THE SYSTEMATIC REVIEW OF MECHANISTIC DATA IN IRIS ASSESSMENTS

Catherine Gibbons, Ph.D.
U.S. EPA

The views expressed in this presentation are those of the author and do not necessarily reflect the views or policies of the U.S. Environmental Protection Agency
• Created in 1985 to foster consistency in the evaluation of chemical toxicity across the Agency

• IRIS assessments contribute to decisions across EPA and other health agencies

• Publishes toxicological information and produces toxicity values
  – Non-cancer: Reference Doses (RfDs) and Reference Concentrations (RfCs)
  – Cancer: Oral Slope Factors (OSFs) and Inhalation Unit Risks (IURs)
Systematic Review Documents

IRIS Handbook: Standard operating procedures for IRIS staff and contractors

Assessment Initiated

Initial Problem Formulation

Assessment Plans (IAP): What the assessment will cover

Protocols: How the assessment will be conducted

IAPs and protocols are released for public comment
What is mechanistic evidence?

- Data from observational and experimental studies that inform biological or chemical events associated with toxic effects but are not generally considered to be adverse outcomes on their own
  - *In vivo* (cellular, biochemical, molecular)
  - *In vitro* or *ex vivo* (human or animal tissues or cells)
  - Non-animal or non-mammalian alternative animal models
  - Big data (‘omics or high-throughput assays) and in silico analyses
  - ADME, TK, physico-chemical properties
- Large, diverse databases
- “The history of science is replete with solid causal conclusions in advance of solid mechanistic understanding” (NAS, 2014)
- **We employ an iterative approach for the evaluation of mechanistic evidence**
Importance in IRIS assessments

- Identify **precursor events** for apical toxicity endpoints
- Inform **susceptibility** (species, strain, or sex differences; at-risk populations or lifestages)
- Inform **human relevance** of animal data (note: the level of analysis will vary depending on the impact of the animal evidence)
- Provide **biological plausibility** (i.e., to human or animal health effect data when evidence is weak or critical uncertainties are identified)
- Establish mechanistic relationships (or lack thereof) across sets of potentially related endpoints/outcomes to inform the consideration of **coherence** during evidence integration
- Aid **extrapolation** (high-to-low dose; short-to-long duration; route-to-route)
- Improve **dose-response** modeling and characterization of uncertainties
Evaluation of mechanistic information requires an iterative approach

To pragmatically incorporate these abundant and heterogenous data, an iterative approach identifies key questions at various stages of review

Focus the topics selected for analysis:

- **Scoping and Problem formulation:**
  - Seek stakeholder input that may narrow scope of assessment
  - Identify ADME/TK information and existing MOAs that may trigger specific analyses (e.g., possible mutagenic MOA)
  - Conduct preliminary literature survey (evidence mapping)
  - Develop assessment plan  →  **IAP public release and comment period**

- **Literature inventory:** Broad literature search and screening
  - Categorize studies by areas of mechanistic relevance (e.g., health effect, key characteristic)
  - Identify mechanistic signals unaddressed in apical human and animal studies
  - Develop refined evaluation plan  →  **Protocol public release and comment period**
Searching and screening literature

**Literature search strategy**

– Initial broad chemical-specific PECO-focused literature search designed to identify primary studies (i.e., original data sources of health effects)
  
  • PBPK models generally considered to meet PECO criteria

– Additional targeted literature searches may be conducted for mechanistic literature

**Literature screening and inventory tools**

– Efficiency enhanced by use of specialized systematic review software, including machine-learning approaches for screening
# Searching and screening literature

## PECO criteria

<table>
<thead>
<tr>
<th>PECO element</th>
<th>Evidence</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Populations</strong></td>
<td>Human: Any population and lifestyle (occupational or general population, including children and other sensitive populations). Animals: Nonhuman mammalian animal species (whole organism) of any lifecycle (including preconception, in utero, lactation, peripubertal, and adult stages).</td>
</tr>
<tr>
<td><strong>Exposures</strong></td>
<td>[Example language that can be included if appropriate.] Relevant forms: Chemical X [CAS number]. Other forms of [Chemical X] that readily disappear (e.g., list any salts, etc.). Metabolites of interest, including. Measures of metabolites used to estimate exposures to [Chemical X]. Studies of the effects of exposure to the metabolites themselves. Indicate whether mixture studies are included. Others determined by the assessment team. Human: Any exposure to [Chemical X] by [oral or inhalation] route(s) if applicable. Specify if certain exposure assessment methods or metrics will NOT be included. Animals: Any exposure to [Chemical X] by [oral or inhalation] route(s). Specify if certain exposures/study designs will NOT be included, or if a minimum number of dose or concentration levels tested in experimental animal studies is indicated. Studies involving exposures to mixtures will be included only if they include exposure to [Chemical X] alone. Other exposure routes, including [dermal or injection], will be tracked during title and abstract as “potentially relevant supplemental information.”</td>
</tr>
<tr>
<td><strong>Comparators</strong></td>
<td>Human: A comparison or referent population exposed to lower levels (or no exposure/exposure below detection limits) of [Chemical X], or exposure to chemical X for shorter periods of time. Case reports and case series will be tracked as “potentially relevant supplemental information.” Animals: A concurrent control group exposed to vehicle-only treatment or untreated control.</td>
</tr>
<tr>
<td><strong>Outcomes</strong></td>
<td>All health outcomes (both cancer and noncancer). (State here if decisions have been made to limit to endpoints related to clinical diagnostic criteria, disease outcomes, histopathological examination, or other aphel/phenotypic outcomes.) May include the following statement, “EPA anticipates that a systematic review for health effect categories other than those identified (i.e., health effect 1, health effect 2...) will not be undertaken unless a significant amount of new evidence is found upon review of references during the comprehensive literature search.”</td>
</tr>
<tr>
<td><strong>PBPK models [an additional criterion to address specific aims]</strong></td>
<td>Studies describing PBPK models for [Chemical X] will be included.</td>
</tr>
</tbody>
</table>

## Potentially Relevant Supplemental Material

<table>
<thead>
<tr>
<th>Category</th>
<th>Evidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mechanistic</td>
<td>Studies reporting measurements related to a health outcome that inform the biological or chemical events associated with phenotypic effects, in both mammalian and nonmammalian model systems, including in vitro, in vivo (by various routes of exposure), ex vivo, and in silico studies.</td>
</tr>
<tr>
<td>Nonmammalian model systems</td>
<td>Studies in nonmammalian model systems (e.g., fish, birds, Caenorhabditis elegans).</td>
</tr>
<tr>
<td>ADMET and toxicokinetic</td>
<td>Studies designed to capture information regarding absorption, distribution, metabolism, and excretion, including toxicokinetic studies. Such information may be helpful in updating or revising the parameters used in existing PBPK models.</td>
</tr>
<tr>
<td>Exposure characteristics</td>
<td>Exposure characteristic studies include data that are unrelated to toxicological endpoints, but which provide information on exposure sources or measurement properties of the environmental agent (e.g., demonstrate a biomarker of exposure).</td>
</tr>
<tr>
<td>Susceptible populations</td>
<td>Studies that identify potentially susceptible groups; for example, studies that focus on a specific demographic, lifestyle, or genotype.</td>
</tr>
<tr>
<td>Mixture studies</td>
<td>Mixture studies that are not considered to meet the PECO criteria because they do not contain an exposure or treatment group assessing only the chemical of interest.</td>
</tr>
<tr>
<td>Routes of exposure not pertinent to PECO</td>
<td>Studies using routes of exposure that fall outside the PECO scope.</td>
</tr>
<tr>
<td>Case studies or case series</td>
<td>In most cases, case reports and case series will be tracked as potentially relevant supplemental information.</td>
</tr>
<tr>
<td>Acute duration exposures</td>
<td>For assessments that focus on chronic exposure, shorter-term exposure durations (i.e., animal studies of less than 28 d) are generally considered supplemental.</td>
</tr>
<tr>
<td>Records with no original data</td>
<td>Records that do not contain original data, such as other agency assessments, informative scientific literature reviews, editorials, or commentaries.</td>
</tr>
<tr>
<td>Others determined by assessment team</td>
<td></td>
</tr>
</tbody>
</table>
Initial Categorization Approach

TIAB, second level TIAB, or full-text
- Based on considerations such as size of evidence base, content knowledge of TIAB screeners
Example of More Detailed Categorization

Typically a second level TIAB or full-text review to ensure engagement of content-specific experts

- KCCs shown here, but it could be any framework to help organize the mechanistic evidence

What characteristics of carcinogens apply? (detailed screening instructions available [here](#))

- [ ] genotoxic
- [ ] alters DNA repair or causes genomic instability
- [ ] electrophilic (or metabolized to electrophile)
- [ ] cell proliferation, cell death, cell nutrition
- [ ] oxidative stress
- [ ] receptor-mediated effects
- [ ] immunomodulation/immunosuppression
- [ ] epigenetic alterations
- [ ] immortalization
- [ ] induces chronic inflammation
- [ ] uncertain
Supplemental Material Categorization

• Tagging approach is pragmatic as it is not always possible to understand potential importance during initial screening

• Being categorized as supplemental material does NOT mean excluded. Studies tagged as supplemental may:
  – Become critical and possibly warrant individual study evaluation, (e.g., selected mutation studies when a mutagenic MOA is postulated)
  – Be a single study that contributes to a well-accepted scientific conclusion and does not need to be evaluated and summarized at the individual study level (e.g., dioxin as an aromatic hydrocarbon receptor (AhR) agonist)
  – Provide key references or context for preparation of certain chapters in an IRIS assessment (e.g., background information on sources, production or use; overview of toxicokinetics)

• It may also be possible to begin deprioritizing mechanistic studies during TIAB screening (e.g., studies using the chemical as a positive control)
Refine areas of focus for the assessment

- **Evidence synthesis and integration**: cross-walk with a detailed mechanistic literature inventory can prioritize impactful qualitative or quantitative analyses
  - Utility of precursor events or other information on biological plausibility when notable uncertainties exist for the available human or animal health effect data
  - Inform decisions related to susceptibility or human relevance of animal data (note: the latter depends on the potential impact of the animal evidence)
  - Evaluate mechanistic relationships across outcomes to inform coherence
  - Targeted evaluation of important data influencing dose-response modeling decisions within or across studies, or informed quantification of uncertainties
Current strategy: For each analysis, continue to narrow the scope to more relevant studies

- Prioritize studies on endpoints relating to the specific question by toxicologic relevance: for example, based on the model systems employed, dose range, or specificity of the assay for the mechanistic event(s) of interest

Tools for mechanistic study evaluations

- IRIS is exploring the use of existing tools
- Identify existing considerations for methods used to measure the selected endpoints

From a pragmatic perspective, evaluating every mechanistic study can be a significant resource issue, especially for large assessments with many studies

- When is individual study evaluation really needed, e.g., when unexplained inconsistency or variability observed?
Mechanistic Evidence Evaluation

B[a]P assessment

• Focused MOA: mutagenicity
• ADME identified key metabolites
• Focused endpoint: DNA-BPDE adduct formation
• Ranked methods of analysis for sensitivity and specificity
• Consistent results; risk of bias for individual studies not determined

### Table D-31. Select PAH-DNA adduct detection methods

<table>
<thead>
<tr>
<th>Adduct detection method</th>
<th>Adduct detection limit (nucleotides)</th>
<th>Quantitation</th>
<th>Adduct identification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Accelerator mass spectroscopy (AMS) (typically $^{14}$C or $^{3}$H); with or without separation</td>
<td>$10^{12}$</td>
<td>Highest sensitivity</td>
<td>High specificity due to radiolabeled chemical exposure (no structural information)</td>
</tr>
<tr>
<td>Dosing with radiolabeled compound (typically $^{14}$C or $^{3}$H) + quantification of radioactive DNA using liquid scintillation counting</td>
<td>$10^{9}$</td>
<td>High to moderate sensitivity (potential isotope artefacts may lower sensitivity)</td>
<td>Moderate specificity (additional characterization may be required)</td>
</tr>
<tr>
<td>$^{32}$P-postlabeling + separation by TLC or HPLC</td>
<td>$10^{9}$</td>
<td>High sensitivity</td>
<td>Low specificity (chemical nature of adducts unknown—additional characterization required)</td>
</tr>
<tr>
<td>Separation by chromatography (GC or LC) + mass spectrometry (MS)</td>
<td>$10^{9}$</td>
<td>High sensitivity</td>
<td>Highest specificity; structural identification possible</td>
</tr>
<tr>
<td>Separation (HPLC or electrophoresis) + fluorescence spectroscopy, electrochemical, or UV detection</td>
<td>$10^{8}$</td>
<td>Moderate to high sensitivity for PAH adducts</td>
<td>High specificity and structural identification (depending on quality of standard)</td>
</tr>
<tr>
<td>Immunoassays using antisera raised against BP-modified DNA or adducts</td>
<td>$10^{8}$</td>
<td>High sensitivity</td>
<td>Broad specificity for family of carcinogenic PAH-DNA adducts</td>
</tr>
<tr>
<td>Immunohistochemistry (in situ detection in intact tissues)</td>
<td>$10^{7}$</td>
<td>Low sensitivity</td>
<td>Broad specificity for family of carcinogenic PAH-DNA adducts</td>
</tr>
</tbody>
</table>
Another example:

- Chemical X is reported by other agency assessments and numerous research publications to be a known male reproductive toxicant

- Evidence:
  - Review of ADME/TK data led to decision to exclude i.p. injection studies from PECO criteria; PBPK models indicated inhalation and oral routes may still reach target tissue
  - Oral and inhalation exposure studies in humans and animals were identified using PECO and evaluated
    - All high and medium confidence studies were negative
    - Some low and critically deficient oral studies did report effects
  - i.p. exposure studies did report male reproductive effects and mechanistic evidence
  - i.p. and in vitro studies demonstrated plausible mechanistic explanation for male reproductive toxicity
    - These mechanistic studies were summarized but not evaluated

- Conclusion: There is inadequate evidence that Chemical X causes male reproductive toxicity in humans
For key analyses, provide detailed documentation of decisions

- IRIS assessments use organizational frameworks to organize and document the analyses and transparently convey conclusions for evidence integration
  
  - EPA’s cancer MOA narrative framework uses *modified* Hill considerations; provides foundation for evidence integration
    
    - Strength, consistency, specificity
    
    - Biological plausibility and coherence
    
    - Temporal and/or dose-response concordance
  
  - Other well-established visual organizational tools (e.g., AOPs or AOP networks) are useful and compatible (e.g., the identification of key events)
Mechanistic Evidence Synthesis

**B[a]P assessment**

- Table summarizes key events in mutagenic MOA and evidence supporting each event.

### Table: Summary of Key Events in Mutagenic MOA

<table>
<thead>
<tr>
<th>Event Description</th>
<th>Evidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Bioactivation of benzo[a]pyrene to DNA-reactive metabolites via three possible metabolic activation pathways.</td>
<td>several in vivo and in vitro studies have observed benzo[a]pyrene-specific mutational spectra (e.g., G→T transversions)</td>
</tr>
<tr>
<td>2. Direct DNA damage by the reactive metabolites, including the formation of DNA adducts and ROS-mediated damage</td>
<td>evidence that benzo[a]pyrene metabolites induce key events: Metabolism of benzo[a]pyrene, leading to increased DNA adducts and ROS injury in tissues.</td>
</tr>
<tr>
<td>3. Formation and fixation of DNA mutations, particularly in tumor suppressor genes or oncogenes associated with tumor initiation</td>
<td>evidence that benzo[a]pyrene metabolites induce key events: Several in vivo and in vitro studies have observed benzo[a]pyrene diole epoxide-specific mutational spectra (e.g., G→T transversions).</td>
</tr>
<tr>
<td>4. Clonal expansion of mutated cells during the promotion and progression phases of cancer development</td>
<td>evidence that benzo[a]pyrene metabolites induce key events: Benzo[a]pyrene has been shown to be a complete carcinogen, in that skin tumors in mice, rats, rabbits, and guinea pigs have been associated with repeated application of benzo[a]pyrene to skin in the absence of exogenous promoters.</td>
</tr>
</tbody>
</table>

**Human Evidence:**

- Humans with CYP polymorphisms have increased levels of diol epoxides, leading to increased DNA damage.

**Detection in human tissues:**

- DNA adducts formed by benzo[a]pyrene have been detected in human tissues.

**Observations:**

- Mice exposed dermally to benzo[a]pyrene for 26 weeks were found to have increased frequencies of H-ras mutations in exposure-induced hyperplastic lesions that were further increased in tumors.

- AhR activation by PAHs (including benzo[a]pyrene) upregulates genes responsible for tumor promotion and increases tumor incidence in mice.
### Mechanistic Evidence Integration

- **Table summarizing weight of evidence for descriptor “Carcinogenic to humans”**

#### a) Strong human evidence of cancer or its precursors
- Increased risk of lung, bladder, and skin cancer in humans exposed to complex PAH mixtures containing benzo[a]pyrene

  **References:** IARC (2004); IARC (2010); Secretan et al. (2009); Baan et al. (2009); Benbrahim-Tallaa et al. (2012)

- Benzo[a]pyrene-specific biomarkers detected in humans exposed to PAH mixtures

  - BPDE-DNA adducts in workers and chimney sweepers
  - BPDE-DNA adducts in smokers

- Benzo[a]pyrene-specific DNA adducts have been detected in target tissues of humans exposed to PAH mixtures

  - BPDE-DNA adducts in human liver and lung tissues of cigarette smokers
  - BPDE-DNA adducts in skin of individuals treated with coal tar.

- Benzo[a]pyrene-specific DNA adducts have been detected in target tissues of human liver and lung tissues of individuals treated with coal tar.

- Benzo[a]pyrene-specific mutations have been identified in PAH-associated tumors.

  - GC→TA transitions at hprt and ras loci
  - Mutations in p53 and K-ras genes

- Increased percentage of G→T mutations at the p53 hotspot in p53 and at K-ras hotspot in ras genes in human tumors associated with PAH exposures.

- Increased percentage of G→T mutations at the p53 hotspot in p53 and at K-ras hotspot in ras genes in human tumors associated with PAH exposures.

#### b) Extensive animal evidence

**Oral exposures**
- Forestomach tumors in male and female rats and in female mice following lifetime exposure
- Forestomach tumors in mice following less-than-lifetime exposures

  **References:** Kroese et al. (2001); Brune et al. (1981); Beland and Culp (1998); Culp et al. (1998)

  Benjamini et al. (1988); Berenblum and Haran (1955); Naeye et al. (1968)

**Inhalation exposures**
- Upper respiratory tract tumors in hamsters following chronic exposure to benzo[a]pyrene

**Dermal exposures**
- Skin tumors in mice following lifetime exposures without a promoter
- Skin tumors in rats, rabbits, and guinea pigs following subchronic exposures

  **References:** Demarini et al. (2001); Keohavong et al. (2003)

  Bennett et al. (1999); Hainaut and Pfeifer (2001); Pfeifer et al. (2002); Pfeifer and Hainaut (2003)

**See ‘Experimental Support for Hypothesized Mode of Action’ section**

**Identification of key precursor events have been identified in animals**

- Bioactivation of benzo[a]pyrene to DNA-reactive metabolites has been shown to occur in multiple species and tissues by all routes of exposure.
- Direct DNA damage by the reactive metabolites, including the formation of DNA adducts and ROS-mediated damage.
- Formation and fixation of DNA mutations, particularly in tumor suppressor genes or oncogenes associated with tumor initiation.

**Strong evidence that the key precursor events are anticipated to occur in humans**

- Mutations in p53 or ras oncogenes have been observed in forestomach or lung tumors from mice exposed to benzo[a]pyrene
  - G→T transitions in ras oncogenes or the p53 gene have been observed in lung tumors of human cancer patients exposed to coal smoke.
  - Higher frequency of G→T transitions in lung tumors from smokers versus nonsmokers.
Specific needs and questions

- Increased transparency in iterative process of focusing the mechanistic analyses

- Evaluating mechanistic data
  
  • Individual study review: Reporting quality, risk of bias/internal validity, sensitivity/specificity of assay, other considerations?
  
  • Currently no pre-specified language for describing confidence at the endpoint, study, mechanistic event, or pathway/MOA level
  
  • Many human and animal studies reporting primary health effects data also report mechanistic data—should the study-level confidence determinations for these endpoints carry over into mechanistic syntheses?

- Clear frameworks and improved transparency for the integration of mechanistic evidence with epidemiologic and toxicologic evidence
Acknowledgements

NCEA SR Approaches
Xabier Arzuaga
Laura Dishaw
Catherine Gibbons
Barbara Glenn
Karen Hogan
Andrew Kraft
April Luke
Beth Radke
Kris Thayer
George Woodall
Erin Yost

IRIS Program Planning
James Avery
Tina Bahadori
Emma Lavoie
Dahnish Shams
Vicki Soto
Kris Thayer

Automation Tools
Michelle Angrish
Audrey Galizia
Amanda Persad
Sue Rieth
Michele Taylor
Andre Weaver