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Risk Assessment of Carcinogenic Potential Based on the Current State of Knowledge of Carcinogenesis in Humans

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Declaration of Interests

- Research funded by NIH and private industry
- Member, US EPA Science Advisory Board
- Member, FEMA GRAS Expert Panel
- Consultant for several companies

Non-Genotoxic Carcinogens

- Many have MOA in rodents not relevant to humans
- Always involves increased cell proliferation as key event
- Always involves a precursor non-cancer key event
- Always involves a threshold
- Protecting against precursor non-cancer event will protect against cancer

Basic Assumptions in Use of Bioassays for Human Risk Assessment

1. Carcinogenic effects at doses used in bioassay (high) will also occur at doses humans are exposed (low)
   (dose extrapolation)

2. Chemicals that cause cancer in rodents will cause cancer in humans
   (species extrapolation)

What We Know

- Genetic alterations required for cancer formation
- More than one genetic alteration required
- DNA replication fidelity is not 100%
- Cancer arises from stem cell population
- Cancers are clonal
- Carcinogenesis is stochastic process

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)

Modes of Action of Human Carcinogens

- DNA Reactive
- Immunosuppressive
- Estrogenic
- Cytotoxicity and regeneration

Two Year Rodent Bioassay

- Cost: time, money, animals
- Dose response: limited
- Mode of action: not determined
- Human relevance: can’t evaluate
- Poor predictive value for human cancer

WHO/IPCS Human Relevance Framework (2006)

1. Is the weight of evidence sufficient to establish the MOA in animals?
2. Can human relevance of the MOA be reasonably excluded on the basis of fundamental qualitative differences in key events between experimental animals and humans?
3. Can human relevance of MOA be reasonably excluded on the basis of quantitative differences in either kinetic or dynamic factors between experimental animals and humans?
4. Statement of confidence; analysis; and implications

General Approach

- Screen for DNA reactivity, immunosuppression, estrogenic activity
- Screen for organ specific effects
- Evaluate mode of action
- Evaluate human relevance
- Evaluate dose response

Modes of Action for Hepatocellular Carcinogenesis

- DNA Reactivity

  Metabolic activation → DNA adducts → DNA damage

- Increased cell proliferation
  - A. Receptor mediated
    1. PPARα (peroxisome proliferation)
    2. Enzyme induction (CAR, PXR, AHR)
    3. Estrogen
    4. Statins
    5. Other
  - B. Non-receptor mediated
    1. Cytotoxicity
    2. Viral
    3. Iron overload
    4. Increased apoptosis (e.g. fumonisin B1)
    5. Other

Key Events in the Induction of Liver Tumors by PPARα Agonists

Key Events	Associated Events
Metabolic activation (if necessary)	Peroxisome proliferation
PPARα activation	Oxidative damage
Increased cell proliferation	Acyl CoA oxidase

Cytotoxicity – Chloroform

Key Event	Rodent	Human
Generation of phosgene/HCl by CYP2E1	Yes	Yes
Cytotoxicity	Yes	Yes
Regeneration/Proliferation	Yes	No data – possible
Tumors	Yes	Inadequate data – possible

Cytotoxicity – Chloroform

Implications for Risk Assessment

- Mode of action possible in humans
- High dose phenomenon – threshold
- Sustained exposure required
- Cannot be sustained in humans

90 Day Screen for Rodent Hepatocarcinogens

- Hepatocellular necrosis
- Hepatocellular hypertrophy
- Hepatocellular cytomegaly
- Increased liver weight
- All NTP bioassay hepatocarcinogens had one or more of these findings in 90 day study

Allen et al., 2004

Rodent Liver Carcinogenesis Screening and Implications for Humans

90 Day Screen – Allen et al., 2004 criteria

No → **Not Hepatocarcinogen**

Yes

DNA Reactivity

No → **Mechanistic Screen**

1. Histopathology
2. Serum enzymes
3. Acyl Co-A oxidase (or TEM)
4. CYP induction
5. AHR binding
6. Estrogen receptor binding (or histologic indication of estrogenic activity in other tissues)
7. Iron stain
8. Reversibility

Yes

Metabolic activation

Kinetics

Follow-up detailed studies

1. CAR, PXR, AHR
2. Metabolic activation
3. Detailed dose response

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Screening for Hepatocellular Carcinogenesis

- Initial screen (Allen et al.)
- Evaluate for DNA reactivity, immunosuppression, estrogenic activity
- Mode of action evaluation to determine human relevance
- If human relevant MOA, evaluate dose response
- Two year bioassay unnecessary

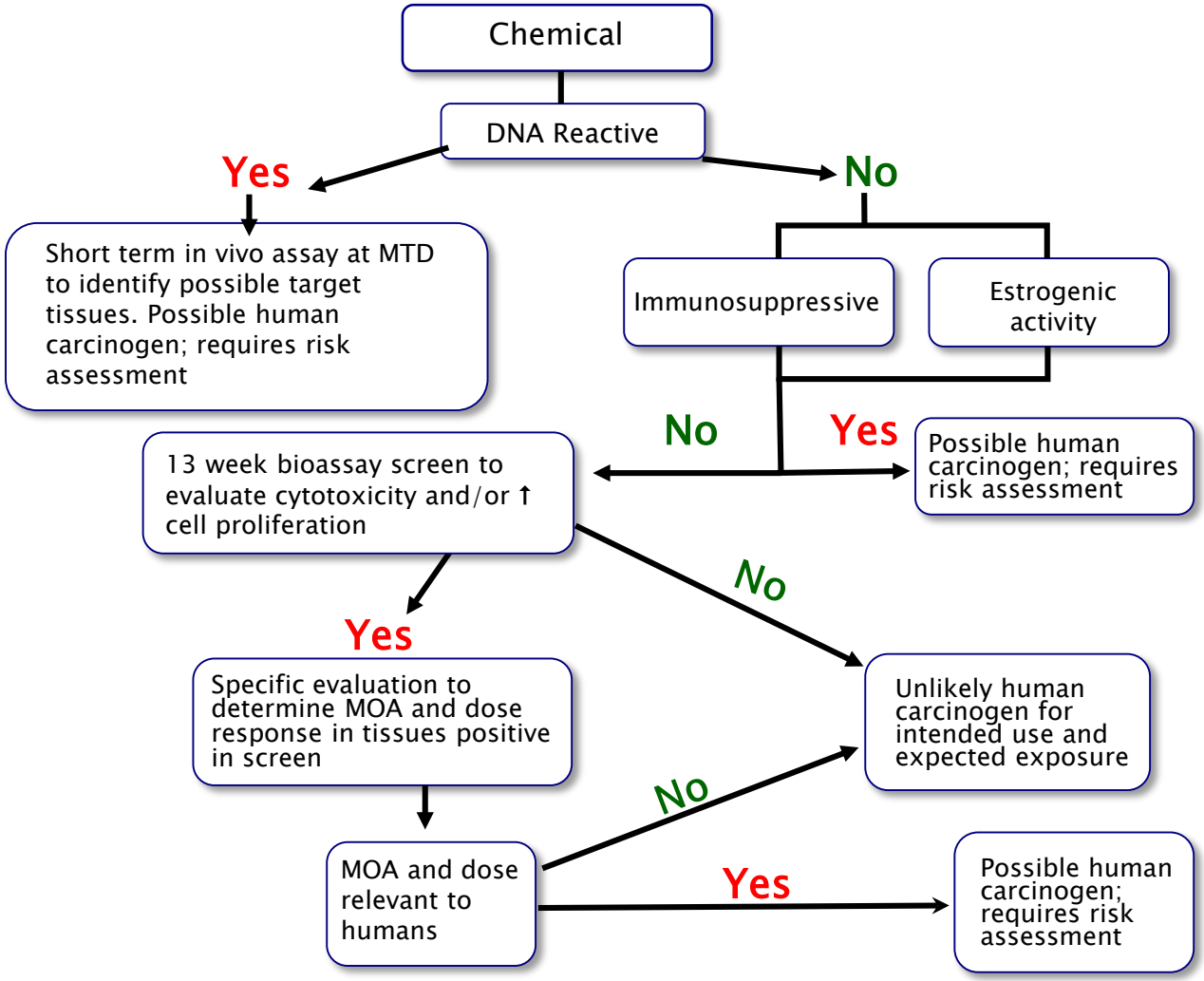
Overall Detailed 1, 4 & 13- Week screening Bioassays

- Organ Weights
- Histologic Evidence of Toxicity and/or Proliferation
- Blood and Urine Chemistries
- DNA Labeling Indices
- Specialized Studies
  - Immunohistochemistry
  - Omics?

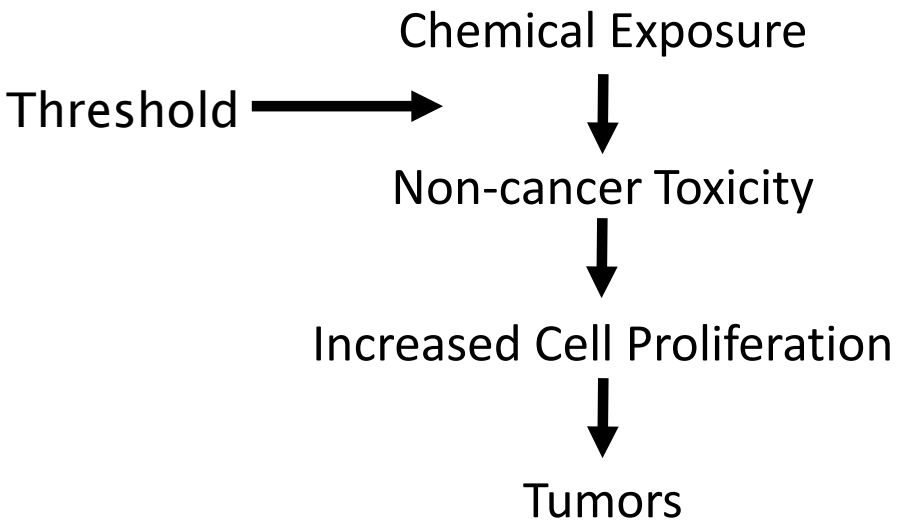
Rodent Tumors Not Relevant to Humans

- Rodent organs without human counterpart
  - Zymbal's gland
  - Harderian gland
  - Forestomach
- Rodent tumors without human analog
  - Splenic mononuclear cell leukemia (rat)
  - Mouse submucosal mesenchymal lesion of bladder (seminal vesicles, uterus)
- Tumors not relevant to humans
  - Rat pancreas
  - Mouse lymphoma
  - Mouse lung?
  - Mouse liver?
- Endocrine organs
  - Thyroid
  - Adrenal cortex
  - Adrenal medulla
  - Pituitary – anterior
  - Pituitary – posterior
  - Parathyroid
  - GI endocrine cells
  - Pancreatic islets
- Reproductive endocrine tumors
  - Ovary – granulosa cell
  - Testis – Leydig cell (? Mesothelioma)
  - Endometrium
  - Prostate
  - Rat mammary gland

Screening for Carcinogenesis



Non-Genotoxic Carcinogens



- **Protecting against non-cancer toxicity will protect for cancer risk**
- For non-DNA reactive carcinogens, default assumption should be threshold effect

Non-Genotoxic Carcinogen Risk Assessment

- Involves threshold
- Protection against non-cancer toxicity will protect against cancer
- To implement change from 2-year bioassay requires change in laws/guidelines

It’s Time to Stop Doing 2- Year Rodent Bioassays