Parma, 24th June 2019

Ad-hoc meeting with industry representatives on smoke flavourings and flavourings other than flavouring substances for use in food and feed

Scientific Evaluation of Regulated Products Department

Trusted science for safe food

efsa
European Food Safety Authority

Agenda

- **Scope of the *ad hoc* meeting**
- **Update from EFSA on the recent scientific requirements for the genotoxicity assessment of chemical mixtures**
- **Open discussion and feedback from industry**

Scope of the *ad hoc* meeting

What?

- Initiative implemented by EFSA to **support applicants** submitting applications for food/feed regulated products (see EFSA Catalogue of supportive initiatives)
- **Set-up an open dialogue** and exchange information between EFSA and industry representatives in the area of **smoke flavourings** and **flavourings mixtures**
- Raise awareness on the most recent **EFSA Scientific Committee guidance documents**, particularly in the area of **genotoxicity** and **chemical mixtures**

Scope of the *ad hoc* meeting

Why now?

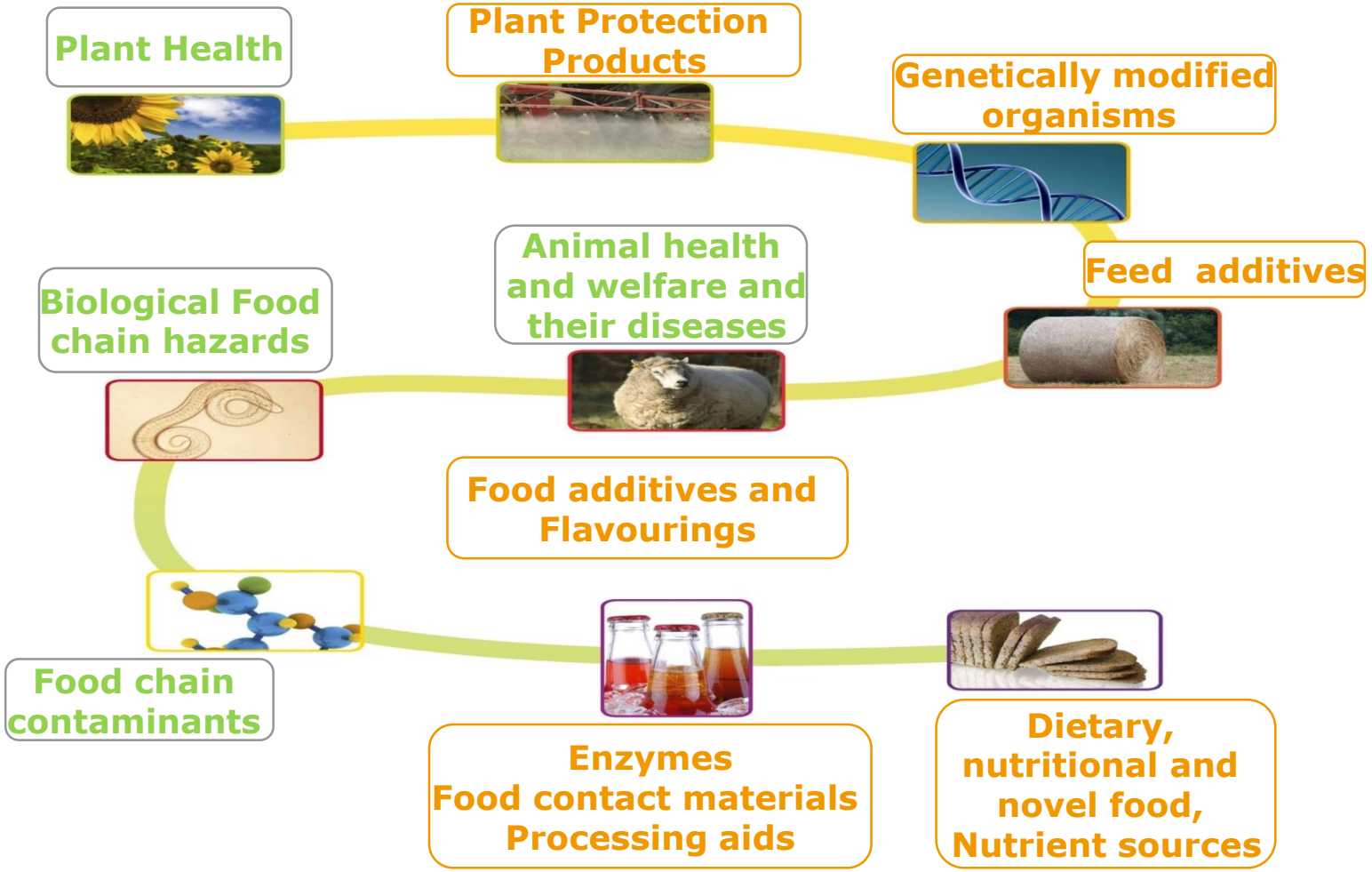
- Upcoming **renewal of authorisation** for smoke flavourings after 10-year marketing period, according to Art 12 Regulation (EC) No 2065/2003. Application dossiers to be submitted to the EC by authorisation holders **by 30 June 2022**
- A number of **horizontal guidance documents** recently issued (2017-2019) by the EFSA Scientific Committee that have an impact on smoke flavourings and flavourings mixtures

Scope of the *ad hoc* meeting

Who?

- **30 industry representatives** in the area of food/feed **flavourings** and **smoke flavourings** (17 attending physically and 13 via TC)
- **7 EFSA staff members** from the EFSA's Scientific Evaluation of Regulated Products (REPRO) Department
- **5 scientific experts** from two EFSA Scientific Panels (Panel on Food Additives and Flavourings (FAF) and Panel on Food Contact Materials, Enzymes and Processing Aids (CEP))
- **6 Member States** representatives (via TC)
- **1 EC DG-SANTE** representative

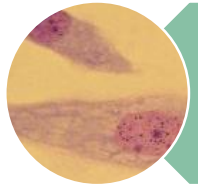
EFSA - independent scientific advice across the food chain



Scientific Committee and Panels

Cross-sectional guidance on genotoxicity and mixtures

SC Opinion (2011): Genotoxicity testing strategies



SC Statement (2017): Clarification on some aspects of genotoxicity assessment (in *vivo* UDS, bone marrow, WoE approach)

SC Statement (2019): Genotoxicity of chemical mixtures

SC Guidance (2019): Harmonised methodologies for human and animal health and ecological risk assessment of combined exposure to multiple chemicals

SC Statement (ongoing): Aneugenicity

Genotoxicity testing strategies

Genotoxicity in the risk assessment of food/feed

- Genotoxicity ***per se*** **is an end-point**: genetic damage in somatic or germ cells is associated with serious detrimental health effects, including cancer, heritable diseases and degenerative conditions
- Under the EU legislation, substances that are classified as mutagenic **should not** be deliberately added to food and feed chain, at any dose level
- Consequently, all regulated food/feed substances (as well as food contact materials) **have to be evaluated for genotoxicity before their approval**
- According to Regulation 178/2002, this task is performed by the European Food Safety Authority, that provides scientific advice to the European Commission

Genotoxicity testing strategy (SC, 2011)

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.

https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2011.2379

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 (MN)** (OECD TG 487). End-points considered: **structural and numerical chromosome aberrations**

- Outcome:
  - Negative: No further testing*
  - Positive: *In vivo* testing

*unless available information indicate the inadequacy of *in vitro* systems

Genotoxicity testing strategy (SC, 2011)

Tier 2: Follow-up*of positive results for

- **Gene mutation:**
  - Transgenic rodent somatic and germ cell gene mutation assays (OECD TG 488)
  - *In Vivo* Mammalian Alkaline Comet Assay (OECD TG 489)
- **Chromosome aberration:**
  - **Structural**
  - *In Vivo* Mammalian Alkaline Comet Assay (OECD TG 489)
  - Mammalian erythrocyte micronucleus test (MN) (OECD TG 474)
  - **Numerical**
  - Mammalian erythrocyte micronucleus test (MN) (OECD TG 474)

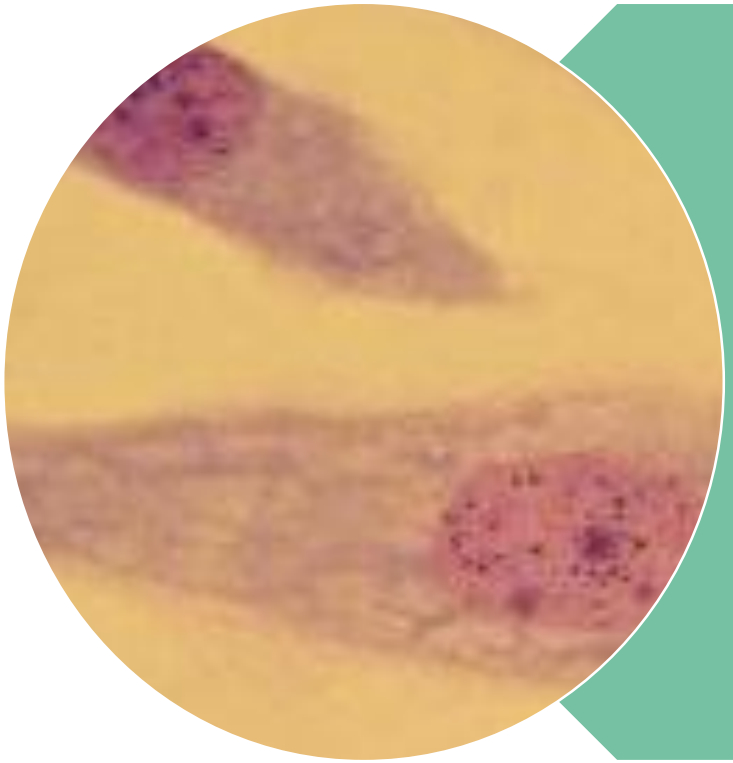
*to be selected case-by-case based on *in vitro* test results, SAR, metabolic and toxicokinetic considerations…

Genotoxicity testing strategy (SC, 2011)

Outcomes of *in vivo* genotoxicity testing:

- Negative*: No further testing
- Positive: Genotoxic hazard

* With evidence of **target tissue exposure**



Clarification on some aspects of genotoxicity assessment

SC Opinion 2017

SCIENTIFIC OPINION

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

https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2017.5113

SC Opinion 2017

European Commission requested EFSA to provide advice on:

1) the adequacy of the **Unscheduled DNA Synthesis (UDS) assay** to follow-up positive results in the *in vitro* gene mutation tests;

2) the adequacy to demonstrate **target tissue exposure** in *in vivo* studies, particularly in the mammalian erythrocyte micronucleus (MN) test;

3) the use of data in a **weight-of-evidence (WoE) approach** to conclude on the genotoxic potential of substances and the consequent setting of health-based reference values for use in human health risk assessment.

SC Opinion 2017 – EC question 1: *in vivo* UDS

1a

The adequacy of the Unscheduled DNA Synthesis (UDS) assay to follow-up positive results in the *in vitro* gene mutation tests

- UDS (OECD TG 486, 1997)
- Tissues other than the liver may in theory be used, but most of experimental experience is related to its application in rat liver

- UDS is indicative of DNA adduct removal by nucleotide excision repair in liver cells;

- DNA damage processed by other mechanisms, as well as unrepaired genetic damage are not detected with this assay

Negative *in vivo* UDS **is insufficent alone to rule out** *in vivo* genotoxic potential

SC Opinion 2017 – EC question 1: *in vivo* UDS

1b

The adequacy of the Unscheduled DNA Synthesis (UDS) assay to follow-up positive results in the *in vitro* gene mutation tests

- Until the publication of:
- OECD TG 488 (2011) for TGR gene mutation assay and
- OECD TG 489 (2014) for comet assay

UDS was the only test method applicable to somatic tissues other than the erythropoietic system

→ it was frequently used in the follow-up of substances positive in gene mutation tests *in vitro*

- The SC guidance (2011) states:…" *However, UDS has a limited use for cells other than liver and its **sensitivity** has been questioned…"*

- low sensitivity confirmed and the lower predictive value of the UDS compared with TGR and *in vivo* comet assays

SC Opinion 2017 – *in vivo* UDS

- The usefulness of the ***in vivo* UDS** is discussed in both a retrospective and a prospective way

- Future assessments: the EFSA is 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

- Re-assessments: existing UDS results may be considered as **adequate only in 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

SC Opinion 2017 - EC question 2: target tissue exposure

2a

The adequacy to demonstrate target tissue exposure in *in vivo* studies, particularly in the micronucleus (MN) test

Regarding bone marrow exposure, the OECD TG 474 (July 2016), states:

- ***Target tissue exposure:*** *"A blood sample should be taken at appropriate time(s) in order to permit investigation of the plasma level of the test substances for the purposes of demonstrating that exposure of the bone marrow occurred, where warranted and where other exposure data do not exist"*

- **Evaluation and interpretation of results:** *"Evidence of exposure of the bone marrow to a test substance may include* ***a depression of the immature erythrocyte ratio*** *or measurement of the plasma or blood level of the substance. In case of intravenous administration, evidence of exposure is not needed. Alternatively, ADME data, obtained in an independent study using the same route and same species, can be used to demonstrate bone marrow exposure. [. . .]"*

SC Opinion 2017 - EC question 2: target tissue exposure

2b

The adequacy to demonstrate target tissue exposure in *in vivo* studies, particularly in the micronucleus (MN) test

Demonstration of target tissue exposure needed **if BM MN negative**

- The following **lines of evidence*** can be considered (if evaluated as test substance–related) :

1. toxicity to the BM observed in the *in vivo* MN study
2. toxicity to the BM observed in toxicity studies, using the same route and the same species as in the MN study
3. test substance (and/or metabolites) detected in BM in a toxicokinetic study
4. systemic toxicity observed in the BM MN test (e.g. signs related to CNS)

* A line of evidence is a set of evidence of similar type (SC, 2017)

SC Opinion 2017 - target tissue exposure

lines of evidence of bone marrow exposure (Cont'd):

5. systemic toxicity observed in toxicity studies

6. test substance (and/or metabolites) detected systemically in a toxicokinetic study

7. substance detected systemically in a specific blood/plasma analysis, to consider e.g.:

- Detection above the quantification limit
- Sampling time
- Analytical method
- Consistency among animals of the same group

SC Opinion 2017 - EC question 3: WoE approach

3a The use of data in a WoE approach to conclude on the genotoxic potential of substances and the setting of health-based reference values for use in human health risk assessment

- Evaluation of all available *in vitro and in vivo* data according to the SC guidance on gentoxicity testing strategies (2011) and according to the SC Guidance on the use of WoE (2017)
- If the dataset includes UDS or in vivo BM MN studies, the advice provided in answering those questions should be followed
- If it is not possible to conclude with confidence, in a second step any additional available data that may assist in reducing the uncertainty (e.g. MOA, structural alerts, read across from structurally related substances) might be considered.
- If still not possible to conclude, additional information would be needed to reduce the uncertainty.

3b

WoE approach and possible setting of Reference Values

- Establishing a Health Based Guidance Value (HBGV) might be possible when the overall evaluation leaves no concern for genotoxicity *in vivo*

- If, based on the overall assessment, concern for genotoxicity remains, derivation of a HBGV is not considered appropriate, neither fixed nor provisional.

- In cases where a HBGV had been previously established for a substance and cannot be confirmed due to newly identified concerns about genotoxicity, then new data might be needed to clarify the concerns.

Genotoxicity assessment of chemical mixtures

SC Statement 2019

STATEMENT

EFSA Journal

ADOPTED: 22 November 2018

doi: 10.2903/j.efsa.2019.5519

Genotoxicity assessment of chemical mixtures

EFSA Scientific Committee,
Simon More, Vasileios Bampidis, Diane Benford, Jos Boesten, Claude Bragard, Thorhallur Halldorsson, Antonio Hernandez-Jerez, Susanne Hougaard-Bennekou, Kostas Koutsoumanis, Hanspeter Naegeli, Søren Saxmose Nielsen, Dieter Schrenk, Vittorio Silano, Dominique Turck, Maged Younes, Gabriele Aquilina, Riccardo Crebelli, Rainer Gürtler, Karen Ildico Hirsch-Ernst, Pasquale Mosesso, Elsa Nielsen, Roland Solecki, Maria Carfì, Carla Martino, Daniela Maurici, Juan Parra Morte and Josef Schlatter

https://www.efsa.europa.eu/en/efsajournal/pub/5519

Type of mixtures in food and feed

- Smoke flavourings
- Flavourings other than flavouring substances (e.g. flavouring preparations, thermal process flavourings, grill flavours)
- Botanicals and botanical preparations
- Enzymes
- Food contact materials

Chemical characterization

Chemical characterisation of mixtures
(demonstration of *identity* and *stability*)

Chemically Fully defined

Mixtures containing a substantial fraction of unidentified components

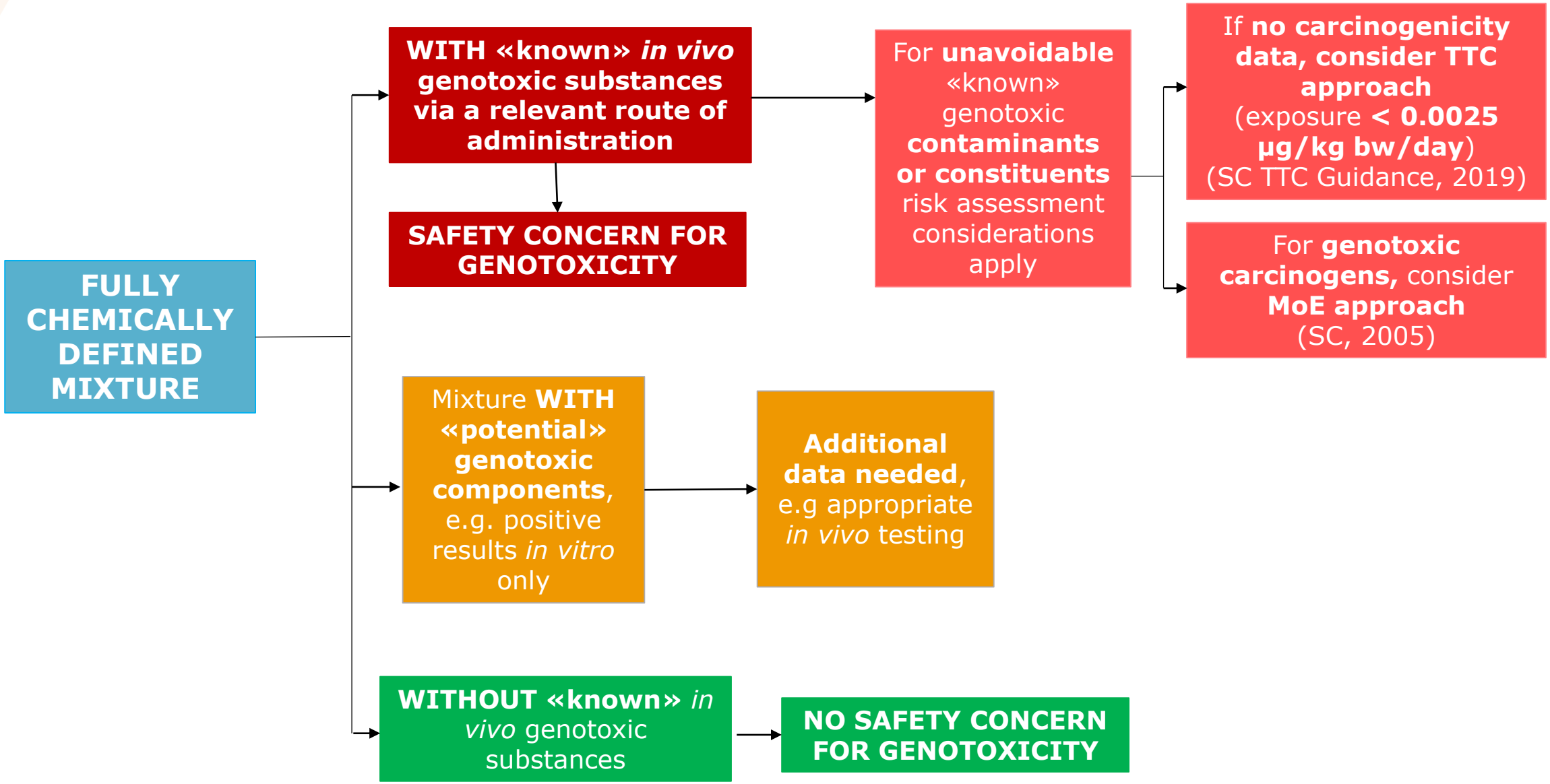
Chemical characterization

- **State-of-the-art analytical methodologies** should be applied in the characterisation, which should be able to detect and quantify constituents at LOD and LOQ
- **Not possible to establish a generic 'cut-off' value** (i.e. percentage of unidentified components considered acceptable without further testing) as this depends on the nature of the mixture
- **Qualitative and quantitative analysis of the components** is required for a clear and unambiguous identification of the components (CAS nr, chemical name, synonyms, isomerism, etc to be provided for each component)

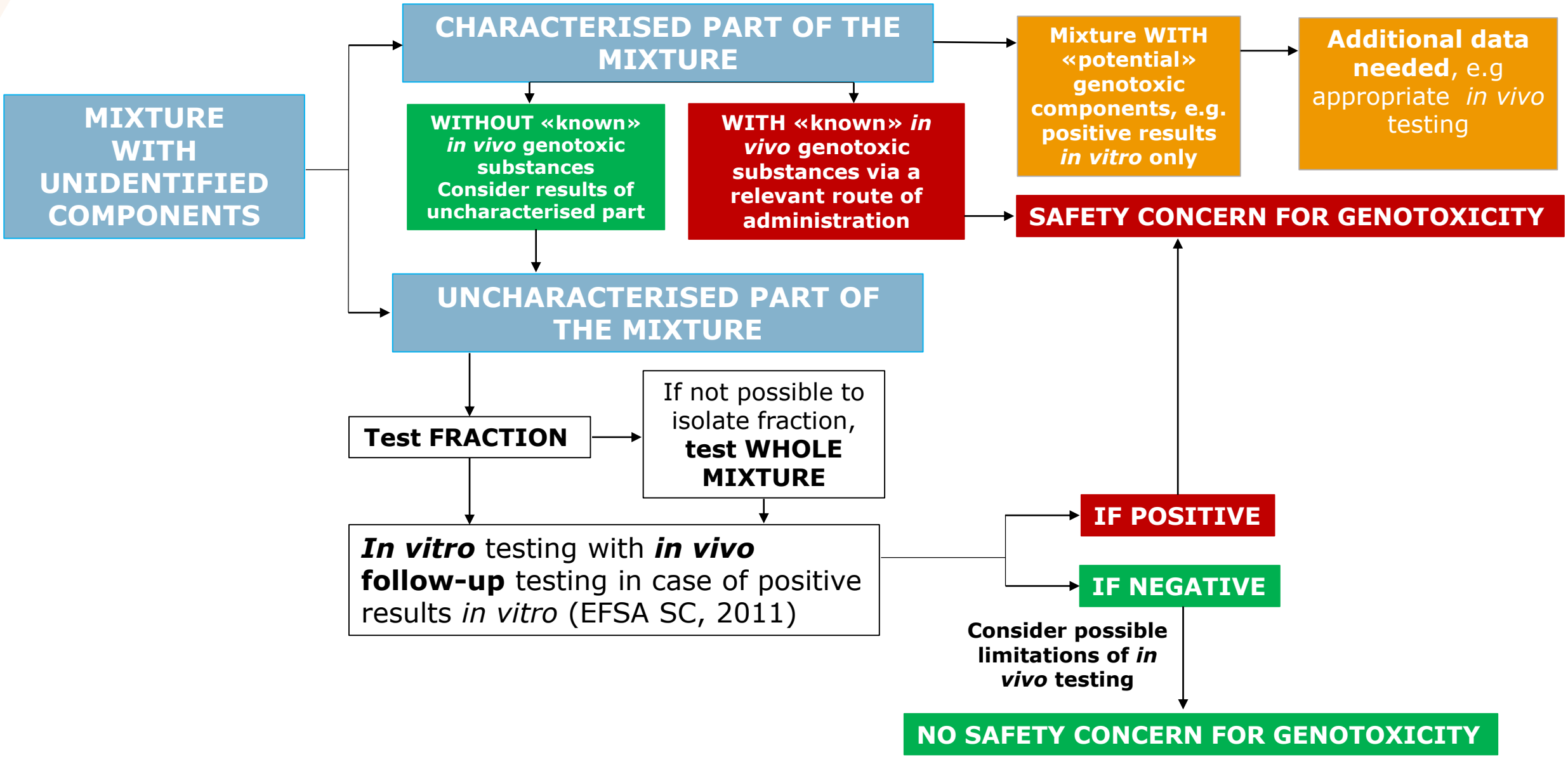
Genotoxicity of mixtures

- **Chemically fully defined mixtures**
  - Assessment of all the components, using all available information (e.g. QSAR analysis, read-across, reliable and relevant literature data, genotoxicity data in line with SC testing strategy): *component-based approach*
- **Mixtures containing substantial fraction of unidentified components**
  - Identified components assessed individually: *component-based approach*
  - Unidentified fraction should be tested as first option. If not feasible, testing of the whole mixture should be undertaken: *whole-mixture approach*

FULLY CHEMICALLY DEFINED MIXTURES: COMPONENT BASED APPROACH



MIXTURE WITH UNIDENTIFIED COMPONENTS



SC Guidance (2019): Harmonised methodologies for human and animal health and ecological risk assessment of combined exposure to multiple chemicals

SC Guidance 2019

EFSA Journal

GUIDANCE

ADOPTED: 20 February 2019

doi: 10.2903/j.efsa.2019.5634

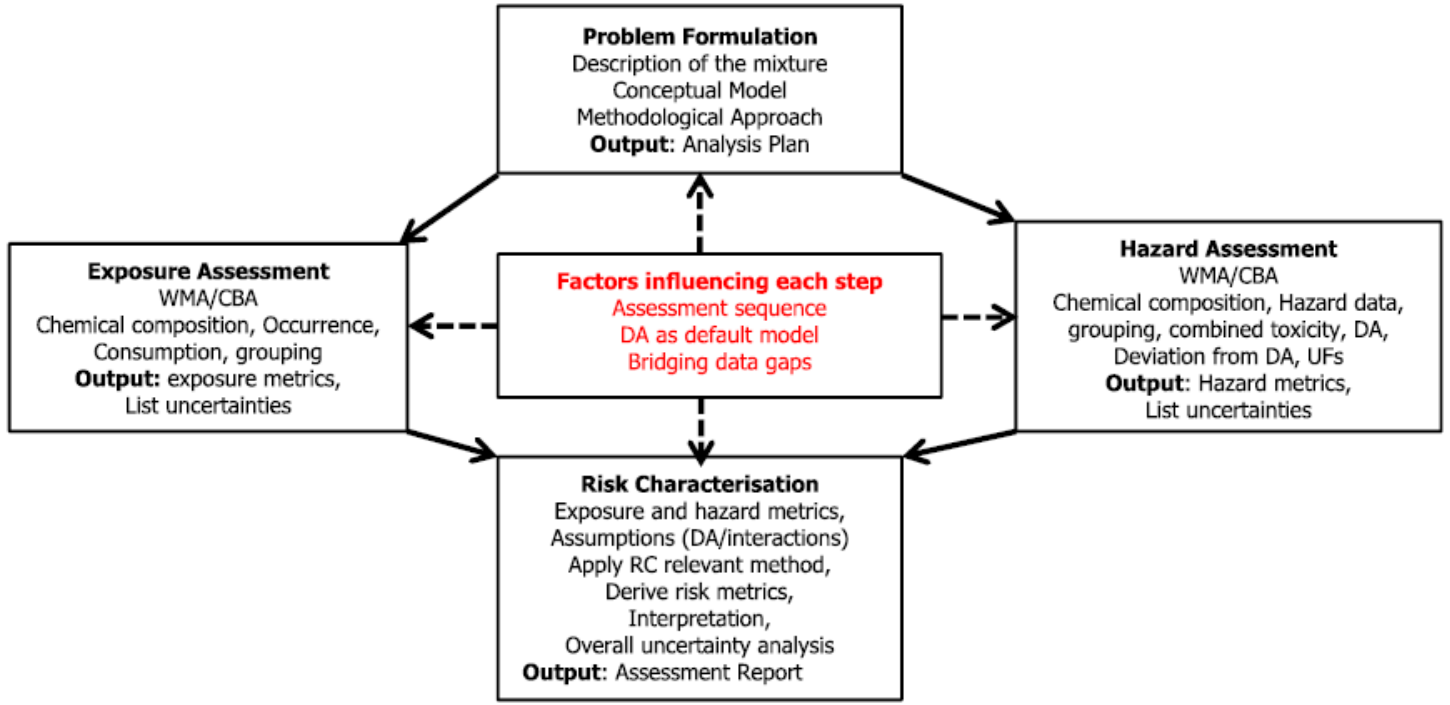
Guidance on harmonised methodologies for human health, animal health and ecological risk assessment of combined exposure to multiple chemicals

EFSA Scientific Committee,
Simon John More, Vasileios Bampidis, Diane Benford, Susanne Hougaard Bennekou, Claude Bragard, Thorhallur Ingi Halldorsson, Antonio F Hernández-Jerez, Konstantinos Koutsoumanis, Hanspeter Naegeli, Josef R Schlatter, Vittorio Silano, Søren Saxmose Nielsen, Dieter Schrenk, Dominique Turck, Maged Younes, Emilio Benfenati, Laurence Castle, Nina Cedergreen, Anthony Hardy, Ryszard Laskowski, Jean Charles Leblanc, Andreas Kortenkamp, Ad Ragas, Leo Posthuma, Claus Svendsen, Roland Solecki, Emanuela Testai, Bruno Dujardin, George EN Kass, Paola Manini, Maryam Zare Jeddi, Jean-Lou CM Dorne and Christer Hogstrand

https://www.efsa.europa.eu/it/efsajournal/pub/5634

SC Guidance: General principles

- **General Principles**
  - Whole mixture (WMA) *vs* components-based approach (CBA)
  - Tiering: purpose of the assessment, data availability
  - Harmonised overarching framework for human, animal and ecological risk assessment
    - Problem formulation
    - Exposure assessment
    - Hazard assessment
    - Risk characterisation
  - Dose addition



Component-based approach

- **Grouping chemicals into assessment groups**
  - definition of assessment groups in **problem formulation**
    - regulatory requirements, exposure
    - physicochemical characteristics, biological and toxicological properties
  - refinement of assessment groups in **hazard characterisation**
    - using weight of evidence, dosimetry, mode of action
  - collection of hazard data to derive reference values for the individual components or for the group
    - handling data gaps within an assessment group (read across, *in silico*)
    - in the absence of interactions, response/*dose addition* as default
  - application of dose addition within assessment groups in **risk characterisation**
    - e.g. hazard index (HI), combined (total) margin of exposure (MOET)

Genotoxic and carcinogenic substances

- **For substances that are genotoxic and carcinogenic**
  - The EFSA SC (2005) advises that a **Margin of Exposure** (MOE) ≥ 10,000, when comparing estimated exposure with a $BMDL_{10}$ from a rodent carcinogenicity study, would be of ***low concern*** from a public health point of view
  - Such a judgement is ultimately a matter for risk managers and a MOE of that magnitude should not preclude risk management measures to reduce or prevent human exposure to genotoxic carcinogens
  - This also applies to **whole mixtures** that are genotoxic and carcinogenic, both for humans and companion animals
  - Genotoxicity and carcinogenicity are generally not considered to be of similar concern for farm animals and the ecological area because of differences in protection goals and lifespan

Combined margin of exposure (MOET)

- **For component-based approach**
  - If one or more components of a mixture are **genotoxic and carcinogenic**, then the combined margin of exposure (MOET) for the mixture when calculated based on a $BMDL_{10}$ from an animal study ≥10,000 is considered of ***low concern***
  - If BMDL values are available for all of the genotoxicants in the mixture, then the MOET can be calculated as the reciprocal of the sum of the reciprocals of the MOE of the individual chemical substances, applying the default assumption of dose addition

Combined (total) margin of exposure (MOET)

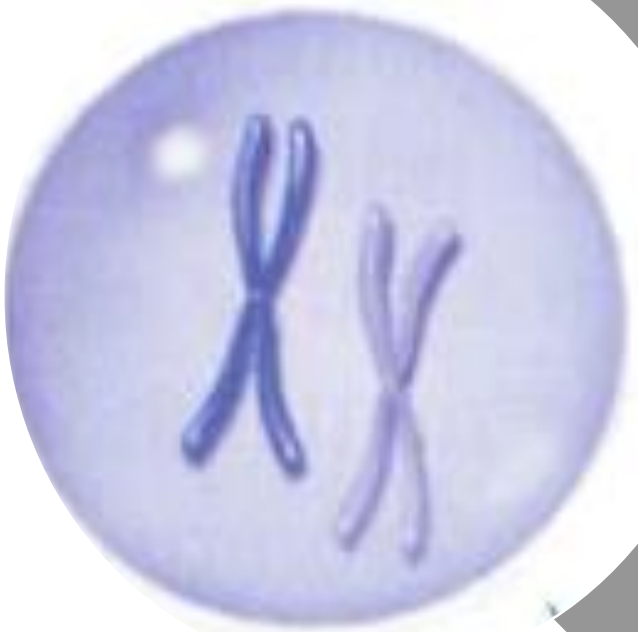
$$MOE = BMDL_{10}/Exposure$$

$$MOET_{(1\text{-}2)} = 1/[(1/MOE_1) + (1/MOE_2)]$$

$$=1/[/1/19{,}125)+(1/27{,}250)]=$$

$$=1/(0.00005+0.0004) = \mathbf{11{,}280}$$

	Intake	$BMDL_{10}$	MOE
	mg/kg bw		
C1	0.0008	15.3	19,125
C2	0.00012	3.3	27,250



SC Statement (ongoing): Aneugenicity

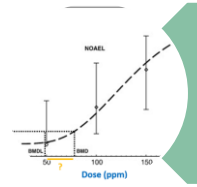
Aneugenicity

- **SC Statement on Aneugenicity is under preparation**
  - What is the most appropriate *in vivo* follow-up for substances that are aneugenic *in vitro*?
  - How should risk to human health be assessed for a substance exhibiting aneugenicity?

- **Timelines**
  - Draft guidance for public consultation by end 2019
  - Finalisation of the guidance **by spring 2020**

Additional cross-sectional issues to be considered

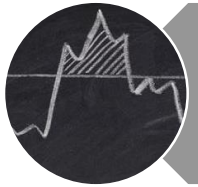
Dietary exposure
EFSA Comprehensive EU Food Consumption Database



Benchmark dose (2017)

Weight of evidence and biological relevance (2017)

Uncertainty (2018)



Threshold of Toxicological Concern (2019)

In summary

- Particularly in the area of **genotoxicity** and **chemical mixtures,** new scientific requirements relevant for the safety assessment of flavourings and smoke flavourings apply

- These requirements are described in detail in the **horizontal SC guidance documents**

- Regardless the update of each **sector-specific** EFSA guidance documents, **these requirements should be already taken into account** by authorisation holders when preparing applications for authorisation/renewal

Discussion and feedback from industries

- Feedback regarding the **level of characterisation** of your products
- State-of-the-art on the available **analytical methods** used to characterise your products
- Possibility to improve the characterisation of the **unidentified fraction** of a complex mixture
- Feasibility of **isolating** the **unidentified fraction** of a complex mixture to be tested experimentally

Thank you for your attention!

Feedback could be sent to
fip@efsa.europa.eu