Toxicology

EXAMPLES OF HEPATOTOXICANTS

Lab. 2

4th stage

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Carbon Tetrachloride

- It is a classic example of a chemical **activated by CYPs** to **form** a **highly reactive free radical.**

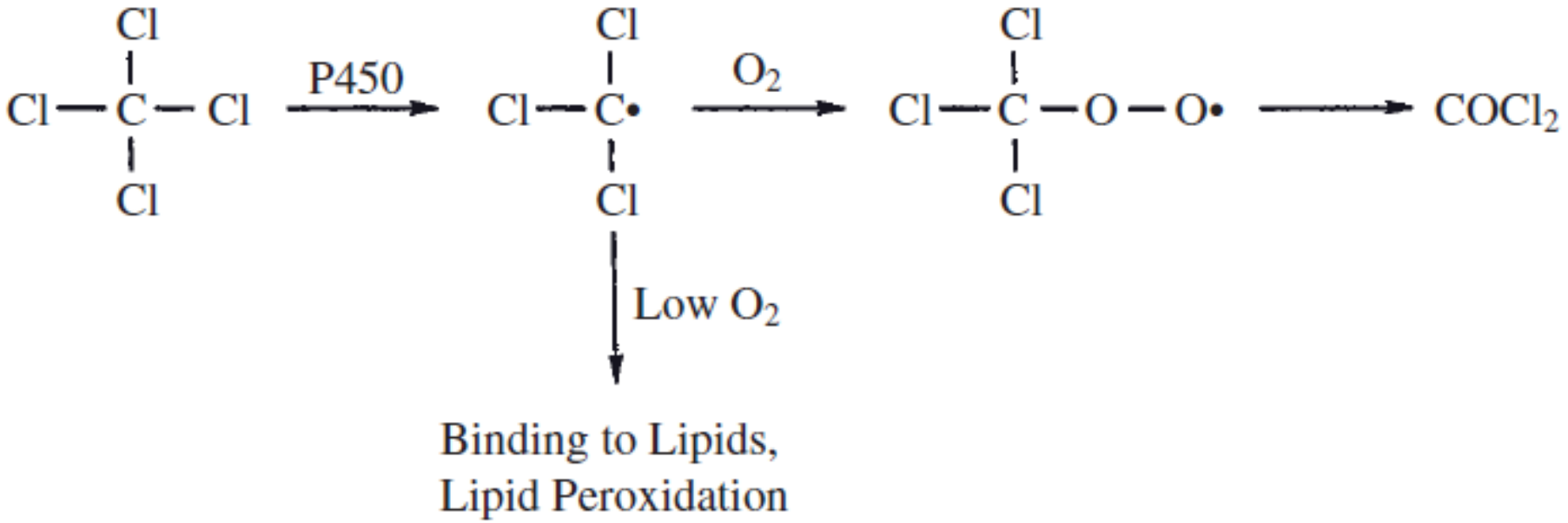


Figure 13.4 Metabolism of carbon tetrachloride and formation of reactive metabolites.

➢**First**, **CCl 4** is converted to the **trichloromethyl radical** (CCl 3 •).

➢Then **to** the trichloromethylperoxy radical (CCl 3 O 2 •).

- Such radicals are highly reactive. And generally have a small radius of action.

❖**For this reason**, the *necrosis* induced **by** CCl 4.

➢It is most *severe* in the centrilobular liver cells that *contain the highest concentration of the CYP isozyme responsible for CCl 4 activation.*

- Typically free radicals may participate in a number of events such as:

(*covalent binding to lipids, proteins, or nucleotides as well as*

lipid peroxidation.)

❖It is now thought that :-

I. **$CCl_3^\bullet$**, is responsible for **covalent binding** to macromolecules.

II. **$CCl_3O_2^\bullet$** (*the more reactive*) which is formed when $CCl_3^\bullet$ reacts with oxygen, is the **prime initiator** of lipid peroxidation.

❖**Lipid peroxidation** is the *initiating reaction* in a cascade of events starting with the oxidation of **unsaturated fatty acids** to form ***lipid hydroperoxide***, which then break down to yield a variety of **end products**, mainly **aldehydes**, which can **go on** to *produce* ***toxicity in distal tissues***.

❖**For this reason**, cellular damage **results not** only from the **breakdown of membranes** **such as** those of the *endoplasmic reticulum*, *mitochondria*, and *lysosomes* **but also** from the production of ***reactive aldehydes*** that can travel to other tissues.

❖*It is now thought that many types of tissue injury, including inflammation, may involve* ***lipid peroxidation***.

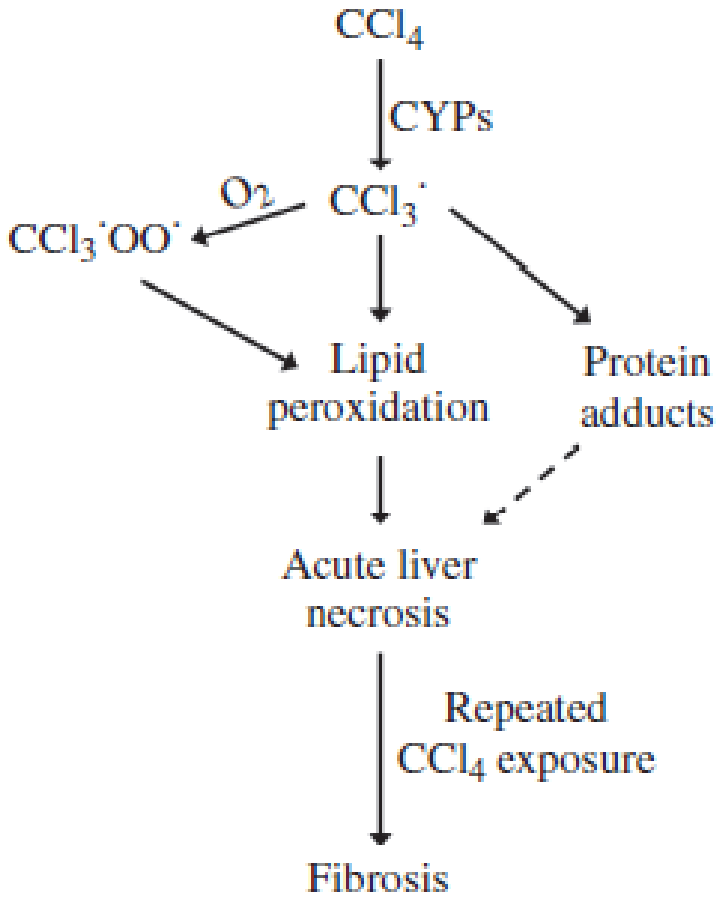
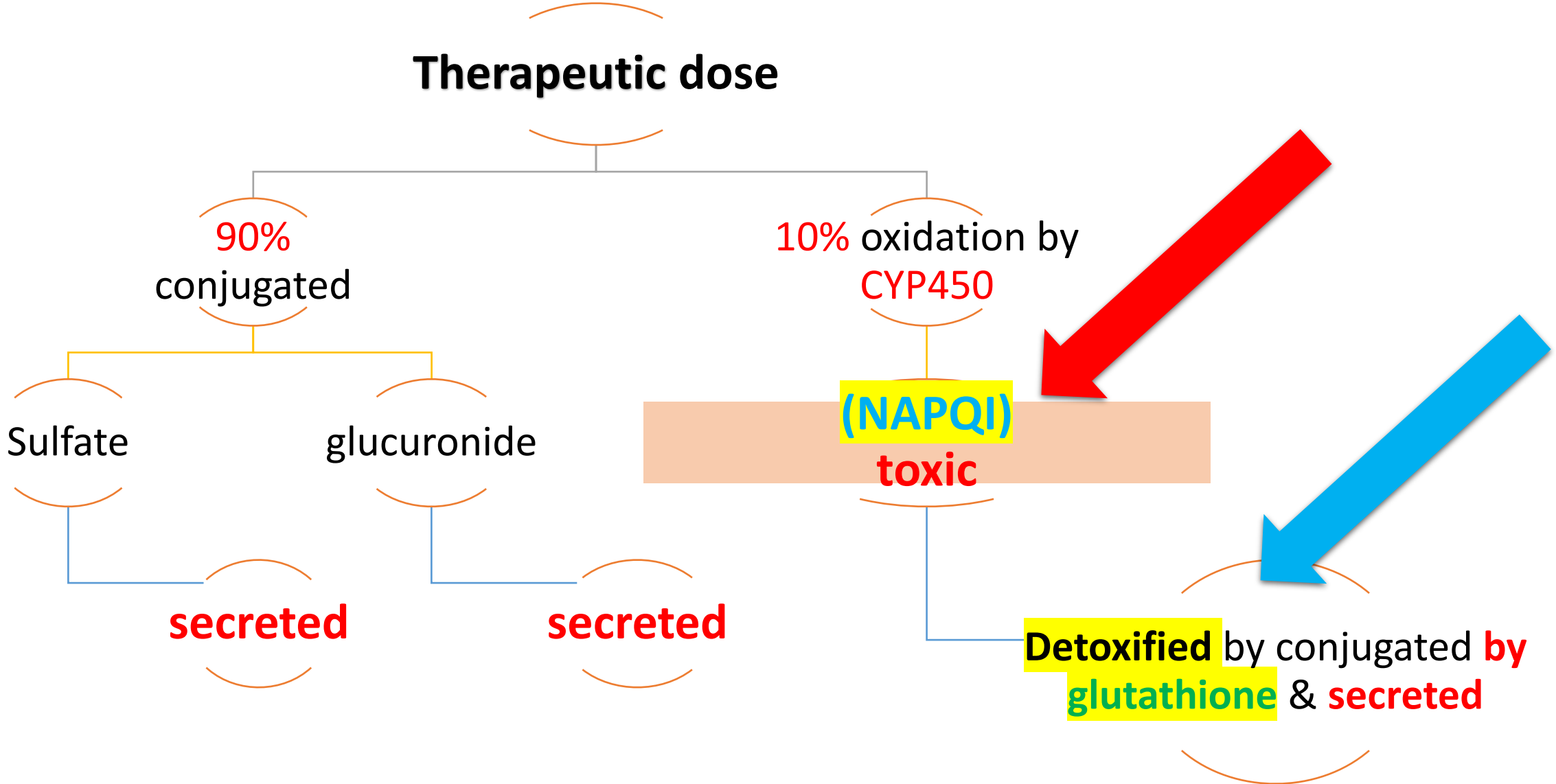


Figure 13-19. *Liver injury from carbon tetrachloride. Left*: CCl_4 is bioactivated by CYPs to the trichloromethyl free radical and in the presence of oxygen to trichloromethyl peroxyl radical. These radicals initiate lipid peroxidation of cell membranes and consequent hepatocellular necrosis. Protein adducts formed with radicals from CCl_4 and from lipid peroxidation also might contribute to liver injury. Repeated CCl_4 exposure leads to fibrosis. *Right*: Necrosis and fibrosis in livers of CCl_4-treated mice. Mice were treated twice per week for 10 weeks with CCl_4. *Top*: Hematoxylin and eosin staining of livers from control (CON) mice and mice treated with CCl_4. In the latter, note the centrilobular hepatocellular necrosis and presence of inflammatory cells within the lesion. *Bottom*: Sirius red staining. Note red staining of excess collagen in septa of lobules in the liver from the CCl_4-treated mouse. (Photomicrographs from Hyun et al. [2016].)

Acetaminophen

- It is one of the **most widely used analgesics.**
- Acetaminophen (N-acetyl p- aminophenol; APAP) is a safe drug when used at therapeutically recommended doses.
- **However**, an **overdose** can ***cause severe liver injury*** and even ***liver failure*** in experimental animals and in humans.
- **APAP poisoning** results in ***centrilobular hepatocellular necrosis***.

Mechanism of Paracetamol Metabolism



- **At therapeutic doses**,

  - approximately **90% of APAP** is **conjugated** with **sulfate** **or** **glucuronide** and excreted.

  - **10% metabolize** by CYPs of a reactive, that produce a **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.***
- **In addition**, the low levels of protein adducts formed after therapeutic doses are removed by **autophagy.**

❖**Thus,** therapeutic doses of APAP pose minimal risk for **liver injury**. why?

❖long-term studies **with paracetamol** in *osteoarthritis* patients did not reveal evidence of liver dysfunction **or** cell injury **even** in **patients** consuming the **maximal recommended** daily dose of APAP for 12 months, **why?**

- **In contrast, after an overdose**, **overwhelmed** sulfate and glucuronide conjugation pathways **lead to break-through** *formation of large amounts of* ***NAPQI.***
- This, **resulting in severe depletion** of *cellular GSH stores needed for NAPQI inactivation.*
- **Thereby** allowing extensive covalent binding of NAPQI to intracellular proteins.

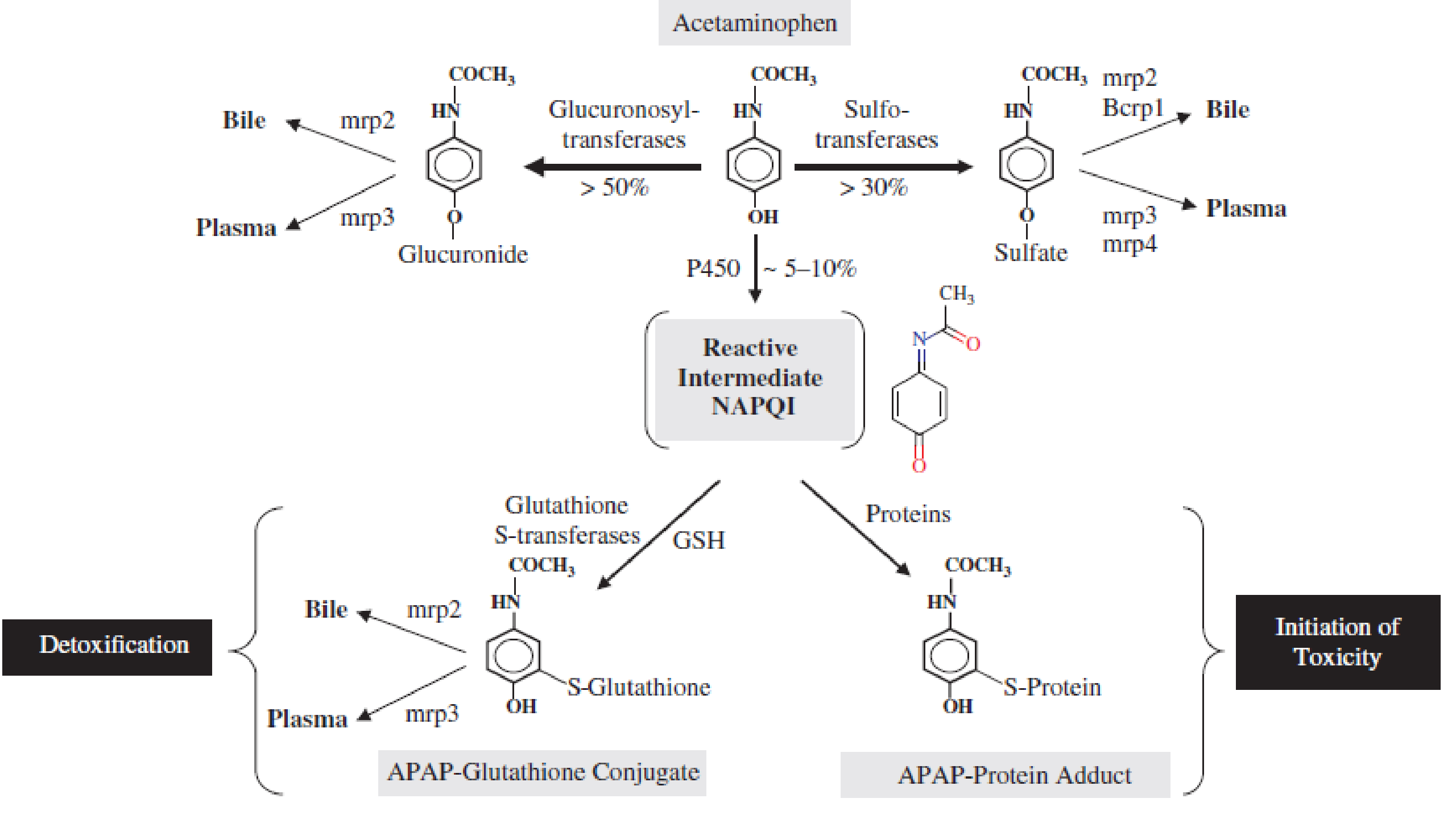
- The generally greater concentration of bioactivating **CYPs** combined with the **lesser GSH** concentration *in centrilobular HPCs are the main reasons for the predominantly centrilobular* ***necrosis*** *observed after* ***paracetamol poisoning***.

❖**Because** protein binding can be prevented by conjugation of NAPQI with GSH,

- **any** manipulation that reduces hepatic GSH levels,

  for example, **fasting, protein malnutrition**, or, Induction of CYP isozymes responsible for the activation of acetaminophen *enhances the toxicity of acetaminophen*.

- **In contrast**, The early administration of **sulfhydryl compounds** such as **cysteamine**, **methionine**, and ***N* - acetylcysteine** is very effective in **preventing** **liver damage**, and **death** that would otherwise follow an acetaminophen overdose.
- These agents are thought to **act** *primarily by stimulating glutathione synthesis.*
- N-acetylcysteine (NAC) was **introduced** into the **clinic** **as** **intervention therapy**


Acetaminophen
Bile
mrp2
Plasma
mrp3
Glucuronide
Glucuronosyl-transferases
> 50%
Sulfo-transferases
> 30%
Sulfate
mrp2
Bcrp1
Bile
mrp3
mrp4
Plasma
P450
~ 5–10%
Reactive Intermediate NAPQI
Glutathione S-transferases
GSH
Proteins
Bile
mrp2
Plasma
mrp3
S-Glutathione
S-Protein
APAP-Glutathione Conjugate
APAP-Protein Adduct
Detoxification
Initiation of Toxicity