Chemical-Induced Carcinogenesis
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, 2006)

Age-adjusted Cancer Death Rates, by Site, US, 1930-2005

http://apps.nccd.cdc.gov/uscs/
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
• Carcinogenic to humans (group 1) – 107 agents to date
• Probably carcinogenic to humans (group 2A) – 58
• Possibly carcinogenic to humans (group 2B) – 249
• Not classifiable as to its carcinogenicity to humans (group 3) – 512
• Probably not carcinogenic to humans (group 4) – 1

• 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
IARC: Evaluation of the weight of the evidence

Cancer in humans
- Sufficient evidence
- Limited evidence
- Inadequate evidence
- Evidence suggesting lack of carcinogenicity

Cancer in experimental animals
- Sufficient evidence
- Limited evidence
- Inadequate evidence
- Evidence suggesting lack of carcinogenicity

Mechanistic and other relevant data
- Mechanistic data “weak,” “moderate,” or “strong”?
- Mechanism likely to be operative in humans?

Overall evaluation
- Group 1: Carcinogenic to humans
- Group 2A: Probably carcinogenic to humans
- Group 2B: Possibly carcinogenic to humans
- Group 3: Not classifiable as to its carcinogenicity to humans
- Group 4: Probably not carcinogenic to humans

Slide courtesy of V. Cogliao (IARC)
A tour of IARC’s classifications
Preamble, Part B, Section 6(d)

EVIDENCE IN EXPERIMENTAL ANIMALS
- Sufficient
- Limited
- Inadequate
- ESLC

EVIDENCE IN HUMANS
- Sufficient
- Limited
- Inadequate
- ESLC

Group 1
- Sufficient
- Limited
- Inadequate
- ESLC

Group 2A
- Limited
- Inadequate
- ESLC

Group 2B (exceptionally, Group 2A)
- Inadequate
- ESLC

Group 3
- Inadequate
- ESLC

Group 4
- ESLC

Slide courtesy of V. Cogliano (IARC)
Mechanistic data can be pivotal when the human data are not conclusive

<table>
<thead>
<tr>
<th>EVIDENCE IN EXPERIMENTAL ANIMALS</th>
<th>Group 1</th>
<th>Group 2B</th>
<th>Group 3</th>
<th>Group 4</th>
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<tr>
<td><strong>Sufficient</strong></td>
<td>✓2A belongs to a mechanistic class where other members are classified in Groups 1 or 2A</td>
<td>✓2A belongs to a mechanistic class</td>
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<td>✓2A belongs to a mechanistic class</td>
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<td>Group 2B (exceptionally, Group 2A)</td>
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<tr>
<td><strong>Limited</strong></td>
<td>✓1 strong evidence in exposed humans ... agent acts through a relevant mechanism</td>
<td>✓2B with supporting evidence from mechanistic and other relevant data</td>
<td>✓2B with strong evidence from mechanistic and other relevant data</td>
<td>✓4 consistently and strongly supported by a broad range of mechanistic and other relevant data</td>
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<tr>
<td><strong>Inadequate</strong></td>
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<td>✓2A belongs to a mechanistic class</td>
<td>✓2A belongs to a mechanistic class</td>
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<tr>
<td><strong>ESLC</strong></td>
<td>✓3 strong evidence ... mechanism does not operate in humans</td>
<td>Group 3</td>
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<td>Current evaluation</td>
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<td>L</td>
<td>S</td>
<td>2A</td>
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<tr>
<td>4-Chloro-ortho-toluidine</td>
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<td></td>
<td>I (ND)</td>
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<tr>
<td>5-Chloro-ortho-toluidine</td>
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<tr>
<td>Cinnamyl anthranilate</td>
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<td>L</td>
<td>3</td>
<td>I (ND)</td>
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<td>Coumarin</td>
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<td>L</td>
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<tr>
<td>2,3-Dibromopropan-1-ol</td>
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<tr>
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<td>Ethyl benzene</td>
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<td>I</td>
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<td>I (ND)</td>
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<td>Nitromethane</td>
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<td>I (ND)</td>
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<tr>
<td>N-Nitrosodiethanolamine</td>
<td>I (ND)</td>
<td>S</td>
<td>2B</td>
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<td>Pyridine</td>
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<td>I</td>
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<tr>
<td>ortho-Toluidine</td>
<td>I</td>
<td>S</td>
<td>2B</td>
<td>L</td>
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<tr>
<td>Triethanolamine</td>
<td>I</td>
<td>I</td>
<td>3</td>
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</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 Assessments in IRIS

- Assign cancer descriptor
  - Weight-of-Evidence (WOE) for Carcinogenicity. The approach outlined in EPA’s Guidelines for Carcinogen Risk Assessment (2005) considers all scientific information in determining whether and under what conditions an agent may cause cancer in humans, and provides a narrative approach to characterize carcinogenicity rather than categories.
  - Five standard weight-of-evidence descriptors are used as part of the narrative.

- Identify available key human studies and cancer bioassays.

- Attempt to identify carcinogenic mode(s) of action.
  - Where data are sufficient, select and apply extrapolation methods to develop a slope factor and/or inhalation unit risk.

- For carcinogens with a mutagenic mode of action, the application of age-dependent adjustment factors is recommended as per the Supplemental Guidance for Early Life Exposures.

<table>
<thead>
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<tr>
<td>A: Human carcinogen</td>
<td>Carcinogenic to humans</td>
<td>Carcinogenic to humans</td>
</tr>
<tr>
<td>B1: Probable human carcinogen (limited human data)</td>
<td>Likely to be carcinogenic to humans</td>
<td>Likely to be carcinogenic to humans</td>
</tr>
<tr>
<td>B2: Probable human carcinogen (inadequate or no human data)</td>
<td>Suggestive evidence of carcinogenicity, but not sufficient to assess human carcinogenic potential</td>
<td>Suggestive evidence of carcinogenic potential</td>
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<tr>
<td>C: Possible human carcinogen</td>
<td>Data inadequate for assessment of human carcinogenic potential</td>
<td>Inadequate information to assess carcinogenic potential</td>
</tr>
<tr>
<td>D: Not classifiable</td>
<td>Not likely to be carcinogenic to humans</td>
<td>Not likely to be carcinogenic to humans</td>
</tr>
</tbody>
</table>

[www.epa.gov/iris](http://www.epa.gov/iris)

Cancer Cases Attributable to Environmental Carcinogens (Worldwide, 1990)

- Infections (viruses, parasites, *H. pylori*) 16%
- Tobacco (smoked and smokeless) 14%
- Occupation 4%
- Alcohol drinking 3%
- Diet and dietary components including contaminants 25%
- Pollution 2%
- Reproductive factors 2%

Total 37%
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) → Cell Proliferation

Second Mutating Event → Cell Proliferation

"N" Mutating Event → Cell Proliferation

Initiation → 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

Detoxication

DNA

RNA

Protein (Biomarker)

Biologically Effective Dose

Efficiency of Mispairing

Cell Proliferation

Initiation
GENETIC AND EPIGENETIC MODELS OF THE CANCER INITIATION

**ACQUISITION OF ADDITIONAL RANDOM MUTATIONS**

- Clonal selection and expression of initiated cells
- Mutator phenotype cells
- Cancer cells

**ALTERATIONS IN CELLULAR EPIGENOME**

- Epigenetically reprogrammed cells
- Mutator phenotype cells
- Cancer cells
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
1. $X \rightarrow$ No Tumors
2. $\text{Tumors}$
3. $\text{Tumors}$
4. $\text{No Tumors}$
5. $\text{No Tumors}$

$X = \text{Application of Initiator}$  $\downarrow = \text{Application of Promoter}$

Time
Basophilic Focus → Adenoma → Carcinoma

Promotion → Regression → Progression

\( M_1 \) → \( M_N \)

\( \uparrow \) = Application of Promoter

No Tumors → Tumors

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**

- **Chemical**: Aflatoxin B1, Ethanol/Smoking, Vinyl Chloride
  - Enzyme activation
  - Conjugation Enzymes
  - Deactivation/Excretion

- **Radiation**: Thorotrust

- **Virus**: HBV, HCV

- ** NORMAL CELL**
  - Conjugation Enzymes
  - Deactivation/Excretion

- **Initiated Cell**
  - Genetic and Epigenetic Changes
  - Selective Clonal Expansion

- **Pre-neoplastic Lesion**
  - Necroinflammatory Liver Disease/Cirrhosis
  - Reactive Oxygen/Nitric Oxide Species

- **Malignant Tumor**

- **Clinical Liver Cancer**

- **Invasion and Metastasis**
  - Integrins
  - E-cadherin
  - β-catenin
  - CMAR
  - NM23, KAI1

- **Genetic Predispositions**
  - e.g., Hemochromatosis
  - Wilson’s Disease
  - α1-Antitrypsin
  - Metabolic Genotypes

- **Telomerase Activation**

- **Genetic Change**

- **20-60 years**

- **Activation of Proto-Oncogenes** (e.g., N-ras, c-myc, c-fos)/Growth Factors (e.g., IGF-1, IGF-2, TGF-α, TGF-β)
- **Inactivation of Tumor Suppressor Genes** (e.g., p53, p16, Rb, LOH 1p, 1q, 2q, 3q, 4q, 5q, 6q, 7q, 8p, 8q, 9p, 9q, 10q, 11p, 13q, 16p, 16q, 17p, 22/APC for hepatoblastoma)

**Hussain et al., Oncogene, 2007**
Classification of Carcinogens According to the Mode of Action

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

**Initiation**
- Initiating Event
- Cell Proliferation (clonal expansion)

**Promotion**
- Second Mutating Event
- Cell Proliferation

**Progression**
- Third Mutating Event
- Cell Proliferation

**Malignancy**
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 showing DNA adduct formation and progression to cancer](image)
Mechanism of Carcinogenesis: Genotoxic Carcinogens

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

Chemical → "Activated" carcinogen → DNA Repair → APOPTOSIS

"inactivated" carcinogen → CYP450s
Schematic diagram showing the mechanism through which exposure to polycyclic aromatic hydrocarbons is thought to cause cancer

Possible pathways of activation of suspected human carcinogens

Heterocyclic amines e.g. IQ, PhIP

Aromatic amines e.g. 4-ABP

PAHs e.g. B[a]P, DMBA

DNA-reactive products of metabolic activation

NH+

C+

Dihydrodiol epoxide

Electrophile

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
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

- Proliferation
- Tissue
- Cell Death
- Mitogenic Agent
- Cytotoxic Agent
Mechanism of Carcinogenesis: Non-Genotoxic Carcinogens

Cell proliferation (to fix “spontaneous” mutation)

CANCER
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 ≠ Carcinogenesis

Cell Proliferation ≠ Carcinogenesis

Toxicity ≠ 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

Nebert & Dalton *Nat Rev Cancer* 2006
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

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### 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>
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<th>Sexes</th>
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<th>Test Groups</th>
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<tr>
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<td>5 x</td>
<td>2 x</td>
<td>2 x</td>
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<td>Controls</td>
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<td>2 x</td>
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</tr>
<tr>
<td>Total</td>
<td></td>
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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.
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>
<thead>
<tr>
<th>Test</th>
<th>Animals</th>
<th>Sexes</th>
<th>Species</th>
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<td>Special controls for clinical lab studies</td>
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**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 x 2 x 2 x 3 = 600</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>50 x 2 x 2 x 1 = 200</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sentinel Animals</td>
<td>15 x 2 x 1 x 2 = 60</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>860</strong></td>
<td></td>
<td></td>
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</tbody>
</table>
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 studya</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mutagenicity:</strong></td>
<td></td>
</tr>
<tr>
<td>Drosophila</td>
<td>$11,083</td>
</tr>
<tr>
<td>Salmonella</td>
<td>3,328</td>
</tr>
<tr>
<td>Cytogenetics</td>
<td>12,932</td>
</tr>
<tr>
<td>Mouse lymphoma</td>
<td>6,500</td>
</tr>
<tr>
<td><strong>Fertility &amp; Reproduction:</strong></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><strong>Teratology:</strong></td>
<td></td>
</tr>
<tr>
<td>Conventional</td>
<td>68,000</td>
</tr>
<tr>
<td>Inhalation</td>
<td>350,000</td>
</tr>
<tr>
<td><strong>Prechronic Studies:</strong></td>
<td></td>
</tr>
<tr>
<td>Dosed feed/dosed water</td>
<td>440,000</td>
</tr>
<tr>
<td>Gavage</td>
<td>505,000</td>
</tr>
<tr>
<td>Skin paint</td>
<td>520,000</td>
</tr>
<tr>
<td>Inhalation</td>
<td>655,000</td>
</tr>
<tr>
<td><strong>Chronic:</strong></td>
<td></td>
</tr>
<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>

aCosts 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