TOXIC RESPONSES OF THE LIVER

Part Two

General Toxicology

4th Stage / 2nd Semester

University of Mustansiriyah/College of Pharmacy

Department of Pharmacology & Toxicology

Lecturer

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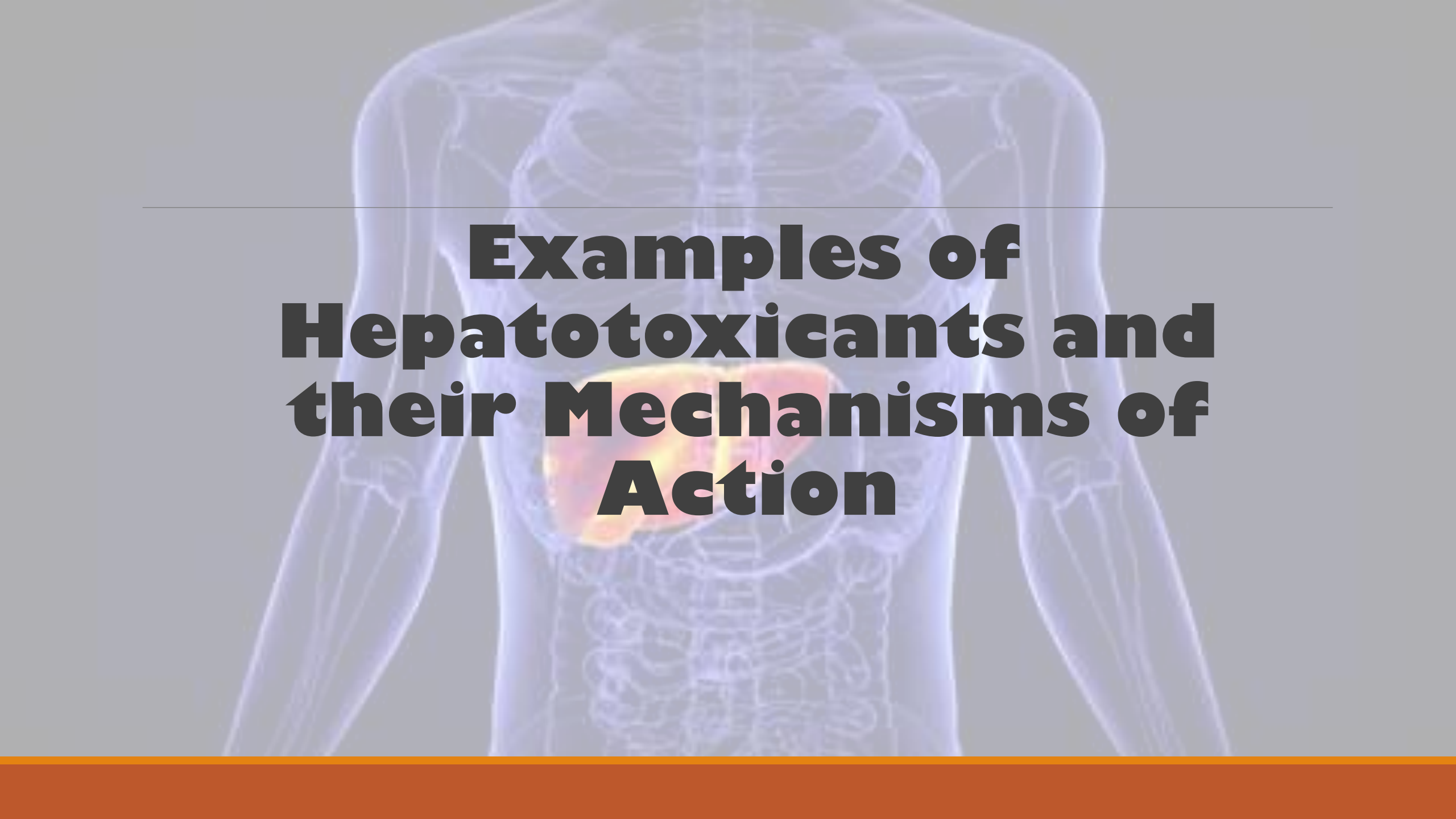
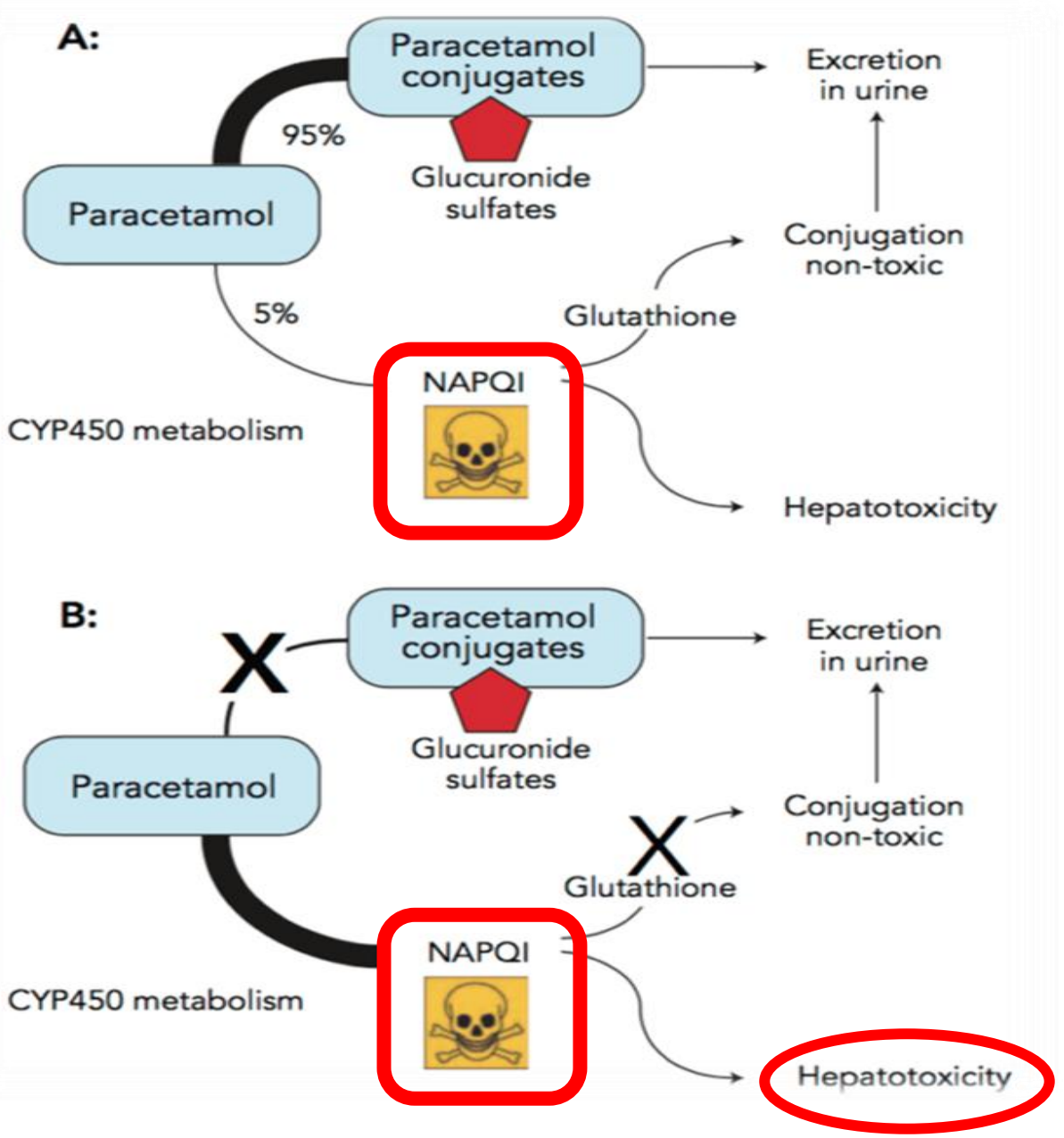


Examples of Hepatotoxicants and their Mechanisms of Action

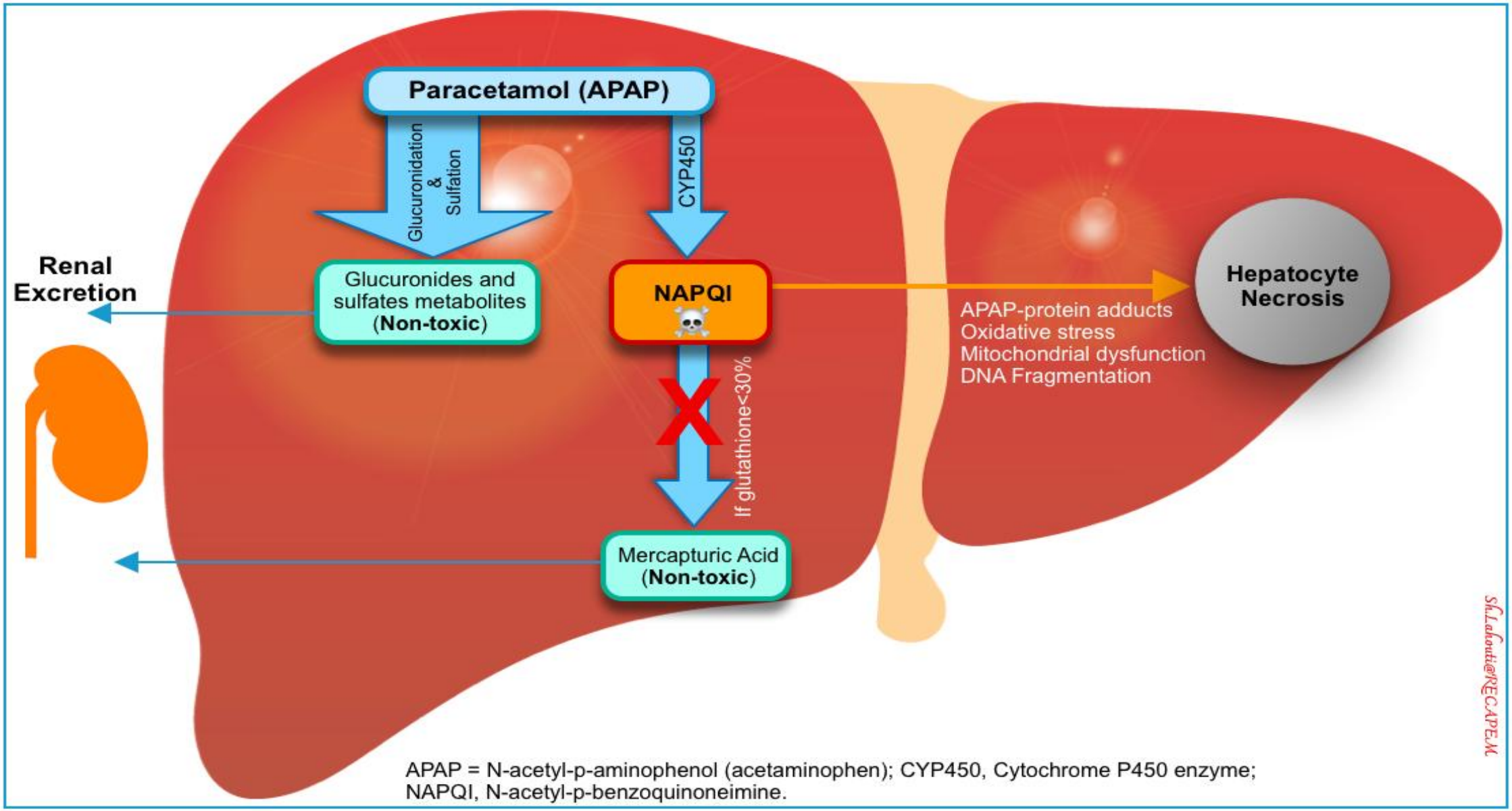
- **Acetaminophen:**
  - One of the most widely used analgesics acetaminophen (*N*-acetyl*p*-aminophenol APAP) is a safe drug when used at therapeutically recommended doses.
  - Overdose can cause severe hepatotoxicity, and certain acquired **factors can enhance hepatotoxicity including (e.g., diet, drugs, alcoholics, diabetes, and obesity).**
  - A significant sensitivity factor in APAP hepatotoxicity can be the consumption of alcoholic beverages, In addition to potential malnutrition in alcoholic individuals.
  - Ethanol is a potent inducer of CYP2E1, which is the main enzyme responsible for the metabolic activation of APAP in humans.
  - This acquired enhancement has widely been attributed to **accelerated bioactivation of acetaminophen to the electrophilic N-acetyl-p-benzoquinone imine (NAPQI) intermediate.**

Acetaminophen:

- At therapeutic doses, approximately 90% of APAP is conjugated with sulfate or glucuronide and excreted.
- Which limits formation of a reactive, toxic metabolite, *N*-acetyl*p*-benzoquinone imine (NAPQI).
- Most of the NAPQI is detoxified by conjugation with glutathione (GSH), thereby limiting its covalent binding to cellular proteins, which is the initiating event for HPC damage.
- APAP poisoning results in centrilobular hepatocellular necrosis



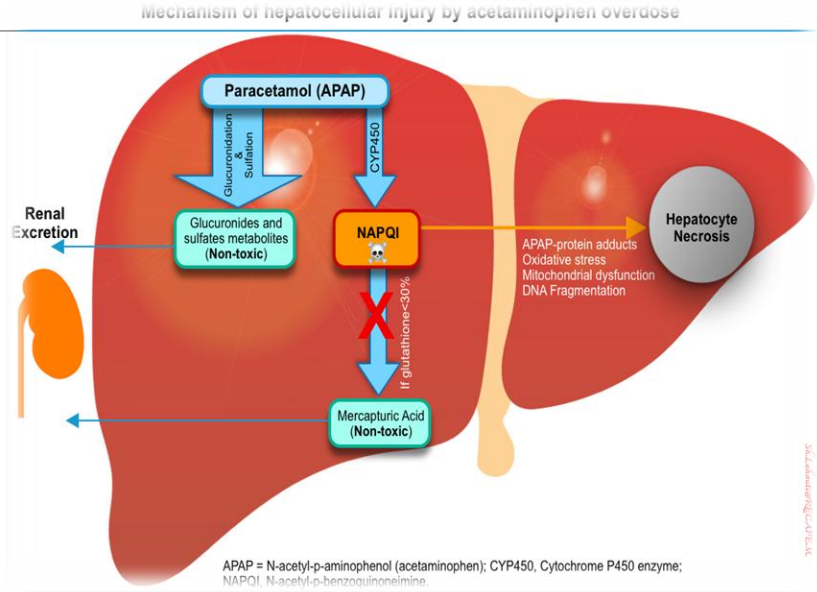
Mechanism of hepatocellular injury by acetaminophen overdose



APAP = N-acetyl-p-aminophenol (acetaminophen); CYP450, Cytochrome P450 enzyme; NAPQI, N-acetyl-p-benzoquinoneimine.

• Acetaminophen:

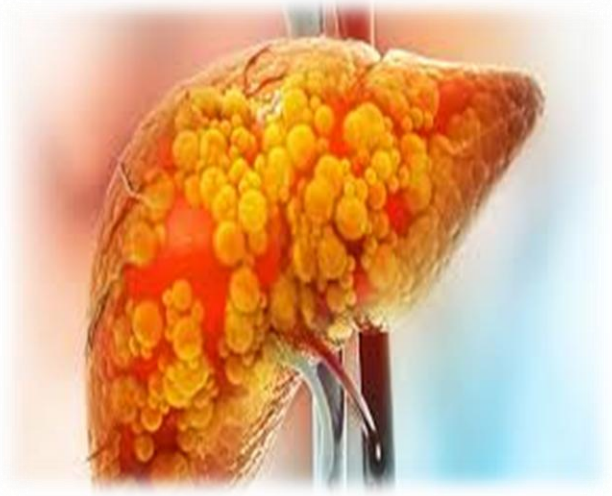
- Injury after large doses of acetaminophen is enhanced by fasting and other conditions that deplete glutathione and is minimized by treatments with N-acetylcysteine that enhance hepatocyte synthesis of glutathione
- The generally greater concentration of bioactivating CYPs combined with the lesser GSH concentration in centrilobular HPCs are the main reasons for the predominantly centrilobular necrosis observed after APAP poisoning.



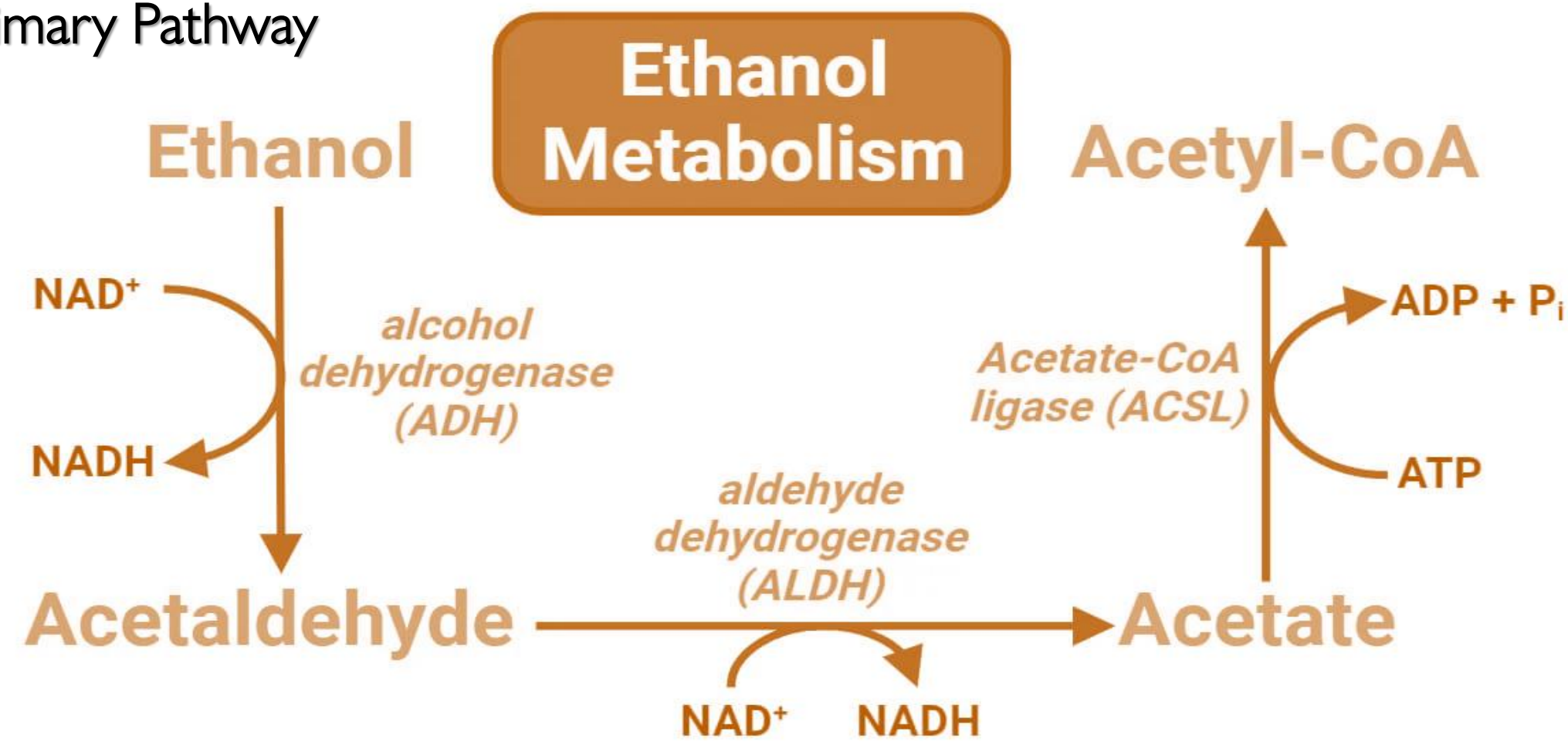
• Acetaminophen:

- In contrast, interventions such as the supply of cysteine, the rate-limiting amino acid for GSH synthesis, promote the detoxification of NAPQI and limit cell injury.
- Based on this fundamental insight into the mechanism of APAP hepatotoxicity, *N*-acetylcysteine (NAC) was introduced into the clinic as intervention therapy.
- More recent evidence indicates that NAC treatment not only promotes cytosolic GSH synthesis to detoxify NAPQI but also replenishes the depleted mitochondrial GSH.
- In addition, excess NAC is degraded and supports mitochondrial ATP generation.

- Ethanol:
  - Morbidity and mortality associated with the consumption of alcohol are mainly caused by the toxic effects of ethanol on the liver.
  - This targeted toxicity is because> 90% of a dose of ethanol is metabolized in the liver.
  - Three principal pathways of ethanol metabolism are known:
    - ✓Oxidation/reduction reaction (primary and most important one)
    - ✓Oxidation
    - ✓Electron donation



Primary Pathway



- **Primary Pathway:**
  - Ethanol + (alcohol dehydrogenase) → acetaldehyde + (aldehyde dehydrogenase) → acetate

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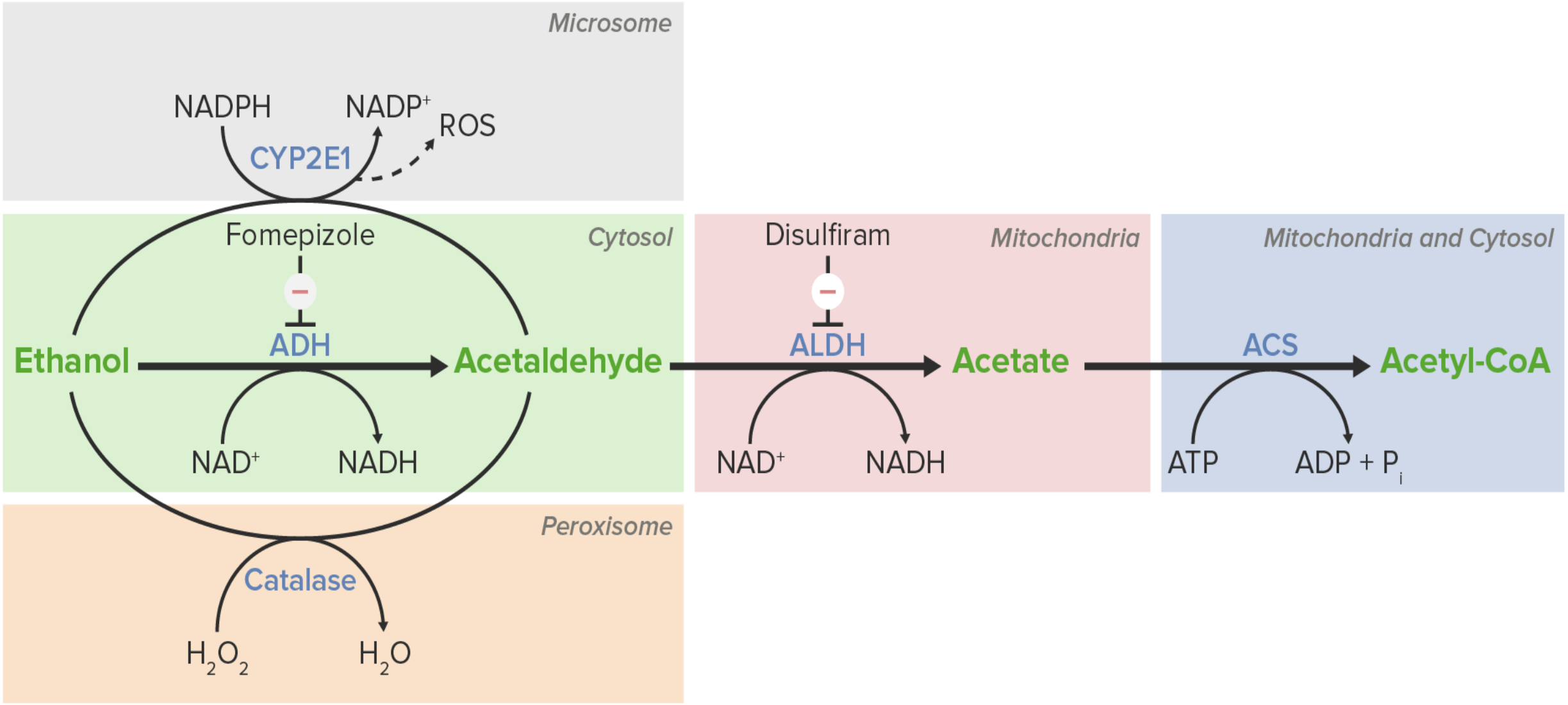
- Both enzymes exhibit genetic polymorphisms that result in higher concentrations of acetaldehyde.
- "fast" activity isozyme of alcohol dehydrogenase and a physiologically very "slow" mitochondrial isozyme of aldehyde dehydrogenase
- Approximately 50% of Asian populations but virtually no Caucasians have the slow aldehyde dehydrogenase.
- alcohol consumption by people with this slow polymorphism leads to uncomfortable symptoms of flushing and nausea due to high systemic levels of acetaldehyde.

- **Second pathway:**
  - involves the alcohol-inducible enzyme CYP2E1
  - Ethanol + (CYP2E1 Oxidase) → acetaldehyde
  - This enzyme requires oxygen and NADPH
  - Due to the nature of the enzyme, this reaction is most relevant for high doses of ethanol and chronic alcoholism.

- **Third pathway:**
  - Ethanol functions as an electron donor for the reduction of hydrogen peroxide to water.
  - Ethanol + (catalase) + H_2O_2 → $2H_2O$
  - the capacity of this pathway is limited due to the low levels of hydrogen peroxide.
  - Less than 2% of ethanol is metabolized through this pathway

The three principal pathways of Ethanol metabolism



- Ethanol:

The early stage of ethanol abuse is associated with Fatty Liver (steatosis).

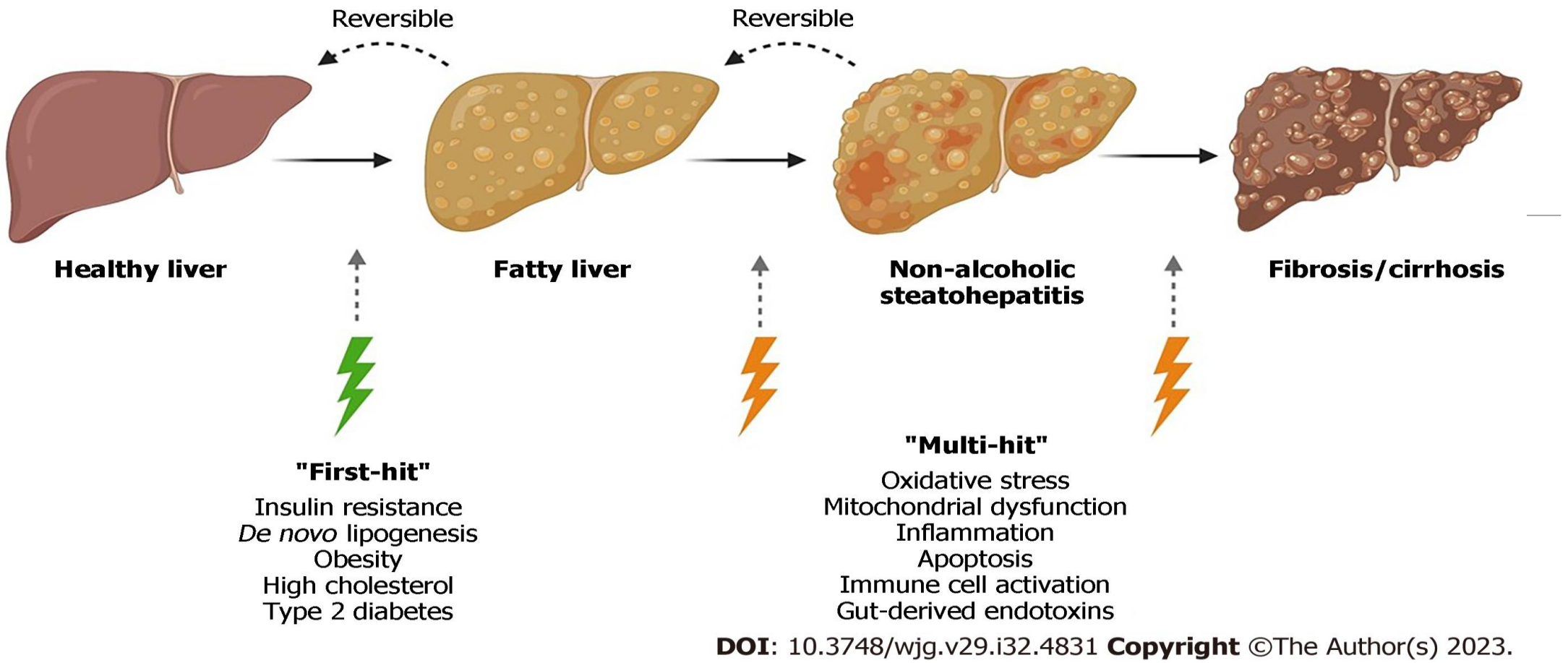
As it progresses, cell death occurs alongside increasing hepatic inflammation (i.e., steatohepatitis). If left unchecked, these pathologic processes drive the replacement of functional liver mass with scar tissue.

People with hepatic cirrhosis due to chronic alcohol abuse frequently become deficient at detoxifying both the ammonia formed by catabolism of amino acids and the bilirubin derived from breakdown of hemoglobin.

Such hepatic dysfunction combined with defects in synthesis of key proteins, such as albumin and clotting factors, can ultimately drive multiple organ dysfunction and death.

- Ethanol:

- Alcohol-mediated steatosis is caused by the excessive supply of acetate and NADH, which increases fatty acid synthesis.
- In addition, **both ethanol and acetaldehyde** disrupt the constitutive regulation of fatty acid metabolizing enzymes by impairing DNA binding of the transcription factor, peroxisome **proliferator-activated receptor-α (PPAR-α)**.
- In addition to this imbalance favoring increased fatty acid synthesis, ethanol exposure **inhibits the transfer of triglycerides from the liver to adipose tissue**.
- CYP2E1 is a source of reactive oxygen formation during ethanol metabolism.
- The resultant intracellular oxidant stress in HPCs can ultimately induce mitochondrial dysfunction and cell death of HPCs, but it also activates stellate cells and promotes fibrosis.
- Ethanol inhibits hepatic NK cells. Because NK cells can kill HSCs, the major cell type promoting hepatic fibrosis, ethanol may indirectly support fibrogenesis by preventing the elimination of activated stellate cells.



DOI: 10.3748/wjg.v29.i32.4831

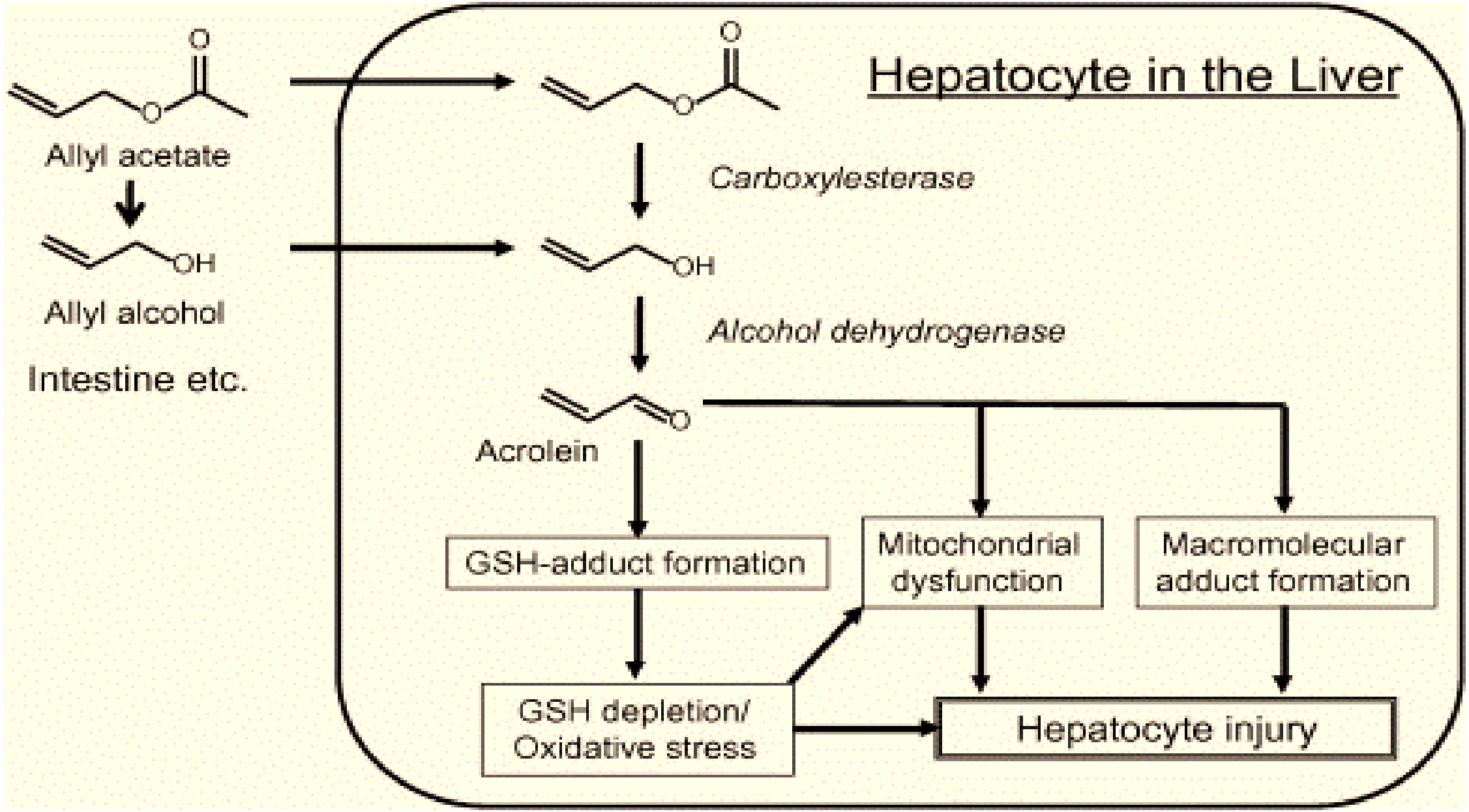
In the two-hit hypothesis of alcoholic liver disease, steatosis is considered the "first hit," which requires a "second hit" to drive simple steatosis to the necro inflammatory change observed in severe alcoholic liver injury .

- Allyl Alcohol:
  - An industrial chemical used in the production of resins, and plastics.
  - Allyl alcohol + alcohol dehydrogenase(ADH) → acrolein + aldehyde dehydrogenase(ALDH) → acrylic acid

  - Variations in the balance between alcohol dehydrogenase and aldehyde dehydrogenase expression can explain age and gender differences in allyl alcohol hepatotoxicity.

- The observations that the toxicity depends on the depletion of hepatic GSH and is prevented by inhibitors of ADH but enhanced by inhibitors of ALDH suggest that acrolein formation is the critical event in liver injury.

- The occurrence of allyl alcohol injury preferentially in zone 1 HPCs is caused by the predominant uptake of allyl alcohol in the periportal region.


Hepatocyte in the Liver
Allyl acetate
Allyl alcohol
Intestine etc.
Carboxylesterase
Alcohol dehydrogenase
Acrolein
GSH-adduct formation
Mitochondrial dysfunction
Macromolecular adduct formation
GSH depletion/ Oxidative stress
Hepatocyte injury

Carbon Tetrachloride CCl4:

- human exposure to CCl4 has been restricted due to its hepatotoxicant effect.
- Acute exposure to CCl4 causes centrilobular necrosis .
  - CCL4 +(Cytochrome P450) → •CCl3 + (oxidation) → CCl3OO•
- Conversion of CCl 4 to trichloromethyl free radical (•CCl3) and then to the trichloromethyl peroxyl radical (CCl3OO•) is a classic example of xenobiotic bioactivation to a free radical capable of initiating lipid peroxidation by abstracting hydrogen atoms from polyunsaturated fatty acids in phospholipid membranes.
- Metabolic activation of CCl4 in vivo involves primarily CYP2E1.
- CCl4-induced lipid peroxidation increases the permeability of the plasma membrane to Ca2+, leading to severe disturbances in calcium homeostasis and consequent necrotic cell death.
- In contrast, treatment with agents such as ethanol that induce CYP2E1 enhances CCL4 liver injury.

Aflatoxins:

- Aflatoxins are mycotoxins produced by *Aspergillus* molds that grow on nuts and crops such as corn, wheat, and rice, and consumption of these foods results in exposure to humans and animals

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- Aflatoxins have also been detected in the milk of animals that consume contaminated crops, and this can lead to exposure of young children.
- various isoforms of CYPs oxidize AFB1. Although most of the products lack significant toxicity, oxidation of the furan ring leads to a reactive epoxide that alkylates DNA and that can be further metabolized to a dialdehyde that alkylates proteins.
- Conjugation with GSH is an essential pathway for detoxification of the reactive epoxide, which is also hydrolyzed both spontaneously and enzymatically by epoxide hydrolases.
- In this regard, combinations of polymorphisms in several genes encoding enzymes that metabolize AFB1 are associated with an increased risk of developing hepatocellular carcinoma.
- In addition, there exists a pronounced synergy between AFB exposure and infection with hepatitis B virus in causing hepatocellular carcinoma in humans.

Mushroom Toxins:

- Amanita phalloides ("death cap") has been of greatest concern due to its ability to cause life-threatening toxicity

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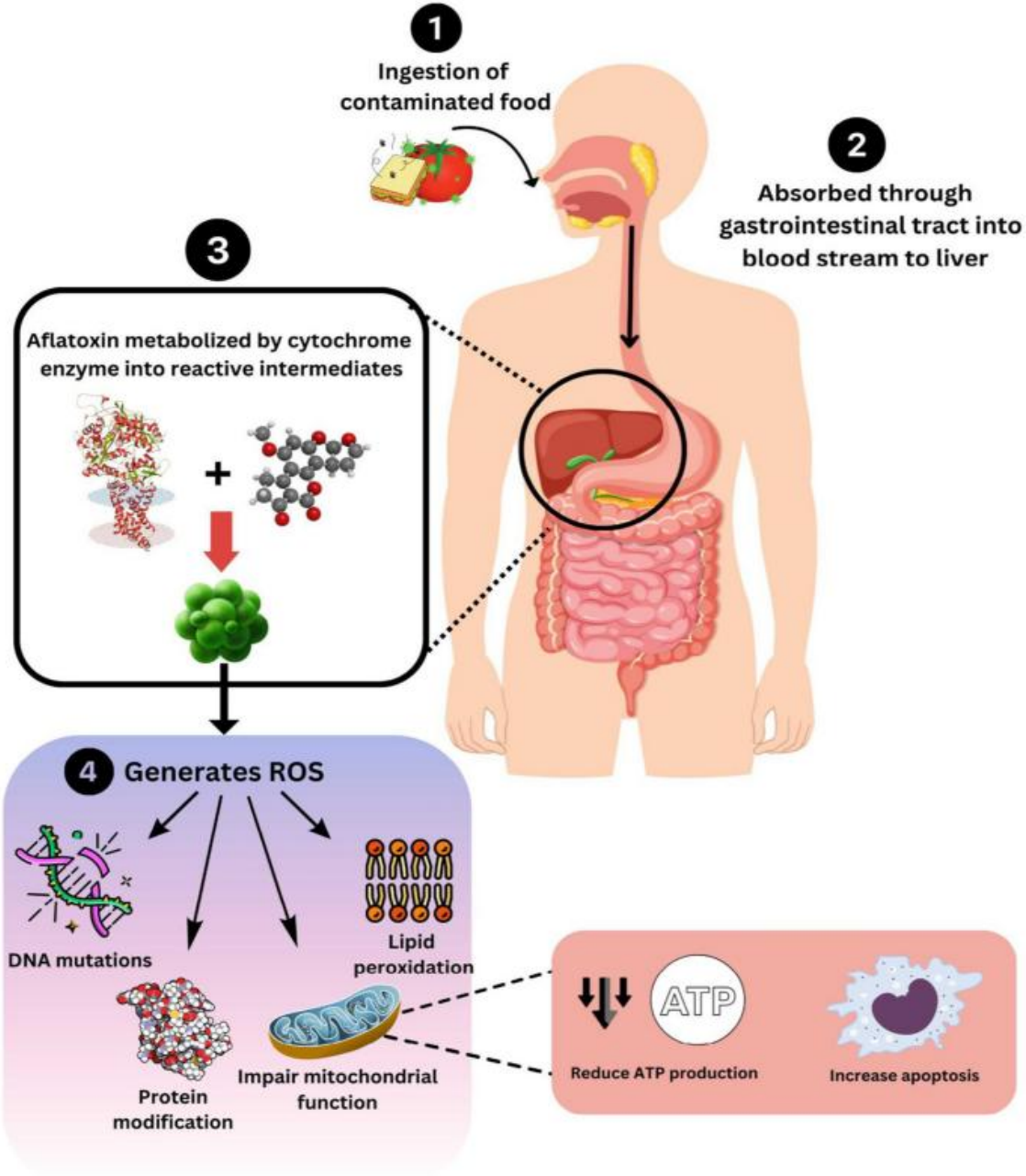
- This mushroom produces several bicyclic heptapeptides known as phallotoxins and octapeptides known as amatoxins.
- Phalloidin is taken into HPCs by transporters and causes cholestasis and hemorrhagic necrosis in livers.
- Unlike phalloidin, α-amanitin is absorbed from the gastrointestinal tract and is rapidly eliminated in the urine.
- α-amanitin causes centrilobular hepatocellular necrosis and hemorrhage accompanied by hepatorenal syndrome characterizes severe poisoning in humans.
- A primary mechanism of toxicity of α-amanitin derives from its ability to bind to nuclear RNA polymerase II, thereby inhibiting RNA elongation with resultant impaired protein synthesis and cell death.


1
Ingestion of contaminated food
2
Absorbed through gastrointestinal tract into blood stream to liver
3
Aflatoxin metabolized by cytochrome enzyme into reactive intermediates
4 Generates ROS
DNA mutations
Lipid peroxidation
Protein modification
Impair mitochondrial function
ATP
Reduce ATP production
Increase apoptosis

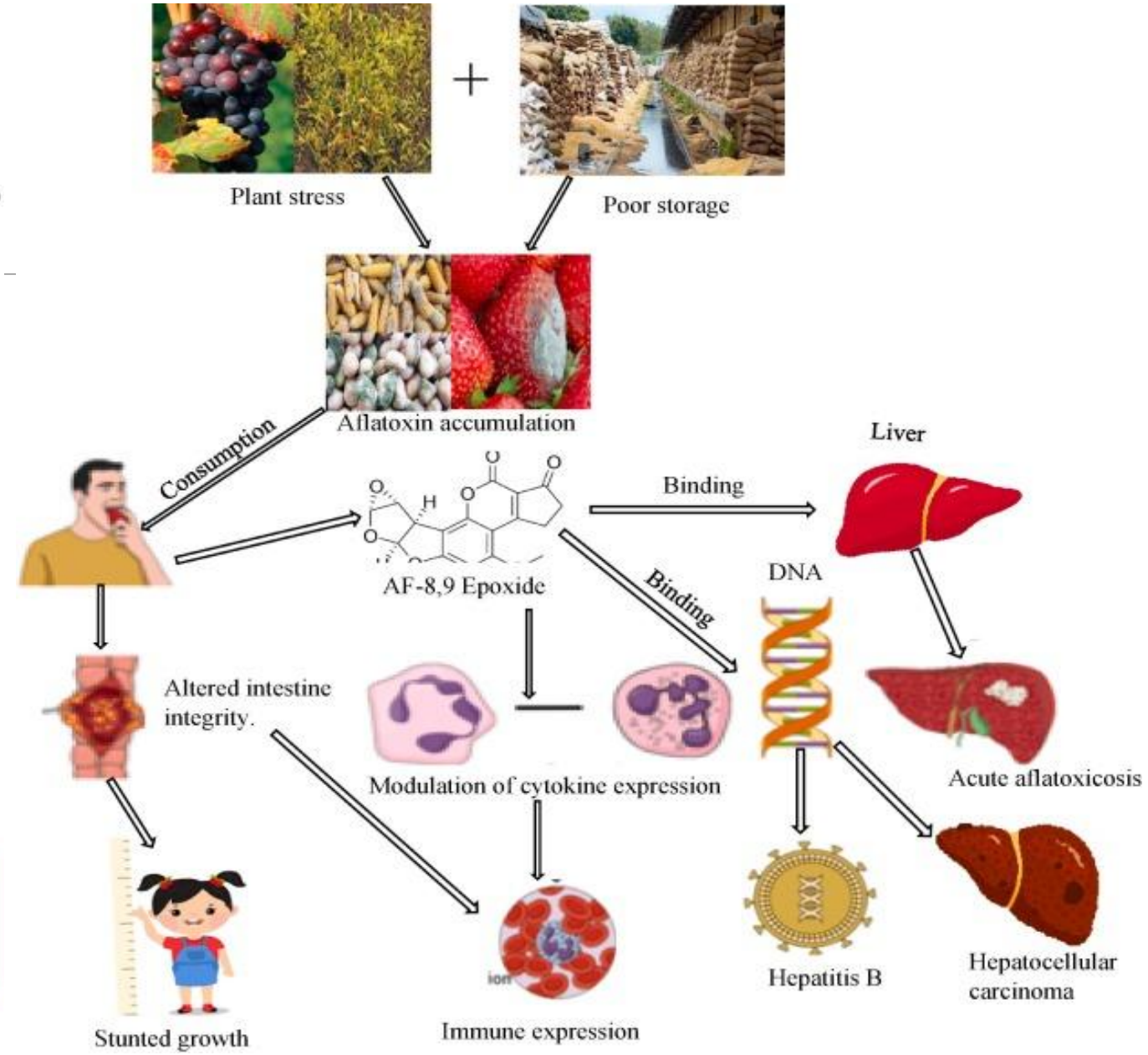

Plant stress
Poor storage
Aflatoxin accumulation
Consumption
AF-8,9 Epoxide
Binding
Liver
DNA
Binding
Altered intestine integrity.
Modulation of cytokine expression
Acute aflatoxicosis
Stunted growth
Immune expression
Hepatitis B
Hepatocellular carcinoma

TABLE 13–2 Types of hepatobiliary injury.

Type of Injury or Damage	Representative Toxins
Fatty liver	Amiodarone, CCl_4, ethanol, fialuridine, tamoxifen, valproic acid
Hepatocyte death	Acetaminophen, allyl alcohol, Cu, dimethylformamide, ethanol
Immune-mediated response	Diclofenac, ethanol, halothane, tienilic acid
Canalicular cholestasis	Chlorpromazine, cyclosporin A, 1,1-dichloroethylene, estrogens, Mn, phalloidin
Bile duct damage	Alpha-naphthylisothiocyanate, amoxicillin, methylene dianiline, sporidesmin
Sinusoidal disorders	Anabolic steroids, cyclophosphamide, microcystin, pyrrolizidine alkaloids
Fibrosis and cirrhosis	CCl_4, ethanol, thioacetamide, vitamin A, vinyl chloride
Tumors	Aflatoxin, androgens, arsenic, thorium dioxide, vinyl chloride

TABLE 13–3 Factors in the site-specific injury of representative hepatotoxicants.

Site	Representative Toxicants	Potential Explanation for Site Specificity
Zone 1 hepatocytes (versus zone 3)	Fe (overload)	Preferential uptake and high oxygen levels
	Allyl alcohol	Higher oxygen levels for oxygen-dependent bioactivation
Zone 3 hepatocytes (versus zone 1)	CCl_4	More P450 isozyme for bioactivation
	Acetaminophen	More P450 isozyme for bioactivation and less GSH for detoxification
	Ethanol	More hypoxic and greater imbalance in bioactivation/detoxification reactions
Bile duct cells	Methylene dianiline, sporidesmin	Exposure to the high concentration of reactive metabolites in bile
Sinusoidal endothelium (versus hepatocytes)	Cyclophosphamide, monocrotaline	Greater vulnerability to toxic metabolites and less ability to maintain glutathione levels
Kupfer cells	Endotoxin, $GdCl_3$	Preferential uptake and then activation
Stellate cells	Vitamin A	Preferential site for storage and then engorgement
	Ethanol (chronic)	Activation and transformation to collagen-synthesizing cell

TABLE 13–4 Examples of drugs with known idiosyncratic hepatotoxicity.

A. Immune-mediated (allergic) idiosyncratic hepatotoxicity

- Diclofenac (analgesic)
- Halothane (anesthetic)
- Nitrofurantoin (antibiotic)
- Phenytoin (anticonvulsant)
- Tienilic acid (diuretic)

B. Nonimmune-mediated (nonallergic) idiosyncratic hepatotoxicity

- Amiodarone (antiarrhythmic)
- Bromfenac (analgesic)—withdrawn from market
- Diclofenac (analgesic)
- Disulfiram (alcoholism)
- Isoniazid (antituberculosis)
- Ketoconazole (antifungal)
- Rifampicin (antimicrobial)
- Troglitazone (antidiabetes)—withdrawn from market
- Valproate (anticonvulsant)

THANK YOU