Trusted science for safe food

7th meeting of the FCM Network, 6-7 November 2019

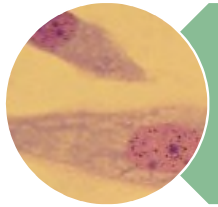
Genotoxicity assessment of chemical mixtures

Scientific Evaluation of Regulated Products Department

efsa
European Food Safety Authority

Scientific Committee guidance documents on genotoxicity

SC Opinion (2011): Genotoxicity testing strategies



SC Opinion (2017): Clarification on some aspects of genotoxicity assessment (in *vivo* UDS, target tissue exposure, WoE approach)

SC Statement (2019): Genotoxicity of chemical mixtures

SC Statement (ongoing): Aneugenicity

Examples 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

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

- ✓ Developed in parallel to the SC *"Guidance on harmonised methodologies for human health, animal health and ecological risk assessment of combined exposure to multiple chemicals";*
- ✓ This statement addresses primarily specific issues related to hazard identification of genotoxicity of mixtures

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)

Chemical characterization

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

batch-to-batch variability as well as stability over time→ Representative of the mixture to be placed on the market

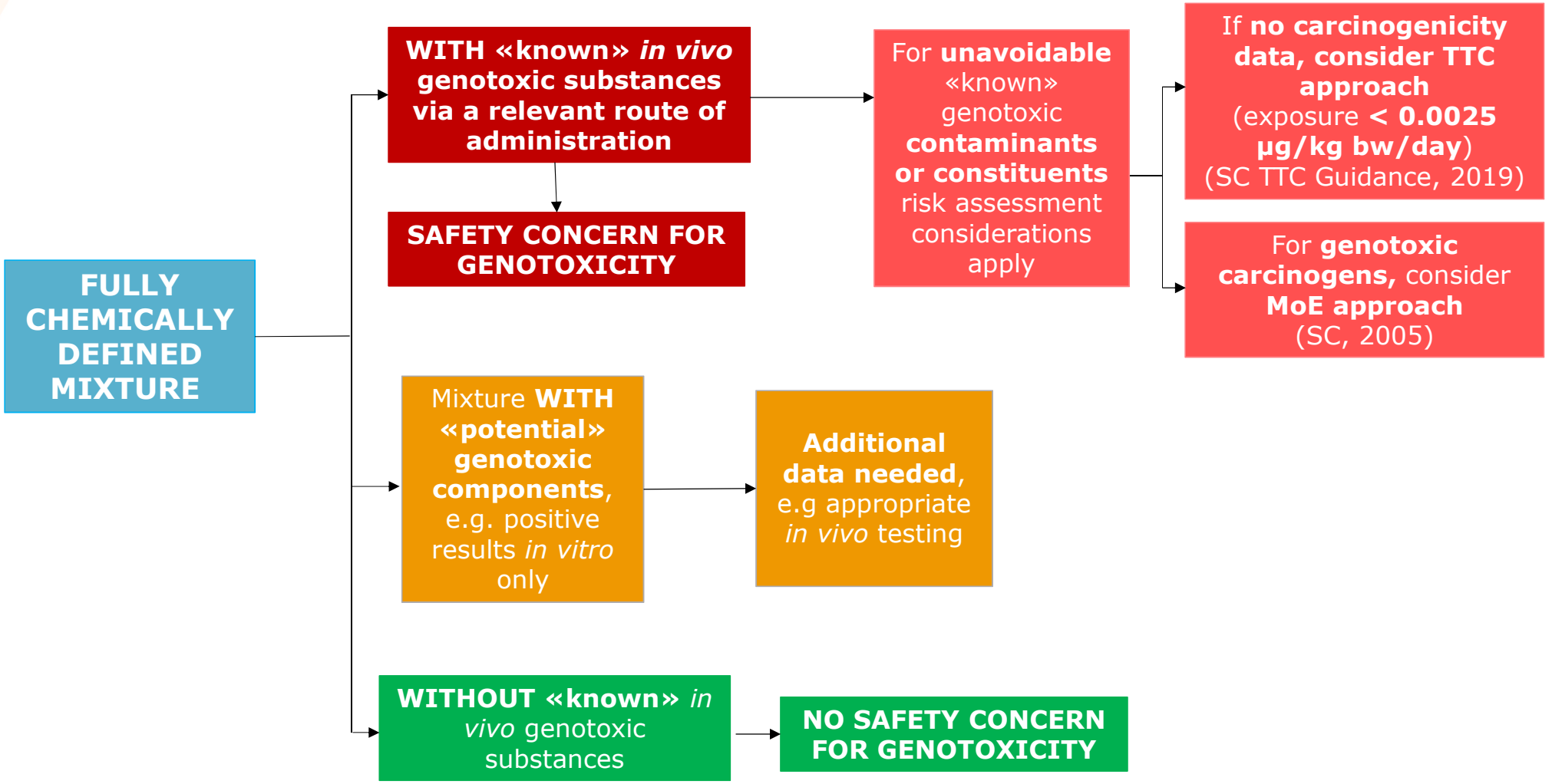
Chemically Fully defined

Mixtures containing a substantial fraction of unidentified components

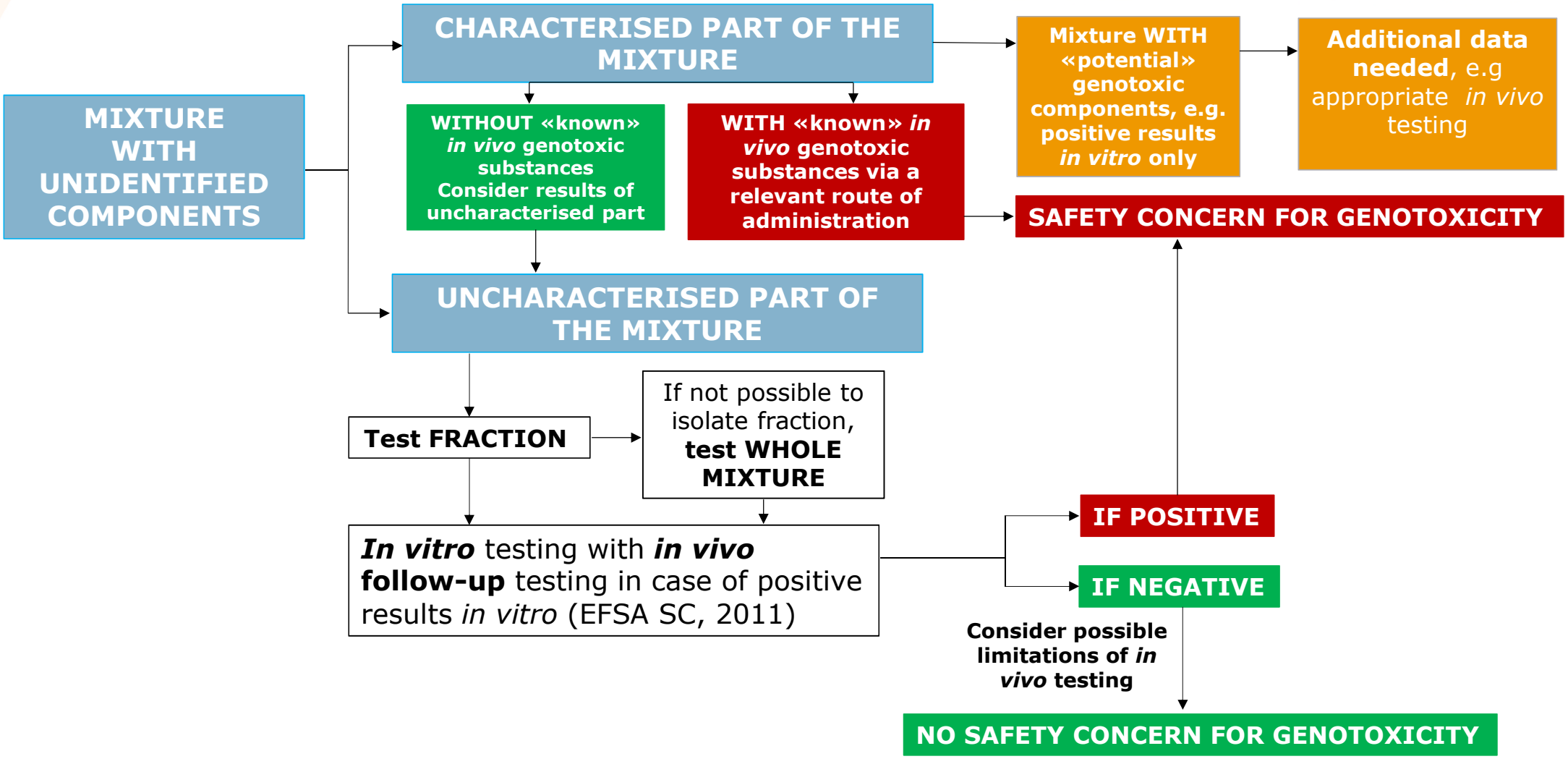
Genotoxicity of mixtures

- **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*

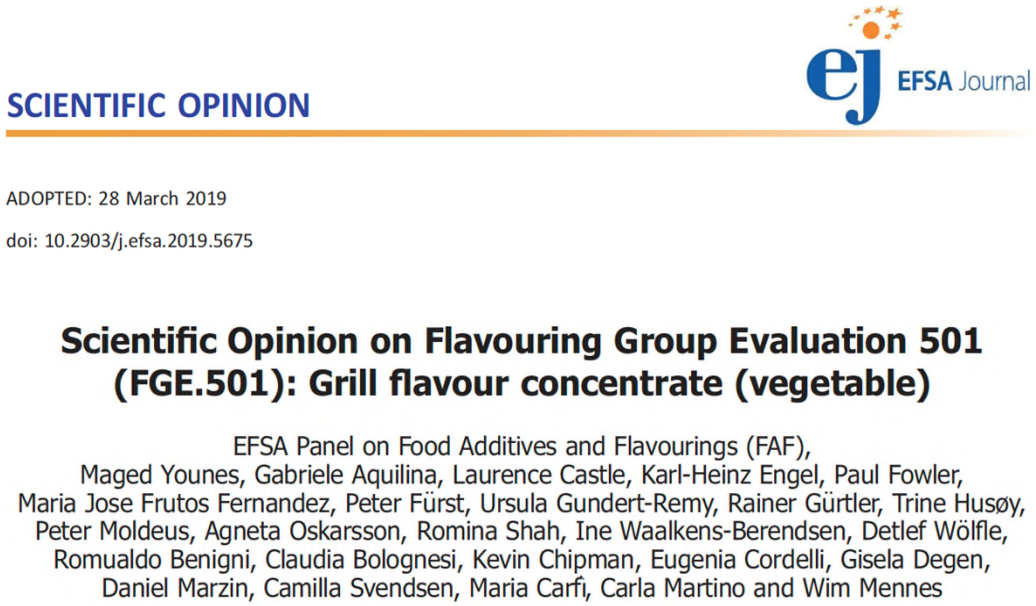
FULLY CHEMICALLY DEFINED MIXTURES: COMPONENT BASED APPROACH



MIXTURE WITH UNIDENTIFIED COMPONENTS



FGE.501 - Grill flavour concentrate (vegetable)



SCIENTIFIC OPINION

EFSA Journal

ADOPTED: 28 March 2019

doi: 10.2903/j.efsa.2019.5675

Scientific Opinion on Flavouring Group Evaluation 501 (FGE.501): Grill flavour concentrate (vegetable)

EFSA Panel on Food Additives and Flavourings (FAF),
Maged Younes, Gabriele Aquilina, Laurence Castle, Karl-Heinz Engel, Paul Fowler, Maria Jose Frutos Fernandez, Peter Fürst, Ursula Gundert-Remy, Rainer Gürtler, Trine Husøy, Peter Moldeus, Agneta Oskarsson, Romina Shah, Ine Waalkens-Berendsen, Detlef Wölfle, Romualdo Benigni, Claudia Bolognesi, Kevin Chipman, Eugenia Cordelli, Gisela Degen, Daniel Marzin, Camilla Svendsen, Maria Carfì, Carla Martino and Wim Mennes

- It is a new «other flavouring» submitted under Reg (EC) No 1334/2008 and Reg (EC) No 1331/2008
- It is a complex mixture of volatile constituents (16% of unidentified fraction) derived from canola oil (from the seeds of *Brassica napus*)
- The EFSA SC Statement on genotoxicity assessment of chemical mixtures was considered in this assessment

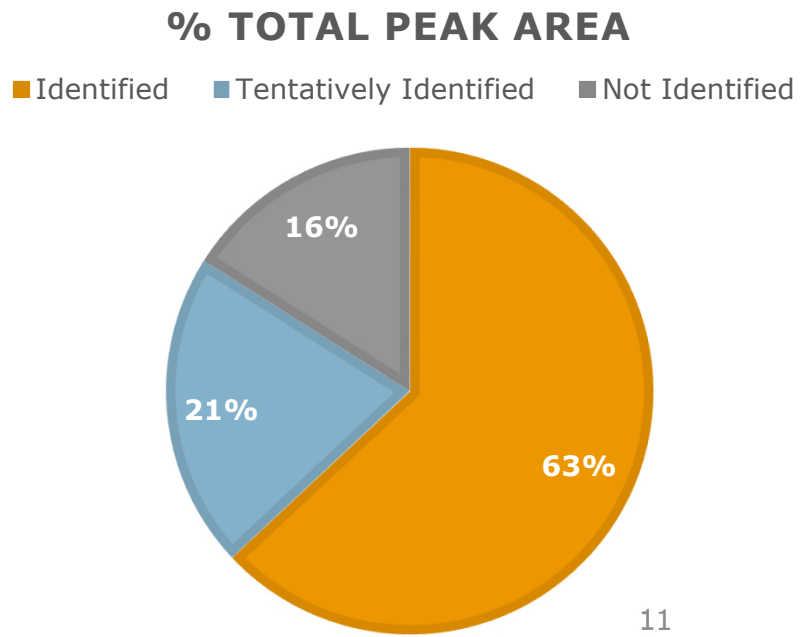
FGE.501 – compositional data

- ✓ GC-FID performed on 5 batches:
  - distributions of the volatiles sufficiently consistent
  - good reproducibility of the manufacturing process
- ✓ GC-MS analysis performed on 1 batch:
  - estimation of relative peak area of single components (average %)

Table 2: Overall composition of Grill flavour concentrate expressed as percentage peak areas determined by GC/MS

Fraction	Number of peaks	% of total peak area	Relative peak area of single components	
			Average % of peak area	Max % of peak area
All peaks	630	100	0.16	5.4
Identified[(a)]	156	63	0.39	5.4
Tentatively identified[(b)]	88	21	0.22	2.1
Not identified	386	16	0.05	1.1

GC/MS: gas chromatography/mass spectrometry.
(a): By means of MS/Reference library.
(b): Compared with fragmentation pattern of homologous compounds.



FGE.501 - compositional data

Table 3: The 20 principal identified constituents of 'Grill flavour concentrate (vegetable)', expressed as percentage of total peak area determined by GC/MS in batch no. 202509

Constituent	% of total peak area
Octanoic acid	5.4
Decanoic acid	5.0
Heptanoic acid	3.9
6-Heptenoic acid	3.5
(*Z*)-8-Heptadecene	2.7
Nonanoic acid	2.6
Hexanoic acid	2.4
7-Octenoic acid	1.9
(*E*)-2-Decenal	1.9
5-Hexenoic acid	1.4
Nonanal	1.2
(*E*)-2-Undecenal	1.1
9-Decenoic acid	0.8
Pentanoic acid	0.8
2-Octylfuran	0.8
4-Pentenoic acid	0.7
Pentadecane	0.7
Heptadecane	0.7
10-Undecenoic acid	0.7
Decan-2-one	0.4
Total	**38.6**

GC/MS: gas chromatography/mass spectrometry.

Analysis of the constituents:

33-34% → saturated and unsaturated short/medium chain (C4-C11) fatty acids
2-4% → long chain fatty acids
20-25% → aliphatic saturated and unsaturated hydrocarbons
9-10% → aldehydes and ketones
1-2% → aromatic compounds

Stability analysis:

no significant change in the overall composition of the flavouring

FGE.501 – genotoxicity data

1. Identified constituents

The 156 identified components of the mixture were evaluated both:

→ *in-silico* (OECD QSAR toolbox) to identify structural alerts related to genotoxicity and derive predictions for specific genotoxicity testing (i.e. Ames test, *in vitro* chromosomal aberration and micronucleus tests profilers)

→ via experimental genotoxicity data (relevant for 79 constituents) made available by industry and assessed by EFSA in the context of other opinions on flavouring substances

FGE.501 – genotoxicity data

Outcome

- *In silico* analysis:
  - Structural alerts (alpha-beta unsaturated carbonyls and simple aldehyde) were identified in 23 substances:
    - For 21 substances the genotoxicity concern identified *in silico* was ruled out by the experimental data available on the substances or on structurally related substances in the context of genotoxicity evaluation of flavourings
    - For 2 substances (2-decen-1,4-lactone, 2-undecen-4-one) the evaluation of their genotoxic potential was still pending as additional genotoxicity data have been requested in the context of ongoing opinions on flavourings

- Missing genotoxicity data:
  - 4 substances (2-pentylfuran, 2-heptylfuran, 2-octylfuran and 2-hexylfuran), although no structural alerts were identified *in silico*, are still under evaluation for their potential genotoxicity in the context of ongoing opinions on flavourings

FGE.501 – genotoxicity data

2. Tentatively identified constituents

- in the absence of fully confirmatory chemical data, the Panel considered the tentatively identified part of the mixture **as uncharacterised**

3. Unidentified fraction

- the flavouring has been **characterised at the maximum extent**, not possible to isolate and/or further characterise the unidentified part of the mixture
- the Panel considered that the **separation and testing** of the unidentified and/or of the tentatively identified part of the mixture would **not be technically feasible**

Therefore, the Panel considered not only the available information on individual constituents of the chemically characterised fraction, but also the experimental data on the whole mixture for the genotoxicity assessment of the flavouring

FGE.501 – genotoxicity data

Experimental data on the whole mixture

- Ames test → negative
- *In vitro* micronucleus assay → negative
- No need of *in vivo* follow-up testing

FGE.501 – conclusion on genotoxicity

- ✓Experimental data obtained with the whole mixture do not indicate a concern for genotoxicity
- ✓Except for six substances (i.e. 2-decen-1,4-lactone, 2-undecen-4-one, 2-pentylfuran, 2-heptylfuran, 2-octylfuran and 2-hexylfuran), the assessment of individual components does not raise a concern for genotoxicity
- ✓For these six substances there is an indication for genotoxicity. Therefore, these six substances have to be evaluated. Until these evaluations have been finalised the safety of Grill flavour concentrate (vegetable) cannot be fully assessed.

Thank you for your attention!