The Two-Year Bioassay is No Longer Necessary for Carcinogenicity Screening

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CropLife America and RISE Regulatory Conference
Arlington, VA
April 5, 2019
Declaration of Interests

Member, EPA Science Advisory Board
Member, FEMA Expert Panel for flavoring ingredients
HESI Editor-in-Chief
Funding from: Texas Commission on Environmental Quality
  NIH
  Sumitomo Chemical Co.
  Lyondell Basell
  Arsenic Science Task Force
  Grocery Manufacturers Assoc. (contract with Michigan State Univ.)
Consult for numerous companies
Outline

• Two-year bioassay
• Basic concepts of carcinogenesis
• Mode of action-based approach to carcinogenicity testing
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
• Increase number of cell divisions
Increasing Cell Proliferation

• Increase cell births
  – Direct mitogenesis (hormones, growth factors)
  – Toxicity and regeneration

• Decrease cell deaths
  – Inhibit apoptosis
  – Inhibit differentiation
<table>
<thead>
<tr>
<th>DIRECT MITOGENICITY</th>
<th>CYTOTOXICITY &amp; REGENERATION</th>
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</thead>
<tbody>
<tr>
<td>CAR, PXR activation</td>
<td>Urinary solids</td>
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<tr>
<td>PPARα activation</td>
<td>Hepatocellular necrosis</td>
</tr>
<tr>
<td>AhR activation</td>
<td>Kidney necrosis</td>
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<tr>
<td>T4, T3 metabolism → TSH stimulation</td>
<td>α₂u-globulin → toxicity</td>
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<tr>
<td>Cholecystokinin activation</td>
<td>Chronic progressive nephropathy</td>
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<tr>
<td>Proton pump inhibition → Gastrin activation</td>
<td>Forestomach irritation</td>
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<tr>
<td>Prolactin activation</td>
<td>Cytotoxic urinary metabolites</td>
</tr>
<tr>
<td>Estrogen increase</td>
<td>Iron accumulation</td>
</tr>
<tr>
<td>LH stimulation</td>
<td>Tubular apoptosis</td>
</tr>
<tr>
<td>Club cell mitogenesis</td>
<td>Bronchoalveolar necrosis</td>
</tr>
</tbody>
</table>

- liver
- thyroid
- rat pancreas acinar cell
- gastric carcinoid
- rat mammary
- mammary
- rat testicular Leydig cell
- mouse lung
- bladder
- liver
- kidney
- rat kidney
- rat kidney
- forestomach
- bladder
- liver
- kidney
- lung
Basic assumptions of animal 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).

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 the 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
How To Do Risk Assessment
Known Modes of Action for Human Carcinogenesis

- DNA reactivity
- Increased cell proliferation
- Immunosuppression
- Estrogenic activity
Detailed 1, 4 & 13-Week Bioassays

- Organ Weights
- Histologic Evidence of Toxicity and/or Proliferation
- Blood and Urine Chemistries
- DNA Labeling Indices
- Specialized Studies
  - Colon Roll – Aberrant Crypt Foci
  - Immunohistochemistry
  - Omics
Rodent Tumors Not Relevant to Humans

• Rodent organs without human counterpart
  – Zymbal’s gland
  – Harderian gland
  – Foreostomach

• Rodent tumors without human analog
  – Spenic mononuclear cell leukemia
  – Mouse submucosal mesenchymal lesion of bladder (seminal vesicles, uterus)

• Mouse lung, liver, vascular tumors?

• 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
Screening and Evaluation for Urinary Bladder Carcinogenicity

Cohen, 2018
Screening and Evaluation for Carcinogenicity

Fig. 1. Overview of suggested carcinogenicity assessment process.

Cohen et al., 2019
Follow-Up Detailed Studies

• Dose Response (Expand Number of Doses)
• Metabolism – Non-linearities?
• Toxicokinetics
• Mechanism of Action (Mode)
Non-cancer & Cancer Endpoints

• For non-genotoxic chemicals, precursor proliferative toxicity leads to cancer.

• If precursor lesion does not occur, cancer will not occur (threshold).

• Setting safety margin for non-cancer toxicity will also be protective of cancer.
Evaluation of Carcinogenic Activity

- Screen for genotoxicity (DNA reactivity), immunosuppression, estrogenic activity.
- For non-DNA reactive chemicals, screen for precursor proliferative lesions.
- Evaluate mode of action/human relevance and dose response relative to human exposures.
- For non-DNA reactive chemicals, set margin of exposure for cancer based on non-cancer endpoints (NOAEL/100 or BMD evaluation).
It’s Time to Stop Doing 2-Year Rodent Bioassays