Stakeholder Workshop on EFSA's Genotoxicity Guidance Revision

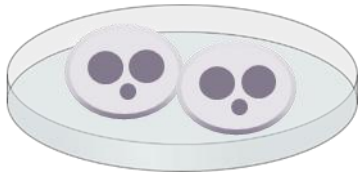
3-4 November 2025, Brussels

QUANTITATIVE GENOTOXICITY ASSESSMENT DRAFT PROPOSAL

Birgit Mertens

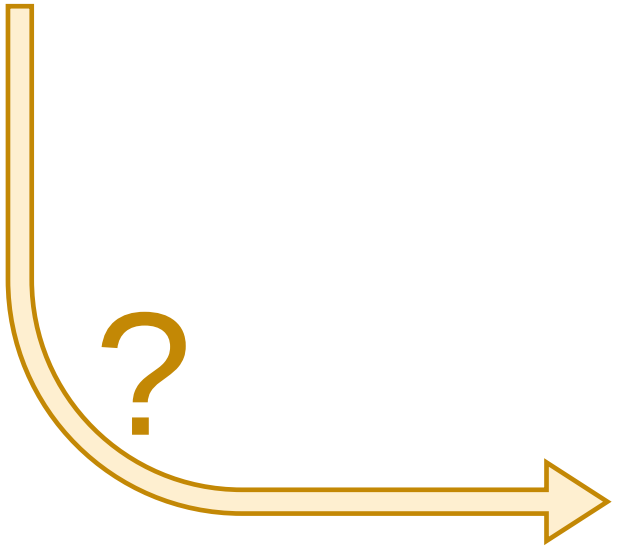
EUROPEAN FOOD SAFETY AUTHORITY

FROM QUALITATIVE TO QUANTITATIVE GENOTOXICITY ASSESSMENT



+ Follow-up testing in adequate *in vivo* test(s)

Hazard identification

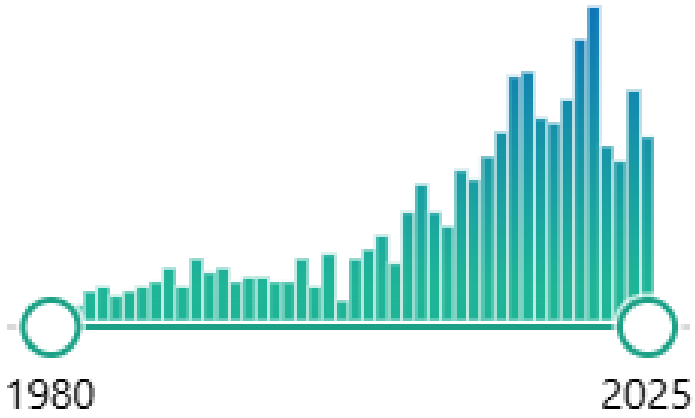


Hazard Characterization

GROWING INTEREST IN QUANTITATIVE GENOTOXICITY ASSESSMENT

Number of manuscripts in PubMed

'Quantitative genotoxicity assessment'



Received: 11 July 2022 | Revised: 28 September 2022 | Accepted: 1 November 2022
DOI: 10.1002/em.22517

RESEARCH ARTICLE

Environmental and Molecular Mutagenesis
WILEY

Establishing a quantitative framework for regulatory interpretation of genetic toxicity dose–response data: Margin of exposure case study of 48 compounds with both in vivo mutagenicity and carcinogenicity dose–response data

Nikolai Chepelev[1] | Alexandra S. Long[2] | Marc Beal[3] | Tara Barton-Maclaren[3] | George Johnson[4] | Kerry L. Dearfield[5] | Daniel J. Roberts[6] | Jan van Benthem[7] | Paul White[1]

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

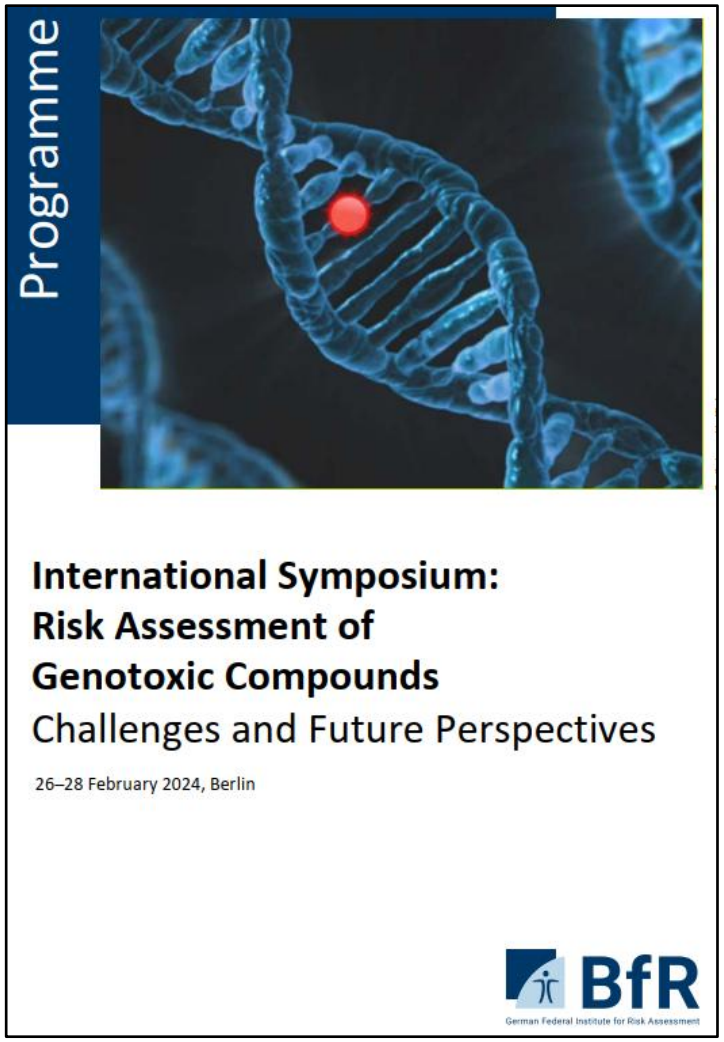
REVIEW ARTICLE

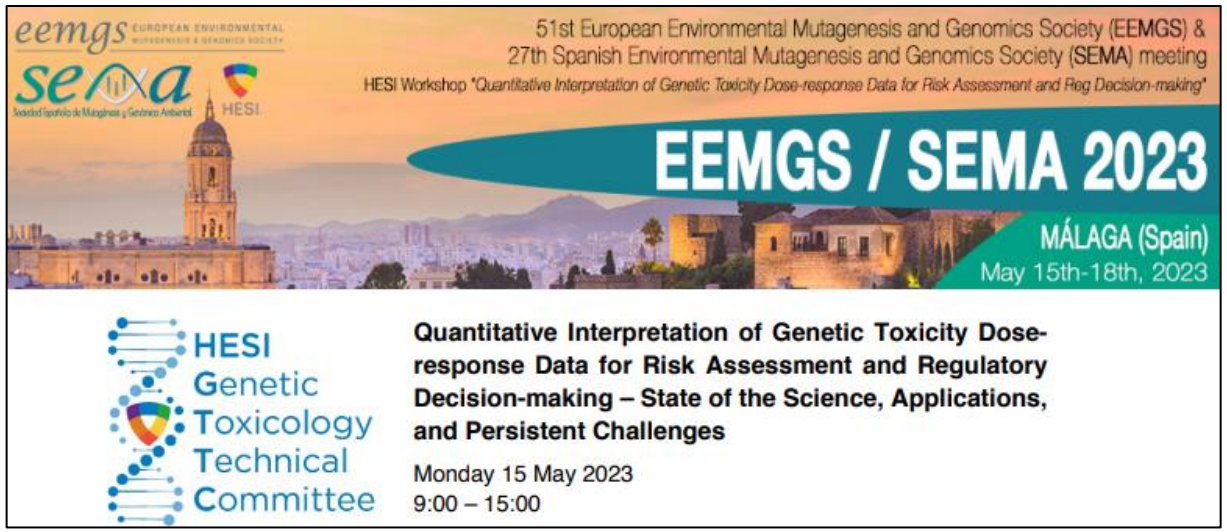
Check for updates

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]

GROWING INTEREST IN QUANTITATIVE GENOTOXICITY ASSESSMENT





9th International Workshop on Genotoxicity Testing (IWGT)

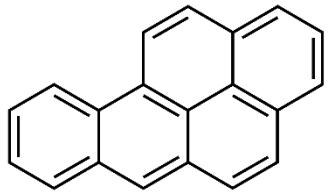
Dates: Tue 1 – Fri 4 September 2026

Methodological Advances | Risk Assessment Innovation | **Next-Generation Approaches**

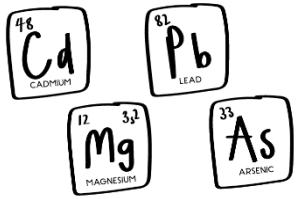
Next-Generation Approaches

- **Quantitative Genotoxicology 2.0**
  Opportunities, challenges and perspectives for quantitative evaluations of dose-response data
- **Error-Corrected Sequencing (ECS)**
  Provide expert, referenceable guidance on application of ECS for regulatory purposes

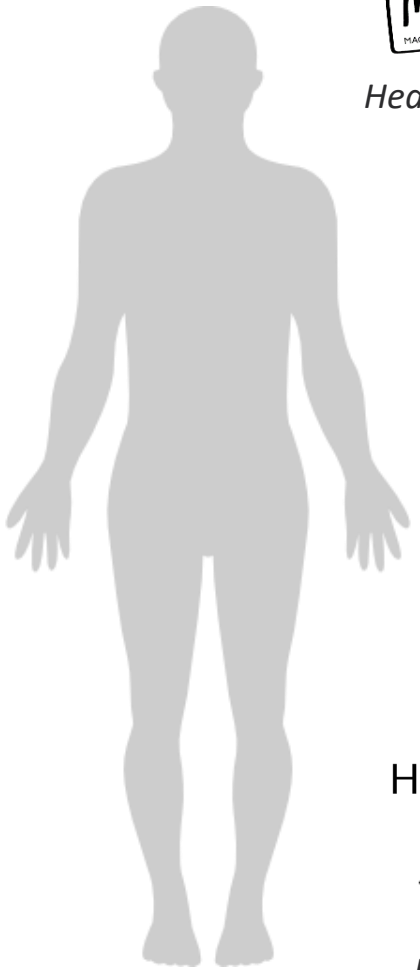
WHY DO WE NEED QUANTITATIVE GENOTOXICITY ASSESSMENT?



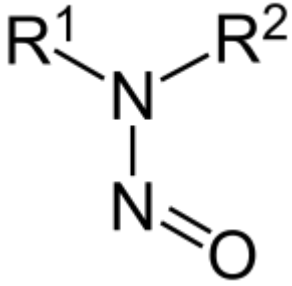
Polycylic aromatic hydrocarbons



Heavy metals

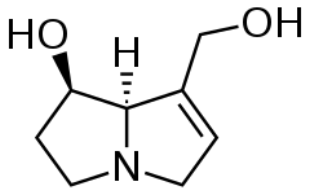


Unavoidable exposure to genotoxic contaminants



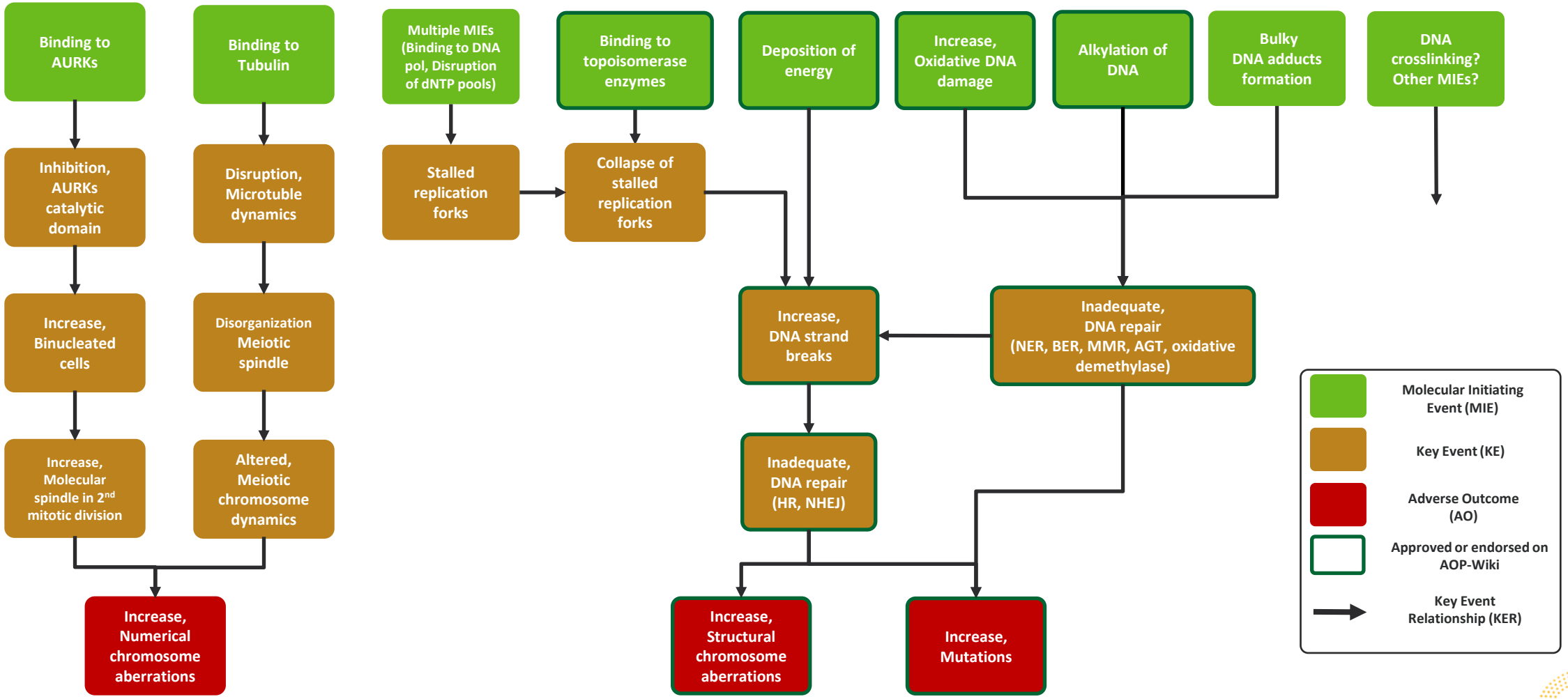
Nitrosamines

Mycotoxins



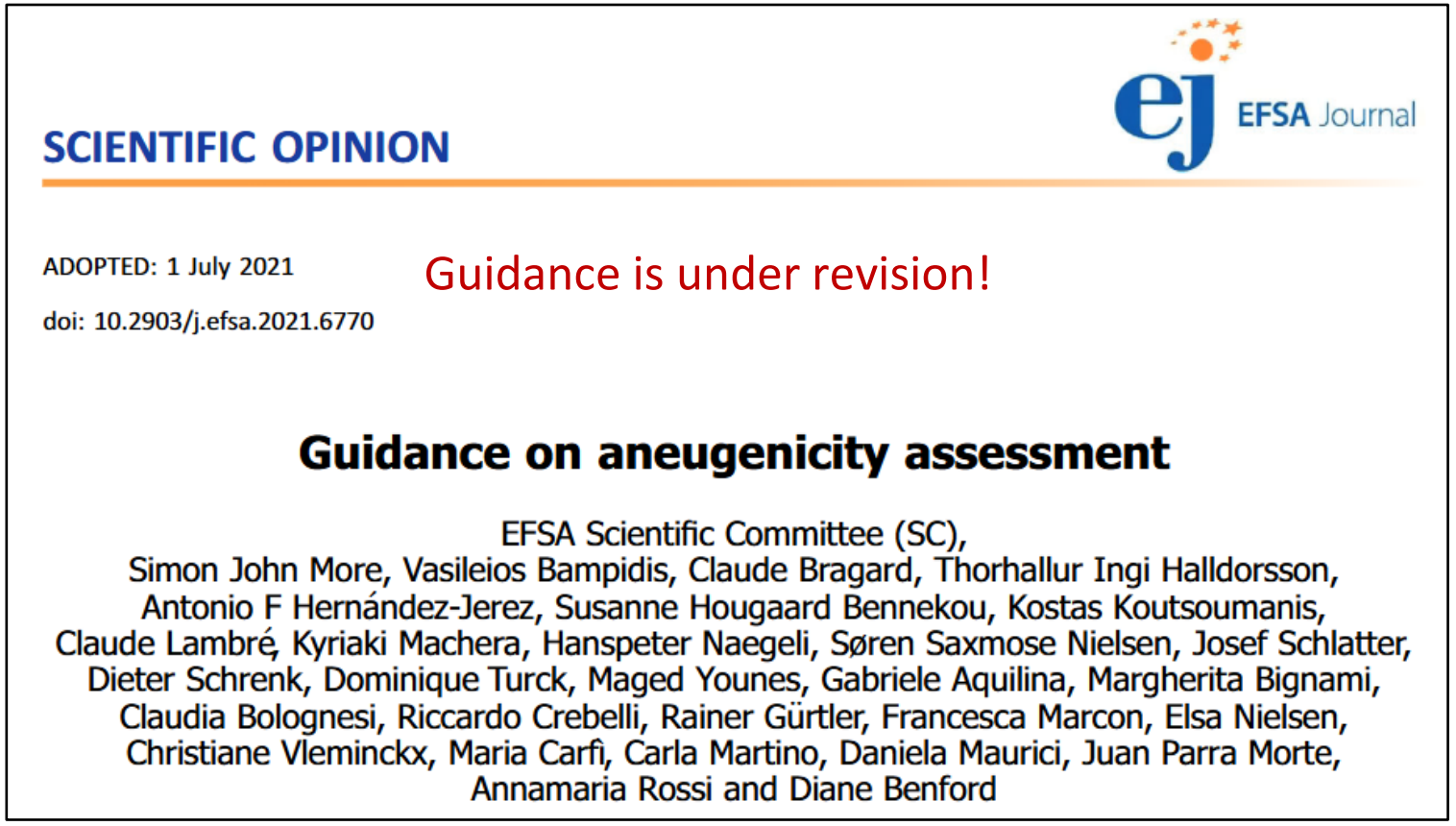
Pyrrolizidine alkaloids

WHY DO WE NEED QUANTITATIVE GENOTOXICITY ASSESSMENT?



Different types of genotoxic effects – DNA-reactive versus non-DNA-reactive

CURRENT EXPERIENCE



SCIENTIFIC OPINION

EFSA Journal

ADOPTED: 1 July 2021

doi: 10.2903/j.efsa.2021.6770

Guidance is under revision!

Guidance on aneugenicity assessment

EFSA Scientific Committee (SC),
Simon John More, Vasileios Bampidis, Claude Bragard, Thorhallur Ingi Halldorsson, Antonio F Hernández-Jerez, Susanne Hougaard Bennekou, Kostas Koutsoumanis, Claude Lambré, Kyriaki Machera, Hanspeter Naegeli, Søren Saxmose Nielsen, Josef Schlatter, Dieter Schrenk, Dominique Turck, Maged Younes, Gabriele Aquilina, Margherita Bignami, Claudia Bolognesi, Riccardo Crebelli, Rainer Gürtler, Francesca Marcon, Elsa Nielsen, Christiane Vleminckx, Maria Carfì, Carla Martino, Daniela Maurici, Juan Parra Morte, Annamaria Rossi and Diane Benford

Some experience available with aneugens

ANEUGENS – EFSA GUIDANCE

- A critical number of target sites must be affected for aneugenicity
- Non-linear and steep dose-response curve
- Quantification of biological response using *in vivo* data to determine a **point of departure** to support MOE approach or, in certain occasions, to derive **a HBGV**
- In absence of (adequate) *in vivo* data, a MOE approach may still be possible (if aneugen does not undergo metabolic activation):
  - Comparison between the concentrations resulting in aneugenicity *in vitro* and the concentrations estimated to be present in the GI tract from ingestion of food or beverage
  - Concentrations in the same order of magnitude = Concern

Two examples will be presented in breakout session on Quantitative genotoxicity assessment

OTHER NON-DNA-REACTIVE GENOTOXIC SUBSTANCES

- Indirect Modes of Action *(no direct DNA reactivity involved)*:
  - Secondary oxidative DNA damage
  - Topoisomerase inhibition (but NOT as a poison stabilizing the DNA-topoisomerase complex)
  - Inhibition of error-free DNA repair systems
- A “practical threshold” can be considered allowing to determine a point of departure and the application of the MOE approach
- Note: Essential to discount a direct effect - A simple demonstration of e.g. oxidative stress and DNA oxidation would not be sufficient as more than one mechanism may occur

Weight of Evidence - Possible role for AOPs and NAMs

DNA-REACTIVE GENOTOXIC SUBSTANCES

- Current strategy assumes no “safe” exposure “thresholds” for DNA-reactive substances
- In cases where data are limited, TTC has been employed (for contaminants, not for compounds deliberately added to the food) – Note: TTC is not risk-free

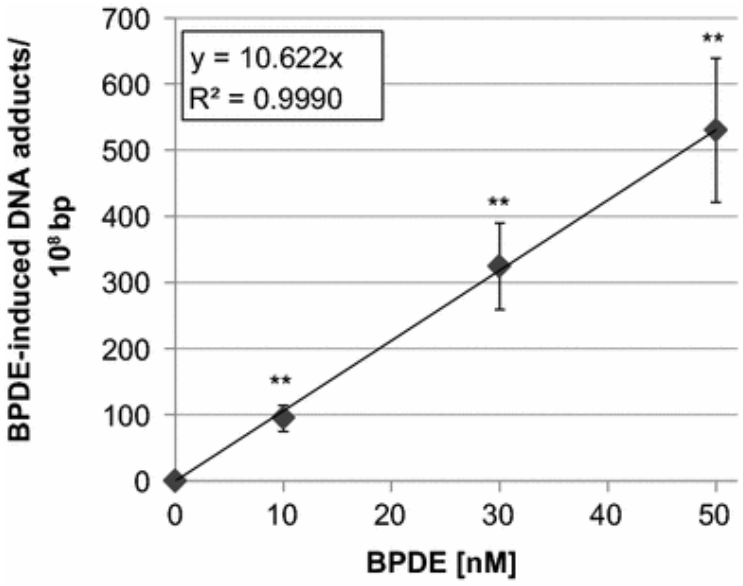
HOWEVER……..

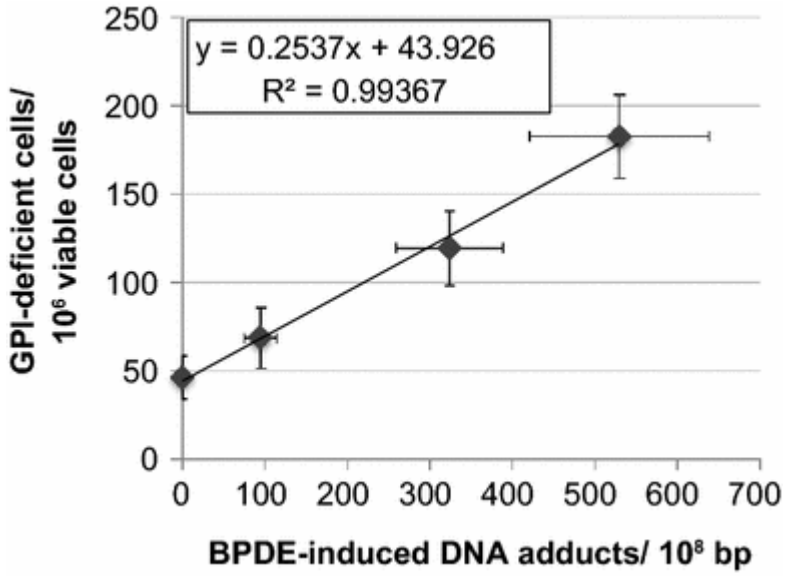
- Experimental and theoretical evidence questions the assumption of linearity and lack of a “threshold”
- Several processes may restrict DNA reactivity or conversion of DNA damage into mutagenicity, thus contributing to non-linear dose responses:
  - DNA Repair
  - Error-free translesion DNA synthesis
  - Metabolic detoxication
  - Antioxidant/Protective systems

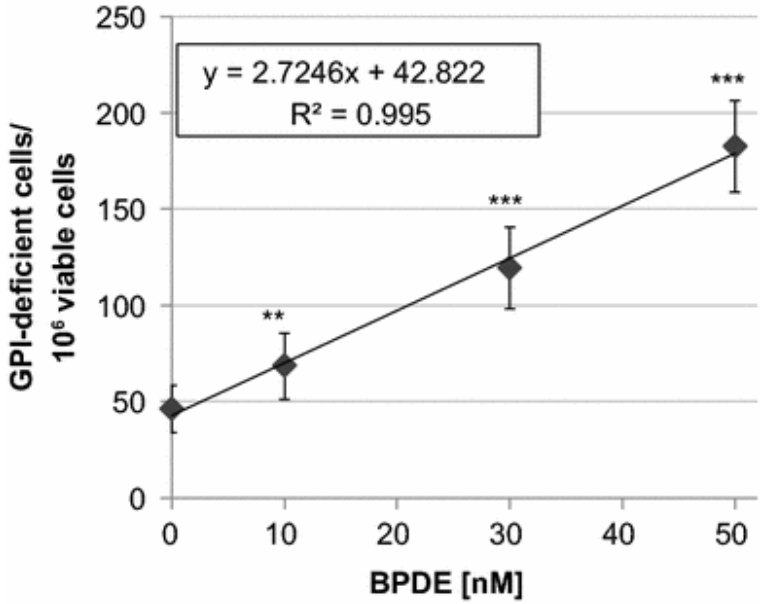
DNA-REACTIVE GENOTOXIC SUBSTANCES

Benzo[a]pyrene diol epoxide (BPDE)

Piberger et al ., 2018 Arch Toxicol. 92, 541-551



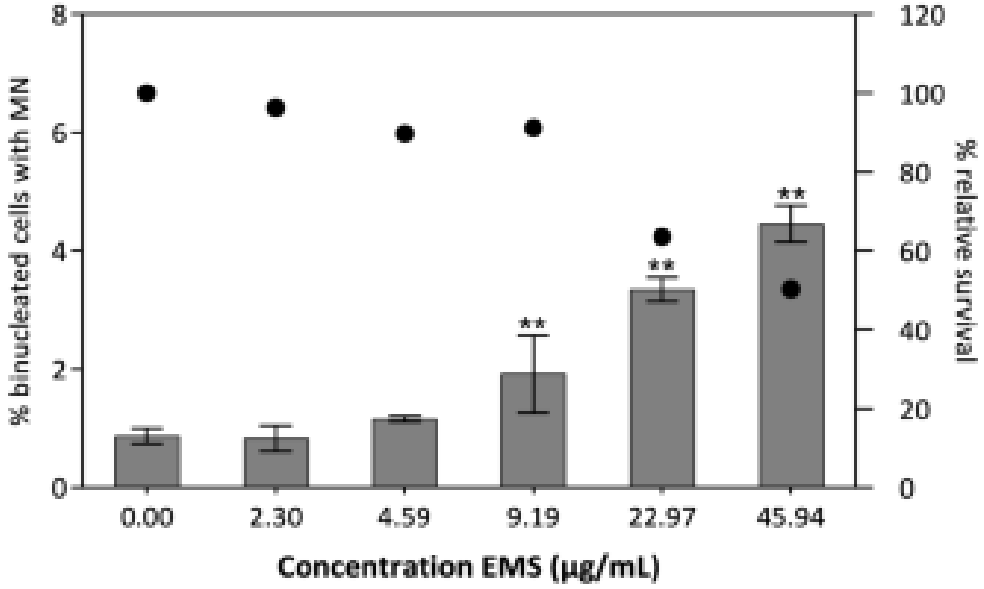




DIRECT ACTING GENOTOXIC AGENTS – NON-LINEAR RESPONSES

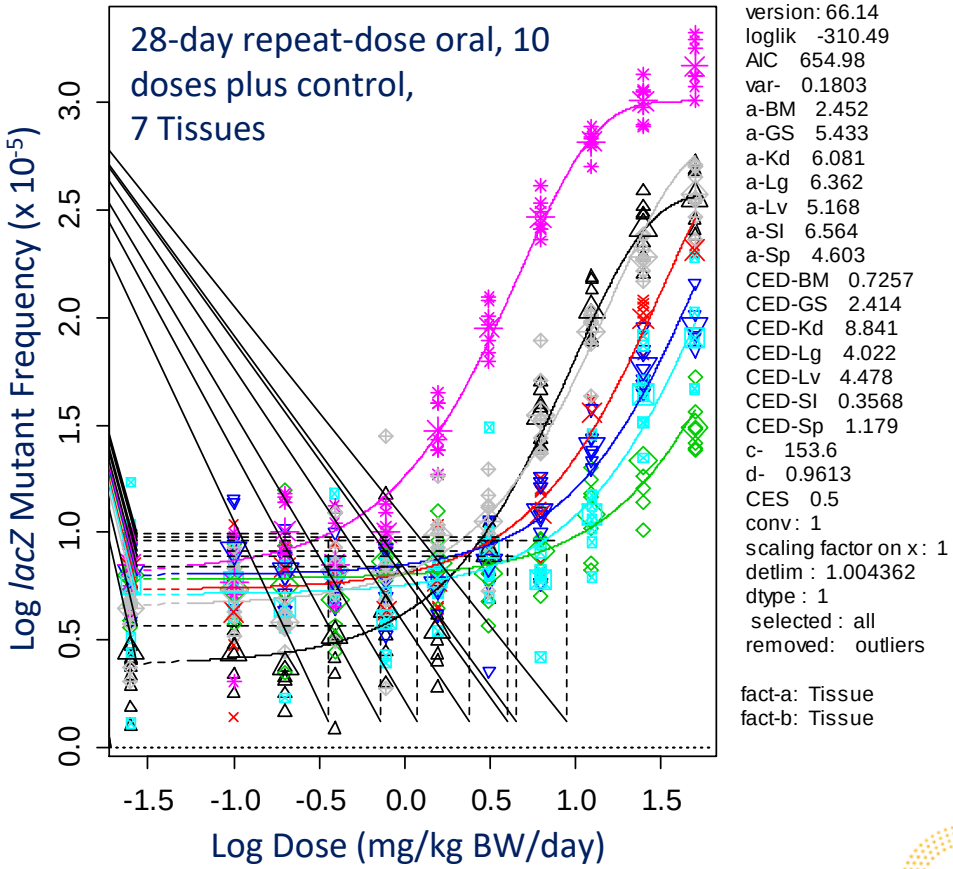
Ethyl methanesulfonate (EMS)

Sanders et al., 2025 Arch Toxicol. 99:1581-1589



Benzo[*a*]pyene

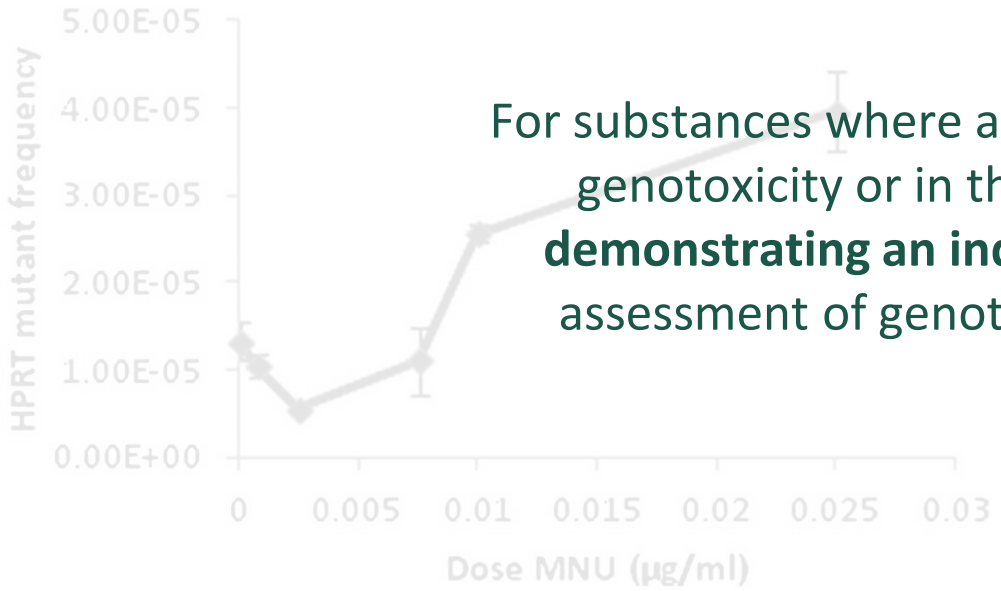
Long et al., 2018 Arch Toxicol. 92(2):967-982



Dose-response may depend on type of DNA lesion and DNA repair to remove lesions

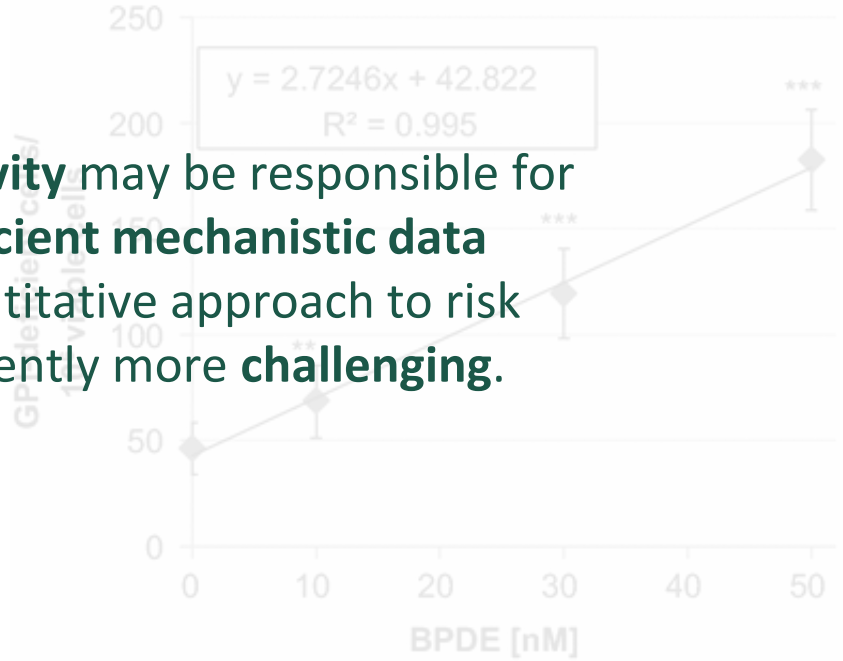
DIRECT ACTING GENOTOXIC AGENTS

Example of non-linear response



Johnson et al ., 2012 Genes and Environment

Example where non-linear response is not evident

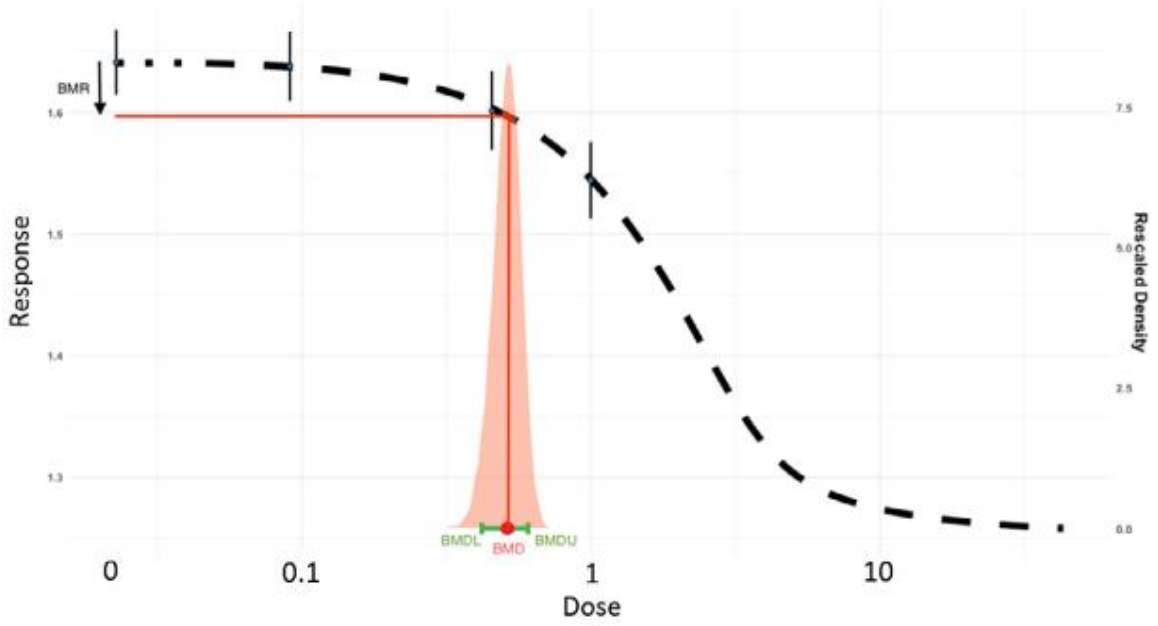


Piberger et al ., 2018 Arch Toxicol.

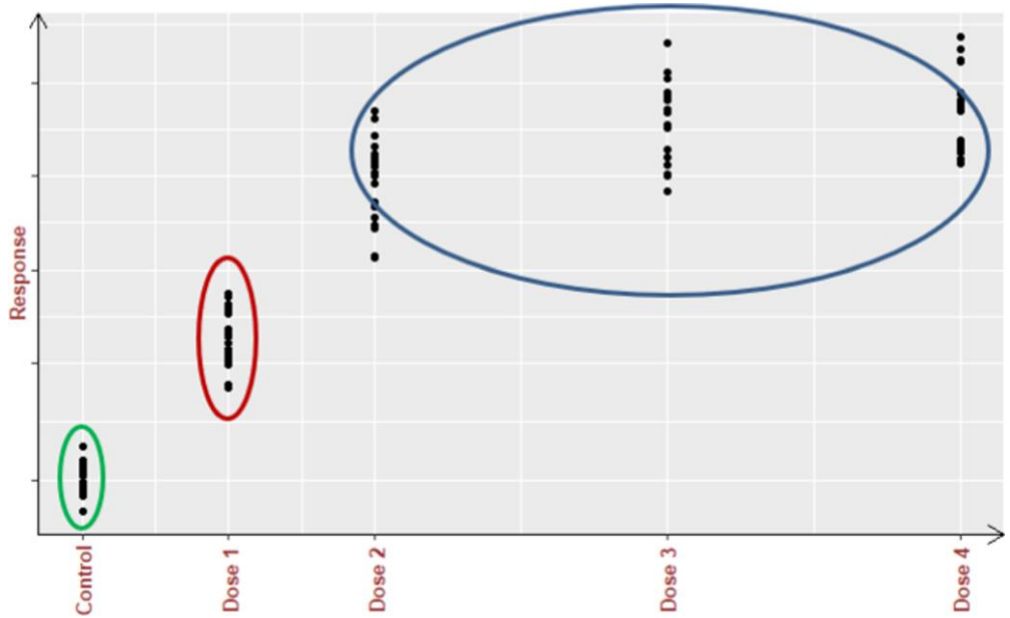
For substances where a **direct DNA reactivity** may be responsible for genotoxicity or in the **absence of sufficient mechanistic data demonstrating an indirect MoA**, a quantitative approach to risk assessment of genotoxicity data is currently more **challenging**.

Dose-response may depend on type of DNA lesion and DNA repair to remove lesions

HOW TO DO THE QUANTITATIVE GENOTOXICITY ASSESSMENT?



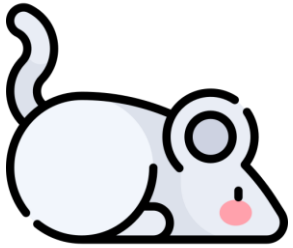
BMD analysis preferred approach



Experimental design is crucial

Sufficient data points & revised study design

BMD ANALYSIS WITH IN VIVO DATA



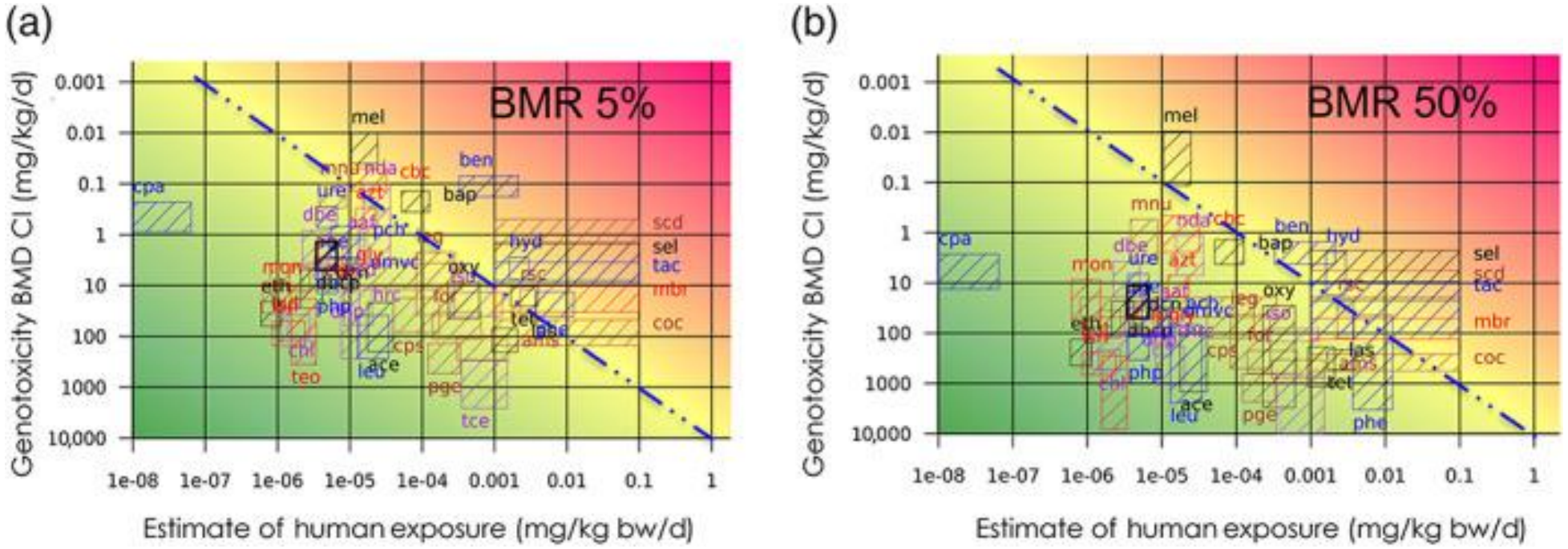
- What is the most appropriate BMR value?

  Current considerations focus on 50% (White et al. 2023 EMM, 66, 172)

- What is the most appropriate parameter from BMD analysis to be taken as point of departure (BMDL or other parameter)?

- More data are required on the comparison of measured BMD/BMDL values obtained from *in vivo* genotoxicity studies with equivalent values from carcinogenicity data on the same agents

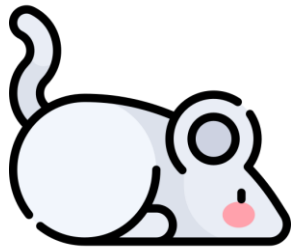
BMD ANALYSIS WITH IN VIVO DATA



"The results indicate that regulatory decisions based on in vivo genotoxicity dose-response data would be consistent with those based on carcinogenicity dose-response data; in some cases, genotoxicity-based decisions would be more conservative. Going forward, and in the absence of carcinogenicity data, in vivo genotoxicity assays (MN and TGR) can be used to effectively prioritise substances for regulatory action."

Chepelev et al ., 2023 EMM 92, 64(1):4-15

BMD ANALYSIS WITH IN VIVO DATA



- What is the most appropriate BMR value?

  Current considerations focus on 50% (White et al. 2023 EMM, 66, 172)

- What is the most appropriate parameter from BMD analysis to be taken as point of departure (BMDL or other parameter)?
- More data are required on the comparison of measured BMD/BMDL values obtained from *in vivo* genotoxicity studies with equivalent values from carcinogenicity data on the same agents
- What uncertainty factor would be required to allow a MOE assessment for *in vivo* genotoxicity?
- How can combined exposure to genotoxicants be taken into account?

BMD ANALYSIS WITH IN VITRO DATA

- What is the most appropriate BMR value?

  Less consensus

- What is the most appropriate parameter from BMD analysis to be taken as point of departure (BMDL or other parameter)?

- More data are required on the comparison of measured BMD/BMDL values obtained from in vitro genotoxicity studies with equivalent values from *in vivo* genotoxicity/carcinogenicity data on the same agents

- What uncertainty factor would be required to allow a MOE assessment for *in vitro* genotoxicity?

- How can combined exposure to genotoxicants be taken into account?

  + *In vitro* to *in vivo* extrapolation needed

SUMMARY

Aneugens and other (indirect acting) genotoxic agents

- In cases where genotoxicity data are demonstrating either aneugenicity or another indirect, clearly defined Mode of Action (MoA) with support of mechanistic data (*in vivo*) that does not imply direct DNA reactivity, quantitation of the response to determine a practical threshold and point of departure could allow a MoE approach for risk assessment.
- Such MoAs may include secondary oxidative DNA damage, inhibition of DNA repair or topoisomerase inhibition.

Genotoxic agents for which a direct DNA reactivity may be responsible

- For substances where a direct DNA reactivity may be responsible for genotoxicity or in the absence of sufficient mechanistic data demonstrating non-DNA-reactive mechanisms, a quantitative approach to risk assessment of genotoxicity data is currently considered more challenging.
- The information necessary for the potential future application of quantitative genotoxicity approach will be identified in the guidance.

STAY CONNECTED

SUBSCRIBE TO
efsa.europa.eu/en/news/newsletters
efsa.europa.eu/en/rss
Careers.efsa.europa.eu – job alerts

LISTEN TO OUR PODCAST
Science on the Menu –Spotify, Apple Podcast and YouTube

FOLLOW US ON BLUESKY
@efsa.bsky.social
@efsa-animals.bsky.social @efsa-plants.bsky.social

FOLLOW US ON LINKEDIN
Linkedin.com/company/efsa

FOLLOW US ON INSTAGRAM
@onehealth_eu

CONTACT US
efsa.europa.eu/en/contact/askefsa

EUROPEAN FOOD SAFETY AUTHORITY