Hepato and Renal Toxicology

James D. Yager, PhD
Johns Hopkins University
Section A

Liver: Structural organization
Liver: Structural Organization

<table>
<thead>
<tr>
<th>PRESSURE</th>
<th>mmHg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vena Cava</td>
<td>2–5</td>
</tr>
<tr>
<td>Hepatic v.</td>
<td>3–6</td>
</tr>
<tr>
<td>Hepatic a.</td>
<td>120</td>
</tr>
<tr>
<td>Portal v.</td>
<td>7–10</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>BLOOD FLOW</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hepatic a.</td>
<td>20–25</td>
</tr>
<tr>
<td>Portal v.</td>
<td>75–80</td>
</tr>
<tr>
<td>Spleen</td>
<td>25</td>
</tr>
<tr>
<td>Intestine</td>
<td>75</td>
</tr>
</tbody>
</table>
### Structural Organization of the Liver: Cellular Composition as % Liver Volume

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hepatocytes</td>
<td>78</td>
</tr>
<tr>
<td>Sinusoidal cells</td>
<td>6</td>
</tr>
<tr>
<td>Endothelial cells</td>
<td>3</td>
</tr>
<tr>
<td>Kupffer cells</td>
<td>2</td>
</tr>
<tr>
<td>Fat Storing (Ito) cells</td>
<td>1</td>
</tr>
<tr>
<td>Spaces</td>
<td>16</td>
</tr>
<tr>
<td>Disse space</td>
<td>6</td>
</tr>
<tr>
<td>Sinusoidal lumen</td>
<td>11</td>
</tr>
<tr>
<td>Bile canaliculi</td>
<td>0.5</td>
</tr>
</tbody>
</table>

---

*Data from Blouin, 1977. Values are percentages*
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

- Sinusoidal lumen
- RBC
- Endothelial cell
- Kupffer cell
- Space of Disse
- Ito cell
- Hepatocyte
- Bile canaliculus
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
<table>
<thead>
<tr>
<th>Zonal Localization of Metabolic Processes</th>
<th>Predominantly Acinar Zone 1 (Periportal)</th>
<th>Predominantly Acinar Zone 3 (Centrilobular)</th>
<th>Distributed Equally</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxidative energy metabolism</td>
<td>Glucose uptake</td>
<td>Metabolism of Ethanol Acetaldehyde</td>
<td></td>
</tr>
<tr>
<td>Fatty acid oxidation</td>
<td>Glycolysis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Respiratory chain</td>
<td>Glycogen synthesis from glucose</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose release</td>
<td>Glycogen degradation to lactate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose synthesis from lactate</td>
<td>Ketogenesis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amino acid utilization</td>
<td>Lipogenesis including bile acid synthesis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amino acid conversion to glucose</td>
<td><strong>Biotransformation</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amino acid degradation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urea formation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Secretion</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bile acids</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bilirubin</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

# Hepatotoxicity

<table>
<thead>
<tr>
<th>Type of Injury/Damage</th>
<th>Representative Toxins</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fatty Liver (Steatosis)</td>
<td>( \text{CCl}_4, \text{ethanol}, \text{fialuridine (anti-viral)}, \text{valproic acid (anti-epileptic)} )</td>
</tr>
<tr>
<td>Hepatocyte Necrosis (cell death)</td>
<td>( \text{acetaminophen, ethanol, chloroform} )</td>
</tr>
<tr>
<td>Canaliculnar cholestasis</td>
<td>( \text{estrogens, chlorpromazine} )</td>
</tr>
<tr>
<td>Bile duct damage</td>
<td>( \text{amoxicilin, } \alpha\text{-naphthyl-isothiocyanate (cholestatic chemical)} )</td>
</tr>
<tr>
<td>Sinusoidal damage</td>
<td>( \text{anabolic steroids, cyclophosphamide} )</td>
</tr>
<tr>
<td>Fibrosis &amp; cirrhosis</td>
<td>( \text{ethanol, vinyl chloride, vitamin A} )</td>
</tr>
<tr>
<td>Tumors</td>
<td>( \text{aflatoxin, vinyl chloride, synthetic estrogens, androgens} )</td>
</tr>
</tbody>
</table>
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

<table>
<thead>
<tr>
<th>Site</th>
<th>Toxicant</th>
<th>Mechanism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zone 1</td>
<td>Fe overload</td>
<td>Preferential uptake, high $O_2$</td>
</tr>
<tr>
<td></td>
<td>allyl alcohol</td>
<td>High $O_2$ (oxidative bioactivation)</td>
</tr>
<tr>
<td>Zone 3</td>
<td>CCl$_4$</td>
<td>P450-dependent bioactivation</td>
</tr>
<tr>
<td></td>
<td>acetaminophen</td>
<td>P450-dependent bioactivation and lower GSH</td>
</tr>
<tr>
<td></td>
<td>ethanol</td>
<td>Lower $O_2$ and bioactivation/detox. imbalance</td>
</tr>
</tbody>
</table>
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 tubulues
  - 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 ducts
- 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 – Ca$^{2+}$ regulation
# Kidney Structures and Functions

<table>
<thead>
<tr>
<th>Structure</th>
<th>Functions</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Vasculature</strong></td>
<td>• afferent arteriole: Deliver blood to glomerulus</td>
</tr>
<tr>
<td></td>
<td>• efferent arteriole: Drains glomerulus</td>
</tr>
<tr>
<td><strong>Glomerulus</strong></td>
<td>Filtration of blood (size and charge-selective filter); Filtration rate = 125 ml/min (180 L/day)</td>
</tr>
<tr>
<td><strong>Tubules</strong></td>
<td>Selectively reabsorb 98-99% salts, H2O, glucose, amino acids</td>
</tr>
<tr>
<td></td>
<td>• Proximal: Reabsorption: water, glucose, Na, K, PO₄, SO₄, amino acids, low molecular weight proteins</td>
</tr>
<tr>
<td></td>
<td>Secretion: organic anionic (-) and cationic (+) compounds</td>
</tr>
<tr>
<td></td>
<td>• Loop of Henle: Urinary concentration</td>
</tr>
<tr>
<td></td>
<td>Descending portion: H₂O leaves filtrate</td>
</tr>
<tr>
<td></td>
<td>Ascending: H₂O impermeable; Na &amp; Cl transport</td>
</tr>
</tbody>
</table>
Kidney Structures and Functions

Structure
Tubules cont’d
  • Distal Tubule & Collecting Duct

Functions
Selectively reabsorb 98-99% salts, H₂O,
Urine formation: final regulation and fine tuning of urine composition

<table>
<thead>
<tr>
<th>Substance</th>
<th>Filtered/day</th>
<th>% Reabsorbed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (g/day)</td>
<td>180</td>
<td>100</td>
</tr>
<tr>
<td>Bicarbonate (meq/day)</td>
<td>4,320</td>
<td>&gt;99.9</td>
</tr>
<tr>
<td>Na⁺ (meq/day)</td>
<td>25,560</td>
<td>99.4</td>
</tr>
<tr>
<td>Cl⁻ (meq/day)</td>
<td>19,440</td>
<td>99.1</td>
</tr>
<tr>
<td>H₂O (L/day)</td>
<td>169</td>
<td>99.1</td>
</tr>
<tr>
<td>Urea (g/day)</td>
<td>48</td>
<td>50</td>
</tr>
<tr>
<td>Creatinine (g/day)</td>
<td>1.8</td>
<td>0</td>
</tr>
</tbody>
</table>
Kidney (nephro) Toxicants

**Metals**
- Cadmium
- Mercury
- Lead

**Halogenated Hydrocarbons**
- $\text{CCl}_4$
- 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

Injury to Nephron

Uninjured Cells
- Hypertrophy
- Cellular Adaptation

Injured Cells → Death
- Proliferation
- Repair

Re-Epithelialization
- Differentiation
- Structural and Functional Recovery

Cellular Adaptation
Section E

Case Study: Hepatotoxicity of Ethanol
Pathogenesis of Ethanol Toxicity

- Alcohol—a food and a drug

Adapted from Liebler, CS, Alcohol 34 (2004) 9-19
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**
  - *Hepatocyte*
    - **Cytosol ADH (alcohol dehydrogenase)**
    - **Increased Reducing Power**
    - **Fat Accumulation**
  - **Acetaldehyde**
    - **Acetate**
    - **MEOS (microsomal ethanol metabolizing system)**
    - **CYP 2E1**
    - **Alcohol dehydrogenase (ADH)**
    - **Aldehyde dehydrogenase (ALDH)**
    - **Covalent Binding to Proteins**
    - **NAD**
      - **NADH**
      - **NADPH**

This diagram illustrates the metabolic pathways of ethanol in the liver, highlighting the direct toxic effects through the formation of acetaldehyde and its reactions with various enzymes and substrates.
Ethanol-Drug Interactions: Ethanol & Acetaminophen

Acetaminophen

\[
\text{NADPH} \rightarrow \text{Cytochrome P-450}
\]

\[\text{O}_2\]

\[\text{NCOCH}_3\]

\[N\text{-Acetyl-}\delta\text{-Benzoquinoneimine}\]

\[\text{UDP-Glucuronosyl Transferase}\]

\[\text{UDP-GA}\]

\[\text{PAPS}\]

\[\text{Sulfotransferase}\]

\[\text{Conjugate}\]

\[\text{GSH}\]

\[\text{Nucleophilic Cell Macromolecules}\]

\[\text{Mercapturic Acid}\]

\[\text{Cell Macromolecules}\]
Principle of Ethanol-Drug Interactions

A

Blood

Liver

Alcohol

Alcohol Dehydrogenase

Acetaldehyde

Microsomes

Metabolites

Drugs

B

Blood

Liver

Alcohol

Alcohol Dehydrogenase

Acetaldehyde

Microsomes

Metabolites

Drugs

C

Blood

Liver

Alcohol

Alcohol Dehydrogenase

Acetaldehyde

Microsomes

Metabolites

Drugs

D

Blood

Liver

Alcohol

Alcohol Dehydrogenase

Acetaldehyde

Microsomes

Metabolites

Drugs
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 - $\text{CCl}_4$
A. Human Exposure:

1. Properties

colorless, volatile, high density, sweet smelling liquid which does not burn or conduct current
2. Sources of exposure to \( \text{CCl}_4 \)

   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

<table>
<thead>
<tr>
<th>Concentration (ppm)</th>
<th>Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>21-79</td>
<td>Odor threshold</td>
</tr>
<tr>
<td>200</td>
<td>Severe toxic effects</td>
</tr>
<tr>
<td>1,000-2,000</td>
<td>Lethal</td>
</tr>
</tbody>
</table>
CCl₄ Metabolism

- **Cytochrome P450 2E1**
  - CCl₄ → CCl₃

- **Trichlormethyl Radical**
  - CCl₃ → CCl₃OO⁺

- **O₂**
  - CCl₃OO⁺ → CCl₂O → CO₂

- **Low O₂**
  - CCl₃ → Toxicity

- **Covalent Binding to Lipids**
  - CCl₃ → Lipid Peroxidation

- **Lipid Peroxidation**
  - CCl₃ → Toxicity

- **Chloroform (CHCl₃)**
  - CCl₄ → CCl₃

- **Trichlormethylperoxy Radical**
  - CCl₃OO⁺ → Toxicity
Figure 1. Initiation of lipid peroxidation

Potentiation of Haloalkane-Induced Hepatotoxicity

**Figure 10.3** - Potentiation of Haloalkane-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)

<table>
<thead>
<tr>
<th>Concentration (ppm)</th>
<th>Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>200-300</td>
<td>odor threshold</td>
</tr>
<tr>
<td>4,100</td>
<td>nausea, fainting</td>
</tr>
<tr>
<td>14,000-16,000</td>
<td>narcotic</td>
</tr>
</tbody>
</table>
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

Major Aerobic Pathway

- H-CCl₃ + P450, O₂ → [HOCCl₃]⁻HCl
- O-CCl₂ → Phosgene
- CO → Protein Acceptor
- Phosgene + H₂O → 2H₂O CO₂
- Phosgene + Cysteine Condensation
- 2-Oxothiazolidine-4-Carboxylic Acid
- Glutathione Conjugates?
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
<table>
<thead>
<tr>
<th>Gender/Species</th>
<th>Dose (mg/kg/day)</th>
<th>Liver Tumor Incidence (%)</th>
<th>Kidney Tumor Incidence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Female mouse</strong></td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>238</td>
<td>80</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>477</td>
<td>95</td>
<td>0</td>
</tr>
<tr>
<td><strong>Male mouse</strong></td>
<td>0</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>138</td>
<td>36</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>277</td>
<td>98</td>
<td>4</td>
</tr>
<tr>
<td><strong>Female rat</strong></td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td><strong>Male rat</strong></td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>180</td>
<td>2</td>
<td>24</td>
</tr>
</tbody>
</table>

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

* 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).

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.

Hepatocyte labelling index in female mice given chloroform in drinking water for 4 days or 3 weeks

Mechanistically-Based Risk Assessment

Toxicokinetics

PB-PK model simulation of rates of liver metabolism in the female B6C3F₁ mouse following a single gavage dose of chloroform in corn oil derived from the model parameters of Corley et al. (1990).

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
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
- 60 ppb
- 1-in-100,000 increased lifetime cancer risk
- Male rat kidney tumor response (U.S. EPA, 1985)
- Current EPA standard (U.S. EPA 1994)

- Cytotoxic/Nongenotoxic mode of action
- 25,100 ppb
- 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)
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
Default LMS model
1-in-1,000,000 increased lifetime cancer risk

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)