Eminent Toxicologist Lecture Series

Chemical Carcinogenesis

Society of Toxicology

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Chemical Carcinogenesis

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Society of Toxicology

Conflict of Interest Declaration

- Consult for Numerous Companies
- Research funded by NIH, Private Industry
- FEMA Expert Panel

Outline

- Basic Principles of Carcinogenesis
- Carcinogenic Chemicals
- Carcinogenicity Testing
- Model of Carcinogenesis
- Mode of Action/Human Relevance

History of Chemical Carcinogenesis

- John Hill–Cancer of nose and snuff users, 1761.
- Sir Percival Pott–Chimney sweeps (scrotal cancer), 1775.
- Rehn–Bladder cancer in aniline dye industry, 1895.
- Kennaway and Cook–First pure chemical carcinogen, dibenz(a,h) anthracene, 1930.
- Hueper–2-naphthylamine as bladder carcinogen in dogs, latency in carcinogenesis, 1937.
- Miller and Miller–Ultimate carcinogenic metabolites are electrophiles adducting DNA, 1967.

Cancer Requires Genetic Alterations

- Usually occur as somatic alterations
- Can occasionally be inherited
- Multiple alterations are required

Inherited Diseases with High Tumor Incidence

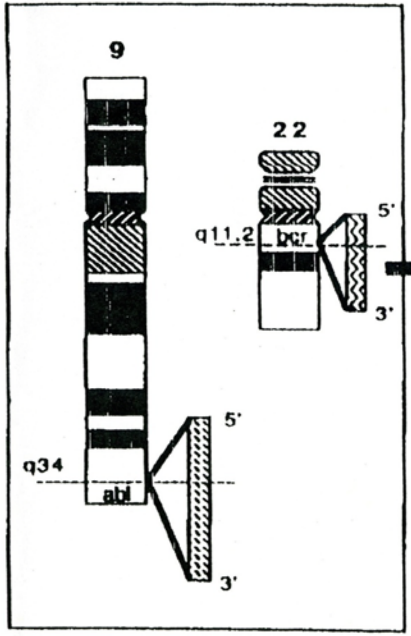
- Retinoblastoma
- Multiple polyposis coli
- Thyroid medullary carcinoma
- Multiple endocrine adenomas
- Von Recklinghausen's disease

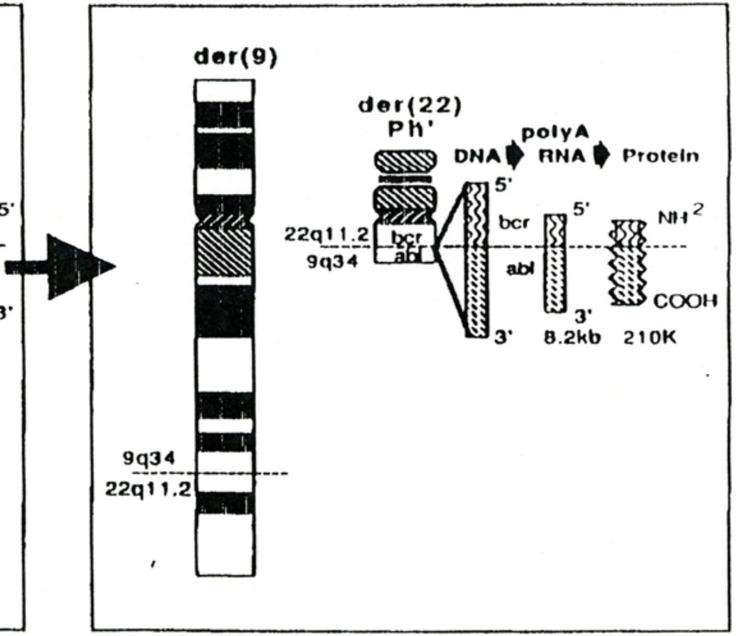
Diseases Associated with Increased Cancer Risk

- UV Radiation
  - Albinism
  - Xeroderma pigmentosm
- Chromosome fragility syndrome
  - Bloom's syndrome
  - Fanconi's syndrome
- Immunodeficiencies
  - X-linked lymphoproliferative disease (XLP)
  - Ataxic-telangiectasia
  - Severe combined immunodeficiency
  - Wiskott-Aldrich Syndrome

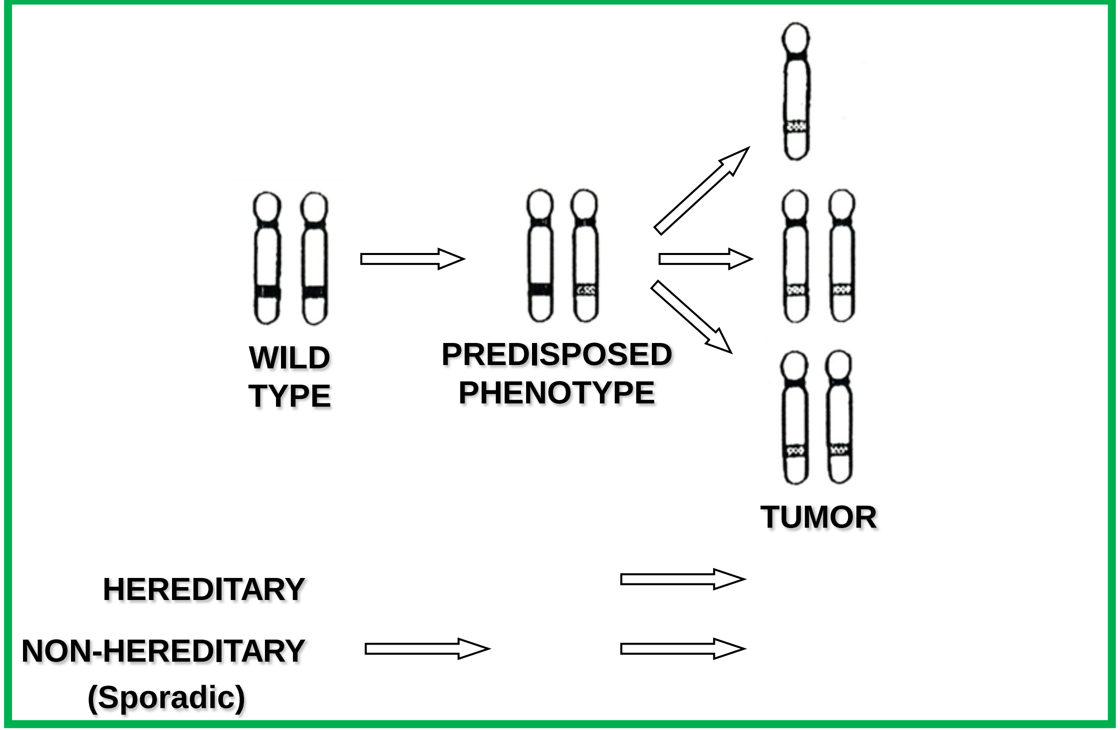
Chronic Myelogenous Leukemia: Philadelphia Chromosome

Normal Configuration of Chromosomes 9 & 22	Rearranged Chromosomes 9 (9q+) & 22 (Ph)





Tumor Suppressor Genes



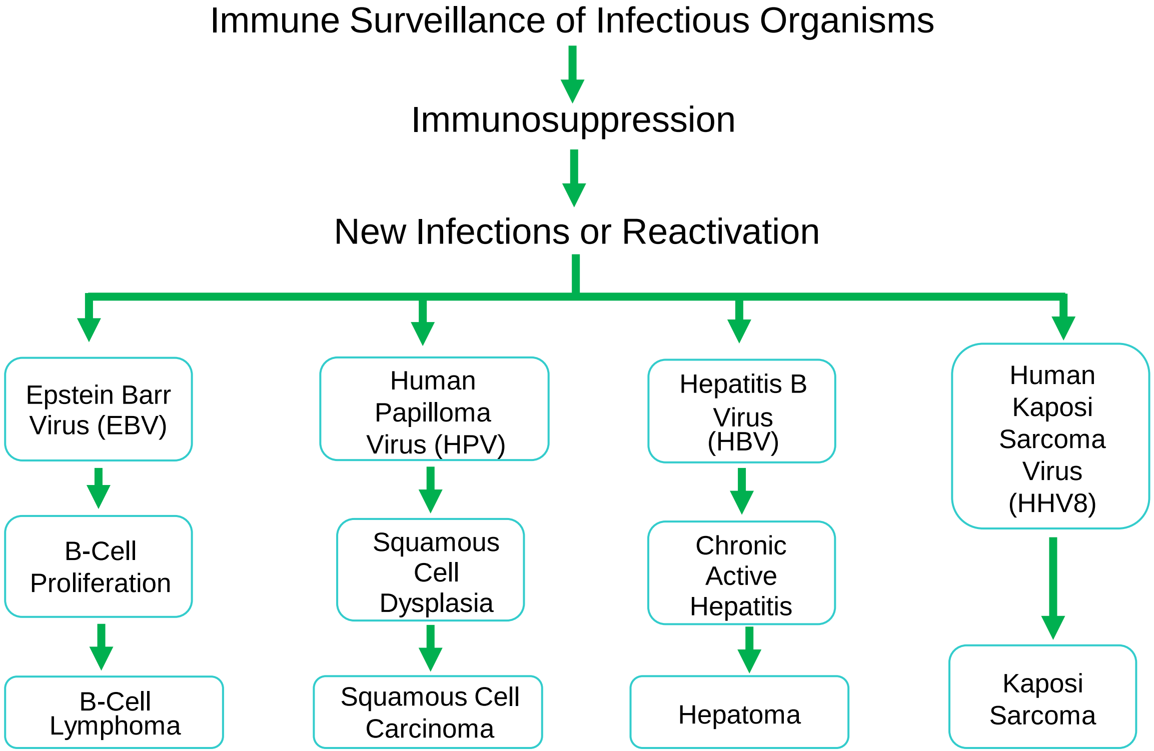
Oncogenes and Suppressor Genes

- Growth factors
- Growth factor receptors
- Cellular growth signal transducers
- Nuclear proteins regulating cell division
- Nuclear proteins regulating replication mechanics
- Apoptosis regulators

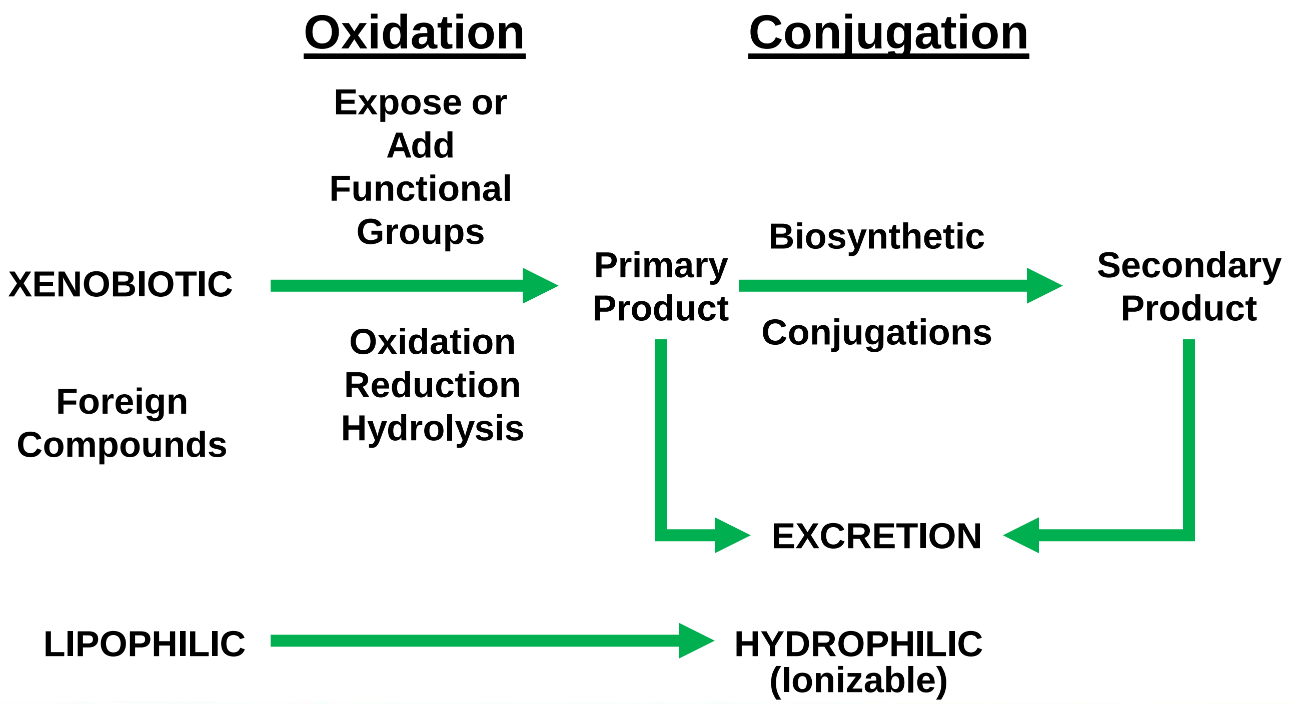
Immunosurveillance and Cancer

- Tumor specific transplantation antigens
  - Viral specific in mice
  - No tumor specific antigens in humans
- Carcinogens are immunosuppressive
  - Frequently only at doses >>carcinogenic dose
- Increased incidence of tumors in immunodeficient patients
  - Only a few specific types of cancer
- Neoplastic clone escapes immune surveillance

Immunosurveillance and Cancer



Xenobiotic Metabolism



Initiation-Promotion

1) No Tumors

2) No Tumors

3) Many Tumors

4) No Tumors

5) Many Tumors

6) No Tumors

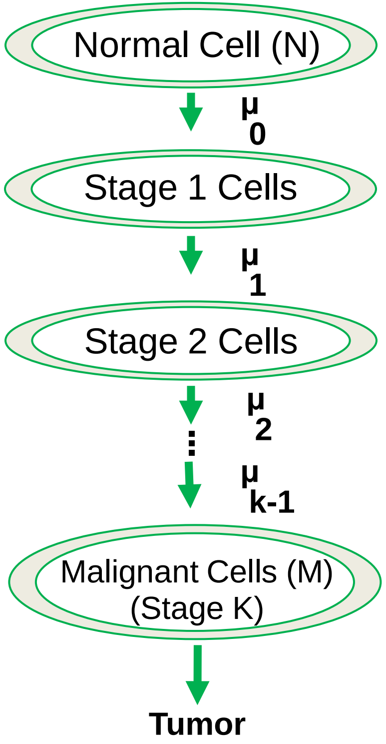
Symbols: Time

Initiator Promoter

Limitations of Initiation-Promotion Model

- Empirically defined – model dependent
- Based on short-term studies
- Assumes intermediate benign clonal expression
  - Many human tumors do not have this
- Sequential administration
  - Humans frequently exposed to agents concurrently
- Terms have become used with a variety of meanings, rarely specified and usually unclear

Armitage-Doll Multistep Cancer Model



Armitage-Doll Multistage Model

$I(t) = N \lambda_0 \lambda_1 \ldots \lambda_{n-1} t^{n-1} / (n-1)!$

I(t) = incidence at time of t

N = number of normal stem cells

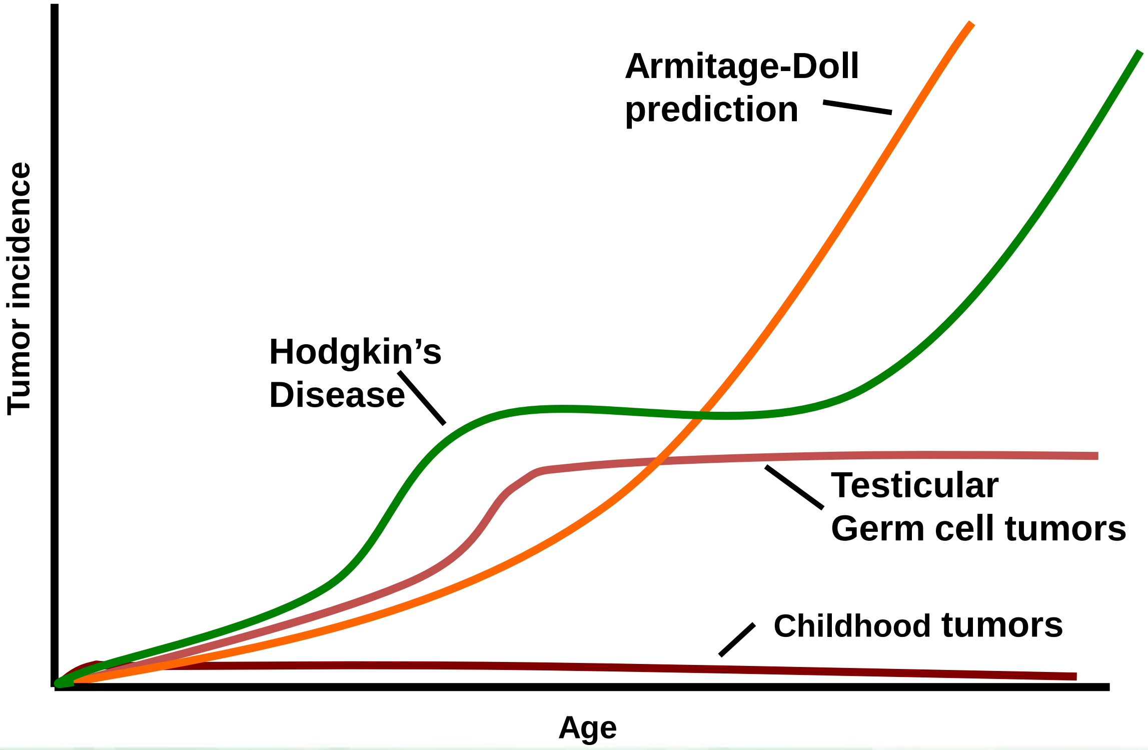
λ = rate of transition between stages

n = number of stages

N, λ assumed to be constant

Cell proliferation rates assumed to be constant

Age-Related Cancer Incidence



Cancer Arises Due to "Bad Luck"

Variation in cancer risk among tissues can be explained by the number of stem cell divisions

Cristian Tomasetti and Bert Vogelstein

Science 347:78-81, 2015

Substantial contribution of extrinsic risk factors to cancer development

Song Wu, Scott Powers, Wei Zhu & Yusuf A. Hannun

Nature 529:43-47, 2016

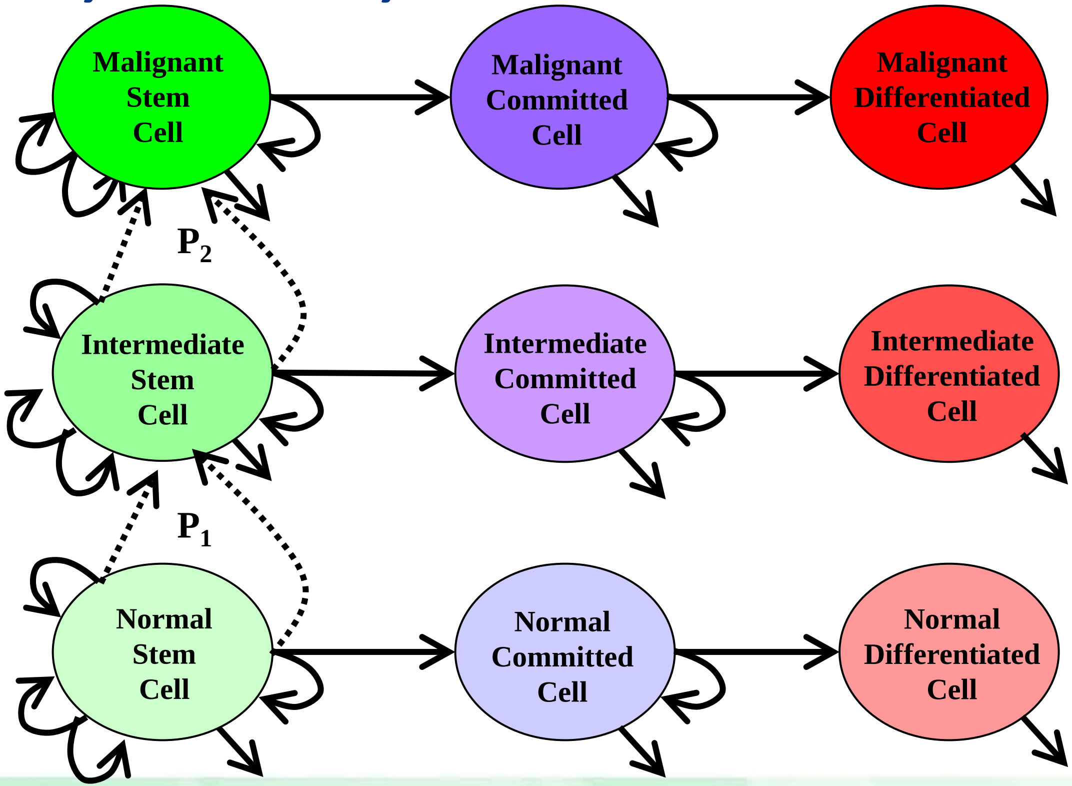
What We Know

- Genetic alterations required for cancer formation
- More than one genetic alteration required
- DNA replication fidelity is not 100%

Means of Increasing Risk of Cancer

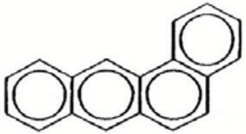
- Increase Rate of DNA Damage Per Cell Division (DNA Reactive)
- Increase Number of Cell Divisions (Non-DNA Reactive; Increased Cell Proliferation)

Cohen, Ellwein, and Greenfield Model

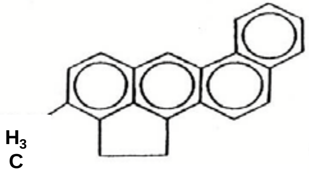


Polycyclic Aromatic Hydrocarbons

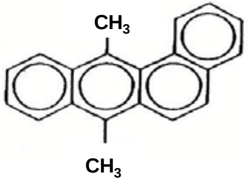
Anthracene



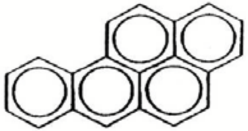
Benz(a)anthracene



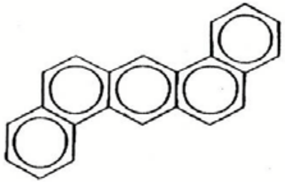
3-Methylcholanthrene



7,12-Dimethylbenz(a)anthracene

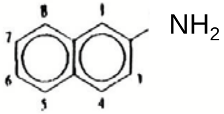


Benzo(a)pyrene

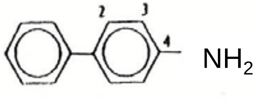


Dibenz(a,h)anthracene

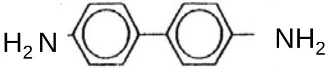
Aromatic and Heterocyclic Aromatic Amines



2-Naphthylamine

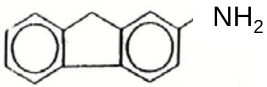


4-Aminobiphenyl

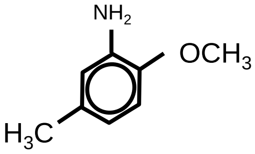


Benzidine

(4,4'-diaminobiphenyl)

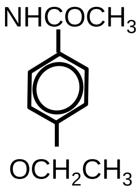


2-Aminoflourene

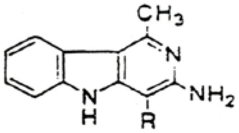


NH2

p-Cresidine

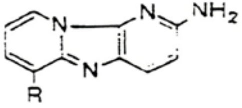


Phenacetin



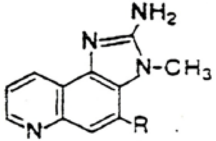
Trp-P-1 R = CH_3

Trp-P-2 R = H



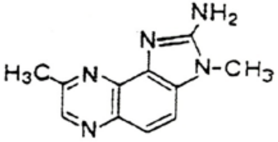
Glu-P-1 R = CH_3

Glu-P-2 R = H

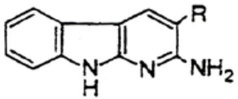


IQ R = H

= CH_3



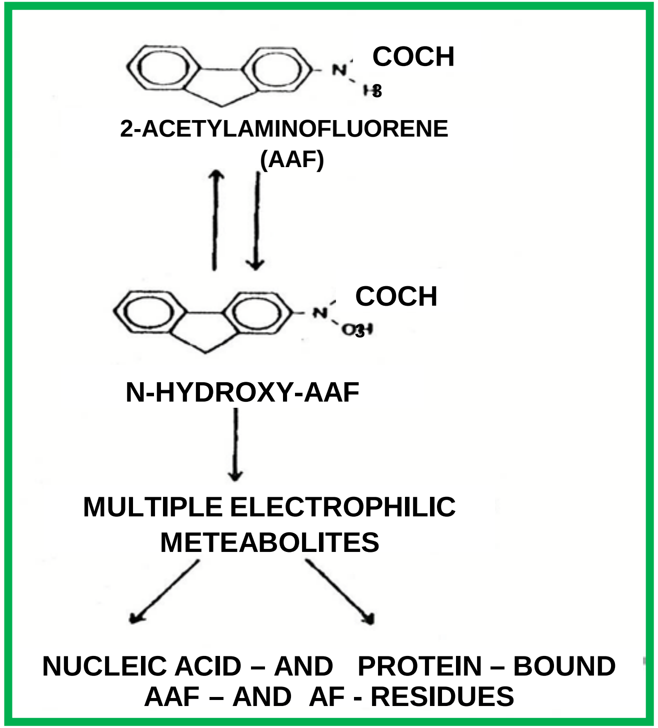
MeIQx



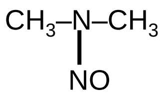
AαC R = H

MeAαC R = CH_3

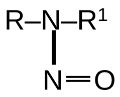
Metabolic Activation of 2-AAF



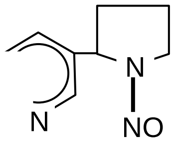
N-Nitroso and Related Chemicals



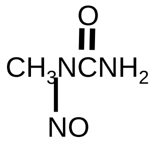
Dimethylnitrosamin
e
or *N*-
nitrosodimethyl-
amine



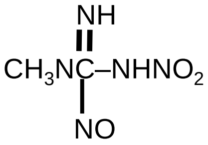
Dialkyl
nitrosamine



N-nitrosonor
-nicotine



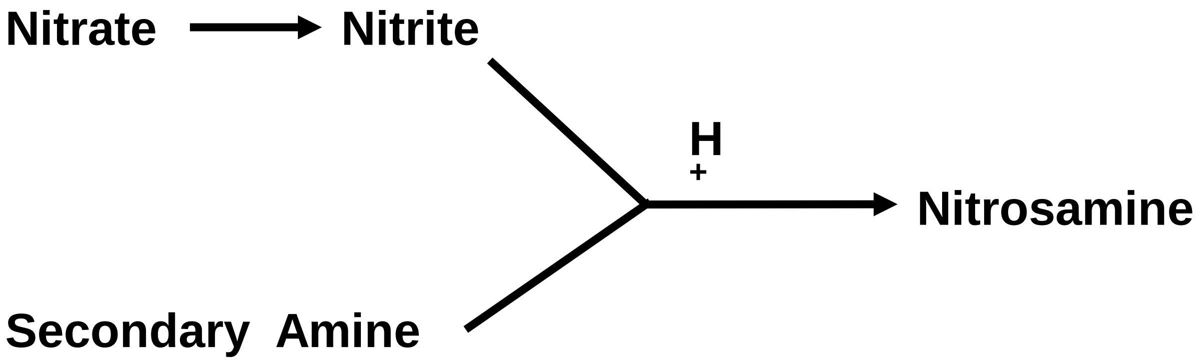
N-Methyl-N-nitroso-
urea



N-Methyl-N[1]-nitro-
N-
nitrosoguanidine

Dimethylhydrazine

Endogenous Formation of N-Nitrosamines



Macronutrients and Cancer

- Fat (obesity)–colon, breast, pancreas, prostate, endometrium, kidney, esophagus (adenocarcinomas)
- Fruits and vegetables
- Meat–colon
- Salt–stomach

Ethanol and Cancer

- Liver
- Esophagus
- Oral Cavity and Pharynx
- Breast
- Colon (beer)
- Larynx

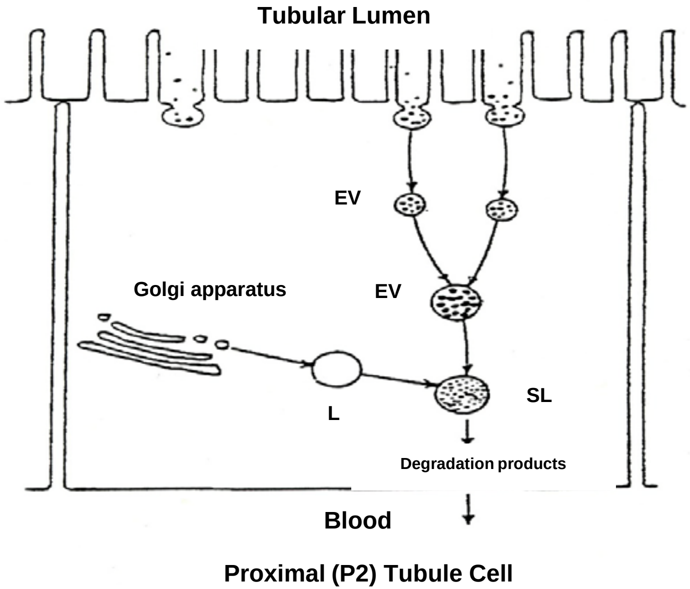
High Dose Only

Cigarette Smoking

Approximately one-third of cancer deaths in United States

Lung
Larynx
Oral cavity & pharynx
Paranasal sinuses
Esophagus
Stomach
Urinary bladder
Ovary (mucinous tumors)
Pancreas
Liver
Kidney
Ureter
Uterine cervix
Bone marrow (leukemia)
Colorectal
Salivary gland (Warthin's tumor)

Normal Processing of α_{2u}–Globulin



Processing of Chemical Bound α_{2u}–Globulin

Tubular Lumen

Alpha –globulin$_{2u}$ + C: GA

endocytic vesicle

lysosome

hyaline droplets

secondary lysosome

Blood

Proximal (P2) Tubule Cell

Incidence of Bladder Carcinoma in Mice Implanted with Paraffin Wax Pellets

Time (wks)	Incidence (%)
40-50	10.6
70-80	26.7
100-110	53.8

Substances Producing Urinary Calculi

Uracil
Melamine
Uric Acid
Homocysteine
Cysteine
Calcium oxalate
Calcium phosphate
Diethylene glycol
Biphenyl
HIV Protease inhibitors
PPARγ agonists

4-Ethylsulfonylnaphthalene-1-sulfonamide
Oxamide
Acetazolamide
Terephthalic acid
Dimethyl terephthalate
Nitrilotriacetate
Polyoxyethylene-8-stearate
Glycine
Orotic acid
Sodium saccharin

Newer Alternatives to Carcinogenicity Testing

- P53 +/- Transgenic Model (6 months)
  - Only used to address genotoxicity
- TG.AC Transgenic Model
  - No longer used
- Neonatal Mouse Model (1 year)
- TGHras2 Model (6 months)
- XPA-1- Repair Deficient Model (9 months)
  - Combined with p53 +/-
- Ito Medium Term Rat Assay
  - Complex regimen

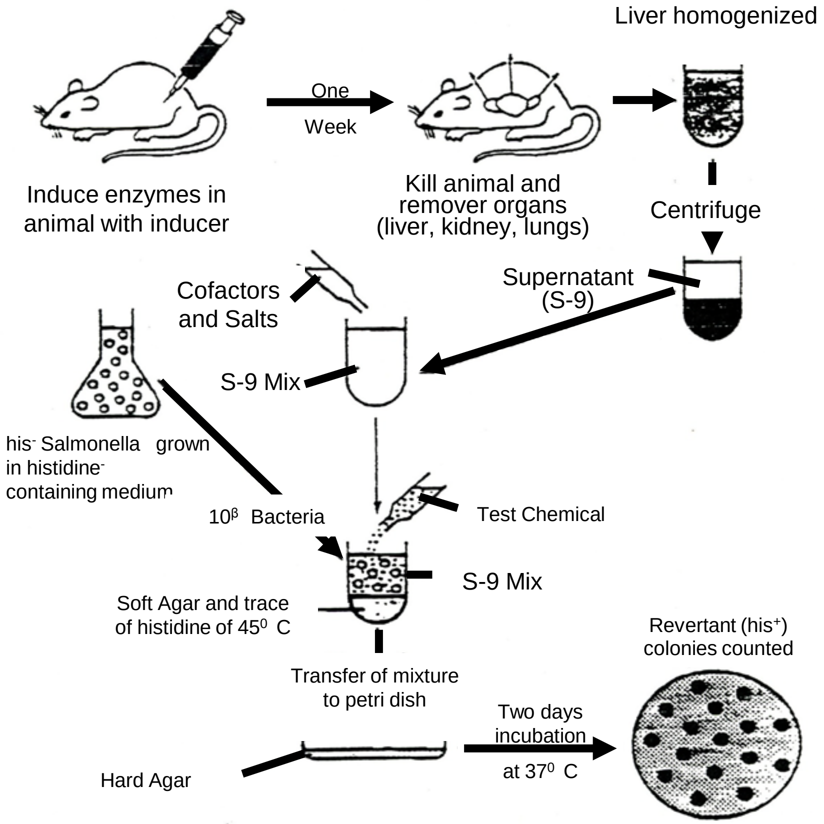
Do not address human carcinogenicity!

Lawyers As A Test Species

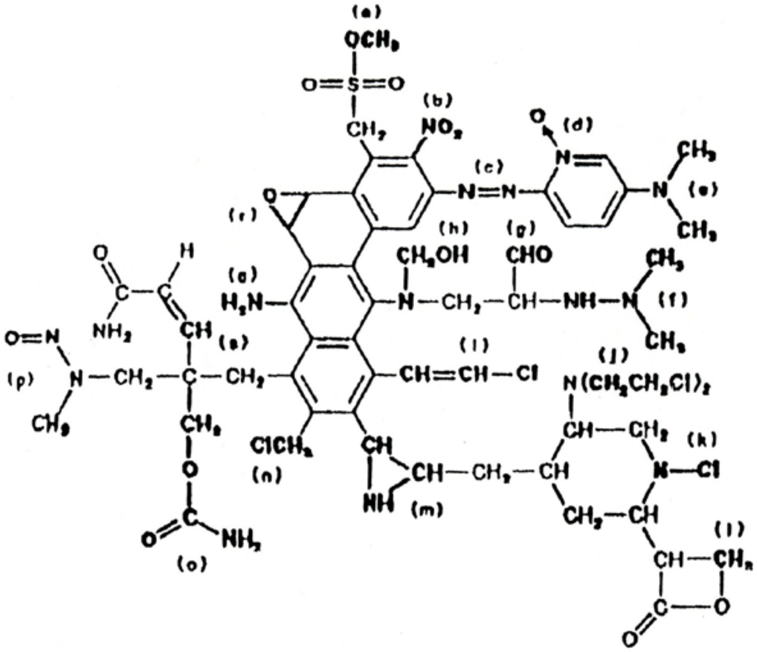
Pros:
- Plenty of Them
- Will do anything, some things even a rat won't do
- No problem with animal rights activists
- Multiply rapidly
- Don't become emotionally attached to them

Cons:
- Expensive
- Still left with interspecies extrapolation problem

Genotoxicity Screens



Structure Activity Relationships (SAR)



(t)

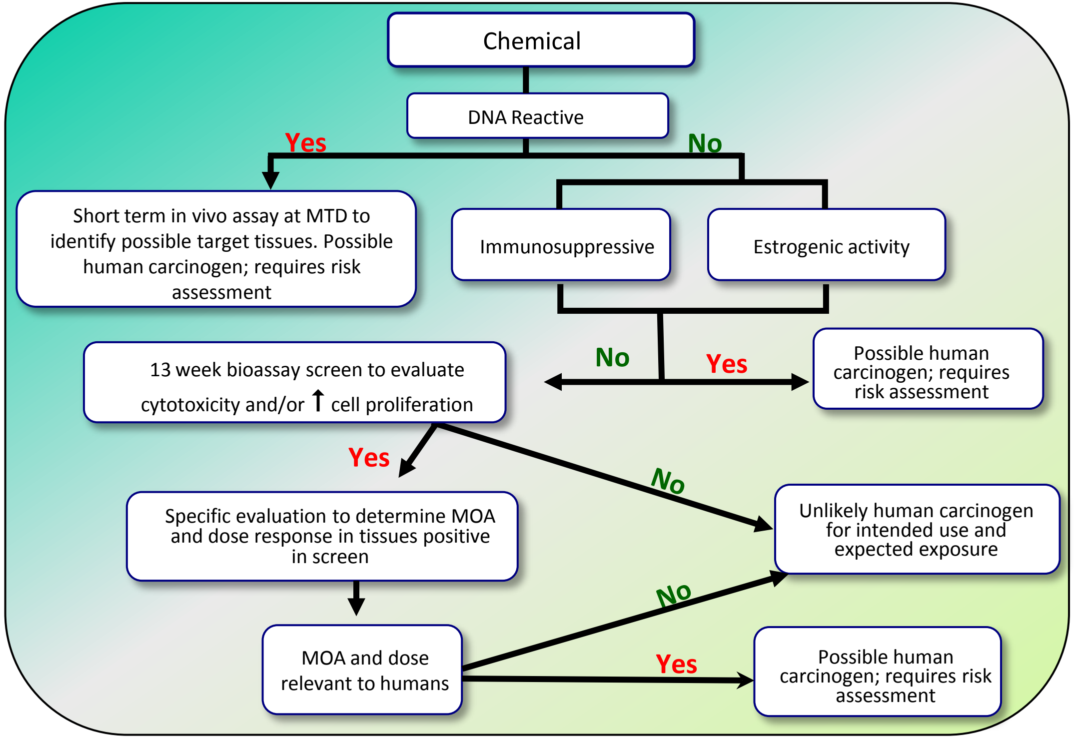
Halogenated methanes

C(X)

X = H,F,Cl, Br,1

In any combination

Screening for Carcinogenesis



MODELS: ALL ARE WRONG,

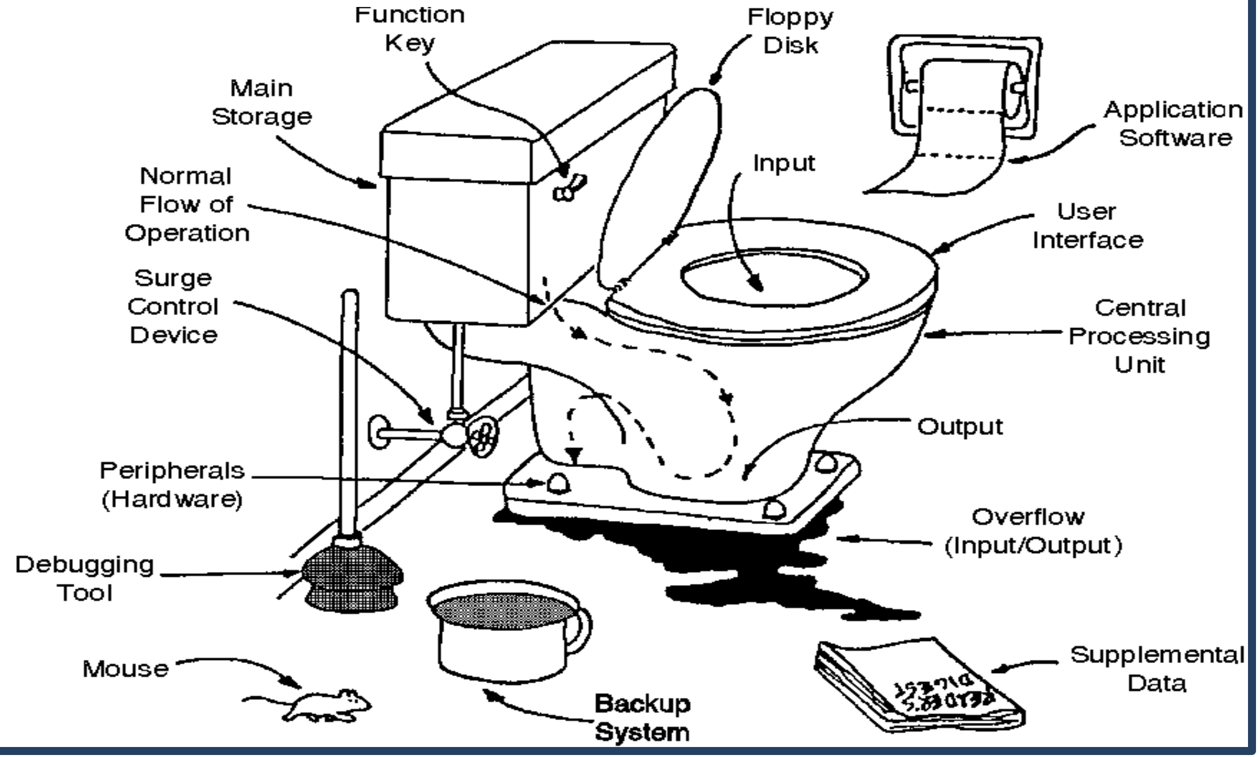
SOME ARE USEFUL.

GEORGE BOX

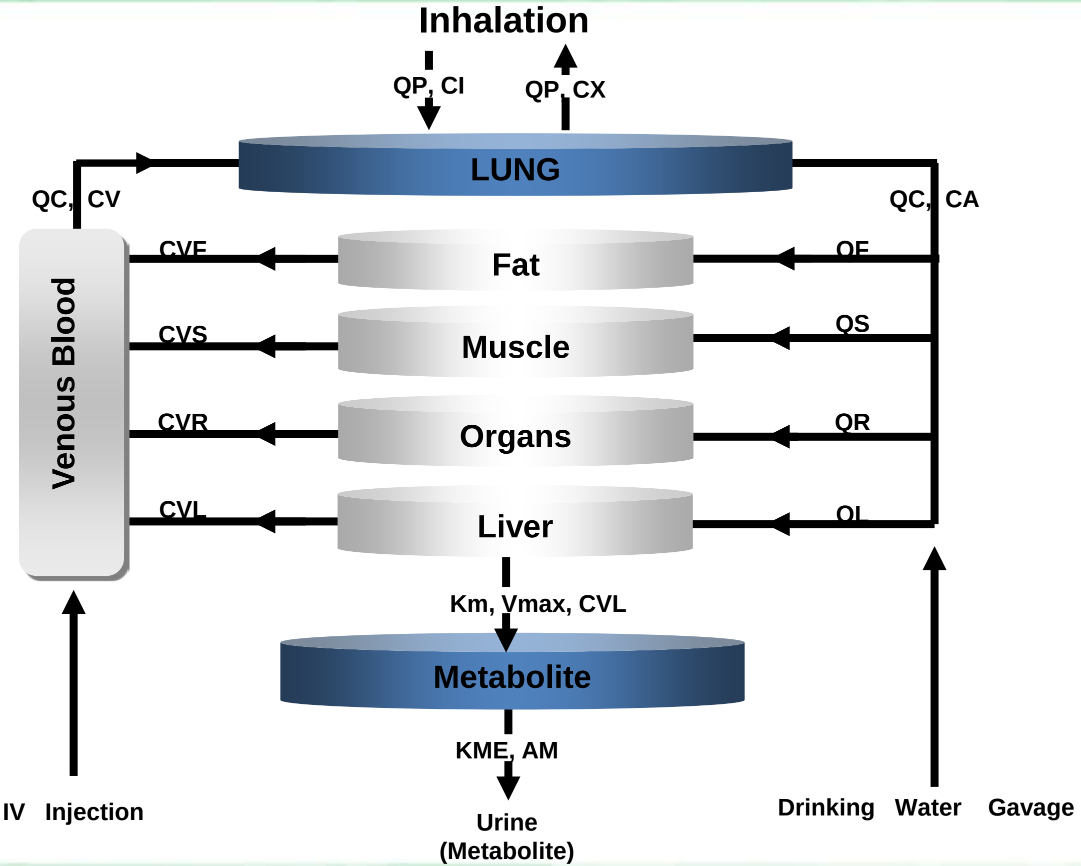
Basic Assumptions of Use of Bioassays for Human Risk Assessment

1. Carcinogenic effects at high doses will also occur at low doses (dose extrapolation).
2. Chemicals that cause cancer in rodents will cause cancer in humans (species extrapolation)

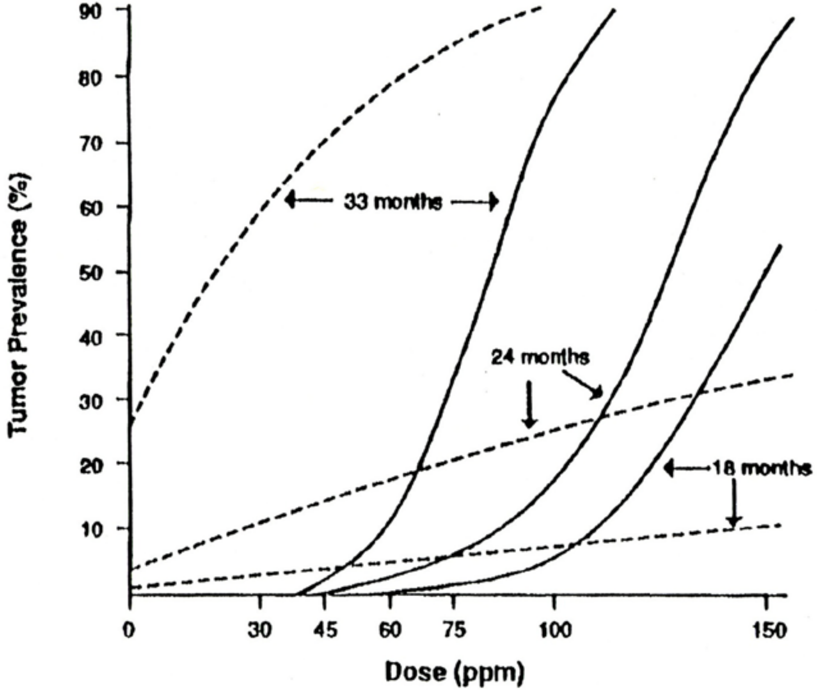
UNDERSTANDING COMPUTER TECHNOLOGY



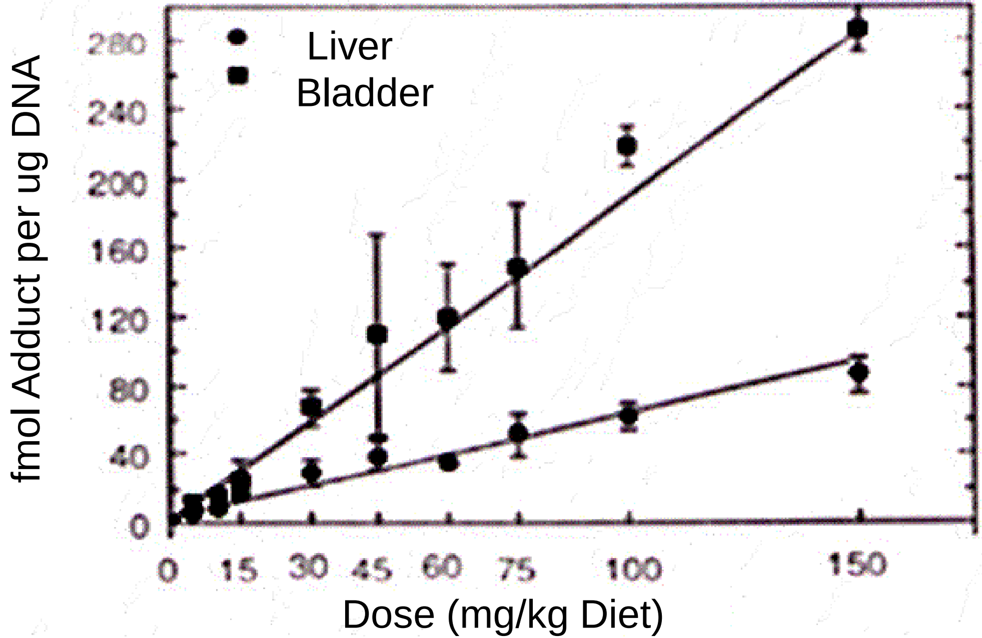
PBPK Modeling



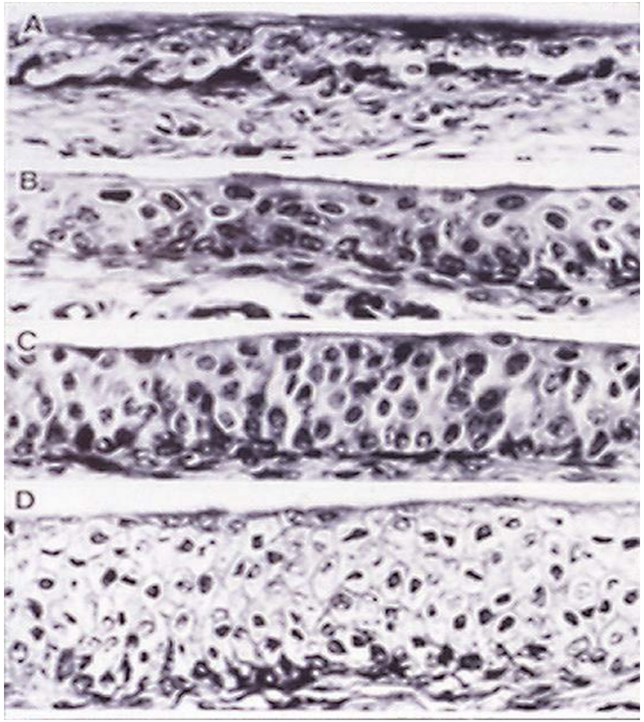
ED01 Megamouse Experiment (2-AAF)



AAF-DNA Adducts



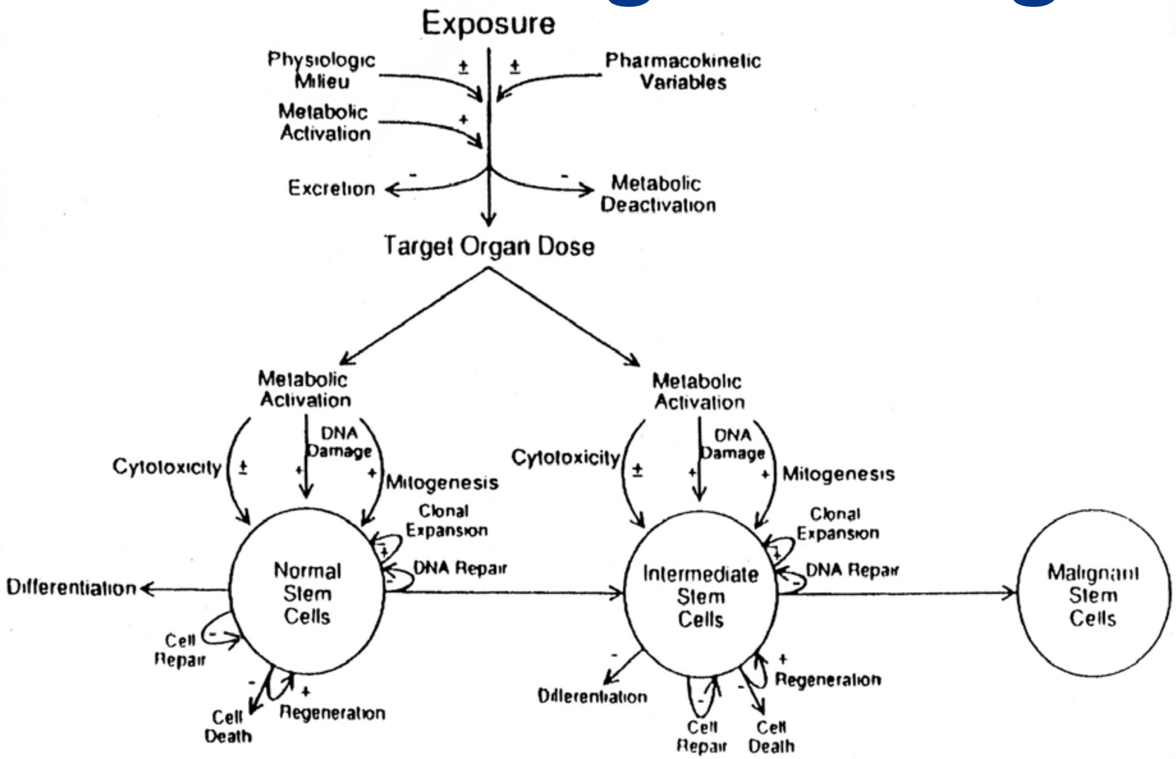
AAF-Induced Bladder Hyperplasia



Synergies Between Genotoxic and Proliferating Agents

Cigarette Smoke	Lung, Bladder
Hepatitis Virus & Aflatoxin	Liver
Papilloma Virus & Cigarette Smoke	Cervix
Helicobacter & N-Nitroso Compounds	Stomach

Factors Influencing Carcinogenesis



The Human Relevance Framework

1. Is the weight of evidence sufficient to establish the MOA in animals?
    a. Postulated MOA
    b. Identification of key events
    c. Animal evidence
    d. Application of DPA/IPCS animal MOA guidance (Table 2)

2. Are key events in the animal MOA plausible in humans?
    a. Concordance analysis of animal and human responses
    b. Statement of confidence

3. Taking into account kinetic and dynamic factors, is the animal MOA plausible in humans?
    1. Concordance analysis of animal and human responses
    2. Statement of confidence

4. Statement of confidence; analysis; implications

Crit. Rev. Toxicol., 33: 593, 2003

Overview/Objectives

1. Principles of Carcinogenesis
2. DNA Reactive and Non-DNA Reactive Carcinogens
3. Methods for Screening for Carcinogenicity
4. Model of Carcinogenesis Incorporating DNA Damage and Increased Cell Proliferation
5. Mode of Action Analysis and Extrapolation of *In Vitro* and Animal Studies to Humans

References

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