Stakeholder Workshop on EFSA's Genotoxicity Guidance Revision

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GENOTOXICITY TESTING STRATEGY DRAFT PROPOSAL

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EUROPEAN FOOD SAFETY AUTHORITY

THE CURRENT EFSA GENOTOXICITY TESTING STRATEGY (EFSA SC, 2011)

A tiered approach is recommended

- **Tier 1 – in vitro testing**. The following two assays are recommended:
  - Bacterial reverse mutation test (Ames test) (OECD TG471)*
  - In vitro micronucleus test (OECD TG487)

If the results of Tier 1 are

- **negative**: no further testing is required
- **equivocal**: further in vitro or in vivo testing may be required to clarify the results obtained
- **positive** (any test): an in vivo follow up is required (Tier 2)

* mammalian cell gene mutation (MCGM) assay for nanomaterials

THE CURRENT EFSA GENOTOXICITY TESTING STRATEGY (EFSA SC, 2011)

- **Tier 2 – in vivo testing**. The following assays are recommended*:
  - Mammalian erythrocyte micronucleus test (OECD TG 474)
  - Transgenic rodent gene somatic and germ cell gene mutation assays (OECD TG 488)
  - In vivo mammalian alkaline comet assay (OECD TG 489)

*The choice of the in vivo tests to be performed (one or more) is guided by the following criteria:

- The in vivo test should cover the same end-point(s) tested positive in vitro
- Adequate exposure of the target tissue should be achieved under the test conditions

If the results of Tier 2 are

- **negative**: no further testing is required (no genotoxic hazard)
- **positive** (any test): generally, no further tests (e.g. in germ cells) are requested. A genotoxic hazard is identified irrespective of carcinogenicity data

ISSUES UNDER REVISION/DISCUSSION IN THE REVISED EFSA GENOTOXICITY TESTING STRATEGY

1. The tiered approach for genotoxicity assessment
2. The basic test battery (Tier 1)
3. The role of NAMs and QSAR
4. The in vivo follow-up (Tier 2)
5. Aneugenicity assessment
6. Bone marrow and target tissue exposure
7. Evaluation of nanomaterials *(not addressed in this presentation)*
8. New in vivo test methods
9. The evaluation of the scientific evidence
10. The quantitative evaluation of genotoxicity data *(discussed separately)*

1. THE APPROACH TO GENOTOXICITY ASSESSMENT

A **tiered approach** (in vitro/in vivo) to the genotoxicity assessment of food chemicals was recommended by the Scientific Committee on Food (2001) and endorsed by EFSA in the 2011 Guidance.

The tiered approach is maintained in the revised testing strategy based on:

- **Available experimental evidence** (low incidence of unique in vivo mutagens)
- **Animal protection criteria** (because in the tiered approach no in vivo testing is normally considered when the results in the base set of in vitro tests are clearly negative)

However, emphasis is given in the revised guideline to the **need to carefully evaluate whether the in vitro test conditions were appropriate for the test chemical**, especially the metabolic activation conditions, considering chemical structure and QSAR data, when available.

2. THE BASIC TEST BATTERY (IN VITRO TESTS FOR TIER 1 TESTING)

Two vs three tests

- The Genotoxicity WG considered that the currently recommended **two test battery** (Ames plus in vitro micronuleus test) **provides sufficient information**, covering the main three genotoxicity end-points (mutagenicity, clastogenicity and aneuploidy) with the minimum use of resources.
- The WG also noted that based on available evidence **the addition of further mammalian cell in vitro tests reduces specificity with no substantial gain of sensitivity**. Thus far the WG does not have a good reason to deviate from a two-test base set.

Ames vs. mammalian cell gene mutation (MCGM) assay

- It is already recommended to apply MCGM instead of the AMES test for nanomaterials and substances with bactericidal properties.
- Application of gene mutation test in mammalian cells (MCGM, either OECD TG476 or TG490) as a potential alternative to the Ames test (OECD TG471) generally for soluble materials is **under discussion.**

MCGM VS. AMES TEST

↑ Pros

- Higher biological relevance for human risk assessment
- Wider applicability (nanomaterials, metals, larger particles, insoluble materials and compounds with biocidal properties)
- Less sensitive to bias from food components (e.g. amino acid residuals in complex food or natural matrix)
- Other?

↓ Cons

- Lower end-point specificity (esp. OECD TG490), with consequent difficulties in deciding the appropriate in vivo follow up?
- Other?

3. THE ROLE OF NAMs AND QSAR

- In the 2011 EFSA Guidance, the SC recommended that «*Before embarking on any testing, it is important for the appropriate conduct of the tests to consider other relevant knowledge on the substance under consideration such as its physico-chemical properties and experimental data on its toxicokinetics*»

- Since then, considerable progress has been made in the development of *in silico* and *in vitro* methods based on the application of new technologies (e.g. 3D models, transcriptomics, reporter-based systems), collectively referred to as New Approach Methodologies (NAMs).

- **The revised guideline supports the integration of toxicokinetic (TK), *in silico* and NAMs data in an IATA (Integrated Approaches for Testing and Assessment) approach**.

3. THE ROLE OF NAMs AND QSAR

In the updated guideline, **information on Mode of Action (MoA) provided by NAMs**, integrated in an AOP framework, **could support the follow-up of *in vitro* positives** with the aim to **optimize** the approach and **reduce**, or even **eliminate**, the **reliance on animal data**.

In particular, guidance is provided on:

- **The actual need for an *in vivo* follow-up** (e.g. in case it can be demonstrated that the MoA is not relevant for human risk assessment)
- **The choice of the appropriate *in vivo* assay(s)**, addressing the most relevant genetic end-point
- **The appropriate study design** (target tissue, type of exposure - e.g. single vs. repeated, dose levels, etc.)

4. THE IN VIVO FOLLOW-UP (TIER 2)

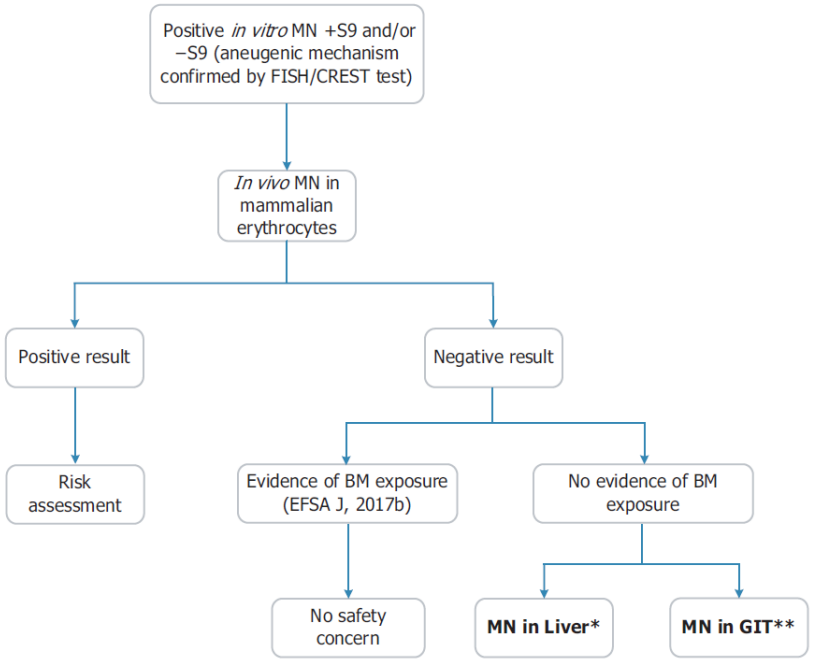
The set of test methods recommended for the *in vivo* follow-up of *in vitro* positives is updated with the inclusion of the new OECD Test Guideline on Pig-A (TG470):

- In vivo mammalian erythrocyte (bone marrow or peripheral blood) micronucleus test (OECD TG 474)
- In vivo bone marrow chromosomal aberration test (OECD TG 475)
- Transgenic rodent somatic and germ cell gene mutation assays (OECD TG 488)
- Mammalian erythrocyte Pig-A gene mutation assay (OECD TG 470) (new)
- In vivo mammalian alkaline comet assay (OECD TG 489)

Emphasis is given to the **integration of multiple assays in one study**, including repeat dose toxicity assays, in order to **minimize the use of animals**.

5. ANEUGENICITY ASSESSMENT

- The approach to the **follow-up of substances aneugenic in vitro** (i.e. tested positive in the in vitro MN test with CREST/FISH analysis of MN) proposed in the EFSA Guidance on Aneugenicity (EFSA, 2021) is revised



EFSA, 2021

Based on recent experience with aneugenic food chemicals, the proposal to perform an in vivo micronucleus test in bone marrow as first option is not recommended any longer.

For direct acting substances, the effects at the site of contact should be considered first. This could rely on test methods applicable in the G.I. tract (e.g. micronucleus) when available, or on the application of a MOE approach based on the in vitro RP for aneugenicity.

For indirect acting aneugens, the use of the liver MN test, currently under validation, is supported.

6. BONE MARROW AND TARGET TISSUE EXPOSURE

Criteria to demonstrate bone marrow exposure in tests in erythropoietic cells (micronucleus, Pig-A, CA assays) set out in the EFSA 2017 opinion are further expanded and their implications in the in vivo follow-up clarified:

- As a well-perfused tissue, bone marrow is considered representative of all tissues systemically exposed to a substance following oral exposure. Consequently, in case bone marrow exposure is clearly demonstrated, a negative outcome in the micronucleus assay is considered sufficient to rule out a concern for systemic genotoxicity.
- However, if bone marrow exposure is not adequately demonstrated, further testing is required:
  - For test items positive in vitro in the absence of metabolic activation, potential effects in GI tract are to be investigated by Comet assay, transgenic rodent assay or micronucleus in GI tract (when validated)
  - For test items positive in vitro only in the presence of metabolic activation, both liver and duodenum investigation is recommended (considering enzymatic activity of enterocytes)

8. NEW IN VIVO TEST METHODS

- The updated guidance relies on the possible future application of the in vivo micronucleus assays in tissues other than erythropoietic cells (liver and gastro-intestinal tract), especially in the assessment of in vivo aneugenicity.
- Concerning other emerging methods, the application of next-generation sequencing (NGS) technologies in genotoxicity is likely to have a major impact on the current approach to in vivo genotoxicity assessment, providing information on mutational spectra across the entire genome out of reach of current test methods. However, further work to define strengths, limits and applicability of these NGS technologies is needed before their inclusion among recommended test methods in the updated guidance.
- Data obtained with the application of such emerging methods (ec-NGS, Duplex sequencing) are however considered as supporting information on a case-by-case basis.

9. THE EVALUATION OF THE SCIENTIFIC EVIDENCE

Criteria for evaluation of the quality and reliability of genotoxicity data in the WoE established in the EFSA 2011 guidance are expanded and refined in the updated document based on the recent EFSA Technical Report (2023).

- **Studies** are evaluated for **Reliability** in 4 categories based on Klimisch criteria considering the compliance to validated protocols and completeness of reporting
- **Test methods** applied are evaluated for **Relevance** (High, Limited, Low) considering the genetic end-point and status of validation
- **Test results** are evaluated for **Relevance** considering the reliability of the study, relevance of test system and other aspects (e.g. route of administration).

Only test results with sufficient (high or limited) relevance are included in the Weight of Evidence.

MAIN ISSUES FOR POTENTIAL DISCUSSION

1. **Composition of the base set of in vitro tests (Tier 1):**
   - MCGM as an alternative/complementary to the Ames test (also for substances other than nanomaterials)
2. **The role of NAMs and QSAR**
   - As supporting information in Tier 1 (in vitro hazard identification)
   - As supporting information in Tier 2 (on MoA and appropriate *in vivo* follow up)
3. **Aneugenicity assessment**
   - The in vivo follow-up
   - Risk characterisation (discussed separately)
4. **The role of new molecular in vivo methods**

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and feed-back!

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