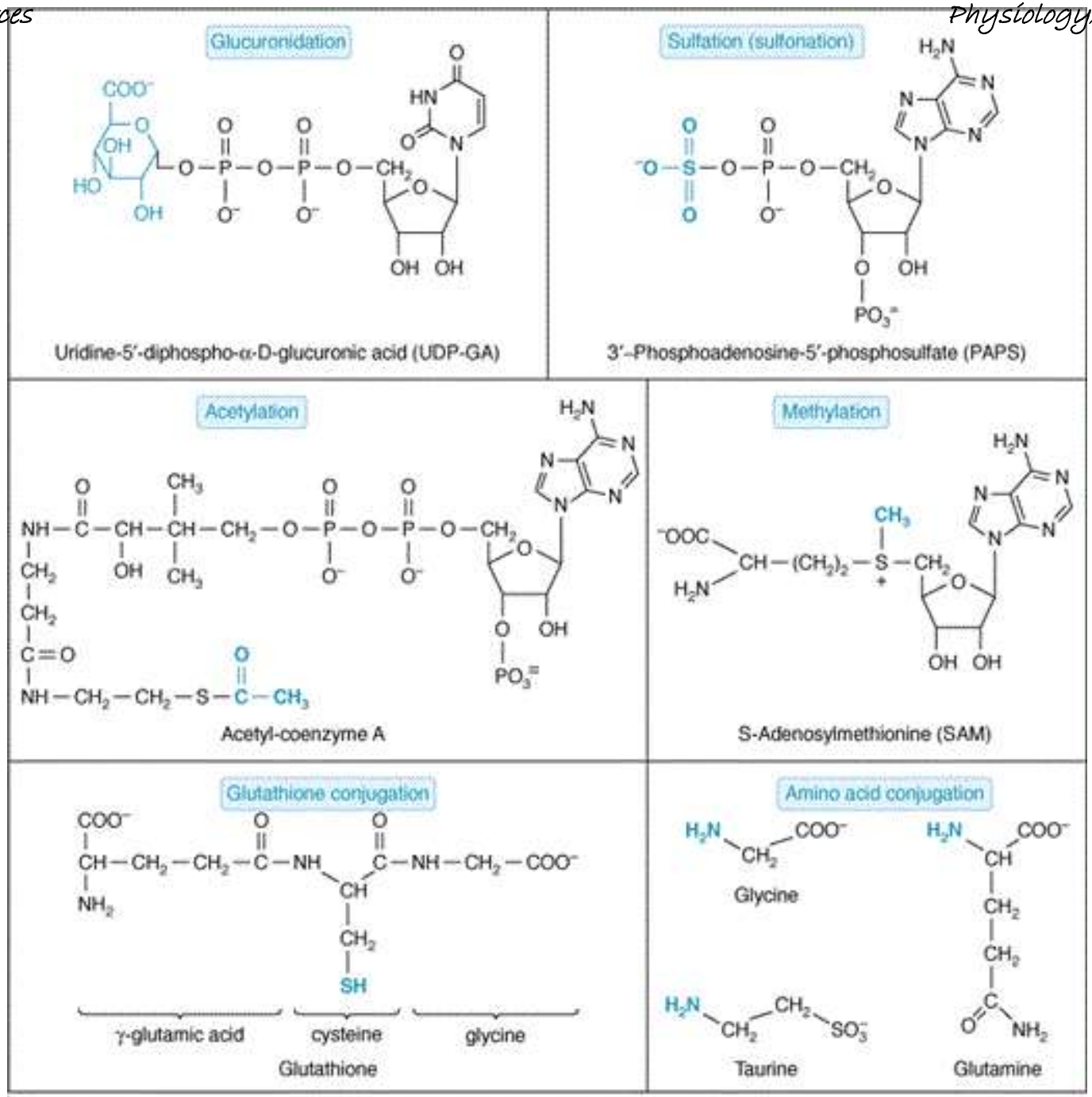
Figure 1.26 Hydrolysis of procaine.

- Sites: Liver, intestines, plasma and other tissues
- Examples: Choline esters, Procaine, Isoniazid,

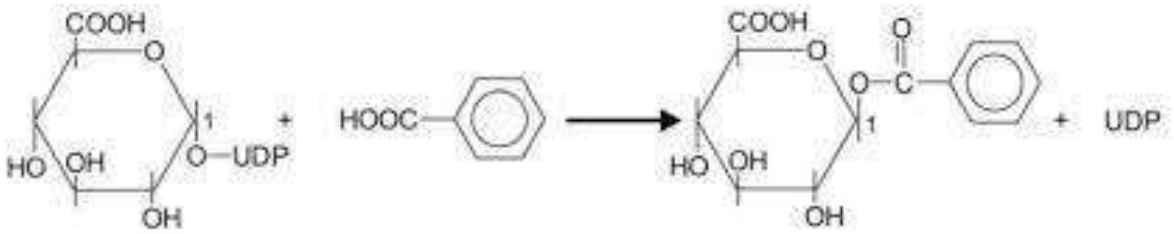
Conjugation biotransformation reactions include glucuronidation, sulfonation (more commonly called sulfation), acetylation, methylation, conjugation with glutathione (mercapturic acid synthesis), and conjugation with amino acids (such as glycine, taurine, and glutamic acid). Most result in a large increase in xenobiotic hydrophilicity; hence, they greatly promote the excretion of foreign chemicals. In these so-called 'conjugation reactions', mediated by the appropriate enzymes, the drug becomes linked to an endogenous moiety tA characteristic of most conjugation reactions is the replacement of a hydrogen atom present in a hydroxyl, amino or carboxyl group, by the conjugating agent. In general, the resulting conjugated metabolites have no pharmacological activity, are highly water-soluble and therefore subsequently readily excreted in the urine

(iii) Glutathione-S-transferases It is one of the most important enzymes of phase II biotransformation reaction. Glucuronidation represents the major route of sugar conjugation, although conjugation with xylulose and ribose are also possibleThe initial step of glutathione conjugation to xenobiotics is catalyzed by a group of enzymes called as glutathione-S-transferases. These enzymes are present both in the soluble and microsomal fractions of tissues. But, their high concentration occurs principally in the soluble fractions. These enzymes are widely distributed in the animal kingdom, for instance, in protozoans (e.g. Acanthamoeba), insects, aquatic invertebrates, fishes and mammals. Besides, they are also known to be present in certain bacteria. Various forms of glutathione-S-transferases have been reported from the liver of rats, mice and human-beings as also in insects. At least five different forms of enzyme have been reported from the soluble fraction of rat- and human liver.

With the exception of methylation and acetylation, conjugations result in a large increase in xenobiotic hydrophilicity, which greatly facilitates excretion of foreign chemicals. Glucuronidation, sulfation, acetylation, and methylation involve reactions with activated or "high-energy" cosubstrates, whereas conjugation with amino acids or glutathione involves reactions with activated xenobiotics. Except for the glucuronosyltransferases, most conjugation enzymes are mainly located in the cytosol


Glucuronidation
Uridine-5′-diphospho-α-D-glucuronic acid (UDP-GA)
Sulfation (sulfonation)
3′-Phosphoadenosine-5′-phosphosulfate (PAPS)
Acetylation
Acetyl-coenzyme A
Methylation
S-Adenosylmethionine (SAM)
Glutathione conjugation
γ-glutamic acid
cysteine
glycine
Glutathione
Amino acid conjugation
Glycine
Taurine
Glutamine

1. The glucuronidation of bilirubin is discussed in Chapter 31; xenobiotics are glucuronidated in the same way, using UDP-glucuronic acid, catalyzed by a variety of glucuronosyltransferases, present in both the endoplasmic reticulum and cytosol. Molecules such as 2-acetylaminofluorene (a carcinogen), aniline, benzoic acid, meprobamate (a tranquilizer), phenol, and many steroids are excreted as glucuronides. The glucuronide may be attached to oxygen, nitrogen, or sulfur groups of the substrates. Glucuronidation is probably the most frequent conjugation reaction. Glucuronidation requires the cosubstrate uridine diphosphate-glucuronic acid (UDP-glucuronic acid), and the reaction is catalyzed by UDP-glucuronosyltransferases (UGTs). The site of glucuronidation is generally an electron-rich nucleophilic heteroatom (O, N, or S) as found in aliphatic alcohols and phenols, carboxylic acids, primary and secondary aromatic and aliphatic amines, and free sulfhydryl groups. Endogenous substrates for glucuronidation include bilirubin, steroid hormones, and thyroid hormones. Glucuronide conjugates of xenobiotics and endogenous compounds are polar, water-soluble metabolites. Whether glucuronides are excreted from the body in bile or urine depends on the size of the aglycone (parent compound or unconjugated metabolite). The carboxylic acid moiety of glucuronic acid, which is ionized at physiologic pH, promotes excretion because (1) it increases the aqueous solubility of the xenobiotic and (2) it is recognized by the biliary and renal organic anion transport systems, which enables glucuronides to be secreted into urine and bile



2. sulfonation: Many xenobiotics and endogenous substrates undergo sulfonation. Sulfate conjugation is catalyzed by sulfotransferases, a multigene family of enzymes that generally produces a highly water-soluble sulfuric acid ester. The cosubstrate for the reaction is 3′-phosphoadenosine-5′-phosphosulfate (PAPS; see Figure 6–13).

Sulfate conjugation involves the transfer of sulfonate, not sulfate (i.e., SO_3^-, not SO_4^-) from PAPS to the xenobiotic. Sulfate conjugates of xenobiotics are excreted mainly in urine. PAPS is synthesized from inorganic sulfate (SO_4^{2-}) and ATP in a two-step reaction.

Some alcohols, arylamines, and phenols are sulfated. The **sulfate donor** in these and other biologic and sulfation reactions (eg, sulfation of steroids, glycosaminoglycans, glycolipids, and glycoproteins) is **adenosine 3′-phosphate-5′-phosphosulfate (PAPS)** (see Chapter 24) – so-called “active sulfate.”

3. Methylation, a minor pathway of biotransformation, generally decreases the water solubility of xenobiotics and masks functional groups that might otherwise be conjugated by other enzymes. Methylation can also lead to increased toxicity. The cosubstrate for methylation is *S*-adenosylmethionine (SAM). The *O*-methylation of phenols and catechols is catalyzed by two different enzymes known as phenol *O*-methyltransferase (POMT) in microsomes and catechol-*O*-methyltransferase (COMT) in cytosol and microsomes.

O-and N-methylation are common biochemical reactions but appear to be of greater significance in the metabolism of endogenous compounds than for drugs or other xenobiotics Reaction results mainly in the formation of O-methylated, N- methylated, and S-methylated products.

The process of O-methylation is catalysed by a magnesium-dependent enzyme, generically designated as catechol-O-methyltransferase (COMT). The reaction involves the transfer of a methyl group to either the meta- or less frequently, to the para-phenolic hydroxyl group of catecholamines,

The N-methylation of various amines is among several conjugate pathways for metabolising amines. The transfer of active methyl groups from SAM to the acceptor substrate is catalysed by specific N-methyltransferases. There are three important N-methyltransferases, namely: histamine N-methyltransferase (HMT), a cytoplasmic enzyme that methylates histamine, phenylethanolamine N-methyltransferase (PNMT), requiring the presence of phenylethanolamine compounds as substrate acceptors and amine-N-methyltransferases (also known as indolethylamine N-methyltransferases), which catalyse the transfer of a methyl group from SAM to the amino group of indoleamines.

Other substrates for methylation reactions include thiols, which are generally considered as toxic. Thiol S-methyl transferases thus play a role among other detoxication pathways for these compounds. thiol-methyl transferase (TMT), thioether-S-methyltransferase (TEMT), and • the soluble thiopurine-methyl transferase (TPMT)

4. acetylation:

N-Acetylation is a major route of biotransformation for xenobiotics containing an aromatic amine ($R—NH_2$) or a hydrazine group ($R—NH—NH_2$), which are converted to aromatic amides ($R—NH—COCH_3$) and hydrazides ($R—NH—NH—COCH_3$), respectively. *N*-Acetylation masks an amine with a nonionizable group, so that many *N*-acetylated metabolites are less water soluble than the parent compound. Nevertheless, *N*-acetylation of certain xenobiotics, such as isoniazid, facilitates their urinary excretion.

Xenobiotic *N*-acetylation catalyzed by cytosolic *N*-acetyltransferases requires the cosubstrate acetyl-coenzyme A (acetyl-CoA; Figure 6–13). The two-step reaction involves: (1) transfer of the acetyl group from acetyl-CoA to an active site cysteine residue within the enzyme with release of coenzyme A and (2) subsequent transfer of the acetyl group from the acylated enzyme to the amino group of the substrate with regeneration of the enzyme. As for other acetylation reactions, **acetyl-CoA** is the acetyl donor. These reactions are catalyzed by **acetyltransferases** present in the cytosol of various tissues, particularly liver. The drug **isoniazid**, used in the treatment of tuberculosis, is subject to acetylation Acetylation is a Phase II reaction of amino groups and it involves the transfer of acetyl-coenzyme A (acetyl CoA) to an aromatic primary or aliphatic amine, amino acid, hydrazine, or sulphonamide group. The primary site of acetylation is the liver, although extrahepatic sites have been identified as well (e.g. spleen, lung and gut). Acetylation reactions require a specific co-factor, acetyl-CoA

5. Aminoacid conjugation:

Two principal pathways by which xenobiotics are conjugated with amino acids are illustrated in Figure 6–18. The first involves conjugation of xenobiotics containing a carboxylic acid group with the amino group of amino acids such as glycine, glutamine, and taurine (see Figure 6–13). After activation of the xenobiotic by conjugation with CoA, the acyl-CoA thioether reacts with the *amino group* of an amino acid to form an amide linkage. The second pathway involves conjugation of xenobiotics containing an aromatic hydroxylamine with the *carboxylic acid group* of such amino acids as serine and proline. This pathway involves activation of an amino acid by aminoacyl-tRNA synthetase, which reacts with an aromatic hydroxylamine to form a reactive *N*-ester. Amino acid conjugates of xenobiotics are eliminated primarily in urine.

Such conjugation with amino acids represents an important metabolic pathway in the eventual elimination of drug (and other xenobiotic) carboxylic acids. The substrates may be aromatic, arylaliphatic, and heterocyclic carboxylic acids and the resulting metabolites are water-soluble ionic conjugates. Usually, these amino acid conjugates are less toxic then their precursor acids and are readily excreted into the urine or bile Besides glycine conjugation, other amino acids yielding conjugated metabolites are glutamine and cysteine

5. Glutathione conjugation:

Conjugation of xenobiotics with glutathione includes an enormous array of electrophilic xenobiotics, or xenobiotics that can be biotransformed to electrophiles. The tripeptide glutathione comprises of glycine, cysteine, and glutamic acid (Figure 6–13). Glutathione conjugates are thioethers, which form by nu cleophilic attack of glutathione thiolate anion (GS^-) with an electrophilic carbon, oxygen, nitrogen, or sulfur atom in the xenobiotic. This conjugation reaction is catalyzed by a family of glutathione *S*-transferases that are present in most tissues, where they are localized in the cytoplasm (>95 percent) and endoplasmic reticulum (<5 percent). Like glutathione, the glutathione *S*-transferases are themselves abundant cellular components, accounting for up to 10 percent of the total cellular protein.

As shown in Figure 6–19, substrates for glutathione conjugation can be divided into two groups: those sufficiently electrophilic to be conjugated directly and those that must first be biotransformed to an electrophilic metabolite prior to conjugation. The conjugation reactions themselves can be divided into two types: *displacement reactions,* in which glutathione displaces an electron-withdrawing group, and *additionreactions,* in which glutathione is added to an activated double bond or strained ring system.

Glutathione can also conjugate xenobiotics with an electrophilic heteroatom (*O, N,* and *S*). In each of the examples shown in Figure 6–20, the initial conjugate formed between glutathione and the heteroatom is cleaved by a second molecule of glutathione to form oxidized glutathione (GSSG). The initial reactions are catalyzed by glutathione *S*-transferase, whereas the second reaction (which leads to GSSG formation) generally occurs nonenzymatically.

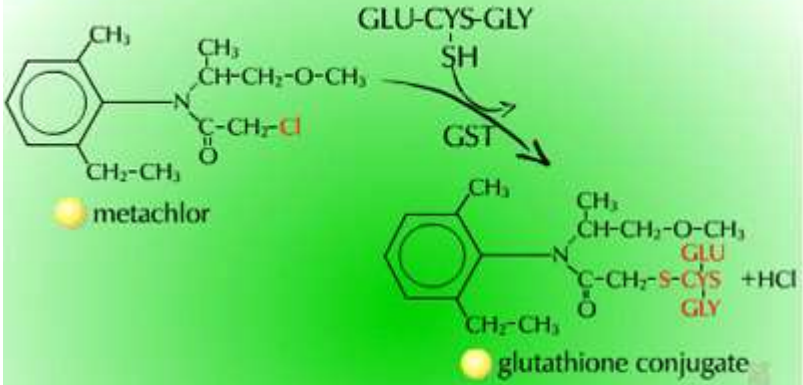
Glutathione conjugates formed in the liver can be effluxed into bile and blood, and they can be converted to mercapturic acids in the kidney and excreted in urine. As shown in Figure 6–21, the conversion of glutathione conjugates to mercapturic acids involves the sequential cleavage of glutamic acid and glycine from the glutathione moiety, followed by *N*-acetylation of the resulting cysteine conjugate.

Glutathione (GSH) is present at highest concentration in the liver, with higher values in the cortex than in the medulla, but is also present in cytosol, mitochondria and nucleus [29,30]. In the blood, it is present at a relative concentration of about 20 μM. Glutathione [N-(N-L-γ-glutamyl-L-cysteinyl)glycine], an atypical tripeptide (Figure 3.10), is an endogenous compound, recognized as playing a protective role within the body in removal of potentially toxic electrophilic compounds. GSH conjugation involves the formation of a thioether link between the GSH and electrophilic compounds. The reaction can be considered as the result of nucleophilic attack by GSH on electrophilic carbon atoms, Thus, conjugation with glutathione usually results in detoxication of the electrophilic compounds by preventing their reaction with nucleophilic centres in macromolecules such as proteins and nucleic acids

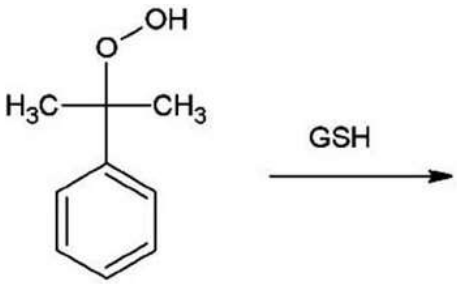
Glutathione conjugation

Glutathione (GSH is triptide of the three amino acids glutamate, cysteine and glycine (GLU-CYS-GLY) attached through peptide bonds.

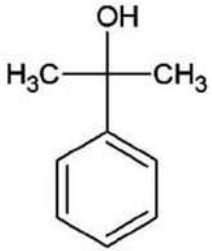
Reduced glutathione
(γ-Glutamylcysteinylglycine)
(GLU–CYS–GLY)
SH



The glutathione moves into the vacuole via a membrane protein which recognizes and transports the GSH conjugate.



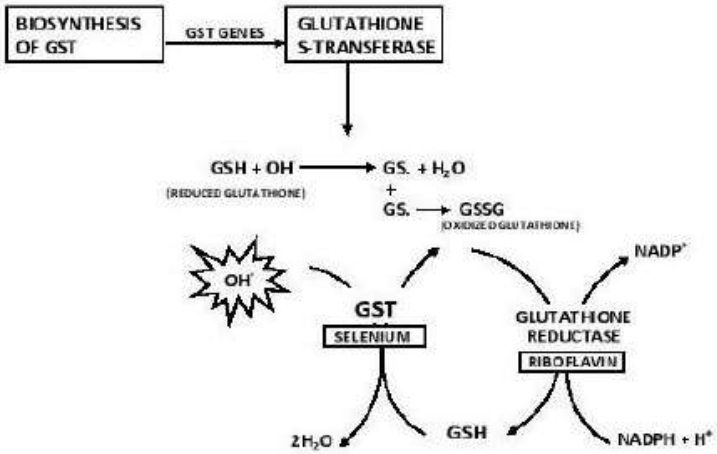
GSH

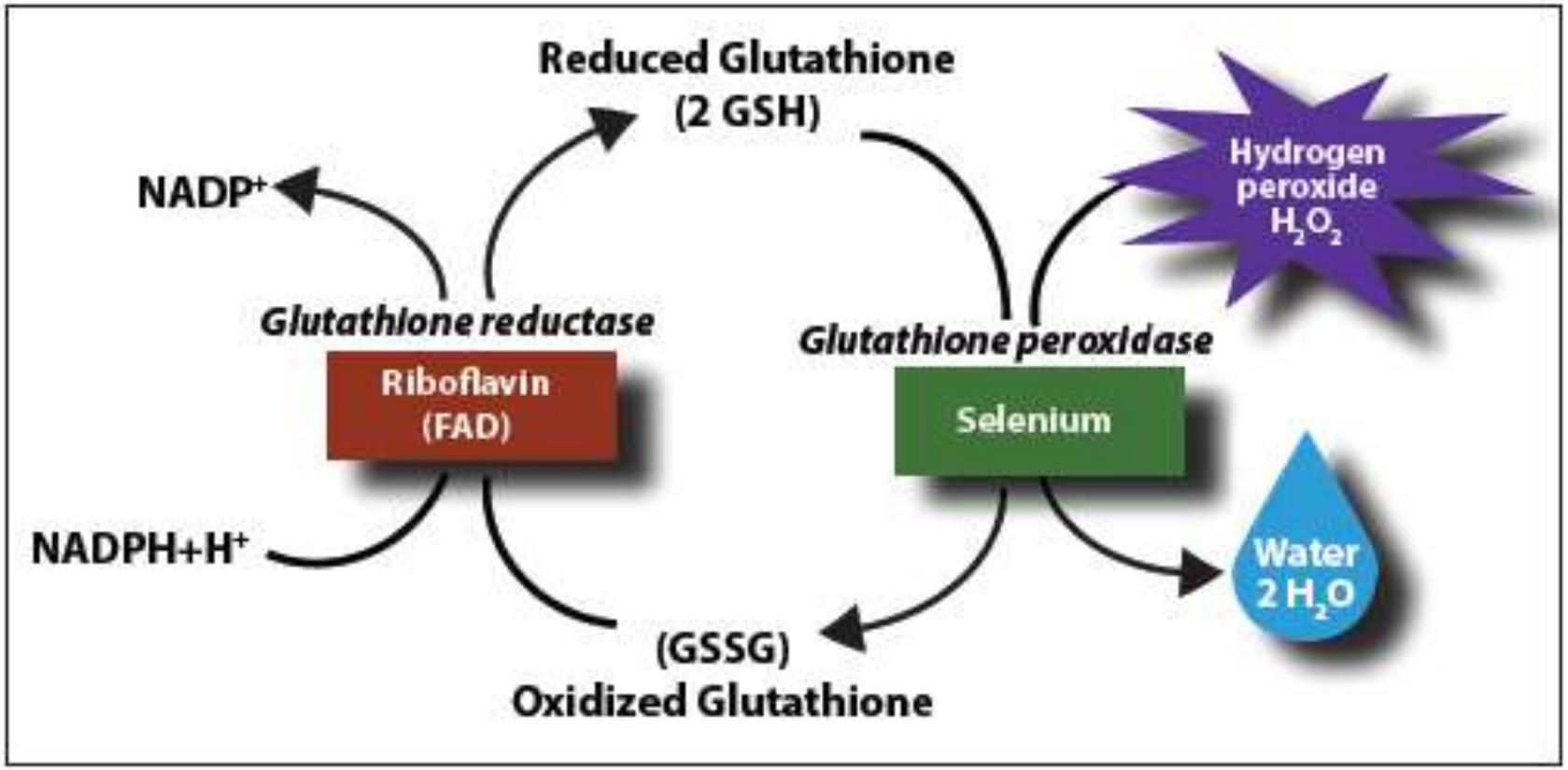


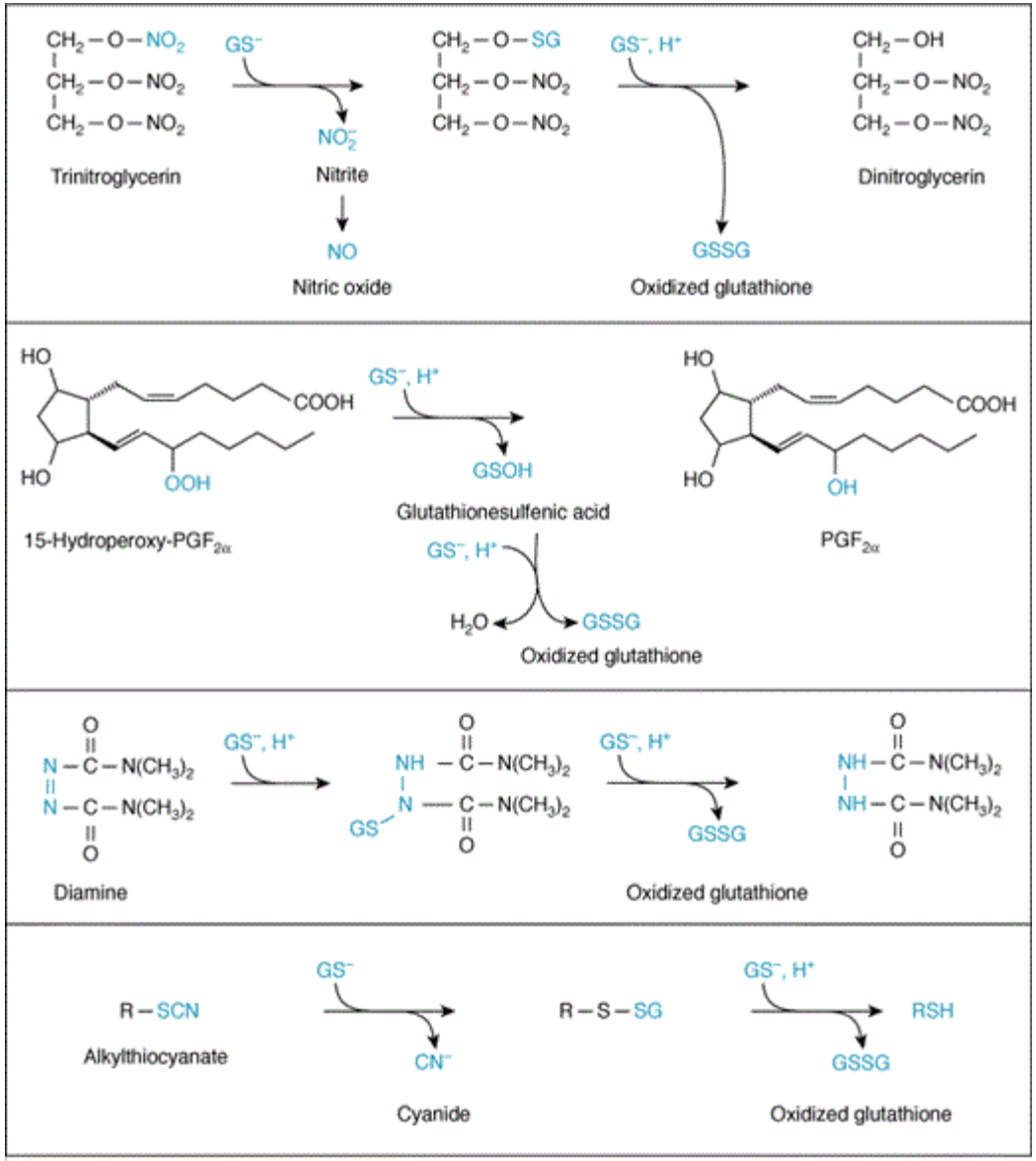
+ GS-OH

GSH

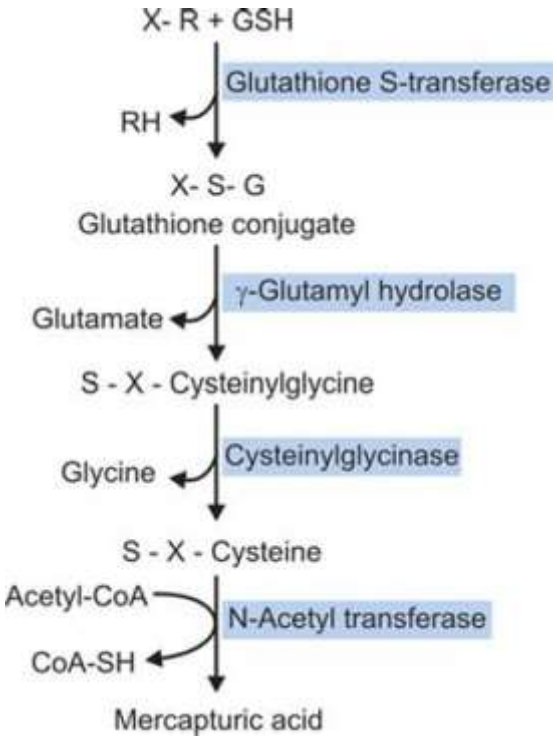
GSSG + H_2O




Reduced Glutathione
(2 GSH)
NADP+
Hydrogen peroxide
H2O2
Glutathione reductase
Riboflavin
(FAD)
Glutathione peroxidase
Selenium
NADPH+H+
Water
2 H2O
(GSSG)
Oxidized Glutathione



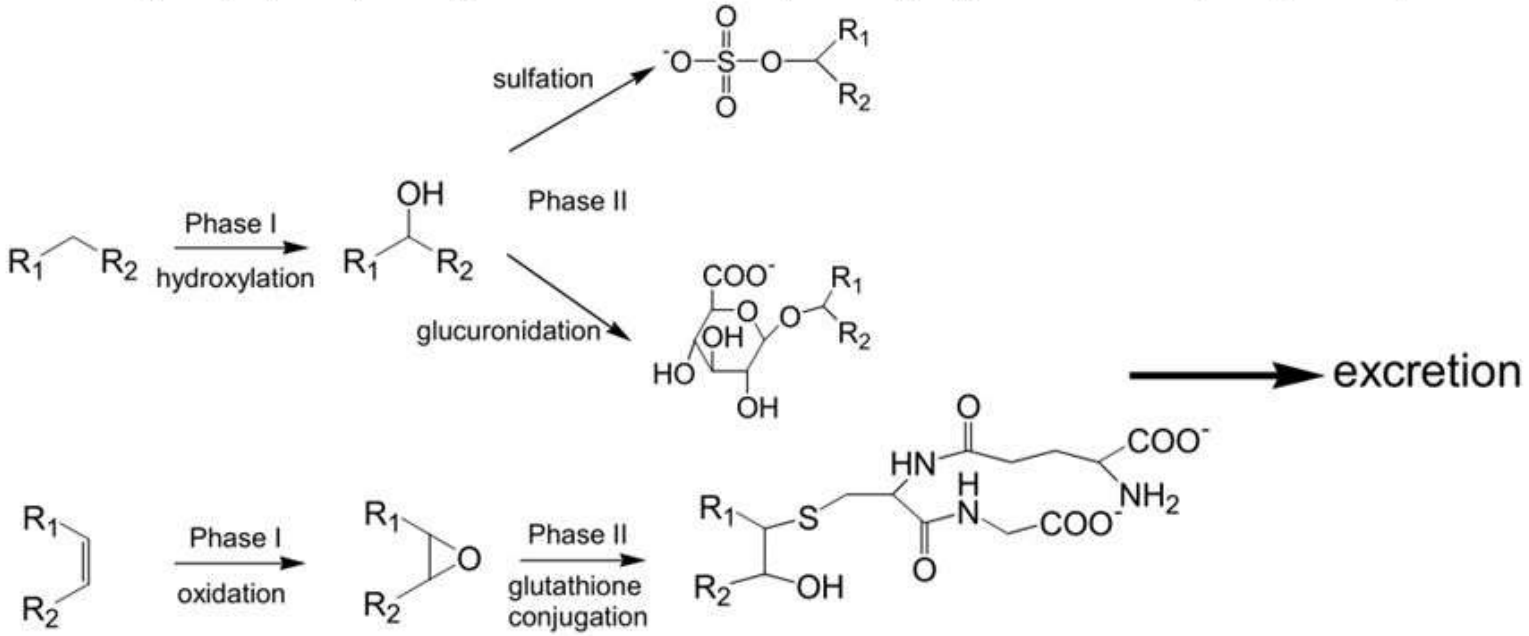
Because glutathione *S*-transferases also bind a number of ligands that are not substrates, including bilirubin, steroid hormones and some carcinogens and their metabolites, they are sometimes known as **ligandin**. Glutathione *S*-transferase binds bilirubin at a site distinct from the catalytic site, transporting it from the bloodstream to the liver, then to the endoplasmic reticulum for conjugation with glucuronic acid, and excretion in the bile



Phase I and Phase II Biotransformation

Reactions catalyzed by xenobiotic biotransforming enzymes are generally divided into two groups: Phase I and phase II.

1. Phase I reactions involve hydrolysis, reduction and oxidation, exposing or introducing a functional group (-OH, -NH_2, -SH or –COOH) to slightly increase hydrophilicity.



2. Phase II reactions include glucuronidation, sulfation, acetylation, methylation, conjugation with glutathione, and conjugation with amino acids (glycine, taurine and glutamic acid) that largely increase hydrophilicity.

Hofmann elimination

Inactivation of the drug in the body fluids by spontaneous molecular re arrangement without the agency of any enzyme

e.g. Atracurium.