Toxic Effects of Hydrocarbons and Alcohols
Aliphatic Hydrocarbons:

Non-Halogenated (Hexane)

Halogenated:

Chlorinated Hydrocarbons (Trichloroethylene)
Brominated Hydrocarbons (Halothane)
Fluorinated Hydrocarbons (Methoxyflurane)

Cyclic Hydrocarbons (Cyclohexane)

Aromatic Hydrocarbons:

Monocyclic (Benzene)
Polycyclic (Benzo(a)pyrrole)

Alcohols (Ethanol)
TRICHLOROETHYLENE
CAS No. 79-01-6
First listed in the Ninth Report on Carcinogens

- High production volume chemical
- Colorless liquid with a sweet, chloroform-like odor
- Common contaminant in more than ½ of Superfund Sites (EPA National Priority List hazardous waste sites)
- Used as an industrial solvent (furniture and fixtures, fabricated metal products, electrical and electronic equipment, transport equipment, and miscellaneous manufacturing industries)
- Exposure: Mainly by inhalation in the workplace, but also in general population via food, water, and air (approximately 10% of the population has detectable levels of TCE in their blood)

http://ehp.niehs.nih.gov/roc/toc10.html
• TCE is rapidly absorbed from the stomach, intestines, and lung

• After absorption, TCE is distributed throughout the body and concentrates in fatty tissues, such as the liver, brain, and body fat

• TCE is metabolized primarily through oxidation by cytochrome P-450 and conjugation with GSH

• TCE metabolism in mice, rats, and humans is qualitatively similar, producing the same primary metabolites.

Figure 24-5. Scheme of metabolism of TCE.

Metabolites marked with an asterisk are known urinary metabolites. 1 = TCE; 2 = DCVG; 3 = DCVC; 4 = 1,2-dichlorovinylthiol; 5 = NacDCVC; 6 = TCE-P450 or TCE-oxide intermediate; 7 = N-(hydroxyacetyl)-aminoethanol; 8 = oxalic acid; 9a = chloral; 9b = chloral hydrate; 10 = dichlooroacetic acid; 11 = trichloroacetic acid; 12 = trichloroethanol; 13 = trichloroethanol glucuronide; 14 = monochloroacetic acid. [Used with permission of Lash et al. (2000).]
Cancer Classification (EPA):

TCE is *reasonably anticipated to be a human carcinogen (? 2010 re-assessment in the works)* based on limited evidence of carcinogenicity from studies in humans, sufficient evidence of carcinogenicity from studies in experimental animals, which indicates there is an increased incidence of malignant and/or a combination of malignant and benign tumors at multiple tissue sites in multiple species of experimental animals, and information suggesting TCE acts through mechanisms that indicate it would likely cause cancer in humans.

**Human studies** found that occupational exposure to TCE was associated with excess incidences of liver cancer, kidney cancer, non-Hodgkin’s lymphoma, prostate cancer, and multiple myeloma, with the strongest evidence for the first three cancers. Elevated risks of death from Hodgkin’s disease, multiple myeloma, cervical cancer, and liver cancer also were observed.

**In experimental animals** tumors occurred at several of the sites as in humans. In mice, TCE induces benign and malignant tumors of the liver, lung, and blood (lymphoma). In rats, TCE induces kidney cancer, interstitial-cell tumors of the testis, and possibly leukemia.

http://ehp.niehs.nih.gov/roc/toc10.html
Mechanisms of Carcinogenesis by TCE

Liver Cancer (M and F B6C3F1 mice, but not Fisher 344 rats):

• Major contribution by P450 metabolism of TCE (CH, TCA, DCA)

• Initiating effects:
  ambiguous data on direct genotoxicity of TCE or metabolites

• Promotional effect on spontaneously initiated cells in B6C3F1 mice via:
  peroxisome proliferation and oxidative stress;
  cytotoxicity and compensatory regeneration;
  reduction of apoptosis;
  perturbation of other cell signaling pathways.
Mechanisms of Carcinogenesis by TCE

Kidney Cancer (M Fisher 344 rats only):

- Major contribution by GSH conjugation metabolism of TCE:
  (DCVC, S-(1,2-dichlorovinyl)-L-cysteine)

- Initiating effects:
  DCVC is metabolically activated in proximal renal tubules (β-lyase) to 1,2-dicholovinylthiol that is unstable and gives raise to alkylating species that damage DNA

- Promotional effects:
  cytotoxicity and compensatory regeneration;
  oxidative stress, ATP depletion, Ca^{2+}-perturbations, etc.;
  Alpha-2u-globulin nephropathy.
$\alpha_{2u}$-Globulin Nephropathy

- $\alpha_{2u}$ is the major component of the urinary protein load in male rats and is unique to male rats, although homologous proteins exist in other species, including humans;

- Renal proximal tubules reabsorb protein from the glomerular filtrate, and toxicants or pathological conditions that interfere with this process cause an excessive accumulation of $\alpha_{2u}$ in lysosomes of renal proximal tubular cells;

- Similar phenomenon has not been observed in female rats or in other species;

- A number of chemicals, many of them halogenated organic solvents, have been shown to cause the so-called hyaline (protein) droplet nephropathy in male rats.
Mechanism for the induction of nephropathy and renal tumors by chemicals that induce $\alpha_{2u}$ nephropathy

- Protein droplets containing $\alpha_{2u}$ increase in number and size in renal proximal convoluted tubular cells of male rats exposed to certain halogenated hydrocarbons. $\alpha_{2u}$ is a low molecular weight protein that is synthesized only in the liver of mature male rats under androgenic control. Hydrocarbons or their metabolites that induce the response bind irreversibly to $\alpha_{2u}$, resulting in the lysosomal degradation of the complex.

- The excessive accumulation of reabsorbed proteins in secondary lysosomes of the renal proximal convoluted tubules (S2 segment) is then thought to cause lysosomal dysfunction and cellular necrosis.

- Intratubular granular casts of necrotic cellular debris then accumulate at the junction of the pars recta of the proximal tubules (S3 segment) and the thin loop of Henle.

- Regenerative cellular proliferation is then induced in response to the loss of cells from the S2 segment of the proximal tubules.

- The increased cellular proliferation is then thought to cause development of renal-cell tumors due to increases in DNA damage in replicating cells.
Mechanisms of Carcinogenesis by TCE

**Lung Cancer** (only by inhalation exposure in mice, but not in rats):

- Major contribution by P450 metabolism of TCE in Clara cells:
  
  (CH accumulation due to low activity of alcohol dehydrogenase that rapidly converts CH to TCOH in liver and lack of glucoronosyltransferase that produces TCOH glucuronide)

- **Initiating** effect:

  CH is most genotoxic of all other TCE metabolites

- **Promotional** effect:

  cytotoxicity and compensatory regeneration;
  
  reduction of apoptosis;
  
  perturbation of other cell signaling pathways.
Human Risk Assessment of Carcinogenesis by TCE

Liver cancer:
Metabolism similar to rodents (+)
Peroxisome proliferation is questionable in humans (-)

Kidney cancer:
GSH conjugation metabolism in rats is greater than in humans (-)
Alpha-2u-globulin accumulation is strictly a male rat event (-)

Lung cancer:
Humans have much fewer Clara cells than mice (-)
P450 content of human Clara cells is much less than in mice (-)
Evidence from experimental, mechanistic, and epidemiologic studies supports the conclusion that TCE is a potential kidney carcinogen.

National Research Council Report 2006

"Assuming a mitogenic mode of action for DCA as a rodent liver carcinogen, genotypic species differences between mice and humans suggest that humans would be much less susceptible to liver carcinogenesis."
Key Issue: Draft Carcinogenicity Characterization as *Carcinogenic to humans*

TCE and Kidney Cancer

<table>
<thead>
<tr>
<th>Study name</th>
<th>Risk ratio</th>
<th>Lower limit</th>
<th>Upper limit</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anttila 1995</td>
<td>0.870</td>
<td>0.391</td>
<td>1.937</td>
<td>0.7330</td>
</tr>
<tr>
<td>Axelson 1994</td>
<td>1.160</td>
<td>0.521</td>
<td>2.682</td>
<td>0.7162</td>
</tr>
<tr>
<td>Boice 1999</td>
<td>0.990</td>
<td>0.472</td>
<td>2.077</td>
<td>0.9788</td>
</tr>
<tr>
<td>Greenland 1994</td>
<td>0.990</td>
<td>0.298</td>
<td>3.293</td>
<td>0.9869</td>
</tr>
<tr>
<td>Hansen 2001</td>
<td>1.100</td>
<td>0.413</td>
<td>2.931</td>
<td>0.8488</td>
</tr>
<tr>
<td>Morgan 1998 unp RR</td>
<td>1.143</td>
<td>0.507</td>
<td>2.576</td>
<td>0.7472</td>
</tr>
<tr>
<td>Raaschou-Nielsen 2003 RCC</td>
<td>1.200</td>
<td>0.950</td>
<td>1.516</td>
<td>0.1262</td>
</tr>
<tr>
<td>Radican 2008</td>
<td>1.180</td>
<td>0.472</td>
<td>2.951</td>
<td>0.7234</td>
</tr>
<tr>
<td>Zhao 2005 mort 20 y lag</td>
<td>1.720</td>
<td>0.377</td>
<td>7.853</td>
<td>0.4840</td>
</tr>
<tr>
<td>Bruning 2003</td>
<td>2.470</td>
<td>1.359</td>
<td>4.488</td>
<td>0.0030</td>
</tr>
<tr>
<td>Charbol 2007- high conf re:exp</td>
<td>1.880</td>
<td>0.889</td>
<td>3.976</td>
<td>0.0985</td>
</tr>
<tr>
<td>Dosemeci 1999</td>
<td>1.300</td>
<td>0.895</td>
<td>1.889</td>
<td>0.1687</td>
</tr>
<tr>
<td>Pesch 2000 JTEM</td>
<td>1.240</td>
<td>1.030</td>
<td>1.492</td>
<td>0.0227</td>
</tr>
<tr>
<td>Siemiatycki 1991</td>
<td>0.800</td>
<td>0.287</td>
<td>2.233</td>
<td>0.6700</td>
</tr>
</tbody>
</table>

**Figure 4-1.** Meta-analysis of kidney cancer and overall TCE exposure (the pooled estimate is in the bottom row). Symbol sizes reflect relative weights of the studies. The horizontal midpoint of the bottom diamond represents the pooled RR estimate and the horizontal extremes depict the 95% CI limits.

*random effects model; same for fixed*
TETRACHLOROETHYLENE (PERCHLOROETHYLENE)
CAS No. 127-18-4
First Listed in the Fifth Annual Report on Carcinogen

- Colorless liquid with a sweet, ether-like odor.
- Slightly soluble in water and miscible with alcohol, ether, chloroform, hexane, and benzene. In water, Perc slowly decomposes to form trichloroacetic and hydrochloric acids. Phosgene, a highly toxic gas, may form when Perc vapors are exposed to sunlight or flames.
- High production volume chemical
- Common contaminant in Superfund Sites
- Used as an industrial solvent (dry cleaning, metal cleaning)
- Exposure: inhalation in the workplace, but also in general population via food, water, and air (population has detectable levels of Perc in their blood and breath)
### Short-Term Exposure (Less Than or Equal to 14 Days)

<table>
<thead>
<tr>
<th>Effects in Animals</th>
<th>Conc. in Air (ppm)</th>
<th>Effects in Humans</th>
</tr>
</thead>
<tbody>
<tr>
<td>Death</td>
<td>10,000</td>
<td>Intolerable eye and nose irritation after 1-2 minutes</td>
</tr>
<tr>
<td>Effects on the Unborn</td>
<td>1,000</td>
<td>Kidney toxicity, shortened life</td>
</tr>
<tr>
<td>Liver toxicity, effects on the nervous system</td>
<td>100</td>
<td>Dizziness, headache, sleepiness</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>Liver toxicity</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>Minimal risk for effects other than cancer</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>Minimal risk for effects other than cancer</td>
</tr>
</tbody>
</table>

### Long-Term Exposure (Greater Than 14 Days)

<table>
<thead>
<tr>
<th>Effects in Animals</th>
<th>Conc. in Air (ppm)</th>
<th>Effects in Humans</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1.000</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.01</td>
<td></td>
</tr>
</tbody>
</table>
Metabolism of Perc by the P450 pathway:
*Identified urinary metabolites: 1, Perc; 2, Perc epoxide; 3, trichloroacetyl chloride; 4, trichloroacetate; 5, trichloroethanol; 6, trichloroethanol glucuronide; 7, oxalate dichloride; 8, trichloroacetyl aminoethanol; 9, oxalate; 10, dichloroacetate; 11, monochloroacetate; 12, chloral.

Lash & Parker Pharmacol Rev (2001)

Metabolism of Perc by the GSH conjugation:
*Identified urinary metabolites: 1, Perc; 2, TCVG; 3, TCVC; 4, NAcTCVC; 5, NAcTCVC sulfoxide; 6, 1,2,2-trichlorovinylthiol; 7, TCVCSO; 8, 2,2-dichlorothioketene; 9, dichloroacetate. Enzymes: GST, GGT, dipeptidase (DP), β-Lyase, FMO3, CCNAT, CYP3A1/2, and CYP3A4. Unstable, reactive metabolites are shown in brackets.
Cancer Classification (EPA):

Tetrachloroethylene is reasonably anticipated to be a human carcinogen based on sufficient evidence of carcinogenicity from studies in experimental animals.

Human studies: In several cohort and proportionate mortality studies, excesses have been reported of lymphosarcomas, leukemias, and cancers of the skin, colon, lung, and urogenital tract. Some excess of lymphomas and of cancers of the larynx and urinary bladder was seen in a large cohort of dry cleaners.

When all studies are considered, there is evidence for consistent positive associations between tetrachloroethylene exposure and esophageal and cervical cancer and non-Hodgkin’s lymphoma. While these associations appear unlikely to be due to chance, confounding cannot be excluded; further, the total numbers in the cohort studies combined are relatively small.

In experimental animals: When administered by inhalation, tetrachloroethylene increased the incidences of hepatocellular adenomas and carcinomas in male mice and hepatocellular carcinomas in female mice. By the same route of administration, the compound increased the incidences of mononuclear cell leukemia in rats of both sexes and rare renal tubular cell neoplasms in male rats. When administered by gavage, tetrachloroethylene increased the incidence of hepatocellular carcinomas in mice of both sexes.

http://ehp.niehs.nih.gov/roc/toc10.html
Postulated modes of action of tetrachloroethylene via the cytochrome P450 pathway for hepatotoxicity and hepatocarcinogenicity

Lash & Parker Pharmacol Rev (2001)
Postulated modes of action of tetrachloroethylene via the GSH conjugation pathway for nephrotoxicity and nephrocarcinogenicity

Lash & Parker Pharmacol Rev (2001)
• Colorless liquid with a sweet, chloroform-like odor
• Was a high production volume chemical
• Common contaminant in some Superfund Sites
• Was extensively used as an industrial solvent, cleaning agent, fumigant, in fire extinguishers, etc.
• Exposure: Is ubiquitous in the air (USA) also found in ground water
Acute Effects:

- Acute (short-term) inhalation and oral exposures to carbon tetrachloride have been observed primarily to damage the liver and kidneys of humans. Depression of the central nervous system has also been reported. Symptoms of acute exposure in humans include headache, weakness, lethargy, nausea, and vomiting.

- Delayed pulmonary edema has been observed in humans exposed to carbon tetrachloride by inhalation and ingestion, but this is believed to be due to injury to the kidney rather than direct action of carbon tetrachloride on the lung.

- Acute animal exposure tests, such as the LC50 and LD50 tests in rats, mice, rabbits, and guinea pigs, have demonstrated carbon tetrachloride to have low toxicity from inhalation exposure, low-to-moderate toxicity from ingestion, and moderate toxicity from dermal exposure.

Chronic Effects (Noncancer):

- Inhalation or oral exposure to carbon tetrachloride produces liver and kidney damage in humans and animals.
Trichloromethyl radical

Low doses: CYP 2E1
High doses: CYP 3A

Trichloromethylperoxy radical

Fig. 1. Biotransformation of carbon tetrachloride
(From Harris & Anders, 1981; Anders & Jakobson, 1985; McGregor & Lang, 1996)
Mouse hepatofibrosis was induced by carbon tetrachloride (CCl4 was administered twice a week for 8 weeks, 0.2-1 mL CCl4/kg mouse weight diluted in olive oil)...

The extent of fibrosis was evaluated by quantitative real-time reverse-transcription polymerase chain reaction of fibrosis-related genes, liver hydroxyproline measurement, and Picro-Sirius red staining and collagen immunofluorescence staining.

In pilot experiments, we used a dose of 1 mL CCl4/kg mouse weight as commonly described (26,28). This dose led to marked lethality in FVB/n mice within 4 weeks before the development of significant hepatic fibrosis (not shown). Notably, strain differences in responsiveness to CCl4 have been described (38).
Cancer Classification (EPA):
Carbon Tetrachloride is *reasonably anticipated to be a human carcinogen* based on sufficient evidence of carcinogenicity in experimental animals.

**Human studies:** No adequate data were available from human studies to evaluate the carcinogenicity of carbon tetrachloride in humans (IARC 1979, 1982, 1987, 1999). Three case reports described liver tumors associated with cirrhosis in humans exposed to carbon tetrachloride. A mortality study of laundry and dry cleaning workers exposed to a variety of solvents suggested an excess of respiratory cancers, liver tumors, and leukemia.

**In experimental animals:** When administered by *gavage*, carbon tetrachloride increased the incidences of hepatomas and hepatocellular carcinomas in mice of both sexes. By the same route of administration, the compound increased the incidence of neoplastic nodules of the liver in rats of both sexes. When administered by *subcutaneous injection*, carbon tetrachloride induced hepatocellular carcinomas in male rats and mammary adenocarcinomas and fibroadenomas in female rats. When administered by *inhalation*, carbon tetrachloride induced liver carcinomas in rats. When administered *intrarectally*, the compound induced nodular hyperplasia of the liver in male mice.

http://ehp.niehs.nih.gov/roc/toc10.html
BENZENE
CAS No. 71-43-2
First Listed in the First Annual Report on Carcinogens

- Clear, colorless to light yellow, volatile, flammable liquid with an aromatic odor
- Is a high production volume chemical
- Common environmental pollutant
- Major raw material used extensively as a solvent in the chemical and pharmaceutical industries, as a starting material and intermediate in the synthesis of numerous chemicals, and as a gasoline additive.
- Exposure: Is ubiquitous in the air (USA) also found in ground water

http://ehp.niehs.nih.gov/roc/toc10.html
**Acute Effects:**

- Acute (short-term) inhalation or dermal exposures to benzene have been observed primarily to lead to bone marrow damage.
- Manifestations: anemia, leukopenia, thrombocytopenia, etc.

**Chronic Effects (Noncancer):**

- Chronic depletion of bone marrow cells – bone marrow aplasia, pancytopenia that may lead to fatal outcome
Cancer Classification (EPA):
Benzene is known to be a human carcinogen based on sufficient evidence of carcinogenicity in humans and experimental animals.

Human studies: Many case reports and case series have described the association of leukemia with exposure to benzene, either alone or in combination with other chemicals. Most cases were acute leukemias and lymphomas. A series of epidemiological studies, both cohort and case-control, showed statistically significant associations between leukemia (predominantly myelogenous) and occupational exposure to benzene and benzene-containing solvents. These results were replicated in a number of countries and different industries. In the epidemiological studies of people exposed primarily to benzene, statistically significant excesses of leukemia were observed..

In experimental animals: When administered by gavage, benzene increased the incidences of Zymbal gland carcinomas and oral cavity papillomas and carcinomas in rats of both sexes, as well as skin carcinomas in male rats. When administered by gavage, benzene increased the incidences of Zymbal gland carcinomas, malignant lymphomas, and alveolar/bronchiolar adenomas and carcinomas in mice of both sexes; harderian gland adenomas and carcinomas of the preputial gland in male mice; and ovarian granulos cell tumors and benign mixed tumors and mammary gland carcinomas and carcinosarcomas in female mice.

http://ehp.niehs.nih.gov/roc/toc10.html
Figure 1. Simplified metabolic scheme for benzene showing major pathways and metabolizing genes. GST, glutathione-S-transferase; NQO1, NAD(P)H:quinone oxidoreductase 1; MPO, myeloperoxidase; CYP2E1, cytochrome P450 2E1.
FIGURE 2. Comprehensive metabolic scheme for HQ. Possible input from arbutin (naturally occurring glucose conjugate of HQ), benzene, and phenol is also indicated (long dashed line arrows). Derivatives shown in gray are considered detoxified metabolites. Proposed mechanism of covalent binding and activated oxygen species production from HQ-SG-derived conjugates also shown (short dashed line arrows). Enzyme (or process) associated with each conversion indicated by numbers: (1) spontaneous reaction (slow), cytochrome P450, or various peroxidases; (2) cytochrome P450 or b6 reductase; (3) NQO1 or carbonyl reductase; (4) sulfotransferase; (5) glucuronol transferase; (6) spontaneous reaction or glutathione S-transferase; (7) γ-GT, (8) dipeptidase; (9) N-acetylsulfontransferase. (GSH = glutathione.)
Table 7
Summary comparison of human and animal in vivo genotoxicity results for benzene exposure

<table>
<thead>
<tr>
<th>Test</th>
<th>Results</th>
<th>Humans</th>
<th>Humans</th>
<th>Animals</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>&lt;1 ppm</td>
<td>&gt;1 ppm</td>
<td></td>
</tr>
<tr>
<td>Micronucleus</td>
<td>2/7</td>
<td>2/2</td>
<td>59(1)/65</td>
<td></td>
</tr>
<tr>
<td>Chromosomal aberrations</td>
<td>15(2)/22</td>
<td>13(1)/18</td>
<td>18/20</td>
<td></td>
</tr>
<tr>
<td>Aneuploidy</td>
<td>5/7</td>
<td>5/6</td>
<td>2/2</td>
<td></td>
</tr>
<tr>
<td>Sister chromatid exchange</td>
<td>5/14</td>
<td>5/11</td>
<td>3(4)/7</td>
<td></td>
</tr>
<tr>
<td>DNA damage/MM, DS breaks</td>
<td>3/4</td>
<td>1/1</td>
<td>5/9</td>
<td></td>
</tr>
</tbody>
</table>

a References for these studies are given in Tables 2 and 6.
b Positive tests and weakly positive tests (in parenthesis)/total tests.
c Column excludes studies in which humans were exposed to BZ concentrations of 1 ppm or lower. Studies that contained groups with exposure levels >1 ppm are counted for each group.

Figure 6-29. Role of cytochrome P450 and peroxidases in the activation of benzene to myelotoxic metabolites.

Fig. 2. Urinary metabolites of benzene.

Glucuronide and Sulfate Conjugates

Mercapturic Acids

DNA-based Adducts(s)

Ring-Opened Metabolites(s)
**Polycyclic Aromatic Hydrocarbons: Benzo(a)pyrine**

**Absorption**

In humans, the major routes of uptake of PAH are thought to be through

(i) the lungs and the respiratory tract after inhalation of PAH-containing aerosols or of particulates to which a PAH, in the solid state, has become to be absorbed

(ii) the gastro-intestinal tract after ingestion of contaminated food or water

(iii) the skin as a result of contact with PAH-bearing materials.

**Distribution**

Owing to the high lipophilicity of this class of compounds, their bioavailability after ingestion and inhalation must be considered to be significant:

(i) detectable levels of PAH occur in almost all internal organs,

(ii) organs rich in adipose tissue can serve as storage depots from which the hydrocarbons are gradually released,

(iii) the GI tract contains high levels of hydrocarbon and metabolites, even when PAH are administered by other routes, as a result of mucociliary clearance and swallowing or hepatobiliary excretion.
• PAHs require a multistep metabolic activation by specific enzymes. The enzyme system primarily responsible for PAH metabolism is the **mixed-function oxidase system**, which requires NADH or NADPH and molecular oxygen to convert the nonpolar PAHs into the polar hydroxy derivatives and arene oxides.

• The first reaction is an **epoxidation**. With benzo(a)pyrene, the product is the corresponding 7,8-epoxide that, in turn, is subject of **epoxide hydrolases** to form stereoisomeric dihydrodiols.

• These are converted further to the 7,8-dihydrodiol-9,10-epoxide. The terminal oxidase is **cytochrome P-450 (CYP1A1)**. The diol epoxide can exist in 4 stereoisomeric forms of which the key carcinogenic product is benzo(a)pyrene-r-7,t-8-diol-t-9,10-epoxide.

• PAH epoxides can then be **conjugated with GSH**. This conjugation is regarded as a true detoxification reaction and is mediated by **glutathione transferase (GSTM1)**.

• The epoxides that are not conjugated with GSH are converted into phenols and diols as mentioned above. These PAH metabolites, however, are sometimes not sufficiently polar to be excreted and are therefore **conjugated with glucuronic or sulfuric acids** to enable excretion to occur.

• In addition to conjugation, the hydroxylated derivatives of PAHs may undergo a number of **oxidation and hydroxylation reactions**. These include the conversion of phenols to phenol-epoxides and subsequently to diphenols and triols, diols to tetrols and diol-epoxides, and triols to triolepoxides and pentols.
Benzo(a)pyrene as a model of PAH metabolism (IARC, 1983)

Benzo(a)pyrene

Arene oxides
4,5-
7,8-
9,10-

Phenols
1-
7-
3-
9-
6-

Quinones
1,6-
3,6-
6,12-

GSH conjugates

GSH conjugates

Glucuronides and sulphate esters

Dihydriodols
4,5-
7,8-
9,10-

Phenol diols
9-OH-4,5-diol
6-OH-7,8-diol
1-(3)-OH-9,10-diol

Glucuronides and sulphate esters

GSH conjugates

Dioleopxides
7,8-diol-9,10-epoxide
9,10-diol-7,8-epoxide

Tetraols

H$_2$O $\xrightarrow{\text{Epoxide Hydrolase}}$ (+) BP-7,8-epoxide

H$_2$O $\xrightarrow{\text{Epoxide Hydrolase}}$ (-) BP-7,8-epoxide

O$_2$ $\xrightarrow{P-450}$ (+) BP-7,8-diol

O$_2$ $\xrightarrow{P-450}$ (-) BP-7,8-diol

(+)-syn BP 7,8-diol

(-)-anti BP 7,8-diol

(-)-anti BP 7,8-diol

(+)-syn BP 7,8-diol

FIGURE 1. The stereoselective metabolism of benzo(a)pyrene (BP) is optically active dial epoxides.

(+)-anti

70% of DNA adducts

(-)-anti

The stereospecific requirements of these adducts are critical to the formation of a stable and reactive DNA adduct.
Figure 2. Air phenanthrene concentration (ug/m³) vs total PAH concentration (ug/m³) (minus phenanthrene concentration) in aluminium-production facilities (A) and coke-production facilities (B).

Table 1

<table>
<thead>
<tr>
<th>Source of PAHs</th>
<th>Job category</th>
<th>No of subjects</th>
<th>Naphthalene level (ng/l)</th>
<th>Phenanthrene level (ng/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Pre-shift</td>
<td>Post-shift</td>
</tr>
<tr>
<td>Diesel exhausts</td>
<td>Dock workers</td>
<td>27</td>
<td>21.2 (1.69)</td>
<td>17.1 (1.71)</td>
</tr>
<tr>
<td></td>
<td>Office workers</td>
<td>4</td>
<td>14.9 (1.51)</td>
<td>18.0 (1.42)</td>
</tr>
<tr>
<td></td>
<td>Shop workers</td>
<td>8</td>
<td>13.0 (1.55)</td>
<td>19.6 (2.28)</td>
</tr>
<tr>
<td>Asphalt emissions</td>
<td>Road milling workers</td>
<td>6</td>
<td>33.3 (1.30)</td>
<td>34.4 (2.01)</td>
</tr>
<tr>
<td></td>
<td>Road paving workers</td>
<td>20</td>
<td>32.3 (1.00)</td>
<td>89.1 (1.00)</td>
</tr>
<tr>
<td>Coke-oven emissions</td>
<td>Office and hospital workers</td>
<td>22</td>
<td>NA</td>
<td>765 (2.31)</td>
</tr>
<tr>
<td></td>
<td>(factory controls)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Coke-oven workers (side and</td>
<td>13</td>
<td>NA</td>
<td>1710 (3.39)</td>
</tr>
<tr>
<td></td>
<td>bottom)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Coke-oven workers (top)</td>
<td>15</td>
<td>NA</td>
<td>3450 (5.14)</td>
</tr>
</tbody>
</table>

*Geometric mean (geometric standard deviation) levels are displayed.

NA, not available.

Cancer Classification (EPA):

PAHs are reasonably anticipated to be human carcinogens based on sufficient evidence of carcinogenicity from studies in experimental animals.

**Human studies**: There is inadequate evidence for the carcinogenicity of PAHs in humans. Workers exposed to creosote containing numerous PAHs developed skin tumors. Exposures to other chemical mixtures that contain PAHs, such as cigarette smoke, coal tar, coal tar pitch, and bitumens, have been associated with increased incidences of lung cancer in humans. Dermal exposure to coal tar and shale oils containing PAHs have been associated with increased incidences of skin tumors in humans.

**In experimental animals**: Target organs (sites): stomach, lung, liver, skin, mammary gland

Ethanol Metabolism

Partial: $\text{CH}_3\text{CH}_2\text{OH} + \text{O}_2 \rightarrow \text{CH}_3\text{COOH} + 3\text{H}_2\text{O}$

Total: $\text{CH}_3\text{CH}_2\text{OH} + 3\text{O}_2 \rightarrow 2\text{CO}_2 + 3\text{H}_2\text{O}$
Alcohol-Induced Liver Injury

ETHANOL

INDUCTION OF ALCOHOL METABOLISM
Alcohol dehydrogenase, catalase, microsomal enzymes (CYP 2E1)

TOXIC METABOLITES:
- acetaldehyde
- free radicals
- lipid peroxides

INJURY
Cell-cell interactions in the mechanism of alcohol-induced liver injury

- Stellate cells
- Endothelial cells
- T (CD4/8) cells
- B cells
- Antigens
- NO·
- Fibrous scars

Pathways:
- Endotoxin
- ETHANOL
- Bacteria
- IgA
ETHANOL

- CYP 2E1
- P450s
- NADPH Ox.
- Xanthine Ox.
- Mitochondria

Reactive Oxygen Species

- DNA Damage
- Lipid Peroxidation
- Protein Adducts
- Protein Carbonyls

Fatty Liver, Inflammation, Necrosis, Fibrosis, Cirrhosis, Cancer
The occurrence of malignant tumors of the oral cavity, pharynx, larynx, esophagus, liver, female breast and colorectum is causally related to the consumption of alcoholic beverages. There is sufficient evidence for the carcinogenicity of alcoholic beverages and ethanol in alcoholic beverages in humans. There is sufficient evidence for the carcinogenicity of ethanol and of alcoholic beverages in experimental animals. Alcoholic beverages and ethanol in alcoholic beverages are carcinogenic to humans (Group 1).
Mechanisms of Ethanol-induced carcinogenesis

The precise mechanism of action is unknown, but is thought to include:

**Initiation:**
- Production of acetaldehyde, the first and most toxic metabolite of ethanol and its binding to DNA
- Generation of oxidants via induced CYP 2E1 and other enzymes
- Increased activation environmental precarcinogens, especially of nitrosamines by CYP 2E1

**Promotion:**
- Increased cell proliferation: direct cytotoxicity, production of mitogenic cytokines, elevated production of eicosanoids
- Activation of MAP kinases
- Activation of insulin-like growth factors
- Interaction between ethanol metabolism and the metabolism of retinol and retinoic acid;
- Alteration of the DNA repair systems
- Concomitant dietary deficiency, which may play a role in carcinogenesis (e.g., folate deficiency, which may lead to hypomethylation of DNA)
- Elevation of levels of sex hormones
- Enhanced fibrogenesis and cirrhosis in liver

**Progression:**
- Immunosupression and immunodeficiency
<table>
<thead>
<tr>
<th>Organ site</th>
<th>Relative Risk increase for consumption of 50 g/day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oral Cavity, Pharynx, Larynx, Oesophagus</td>
<td>2-3 x</td>
</tr>
<tr>
<td>Liver</td>
<td>Difficult to quantify</td>
</tr>
<tr>
<td>Breast</td>
<td>1.5 x</td>
</tr>
<tr>
<td>Colorectum</td>
<td>1.4 x</td>
</tr>
</tbody>
</table>

**Risk linked to alcohol, not to type of drink**

As these associations were observed with different types of alcoholic beverages, and given the carcinogenicity of ethanol in animals, ethanol in alcoholic beverages was classified as "carcinogenic to humans (Group 1)".

*Genetic susceptibility*

The major alcohol-metabolising enzymes in humans are the alcohol dehydrogenases (ADH) that oxidise ethanol to acetaldehyde, and the aldehyde dehydrogenases (ALDH) that detoxify acetaldehyde to acetate. The variant allele ALDH2*2, which encodes an inactive subunit of the enzyme ALDH2, is dominant and highly prevalent in certain eastern-Asian populations (28–45%), but rare in other ethnic groups. Most homozygous carriers of this allele (ALDH2*2/*2) are abstainers or infrequent drinkers, because the enzyme deficiency would cause a strong facial flushing response, physical discomfort, and severe toxic reactions. In heterozygous carriers (ALDH2*1/*2, with about 10% residual ALDH2 activity) these acute adverse effects are less severe, but when they consume alcohol these carriers are at high risk for several alcohol-related aerodigestive cancers. For example, genetic epidemiological studies provide strong evidence that the heterozygous ALDH2*1/*2 genotype contributes substantially to the development of esophageal cancer related to alcohol consumption, with relative risks — compared with carriers of the homozygous ALDH2*1/*1 genotype, which encodes the active enzyme — of up to 12 for heavy drinkers. Compared with those with the ALDH2*1/*1 genotype, the heterozygous carriers have higher levels of acetaldehyde in blood and saliva after alcohol drinking, and in a recent study higher levels of acetaldehyde-related DNA adducts have been measured in their lymphocytes.