Strategic Adverse Outcome Pathway Analysis to Inform Human Health Risk Assessment: An Example with Inorganic Arsenic

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Disclaimer: The views expressed are those of the authors and do not necessarily represent the views or policies of the U.S. EPA
Context: Inorganic arsenic (iAs)

- Ubiquity of arsenic
  - Multiple organizations, agencies

- Potential exposures
  - Water, food, juice
  - Susceptible populations

- Health effects*

**Cancer**
- Lung†
- Skin†
- Bladder†
- Prostate
- Pancreatic

**Noncancer**
- Renal
- Liver
- Ischemic heart disease
- Skin lesions
- Diabetes
- Nonmalignant respiratory disease
- Pregnancy outcomes

* NRC 2013, †IARC 2012
Context: Risk Assessment & Management of iAs

- Risk management
  - Guidance, restrict, label food products
  - Drinking water limits

- Risk assessment
  - FDA draft: Apple juice
  - EPA: Integrated Risk Information System
    - Hazard identification and dose-response
    - Stakeholder and partner recommendations
    - National Research Council recommendations
Approach: AOP analysis in iAs IRIS Assessment

1. Provide problem formulation statement (Develop Populations, Exposures, Comparators, Outcomes [PECO] statement for AOP analysis)

2. Tabulate adverse outcome data (supporting & conflicting)

3. Provide pharmacokinetic data for each adverse outcome & its precursors (exposure & temporal ranges)

4. List modes of action for each adverse outcome (link pharmacokinetic & pharmacodynamic data to adverse outcome in exposure & temporal manner)

5. Construct concordance table (strengths, weakness of each MOA by species, population, subpopulation)

NRC, 2013

“The mode-of-action framework (Boobis et al. 2006, 2008; Carmichael et al. 2011) in conjunction with the human-relevance framework (Meek et al. 2003) provides a transparent method of organizing information for hazard identification and risk assessment that includes exposure information, dose–response information, a clear conclusion, identified data gaps, and potentially susceptible populations.”
Approach: AOP analysis in iAs IRIS Assessment

- **Ideal world**: established AOPs for iAs-associated health effects

- **Reality**: many hypothesized mechanisms of action for iAs-associated health effects

- **Solutions**:
  - systematic review of mechanistic data per NRC guidance (short-term)
  - scientific community (research & regulatory) → develop & validate AOPs (long-term)
Adverse Outcome Pathway analysis: PECO statement development

Goal: Clearly define what AOP analysis can inform & how data would influence assessment

AOP Analysis Key Question: For dose-response analysis of an iAs health effect in humans (including susceptible populations), do mechanistic data increase or decrease confidence in the: 1) response metric selection, 2) dose-metric selection, or 3) model selection (e.g., linear low-dose, high-dose plateau), or 4) human variability?

Draft Dose-Response Confidence Evaluation:

Confidence in Dose-Response Analysis

Initial Confidence based upon Key Features of Analyses

| HIGH (++++) | MODERATE (+++) | LOW (++) | VERY LOW (+) |

Mechanistic Data Factors: ↑/↓ Confidence

- Biological concordance
- Key Event Essentiality
- Temporal concordance
- Dose-response concordance
- Consistency

Confidence in Dose-Response Analysis with Mechanistic Data

| HIGH (++++) | MODERATE (+++) | LOW (++) | VERY LOW (+) |

Adverse Outcome Pathway analysis: PECO statement development

**iAs-induced bladder cancer example**

- **Example PECO Question:** Studies in human populations show mixed results when examining the relationship between low level (<10 ug/L) iAs exposures and bladder cancer in smokers. Do mechanistic data ↑/↓ confidence that iAs interacts with other causes of bladder cancer (e.g., smoking) in different populations (e.g., selection of additive vs. relative risk models)?

**Example analysis of iAs bladder cancer risk in ever smokers:**

<table>
<thead>
<tr>
<th>Study Name</th>
<th>Subgroup within Study</th>
<th>Relative Risk and 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bates et al. (1995)</td>
<td>19–33 mg cumulative</td>
<td></td>
</tr>
<tr>
<td></td>
<td>33–53 mg</td>
<td></td>
</tr>
<tr>
<td></td>
<td>≥53 mg</td>
<td></td>
</tr>
<tr>
<td>Steinmaus et al. (2003)</td>
<td>6.4–82.8 mg cumulative</td>
<td></td>
</tr>
<tr>
<td></td>
<td>&gt;82.8 mg</td>
<td></td>
</tr>
<tr>
<td>Karagas et al. (2004)</td>
<td>0.060–0.086 µg/g toenail</td>
<td></td>
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<tr>
<td></td>
<td>0.087–0.126 µg/g</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.127–0.193 µg/g</td>
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<tr>
<td></td>
<td>0.194–0.277 µg/g</td>
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<tr>
<td></td>
<td>0.278–0.330 µg/g</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.331–2.484 µg/g</td>
<td></td>
</tr>
<tr>
<td>Melliker et al. (2010)</td>
<td>1–10 µg/L</td>
<td></td>
</tr>
<tr>
<td></td>
<td>&gt;10 µg/L</td>
<td></td>
</tr>
<tr>
<td>Bates et al. (2004)</td>
<td>1.1–17 µg/L</td>
<td></td>
</tr>
<tr>
<td></td>
<td>18–80 µg/L</td>
<td></td>
</tr>
<tr>
<td></td>
<td>&gt;80 µg/L</td>
<td></td>
</tr>
<tr>
<td>Michaud et al. (2004)</td>
<td>0.05–0.105 µg/g toenail</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.106–0.161 µg/g</td>
<td></td>
</tr>
<tr>
<td></td>
<td>&gt;0.161 µg/L</td>
<td></td>
</tr>
<tr>
<td>Kurttio et al. (1999)</td>
<td>0.1–0.5 µg/L</td>
<td></td>
</tr>
<tr>
<td></td>
<td>≥5 µg/L</td>
<td></td>
</tr>
</tbody>
</table>

**SRRE = 1.18 (95% CI: 0.97–1.44)**

P-Heterogeneity = 0.034

Tsuji et al. 2014.
AOP Analysis: PECO statement development
(Literature Review)

1. Filters
   - PubMed: None
   - Toxline, TSCATS, & DART: Not PubMed
   - Web of Science: Research Areas

2. Computerized Keyword Search & Screen
   - PubMed: 21,489 Articles
   - Toxline, TSCATS, & DART: 13,340 Articles
   - Web of Science: 18,210 Articles

3. Identified from other sources: +53
   - 2014 June lit searches: +166

4. Identified from health effect clusters as relevant MOA
   - 379 MOA Seeds: +387

5. Identified from MOA clusters as relevant MOA
   - 5,105 Articles

6. 2014 June lit searches
   - 2,537 Articles
     - Health Effects
   - 2,227 Articles
     - Susceptibility

7. 1,745 Articles
   - Mechanistic categories

8. Articles specific to health effect, MOA, susceptibility factor(s)
   - (e.g., bladder cancer, cytotoxicity & regenerative proliferation, smoking)

* MOA studies identified using references included in MOA sections of recent draft IRIS Toxicological Reviews
** Key words used to categorize studies based on title and abstract
Adverse Outcome Pathway analysis: Data visualization—Hypothesized Key Events & Modifying Factors

**iAs-induced bladder cancer example**

**Molecular Initiating Event**

- **Active iAs metabolites**
  - iAs(III), MMA(III), thiolated species

**Multiple Sulfhydryl Protein Targets**

Examples:
- Tubulin, thioredoxin, DNA repair proteins (PARP-1)

**Multiple Possible Biochemical**

Examples:
- DNA damage, reactive oxygen species generation

**Cellular Response**

- Cytotoxicity, Regenerative Proliferation

**Apical Outcomes**

- **Individual Response**
  - Tumor formation
- **Population Response**
  - Bladder Cancer

- **Potential Modifying Factor**
  - Nutrition
  - Sex
  - Genetic polymorphisms
  - Life stage (children)
  - Co-exposure (cadmium)
  - Co-exposure (alcohol)
  - Life stage (in utero)
  - Smoking
  - Co-exposure (alcohol)

- One of several hypothesized AOPs
- Not necessarily simple progression of Key Events
  - How to consider confidence in modifying factors at relevant exposure levels?
  - How to consider confidence in interactions (or lack thereof) between modifying factors?

Simon et al, 2014; See reference list for iAs references
Adverse Outcome Pathway analysis: PECO statement development

**iAs-induced developmental neurotoxicity example**

- **Example PECO Question:** Human and animal data suggest that early life exposures to iAs may result in developmental neurotoxicity, yet measures of exposure and response are not consistent between animal and human studies. Do mechanistic data ↑/↓ confidence in selection of IQ measures as a response metric for developmental neurotoxicity (i.e., response metric selection)?

![Graph showing latency to novel object between control and arsenic exposure.](image)
Adverse Outcome Pathway analysis in iAs IRIS Assessment: Challenges for Discussion

• Developing an AOP vs. querying an AOP for relevance to assessment of a particular chemical
  – Pursuit of data for scientific vs. assessment interests
  – Confidence in data to “validate” an AOP vs. inform an assessment
  – Methods to determine confidence in key events, modifying factors, AOP, chemical-specific data relevant to KE, MFs, AOP
  – Communication tools: process and outcomes of confidence determination

• Systematic review of mechanistic literature
  – Generally larger body of studies compared to health effects
  – Terminology varies in literature
  – Lack of methods for study quality review
    • Internal validity, external validity, risk of basis
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  – Lauren Joca
  – Ryan Jones
  – Connie Meacham
  – Reeder Sams

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  – Audrey Turley
References (continued)

• Tokar et al., Carcinogenic effects of “whole-life” exposure to inorganic arsenic in CD1 mice. Toxicol Sci (2011) 1: 73083.

Other relevant references: