Use of Mode of Action in Human Health Risk Assessment

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

MoA Analyses by Office of Pesticide Programs

- Chloro-s-triazines (neuroendocrine disruption & developmental/reproductive effects & mammary gland tumors)
- Conazole fungicides (CAR activation & liver tumors)
- Diphenyl ethers (PPARα agonism & liver tumors)
- Dicarboximide fungicides (inhibition of androgen receptor & reproductive malformations/leydig cell tumors)
- Organophosphorus pesticides (cholinesterase inhibition & cholinergic toxicity)
- Chloroacetanilide herbicides (cytotoxicity/regenerative proliferation & nasal tumors)
- Different classes (disruption of Thyroid hormone homeostasis & neurodevelopmental effects & follicular cell tumors)
- AND SO FORTH . . . . . . . . . . . . . .
How Do We Assess Human Health Risks?

NAS 4-Step Paradigm

- Hazard Assessment & Characterization
- Exposure Assessment & Characterization
- Risk Assessment & Characterization
- Dose Response Assessment & Characterization

FQPA 10 X Safety Factor, Aggregate Exposure & Cumulative Risk

How Do We Assess Human Health Risks?

- Relies heavily on laboratory animal data
- Relies on extrapolations, inference methods, safety factors, etc
  - Animal Biology = Human Biology
  - Effects found at high animal doses predict effects at environmental levels of exposure
  - Current animal assays provide adequate coverage for predicting effects on human health including susceptible groups (?)
Mode of Action (MoA) Data in Risk Assessment

**Inform**
- Human Relevance
- Dose Response
- Susceptibilities

**Identify Key Biological Events**
- Toxicity Pathways

**Understand**
- Chemical Mixture
- or Cumulative Risk

**Promote**
- Harmonized
- Approach For All
- Health Endpoints

MoA/Human Relevance Framework

- **History**
  - USEPA '96 draft Cancer Guidelines
    - Defines MoA & contrasts with mechanism
  - USEPA '99 draft Cancer Guidelines
    - Introduces Framework
      - Bradford Hill criteria for causality for weight of evidence for MoA

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

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

History continued
- ILSI RSI develops human relevance (HR) component & case studies for cancer and noncancer outcomes
  - *Critical Reviews in Toxicology* (Meek et al., 2003; Cohen et al., 2004; Seed et al., 2005)
- IPCS refines & adds case studies
  - *Critical Reviews in Toxicology* (Boobis et al., 2006; Boobis et al., 2008)

Human Relevance Framework for Analyzing MoA

- Provides Transparency
  - clarifies extent of weight of evidence as a basis for decision making
  - not prescriptive but designed to organize information
- Ensures Rigor of Evaluations & Consistency of Documentation
- Aids in Identification of Critical Data or Research Needs
  - impact on conclusions
  - basis for iterative dialogue between risk assessors/researchers

Why?
Is the WoE Sufficient to establish a MoA in animals?

MoA Not Relevant

Are the key events in the animal MoA (qualitatively) plausible in humans?

Comparability analysis of "Key Events" & relevant biology between animals & humans

MoA Not Relevant

Taking into account Kinetic & dynamic factors, is the animal MoA plausible in humans?

Proceed with Risk Assessment

MoA: " Sufficiency of Evidence"

Interdisciplinary Teams

Critical Analysis → How much is enough?

Peer Engagement

* Burden of proof is reduced for well established MoAs
Q2. & 3. The Concordance Analysis

<table>
<thead>
<tr>
<th>Key Event</th>
<th>Animals</th>
<th>Humans</th>
<th>Strength</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat mode of action for mammary gland tumors not expected to occur in humans but mode of action should be assumed relevant for effects on reproductive development (FIFRA Science Advisory Panel, 2000)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
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Atrazine as an Example
MoA/Human Relevance Framework

**MoA/Human Relevance Framework: Q1. Is the WoE Sufficient to Establish MoA in Animals?**

**Bradford Hill Criteria**

- Postulated mode of action
  - Identify sequence of key events on the path to cancer
- Experimental support
  - Concordance of dose-response for key events with that for tumors
  - Temporal relationships for key events & tumors
- Biological plausibility & Coherence
- Strength, consistency & specificity
- Other modes of action
- Identify uncertainties
- Conclusion
Postulated MoA: Consider Normal Biological Process

- Reproductive Aging (prolong estrus) in SD female Rats plays role in high mammary gland tumor incidence
- Establishes a hormonal environment conducive to tumor development
  - prolonged exposure to endogenous estrogens

MoA/Human Relevance Framework: Q1. Weight of Evidence Sufficient to Establish MoA?

- Postulated MoA - Description of Key Events

  ↓ Latency/ ↑ Incidence
  Mammary Gland Tumors

  ↑ Exposure to estrogens
  Anovulation (extended estrus)
  ↓ Pituitary LH Surge
  ↓ GnRH

Atrazine Alters Hypothalamic Control of Pituitary-Ovarian Function
Human Relevance Framework: Q 1. Weight of Evidence Sufficient to Establish MoA

- Experimental Support
  - Dose-Response Relationship
    - Concordance of ↓LH, extended estrus,
      ↑latency/incidence of mammary tumors
  - Temporal Relationship
    - ↓LH, disrupted cyclicity precede tumors

<table>
<thead>
<tr>
<th>Effect</th>
<th>LOAEL mg/kg/d</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>↓LH (6 mo.)</td>
<td>3.65</td>
<td>Morseth, 1996</td>
</tr>
<tr>
<td>↑Days in Estrus (6 mo.)</td>
<td>3.65</td>
<td>Morseth, 1996</td>
</tr>
<tr>
<td>↓Latency in Mammary Tumors (12 mo.)</td>
<td>3.79</td>
<td>Thakur, 1997</td>
</tr>
<tr>
<td>↑Incidence in Mammary Tumors (24 mo.)</td>
<td>3.5</td>
<td>Mayhew, 1986</td>
</tr>
</tbody>
</table>

- Strength, Consistency, Specificity of Association
  - Consistent evidence from many studies
    - Strong correlation between dose & effect (↓LH, estrous cycle disruption, mammary gland tumors)
    - ATZ-treated OVX females do not develop mammary tumors, ectopic pituitary & GnRH experiments support hypothalamus as a primary site
  - Biological Plausibility & Coherence
    - Substantial literature on role of estrogen in pathogenesis of mammary tumors in rats
      - Morphological changes indicative of estrogen stimulation
    - Other LH-dependent events affected
      - pregnancy loss, delayed puberty
  - Assessment of Uncertainties
    - LH data variable, acute studies for GnRH levels, limited data on estrogen levels
    - Do not discount postulated neuroendocrine MoA
**Other MoAs**
- Not a DNA reactive mutagenic
- No intrinsic estrogen potential
- Induction of aromatase activity but only in vitro
  - No support in vivo (rat)
- Disruption of adrenal steroidogenesis in rats
  - Higher doses than those required to ↓ LH

**MoA/Human Relevance Framework**

1. Is the WoE Sufficient to establish a MoA in animals? **No** → Proceed with Risk Assessment
   **Yes**
   - Are the key events in the animal MoA (qualitatively) plausible in humans? **No** → MoA Not Relevant
     **Yes**
     - Taking into account kinetic & dynamic factors, is the animal MoA plausible in humans? **No** → MoA Not Relevant
       **Yes** → Proceed with Risk Assessment
### Q2. Qualitative Concordance Analysis

<table>
<thead>
<tr>
<th>Key Event</th>
<th>Rat (SD)</th>
<th>Humans</th>
</tr>
</thead>
<tbody>
<tr>
<td>↓ GnRH pulses</td>
<td>Yes, Ectopic Pituitary &amp; GnRH expts; GnRH restores LH in atrazine-blocked rats</td>
<td>Unknown, possible</td>
</tr>
<tr>
<td>↓ of LH surge</td>
<td>Yes, several expts in rats at different dose/durations</td>
<td>Unknown, possible</td>
</tr>
<tr>
<td>↑ % days in estrus</td>
<td>Yes, several expts in rats at different dose/durations</td>
<td>Unknown, possible</td>
</tr>
<tr>
<td>↑ exposure to estrogen</td>
<td>Yes, questionable data</td>
<td>Unknown, NOT plausible</td>
</tr>
<tr>
<td>Mammary Gland Tumors</td>
<td>Yes, ↑ incidence &amp; early onset</td>
<td>Not plausible, no convincing evidence that atrazine results in cancer in humans</td>
</tr>
</tbody>
</table>

### MoA/Human Relevance Framework

1. **Is the WoE Sufficient to establish a MoA in animals?**
   - Yes
   - No: Proceed with Risk Assessment

2. **Are the key events in the animal MoA (qualitatively) plausible in humans?**
   - Yes
   - No: Developmental effects should be assumed

3. **Taking into account kinetic & dynamic factors, is the animal MoA plausible in humans?**
   - Yes
   - No: Proceed with Risk Assessment
MoA: Implications for Dose Response

Consider “Key Events” to protect human health from neuroendocrine effects of Atrazine

PoD Based on LH Suppression

PoD = Point of Departure
RfD = Reference Dose
MoE = Margin of Exposure

Cumulative Risk of Compounds that Share a Common Mechanism of Toxicity (FQPA 96’)

Pesticide Inventory

Triazines: Reproductive & developmental effects based on GnRH & LH suppression
Contribution of MoA Human Relevance Framework

- Transparent Consideration of Weight of Evidence Basis for MoA
- Promotes Use of All Relevant (including non chemical specific) Data
- Considers of Lifestage Responses
- MoA Defines the “Key Events” (Critical Endpoints) Relevant to Risk Assessment
  - dose response extrapolation method & point of departure
- Concordance Tables Help Identify Kinetic & Dynamic Differences Between Species (interspecies scaling)
- Delineates Types of Data that are Preferred over Defaults

Next Steps

- Paradigm Change
  - Reinforced by NRC, 2007 (Toxicity Testing in the 21st Century)
  - Move toward hypothesis/tiered approaches
    - Use knowledge of toxicity pathways
    - Use computational approaches, genomics, HTP, in vitro technologies for tailoring in vivo testing
    - In vivo testing focuses on relevant endpoints, mode of action & kinetics/metabolism