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THE SYSTEMATIC REVIEW OF MECHANISTIC DATA IN IRIS ASSESSMENTS

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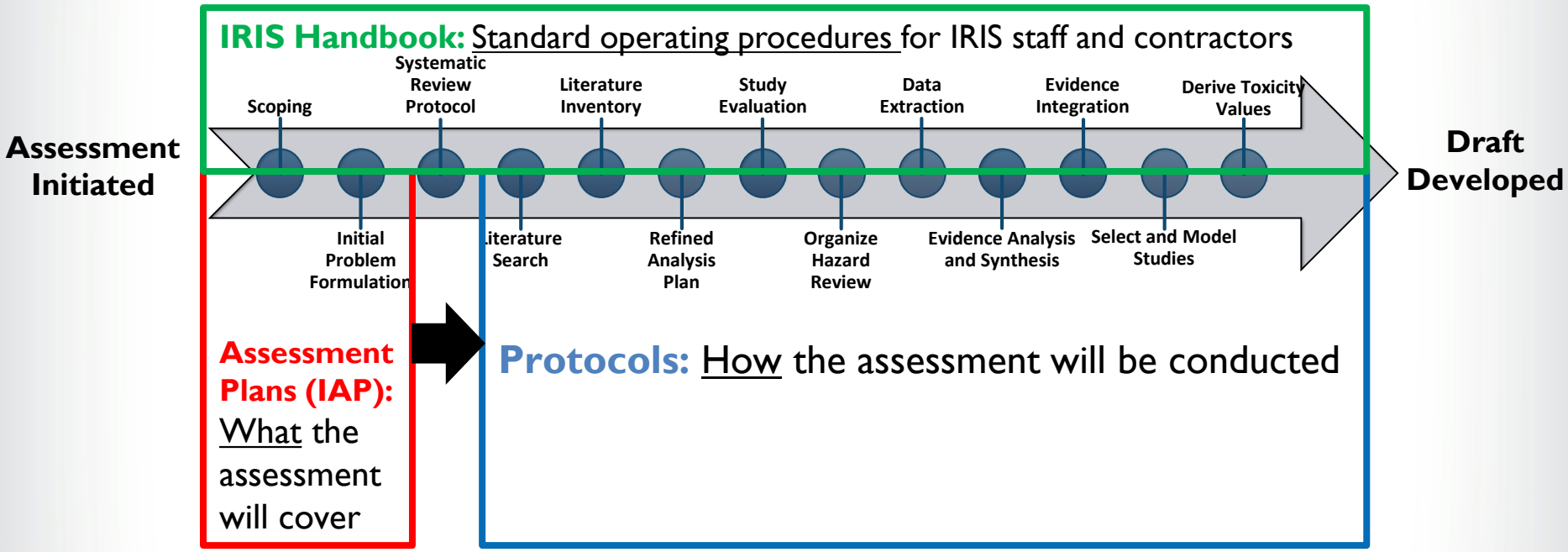
Office of Research and Development
NCEA, IRIS

- **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



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

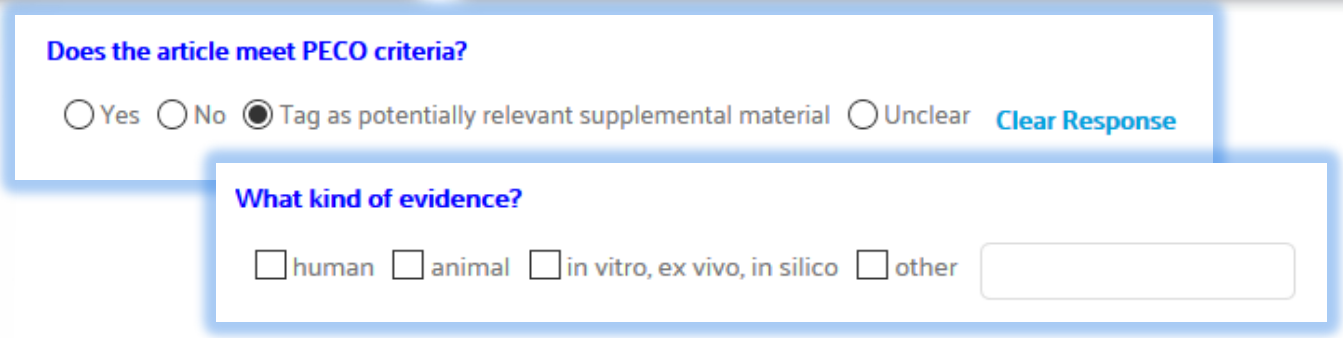
PECO element	Evidence
Populations	**Human:** Any population and lifestage (occupational or general population, including children and other sensitive populations). **Animal:** Nonhuman mammalian animal species (whole organism) of any lifestage (including preconception, in utero, lactation, peripubertal, and adult stages).
Exposures	*[Example language that can be included if appropriate.]* Relevant forms: [chemical *X*] (*CAS number*). *Other forms of* [chemical *X*] *that readily dissociate (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] [via [oral or inhalation] route[s] if applicable]. *Specify if certain exposure assessment methods or metrics will NOT be included.* **Animal:** Any exposure to [chemical X] via [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."
Comparators	**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." **Animal:** A concurrent control group exposed to vehicle-only treatment or untreated control.
Outcomes	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 apical/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."
PBPK models *[an additional criterion to address specific aims]*	Studies describing PBPK models for [chemical *X*] will be included.

Potentially Relevant Supplemental Material

Category	Evidence
Mechanistic	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.
Nonmammalian model systems	Studies in nonmammalian model systems (e.g., fish, birds, *Caenorhabditis elegans*).
ADME and toxicokinetic	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.
Exposure characteristics	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).
Susceptible populations	Studies that identify potentially susceptible groups; for example, studies that focus on a specific demographic, lifestage, or genotype.
Mixture studies	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.
Routes of exposure not pertinent to PECO	Studies using routes of exposure that fall outside the PECO scope.
Case studies or case series	In most cases, case reports and case series will be tracked as potentially relevant supplemental information.
Acute duration exposures	For assessments that focus on chronic exposure, shorter-term exposure durations (i.e., animal studies of less than 28 d) are generally considered supplemental.
Records with no original data	Records that do not contain original data, such as other agency assessments, informative scientific literature reviews, editorials, or commentaries.
Others determined by assessment team	

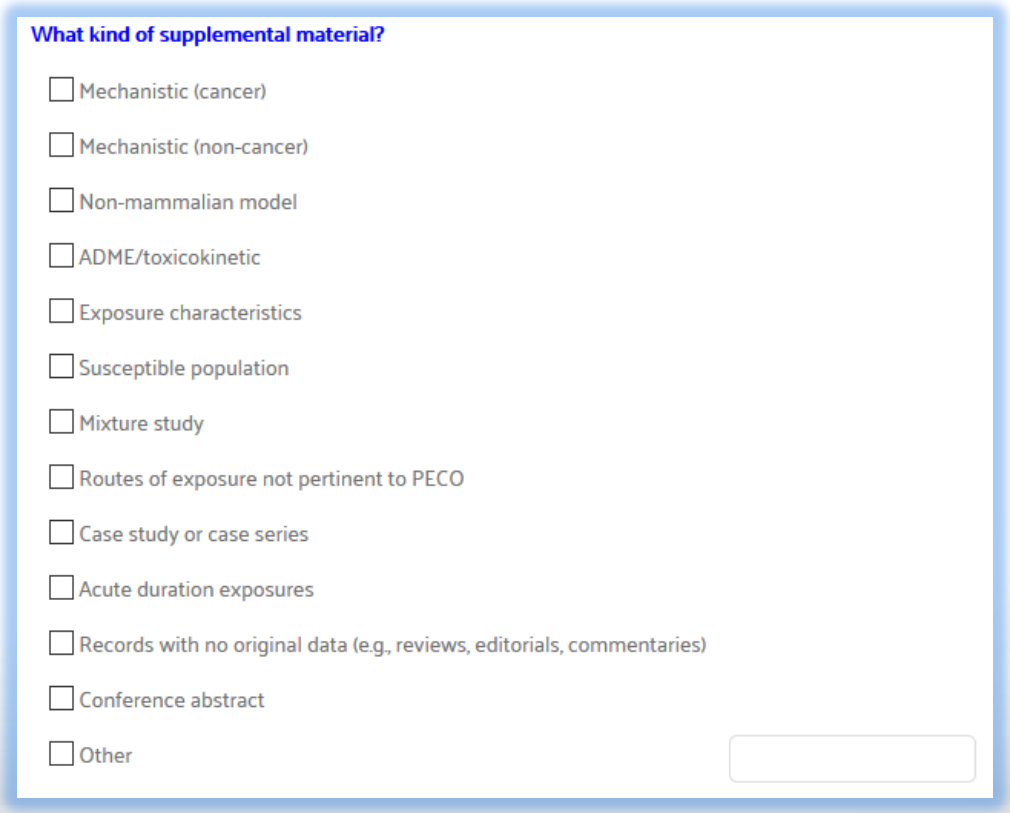
Initial Categorization Approach

TIAB



TIAB, second level TIAB, or full-text

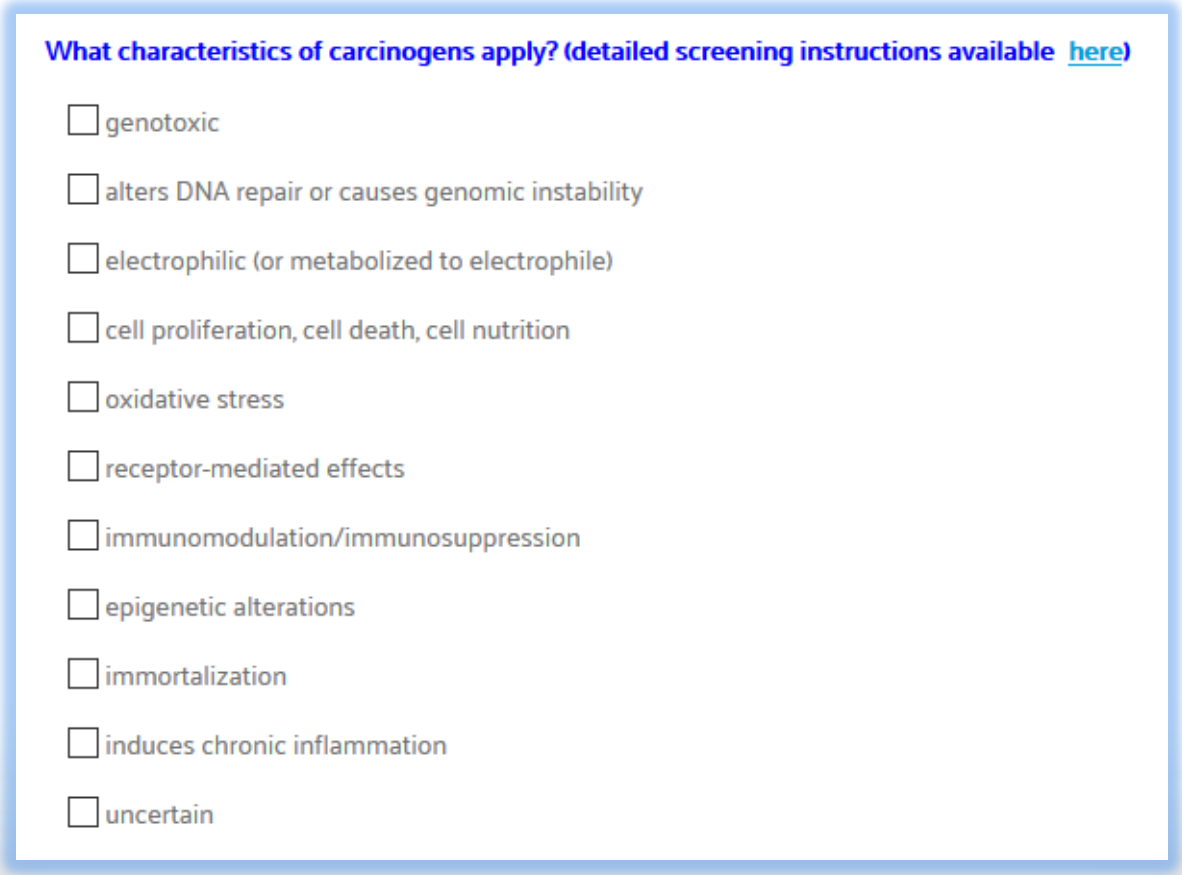
- Based on considerations such as size of evidence base, content knowledge of TIAB screeners



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



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

Mechanistic Evidence Evaluation

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[a]

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

Mechanistic Evidence Evaluation

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

Mechanistic Evidence Synthesis and Integration

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

2. Direct DNA damage by the reactive metabolites, including the formation of DNA adducts and ROS-mediated damage

Evidence that benzo[a]pyrene metabolites induce key events:

1. Bioactivation of benzo[a]pyrene to DNA-reactive metabolites via three possible metabolic activation pathways: a diol epoxide pathway, a radical cation pathway, and an *o*-quinone and ROS pathway

Evidence that benzo[a]pyrene metabolites induce key events:

- Metabolism of benzo[a]p… studies, and the diol epo… in in vivo studies in huma…
- Multiple in vivo studies i… to target tissues

Human evidence that key events …

- Humans with CYP polymorph… diol epoxides, leading to incre… 2007; Pavanello et al., 2005; …
- Benzo[a]… cause ox… 1997; Flo…

in vitro
: mouse assay,
nd DNA
)

3. Formation and fixation of DNA mutations, particularly in tumor suppressor genes or oncogenes associated with tumor initiation

Evidence that benzo[a]pyrene metabolites induce key events:

- Several in vivo exposure studies have observed benzo[a]pyrene diol epoxide-specific mutational spectra (e.g., G→T transversion… 2000; Nesnow et al., 19…
- Multiple studies in vivo … and *p53* in target tissue… (Chakravarti et al., 199…

Human evidence that key events …

- DNA adducts formed by … predominantly to G→T … PAH-associated tumors … suppressor genes (*p53*) … 2002; Demarini et al., 2… Puisieux et al., 1991; M…

Human evidence …

- Detectio… in humar…
- These be… have been detected in tissues of humans … 2007; Rojas et al., 2004; Godschalk et al., 2002; Li et al., 2001; Pavanello et al., 1999; Rojas et al., 1998; Andreassen et al., 1996; Alexandrov et al., 1992)

4. Clonal expansion of mutated cells during the promotion and progression phases of cancer development

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 (IPCS, 1998; Sivak et al., 1997; ATSDR, 1995; Grimmer et al., 1984; Habs et al., 1984; Grimmer et al., 1983; IARC, 1983; Habs et al., 1980; Schmähl et al., 1977; IARC, 1973; Schmidt et al., 1973; Roe et al., 1970; Poel, 1963, 1959)
- 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 (Wei et al., 1999)
- AhR activation by PAHs (including benzo[a]pyrene) upregulates genes responsible for tumor promotion and increases tumor incidence in mice (Ma and Lu, 2007; Talaska et al., 2006; Shimizu et al., 2000)

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	IARC (2004); IARC (2010); Secretan et al. (2009);Baan et al. (2009); Benbrahim-Tallaa et al. (2012)
• Benzo[a]pyrene-specific biom humans exposed to PAH mix with increased risk of cancer	
– BPDE-DNA adducts in W workers and chimney sw	
– BPDE-DNA adducts in sn	
• Benzo[a]pyrene-specific DNA been detected in target tissu exposed to PAH mixtures	
– BPDE-DNA adducts in nc tissues of cigarette smol cancer and in skin of ecz treated with coal tar	
– BPDE-DNA adduct forma human cells in vitro corr mutational hotspots at g human lung tumors	
• Benzo[a]pyrene-specific mut identified in PAH-associated	
– GC→TA transversions ar transitions at hprt locus of humans with lung car	
– G→T transversions in ex knock-in mouse fibrobla mutational hotspot in *p5* related lung tumors in h	
– G→T transversions at th hotspot in *p53* and *K-ras* tumors associated with exposures	
– Increased percentage of transversions in *p53* in s nonsmokers	

b) Extensive animal evidence	
Oral exposures	
• Forestomach tumors in male and female rats and in female mice following lifetime exposure	Kroese et al. (2001); Brune et al. (1981); Beland and Culp (1998); Culp et al. (1998)
• Forestomach tumors in mice following less-than-lifetime exposures	Benjamin et al. (1988); Berenblum and Haran (1955);
• Alimentary tract and liver tumors female rats following lifetime exp	
• Kidney tumors in male rats follov exposure	
• Auditory canal tumors in male ar following lifetime exposure	
• Esophageal, tongue, and larynge female mice following lifetime ex	
• Lung tumors in mice following le lifetime exposure	
Inhalation exposures	
• Upper respiratory tract tumors ir hamsters following chronic expo	
Dermal exposures	
• Skin tumors in mice following life exposures without a promoter	
• Skin tumors in rats, rabbits, and following subchronic exposures	

c) 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	See 'Experimental Support for Hypothesized Mode of Action' section
• 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	

d) 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	Culp et al. (2000); Mass et al. (1993); Nesnow et al. (1998a); Nesnow et al. (1998b); Nesnow et al. (1995); Nesnow et al. (1996)
– G→T transversions in *ras* oncogenes or the *p53* gene have been observed in lung tumors of human cancer patients exposed to coal smoke	Demarini et al. (2001); Keohavong et al. (2003)
– Higher frequency of G→T transversions in lung tumors from smokers versus nonsmokers	Bennett et al. (1999); Hainaut and Pfeifer (2001); Pfeifer et al. (2002); Pfeifer and Hainaut (2003)

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**

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