Chemical Carcinogenesis
### Proportion of chemicals evaluated as carcinogenic

<table>
<thead>
<tr>
<th>Category</th>
<th>Count</th>
<th>Proportion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemicals tested in both rats and mice</td>
<td>350/590</td>
<td>59%</td>
</tr>
<tr>
<td>Naturally occurring chemicals</td>
<td>79/139</td>
<td>57%</td>
</tr>
<tr>
<td>Synthetic chemicals</td>
<td>271/451</td>
<td>60%</td>
</tr>
<tr>
<td>Chemicals tested in rats and/or mice</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chem. in Carcinogen. Potency Database</td>
<td>702/1348</td>
<td>52%</td>
</tr>
<tr>
<td>Natural pesticides</td>
<td>37/71</td>
<td>52%</td>
</tr>
<tr>
<td>Mold toxins</td>
<td>14/23</td>
<td>61%</td>
</tr>
<tr>
<td>Chemicals in roasted coffee</td>
<td>21/30</td>
<td>70%</td>
</tr>
<tr>
<td>Innes negative chemicals retested</td>
<td>17/34</td>
<td>50%</td>
</tr>
<tr>
<td>Physician’s desk reference PDR</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Drugs with reported cancer tests</td>
<td>117/241</td>
<td>49%</td>
</tr>
<tr>
<td>FDA database of drug submissions</td>
<td>125/282</td>
<td>44%</td>
</tr>
</tbody>
</table>

Sources:
CANCER:

“A multicausal, multistage group of diseases the mechanisms of which are still only partially known” (IARC Scientific Publications, 1992)

“Cancer is a group of diseases characterized by uncontrolled growth and spread of abnormal cells […] that can result in death” (American Cancer Society, 2002)
• **Benign** tissue *is not cancer*. Although the cell growth is moderately increased, the cells do not invade nearby tissue or spread to other parts of the body.

• **Malignant** tissue *is cancer*. The cancer cells divide out of control. They can invade and destroy nearby healthy tissue. Also, cancer cells can break away from the tumor they form and enter the bloodstream and lymphatic system.

• **Metastasis**: the spread of cancer beyond the organ of origin.
WHAT MAY CAUSE CANCER?

- Hereditary disorders
- Chemicals
- Viruses
- Chronic inflammation
- ???

Interaction of Genes and Environment

From http://www.cancersupportivecare.com/riskintro.html
History of Chemical Carcinogenesis

• Chemical carcinogenesis was first suggested by clinicians 200 years ago
  – Scrotal cancer in chimney sweeps - Potts
  – Nasal cancer and snuff dipping - Hill
  – Today, >50 chemicals are recognized as human carcinogens

• First experimental studies in animals were done ~80 years ago
History of Chemical Carcinogenesis

- Large numbers of chemicals were tested for carcinogenic potential in the 1970-1990s
  - Maximum Tolerated Doses (MTD) were used.
  - 60% of rodent carcinogens were genotoxic
  - 40% of rodent carcinogens were nongenotoxic
  - Some chemicals were single site, single species carcinogens
  - Others were multisite, multispecies carcinogens
  - Dose-response varies from <1/2 MTD to <1/1000 MTD

- Most regulations use straight mathematical extrapolation of high dose rodent data to predict risks
IARC (2004)
• Carcinogenic to humans (group 1)
• Probably carcinogenic to humans (group 2A)
• Possibly carcinogenic to humans (group 2B)
• Not classifiable as to its carcinogenicity to humans (group 3)
• Probably not carcinogenic to humans (group 4)

• Carcinogenic to humans
• Likely to be carcinogenic to humans
• Suggestive evidence of carcinogenic potential
• Inadequate information to assess carcinogenic potential
• Not likely to be carcinogenic to humans

• Known to be a human carcinogen
• Reasonably anticipated to be a human carcinogen

• Known to the state to cause cancer

Cogliano et al, 2004
# IARC Monographs Working Group - Volume 77: Some Industrial Compounds

<table>
<thead>
<tr>
<th>Agent</th>
<th>Previous evaluation</th>
<th>Current evaluation</th>
<th>Overall evaluation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Degree of evidence</td>
<td>Degree of</td>
<td>Degree of</td>
</tr>
<tr>
<td></td>
<td>in humans</td>
<td>evidence in</td>
<td>evidence in</td>
</tr>
<tr>
<td></td>
<td></td>
<td>animals</td>
<td>humans</td>
</tr>
<tr>
<td>2,2-Bis(bromomethyl)propan-1,3-diol</td>
<td>I (ND)</td>
<td>L</td>
<td>I (ND)</td>
</tr>
<tr>
<td>4-Chloro-ortho-toluidine</td>
<td>L</td>
<td>S</td>
<td>L</td>
</tr>
<tr>
<td>5-Chloro-ortho-toluidine</td>
<td>I (ND)</td>
<td>L</td>
<td>I (ND)</td>
</tr>
<tr>
<td>Cinnamyl anthranilate</td>
<td>I (ND)</td>
<td>L</td>
<td>I (ND)</td>
</tr>
<tr>
<td>Coumarin</td>
<td>I (ND)</td>
<td>L</td>
<td>I (ND)</td>
</tr>
<tr>
<td>2,3-Dibromopropan-1-ol</td>
<td>I (ND)</td>
<td>L</td>
<td>I (ND)</td>
</tr>
<tr>
<td>Diethanolamine</td>
<td>I</td>
<td></td>
<td>I</td>
</tr>
<tr>
<td>Diis(2-ethylhexyl) adipate</td>
<td>I (ND)</td>
<td>L</td>
<td>I (ND)</td>
</tr>
<tr>
<td>Diis(2-ethylhexyl) phthalate</td>
<td>I (ND)</td>
<td>S</td>
<td>I</td>
</tr>
<tr>
<td>Ethyl benzene</td>
<td>I</td>
<td>S</td>
<td>I</td>
</tr>
<tr>
<td>Glycidol</td>
<td>I (ND)</td>
<td>S</td>
<td>I</td>
</tr>
<tr>
<td>Nitromethane</td>
<td>I (ND)</td>
<td>S</td>
<td>I</td>
</tr>
<tr>
<td>N-Nitrosodiethanolamine</td>
<td>I (ND)</td>
<td>S</td>
<td>I</td>
</tr>
<tr>
<td>Pyridine</td>
<td>I</td>
<td></td>
<td>I</td>
</tr>
<tr>
<td>ortho-Toluidine</td>
<td>I</td>
<td>S</td>
<td>I</td>
</tr>
<tr>
<td>Triethanolamine</td>
<td>I</td>
<td></td>
<td>I</td>
</tr>
</tbody>
</table>

S, sufficient evidence of carcinogenicity; L, limited evidence of carcinogenicity; I, inadequate evidence of carcinogenicity; ND, no data

Group 1: carcinogenicity to humans
Group 2A: probably carcinogenic to humans
Group 2B: possibly carcinogenic to humans
Group 3: cannot be classified as to its carcinogenicity to humans

*Other relevant data taken into consideration
Cancer Cases Attributable to Environmental Carcinogens (Worldwide, 1990)

<table>
<thead>
<tr>
<th>Cause</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infections (viruses, parasites, <em>H. pylori</em>)</td>
<td>16%</td>
</tr>
<tr>
<td>Tobacco (smoked and smokeless)</td>
<td>14%</td>
</tr>
<tr>
<td>Occupation</td>
<td>4%</td>
</tr>
<tr>
<td>Alcohol drinking</td>
<td>3%</td>
</tr>
<tr>
<td>Diet and dietary components including contaminants</td>
<td>25%</td>
</tr>
<tr>
<td>Pollution</td>
<td>2%</td>
</tr>
<tr>
<td>Reproductive factors</td>
<td>2%</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>29%</strong></td>
</tr>
</tbody>
</table>
IARC Group 1 – *Carcinogenic to humans*
Monographs Volumes 1-84 (1972-2002): 89 Agents and Exposures

- Medical drugs and treatments: 24
- Industrial processes: 13
- Infectious agents or processes: 10
- Physical agents: 10
- Industrial chemicals: 7
- Inhaled particulates: 5
- Metals and inorganic salts: 5
- Lifestyle factors (incl. herbal remedies): 7
- Other: 8
## Exposures to Chemicals in the Workplace

<table>
<thead>
<tr>
<th>Agent</th>
<th>Industries and Trades with Proved Excess Cancers and Exposure</th>
<th>Primary Affected Site</th>
</tr>
</thead>
<tbody>
<tr>
<td>p-Aminodiphenyl</td>
<td>Chemical manufacturing</td>
<td>Urinary bladder</td>
</tr>
<tr>
<td>Asbestos</td>
<td>Construction, asbestos mining and milling, production of friction products and cement</td>
<td>Pleura, peritoneum, bronchus</td>
</tr>
<tr>
<td>Arsenic</td>
<td>Copper mining and smelting</td>
<td>Skin, bronchus, liver</td>
</tr>
<tr>
<td>Alkylating agents (mechloroethamine hydrochloride and bis[chloromethyl]ether)</td>
<td>Chemical manufacturing</td>
<td>Bronchus</td>
</tr>
<tr>
<td>Benzene</td>
<td>Chemical and rubber manufacturing, petroleum refining</td>
<td>Bone marrow</td>
</tr>
<tr>
<td>Benzidine, β-naphthylamine, and derived dyes</td>
<td>Dye and textile production</td>
<td>Urinary bladder</td>
</tr>
<tr>
<td>Chromium and chromates</td>
<td>Tanning, pigment making</td>
<td>Nasal sinus, bronchus</td>
</tr>
<tr>
<td>Isopropyl alcohol manufacture</td>
<td>Chemical manufacturing</td>
<td>Cancer of paranasal sinuses</td>
</tr>
<tr>
<td>Nickel</td>
<td>Nickel refining</td>
<td>Nasal sinus, bronchus</td>
</tr>
<tr>
<td>Polynuclear aromatic hydrocarbons from coke, coal tar, shale, mineral oils, and creosote</td>
<td>Steel making, roofing, chimney cleaning</td>
<td>Skin, scrotum, bronchus</td>
</tr>
<tr>
<td>Vinyl chloride monomer</td>
<td>Chemical manufacturing</td>
<td>Liver</td>
</tr>
<tr>
<td>Wood dust</td>
<td>Cabinetmaking, carpentry</td>
<td>Nasal sinus</td>
</tr>
</tbody>
</table>

Modified from Cullen et al. (1990).
# Carcinogenic Risks of Chemical Agents Associated with Medical Therapy and Diagnosis

<table>
<thead>
<tr>
<th>Chemical or Drug</th>
<th>Associated Neoplasms</th>
<th>Evidence for Carcinogenicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkylating agents (cyclophosphamide, melphalan)</td>
<td>Bladder, leukemia</td>
<td>Sufficient</td>
</tr>
<tr>
<td>Inorganic arsenicals</td>
<td>Skin, liver</td>
<td>Sufficient</td>
</tr>
<tr>
<td>Azathioprine (an immunosuppressive drug)</td>
<td>Lymphoma, reticulum cell sarcoma, skin, Karposi’s sarcoma (?)</td>
<td>Sufficient</td>
</tr>
<tr>
<td>Chlornaphazine</td>
<td>Bladder</td>
<td>Sufficient</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>Leukemia</td>
<td>Limited</td>
</tr>
<tr>
<td>Diethylstibesterol</td>
<td>Vagina (clear cell carcinoma)</td>
<td>Sufficient</td>
</tr>
<tr>
<td>Estrogens</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Premenopausal</td>
<td>Liver cell adenoma</td>
<td>Sufficient</td>
</tr>
<tr>
<td>Postmenopausal</td>
<td>Endometrium</td>
<td>Limited</td>
</tr>
<tr>
<td>Methoxypsoralen with ultraviolet light</td>
<td>Skin</td>
<td>Sufficient</td>
</tr>
<tr>
<td>Oxymetholone</td>
<td>Liver</td>
<td>Limited</td>
</tr>
<tr>
<td>Phenacetin</td>
<td>Renal pelvis (carcinoma)</td>
<td>Sufficient</td>
</tr>
<tr>
<td>Phenytoin (diphenyhydantoin)</td>
<td>Lymphoma, neuroblastoma</td>
<td>Limited</td>
</tr>
<tr>
<td>Thorotrast</td>
<td>Liver (angiosarcoma)</td>
<td>Sufficient</td>
</tr>
</tbody>
</table>
## Carcinogenic Factors Associated with Lifestyle

<table>
<thead>
<tr>
<th>Chemical, Physiological Condition or Natural Process</th>
<th>Associated Neoplasm</th>
<th>Evidence for Carcinogenicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alcoholic beverages</td>
<td>Esophagus, liver, oropharynx, larynx</td>
<td>Sufficient</td>
</tr>
<tr>
<td>Aflatoxins</td>
<td>Liver</td>
<td>Sufficient</td>
</tr>
<tr>
<td>Betel chewing</td>
<td>Mouth</td>
<td>Sufficient</td>
</tr>
<tr>
<td>Dietary intake (fat, protein, calories)</td>
<td>Breast, colon, endometrium, gallbladder</td>
<td>Sufficient</td>
</tr>
<tr>
<td>Reproductive history</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Late age at first pregnancy</td>
<td>Breast</td>
<td>Sufficient</td>
</tr>
<tr>
<td>Zero or low parity</td>
<td>Ovary</td>
<td>Sufficient</td>
</tr>
<tr>
<td>Tobacco smoking</td>
<td>Mouth, pharynx, larynx, lung, esophagus, bladder</td>
<td>Sufficient</td>
</tr>
</tbody>
</table>

Modified from Pitot (1986a) and Vainio et al. (1991)
Chemical Carcinogenesis in the 21st Century

New perceptions of previously known carcinogens:

Combined effects of multiple exposures

Examples:
- Alcohol drinking and aflatoxins
- Alcohol drinking and HBV/HBC
- Alcohol drinking and tobacco smoking
- Tobacco smoking and asbestos/arsenic/radon
Stages of Carcinogenesis

Initiating Event → Cell Proliferation (clonal expansion) → Second Mutating Event → "N" Mutating Event → Cell Proliferation → Promotion → Progression → Malignancy
Cellular and Molecular Mechanisms in Multistage Carcinogenesis: INITIATION

Initiating event involves cellular genome – MUTATIONS

Target genes:
- oncogenes/tumor suppressor genes
- signal transduction
- cell cycle/apoptosis regulators

“Simple” genetic changes

From http://newscenter.cancer.gov/sciencebehind/
SOURCES OF MUTATIONS

ENDOGENOUS DNA DAMAGE

Free Radicals
Polymerase Errors
Depurination

EXOGENOUS DNA DAMAGE

Environmental Agents
Life Styles

DNA REPAIR

CELL REPLICATION

MUTATION
Chemical Exposure (air, water, food, etc.)

Internal Exposure

Metabolic Activation

Macromolecular Binding
- DNA
- RNA

Detoxication
- Protein (Biomarker)

Biologically Effective Dose

Efficiency of Mispairing

Cell Proliferation

Initiation
Possible pathways of activation of suspected human carcinogens

Heterocyclic amines
e.g. IQ, PhIP

Aromatic amines
e.g. 4-ABP

PAHs
e.g. B[α]P, DMBA

DNA-reactive products of metabolic activation

NH⁺

C⁺

Dihydrodiol epoxide

Electrophile

Accumulation of mutations during tumor progression

Environmental
Endogenous

DNA repair

DNA damage Mutations Mutations in mutator genes Repetitive selection for mutants & mutators

Selection for malignant phenotype

20 years

Clinical detection

Loeb L.A. Cancer Res. 61:3230-9 (2001)
Cellular and Molecular Mechanisms in Multistage Carcinogenesis: PROMOTION

Reversible enhancement/repression of gene expression:
- increased cell proliferation
- inhibition of apoptosis

No direct structural alteration in DNA by agent or its metabolites

From http://newscenter.cancer.gov/sciencebehind/
1. $X$ \rightarrow \text{No Tumors}

2. $X$ \rightarrow \text{Tumors}

3. $X$ \rightarrow \text{Tumors}

4. \text{No Tumors}

5. \text{No Tumors}

$X = \text{Application of Initiator}$ \hspace{1cm} \text{=} \hspace{1cm} \text{Application of Promoter}$
N Basophilic Focus Adenoma Carcinoma

M 1 MN

Promotion Regression Progression


No Tumors

Tumors

\( \downarrow \) = Application of Promoter

Cellular and Molecular Mechanisms in Multistage Carcinogenesis: PROGRESSION

- Irreversible enhancement/repression of gene expression
- Complex genetic alterations (chromosomal translocations, deletions, gene amplifications, recombinations, etc.)
- Selection of neoplastic cells for optimal growth genotype/phenotype in response to the cellular environment

“Complex” genetic changes

From http://newscenter.cancer.gov/sciencebehind/
Phenotypic characteristics of cancer cells:

- Immortalization
- Transformation
- Loss of contact growth inhibition
- Autonomy of proliferation
- Avoidance of apoptosis
- Aberrant differentiation
- Induction of angiogenesis
Human Tumors and Stages of Carcinogenesis

- Defects in Terminal Differentiation
- Defects in Growth Control
- Resistance to Cytotoxicity

- Activation of Proto-Oncogenes
- Inactivation of Tumor Suppressor Genes
- Inactivation of Antimetastasis Genes
Multiple Stages of Human Colon Cancer

- It is estimated that by age 70, 50% of the population at large have acquired pre-cancerous adenomas in the colon; 10% of this group will progress to malignancy in the following 10 years.

- **Familial Adenomatous Polyposis (FAP)** is linked to the APC gene whose protein is involved in β-catenin signaling. The gene acts as a tumor suppressor, and the loss of function mutation causes development of hundreds to thousands of adenomas, with a consequent high risk of progression to malignancy.

- **Hereditary Non-Polyposis Colon Carcinoma (HNPCC)** is a hereditary predisposition to carcinoma without the prior accumulation of adenoma. HNPCC is caused by a germ line mutation in one set of genes responsible for DNA mismatch repair. To date, there are five genes known to be responsible for causing HNPCC: **MSH2, MSH6, MLH1, PMS1** and **PMS2**. To date, 90% of the inherited mutations in HNPCC are in **MSH2** or **MLH1**.

- **Sporadic colorectal cancer** (i.e., cancer that occurs without any familial predisposition to the disease) is associated with a variety of risk factors. The most prevalent risk factors, besides a personal or family history of colorectal and specific other cancers, are inflammatory bowel disease and age. Most sporadic colorectal cancers occur in women and men over the age of 50. Additional risk factors include diet, less than moderate exercise, and obesity.
## Multiple Stages of Human Colon Cancer

<table>
<thead>
<tr>
<th>Classification</th>
<th>Dukes’ A</th>
<th>Dukes’ B</th>
<th>Dukes’ C</th>
<th>Dukes’ D</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Development of Disease</strong></td>
<td><img src="image" alt="Image of Dukes’ A" /></td>
<td><img src="image" alt="Image of Dukes’ B" /></td>
<td><img src="image" alt="Image of Dukes’ C" /></td>
<td><img src="image" alt="Image of Dukes’ D" /></td>
</tr>
<tr>
<td>Explanation of Cancer Progression</td>
<td>Cancer confined to most superficial cell layers of colon or rectum. (e.g. the top of this polyp).</td>
<td>Cancer may extend completely through wall of colon or rectum, but there is no lymph node involvement.</td>
<td>Cancer may extend completely through wall of colon or rectum and has spread to lymph nodes.</td>
<td>Metastatic disease. The cancer has spread to distant organs, such as the liver.</td>
</tr>
<tr>
<td><strong>Estimated 5-Year Survival Rate</strong></td>
<td>95%</td>
<td>80%</td>
<td>50%</td>
<td>5%</td>
</tr>
<tr>
<td><strong>Percent Diagnosed at Stage</strong></td>
<td>37%</td>
<td></td>
<td>63%</td>
<td></td>
</tr>
</tbody>
</table>
**Multiple Stages of Human Colon Cancer**

**APC protein** (Adenomatous Polyposis Coli) is normally expressed in colorectal epithelial cells, a site of relatively high natural proliferation rates. The epithelium is convoluted into deep recesses called crypts and projections called villi. Crypts contain stem cells for tissue replacement, and the base of the crypt is a site of high mitotic activity. As cells age, they progress up the villus to the tip.

<table>
<thead>
<tr>
<th>Germline APC+/–</th>
<th>Polyps</th>
<th>Adenomas</th>
<th>Progression to cancer</th>
</tr>
</thead>
<tbody>
<tr>
<td>FAP</td>
<td>&gt;90% by age 20</td>
<td>&gt;90% by age 30</td>
<td>50% by age 40</td>
</tr>
<tr>
<td>Germline APC+/+</td>
<td>50% by age 70</td>
<td></td>
<td>5%</td>
</tr>
<tr>
<td>normal</td>
<td></td>
<td>hyperplasia,</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>aneuploidy</td>
<td></td>
</tr>
</tbody>
</table>

Cell accumulation and dysplasia

<table>
<thead>
<tr>
<th>Progression to cancer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenomas</td>
</tr>
<tr>
<td>Polyps</td>
</tr>
<tr>
<td>&gt;90% by age 30</td>
</tr>
<tr>
<td>50% by age 40</td>
</tr>
<tr>
<td>&gt;90% by age 30</td>
</tr>
<tr>
<td>50% by age 70</td>
</tr>
<tr>
<td>hyperplasia, aneuploidy</td>
</tr>
<tr>
<td>proliferating, anti-apoptotic, metastatic, angiogenic</td>
</tr>
</tbody>
</table>

www.chembio.uoguelph.ca
Genetic changes underlying development and progression of prostate cancer

- Normal epithelium
- Prostatic intraepithelial neoplasia
- Histologic prostate cancer
- Localized clinical cancer
- Metastatic cancer
- Hormone-refractory cancer

**Losses**
- 8p
- 13q (RB1)
- 6q
- 7q
- 10q (PTEN, MX11)
- 16q (E-C AD)
- 18q
- 17p (p53)
- 11p (KAI1)
- 5q + others

**Gains and amplification**
- 7 (caveolin)
- 8q (MYC, eIF3-p40)
- Xq (AR) + others

**Mutations and linkage**
- 1q24-25 (HPC-1)
- Xq27-28 (HPC-X)
- 1q42-q43
- 1p36
- RAS
- AR
Histopathological and molecular events leading to esophageal adenocarcinogenesis

- Reflux of gastric and duodenal contents
  - Reflux esophagitis (GERD)
    - Columnar-lined Esophagus (CLE)
      - CLE with Dysplasia
        - Esophageal Adenocarcinoma (EAC)

Local Irritation and inflammation:
- gastric acid
- bile acids
- digestive enzymes

Arachidonic Acid Metabolites (PGE₂, LTB₄, etc.):
- recruitment and activation of inflammatory cells
- direct effects on cells expressing respective receptors

Reactive Oxygen Species:
- DNA strand breaks
- DNA base modifications
- protein oxidation

Genetic and Epigenetic Changes:
- genomic instability & hyperproliferation
- altered gene expression & cell cycle control
- gene mutation & allelic loss
- altered apoptosis
- gene amplification

Chen and Yang, Carcinogenesis 22:1119-29 (2001)
Classification of Carcinogens According to the Mode of Action

**GENOTOXIC**  **NON-GENOTOXIC**

Initiating Event → Cell Proliferation (clonal expansion) → Second Mutating Event → Cell Proliferation → Third Mutating Event → Cell Proliferation → Progression → Promotion → Initiation → Malignancy

Classification of Carcinogens According to the Mode of Action

- GENOTOXIC
- NON-GENOTOXIC
Classification of Carcinogens According to the Mode of Action

**GENOTOXIC:**
- DNA-reactive or DNA-reactive metabolites
- Direct interaction to alter chromosomal number/integrity
- May be mutagenic or cytotoxic
- Usually cause mutations in simple systems

![Diagram of DNA adduct, mutation, and cancer](image)
Mechanism of Carcinogenesis: Genotoxic Carcinogens

1. Carcinogen activation
2. DNA binding
3. Cell proliferation (fix mutation)
4. Gene mutation

CYP450s

Chemical

"Activated" carcinogen

"inactivated" carcinogen

DNA Repair

APOPTOSIS
Interaction of the exo-epoxide of aflatoxin B$_1$ with DNA

Smela et al., Carcinogenesis 22:535-45 (2001)
Classification of Carcinogens According to the Mode of Action

**NON-GENOTOXIC:**
- Do not directly cause DNA mutation
- Mechanism of action is not completely understood
- Difficult to detect - requires rodent carcinogen bioassay

![Diagram showing the process of mutation leading to cancer](image.png)
Non-Genotoxic Carcinogens

1) Mitogens:
   - stimulation of proliferation
   - mutations may occur secondarily to cell proliferation
   - may cause preferential growth of preneoplastic cells

2) Cytotoxicants:
   - cytolethal
   - induce regenerative growth
   - mutations may occur secondarily to cell proliferation
Tissue Changes with Mitogenic and Cytotoxic Agents

Tissue

Cell Death

Proliferation

Mitogenic Agent

Cytotoxic Agent
Mechanism of Carcinogenesis: Non-Genotoxic Carcinogens

Cell proliferation (to fix “spontaneous” mutation)
Mechanisms of Non-Genotoxic Carcinogenesis
(what’s in a “black box” ?)

- Increased cell proliferation
- Decreased apoptosis
- Changes in gene expression
- Induction of metabolizing enzymes
- Activation of receptors (signaling)
- Oxidative stress
- ???
Cell Replication is Essential for Multistage Carcinogenesis

- Decreases time available for DNA repair
- Converts repairable DNA damage into non-repairable mutations
- Necessary for chromosomal aberrations, insertions, deletions and gene amplification
- Clonally expands existing cell populations
Mutagenesis $\neq$ Carcinogenesis

Cell Proliferation $\neq$ Carcinogenesis

Toxicity $\neq$ Cell Proliferation
Apoptosis

Programmed Cell Death (Apoptosis): Active, orderly and cell-type-specific death distinguishable from necrotic cell death (passive process):

- Induced in normal and cancer cells
- Non-random event
- Result of activation of a cascade of biochemical, gene expression and morphological events
- Tissue and cell specific
- Growth factors and mitogens inhibit apoptosis
Alteration of Gene Expression

- Nuclear (hormone-like) receptors
- Kinase cascades
- Calcium-, nitric oxide-mediated signaling
- Transcription factors
- Gene methylation status (hypo -> enhanced gene expression; hyper -> gene silencing)
Induction of Metabolizing Enzymes

- May be a reason for tissue-, and/or species-selectivity of carcinogens
- Metabolites may be ligands for receptors
- Production of reactive oxygen species
Oxidative Stress

- Indirect DNA damage
- Induction of cell proliferation/apoptosis signaling cascades
Early History of Animal Cancer Studies

- Yamagiwa & Ichikawa - 1918
  - Coal tar & SCC of rabbit ears
- Murphy & Sturm - 1925
  - Coal tar skin exposure caused lung tumors in mice
- Cook et al. - 1932
  - PAHs caused skin cancer in mice
- Sasaki & Yoshida - 1935
  - o-Amidoazotoluene caused liver tumors in rats

NCI Bioassay History

- 1962 - First contracted bioassay
- 1969 - Innes et al., study published
  - Selection of B6C3F1 mouse
- 1971 - National Cancer Act
  - Decision made to standardize bioassay testing
- ~1975 - F344 rat selected
  - Small size, vigor & survival, disease resistance
  - Inbred
The National Toxicology Program (NTP) was established in 1978 to coordinate toxicological testing programs within the Department of Health and Human Services, develop and validate improved testing methods, develop approaches and generate data to strengthen scientific knowledge about potentially hazardous substances and communicate with stakeholders.

- Modified the rodent cancer bioassay
  - More doses
  - Incorporation of pharmacokinetics
  - Incorporation of mechanistic studies
  - Standardization of pathology evaluation
  - More emphasis on non-cancer effects

- Re-evaluate existing practices & research portfolio
  - “Doull” report - 1984
  - Mouse strain workshop - ~1985
  - Mechanism conference - 1995
  - NTP Roadmap - August 2003
“The NTP performs appropriate toxicity studies in part to provide dose-setting information for chronic studies and also to address specific deficiencies in the toxicology database for the chemical.”

Toxicology/Carcinogenicity studies generally fall into two categories:

1. **Prechronic Toxicity Studies**
   - 14-day study
   - 13 week (90 day) study

2. **Two-Year Toxicology and Carcinogenesis Rodent Studies**
   - usually - 104 wks
   - sometimes - ~90 wks exposure followed by 10-15 wks of normal diet

### Current NTP Animal Models

- **F344/N@Tac**
  - Inbred rat
- **B6C3F1/N@Tac**
  - Isogenic hybrid mouse
  - F1 generation of C57BL/6- E84 female X C3H/HeN-MTV <-> male
14-Day Toxicity Protocol

The goal of this is to provide a basis for identifying potential target organs and toxicities and to assist in setting doses for the 13-week exposure study.

**Treatment:**
10- to 14-day quarantine period, animals are assigned at random to groups. Five treatment groups each administered a different concentration of test article per sex/species plus a control group. For dosed-feed and dosed-water studies animals are exposed for 14 consecutive days. For inhalation, gavage and dermal studies animals are exposed for 12 treatment days, not including weekends or holidays with at least two consecutive treatment days before the terminal sacrifice day.

<table>
<thead>
<tr>
<th></th>
<th>Animals</th>
<th>Sexes</th>
<th>Species</th>
<th>Test Groups</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>5</td>
<td>x</td>
<td>2</td>
<td>x</td>
<td>100</td>
</tr>
<tr>
<td>Controls</td>
<td>5</td>
<td>x</td>
<td>2</td>
<td>x</td>
<td>20</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td></td>
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<td></td>
<td>120</td>
</tr>
</tbody>
</table>

**Observations:**
Animals are weighed individually on day one, after seven days, and at sacrifice. The animals are observed twice daily, at least six hours apart (before 10:00 AM and after 2:00 PM) including holidays and weekends, for moribundity and death. Animals found moribund or showing clinical signs of pain or distress are humanely euthanized. Observations are made twice daily for clinical signs of pharmacologic and toxicologic effects of the chemical. For dosed-feed or dosed-water studies, food consumption/water consumption shall be measured and recorded weekly.

**Necropsy and Histopathologic Evaluation:**
Liver, thymus, right kidney, right testicle, heart, and lung weights are recorded for all animals surviving until the end of the study. A complete necropsy is performed on all treated and control animals that either die or are sacrificed and all tissues are saved in formalin. Histopathologic evaluation is done only on those organs/tissues showing gross evidence of treatment-related lesions to a no-effect level plus corresponding tissues are evaluated in control animals. If specific targets are required they shall be read in the control and highest treatment group and the remaining groups to a no-effect level.
90-Day Toxicity Protocol

In addition to obtaining toxicological data, the purpose of this study is to determine the treatments for each strain and species to be used in the 2-year toxicology/carcinogenesis study.

**Treatment:** 10- to 14-day quarantine period, animals are assigned at random to treatment groups. Five treatment groups plus a control group. Each group - 10 animals per sex/species. Controls receive untreated water or feed or vehicle alone in gavage and dermal studies. For dosed-feed and dosed-water studies, animals are exposed for 90 days after which they are sacrificed with no recovery period. For inhalation, gavage and dermal studies animals are exposed five times per week, weekdays only until the day prior to necropsy.

<table>
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<tr>
<th>Test</th>
<th>Animals</th>
<th>Sexes</th>
<th>Species</th>
<th>Groups</th>
<th>Total</th>
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<tbody>
<tr>
<td>Treatment</td>
<td>10</td>
<td>x 2</td>
<td>x 2</td>
<td>x 5</td>
<td>200</td>
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<td>Controls</td>
<td>10</td>
<td>x 2</td>
<td>x 2</td>
<td>x 1</td>
<td>40</td>
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<tr>
<td>Special &quot;rats&quot; for clinical lab studies</td>
<td>10</td>
<td>x 2</td>
<td>x 1</td>
<td>x 5</td>
<td>100</td>
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<tr>
<td>Special controls for clinical lab studies</td>
<td>10</td>
<td>x 2</td>
<td>x 1</td>
<td>x 1</td>
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<tr>
<td>Total</td>
<td></td>
<td></td>
<td></td>
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<td>360</td>
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</tbody>
</table>

**Observations:** Animals are weighed individually on day 1, after 7 days, and at weekly periods thereafter. Animals are observed twice daily, at least 6 hours apart, including holidays and weekends, for moribundity and death. Formal clinical observations are performed and recorded weekly. For dosed-feed or dosed-water studies, food/water consumption is measured and recorded weekly.

**Necropsy and Histopathologic Evaluation:**
Liver, thymus, right kidney, right testis, heart, and lung weights are recorded from all animals surviving until the end of the study. A complete necropsy is performed on all treated and control animals that die or are sacrificed.

**Specific Toxicologic Parameters Evaluated in the 13-Week Study**

**Clinical Laboratory Studies:** Blood is collected from both sexes of "special study" rats, at days 4 ± 1 and 21 ± 2 and from the core study rats at the end of the study.

**Blood for Micronuclei:** Blood samples are taken at study termination for micronuclei determinations.

**Sperm Morphology and Vaginal Cytology Evaluations (SMVCE)**
Two-year Carcinogenesis “Bioassay” Protocol

- **Typical NTP Bioassay Design**
  - Animal numbers-- 50 to 100 per dose group
  - Number of doses-- 3 plus control
  - Study duration- 2 years
  - Life stage- young to late adult
  - Dose ranges- MTD, 1/2 to 1/3, 1/3 to 1/9 MTD
  - Pathology- “complete” approximately 40 tissues
  - Statistics- survival adjusted trend tests
  - Route- feed, gavage, drinking water, inhalation, dermal
  - Diet- NIH-07, NTP-2000
  - **Species, strains- F344/N rat, B6C3F1 mouse**

<table>
<thead>
<tr>
<th>Test</th>
<th>Animals</th>
<th>Sexes</th>
<th>Species</th>
<th>Groups</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>50</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>600</td>
</tr>
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<td>Sentinel Animals</td>
<td>15</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>60</td>
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<tr>
<td><strong>Total</strong></td>
<td></td>
<td></td>
<td></td>
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The NTP Vision for the 21st Century:
To support the evolution of toxicology from a predominantly observational science at the level of disease-specific models to a predominantly predictive science focused upon a broad inclusion of target-specific, mechanism-based, biological observations.

Table 4-1.—Costs of NTP Studies of Fiscal Year 1986

<table>
<thead>
<tr>
<th>Type of study</th>
<th>Cost per study²</th>
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</thead>
<tbody>
<tr>
<td>Mutagenicity:</td>
<td></td>
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<tr>
<td>Drosophila</td>
<td>$11,083</td>
</tr>
<tr>
<td>Salmonella</td>
<td>3,328</td>
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<tr>
<td>Cytogenetics</td>
<td>12,932</td>
</tr>
<tr>
<td>Mouse lymphoma</td>
<td>6,500</td>
</tr>
<tr>
<td>Fertility &amp; Reproduction:</td>
<td></td>
</tr>
<tr>
<td>Fertility assessment</td>
<td>80,300</td>
</tr>
<tr>
<td>Sperm morphology</td>
<td>5,300</td>
</tr>
<tr>
<td>Teratology:</td>
<td></td>
</tr>
<tr>
<td>Conventional</td>
<td>68,000</td>
</tr>
<tr>
<td>Inhalation</td>
<td>350,000</td>
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<tr>
<td>Prechronic Studies:</td>
<td>Low range³</td>
</tr>
<tr>
<td>Dosed feed/dosed water</td>
<td>440,000</td>
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<tr>
<td>Gavage</td>
<td>505,000</td>
</tr>
<tr>
<td>Skin paint</td>
<td>520,000</td>
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<tr>
<td>Inhalation</td>
<td>655,000</td>
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<tr>
<td>Chronic:</td>
<td></td>
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<tr>
<td>Dosed feed/dosed water</td>
<td>1,210,000</td>
</tr>
<tr>
<td>Gavage</td>
<td>1,460,000</td>
</tr>
<tr>
<td>Skin paint</td>
<td>1,460,000</td>
</tr>
<tr>
<td>Inhalation</td>
<td>1,960,000</td>
</tr>
</tbody>
</table>

²Costs include actual contract award, support contracts, plus in-house operating costs.
**Roadmap Activities: Toxicology Research Operations**

- Review existing protocols and designs and change as needed
- Expand endpoints targeted in *in vivo* studies to include functional genomics
- Develop a high-throughput capability for mechanistic targets
- Further evaluate and refine the use of non-mammalian animal models
- Improve the use of toxicokinetic information
- Expand the use of imaging technologies

**Roadmap Activities: High-Throughput Screening (HTS)**

**Short-term Activities**
- Catalogue available assays
- Convene working groups to provide advice on selection of assays
- Develop assays
- Identify initial set of chemicals for testing

**Mid-term Activities**
- Continue assay development
- Validate individual assays
- Develop methods for analysis of data
- Develop HTS database
- Review effectiveness

**Long-term Activities**
- Develop mechanisms to make chemical sets and tissue banks available for external researchers
- Evaluate HTS data for predictability of toxicity
- Develop a communication plan
- Review effectiveness