10 October 2024, 14:30-16:30 CET

Ad-hoc meeting with interested food business representatives on Art 8(4) Opinion related to HYDROXYANTRACENE DERIVATIVES (HAD)

EUROPEAN FOOD SAFETY AUTHORITY

WHO'S WHO

- **Nutrition and Food Innovation (NIF) Unit**: Ana Afonso (Head of Unit, Chair), Thibault Fiolet, Leng Heng, Georges Kass, Leonard Matijevic, Yustina Olshevska-Grigorov, Annamaria Rossi, and Ariane Titz
- **Methodology and Scientific Support (MESE) Unit**: Daniela Maurici, Sara Levorato, Alexis Nathanail
- **Engagement and External Relations unit (ENREL) Unit**: Lucia Parrino
- **Legal Affairs Services (LA) Unit**: Citlali Pintado
- **European Commission–SANTE A.1 Unit**: Fruzsina Nyemecz, Petroula Vantsiouri (Observers)

WELCOME TO INTERESTED FOOD BUSINESS OPERATORS' REPRESENTATIVES

REPRESENTATIVE ORGANISATIONS OF PARTICIPANTS	
Italian Society of Phytotherapy	FEDERAZIONE ERBORISTI ITALIANI (FEI) - FEDER BOTANICALS ITALIA – CONFCOMMERCIO *[FEI: Submitter of studies during scrutiny period]*
Ortis *[Submitter of studies during scrutiny period]*	LINNEUS counsulting
Association of the European Self-Care Industry (AESGP)	Finzelberg GmbH & Co.KG *[Submitter of studies during public consultation]*
Bayer	Food Supplement Europe (FSE)
MCM Klosterfrau Vertriebsgesellschaft mbH	ASSOERBE
Pharma Deutschland	LR HEALTH
LABORATORIOS CINFA	HERBALIFE
Laboratorios Normon	Phytolab
IGEPHA	The European Federation of Associations of Health Product Manufacturers (EHPM)
Indena	Lebensmittelverband Deutschland
Fondazione Toscana Life Sciences	HERMES ARZNEIMITTEL GmbH *[Submitter of studies during Public consultation]*

HOUSEKEEPING

- Questions pre-submitted are addressed in the presentation
- Q&A after the sessions on methodology & on results conclusions, and at the end
- Use “raise hand” function to ask the floor during Q&A sessions
- keep your microphone muted & camera off, unless you are giving the floor
- Keep the meeting chat box clean
- If you have problems with the connection, exit the meeting & rejoin

POST MEETING - Publication on EFSA’s website: the presentation including the agenda, & list of participating organisations.

AGENDA

1. WELCOME TO PARTICIPANTS
2. SCOPE OF THE MEETING
3. BACKGROUND & MANDATE
4. GENOTOXICITY ASSESSMENT + Q&A
5. RESULTS AND CONCLUSIONS
6. REMAINING QUESTIONS + Q&A
7. CLOSING REMARKS

SCOPE (1)

- In line with EFSA's commitment to engaging with its stakeholders and improving understanding of its work
- **The meeting objective**: To explain & exchange information on the methodological aspects, scientific requirements, and approach applied for the 2024 **EFSA NDA Panel's Art. 8(4) Opinion related to hydroxyanthracene derivatives (HAD)**

SCOPE (2)

No re-opening the conclusions of:

- 2018 Scientific Opinion of the ANS Panel on the safety of HAD
- 2022 Technical Report on the request for technical assistance in relation to the safety of HAD – which assessed two studies by Galli et al. (2021a, 2021b)
- 2024 EFSA's Art. 8(4) opinion related to HAD

ART 8(4) OPINION

UNDER THE FRAMEWORK OF REG (EC) NO 1925/2006 ON THE ADDITION OF VITAMINS AND MINERALS AND OF CERTAIN OTHER SUBSTANCES TO FOODS:

HYDROXYANTRACENE DERIVATIVES (HAD)

Dr. Thibault Fiolet, Dr. Ariane Titz

EFSA, Nutrition and Food Innovation Unit

Dr. Alexis Nathanail

EFSA, Methodology and Scientific Support Unit

efsa
EUROPEAN FOOD SAFETY AUTHORITY

BACKGROUND & MANDATE

EFSA ANS PANEL OPINION (2018)

2016: initiation of Article 8(2) procedure to evaluate the safety of Hydroxyanthracene derivatives (HAD)

2018: ANS Panel Opinion on HAD

Genotoxic *in vitro*	**Genotoxic *in vivo***	**Carcinogenic**
Emodin, danthron, aloe-extracts, **aloe-emodin**	**Aloe-emodin**	Whole leaf aloe extract, danthron

"The Panel concluded that **hydroxyanthracene derivatives** should be regarded **as genotoxic and carcinogenic** unless there are specific data to the contrary, such as for rhein

And that there is a **safety concern for extracts containing hydroxyanthracene derivatives** although uncertainty persists"

EFSA ANS PANEL OPINION (2018)

HAD terms **restricted** to anthranoid compounds found

- ❑ in the root and rhizome of *Rheum palmatum* L. and/or *Rheum officinale* Baillon and/or their hybrids
- ❑ leaves or fruits of *Cassia senna* L. and *Cassia angustifolia* Vahl
- ❑ bark of *Rhamnus frangula* L.
- ❑ bark of *Rhamnus purshianus* D.C.
- ❑ leaves of *Aloe barbadensis* Miller and/or various aloe species, mainly *Aloe ferox* Miller and its hybrids and further

COMMISSION REGULATION (EU) 2021/468

Amending Annex III of Regulation (EC) No 1925/2006

Part A Prohibited

- **aloe-emodin, emodin** and danthron and all preparations in which these substances are present
- preparations from the leaf of *Aloe* species containing hydroxyanthracene derivatives

Parc C Under union scrutiny

- 'preparations from the root or rhizome of *Rheum palmatum* L., *Rheum officinale* Baillon and their hybrids containing
- hydroxyanthracene derivatives';
- 'preparations from the leaf or fruit of *Cassia senna* L. containing hydroxyanthracene derivatives';
- 'preparations from the bark of *Rhamnus frangula* L., *Rhamnus purshiana* DC. containing hydroxyanthracene derivatives'

EC MANDATE (ARTICLE 8.4) - TERMS OF REFERENCE

On 18 March 2021, pursuant to Article 1(2) of Commission Regulation (EU) 2021/468[2], amending Annex III, Part C, of Regulation (EC) No 1925/2006, the Commission has placed the following entries under scrutiny:

- 'preparations from the root or rhizome of *Rheum palmatum* L., *Rheum officinale* Baillon and their hybrids containing hydroxyanthracene derivatives';
- 'preparations from the leaf or fruit of *Cassia senna* L. containing hydroxyanthracene derivatives';
- 'preparations from the bark of *Rhamnus frangula* L., *Rhamnus purshiana* DC. containing hydroxyanthracene derivatives'

We would like to ask the opinion of EFSA on whether the scientific data contained in the files submitted or the be submitted for evaluation by food business operators, or any other interested parties demonstrate the safety of substances placed under Union scrutiny mentioned above.

SCOPE OF THE MANDATE

Under this framework, there was **no re-assessment of the safety of individual HADs** (aloe-emodin, emodin, danthron) or plants preparations mentioned in Annex III, Part A

Only data submitted during the **scrutiny period** and the **public consultation** were used for the Article 8(4) evaluation

INTERPRETATION OF TERM OF REFERENCE

Recital 11 of Commission Regulation (EU) 2021/468 : 'scientific uncertainty persists about whether such preparations contain the substances listed in Annex III, Part A (aloe-emodin, emodin and danthron)

EFSA was requested to assess:

1) whether the plant preparations under evaluation **contain aloe-emodin and/or emodin** (Part A), and/or contain **other HAD** (ANS Panel 2018 Opinion)

2) in case of the **absence of genotoxic carcinogenic HAD** (as demonstrated by appropriate analytical methods), the genotoxic and carcinogenic potential of the plant preparations (as chemical mixtures) will be evaluated

DATA RECEIVED

PERIOD OF SCRUTINY

- **ORTIS: 3 genotoxicity studies** on Rhubarb extract
- **FEI: *in vitro* studies**: bioavailability, computational prediction of pharmacokinetics of different HADs, cytotoxicity, induction of cytokines, ROS formation on individual HADs and plant extracts + narrative review

PUBLIC CONSULTATION

- **SITOX: 5 *in vitro* micronucleus test** on Extracts of *Rheum palmatum* L., *Rhamnus purshiana* DC., *Rhamnus frangula* L., and *Cassia senna* L
- **Finzelberg Gmbh & Co: 5 Ames tests** on *Senna* leaves and fruit, *Rhamnus purshiana* bark extract, *Rheum palmatum* and *Rhamus frangula*
- **1 publication**: Melzi, Gloria et al. "Lack of genotoxicity of rhubarb (rhizome) in the Ames and micronucleus in vitro tests." Toxicology reports vol. 9 1574-1579. 30 Jul. 2022,

Evaluation by the **cross-cutting WG on Genotoxicity**

The outcome of this assessment was submitted to the NDA Panel for its consideration and decision

GENOTOXICITY ASSESSMENT

GENOTOXICITY IN THE RISK ASSESSMENT OF FOOD/FEED

- Genotoxicity *per se* is an endpoint: genetic damage in somatic or germ cells is associated with serious detrimental health effects, including cancer, heritable diseases and degenerative conditions. Consequently, the risk assessment of food contains a genotoxicity assessment.

- **EFSA's genotoxicity guidance documents:**

  - Scientific Committee (SC) Opinion (2011): Genotoxicity testing strategies
  - SC Statement (2017): Clarification on some aspects of genotoxicity assessment (in vivo UDS, bone marrow, reference values)
  - SC Statement (2019): Genotoxicity assessment of mixtures
  - SC Guidance (2019): Aneugenicity assessment
  - SC Technical Report (2023): Harmonised approach for reporting reliability and relevance of genotoxicity studies

EFSA'S CROSS-CUTTING GENOTOXICITY WORKING GROUP

- Genotoxicity assessment of substances is carried out by the experts of EFSA's Scientific Panels or Member States Authorities (pesticides). These assessments are following EFSA's Genotoxicity Testing Strategies and relevant guidance documents, when applicable.

- The **Cross-cutting (cc) Working Group on Genotoxicity** aims to ensure a harmonised interpretation and implementation of EFSA's genotoxicity testing strategies among Panels and Units:
  - Provides support to the different EFSA Units/Panels in the evaluation of genotoxicity data sets/scientific literature for assessments where different views have been expressed in the respective Panels or within the same Panel.
  - Provides advice on the interpretation of genotoxicity data in the light of the genotoxicity strategy and provides advice on the interpretation of equivocal and complex genotoxicity test results.

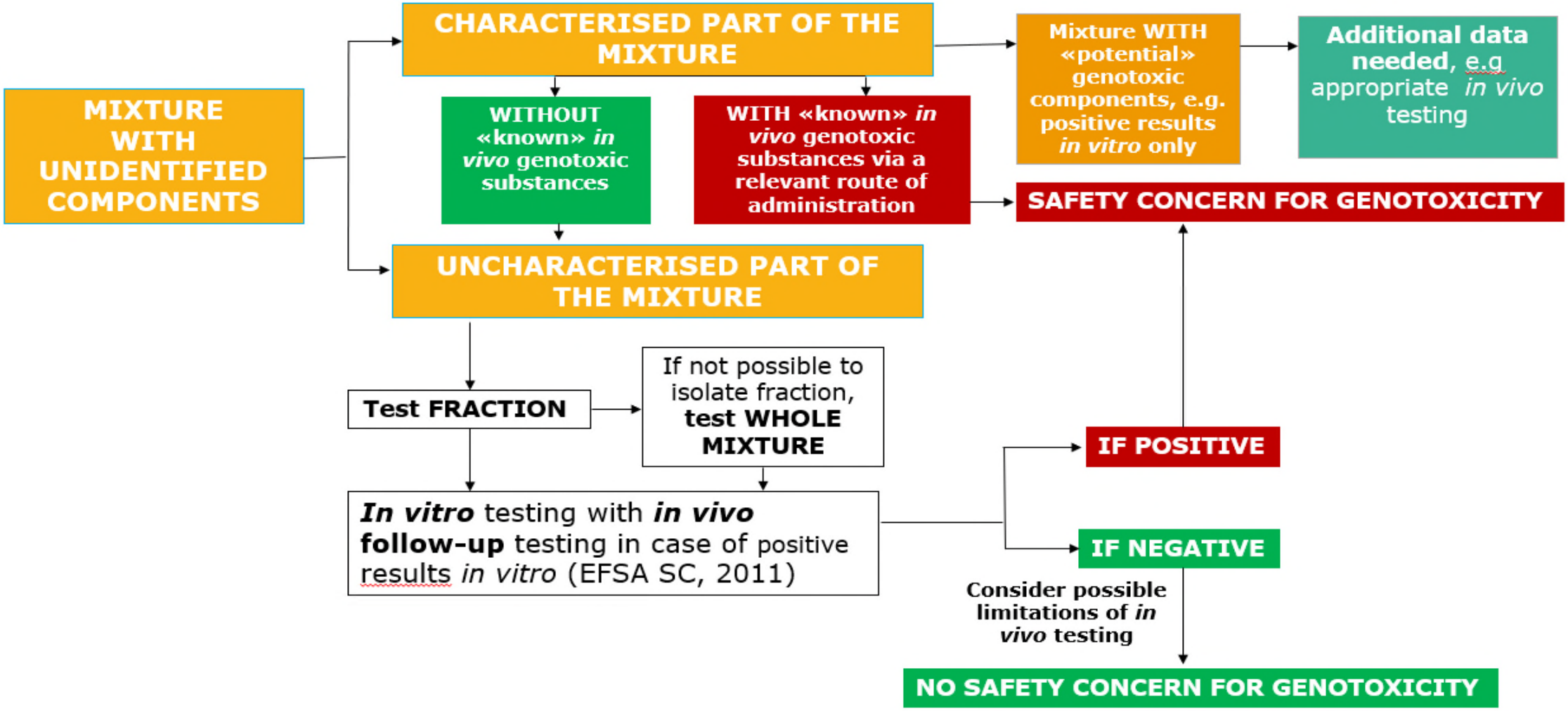
EVALUATION OF RELEVANCE OF INDIVIDUAL GENOTOXICITY STUDIES

- The **relevance of the test system** should be based
  - on the **genetic endpoint.** Bacterial reverse mutation test, micronucleus tests *in vitro* and *in vivo*, comet assay *in vivo are* tests with high relevance for hazard identification. *In vitro* comet assay has a low relevance
  - the status of validation (following OECD guidelines > internationally recommended protocol > validation at national level only)

- The **relevance of the study results** should take into account the route of administration, the biological relevance of the study results (purity of test substance, metabolic capabilities of test system, exposure of target tissue, etc.) and the reliability of the study

EFSA (European Food Safety Authority), Andreoli C, Aquilina G, Bignami M, Bolognesi C, Crebelli R, Dusinska M, Gürtler R, Louro H, Marcon F, Nielsen E, Schlatter J, Vleminckx C, Astuto MC, Nathanail AV and Benford D, 2023. Harmonised approach for reporting reliability and relevance of genotoxicity studies. EFSA supporting publication 2023:EN-8270. 12 pp. doi:10.2903/sp.efsa.2023.EN-8270

MIXTURES WITH UNIDENTIFIED COMPONENTS



EFSA'S GUIDANCE DOCUMENTS – UPCOMING REVISIONS

EFSA is periodically updating its Guidance documents and methodologies, more specifically:

MoE Guidance Revision:

- Scoping Paper Public Consultation completed
- Currently, agreement of terms of reference and drafting to start soon (for a period of 18-24 months)

Genotoxicity Guidance Revision (major revision concerning all relevant guidance documents):

- Scoping paper under development by experts from the ccGenotoxicity WG
- Public Consultation of scoping paper in Q4 2024 or Q1 2025 following approval by EFSA's SC
- Finalisation of terms of reference and start of revision by end of Q1 2025 (for a period of ~2 years)

Botanicals Guidance Revision:

- Need to update the guidance has been identified
- Scoping document to be prepared and presented to the SC for discussion in Q2 2025

RESULTS AND CONCLUSIONS

PRESENCE OF GENOTOXIC HAD IN THE EXTRACTS

	Documents submitted	Number of HADs measured
ORTIS	Certificates of analysis on HADs, nutritional composition (ADR1)	9 HADs quantified + discrepancy on the total HADs content
FEI	Data not considered further because it did not relate to genotoxicity or carcinogenicity	
Finzelberg	Information on the standardization of the extract	1 HAD per extract. No information on aloe-emodin, emodin
SITOX	Letter with an average content on aloe-emodin, emodin, rhein	1 HAD per extract (for example: cascaroside A for cascara extract)
Melzi 2022	HADs and nutritional composition provided	6 HADs

- All the studies **confirmed the presence of aloe-emodin (genotoxic in vivo) and emodin (genotoxic in vitro)**
- Hence, the evidence provided could not establish that they are systematically absent from these plant preparations

UNCERTAINTY ON THE CHARACTERIZATION

- Several assays did not report the scientific names, and the plant part of the extracts tested
- HAD represent a broad category of compounds and **reporting was variable and heterogenous**
- For all the assays, there was uncertainty on the content of **other components than HAD**

The presence of **unidentified or uncharacterised components in the plant mixtures** used in foods represents a major source of **uncertainty**

RESULTS

- Seven *in vitro* bacterial reverse mutation tests
- One combined *in vivo* comet assay and micronucleus test in Sprague-Dawley SD rats
- Six *in vitro* micronucleus tests in human lymphocytes

All the studies reported negative results for extracts from Rheum palmatum L., Rhamnus purshiana DC., Rhamnus frangula L. (bark) and Cassia senna L. (leaves and fruits)

All the studies confirm the presence of aloe-emodin (genotoxic in vivo) → low relevance of the results

CONCLUSION

- On this basis, considering that **aloe-emodin was shown to be genotoxic in vivo**, **the mixture has to be considered of concern for genotoxicity** if in a botanical extract the absence of this component cannot be demonstrated by appropriate analytical methods. This is **independent of the outcome of experiments** conducted on the whole extract.

CONCLUSION

- **The NDA Panel concluded there is a safety concern for genotoxicity of the plant preparations containing HAD included in this mandate**

- According to the 2017 EFSA Scientific Committee opinion on the Clarification of some aspects related to genotoxicity assessment, *"taking all available evidence into account, if the overall evaluation leaves no concern for genotoxicity in vivo, a HBGV may be established. If, based on the overall assessment,* ***concern for genotoxicity remains, establishing an HBGV is not considered appropriate****"*

REMAINING QUESTIONS

QUESTIONS

(in blue = questions addressed)

- **ORTIS**: why the EFSA panel has changed the initial request of the European Commission?
- **FSE/Hermes/FEI/ORTIS:** questions on the presence of genotoxic HAD and the partial and/or insufficient characterisation
- **FSE:** Can EFSA confirm that if a food business operator can demonstrate the absence of genotoxic HAD by appropriate analytical methods such preparations would not be of concern for genotoxicity?

QUESTIONS

(in blue = questions addressed)

Genotoxicity assessment

- ❑ FSE: why EFSA concluded that the relevance of the results of the new studies is low

- ❑ **FSE:** can EFSA explain why it did not consider the margin of exposure approach being suitable for the assessment of these plants or plant preparations containing HAD?

- ❑ **FSE/SISTE/EHPM:** questions on this evaluation based on individual components, not considering the matrix effect

- ❑ **SISTE:** question on natural genotoxic compounds (methyl eugenol, safrole) in common food

- ❑ **SISTE:** what is cover by the term “plant preparations” (beverage, jams or candies)?

The term ‘preparation’ covers all preparations obtained from botanical materials (e.g. whole, fragmented or cut plants, plant parts, algae, fungi and lichens) by various processes (e.g. pressing, squeezing, extraction, fractionation, distillation, concentration, drying up and fermentation) (EFSA Scientific Committee, 2009)

QUESTIONS

The weight of the previous studies

- **SISTE:** consideration of the Galli et al., 2021 study results?
- **EHPM/FSE:** question on the disparity in the methodological quality between current papers and those from 10 to +25 years ago

Other assessment

- **FSE:** question on EMA assessment in relation to Cassia senna L. and Galli et al. 2021 study
- **Finzelberg:** question on the UK interim position on the safety of hydroxyanthracene derivates for the use in food (April 3rd, 2024)

- **FEI:** possibility to submit additional data

Thanks for your attention

Please make sure to subscribe to EFSA's newsletters and email alerts to stay updated on consultation opportunities regarding the guidance document revisions mentioned during the meeting, as well as on general opportunities for engagement with EFSA