INTERNATIONAL SYMPOSIUM: RISK ASSESSMENT OF GENOTOXIC COMPOUNDS.

SESSION I: "CLASSICAL APPROACHES" IN GENOTOXICITY ASSESSMENT

Diane Benford, EFSA Scientific Committee

EUROPEAN FOOD SAFETY AUTHORITY

CONTENTS

- Guidance
- EFSA testing strategy
- Issues arising
  - In vivo UDS
  - Demonstration of bone marrow exposure
  - Aneugenicity
- Reliability and relevance
- Margin of exposure (MOE) for substances that are genotoxic and carcinogenic
- Future developments

- Main focus is on assessment of chemicals in food

RELEVANT GUIDANCE

- EFSA
- Genotoxicity testing strategies (EFSA J. 2011)
- Clarification of some aspects of genotoxicity assessment (EFSA J. 2017)
- Guidance on aneugenicity assessment (EFSA J. 2021)
- Harmonised approach for reporting reliability and relevance of genotoxicity studies (2023) (Technical Report)

- Margin of exposure (EFSA J. 2005)

- WHO
- Environmental Health Criteria vol 240

EFSA OPINION ON GENOTOXICITY TESTING STRATEGIES

European Food Safety Authority

EFSA Journal 2011;9(9):2379

SCIENTIFIC OPINION

Scientific opinion on genotoxicity testing strategies applicable to food and feed safety assessment [1]

EFSA Scientific Committee[2, 3]

European Food Safety Authority (EFSA), Parma, Italy

This Scientific Opinion, published on 3 October 2012, replaces the earlier version published on 30 September 2011.

EFSA OPINION ON GENOTOXICITY TESTING STRATEGIES 2011

Objective of genotoxicity testing:

- Identify substances that could cause heritable damage in humans
- Predict potential genotoxic carcinogens where carcinogenicity data are not available
- Contribute to understanding of mechanism of action of chemical carcinogens

- Testing aims to identify **hazard** in relation to the different genotoxic endpoints:
- Induction of gene mutations
- Structural chromosomal aberrations (clastogenicity)
- Numerical chromosomal aberrations (aneugenicity)

- No single test can simultaneously provide information on all these end-points

EFSA OPINION ON GENOTOXICITY TESTING STRATEGIES 2011

Tier 1: the basic battery:

- Bacterial reverse mutation test in *Salmonella typhimurium* and *Escherichia co*li (OECD TG 471):
  end-point considered - gene mutation.
- *In vitro* mammalian cell micronucleus test – MNT (OECD TG 487):
  end-points considered - structural and numerical chromosome aberrations.

- Outcome:
  - Negative:
    No further testing unless available information indicates the inadequacy of the *in vitro* systems.
  - Positive:
    In vivo testing is required

- Requirements may differ in some sectors.

EFSA OPINION ON GENOTOXICITY TESTING STRATEGIES 2011

Tier 2: Follow-up of positive results

- Selected case-by-case based on, e.g. *in vitro* test results, structure activity relationships (SAR), metabolic and toxicokinetic considerations, potential for site of contact effects
- Endpoint chromosome aberration:
  - Mammalian erythrocyte micronucleus test in bone marrow or peripheral blood (OECD TG 474).
- Endpoint gene mutation:
  - Transgenic rodent gene somatic and germ cell gene mutation assays (OECD TG 488).
  - Mammalian erythrocyte Pig-a gene mutation (OECD TG470
- Indicator test for gene mutation and/or structural chromosome aberration
  - *In vivo* mammalian alkaline Comet assay using a range of tissues (e.g. liver, GI tract plus others as relevant)
    - (OECD TG 489).

EFSA OPINION ON GENOTOXICITY TESTING STRATEGIES 2011

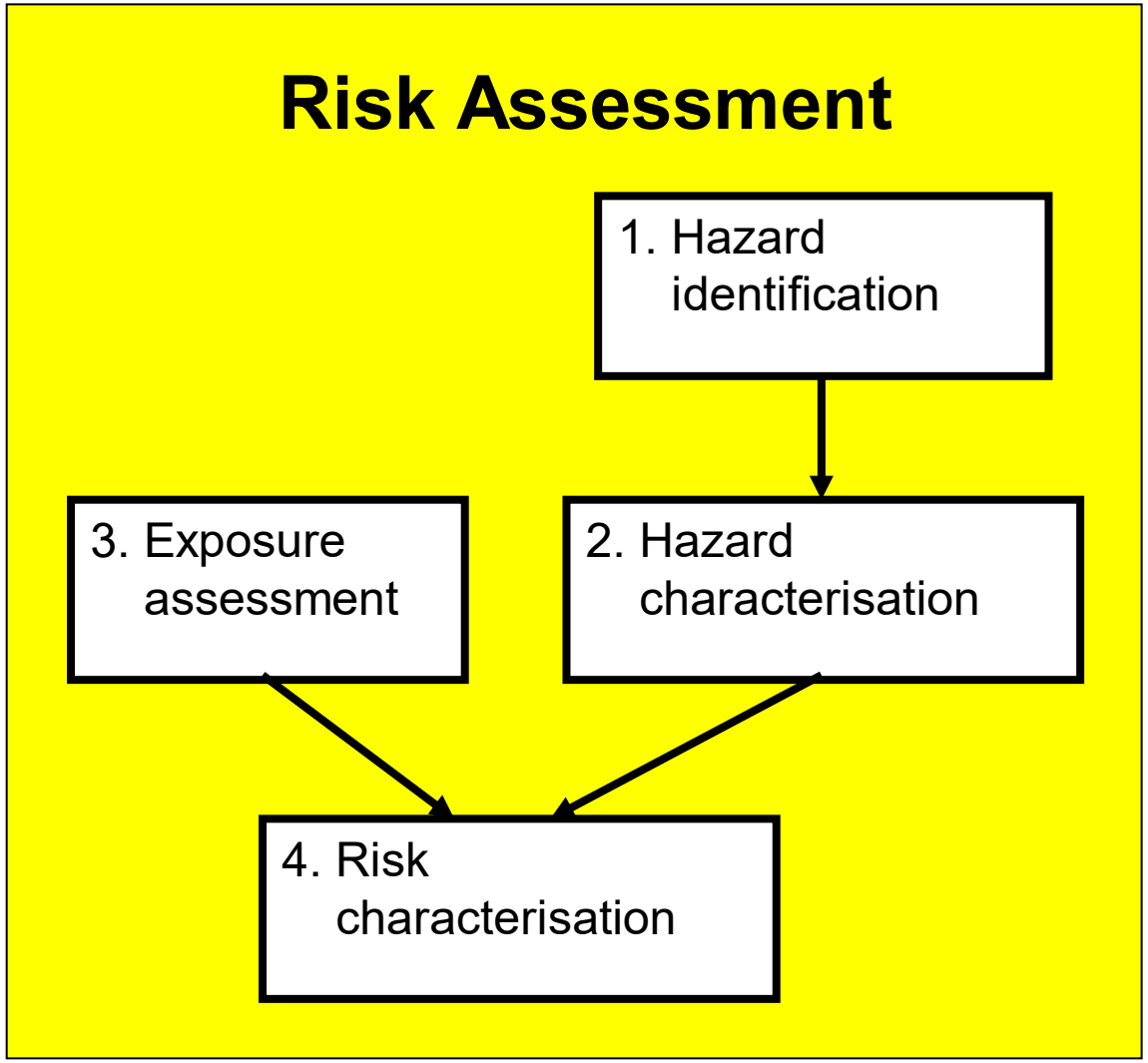
Outcomes of *in vivo* genotoxicity testing:

- Outcome:
  - Negative (with evidence of target cell exposure): No further testing required
  - Positive: Genotoxic hazard: Assessment stops

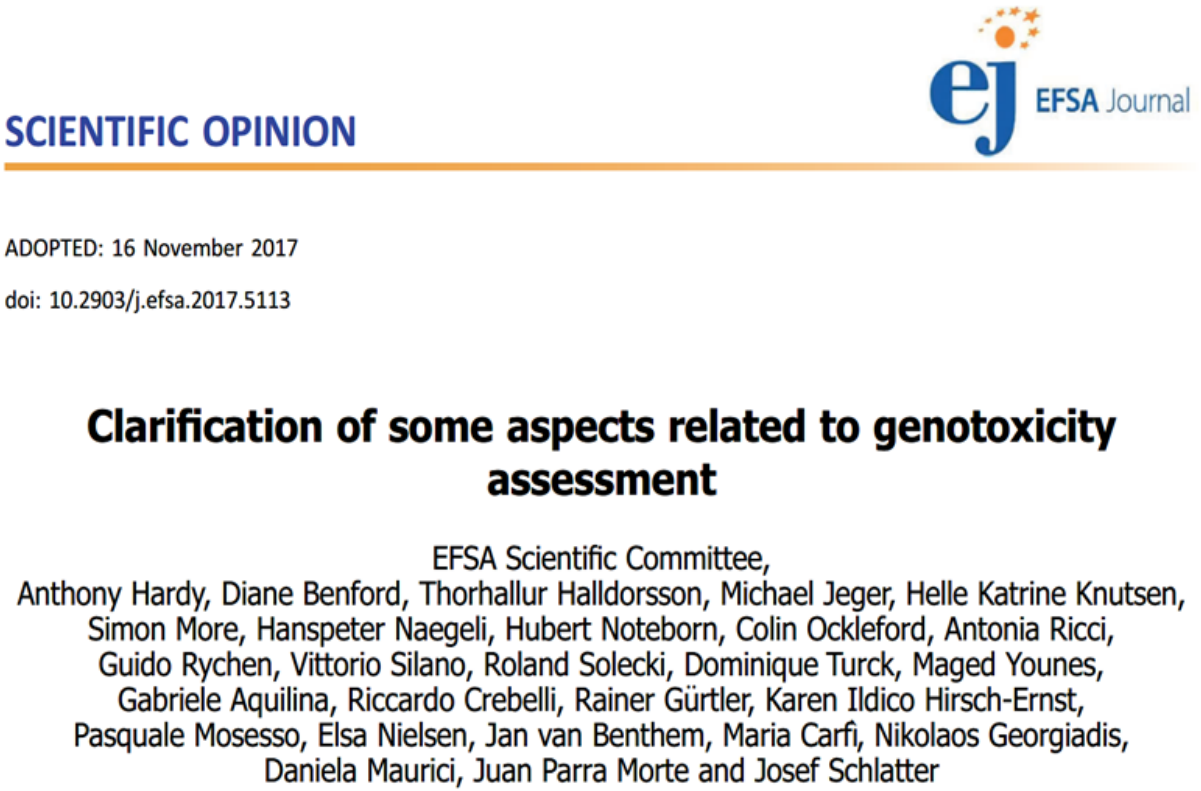
- Substances positive in tests in somatic cells are assumed to reach germ cells and to be germ cell mutagens;
- Even in the presence of negative carcinogenicity data, genotoxicity *in vivo* in somatic cells is considered an adverse effect *per se*.

- A conclusion of genotoxic hazard indicates a health concern
- No quantitative risk assessment is performed

RISK ASSESSMENT PARADIGM



EFSA CLARIFICATIONS (2017)



SCIENTIFIC OPINION

EFSA Journal

ADOPTED: 16 November 2017

doi: 10.2903/j.efsa.2017.5113

Clarification of some aspects related to genotoxicity assessment

EFSA Scientific Committee,
Anthony Hardy, Diane Benford, Thorhallur Halldorsson, Michael Jeger, Helle Katrine Knutsen, Simon More, Hanspeter Naegeli, Hubert Noteborn, Colin Ockleford, Antonia Ricci, Guido Rychen, Vittorio Silano, Roland Solecki, Dominique Turck, Maged Younes, Gabriele Aquilina, Riccardo Crebelli, Rainer Gürtler, Karen Ildico Hirsch-Ernst, Pasquale Mosesso, Elsa Nielsen, Jan van Benthem, Maria Carfi, Nikolaos Georgiadis, Daniela Maurici, Juan Parra Morte and Josef Schlatter

CLARIFICATION FOR GENOTOXICITY ASSESSMENT, 2017

1 When and how is the *in vivo* Unscheduled DNA Synthesis (UDS) test suitable to follow up a positive *in vitro* gene mutation test results?

- UDS detects the induction of unscheduled DNA synthesis (i.e. in cells that are not in the S-phase) in the liver of treated adult rats ➔ test designed to respond to substances that induce a type of **DNA damage repaired by excision repair** but not by other mechanisms and not unrepaired genetic damage
- Negative *in vivo* UDS alone is insufficient to rule out *in vivo* genotoxic potential
- The EFSA SC guidance (2011) states:…" *However, UDS has a limited use for cells other than liver and its sensitivity has been questioned…"*

USEFULNESS OF THE IN VIVO UDS ASSAY

- Assessments of existing data:
- existing UDS results may be considered as **adequate only in the case of positive results**.
- If negative, evaluation in a WoE approach considering all available info on MOA before deciding if more reliable tests (TGR or *in vivo* comet) would be needed to complete the assessment

- Future assessments:
- not aware of situations or chemical classes that can be identified, where the UDS could be considered preferable to TGR or comet assay.
- **Recommendation to no longer perform UDS test**

CLARIFICATION OF GENOTOXICITY ASSESSMENT, 2017

2 How to verify the exposure of the bone marrow (BM) in a negative *in vivo* Micronucleus test (MNT)

- **Lines of evidence of bone marrow exposure**
  1. Toxicity in the BM in the MNT (decreased PCE/NCE)
  2. Toxicity studies demonstrating effects in the BM
  3. ADME study demonstrating substance and/or its metabolites can reach BM)
  4. Systemic toxicity observed in the MNT
  5. Systemic toxicity observed in toxicity studies (same route of administration and same species used in the MNT)
  6. Substance/metabolites detected systemically in ADME studies
  7. Substance detected systemically in an appropriate blood/plasma analysis (e.g. appropriate analytical method)

CLARIFICATION OF GENOTOXICITY ASSESSMENT, 2017

3

Establishing Health-Based Guidance Values

- Establishing a Health Based Guidance Value (HBGV) might be possible when:

1. the overall evaluation leaves no concern for genotoxicity *in vivo*
2. genotoxicity is due to doses resulting in saturation of detoxification pathways
3. substances interact with molecular targets other than DNA

For 2 and 3, robust data on underlying MOA are essential

- If, based on the overall assessment, concern for genotoxicity remains, derivation of a HBGV is not considered appropriate.

- EHC240 also specifies that derivation of a HBGV is not considered appropriate when there is a concern for genotoxicity.

ANEUGENICITY ASSESSMENT, 2021

EFSA Journal

SCIENTIFIC OPINION

ADOPTED: 1 July 2021

doi: 10.2903/j.efsa.2021.6770

Guidance on aneugenicity assessment

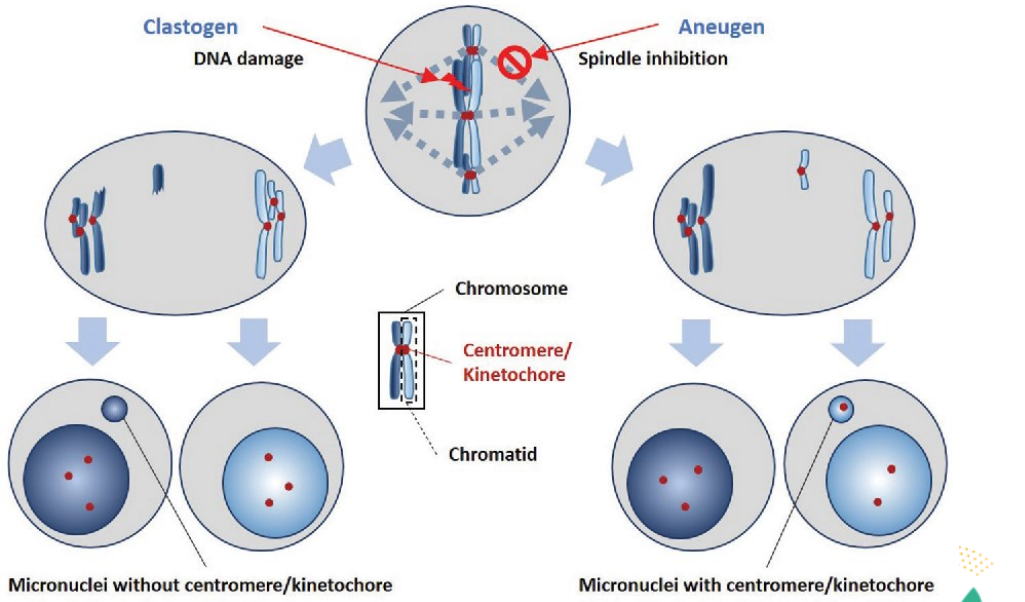
EFSA Scientific Committee (SC),
Simon John More, Vasileios Bampidis, Claude Bragard, Thorhallur Ingi Halldorsson, Antonio F Hernández-Jerez, Susanne Hougaard Bennekou, Kostas Koutsoumanis, Claude Lambré, Kyriaki Machera, Hanspeter Naegeli, Søren Saxmose Nielsen, Josef Schlatter, Dieter Schrenk, Dominique 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

ANEUGENS

- **Clastogenic substances** induce **structural** chromosomal aberrations through **DNA breaks**.

Clastogenicity ≠ Aneugenicity

- **Aneugenic substances** induce **numerical** chromosomal aberrations through **interactions with cellular targets other than DNA**, such as proteins involved in the segregation of chromosomes during mitosis or meiosis.

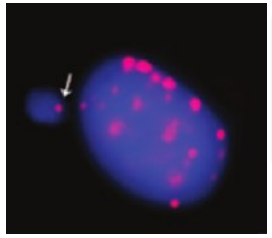


Mishima, 2017

A THRESHOLD FOR ANEUGENS

- It is theoretically possible that substances that induce gene mutations or clastogenicity can interact with DNA with a linear dose-response relationship (i.e. one single molecular interaction with can induce mutations.
  - Therefore a thresholded mechanism cannot be assumed and establishing a health-based guidance value (HBGV) is not considered appropriate.
- Aneugens have non-DNA targets and induce abnormal chromosome segregation, interacting with a variety of molecular and structural targets of the mitotic/meiotic machinery within the cell (e.g. colchicine, carbendazim, mebendazole, nocodazole)
  - A critical number of molecular events must occur for the aneugenic effect
  - A steep dose-response relationship is typically seen
  - A thresholded mechanism is plausible and an HBGV can be established, taking into account the entire toxicological database

EFSA GUIDANCE ON ANEUGENICITY ASSESSMENT



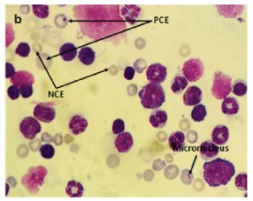
Melo, 2014

OECD TG 487

Positive *in vitro* MN +S9 and/or −S9 (aneugenic mechanism confirmed by FISH/CREST test)

In vivo MN in mammalian erythrocytes

OECD TG 474



3Rs: possible integration in 28day repeated-dose toxicity studies

Positive result

Risk assessment

Negative result

Evidence of BM exposure (EFSA J, 2017b)

No safety concern

No evidence of BM exposure

MN in Liver*

MN in GIT**

Proposed testing scheme for aneugenic substances for which induction of gene mutation and clastogenicity has been already ruled out

*:For a positive *in vitro* MNT**+S9.** In the process of being considered for the development of an OECD TG (Kirkland et al., 2019).

: For a positive *in vitro* MN test in the **absence of S9, and after negative results in an *in vivo* MN with no evidence of bone marrow (BM) exposure, a GIT MN assay would be appropriate, but more work is required for an OECD TG.

POTENTIAL APPLICATION OF MN IN LIVER AND GASTROINTESTINAL TRACT

Aneugenic compounds detected *in vitro* exclusively or predominantly **in the presence of liver S9 fraction**, suggesting the involvement of liver-specific metabolites, **can be evaluated in the liver MN assay**.

Aneugenic substances inducing increased *in vitro* micronuclei frequency in **the absence of S9 fraction, can be evaluated with the MN assay in the GIT.**

Data generated by applying the MN test in the stomach and colon are evaluated **case-by-case as supporting evidence** when obtained using appropriate dosing period and dose levels (Kirkland et al., 2019) in the comprehensive evaluation of all data to assess the *in vivo* aneugenic hazard.

RELIABILITY AND RELEVANCE, 2023

Technical Report

efsa SUPPORTING PUBLICATIONS

APPROVED: 14 September 2023
doi: 10.2903/sp.efsa.2023.EN-8270

Harmonised approach for reporting reliability and relevance of genotoxicity studies

European Food Safety Authority (EFSA),
Cristina Andreoli, Gabriele Aquilina, Margherita Bignami, Claudia Bolognesi, Riccardo Crebelli, Maria Dusinska, Rainer Gürtler, Henriqueta Louro, Francesca Marcon, Elsa Nielsen, Josef Schlatter, Christiane Vleminckx, Maria Chiara Astuto, Alexis V Nathanail and Diane Benford

Abstract

This technical report describes an approach developed by the EFSA cross-cutting Working Group on Genotoxicity for the reporting of reliability and relevance of genotoxicity studies. The scope of this document is to ensure harmonisation and transparency of the approach for evaluation of genotoxicity evidence among EFSA Units dealing with scientific assessments. It is recommended to be used as a template for the drafting of genotoxicity assessments in EFSA Opinions.

RELIABILITY

- “Evaluating the inherent quality of a test report or publication relating to preferably standardised methodology and the way that the experimental procedure and results are described to give evidence of the clarity and plausibility of the findings” (Klimisch et al., 1997)
- Including compliance with the OECD Test Guidelines (TGs) or standardised methodology and the completeness of the reporting

1. Reliable without restriction
2. Reliable with restrictions
3. Not reliable
4. Not assignable

RELEVANCE OF THE TEST SYSTEM (HIGH, LIMITED OR LOW)

- Genetic endpoint: higher relevance is given to studies providing information on apical endpoints, i.e., gene mutations, structural and numerical chromosomal alterations.
- Supporting information may be obtained from indicator assays; exception is the in vivo Comet assay that is considered with high relevance when applied as follow-up to a positive in vitro result).
- Tests with high relevance for hazard identification:
- Bacterial reverse mutation test
- Mammalian cell gene mutation tests in vitro
- Micronucleus tests in vitro and in vivo
- Chromosomal aberration tests in vitro and in vivo
- Comet assay in vivo
- Mutation tests in vivo (e.g., in transgenic rodents and Pig-a)

SUPPORTING STUDIES

- Tests with lesser relevance include:
- Comet assay in vitro
- γH2AX assay,
- Growth Arrest and DNA Damage assay (GADD)
- Changes in the expression and/or function of genes involved in DNA repair
- Unscheduled DNA Synthesis (UDS) in vitro or ex vivo
- Sister Chromatid Exchange (SCE) assay
- Formation of DNA adducts in vitro or in vivo provide useful mechanistic information, in particular to clarify if the genotoxicity is due to a direct DNA reactive mechanism, and should thus, be considered in the weight of evidence (WoE) assessment.

RELEVANCE OF THE STUDY RESULTS (HIGH, LTD OR LOW)

Takes into account:

- Reliability of the results
- Relevance of the test system
- Other possible considerations
  - Route of administration

WEIGHT OF EVIDENCE

- Considers only results with high or limited evidence
- MOA studies as supporting evidence
- Qualitative method base on expert judgement

MARGIN OF EXPOSURE APPROACH

The EFSA Journal (2005) 282, 1-31

Opinion of the Scientific Committee on a request from EFSA related to

A Harmonised Approach for Risk Assessment of

Substances Which are both Genotoxic and Carcinogenic

(Request No EFSA-Q-2004-020)

(ADOPTED ON 18 OCTOBER 2005)

Relates to substances that are genotoxic by a direct mode of action

MOE APPROACH (EFSA, 2005)

- **Margin of Exposure (MoE)**
  - ratio of a defined reference point (RP) or point of departure (PoD) on the dose response curve to estimated human intake
  - Preferred RP is the lower confidence interval of the Bench Mark Dose resulting in 10% increase in tumour incidence (BMDL10) derived from a carcinogenicity study
  - larger the MoE the lower the concern
  - EFSA considered MOE of 10,000 is of “low concern”:
    - 100-fold for species differences and human variability in toxicokinetics and toxicodynamics
    - 100-fold for additional uncertainties in the carcinogenic process, and because the BMDL is not a NOAEL when considering substance that are genotoxic
  - Not viewed as a basis for allowing deliberate addition to foods, or use earlier in the food chain.
  - Provides advice to risk managers

DOSE RESPONSE MODELLING

- Different models give different results
- Results of model averaging is preferred
- Guidance on modelling is available from EFSA (2022) and EHC240

FUTURE ISSUES

- Need to provide risk assessment advice on substances for which it is not possible to calculate a $BMDL_{10}$ for carcinogenicity
  - High incidence of tumours at all doses
  - No carcinogenicity data for a genotoxic substance

- What about germ cell mutagens?

KEY ISSUES FOR MODELLING OF GENOTOXICITY DATA

- Selection of dataset
  - – Which endpoint is most sensitive/relevant?
  - Species/cell type
  - In vivo study design – single/repeat dose?
  - Are data suitable for dose-response modelling?
  - Use of in vitro data with PBTK extrapolation to in vivo?
- Benchmark response (BMR) (also referred to as critical effect size) for genotoxicity (continuous) data
  - "For continuous data, the BMR should reflect the dose where an effect becomes adverse and, therefore, depends on the nature of the endpoint selected (including apical and non-apical endpoints)" (EFSA, 2022)
  - "For continuous data, a biologically meaningful BMR depends on the type of end-point and therefore varies. Ideally, it is set numerically so that the BMR reflects the onset of a human-relevant adverse effect, meaning that a response above the BMR is considered adverse" (EHC 240)
- How do we interpret the MOE?

CONCLUSIONS ON ASSESSMENT OF GENOTOXICITY

- *In vitro* genotoxicity tests aim to identify genotoxic hazard
- *In vivo* genotoxicity tests aim to determine whether the genotoxic hazard identified *in vitro* is expressed in vivo
- There is currently no quantitative (dose response) assessment of the genotoxicity data

CONCLUSIONS ON THE USE OF THE MOE APPROACH

- The MOE approach for substances that are genotoxic and carcinogenic, based on carcinogenic potency, and is well accepted and has been used for almost 20 years
- An analogous MOE approach based on genotoxic potency would provide a valuable tool for risk assessors and regulators
- Depending on the approach taken it is possible to generate many different BMDL values for one substance. Therefore a systematic and transparent approach is required
- Latest guidance on BMD modelling (e.g. EFSA, 2022, EHC240) should be taken into account
- Development of such an approach for genotoxicity data should take into account the lessons learnt from the MOE for carcinogenicity
- A big challenge is how to interpret the value of an MOE, with scientific justification for defining an MOE of low concern

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