Hepato and Renal Toxicology

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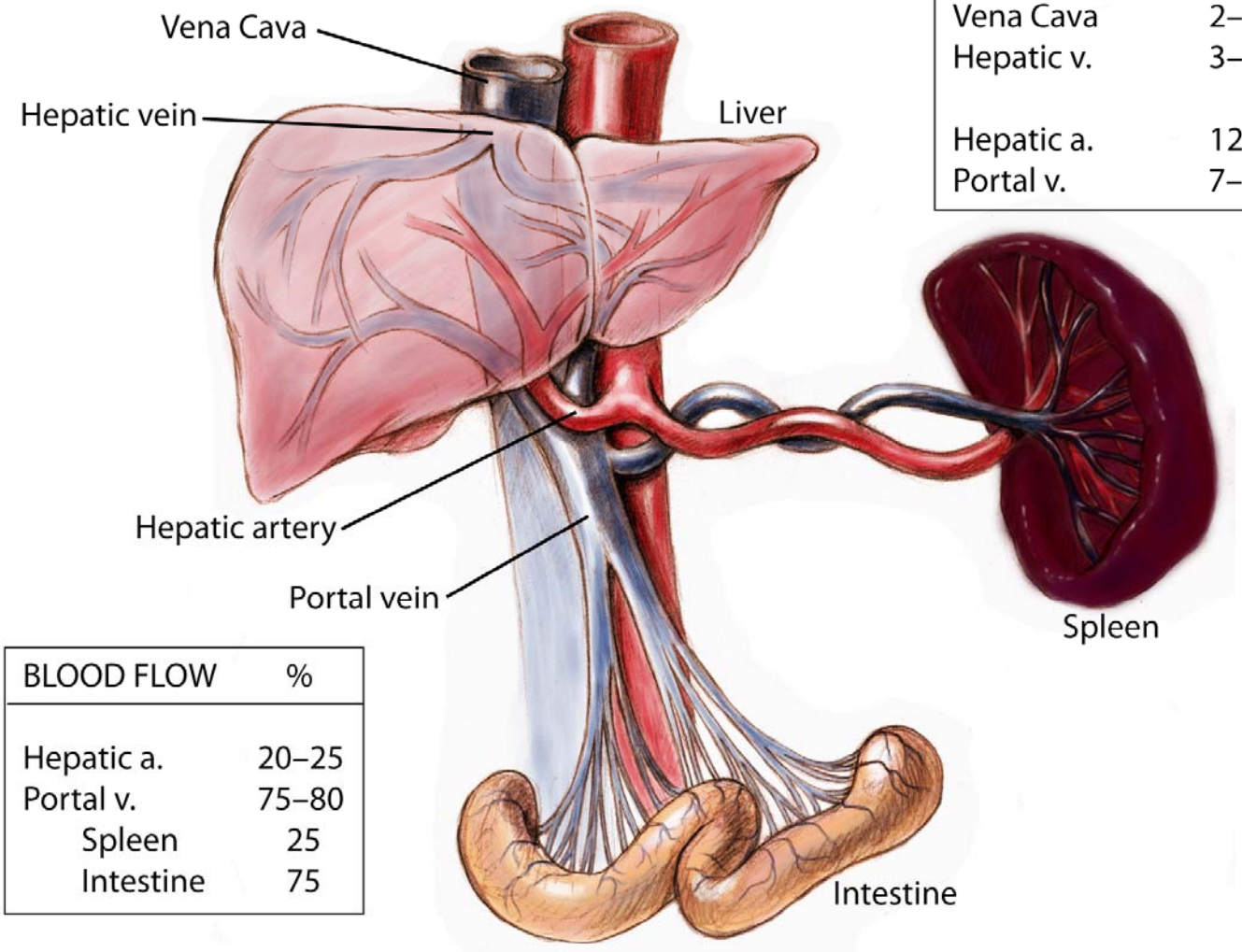
Section A

Liver: Structural organization

Liver: Structural Organization

PRESSURE	mmHg
Vena Cava	2–5
Hepatic v.	3–6
Hepatic a.	120
Portal v.	7–10

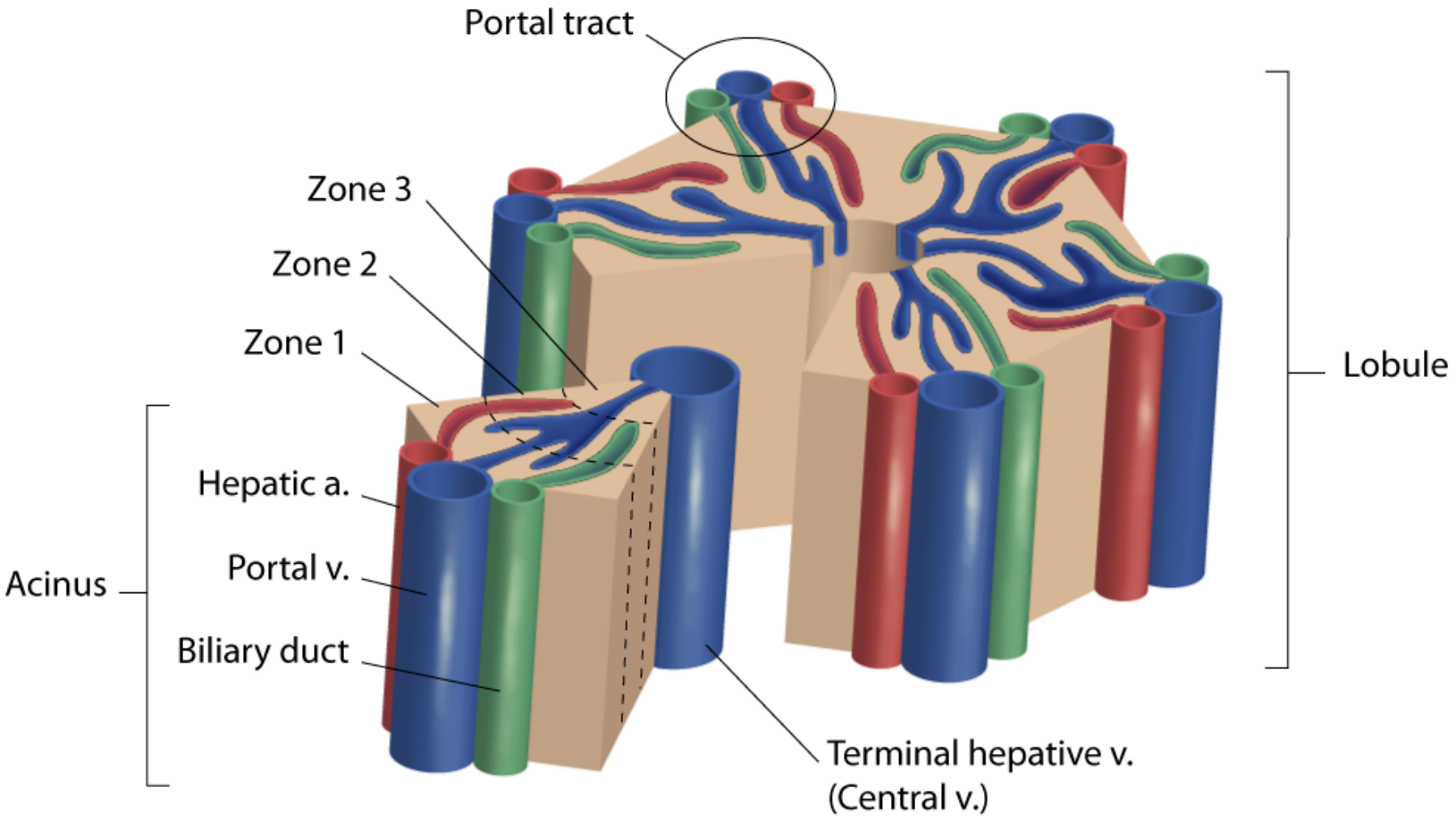


BLOOD FLOW	%
Hepatic a.	20–25
Portal v.	75–80
Spleen	25
Intestine	75

Structural Organization of the Liver: *Cellular Composition as % Liver Volume*	
Hepatocytes	78
Sinusoidal cells	6
Endothelial cells	3
Kupffer cells	2
Fat Storing (Ito) cells	1
Spaces	16
Disse space	6
Sinusoidal lumen	11
Bile canaliculi	0.5

Data from Blouin, 1977. Values are percentages

Hepatic Lobule Organization



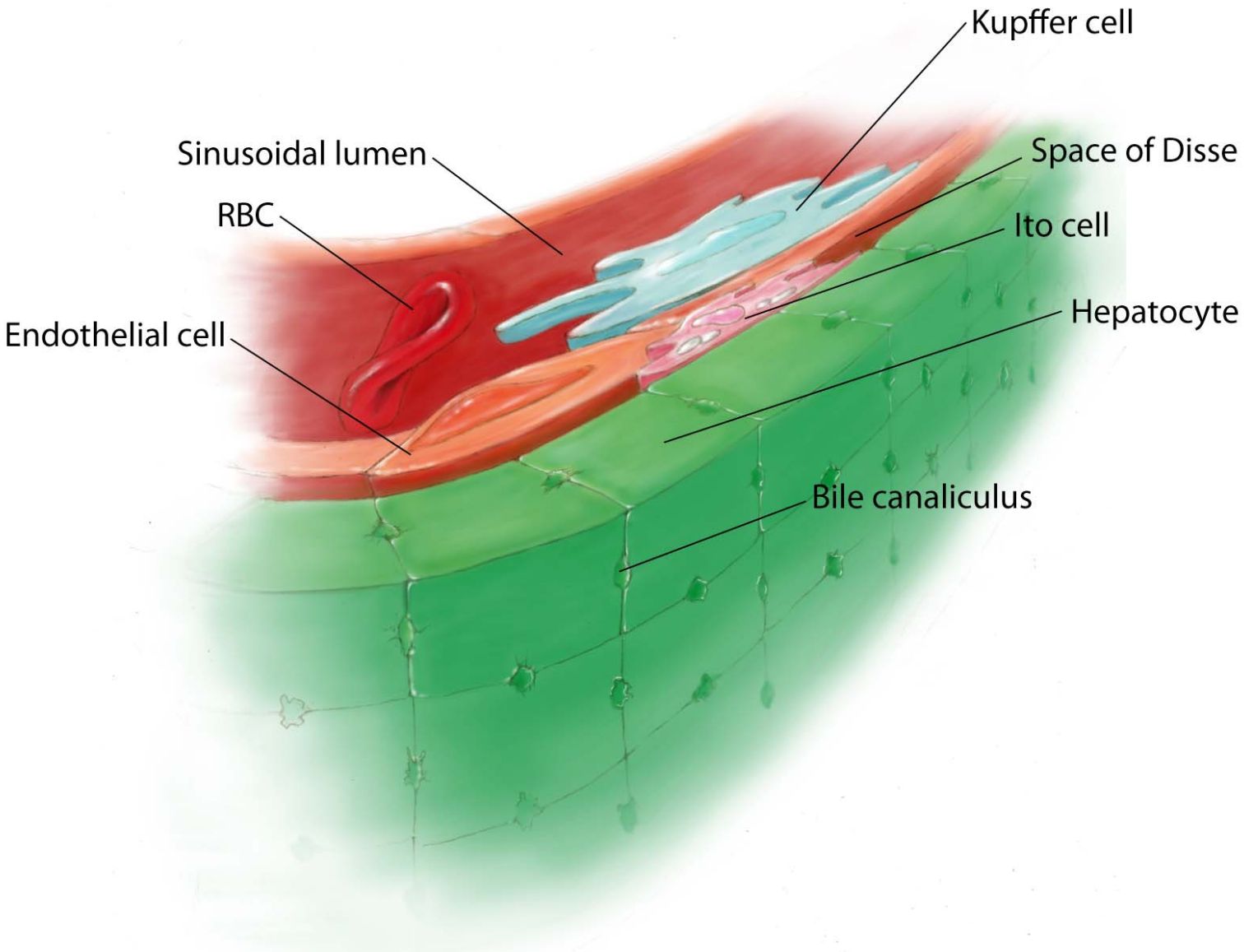
Normal Liver

- Liver is divided histologically into lobules
- The center of the lobule is the **central vein**
- At the periphery of the lobule are **portal triads**

Normal Liver

- Functionally, the liver can be divided into three zones, based upon oxygen supply
  - **Zone 1** encircles the portal tracts where the oxygenated blood from hepatic arteries enters and mixes with portal blood
  - **Zone 3** is located around central veins where blood exits; oxygenation is low
  - **Zone 2** is the area in between Zones 1 and 3

Liver: Structural Organization



Section B

Liver: Functions, Injury, Detection, and Response

Liver Functions

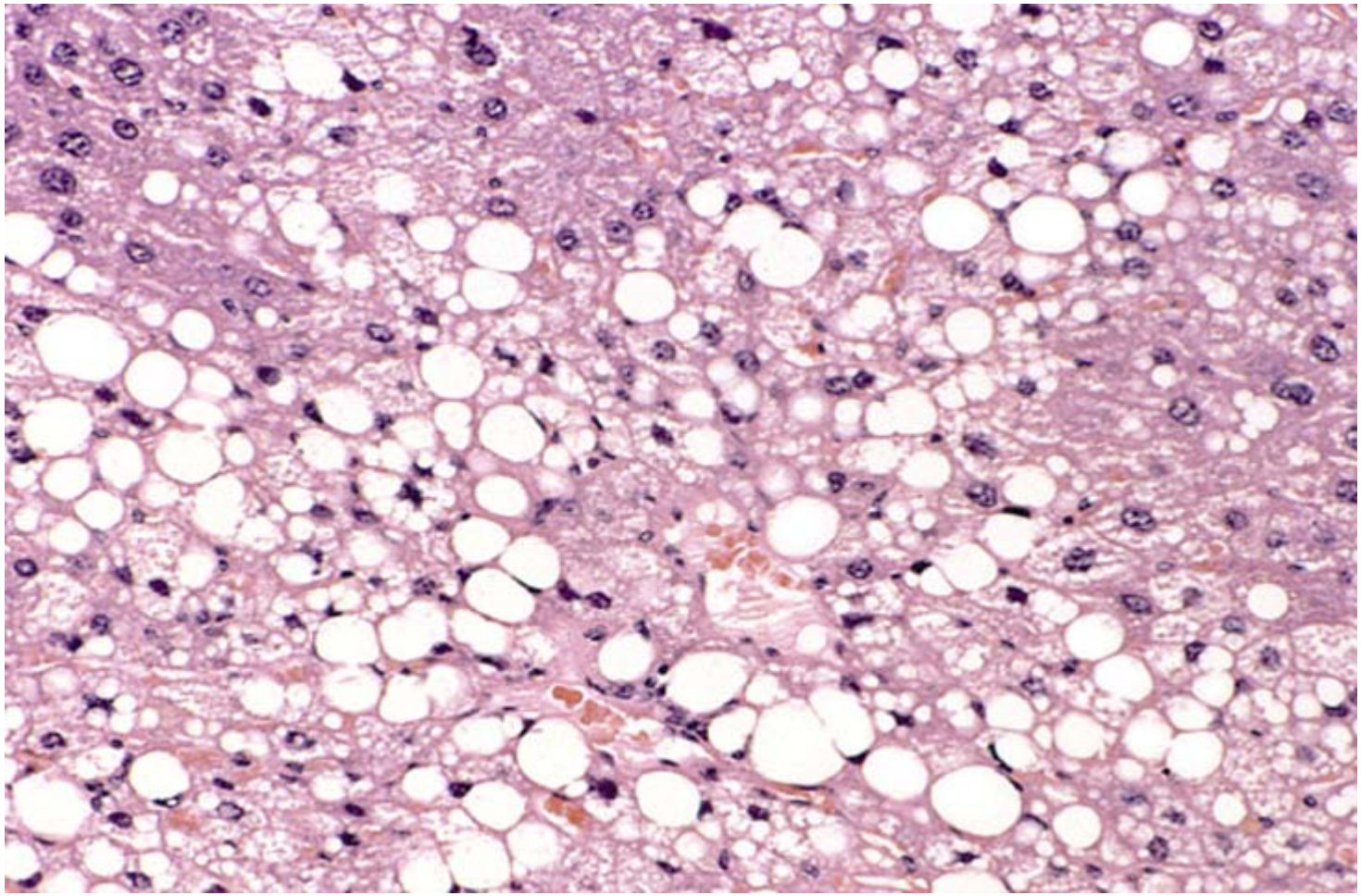
1. **Biotransformation of xenobiotics, endogenous compounds, including hormones**
2. **Carbohydrate metabolism and storage**
3. **Synthesis of blood proteins (albumin, lipoproteins)**
4. **Urea formation**
5. **Fat metabolism**
6. **Bile formation**

Zonal Localization of Metabolic Processes		
Predominantly Acinar Zone 1 (Periportal)	**Predominantly Acinar Zone 3 (Centrilobular)**	**Distributed Equally**
Oxidative energy metabolism	Glucose uptake	Metabolism of Ethanol Acetaldehyde
Fatty acid oxidation	Glycolysis	
Respiratory chain	Glycogen synthesis from glucose	
Glucose release	Glycogen degradation to lactate	
Glucose synthesis from lactate	Ketogenesis	
Amino acid utilization	Lipogenesis including bile acid synthesis	
Amino acid conversion to glucose	**Biotransformation**	
Amino acid degradation		
Urea formation		
Secretion Bile acids Bilirubin		
Modified from Jungermann (1986); Thurman and Kaufman (1985) Traber et al (1988)		

Hepatotoxicity

Type of Injury/Damage	Representative Toxins
Fatty Liver (Steatosis)	**CCl_4, ethanol**, fialuridine (anti-viral), valproic acid (anti-epilectic)
Hepatocyte Necrosis (cell death)	acetaminophen, **ethanol, chloroform**
Canalicular cholestasis	estrogens, chlorpromazine
Bile duct damage	amoxicilin, α-napthyl-isothiocyanate (cholestatic chemical)
Sinusoidal damage	anabolic steroids, cyclophosphamide
Fibrosis & cirrhosis	**ethanol**, vinyl chloride, vitamin A
Tumors	aflatoxin, vinyl chloride, synthetic estrogens, androgens

Liver Steatosis



Histological section of a murine liver showing severe steatosis. The clear vacuoles would have contained lipid in the living cells, however the histological fixation caused it to be dissolved and hence only empty spaces remain. Source: NIEHS, NIH. Public Domain.

Site-specific Hepatotoxicity

Site	Toxicant	Mechanism
Zone 1	Fe overload	Preferential uptake, high O_2
	allyl alcohol	High O_2 (oxidative bioactivation)
Zone 3	CCl_4	P450-dependent bioactivation
	acetaminophen	P450-dependent bioactivation and lower GSH
	ethanol	Lower O_2 and bioactivation/detox. imbalance

Zone 3 Hepatotoxicity: caused by CCl_4, acetaminophen

- Necrosis involves the hepatocytes around the central vein (susceptibility because of higher quantity of P450 enzymes in Zone 3 (centrilobular area)

Detection of Hepatotoxicity

Endpoints/Biomarkers

Symptoms

- Nausea, vomiting, fatigue, hepatomegaly, jaundice

Histopathology

- Fatty liver, cirrhosis, necrosis, fibrosis,
- Hepatocellular tumors

Blood Tests

- Serum hepatic enzymes – ALT, AST, GGT
- Drug clearance
- Clotting times
- Bilirubin

Response to Xenobiotics and Repair of Hepatotoxicity

Liver responds to increased workload by

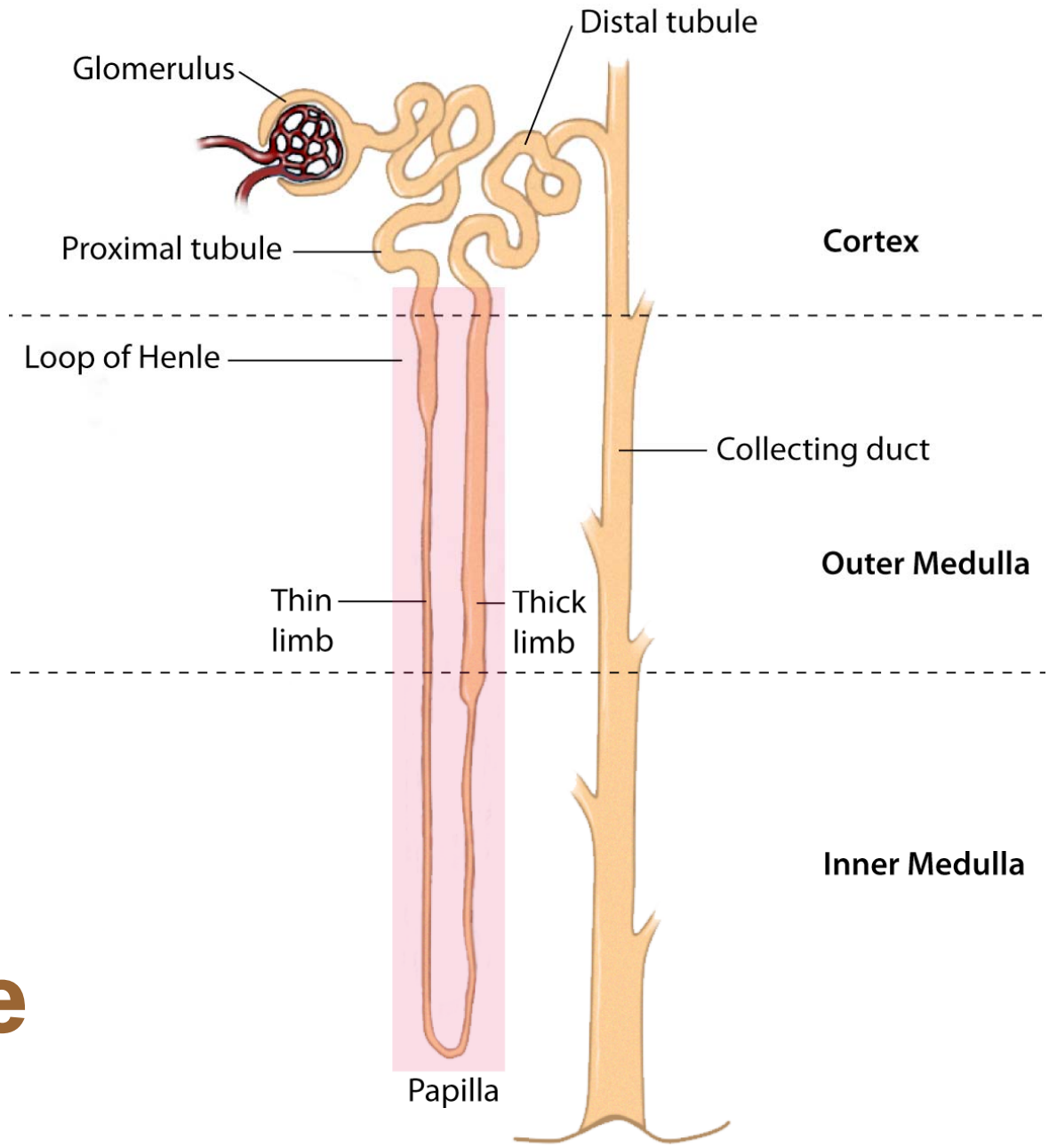
- **Hypertrophy (increased cell size)**
- **Hyperplasia (increased cell number)**

Liver has enormous regenerative capacity

Section C

Kidney: Structure

Nephron Structure



Features of the Renal Cortex

- Glomerulus
- Renal tublues
  - Proximal
  - Distal
- Bowman's capsule
- Bowman's space
- Capillaries
- Mesangium

- Images of normal kidney structures are available at http://www.biologyofhumanaging.com/slides/kidney07.htm

Features of the Medulla

- Collecting duts
- Loops of Henle
  - Thick loop
  - Thin loop

- Images of normal kidney structures are available at http://www.biologyofhumanaging.com/slides/kidney07.htm

Section D

Kidney: Functions, Injury, Detection, and Response

Kidney - Functions

- **Removal and Excretion** of toxic metabolic waste from blood
- **Regulation of homeostasis** of organism
  - **Elimination/conservation** of water and electrolytes
  - **Hormonal functions**:
    - renin production (regulation of blood pressure)
    - erthropoietin production (regulation of Hb synthesis
    - Vit. D (1,25 dihydroxycholecalciferol) formation
    - Parathyroid hormone metabolism – $Ca2^{+}$ regulation

Kidney Structures and Functions

Structure	**Functions**
Vasculature	
• afferent arteriole	Deliver blood to glomerulus
• efferent arteriole	Drains glomerulus
Glomerulus	Filtration of blood (size and charge- selective filter); Filtration rate = 125 ml/min (180 L/day)
Tubules	Selectively reabsorb 98-99% salts, H_20, glucose, amino acids
• Proximal	Reabsorption: water, glucose, Na. K, PO_4, SO_4, amino acids, low molecular weight proteins Secretion: organic anionic (-) and cationic (+) compounds
•Loop of Henle	Urinary concentration Descending portion: H_2O leaves filtrate Ascending: H_2O impermeable; Na & Cl transport

Kidney Structures and Functions

Structure	Functions
Tubules cont'd •Distal Tubule & Collecting Duct	Selectively reabsorb 98-99% salts, H_20, Urine formation: final regulation and fine tuning of urine composition

Substance	Filtered/day	% Reabsorbed
Glucose (g/day)	180	100
Bicarbonate (meq/day)	4,320	>99.9
Na^+ (meq/day)	25,560	99.4
Cl^- (meq/day)	19,440	99.1
H_2O (L/day)	169	99.1
Urea (g/day)	48	50
Creatinine (g/day)	1.8	0

Kidney (nephro) Toxicants

Metals

- •Cadmium
- • Mercury
- • lead

Halogenated Hydrocarbons

- • **CCl_4**
- • **Chloroform**
- • Methoxyflurane (surgical anesthetic)
- • Perchlorethylene

Other Chemicals

- • MTBE (methyl-tert-butyl ether) (Gasoline additive)
- • Acetaminophen
- • Various antibiotics

Specificity of Renal Injury

Various nephrotoxicants cause site-selective injury

Mechanistic Basis

- Complex
- Blood flow
- Transport mechanisms
- Biotransformation capability of various regions
- Physicochemical properties of chemicals
- Specific functions of the cells in region

Detection of Renal Toxicity – Endpoints/Biomarkers

Symptoms

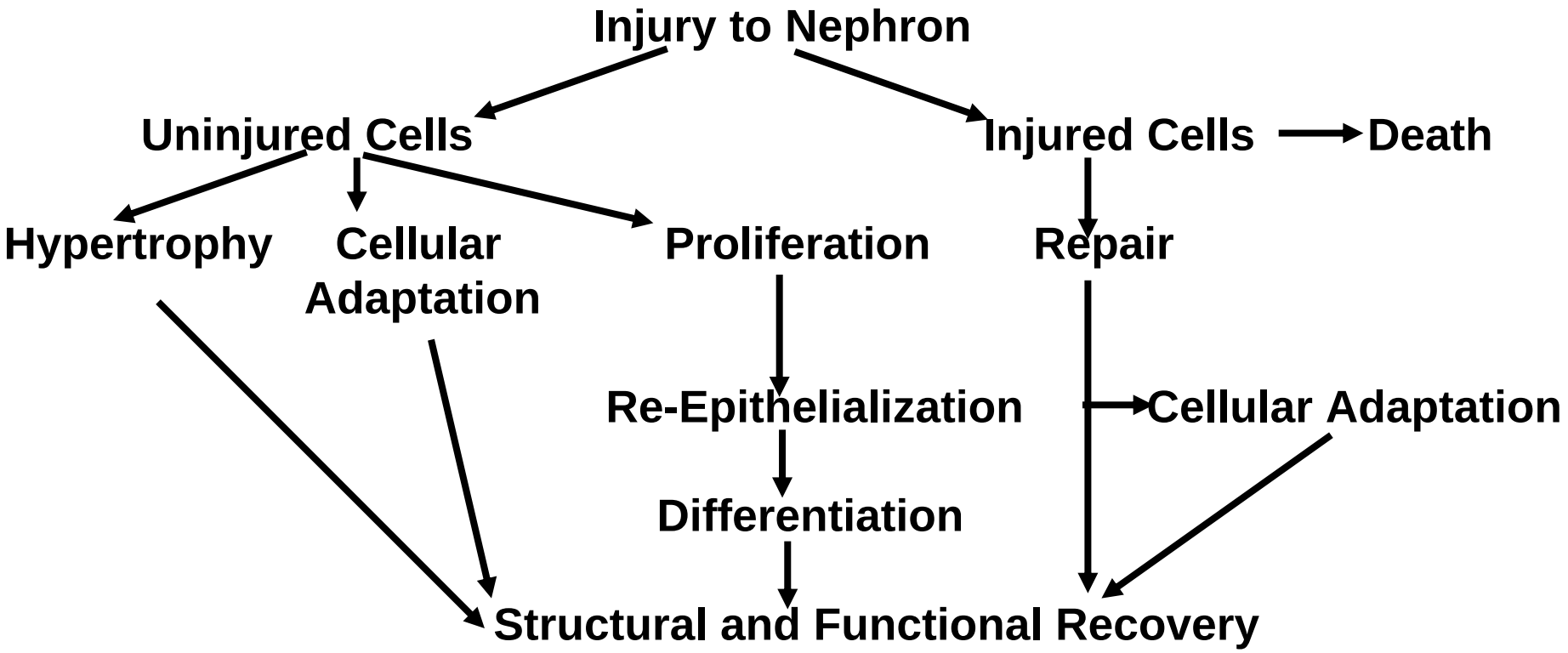
- Acute Renal Failure

Alterations in excretion of wastes

- Glomerular filtration rate
  - Use of inulin (5,200 mwt polymer)
- Renal plasma flow
  - Some organic acids (complete removal from plasma)
- Additional tests
  - pH, volume, glucose, salts (Na, K)

Response to Xenobiotics and Repair of Renal Toxicity

Kidney has regenerative capacity

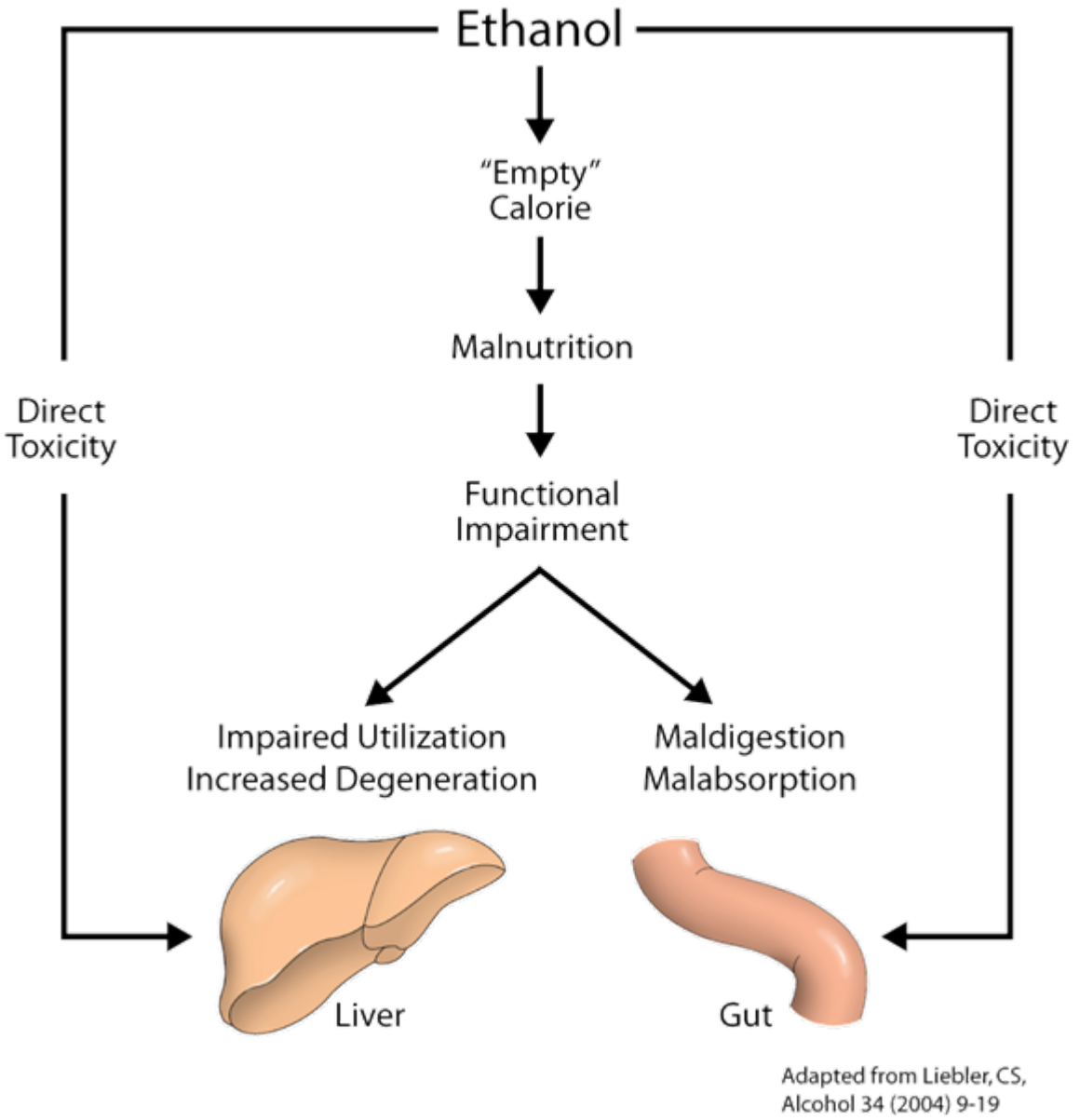


Section E

Case Study: Hepatotoxicity of Ethanol

Pathogenesis of Ethanol Toxicity

- Alcohol—
  a food and
  a drug



Pathogenesis of Ethanol Toxicity

Alcohol—A Food and a Drug

Summary of Pathogenic Mechanisms

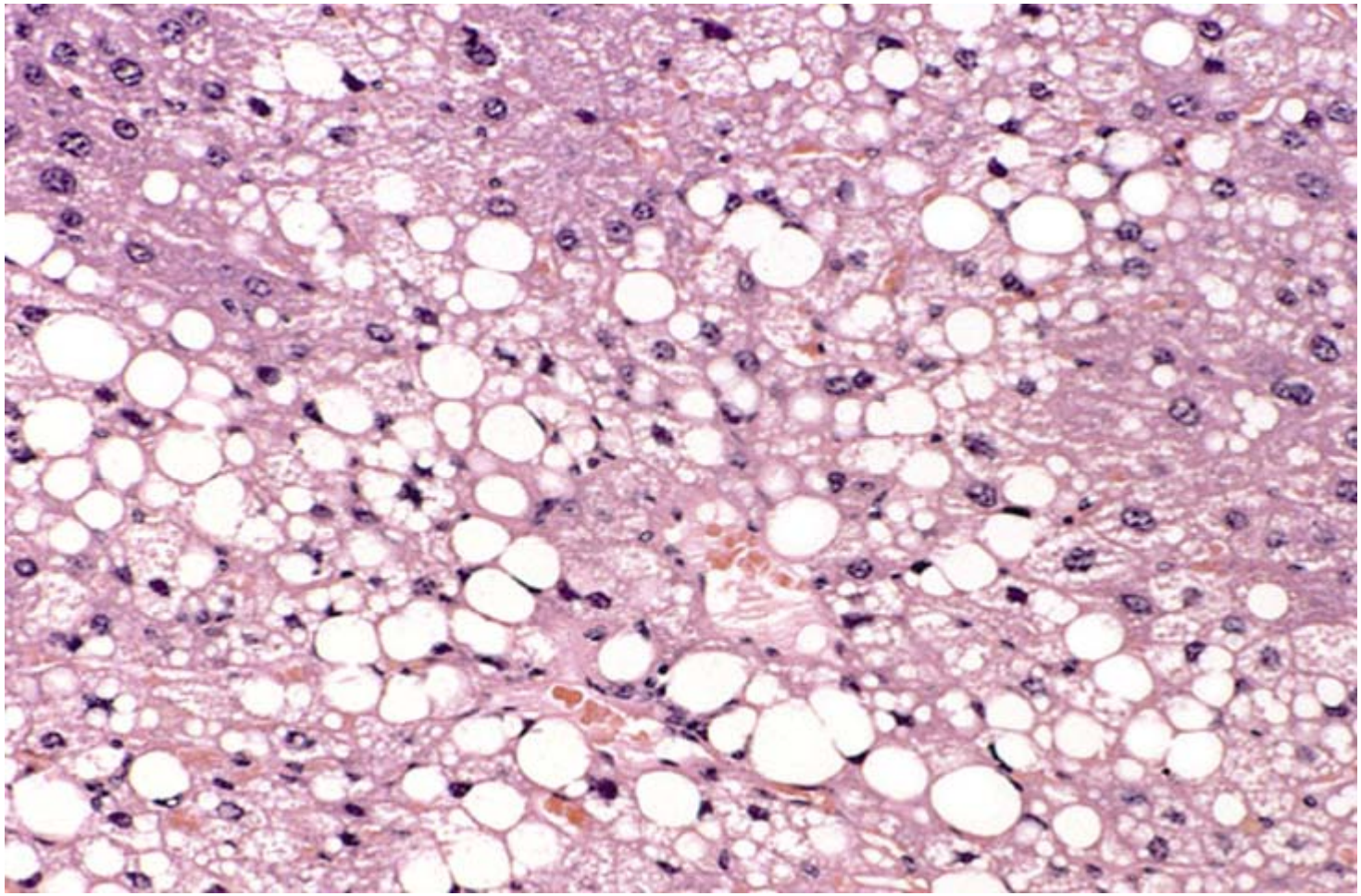
Direct

- Production of reactive acetaldehyde
- Increased levels of reducing co-factors

Indirect

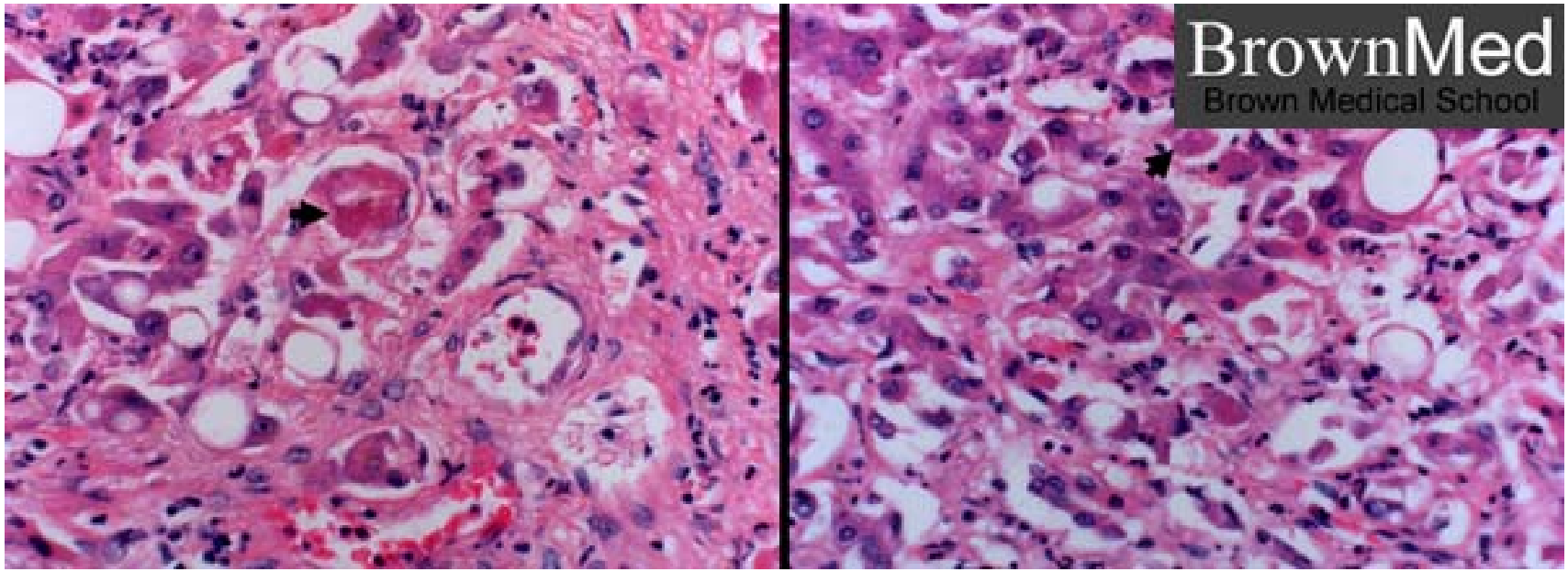
- Affects cell membrane fluidity
- Formation of a unique phospholipid (phosphatidylethanol)
- Formation of toxic fatty acid ethyl esters
- Mitochondrial inner membrane damage
- Promotes formation of Reactive Oxygen Species (ROS)
    - Formation of hydroxymethyl radical
    - ROS produced by CYP2E1

Liver Steatosis



Histological section of a murine liver showing severe steatosis. The clear vacuoles would have contained lipid in the living cells, however the histological fixation caused it to be dissolved and hence only empty spaces remain. Source: NIEHS, NIH. Public Domain.

Necrosis and degeneration (alcohol hepatitis)

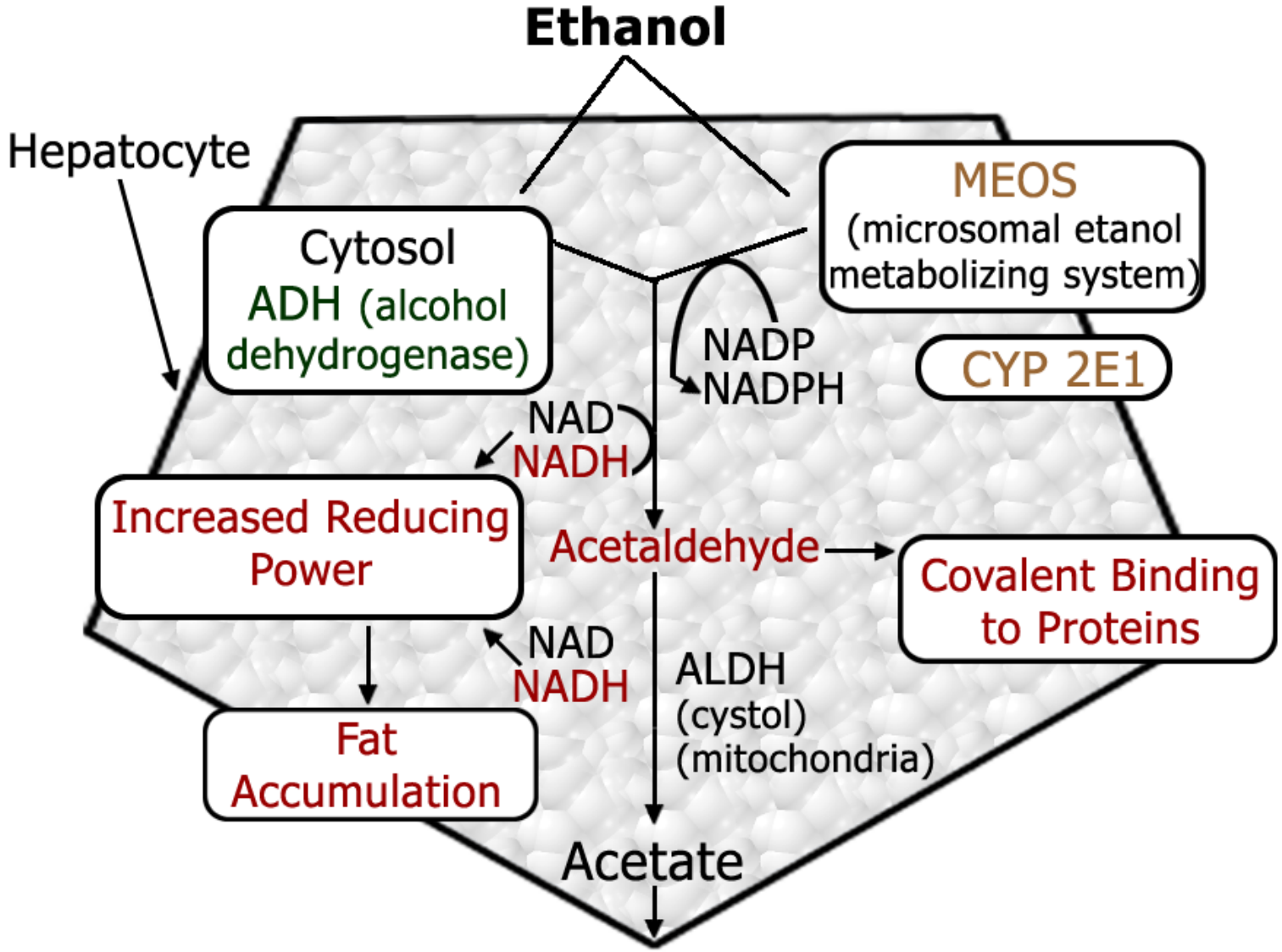


These photos from a case of acute alcoholic hepatitis show the characteristic but nonspecific findings of Mallory bodies (arrows), steatosis, and an inflammatory infiltrate. Mallory bodies (“alcoholic hyalin”) are cytoplasmic inclusions formed by accumulations of keratin intermediate filaments. Images reproduced with permission from Brown Medical School Digital Pathology. All Rights Reserved.

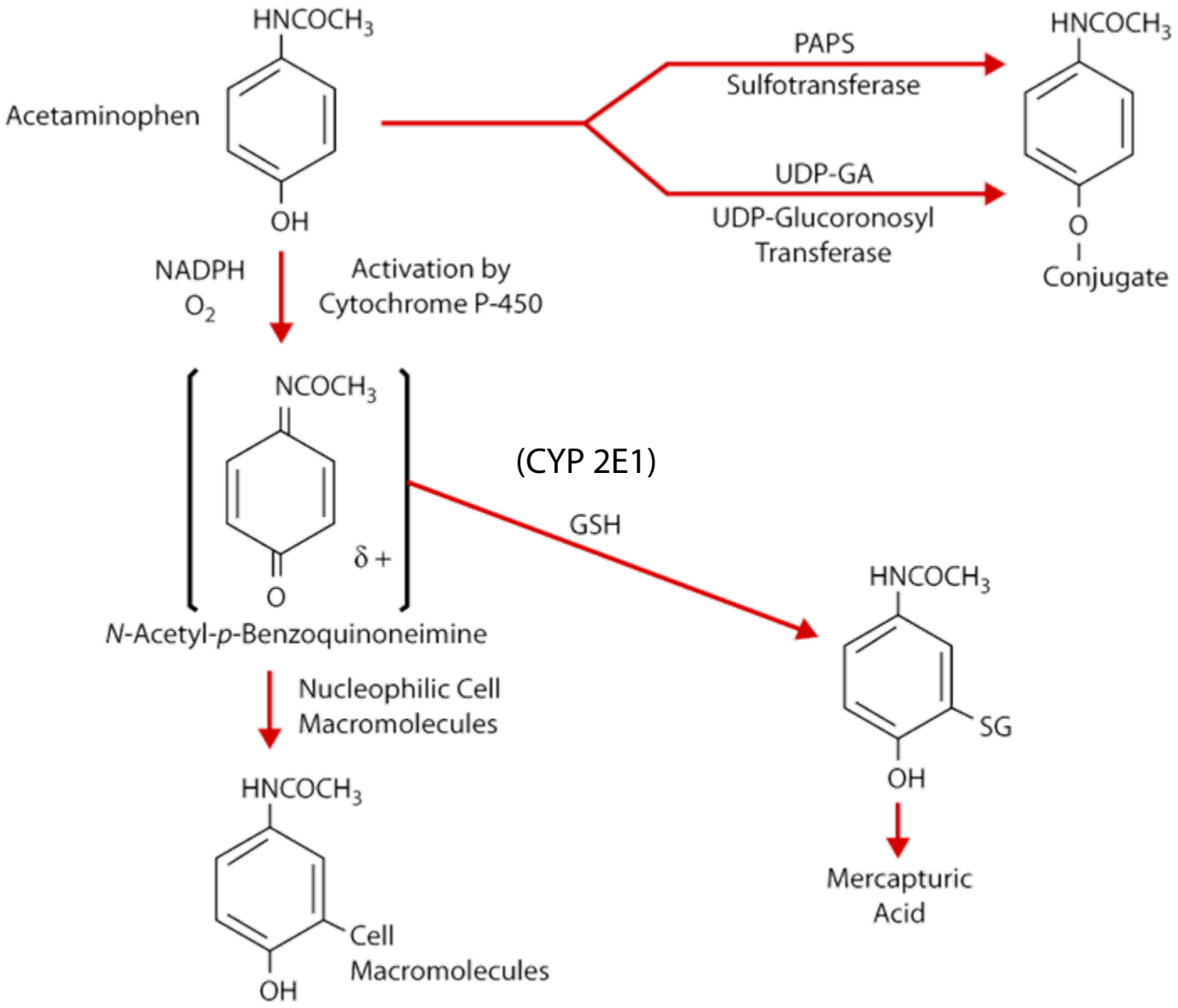
Hepatotoxicity of Ethanol: Liver—Alcohol Cirrhosis

- With cirrhosis, the regenerative nodules of hepatocytes are surrounded by fibrous connective tissue that bridges between portal tracts
- Within this collagenous tissue are scattered lymphocytes as well as a proliferation of bile ducts

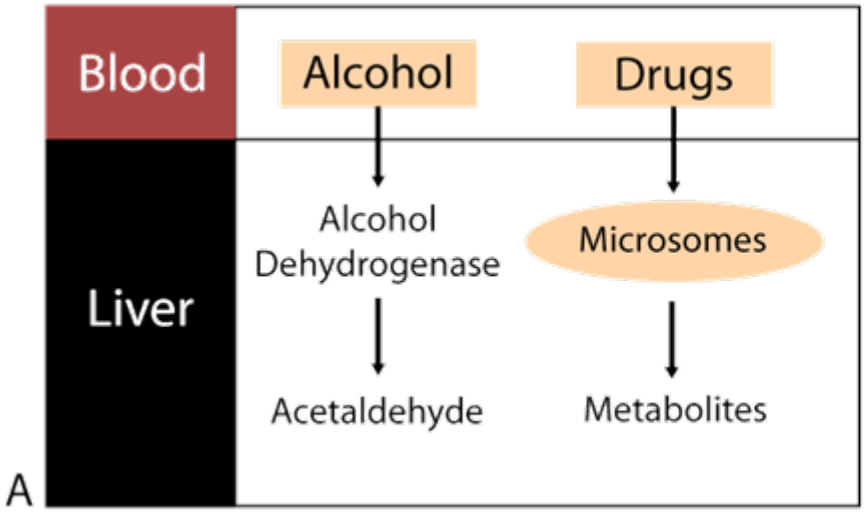
Metabolism of Ethanol in the Liver—Direct Toxicity

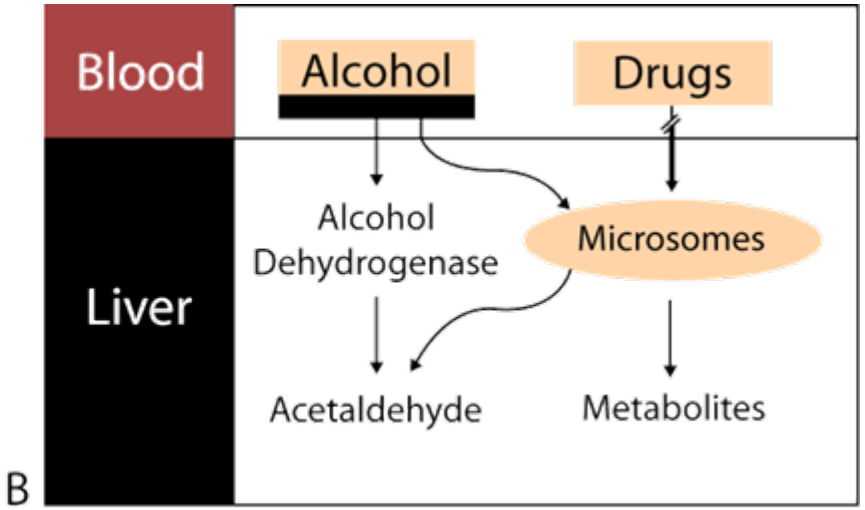


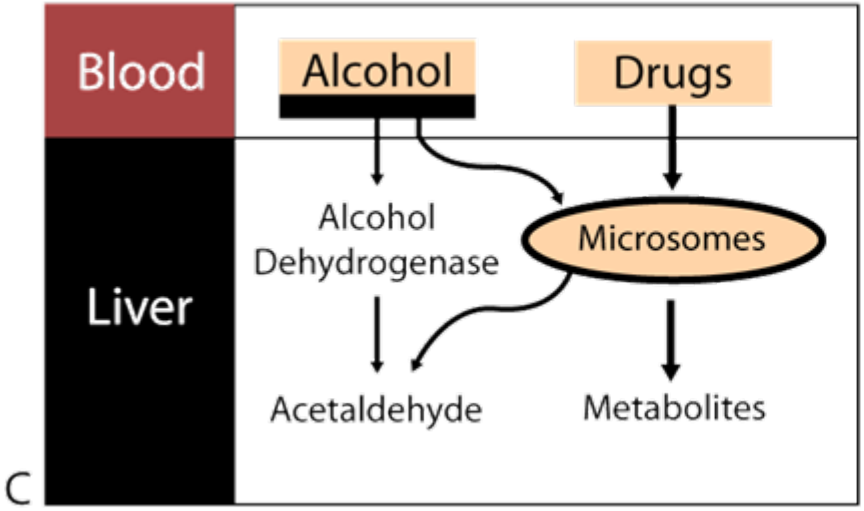
Ethanol-Drug Interactions: Ethanol & Acetaminophen

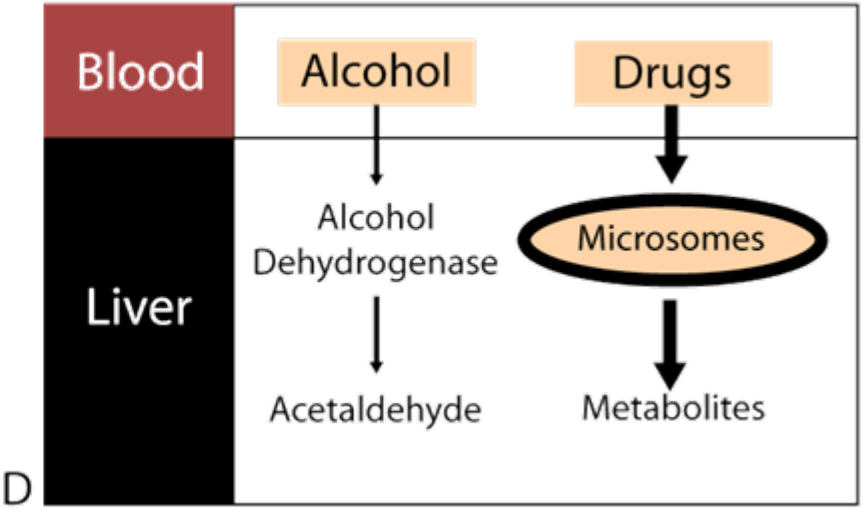


Principle of Ethanol-Drug Interactions









Ethanol Toxicity

Other Effects

- Women more vulnerable to alcoholic liver injury
- Teratogenicity
  - Fetal alcohol syndrome
- Carcinogenicity
  - Oral cavity (pharynx, larynx, esophagus), liver

Section F

Case Study: Hepatotoxicity of Carbon Tetrachloride - CCl_4

CCl_4: a classic hepatotoxin

A. Human Exposure:

1. Properties

colorless, volatile, high density, sweet smelling liquid which does not burn or conduct current

2. Sources of exposure to CCl_4

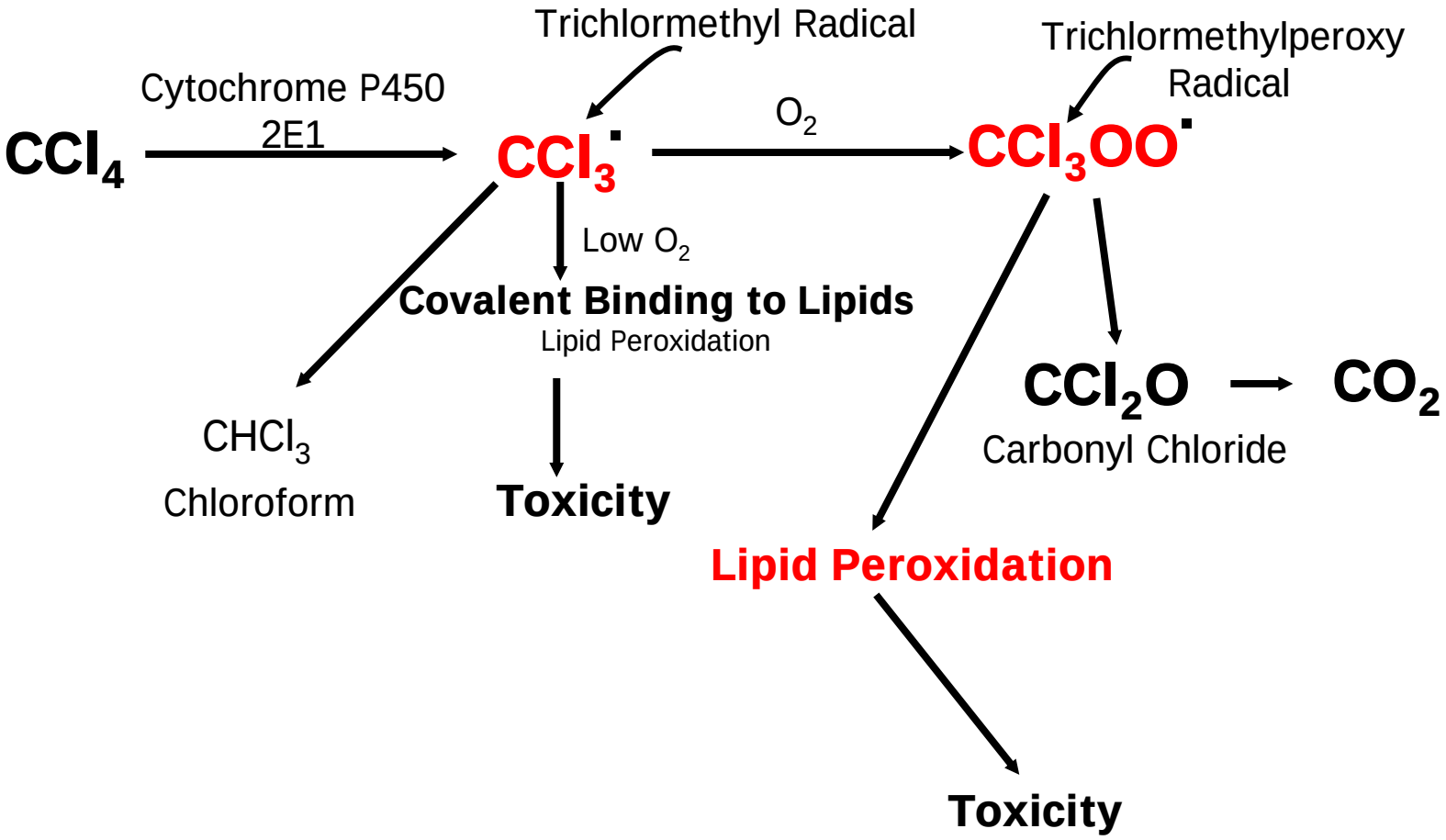
a. past: anesthetic (1800s); shampoo (early 1900's → deaths); hookworm (deaths); fire extinguishers, solvent/cleaning agent

b. consumer use discontinued; still has a number of industrial uses

3. Physiologic Responses

Concentration (ppm)	**Response**
21-79	Odor threshold
200	Severe toxic effects
1,000-2,000	Lethal

CCl_4 Metabolism



Lipid Peroxidation

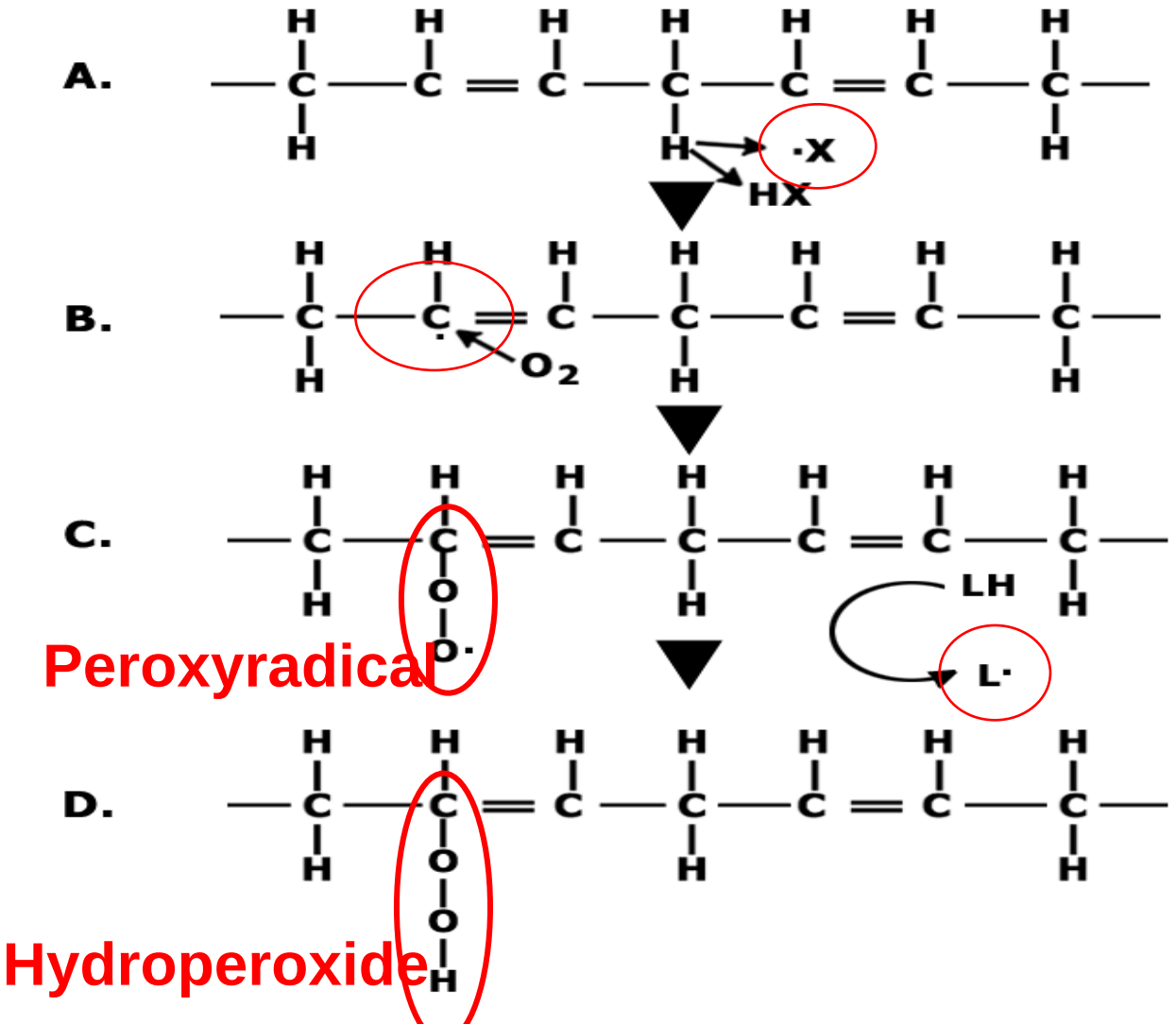


Figure 1. Initiation of lipid peroxidation

Adapted from Figure 1 in Recknagel O, Glende EA. Freed radical damanage and lipid peroxidation. In: *Hepatotoxicology.* Meeks RG, et al. Boca Raton, FL: CRC Press, 1991.

Potentiation of Haloalkane-Induced Hepatotoxicity

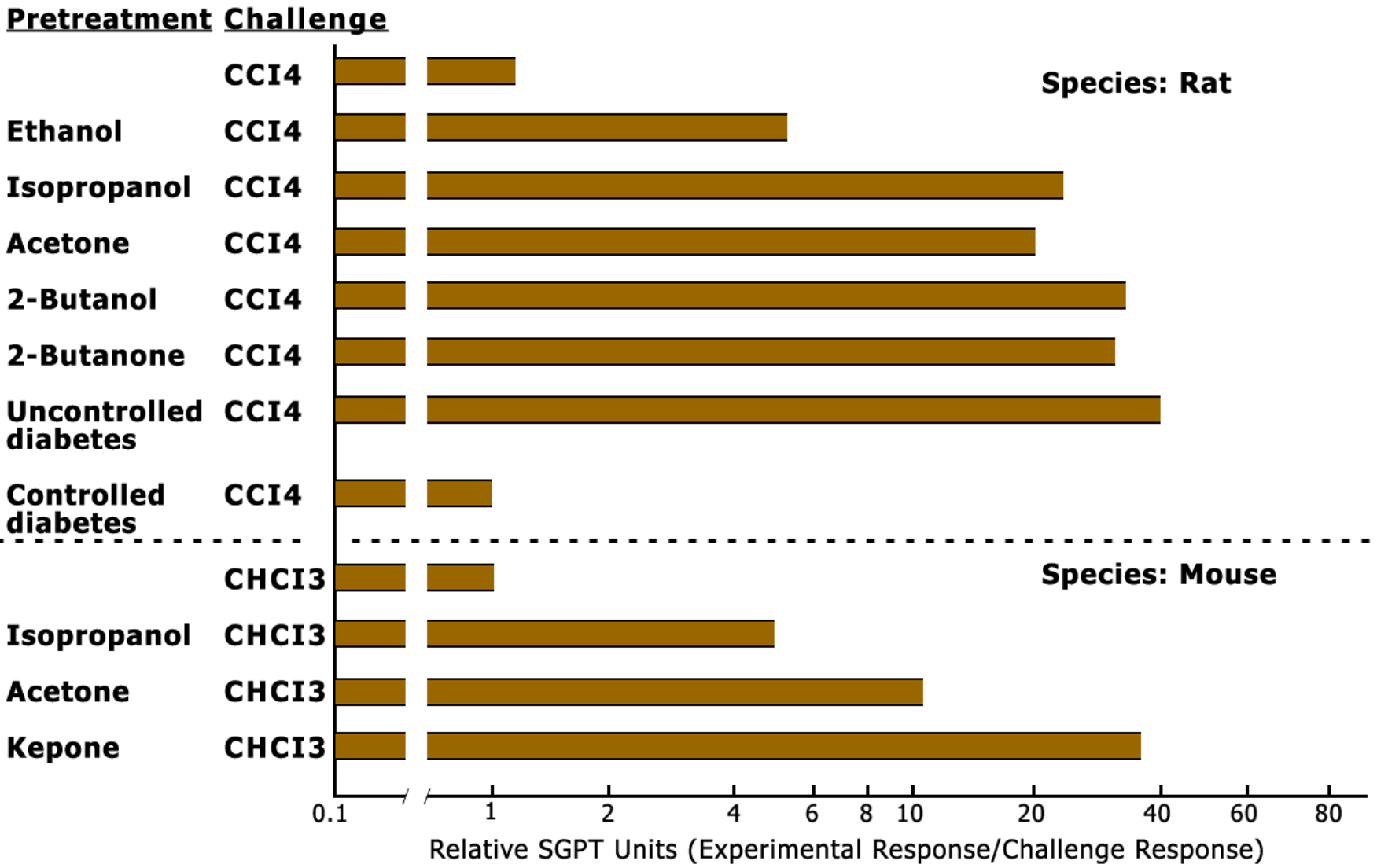


Figure 10.3 - Potentiation of Holoalkane-induced hepatotoxicity.

Section G

Case Study: Hepato and Renal Toxicity of Chloroform

Hepatotoxicity of Chloroform

- Properties
- Human exposure
- Effects of chlorinated chemicals on wildlife and human health
- Metabolism of chloroform
- Risk assessment issues associated with chloroform

Human Exposure to Chloroform

- Properties
  - Volatile, pleasant-smelling, water-soluble liquid
- Past uses
  - Solvent/extraction solvent, spot remover, fire extinguishers, anesthetic

Human Exposure to Chloroform

- Current uses
  - Chemical intermediate used in a wide array of chemicals and plastics
  - A trihalomethane by-product of
    - Chlorination of cooling water in power plants
    - Bleaching of paper
    - Chlorination of drinking water

Physiologic Responses to chloroform exposure (air)

Concentration (ppm)	Response
200-300	odor threshold
4,100	nausea, fainting
14,000-16,000	narcotic

Human Exposure to Chloroform

- Routes of exposure
  - Drinking water : 2-44 ppb in treated drinking water (0.1 - 300; most municipal water supplies < 60 ppb)
  - Swimming pool: 1,000 ppb (1ppm)
  - Air : 0.00001 to 0.0005 ppm

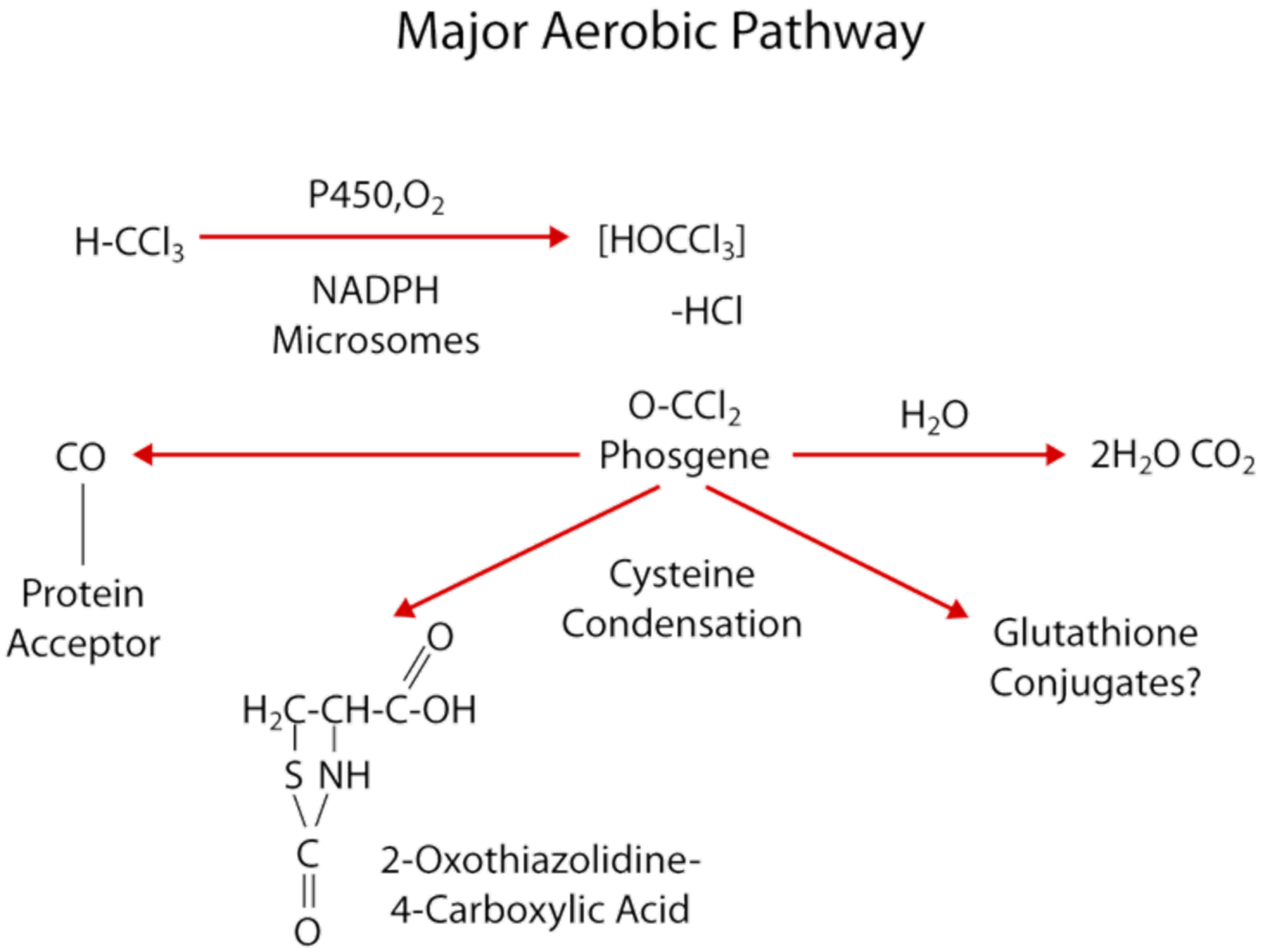
    (air above swimming pool: 0.13 ppm)

    (shower stall: 0.066 ppm)

Potential Effects of Chlorinated Chemicals on Wildlife and Human Health

- Wildlife: Birth defects and reproductive abnormalities
- Rodent bioassays: Liver and renal tumors
- Humans – Exposure associated with:
  - Carcinogenic effects—breast, prostate, stomach, bladder
  - Endometriosis
- Movement to ban use of chlorine and chlorinated chemicals
- Strength of evidence - Weak

Chloroform Biotransformation



Section H

Case Study: Risk Assessment Issues Associated with Chloroform

Risk Assessment Issues Associated with Chloroform

- Virtually safe dose (VSD) estimated by EPA for chloroform
  - Drinking water— 4.3 ppb for a 1/100,000 increased lifetime risk of cancer
  - Airborne—0.000008 ppm for a 1/1,000,000 increased lifetime risk of cancer

Risk Assessment Issues Associated with Chloroform

- Induction of mouse liver tumors and rat kidney tumors by chloroform
  - Administration by Gavage
  - Administration in the drinking water

Chloroform Administered by Gavage in Corn Oil 5 Days/Week to B6C3F1 Mice and Osborne-Mendel Rats (NCI, 1976)			
Gender/Species	Dose (mg/kg/day)	Liver Tumor Incidence (%)	Kidney Tumor Incidence (%)
Female mouse	0 238 477	0 80 95	0 0 0
Male mouse	0 138 277	6 36 98	6 2 4
Female rat	0 100 200	0 0 0	0 0 4
Male rat	0 90 180	0 0 2	0 8 24

Adapted from Franklin J. Poisons of the mind. *CIIT Activities* 1994;14(5):1-6 and from Jorgensen TA, et al. Carcinogenicity of chloroform in drinking water to male Osborne-Mendel rats and female B6C3F1 mice. Fundam Appl Toxicol 1985;5:760-769.

Chloroform Administered *Ad Libitum* in the Drinking Water to B6C3F1 Mice and Osborne-Mendel Rats (Jorgenson *et al*, 1985)

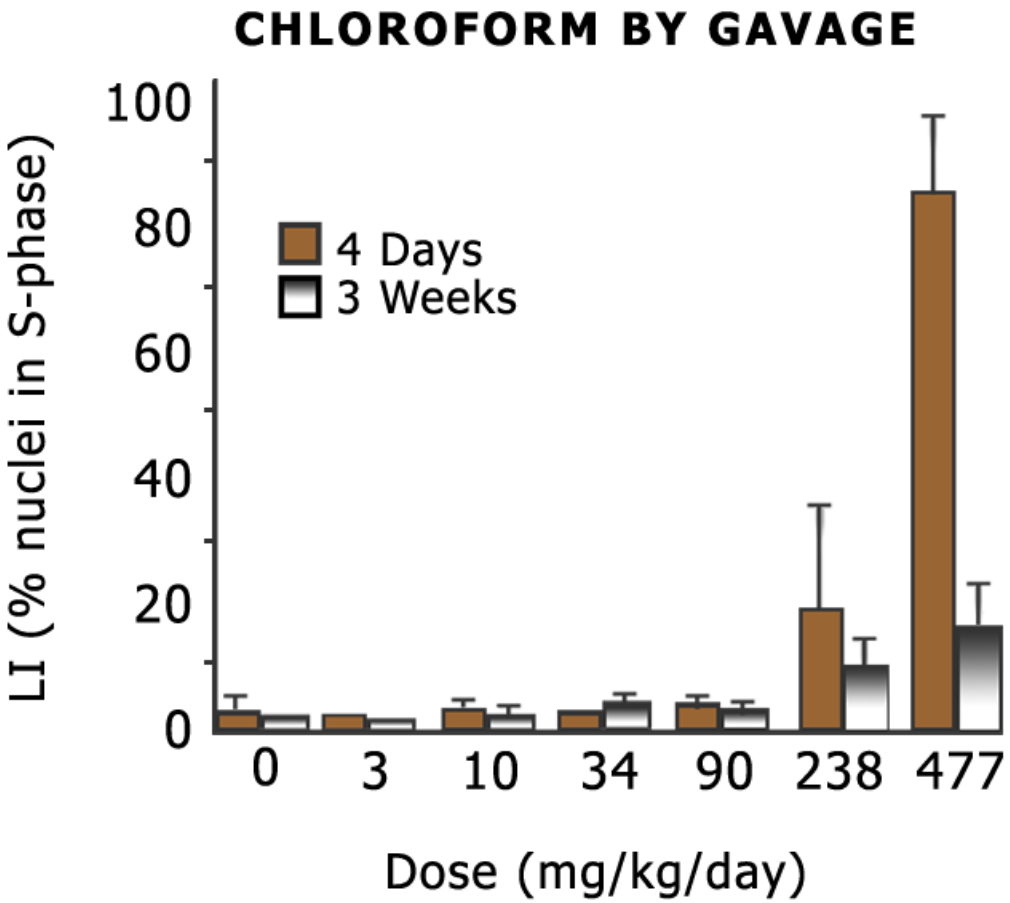
Gender/ Species	Drinking Water Concentration (ppb)	Dose (mg/kg/day)	Liver Tumor Incidence (%)	Kidney Tumor Incidence (%)
Female mouse	0	0	0	*
	200,000	34	4	*
	400,000	65	6	*
	900,000	130	0	*
	1,800,000	263	2	*
Male rat	0	0	*	2
	200,000	19	*	2
	400,000	38	*	5
	900,000	81	*	14
	1,800,000	160	*	14

* Incidence data were not presented in tabular form. However the text noted that these tumors were not increased in chloroform-exposed animals compared to controls (Jorgenson *et.al.*, 1985)

Adapted from Franklin J. Poisons of the mind. *CIIT Activities* 1994;14(5):1-6 and from Jorgensen TA, et al. Carcinogenicity of chloroform in drinking water to male Osborne-Mendel rats and female B6C3F1 mice. Fundam Appl Toxicol 1985;5:760-769.

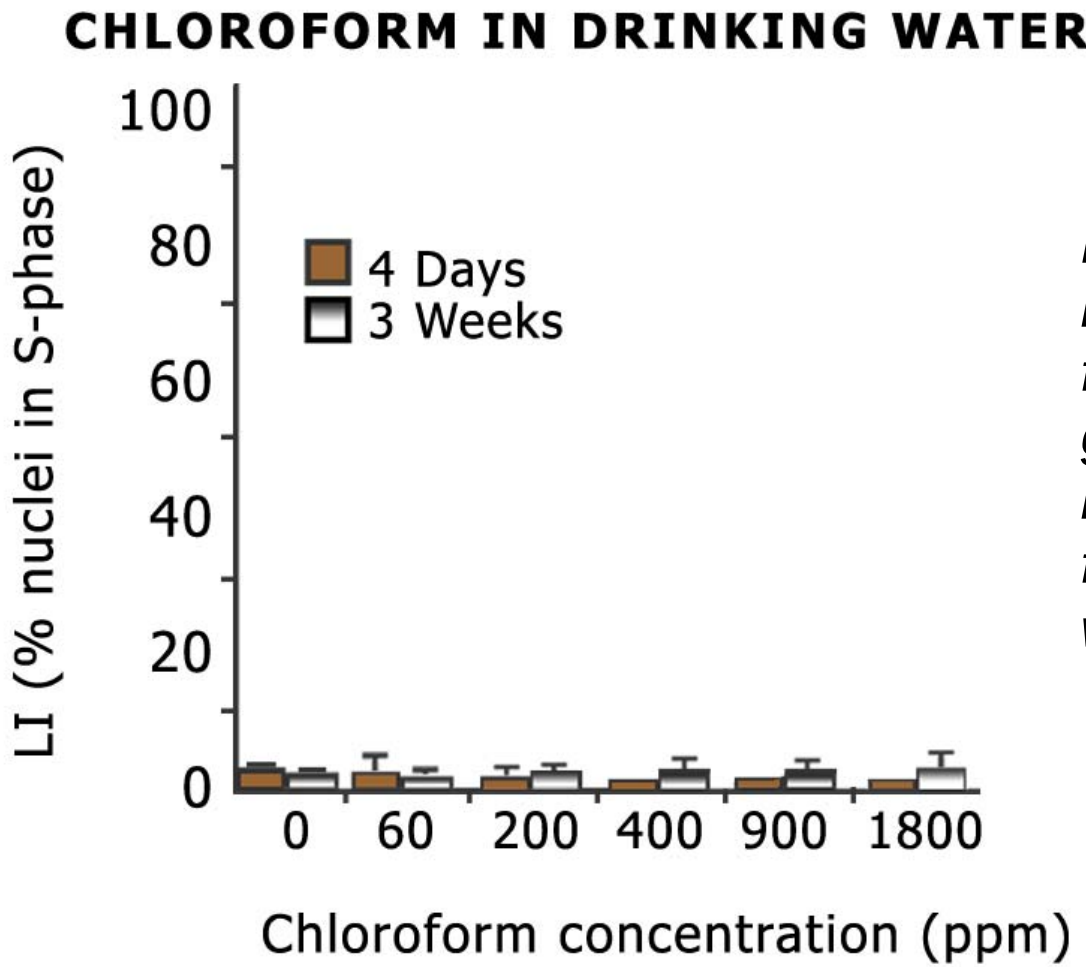
Risk Assessment Issues Associated with Chloroform

- Mechanistically-based risk assessment
  - Genotoxicant?
  - Nongenotoxic-cytotoxicant
  - Enhanced cell proliferation



Hepatocyte labelling index in female mice given chloroform by Gavage for 4 days or 3 weeks

Adapted from Butterwoth BE, et al. Risk assessment issues associated with chloroform-induced mouse liver tumors. *CIIT Activities* 1994;14(2):1-8 and from Larson JL, et al. The toxicity of 1-week exposures to inhaled chloroform in female B6C3F1 mice and male F-344 rats. Fundam Appl Toxicol 1994;22:431-436.



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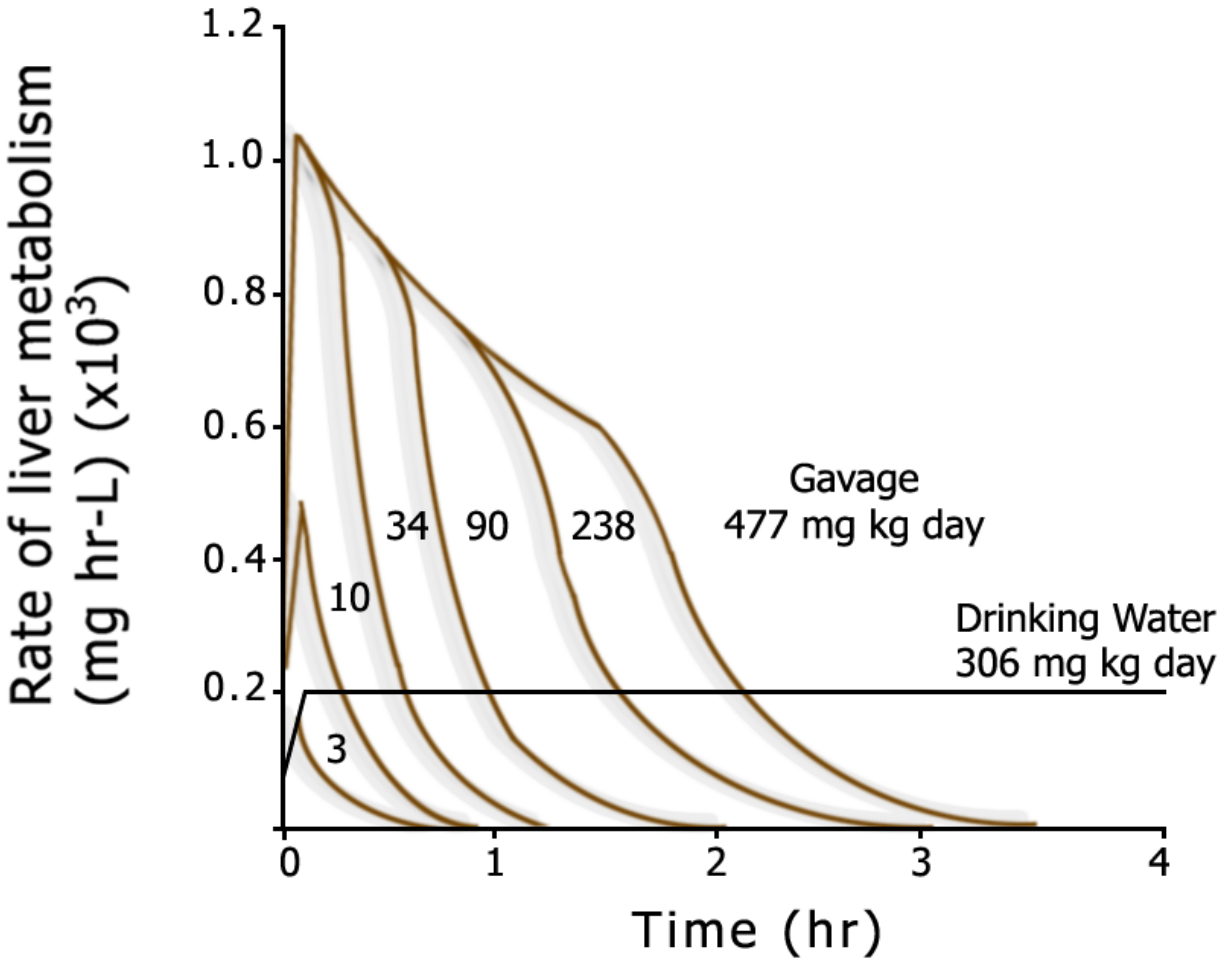
Mechanistically-Based Risk Assessment
Toxicokinetics



PB-PK model simulation of rates of liver metabolism in the female B6C3F$_1$ mouse following a single gavage dose of chloroform in corn oil derived from the model parameters of Corley et al. (1990).

Adapted from Butterwoth BE, et al. Risk assessment issues associated with chloroform-induced mouse liver tumors. *CIIT Activities* 1994;14(2):1-8 and from Larson JL, et al. The toxicity of 1-week exposures to inhaled chloroform in female B6C3F1 mice and male F-344 rats. Fundam Appl Toxicol 1994;22:431-436.

Risk Assessment Issues Associated with Chloroform

- Risk assessment based on cell proliferation data

Mechanistically-Based Risk Assessment: Based on Cell Proliferation Data

Chloroform Drinking Water Risk Assessment

Based on mouse liver tumor data from the gavage study *(NCI, 1976)*

Assumptions	VSD
Default LMS model	4 ppb
1-in-100,000 increased lifetime cancer risk	
Male and female mouseliver tumor response (U.S. EPA, 1985)	

Mechanistically-Based Risk Assessment: Based on Cell Proliferation Data

Chloroform Drinking Water Risk Assessment-

Based on the male rat kidney tumor data from the drinking water study (Jorgenson et al., 1985)

Assumptions	VSD
Default LMS model 1-in-100,000 increased lifetime cancer risk Male rat kidney tumor response (U.S. EPA, 1985) Current EPA standard (U.S. EPA 1994)	60 ppb
Cytotoxic/Nongenotoxic mode of action Model incorporating dosimetry and cell killing 1-in-100,000 increased lifetime cancer risk Uncertainty factor of 1,000 Male rat kidney tumor response (Reitz *et.al.*, 1991)	25,100 ppb

Mechanistically-Based Risk Assessment: Based on Cell Proliferation Data

Chloroform in Air Risk Assessment

Based on mouse liver tumor data from the gavage study (NCI, 1976)

Assumptions	VSD
Default LMS model 1-in-1,000,000 increased lifetime cancer risk Male and female moue liver tumor response (U.S. EPA, 1985; U.S. EPA, 1994)	0.000008 ppm
Cytotoxic/Nongenotoxic mode of action Modified LMS incorporating dosimetry and cell killing 1-in-1,000,000 increased lifetime cancer risk Female mouse liver tumor response Referred to as a Risk Specific Dose (RSD) (Reitz, *et.al.,* 1991)	0.23 ppm