Guidance Document #05
Genotoxicity Testing
April 2021
At Step 7: Steering Committee Endorsement

GENOTOXICITY TESTING

Endorsed by the Steering Committee in
March 2021

It is recommended for the companies willing to submit applications/dossiers for pre-market authorization, to contact the jurisdictions of the countries concern to confirm their acceptance of the current guidance document.

The International Cooperation for Convergence of Technical Requirements for the Assessment of Feed Ingredients (ICCF) was launched in 2017 and aims to develop and establish common guidance documents to provide technical recommendations for the assessment of feed ingredients, including new uses of existing feed ingredients.

This guidance document has been developed by the appropriate ICCF Experts Working Group and was subject to consultation by the Parties, in accordance with the ICCF Process.

The founding members of the ICCF include the Canadian Food Inspection Agency (CFIA), the European Commission (DG SANTE), the U.S. Food and Drug Administration (FDA), as well as the American Feed Industry Association (AFIA), the Animal Nutrition Association of Canada (ANAC), the EU Association of Specialty Feed Ingredients and their Mixtures (FEFANA) and the International Feed Industry Federation (IFIF).

Table of content

GENOTOXICITY TESTING

1. INTRODUCTION

1.1 Objective of the Guidance

In the assessment and evaluation of the safety of feed ingredients, the potential for genotoxicity is a key component in the battery of required toxicity endpoints to be considered. It is important to develop a consistent approach on how to assess the genotoxic potential of feed ingredients.

This document provides guidance to applicants for pre-market assessment on the approach to be taken to characterize the genotoxic potential of a feed ingredient, in accordance with the Section 1.4 (Scope). It has been developed by an international team of experts and considers the best practices for the characterization of the genotoxic hazard linked with the use of a feed ingredient.

While this guidance document supports the acceptability of the approach, applicants are advised to consult the appropriate regulatory authorities or guidelines during the development phase of a new feed ingredient or a new use of an authorized ingredient. This will help to determine whether this assessment is necessary.

1.2 Initial considerations

This guideline was developed after consideration of the current practices for evaluating feed safety in the United States of America (USA), European Union (EU), and Canada. It is one of a series of guidelines developed to facilitate the mutual acceptance of data necessary for the determination of the safety of feed ingredients. The coordination of regulatory requirements for the assessment of feed ingredients strives to eliminate repetitious and unnecessary animal testing. Existing guidance document from national and international jurisdictions were reviewed for best practices. When references are made to those published guidance documents, their latest versions should be referred to at the time the test is run.

The approach proposed in this guidance document should provide an adequate amount of toxicological information to ensure animal health and food safety, while reducing the number of animals used in testing and conserving resources. In all cases, when *in vivo* tests are necessary,

the number of animals should be justified scientifically and consider the tenets of the 3R's principles (replacement, refinement, and reduction) of animal testing. In certain cases, it might be appropriate to combine the proposed tests or to combine them with repeated dose toxicity tests.

When designing and carrying out the relevant tests, the welfare of the test animals should be addressed in accordance with national and international protocols. The use of animals in the tests should adhere to these protocols and should conform to general ethical standards and to the national standards for the use and care of experimental animals.

Note that there may be an obligation in certain jurisdictions for tests to be conducted in accordance with Good Laboratory Practices (GLPs). It is important that the applicant is aware of this requirement.

1.3 Definitions

The following definitions apply solely in the context of this guidance document:

Active substance: Any substance in a feed ingredient that contributes to the intended effect[1].

Aneugenicity[2]: The ability to cause a numerical deviation of the modal number of chromosomes in a cell or organism.

Aneuploidy[2]: Numerical deviation of the modal number of chromosomes in a cell or organism.

Chromosome aberration[2]: Any structural or numerical change of chromosomes.

Clastogenicity[2]: The ability to cause structural changes of chromosomes.

Feed (Feedingstuff)[3]: Any single or multiple materials, whether processed, semi-processed or raw, which is intended to be fed directly to animals.

[1] The intended effect refers to the conditions of use of the additive and not to the potential hazardous effect of the substance.
[2] Adapted from VICH GL 23 ® (1)
[3] Adapted from Codex Alimentarius, Code of Practice on good animal feeding (CAC/RCP 54-2004)

Feed Ingredient[3]: A component part or constituent of any combination or mixture making up a feed, whether or not, it has nutritional value in the animal's diet. Ingredients are of plant, animal, microbial or aquatic origin, or other organic or inorganic substances.

Gene mutation[2]: A detectable permanent change within a single gene or its regulating sequences. The change may be a point mutation, insertion, deletion, etc.

Genotoxicity[2]: A broad term that refers to any deleterious change in the genetic material regardless of the mechanism by which the change is induced.

Micronucleus[2]: Particle in a cell that contains microscopically detectable nuclear DNA; it might contain a whole chromosome or a broken centric or acentric part of chromosome. The size of a micronucleus is usually defined as less than 1/5 but more than 1/20 of the main nucleus.

Mutagenicity[2]: The capacity to cause a permanent or heritable change in the amount or structure of the genetic material in an organism or cell that may result in change in the characteristics of the organism or cell. The alteration may involve changes to the sequence of bases in the nucleic acid (gene mutation), structural changes to chromosomes (clastogenicity) and/or changes to the number of chromosomes in cells (aneuploidy or polyploidy).

Normochromatic erythrocytes (NCE): Mature erythrocytes.

Polychromatic erythrocytes (PCE): Immature erythrocytes also called reticulocytes.

Polyploidy[2]: Numerical changes of complete sets of chromosomes.

1.4 Scope of the Guidance

This guidance document addresses the approach to be taken to evaluate the genotoxic potential of feed ingredients and a description of the tests requested to support this approach. Depending on the jurisdiction and on the feed ingredient, the interpretation of the results of the tests could be used to assess the safety of the feed ingredient for the consumer of the food of animal origin and workers, exposed to the feed ingredient while handling it. It might also be used for the target animal safety assessment.

Tests aimed at evaluating the genotoxicity potential of a feed ingredient are to be considered for purified and/or standardized active substances, except for viable microorganisms.

2. GENERAL PRINCIPLES

Genotoxicity tests are designed to detect feed ingredients that have the potential to induce genetic damage by various mechanisms. These tests enable hazard identification with respect to damage to DNA and its fixation, including in the form of gene mutations, structural and numerical chromosomal damage, or recombination, which are generally considered to be essential for heritable effects. These effects may play a role in the complex multi-step process of carcinogenicity.

Numerical chromosome changes have also been associated with tumorigenesis and can indicate a potential for aneuploidy in germ cells.

In addition, the outcome of genotoxicity tests can be valuable for the interpretation of carcinogenicity tests.

Genotoxicity testing of feed ingredients is usually undertaken in a stepwise approach, as described in ANNEXES I and II:

- an initial screening based on read-across and *in silico* Quantitative Structure-Activity Relationship (QSAR) models
- an assessment *in vitro*, to determine any intrinsic genotoxic activity
- when a positive or equivocal response in the *in vitro* phase is realized, an *in vivo* evaluation to determine if any such activity is expressed in the whole animal.

When tests are set-up, it is recommended to follow internationally accepted guidelines, such as Organization for Economic Co-operation and Development (OECD) guidelines.

A weight-of-evidence approach is recommended to evaluate and interpret genotoxicity data. This must consider the quality and reliability of the data on genotoxicity itself and consider all relevant information and data, including:

- physicochemical characteristics of the feed ingredient
- chemical reactivity of the active substance, which might predispose to effects at the site of first contact in the gastro-intestinal tract of the animals
- structure activity relationships (including structural alerts of genotoxicity and “read-across”, from structurally related substances)
- bioavailability, toxicokinetic and metabolism, any target organ specificity, and
- the outcome of any repeated dose toxicity and carcinogenicity tests.

The conclusions to be drawn from the results of the test are indicative of the potential of the feed ingredient to be or not to be genotoxic.

3. *IN VITRO* TESTING

In vitro tests are commonly used as an initial step for the evaluation of the genotoxicity potential of feed ingredients. The *in vitro* tests aim at evaluating the different endpoints of the genotoxic effects:

- Gene mutation
- Numerical chromosomal aberration
- Structural chromosomal aberration

The recommended *in vitro* tests are:

- Bacterial Reverse Mutation Test (Ames Test) (OECD TG#471)
- *In vitro* Mammalian Cell Micronucleus Test (*in vitro* MNT – OECD TG#487)
- *In vitro* Mammalian Chromosomal Aberration Test (*in* vitro CAT - OECD TG#473)

It is recommended to combine the tests for fulfilling the basic requirements to cover all three (3) above-mentioned genetic endpoints. The use of alternative tests would require clear justification. The three (3) proposed tests provide information on different endpoints. The Ames Test provides information on the gene mutation and the *in vitro* CAT informs on the structural chromosomal changes, while the *in vitro* MNT informs on the structural and numerical chromosomal aberrations.

Three (3) *in vitro* tests are described below.

3.1 Bacterial Reverse Mutation Test (Ames Test)

A bacterial reverse mutation test (Ames Test) is recommended to be performed according to the protocol set out in OECD Test Guidelines #471 (2).

The Ames Test uses amino acid requiring strains of *Salmonella typhimurium* and/or *Escherichia coli* to detect point mutations by base substitutions or frameshifts.

The principle of this test is that it detects mutations which revert mutations present in the test strains and restore the functional capability of the bacteria to synthesize an essential amino acid. At least four different strains of *S. typhimurium* should be used. A fifth strain should be tested, either a fifth *S. typhimurium* strain or one *E. coli* strain.

3.2 In vitro Mammalian Cell Micronucleus Test (*in vitro* MNT)

An *in vitro* Mammalian Cell Micronucleus Test (*in vitro* MNT) is recommended to be performed according to the protocol set out in OECD Test Guidelines #487 (5).

The *in vitro* MNT is used for the detection of micronuclei in the cytoplasm of interphase cells. Micronuclei may originate from acentric chromosome fragments (i.e. lacking a centromere), or whole chromosomes that are unable to migrate to the poles during the anaphase stage of cell division. Therefore, the test detects the activity of feed ingredients that may be clastogenic and aneugenic, causing structural and numerical chromosome aberrations in cells that have undergone cell division during or after exposure to the feed ingredient.

When the *in vitro* MNT test is positive, it can be coupled with fluorescence in situ hybridization (FISH) or using the immunofluorescent antikinetochore (CREST) staining of micronuclei, to characterize the content of micronuclei and provide additional information on the prevalent mode of action (clastogenicity and/or aneugenicity).

3.3 *In vitro* Mammalian Chromosomal Aberration Test (*in vitro* CAT)

An *in vitro* Mammalian Chromosomal Aberration Test (*in vitro* CAT) is recommended to be performed according to the protocol set out in OECD Test Guidelines #473 (3).

The purpose of the *in vitro* CAT is to identify agents that cause structural chromosome aberrations in cultured mammalian somatic cells. Structural aberrations may be of two types: chromosome or chromatid. This test does not cover the endpoint aneuploidy and should therefore be used in combination with one or both other tests, described previously. The *in* vitro CAT has been used extensively for certain types of feed ingredients (e.g., enzyme preparations) (14).

3.4 Modification of the tests

In a few instances, applicant(s) may use alternative tests to those described above, or they may need to modify the protocols of the individual tests undertaken, when justified scientifically. For example, the physicochemical properties of a feed ingredient (e.g., volatility, pH, solubility, stability, etc.) can sometimes make standard test conditions inappropriate, e.g., no exposure to the feed ingredient tested. It is essential that this is given due consideration before tests are

conducted. Modified protocols should be used, where it is evident that standard conditions will give false negative results. The OECD Guidelines for Testing of Chemicals for genotoxicity give some indications on the susceptibility of the individual tests to the physical characteristics of the test material and offer some advice on compensatory measures that may be taken. Feed ingredients tested using alternative batteries of genotoxicity tests or modifying the conditions of the tests will be considered on a case-by-case basis.

3.5 Interpretation of *in vitro* tests

If all *in vitro* endpoints are clearly negative in adequately conducted tests, then it can be concluded with reasonable certainty that the feed ingredient is not genotoxic.

However, the published literature indicates that a limited number of substances that are negative in *in vitro* tests could have positive results in *in vivo* tests. The reasons for this could be that the *in vitro* metabolic activation system does not cover the full spectrum of potential genotoxic metabolites generated *in vivo* or the involvement of specific conditions such as reactions in the gastro-intestinal tract. Therefore, the decision of whether to proceed to *in vivo* testing after negative *in vitro* testing should consider the documented weight of evidence approach, on a case by case basis.

If positive results are observed in one or more *in vitro* tests, the feed ingredient should be tested in the relevant *in vivo* test(s), as described in Section 4.

In the case of one or more equivocal or inconclusive results of the *in vitro* tests, further *in vitro* testing may be performed either by repeating the test with equivocal or inconclusive results, using different conditions or by conducting a different type of *in vitro* test.

4. *IN VIVO* TESTING

The choice of the *in vivo* test will depend on the results obtained in the *in vitro* test (i.e., the relevant endpoint(s)), considering other available information.

The recommended *in vivo* tests are:

- The Transgenic Rodent Somatic and Germ Cell Gene Mutation Assay (TGR, OECD TG #488)
- The *in vivo* Mammalian Alkaline Comet Assay (Comet Assay, OECD TG #489)
- The *in vivo* Mammalian Erythrocyte Micronucleus Test (MNviv, OECD TG #474)

The Unscheduled DeoxyriboNucleic Acid (DNA) Synthesis (UDS) test is not recommended as an *in vivo* follow-up to positive results in *in vitro* gene mutation tests, as indicated in the 2017 EFSA opinion (11).

The three (3) *in vivo* tests are described below.

4.1 Transgenic Rodent Somatic and Germ Cell Gene Mutation Assay (TGR)

An *in vivo* Transgenic Rodent Somatic and Germ Cell Gene Mutation test is recommended to be performed according to the protocol set out in OECD Test Guidelines #488 (6).

The TGR can be used as a follow-up of a positive bacterial reverse mutation test, either alone, when the result of the *in vitro* MNT is negative or in combination with MNviv, in the case of a positive *in vitro* MNT.

The test is based on transgenic rats and mice that contain multiple copies of chromosomally integrated phage or plasmid shuttle vectors that harbor reporter genes. It detects mutation and/or chromosomal rearrangements (plasmid model and Spi-assay) induced *in vivo* by the feed ingredient tested. The TGR measures mutations induced in genetically *neutral marker genes* (i.e. genes that have no immediate consequence to the animal) recovered from virtually any tissue of the rodent. Mutation arising in a rodent are scored by recovering the transgene and analyzing the phenotype of the reporter gene in a bacterial host deficient for the reporter gene.

Alternatively, the Pig A-Assay, detecting gene mutations *in vivo* could be used (9, 14)

4.2 *In vivo* Mammalian Alkaline Comet Assay (Comet Assay)

The *in vivo* Mammalian Alkaline Comet Assay is recommended to be performed according to the protocol set out in OECD Test Guidelines #489 (7).

The Comet Assay can be used as a follow-up test to investigate the relevance of positive *in vitro* tests (gene mutagens and clastogens, but not aneugens). It therefore can be used as a follow-up of a positive bacterial reverse mutation test or structural chromosomal aberration test, either alone, when the result of the *in vitro* MNT is negative or in combination with MNviv because of a positive *in vitro* MNT.

The purpose of the Comet Assay is to identify feed ingredients that cause DNA damage. It detects DNA single and double strand breaks, alkali-labile lesions, as well as DNA strand breaks

arising during the repair of DNA lesions. It has the advantage of being rapid and may be applied to any tissue of animals, usually rodents from which single cell suspensions can be prepared. Besides the liver, for orally administered substances, it would be appropriate to examine effects at the site of direct contact, e.g., the glandular stomach or duodenum/jejunum. Cell division is not required, and a low number of cells is sufficient for the analysis. It is considered an indicator test detecting pre-mutagenic lesions and can be used for mechanistic tests.

The Comet Assay may also be performed using DNA repair enzymes, such as formamido-pyrimidine DNA glycosylase (FPG) to detect DNA base damage in addition to DNA strand breaks.

4.3 In vivo Mammalian Erythrocyte Micronucleus Test (MNviv)

The *in vivo* Mammalian Erythrocyte Micronucleus Test (MNviv) is recommended to be performed according to the protocol set out in OECD Test Guidelines #474 (4).

The MNviv can be used as a follow-up of positive *in vitro* MNT and in combination with either the Comet Assay, using standardized protocol or the TGR test, when the bacterial reverse mutation test is also positive.

The purpose of the MNviv is to identify the feed ingredients that cause structural and numerical chromosomal damage in somatic cells *in vivo.* The damage results in the formation of micronuclei, containing chromosome fragments or whole chromosomes in young erythrocytes sampled in bone marrow and/or reticulocytes of peripheral blood cells of animals.

This test has a long history of use and is still the most widely used *in vivo* genotoxicity test that detects both clastogens and aneugens. The MNviv can be combined with FISH staining to provide additional mechanistic information when results are positive.

4.4 Strategy for *in vivo* test selection

Any *in vivo* test should be selected on a case by case basis, considering the full dataset available for the feed ingredient. *In vivo* tests should relate to the genotoxic endpoint(s) identified *in vitro* and to appropriate target organs and tissues.

As a follow-up for *in vitro* positive results for gene mutation, both the TGR and the Comet Assay are suitable. It should be noted, however, that the TGR is a test that measures gene mutations directly, whereas the Comet Assay is an indicator test for DNA lesions that may or may not result in mutations.

As a follow-up for *in vitro* positive results for clastogenicity, the Comet Assay is suitable.

As a follow-up for *in vitro* positive results for clastogenicity and/or aneugenicity, the MNviv is suitable. For highly reactive feed ingredients and/or metabolite(s), site of contact effects in relevant tissues may be considered.

Some typical scenarios and possible approaches (with different combinations of positive results *in vitro*) are described below and in ANNEX II. These **examples** are illustrative and alternative approaches may be appropriate.

i. bacterial reverse mutation test **positive** and *in vitro* MNT / *in vitro* CAT **negative**

The approach would be to conduct a TGR or Comet Assay. Both tests are also suitable for detection of first site of contact effects. Adequate target tissues, especially site of contact and liver, are selected depending on the reactivity of the feed ingredient or its metabolite with DNA (which might predispose to site contact effects), bioavailability, metabolism, toxicokinetics, and any target organ specificity (if known from repeat dose toxicity studies).

ii. bacterial reverse mutation test **negative** and *in vitro* MNT / *in vitro* CAT **positive**

The selection of appropriate *in vivo* follow-up studies should account for the relevant mode of action for micronuclei induction (e.g., discrimination between clastogenic and aneugenic effects with use of CREST or FISH technologies) and information on the possible involvement of genotoxic metabolites (e.g., if positive tests result only in the presence of rat liver S9 mix). Three (3) different situations could be considered:

a. The appropriate follow-up of an aneugenic effect *in vitro* (i.e., increase in centromere positive nuclei) would be a MNviv (in bone marrow or peripheral blood). If an adequately conducted MNviv (with evidence for significant exposure of the target tissue) is negative, it could be concluded that the feed ingredient is not aneugenic *in vivo*.
b. The appropriate follow-up for a clastogenic effect *in vitro* (i.e., increase in centromere negative micronuclei), detected in the absence of rat liver S9 mix, would be a MNviv (in bone marrow or peripheral blood) and a Comet Assay in the relevant tissues (including site of contact). If an adequately conducted MNviv and Comet Assay (with evidence for significant exposure of the target tissue) is

negative, it could be concluded that the feed ingredient is not an *in vivo* clastogen.

c. The appropriate follow-up for a clastogenic effect *in vitro* detected in the presence of rat liver S9 mix should consider the involvement of liver specific clastogenic metabolites, which is achieved by a single rodent study combining a MNviv (in bone marrow or blood) and a Comet Assay in the liver. If an adequately conducted combined MNviv / Comet Assay (with evidence for significant exposure of the target tissues) is negative, it could be concluded that the feed ingredient or its metabolites are not clastogenic *in vivo*.

iii. both bacterial reverse mutation test and *in vitro* MNT / *in vitro* CAT **positive**

If feed ingredients are positive in both *in vitro* tests, the appropriate follow-up would be the combined MNviv / Comet assay with adequate target tissue selection (see above). If the results are negative, it could be concluded that the feed ingredient is not genotoxic *in vivo*. An alternative option would be to combine a TGR and a MNviv.

4.5 Interpretation of in vivo tests

If all the *in vivo* test endpoints are clearly negative, then it can be concluded that the feed ingredient is not genotoxic.

Some regulatory authorities may require the demonstration of target tissue exposure for *in vivo* tests that give negative results, to provide reassurance that the negative result is not a false negative.

In these cases, possible approaches for demonstrating the *in vivo* exposure could consider any of the following measurements:

i. For cytogenic tests, by obtaining a significant reduction in the proportion of immature erythrocytes among total erythrocytes in the MNviv, i.e. decrease in the ratio PCE/ (NCE + PCE) in bone marrow or peripheral blood,

ii. Evidence that the feed ingredient and/or its metabolite(s) is(are) detected systemically by a valid analytical method in a specific blood sample taken at appropriate time(s) as indicated in the OECD guidelines,

iii. Direct measurement of the feed ingredient and/or its metabolites in the target tissues.

If positive results are observed in one or more *in vivo* test, it is concluded that the feed ingredient presents a genotoxic potential.

There is a growing body of evidence that compound-related disturbances in the physiology of the rodents used in the *in vivo* genotoxicity tests can result in increases in micronucleated cells in the bone marrow that are not related to intrinsic genotoxicity of the feed ingredients under test (16). Hence, the risk assessment of feed ingredients shall be done on a case by case basis.

In the case of one or more equivocal or inconclusive results of the *in vivo* tests, further *in vivo* testing may be performed.

5. DATA EVALUATION AND STATISTICAL ANALYSIS

The data evaluation and the statistical analysis are described in detail in the OECD Technical guidance documents for each of the tests. It is recommended to the applicant to refer to those guidance documents for evaluating and analyzing the data of the different tests, considering the specific acceptability criteria for each test.

For this guidance document, a test is:

- Clearly positive if the following conditions are all met:
  - A treatment group exhibits an increase compared with the concurrent negative controls and
  - The increase is dose-related at least at one experimental condition/sampling time and
  - All results are outside the distribution range of the historical negative control data.
- Clearly negative if the following conditions are all met:
  - None of the groups exhibits an increase compared with the concurrent negative controls and
  - There is no dose related increase at any sample time and
  - All results are inside the distribution range of the historical negative control data.

A test might be considered equivocal or inconclusive, if only one of the above-mentioned conditions is met. As recommended in the respective OECD technical guidance documents, expert judgment and/or follow-up experiments should be decided on a case by case basis.

6. DATA REPORTING

The data reporting is described in detail in the OECD Technical guidance documents for each of the tests. It is recommended to the applicant to refer to those guidance documents for reporting the data from the different tests.

As a summary, the following sections should be included in the report for each test performed:

- Test active substance[4]
- Test active substance preparation (*in vivo*)
- Test system/Test animals
- Test conditions
- Results
- Discussion of the results
- Conclusion

7. BIBLIOGRAPHY

7.1 International Guidelines

1. VICH GL23 ® (Safety) – Genotoxicity – Studies to evaluate the safety of residues of veterinary drugs in human food: genotoxicity testing
2. OECD *Test Guideline No. 471:* Bacterial Reverse Mutation Test, OECD Guidelines for the Testing of Chemicals
3. OECD *Test Guideline* No. 473: *In vitro* Mammalian Chromosomal Aberration Test, OECD Guidelines for the Testing of Chemicals
4. OECD *Test Guideline* No 474: Mammalian Erythrocyte Micronucleus Test, OECD Guidelines for the Testing of Chemicals
5. OECD *Test Guideline* No 487: *In vitro* Mammalian Cell Micronucleus test, OECD Guidelines for the Testing of Chemicals
6. OECD *Test Guideline* No 488: Transgenic Rodent Somatic and Germ Cell Gene Mutation Assays, OECD Guidelines for the Testing of Chemicals

[4] In the case the feed ingredient contains a mixture of active substances, it is preferable to test each active substance individually, when possible (11).

7. OECD *Test Guideline* No 489: *In vivo* Mammalian Alkaline Comet Assay, OECD Guidelines for the Testing of Chemicals
8. ICH S2 (R1) – Guidance on Genotoxicity Testing and Data Interpretation for Pharmaceuticals Intended for Human Use
9. OECD Series on Testing and Assessment No 316: The *in vivo* erythrocyte pig-a gene mutation assay – Part 2: Validation Report

7.2 European Guidance Documents

10. EFSA Scientific Committee; Scientific Opinion on genotoxicity testing strategies applicable to food and feed safety assessment. EFSA Journal 2011;9(9):2379, 69 pp. https://doi:10.2903/j.efsa.2011.2379 .
11. EFSA Scientific Committee, Hardy A, Benford D, Halldorsson T, Jeger M, Knutsen HK, More S, Naegeli H, Noteborn H, Ockleford C, Ricci A, Rychen G, Silano V, Solecki R, Turck D, Younes M, Aquilina G, Crebelli R, Gurtler R, Hirsch-Ernst KI, Mosesso P, Nielsen E, van Benthem J, Carfi M, Georgiadis N, Maurici D, Parra Morte J and Schlatter J, 2017. Scientific Opinion on the clarification of some aspects related to genotoxicity assessment. EFSA Journal 2017;15(12):5113, 25 pp. https://doi.org/10.2903/j.efsa.2017.5113
12. EFSA Scientific Committee, More S, Bampidis V, Benford D, Boesten j, Bragard C, Halldorsson T, Hernandez-Jerez A, Hougaard-Bennekou S, Koutsoumanis K, Naegeli H, Nielssen SS, Schrenk D, Silano V, Turck D, Younes M, Aquilina G, Crebelli R, Gürtler R, Hisch-Ernst KI, Mosesso P, Nielsen E, Solecki R, Carfi, M, Martino C, Maurici D, Parra Morte J and Schlatter J, 2019. Statement on the genotoxicity assessment of chemical mixtures. EFSA Journal 2019;17(1):5519,11pp https://doi.org/10.2093/j.efsa.2019.5519

7.3 Other Publications

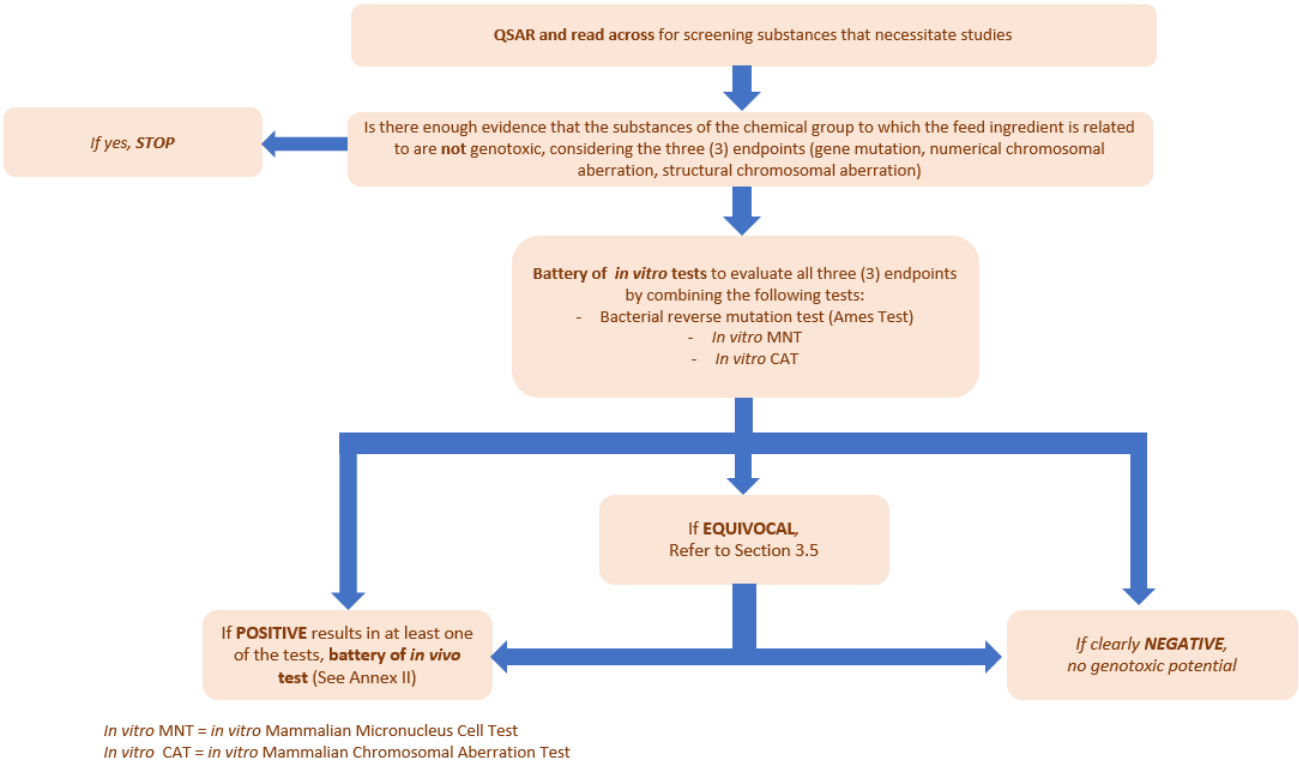
13. Code of Practice on good animal feeding – CAC/RCP 54-2004
14. R.H. Heflich et al: The *in vivo* erythrocyte Pig-a gene mutation assay – Part 1: Detailed review paper and performance assessment – 20 May 2019 – Report for the Organization for Economic Cooperation and Development Working Group of the National Coordinators of the Test Guidelines Program – 133 pages
15. G.S. Ladics, V; Sewalt: Industrial microbial enzyme safety: what does the weight of evidence indicate? – Regulatory Toxicology and Pharmacology 98(2018) 151-154
16. Tweats et al: Report of the IWGT working group on strategies and interpretation on regulatory *in vivo* tests: I Increases in micronucleated bone marrow cells in rodents that do not indicate genotoxic hazards – Mutation Research 627 (2007) 78-91

8. ABBREVIATIONS

CREST	Immunofluorescent antikinetochore
DNA	DeoxyriboNucleic Acid
FISH	Fluorescence in Situ Hybridization
GLP	Good Laboratory Practices
MNT	Mammalian Micronucleus Cell Test
MNviv	*in vivo* Mammalian Erythrocyte Micronucleus Test
OECD	Organization for Economic Co-operation and Development
FPG	Formamidopyrimidine DNA glycosylase
QSAR	*in silico* Quantitative Structure-Activity Relationship
TGR	Transgenic rodent somatic and germ cell mutation Test
UDS	Unscheduled DNA Synthesis

ANNEX I

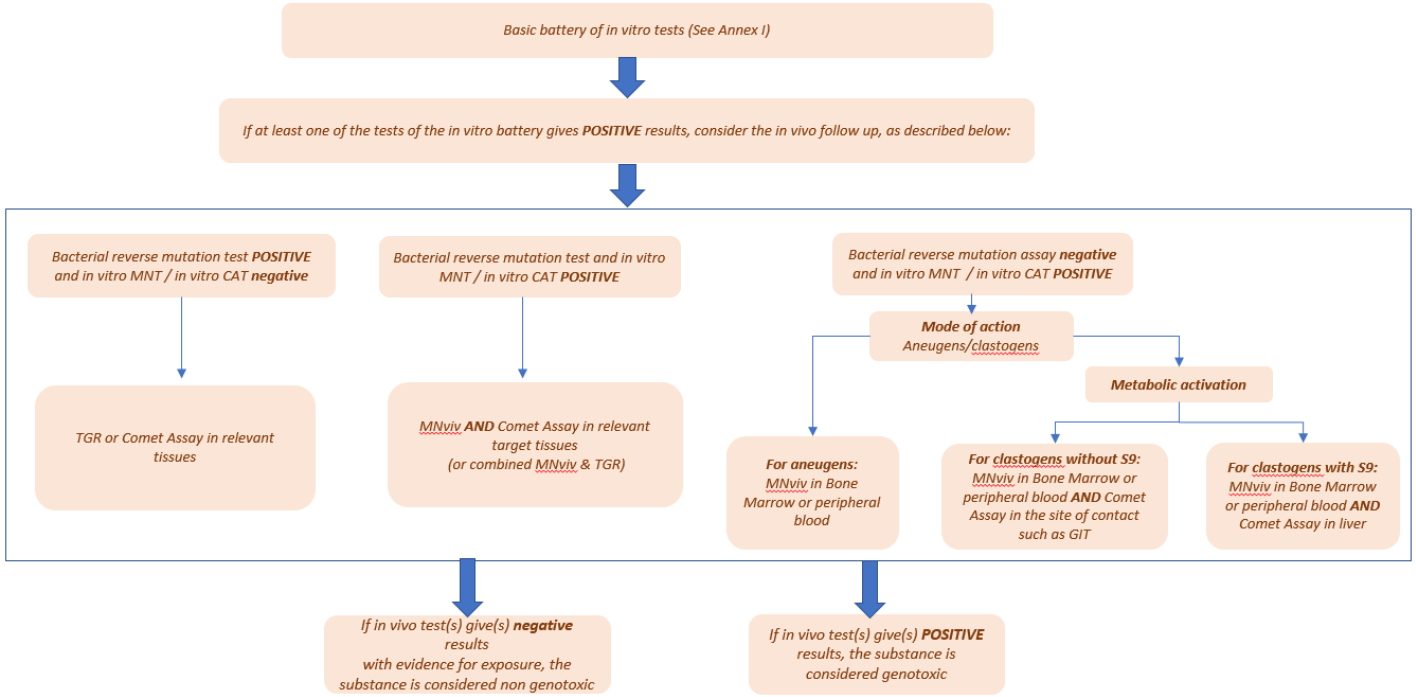
Stepwise approach for in vitro genotoxicity testing



In vitro MNT = *in vitro* Mammalian Micronucleus Cell Test
In vitro CAT = *in vitro* Mammalian Chromosomal Aberration Test

ANNEX II

Strategy for in vivo genotoxicity testing



In vitro MNT = *in vitro* Mammalian Micronucleus Cell Test
In vitro CAT = *in vitro* Mammalian Chromosomal Aberration Test
MNviv = *in vivo* Mammalian Micronucleus Cell Test
TGR = Transgenic Rodent Somatic and Germ Cell Gene Mutation Assay
GIT = Gastro-Intestinal Tract