Mode of Action/Human Relevance Framework: A Hypothesis-Based Weight of Evidence Approach

Vicki L. Dellarco, Ph.D.
Office of Pesticide Programs
U.S. Environmental Protection Agency

This talk represents the view of presenter & does not necessarily represent the decisions or stated policies of the EPA.
Topics

- Tumor profiles in rodents (pesticide database) & humans
- Approach to evaluate the human relevance of an animal mode of action for tumors (and noncancer endpoints)
- Case Example
Mouse Tumor Distribution

Rat Tumor Distribution

219 Pesticides
Value of Mode of Action Data

Data Before Defaults

Hazard Characterization
- Animal endpoints plausible in humans?

Dose Response Analyses
- Shape of the dose response curve?

Quantitative Relevance to Humans
- Toxicological equivalency of exposures to humans?

Exposure Estimation

Risk Characterization
- Hazard X Exposure = Risk

Uncertainty Characterization
How Do You Determine the Weight of Evidence (WoE) for Establishing a Mode of Action (MoA)?

- Needs to be based around specific hypothesis against data

**MoA/Human Relevance Framework**

- Based on Bradford Hill criteria for causality
- Distinguished MoA vs. Mechanism of Action
Approach: MoA/Human Relevance Framework

History


- ILSI 2003
  - Framework for human relevance analysis of information on carcinogenic modes of action

- ILSI 2005
  - Extends Framework to noncancer outcomes & life stage information

- IPCS 2006 & 2008
  - Adopts Human Relevance Framework

MoA = Plausible hypothesis with measured key events (vs detailed molecular description of causality)

Key Event: Critical, Rate Limiting, Quantifiable
Reasons for MoA/Human Relevance Framework

- Provides Transparency
  - Clarifies extent of WoE as a basis for decision making
- Ensures rigor of evaluations & consistency of documentation
- Aids in Identification of Critical Data or Research Needs
  - Basis for iterative dialogue between risk assessors & researchers
- Sufficiency of Evidence
  - Requires expert judgment, peer engagement & review
Q1. Is the weight of evidence sufficient to establish the MoA in animals?

- Yes
- No

Q2. Are the key events in the Animal MoA plausible in humans?

- No
- Yes

Q. Taking into account kinetic & dynamic factors, are key events in Animal MoA plausible in humans?

- No
- Yes

Animal-Human Comparability Indicates Human Relevance

MoA Relevant: Continue with Risk Assessment

Data Insufficient to Characterize MoA

MoA Not Relevant to Humans: No Need to Continue Risk Assessment

MoA Unlikely Due to Quantitative Differences

Specific to Test Species
Question 1: Sufficient Weight of Evidence to Establish MoA in Animals?

- Postulated MoA (theory of the case)
  - Other possible MoAs

- Experimental support for key events
  - Concordance of dose-response relationships
  - Temporal association
  - Strength, consistency and specificity of association of toxicological effect with key events
  - Biological plausibility and coherence

- Uncertainties, inconsistencies, & data gaps
Q1. Is the WoE for MoA in Animals Sufficient?

- Begins with Formulating a Hypothesis
  - Sequence of “Key Events” described
  - Other MOAs?

Critical components (key events) of a toxicity pathway
Other metabolites? DNA reactivity?
### Example of an MoA: It’s an Iterative Process

#### Cacodylic Acid & Induced Bladder Cell Tumors in Rats

<table>
<thead>
<tr>
<th>Possible MOA</th>
<th>Evidence</th>
<th>Initial Conclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mutagenic</strong></td>
<td>Numerous studies do not support direct DNA reactivity</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>[but suggestion of indirect mechanism via ROS - oxidative damage - mostly in vitro]</td>
<td>Possible</td>
</tr>
<tr>
<td><strong>Mitogenic</strong></td>
<td>Studies do not support</td>
<td>No</td>
</tr>
<tr>
<td><strong>Cell injury &amp; regenerative proliferation</strong></td>
<td>Multiple studies DMA(^V\rightarrow DMA^{III})</td>
<td>Possible</td>
</tr>
</tbody>
</table>
Cacodylic Acid: Mode of Action

Measurable Key Events in Target Tissue

DMA\textsuperscript{III} Metabolite

Urothelial Toxicity

Regenerative Proliferation

Hyperplasia

ROS
DNA Damage

Stable Chromosome Aberrations

Sustained

BrdU Labeling

Urinary Bladder Tumors
Q1. Is the WoE for MoA in Animals Sufficient?

- **Experimental Support**
  - Key events characterized & measured in the species/tissue of interest
  - Dose response relationships for key events are compared with one another & with those for adverse outcome
    - key events always observed at doses below or similar to those associated with the adverse outcome
  - Temporal Association
    - Key events & adverse outcome occur in expected order
## Cacodylic Acid: Association of Key Events & Rat Bladder Tumors

### Temporal

<table>
<thead>
<tr>
<th>Dose</th>
<th>Metabolism DMA(^V) ⇄ DMA(^{III})</th>
<th>Urothelial Toxicity</th>
<th>Regenerative Proliferation</th>
<th>Hyperplasia</th>
<th>Tumors</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 ppm</td>
<td>+</td>
<td>slight</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>10 ppm</td>
<td>+</td>
<td>+</td>
<td>slight</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>40 ppm</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>100 ppm</td>
<td>+++</td>
<td>+++</td>
<td>++</td>
<td>++</td>
<td>+</td>
</tr>
</tbody>
</table>
Q1. Is the WoE for the MoA in Animals Sufficient? (Cont’d)

**Strength, Consistency & Specificity**

- Evidence linking key events & outcome
  - Consistency of associations found in repeated experiments within a lab & among different labs
  - Inhibition of DMA\textsuperscript{V} \(\Rightarrow\) DMA\textsuperscript{III} reduced cytotoxicity
  - Cessation of exposure results in recovery of tissue

**Biological Plausibility & Coherence**

- Hypothesized MoA make sense based on broader knowledge & in relation to what is also known for the substance specifically
  - Regenerative proliferation associated with persistent toxicity appears to be a risk factor for bladder cancer in humans
Q1. Is the weight of evidence sufficient to establish the MoA in animals?

Yes \(\rightarrow\) MoA Relevant: Continue with Risk Assessment

No \(\rightarrow\) MoA Not Relevant to Humans: No Need to Continue Risk Assessment

Q2. Are the key events in the Animal MoA plausible in humans?

Yes \(\rightarrow\) Yes

No \(\rightarrow\) No

Q. Taking into account Kinetic & dynamic factors, are key events in Animal MoA plausible in humans?

No \(\rightarrow\) MoA Unlikely Due to Quantitative Differences

Yes \(\rightarrow\) Animal-Human Comparability Indicates Human Relevance

Specific to Test Species
## Q2: Comparative Analysis of Key Events

<table>
<thead>
<tr>
<th>Key Event</th>
<th>Rat</th>
<th>Human</th>
</tr>
</thead>
<tbody>
<tr>
<td>Presence of DMA(^{III}) in urine</td>
<td>Yes, Data</td>
<td>Limited evidence indicates significantly less</td>
</tr>
<tr>
<td>Persistent urothelial cytotoxicity</td>
<td>Yes, Data</td>
<td>Unknown: Potential if sufficient DMA(^{III}) is produced</td>
</tr>
<tr>
<td>Sustained regenerative prolif/hyperplasia</td>
<td>Yes, Data</td>
<td>Unknown: Potential if sufficient cell killing is produced &amp; sustained</td>
</tr>
<tr>
<td>Bladder Tumors</td>
<td>Yes, Data</td>
<td>No epidemiologic data, but Potential Assumed</td>
</tr>
</tbody>
</table>
Cytotoxicity – Cacodylic Acid

Implications for Risk Assessment

✓ MoA plausible in humans
✓ Nonlinear Dose Response

Must be sufficient DMA$^{III}$ to produce sufficient cell killing to lead to regenerative proliferation

Cytotoxicity & enhanced proliferation need to be sustained

Frequency of mutations dependent on enhanced proliferation & possibly on generation of ROS

✓ Sustained exposure required
In Summary

Reasons for MoA/Human Relevance Framework Provides

- Transparent Consideration of Weight of Evidence Basis for MoA
- Promotes Use of All Relevant Data
- Defines the “Key Events” Relevant to Risk Assessment
- Delineates Types of Data that are Preferred over Defaults
- Aids in Identification of Critical Data or Research Needs
For more information: Mode of Action & Human Relevance Framework

- **ILSI Website**
  
  http://www.ilsi.org
  
  

- **IPCS Harmonization Website**
  
  http://www.who.int/ipcs/methods/harmonization/index.html
  
  
  
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