Genotoxicity testing to inform pathway analysis (Adverse Outcome Pathways) and mutagenic modes of action

Stakeholder Workshop on EFSAs Genotoxicity Guidance Revision

Presented by:
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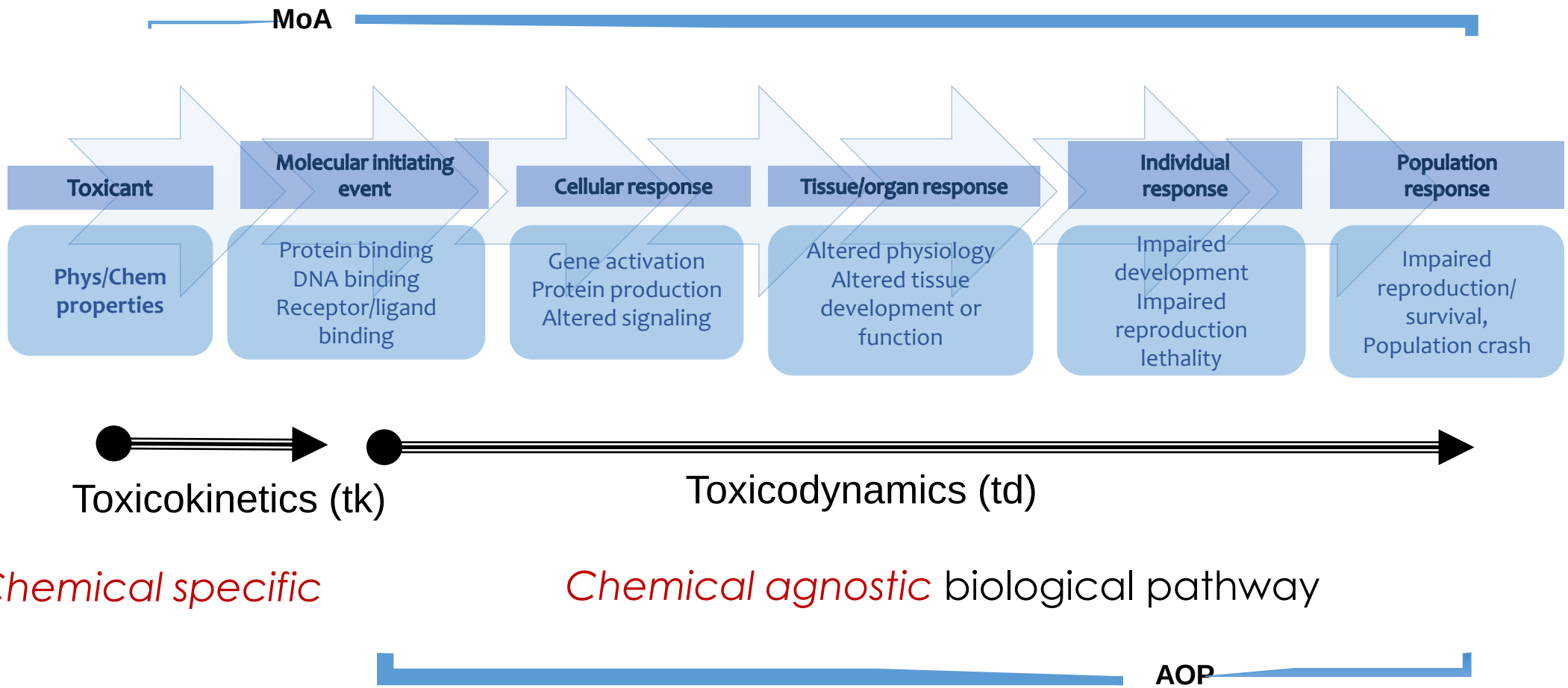
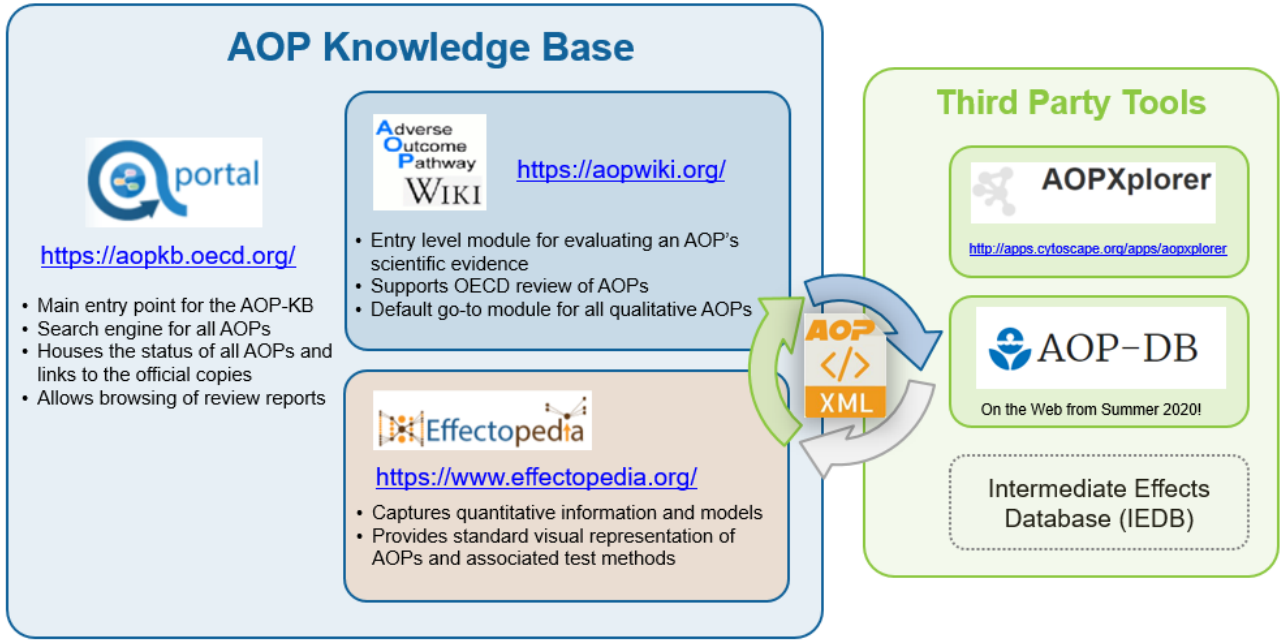
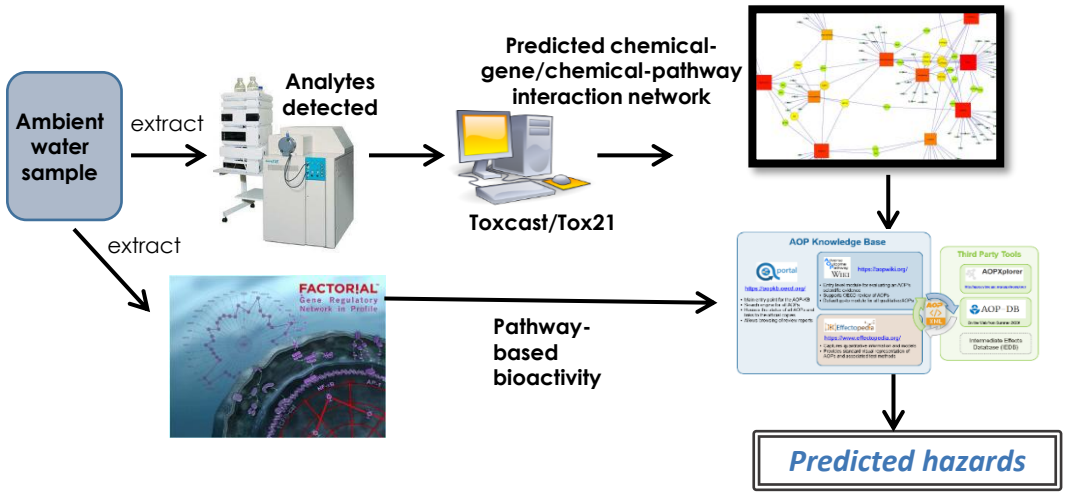
Terminology – Mutagenic Modes of Action
Adverse Outcome Pathways/Mode of Action



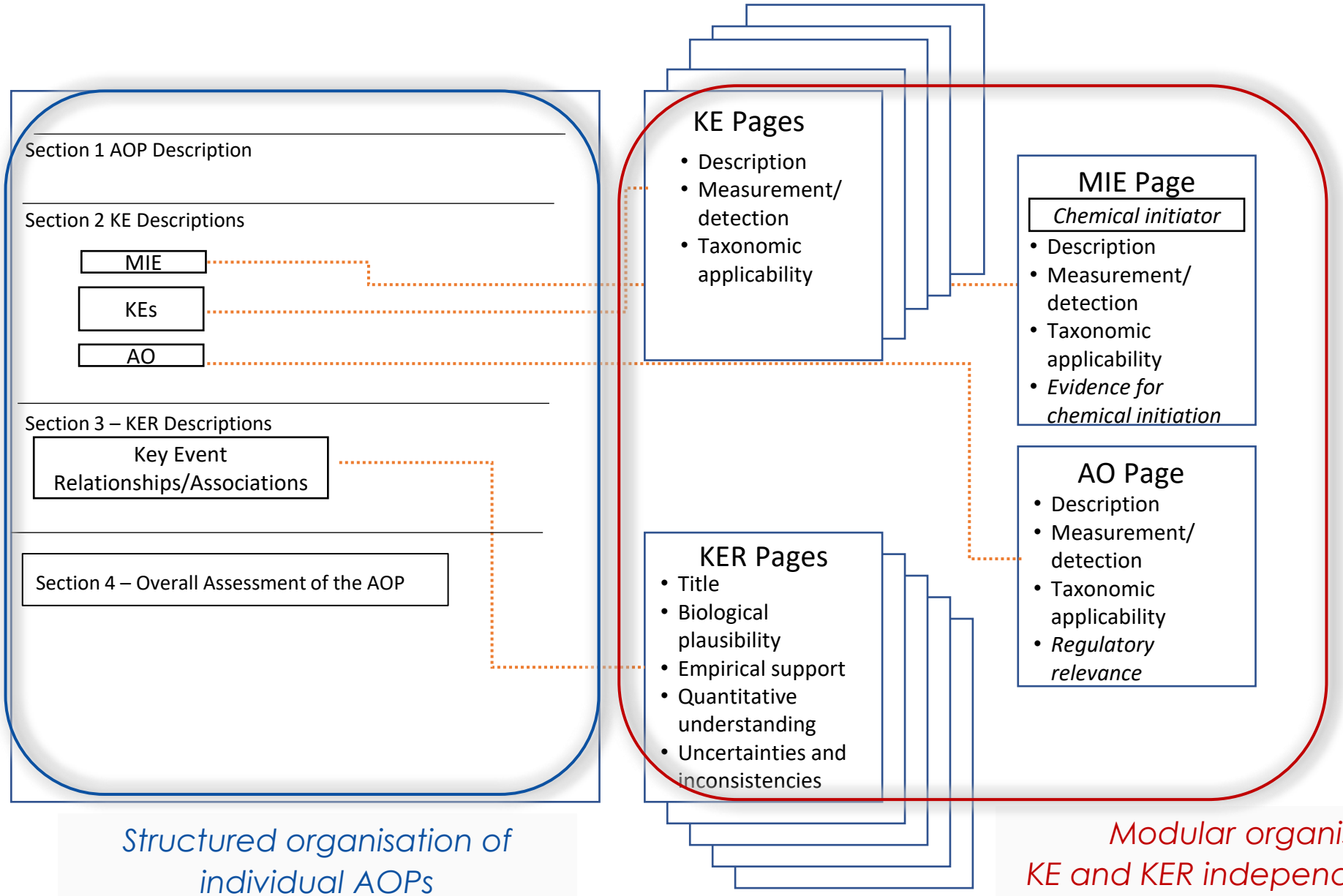
AOP Wiki: Information Storage, Linkage and Evaluation

- Captures and organizes all information and supporting documentation for AOP elements
- Supported by extensive guidance, tutorials and an online course
- Designed to enable rigorous evaluation and scientific review
- Curated data source for Integrated Approaches to Testing and Assessment (IATA) and Mode of Action (MOA)





AOP Wiki Content And Organization

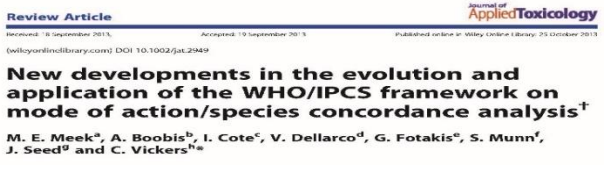


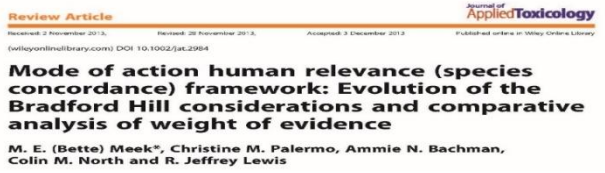
Structured organisation of individual AOPs

Modular organisation
KE and KER independent pages

Weight of Evidence (WOE) for Pathway Descriptions

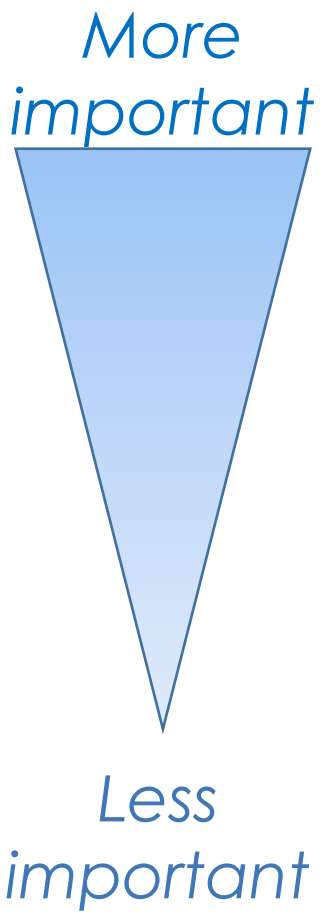
- Based on modified Bradford Hill (B/H)considerations
  - Initially introduced to assess causality of associations observed in epidemiological studies in humans
  - later adapted to impacts on wildlife
- Utility broadly recognized by a range of communities
  - E.g., human health/eco
- Longstanding
- Considerable regulatory experience in their application
- Sufficiently generic that with modification, they're broadly applicable
  - Selected subset send itself very well to MOA/AOP analysis



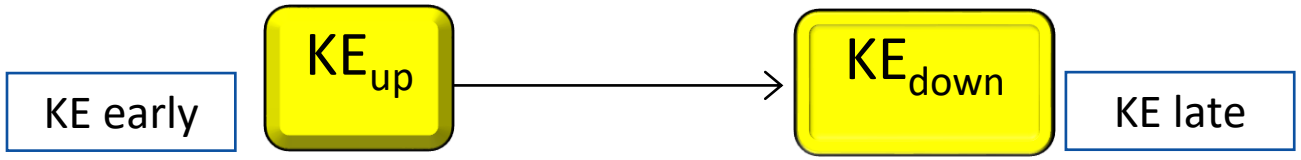


Weight/Extent of the Evidence – Elements

- Biological Plausibility – ***KERs***
  - Extent of knowledge of the biology of the pathway
  - Knowledge of the structural-functional relationships
  - Consistency with prediction - experimental support from disrupting the pathway
- Essentiality – ***KEs***
  - Necessity of Key Events
  - Experimental support normally from specialized studies to block or modify key events, stop/recovery studies
- Empirical Support – ***KERs***
  - Pattern of Quantitative Associations among Key Events
    - Dose Response/Temporality



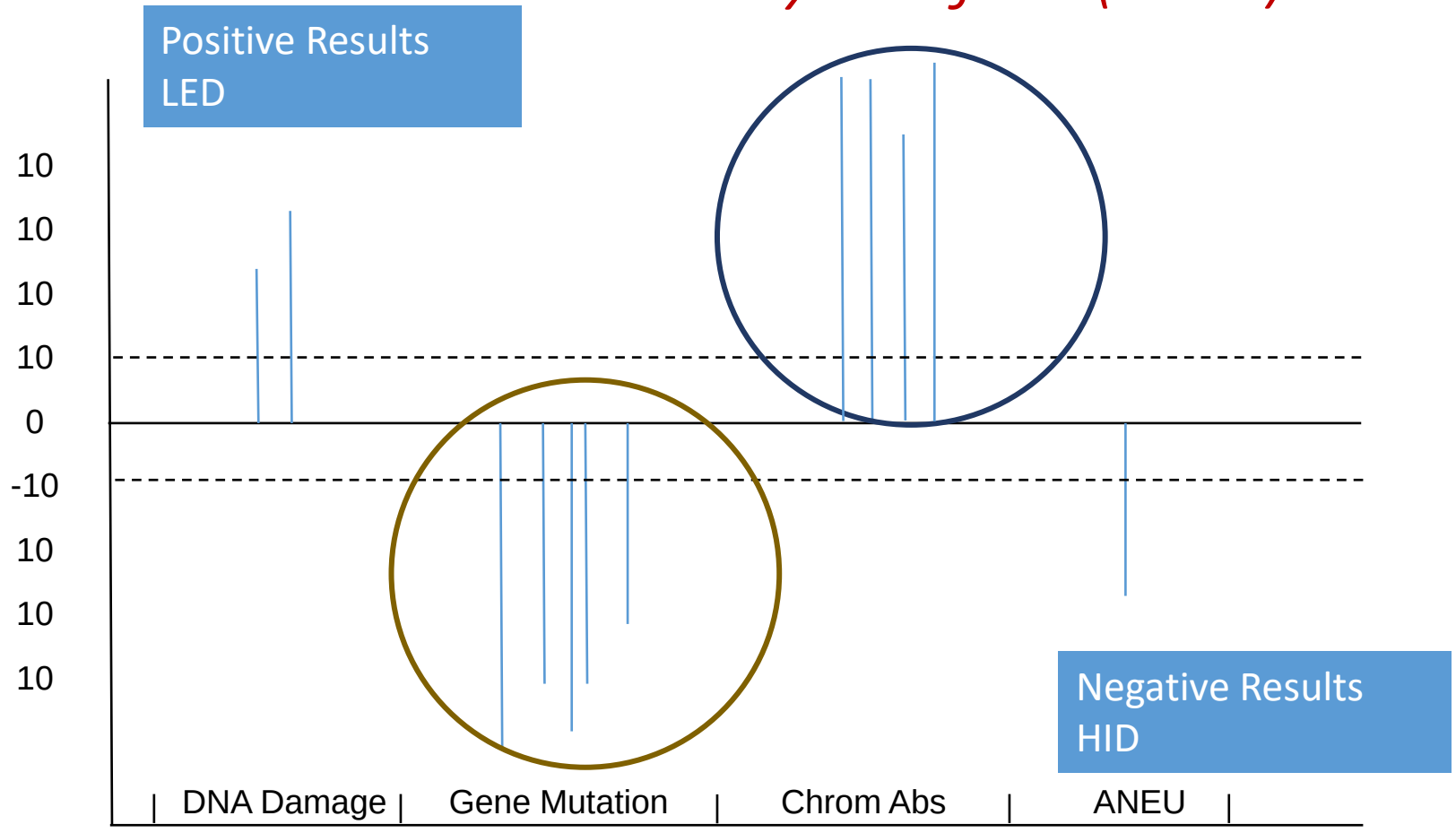
Empirical Support: Expected Patterns



- Temporal Association (Time)
  - Early key events precede hypothesized late key events
- Dose-Response
  - The "severity" of early KEs is less that that for late KEs
    - Impact at increasing levels of biological organization to compromise normal function
      - e.g., cells vs. organs
  - Early key events occur at lower doses than late key events
  - For a given dose, the *incidence* (relative abundance/proportion impacted) of early key events is greater than or equal to that of later key events

Robust dose-response gentox data are critical to assess weight of evidence for mutagenic modes of action

Interpreting "Patterns" of Gentox Data for Mutagenic Modes of Action
Genetic Activity Profiles(GAP)



LED – lowest effective dose; HID = highest ineffective dose

Is it Time to Go Beyond Yes or No?

Archives of Toxicology (2023) 97:2303–2328
https://doi.org/10.1007/s00204-023-03553-w

REVIEW ARTICLE

Genotoxicity assessment: opportunities, challenges and perspectives for quantitative evaluations of dose–response data

Jakob Menz[1] · Mario E. Götz[1] · Ulrike Gündel[2] · Rainer Gürtler[1] · Kristin Herrmann[3] · Stefanie Hessel-Pras[1] · Carsten Kneuer[3] · Franziska Kolrep[4] · Dana Nitzsche[2] · Ulrike Pabel[4] · Benjamin Sachse[1] · Sebastian Schmeisser[2] · David M. Schumacher[4] · Tanja Schwerdtle[5] · Tewes Tralau[3] · Sebastian Zellmer[2] · Bernd Schäfer[1]

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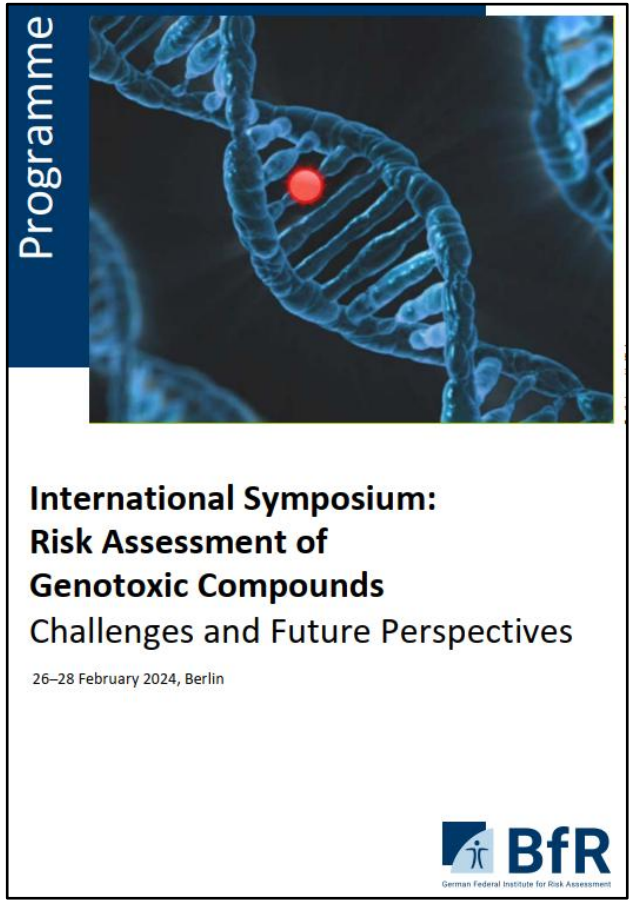


Session I: Introduction (live stream available)
Session Chair: Benjamin Sachse, BfR, Berlin

09:15–09:45	**"Classical approaches" in genotoxicity assessment** Diane Benford, Scientific Committee of the European Food Safety Authority (EFSA), Italy
09:45–10:15	**Quantitative interpretation of *in vivo* mutagenicity dose-response data for risk assessment and regulatory decision-making** Paul White, Health Canada, Canada

Objectives:

- platform to discuss the current opportunities, challenges and perspectives for a more quantitative approach to genotoxicity assessment
- to foster collaboration and knowledge exchange, leading to improved risk assessment strategies for genotoxic substances."

Outcome:

- application of gentox dose–response data for prioritization in margins of exposure promising for routine application, provided that:
  - a generally high concordance between genotoxicity-based reference points and reference points based on downstream apical endpoints are highly concordant for a sufficiently large number of genotoxicants or,
  - observed differences are at least of a constant nature
    - (i.e., reference points based on earlier key events are protective)

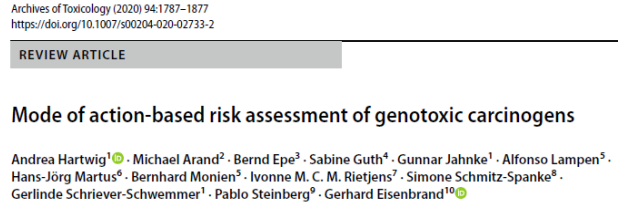
Terminology

- "Genotoxic and carcinogenic" (EFSA 2005 opinion)
  - imprecise (what is "genotoxic"?)
    - one, several, many assays?
  - implies separate assessments
    - rather than being mechanism informed integration of cancer/gentox

- "DNA reactive"
  - implies (almost) only the first key event in a mechanism informed pathway
  - imprecise
    - requires much qualification (i.e., specific DNA lesions; extent of reversibility, nature of repair)

- Mutagenic mode of action
  - maximally mechanistically informed
  - involving interpretation of patterns of results of genotoxicity bioassays in the context of cancer induction (meaningful integration)
  - specifies the *"tripping"* (i.e., irreversible) key event in cancer
    - *fixed changes* in nucleotide sequence
  - consistent with EFSA's leading role/investment in the description and application of mechanistic pathways
  - based on decades of experience in systematic consideration of the extent of the supporting evidence
    - more transparent/reproducible than "expert opinion" alone

A helpful reference: Hartwig et al. (2020), 0.1007/s00204-020-02733-2



Archives of Toxicology (2020) 94:1787–1877
https://doi.org/10.1007/s00204-020-02733-2

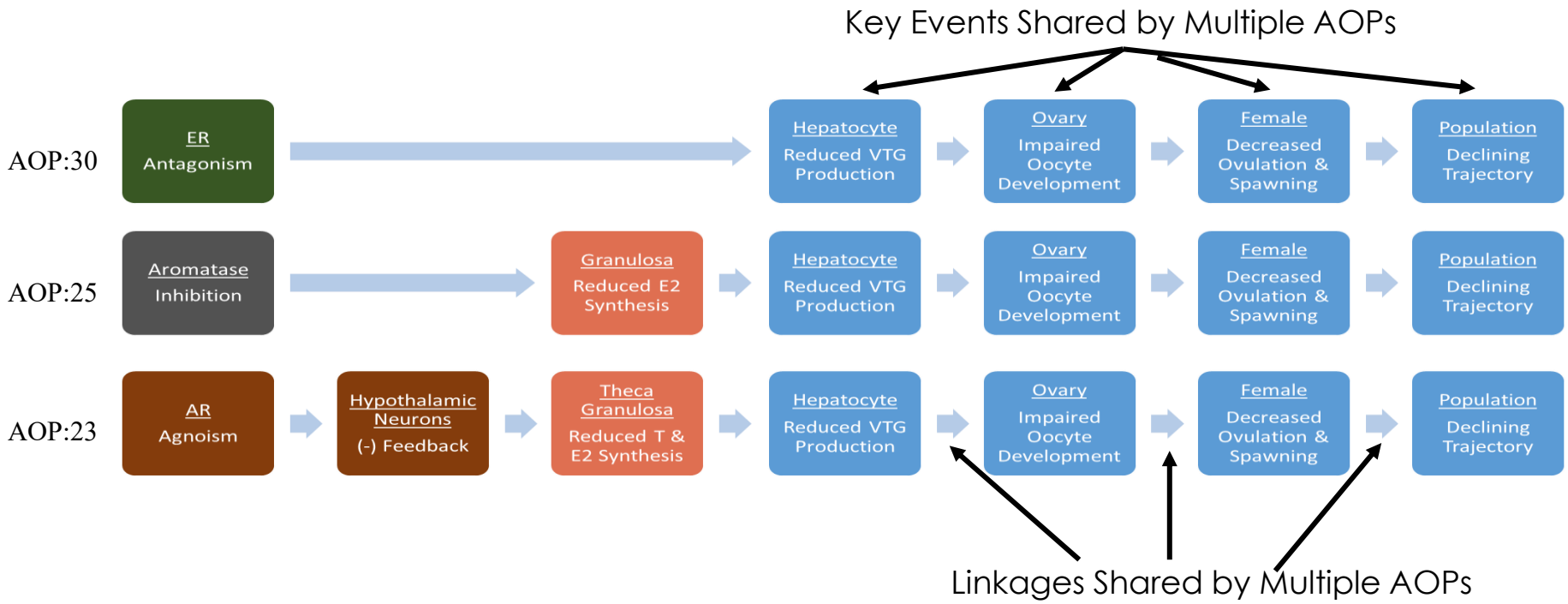
REVIEW ARTICLE

Mode of action-based risk assessment of genotoxic carcinogens

Andrea Hartwig[1] · Michael Arand[2] · Bernd Epe[3] · Sabine Guth[4] · Gunnar Jahnke[1] · Alfonso Lampen[5] · Hans-Jörg Martus[6] · Bernhard Monien[5] · Ivonne M. C. M. Rietjens[7] · Simone Schmitz-Spanke[8] · Gerlinde Schriever-Schwemmer[1] · Pablo Steinberg[9] · Gerhard Eisenbrand[10]

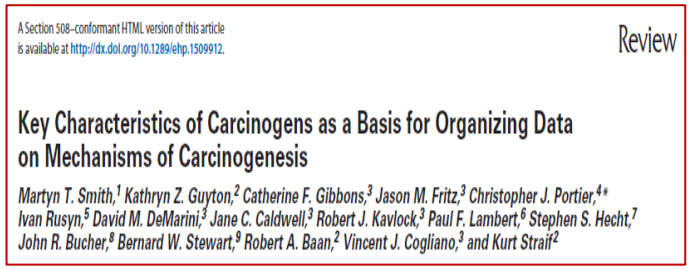
Mutagenic Modes of Action – Draft Definition

- “causing cancer via a mode for which mutation is a primary (early) key event in cancer induction rather than being secondary to DNA damage or toxicity”
  - note: that this does not preclude contribution from several pathways
  - but: there is one mode of action of induction of cancer for which late key events converge



Key Characteristics

- Defined as "generalized categories or properties considered to be associated with *human disease outcome*" *(https://keycharacteristics.org/)*
- Represent various combinations of one or more events at different levels of biological organization
- Developed based on expert consultation
  - E.g., workshops for each endpoint(e.g., cancer, reproductive, etc.)
- System for *"identifying and organizing"* (categorizing)scientific findings relevant to *mechanisms*
  - Considered helpful in streamlining (particularly in vitro) mechanistic data into more systematic review process for chemical specific hazard assessment
- Envisaged potentially to contribute to development of both AOPs and Networks


A Section 508–conformant HTML version of this article is available at http://dx.doi.org/10.1289/ehp.1509912.

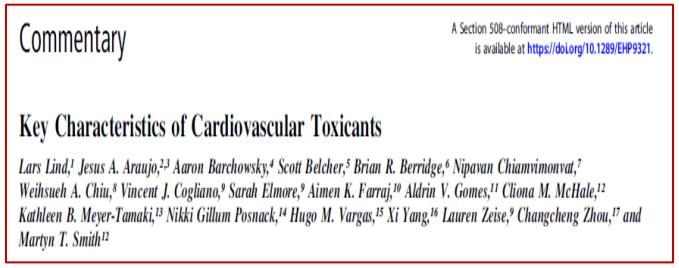
Review

Key Characteristics of Carcinogens as a Basis for Organizing Data on Mechanisms of Carcinogenesis

Martyn T. Smith,[1] Kathryn Z. Guyton,[2] Catherine F. Gibbons,[3] Jason M. Fritz,[3] Christopher J. Portier,[4]* Ivan Rusyn,[5] David M. DeMarini,[3] Jane C. Caldwell,[3] Robert J. Kavlock,[3] Paul F. Lambert,[6] Stephen S. Hecht,[7] John R. Bucher,[8] Bernard W. Stewart,[9] Robert A. Baan,[2] Vincent J. Cogliano,[3] and Kurt Straif[2]


Consensus on the key characteristics of endocrine-disrupting chemicals as a basis for hazard identification

Michele A. La Merrill[1]*, Laura N. Vandenberg[2], Martyn T. Smith[3], William Goodson[4], Patience Browne[5], Heather B. Patisaul[6], Kathryn Z. Guyton[7], Andreas Kortenkamp[8], Vincent J. Cogliano[9], Tracey J. Woodruff[10], Linda Rieswijk[11], Hideko Sone[12], Kenneth S. Korach[13], Andrea C. Gore[14], Lauren Zeise[15] and R. Thomas Zoeller[16]

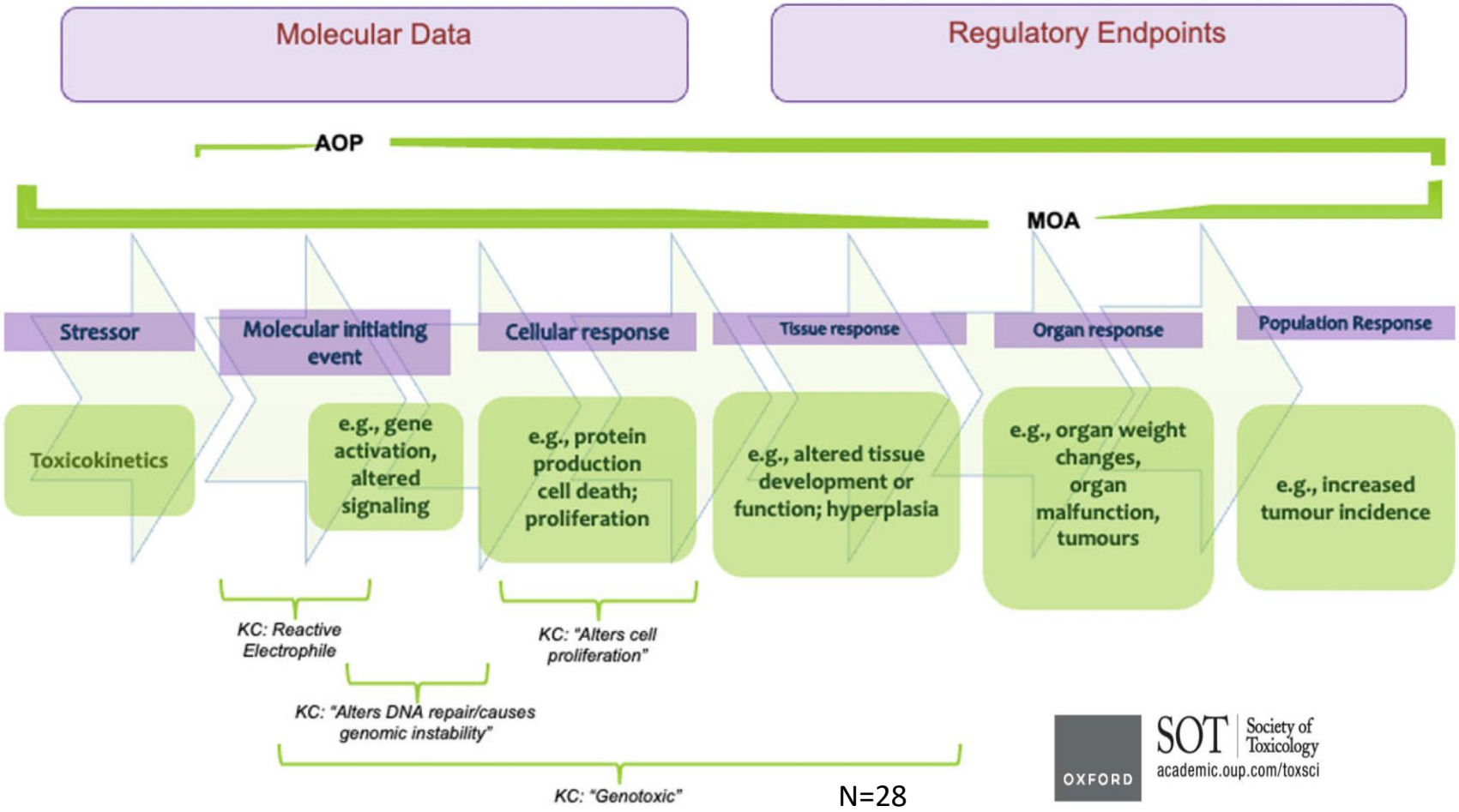

Commentary

A Section 508-conformant HTML version of this article is available at https://doi.org/10.1289/EHP9321.

Key Characteristics of Cardiovascular Toxicants

Lars Lind,[1] Jesus A. Araujo,[2,3] Aaron Barchowsky,[4] Scott Belcher,[5] Brian R. Berridge,[6] Nipavan Chiamvimonvat,[7] Weihsueh A. Chiu,[8] Vincent J. Cogliano,[9] Sarah Elmore,[9] Aimen K. Farraj,[10] Aldrin V. Gomes,[11] Cliona M. McHale,[12] Kathleen B. Meyer-Tamaki,[13] Nikki Gillum Posnack,[14] Hugo M. Vargas,[15] Xi Yang,[16] Lauren Zeise,[9] Changcheng Zhou,[17] and Martyn T. Smith[12]

Where do KCs "fit" Relevant to AOPs? Cross-Mapping in the AOP Wiki



N=28

Meek and Wikoff, https://doi.org/10.1093/toxsci/kfad039

OXFORD SOT Society of Toxicology
academic.oup.com/toxsci

Toxicological Sciences, 2023, 1–10
https://doi.org/10.1093/toxsci/kfad039
Advance Access Publication Date: April 19, 2023
Forum

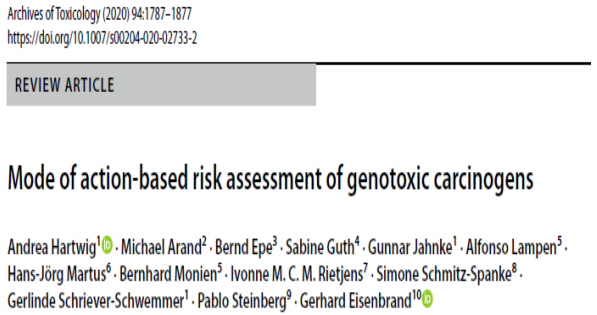
The need for good practice in the application of mechanistic constructs in hazard and risk assessment

M.E. (Bette) Meek,[1,2,*] Daniele Wikoff[3]

Is Mutation Predictive of Cancer?

- very unlikely to have cancer bioassays in future (3Rs, cost, etc.)
- the importance of *pathway approaches* in identifying precursor key events
- conducting a systematic (like) review on the quantitative correlation of mutation (in vivo) and cancer,
  - Considered in previous reviews e.g., (Hartwig et al., 2020)
    - *strongest correlation between the lowest BMD10 from the comet assay or rodent in vivo transgenic data (i.e., lowest genotoxicity BMD10) and the tissue-matched tumour BMD10*
    - *necessarily dependent on the sensitivity of the assays*
- predictable quantitative relationship between mutation and cancer in documented pathways for mutagenic modes of action
  - not 1:1
  - the incidence of early key events > that of late key events

Hartwig et al. (2020), 0.1007/s00204-020-02733-2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REVIEW ARTICLE

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Implications of the Work of the MoE guidance revision for the the update of the Gentox Guidance

- Terminology
  - Mutagenic mode of action
  - “DNA reactive”
  - “Genotoxic and carcinogenic”

- The need for robust characterization of dose-response in gentox assays
  - Hazard characterization rather than hazard identification to support MOA analysis
    - and reliance on earlier key events (e.g., mutation) to protect for cancer
  - For in vivo studies, smaller group sizes (n=5) with more dose levels
- Need to consider “mutagenic” vs. “non mutagenic” modes of action for cancer

Mechanisms of Mutagenesis

Endogenous:

- Errors in DNA Replication
- Errors in DNA Repair Mechanisms
- Spontaneous Base Deamination
- Oxidative DNA Damage
- Base Methylation
- Abasic Sites

Exogenous:*

- Ionizing Radiation: *Direct* (DNA Strand Breaks)& *Indirect* DNA Damage (ROS)
- UV Radiation: Energy transfer distorting DNA helix causing pyrimidine dimers
- Alkylating Agents (Nitrogen Mustard Gas, MMS, EMS, ENU, aflatoxin, nitrosamines): Affinity for nitrogens on nucleotide bases (N3 of adenine and N7 of guanine → abasic sites)
- Aromatic Amines (2-aminofluorene): lesions to the C8 position of guanine → substitution and frameshift mutations
- PAHs, acridine dyes: intercalation into DNA to form adducts → frameshift mutations
- Cyclophosphamide, cisplatin and psoralens: DNA interstrand crosslinking, blocking replication

*& exogenous DNA of viruses integrated into host DNA (insertional mutagenesis)

Durlad and Ahmadian-Moghadam, 2022; www.ncbi.nlm.nih.gov/books/NBK560519/