SH MEETING ON BOTANICALS
5 FEBRUARY 2025

METHODOLOGICAL ASPECTS AND THE STANDARD APPROACH TO ESSENTIAL OILS, PARTICULARLY REGARDING THEIR SAFETY FOR TARGET ANIMAL SPECIES

FEEDAP Working Group on Feed Flavourings

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EUROPEAN FOOD SAFETY AUTHORITY

AIM OF THE MEETING

- To inform applicants on methodological updates in the approach to assess the safety for the target species of botanicals, particularly for essential oils and to have a common understanding of the methodology
- To address in a generic way some scientific requirements requested for the assessment

OUTLINE

- Compounds included in the specifications
- Need to indicate an LOD of the methods for compounds expected but not detected
- Stereoisomers
- Read-across to major components: issues identified
- Grouping of compounds
- Results of tolerance trials
- Uncertainty factor for cats: 500 by default, exceptions
- Outcome of the New Assessment Methodology case study on essential oils: which implications for cats?
- Interpretation of the wording “low concern”
- Status of the re-evaluation of botanical feed flavourings
- Restart of all Botanically Defined Groups (22/01/2025)

COMPOUNDS INCLUDED IN THE SPECIFICATIONS

- Several requests for EC and MS:
- **Juniper oil** (ON-8349): α-pinene and β-pinene assessed at the highest analysed concentrations (50.2%), but up to 67% by specification. When considering sabinene: 59.9% vs 87%
- **Coriander oil** (ON-8349): linalool and camphor assessed at the maximum specifications, the other compounds at the highest analysed concentrations
- *Does the FEEDAP assessment cover all the oils meeting the proposed specifications?*

Table 2: Main constituents of the essential oil from the berries of *Juniperus communis* L. as defined by specifications: batch to batch variation based on the analysis of five batches. The content of each constituent is expressed as the area per cent of the corresponding chromatographic peak (% GC area), assuming the sum of chromatographic areas of all detected peaks as 100%

Constituent	CAS No	FLAVIS No	% GC area		
EU register name			Specification	Mean	Range[(a)]
α-Pinene (pin-2(3)-ene)	80-56-8	01.004	25–45	41.0	39.6–44.2
Myrcene	125-35-3	01.008	3–22	10.6	4.9–12.9
Sabinene (4(10)-thujene)	3387-41-5	01.059	4–20	8.2	7.5–9.7
β-Pinene (pin-2(10)-ene)	127-91-3	01.003	1–12	5.0	4.3–7.3
Total				64.8	63.4–66.1

Table 2: Major constituents of the essential oil from the fruit of *Coriandrum sativum* L. as defined by specification: batch to batch variation based on the analysis of five batches by gas chromatography with flame ionisation detector (GC-FID). The content of each constituent is expressed as the area per cent of the corresponding chromatographic peak (% GC area), assuming the sum of chromatographic areas of all detected peaks as 100%

Constituent	CAS No	FLAVIS No	% GC area		
EU register name			Specification	Mean	Range
Linalool	78-70-6	02.013	65–78	71.7	67.3–76.4
α-Pinene	80-56-8	01.004	3–8.5	6.23	4.93–7.06
γ-Terpinene	99-85-4	01.020	2–7	4.41	3.48–5.07
Camphor[(a)]	76-22-2	–	3–6	4.59	3.84–5.39
Geranyl acetate	105-87-3	09.011	0.5–4.5	2.70	1.33–3.92
d-Limonene	138-86-3	01.045	0.5–5	2.67	1.19–3.69
Geraniol	106-24-1	02.012	0.1–3	1.41	0.72–2.20
Myrcene	123-35-3	01.008	0.1–2	1.05	0.90–1.19
α-Terpineol	98-55-5	02.014	≤ 1.5	0.32	0.18–0.57

COMPOUNDS INCLUDED IN THE SPECIFICATIONS

- **Anise oil** (ON-7976, 2023): estragole included in the specifications (0.1-3.0%)
- Two scenarios assessed:
  - Scenario **(a)**: estragole at the highest specification (3%), Table 6
  - Scenario **(b)**: estragole at the highest analysed concentration (1.08%), Table 7
- Different conclusions for the two oils
  - MOET <10,000 for **(a)**, concern
  - MOET >10,000 for **(b)**, low concern

Table 6: Target animal intake of estragole and total *p*-allylalkoxybenzenes and combined margin of exposure (MOET) calculated at the maximum proposed use level of the additive in feed for an essential oil with a content of estragole of 3%, and other *p*-allylalkoxybenzenes present at the maximum expected concentration (1.0% myristicin, 0.3% dillapiole and 0.03% methyleugenol)

Animal category	Daily feed intake	Body weight	Use level in feed	Estragole intake[(a)]	Combined intake[(a)]	MOET
Long-living and reproductive animals	kg DM/day	kg	mg/kg feed	µg/kg bw per day		
Laying hen	0.106	2	1.9	3.433	4.480	4,480[(b)]
Horse	8	400	5	3.409	4.515	4,512[(b)]
Target species for fattening						
Chicken for fattening	0.158	2	1.5	4.040	5.831	1,715[(c)]
Turkey for fattening	0.176	3	1.7	3.400	4.424	2,038[(c)]

Table 7: Target animal intake of estragole and total *p*-allylalkoxybenzenes and combined margin of exposure (MOET) calculated at the maximum proposed use level of the additive in feed for an essential oil with a content of estragole, myristicin, dillapiole and methyleugenol at the highest analysed concentration (1.08%, 0.627%, 0.207% and 0.015%, respectively)

Animal category:	Daily feed intake	Body weight	Use level in feed	Estragole intake[(a)]	Combined intake[(a)]	MOET
Long-living and reproductive animals	kg DM/day	kg	mg/kg feed	µg/kg bw per day		
Laying hen	0.106	2	1.9	1.236	2.209	10,052[(b)]
Horse	8	400	5	1.227	2.193	10,122[(b)]
Target species for fattening						
Chicken for fattening	0.158	2	1.5	1.454	2.599	3,848[(c)]
Turkey for fattening	0.176	3	1.7	1.224	2.187	4,572[(c)]

COMPOUNDS INCLUDED IN THE SPECIFICATIONS

For consistency and clarity reasons, as of September 2024, the compounds included in the specifications are assessed based on the maximum proposed limit

Initially ***limited to selected*** *components*

- Spanish sage oil (ON-9015): limited to camphor (up to 36%)
- Spanish type origanum oil (ON-9018): limited to carvacrol (75%) and thymol (5%)

Extended to all specified components

- Sage oil (ON-9135): camphor (24.5%), α-thujone (27%), β-thujone (7%) and 1,8-cineole (13%)
- Peppermint oil (ON-9076): menthol (55%), menthone (32%), *d,l*-isomentone (10%), menthyl acetate (10%) and 1,8-cineole (8%)

- **From now on:** For those compounds covered by specifications the maximum limit is used for the calculation of exposure
- Approach presented to the MS (November 2024)

LIMIT OF DETECTION FOR NOT DETECTED COMPOUNDS

- Recurrent request of clarification concerning the need to indicate the limit of detection (LOD) for components not detected
- Particularly relevant for substances of concern (e.g. *p*-allylalkoxybenzenes, furocoumarins, perillaldehyde, thujones)
- When not detected the LOD may be included in the recommendation
- In general, for methyleugenol, safrole, estragole LOD of 0.01% (%GC area)
- In few cases, a lower LOD, ranging between 0.001% and 0.005%
- Different LODs for *p*-allylakoxybenzenes (0.0015-0.01%) in caraway oil, provided by the different laboratories
- **For action:** to provide the LOD with the dataset

STEREOISOMERS

For several components, stereochemistry is not indicated in the dataset provided

- Camphor, e.g. in sage oil
  - Footnote: Present in the additive as a mixture of enantiomers (*d,l*-camphor), the ratio between *d*- and *l*-stereoisomers not given
  - Read-across from *d*-camphor tested in tolerance trials to *l*-camphor

Information from literature, monographs may complement analytical data

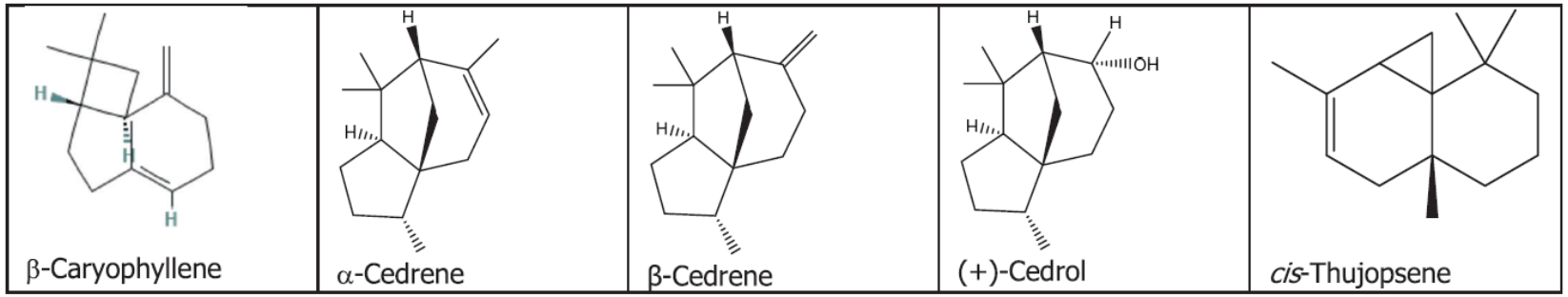
- Limonene, e.g. in sage oil
  - Footnote: Stereochemistry not given, however considering that the naturally occurring limonene is typically *d*-limonene, it is assumed that this form also occurs in sage oil
  - Read across from *d*-limonene [01.045] in CG 31,III
- Carvone and limonene in caraway oil
  - The FEEDAP Panel notes that the main components of caraway oil are *d*-carvone and *d*-limonene (EMA, 2015e; PhEur Commentary, 2020). Therefore, the current assessment will refer to these enantiomers
  - $BMDL_{10}$ of 60 mg/kg bw per day for *d*-carvone, tolerance trials for *l*-carvone (10 mg/kg)

APPLICATION OF READ-ACROSS: ISSUES IDENTIFIED

- Read-across within an assessment group (and across groups) has been widely applied to essential oils since 2019
- In the majority of cases, reference values were available for the major components and read-across has been applied to **minor components**
- In 2024, two assessments challenged the approach, with a different outcome
  - Cedarwood Texas oil (ON-8799)
  - Tea tree oil (ON-9026)
- Common issue: reference values not available for the major components accounting for about 80% of the % GC area
- High uncertainty when read-across is made to major components

APPLICATION OF READ-ACROSS: CEDARWOOD TEXAS OIL

- The main constituents cis-thujopsene (up to 35%), (+)-cedrol (>20%), α- cedrene (up to 25%) and β-cedrene (up to 8%) account for 78% of the % GC area
- Partial evaluation as flavourings of (+)-cedrol [02.120] and α- cedrene [01.122]
- Reference points not available for the four specified components
- Read-across from β-caryophyllene (NOAEL of 222 mg/kg bw) *is possible?*

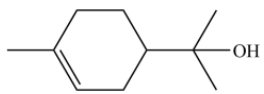
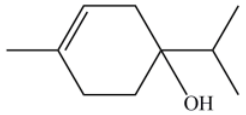
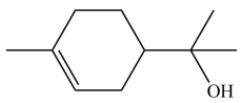
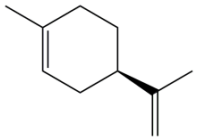
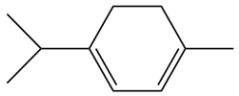
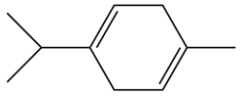
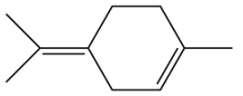


- Expert judgement (structural and metabolic similarity) and computational tools (VEGA, Janus, ADMETlab 2.0 and swissADME)
- Case study presented to the SC Working Group on read-across (03/2024)

https://doi.org/10.2903/j.efsa.2024.8799

TEA TREE OIL

- The main constituents 4-terpinenol (up to 48% by specification), γ-terpinene (up to 28%), and α-terpinene (up to 13%) account for up to 89% of the % GC area
- Other terpinyl derivatives present: α-terpineol (up to 7%), terpinolene (3.6%)

Terpineol [02.230]	**4-terpineol** [02.072]	α-terpineol [02.014]	d-limonene [01.045]	**α-terpinene** [01.019]	**γ-terpinene** [01.020]	Terpinolene [01.005]
						

- Terpineol isomers in CG 6, terpinyl derivatives in CG 31,III
- Tea tree oil: parallel assessment for an oil with the same composition as pesticide
- Limited data *vs*. extensive data: different data requirements
- Methyleugenol: minor component of the feed additive and impurity (pesticide) assessed based on the same approach, the MOE

COMPONENT-BASED OR WHOLE MIXTURE APPROACH?

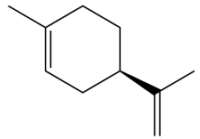
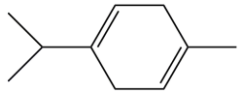
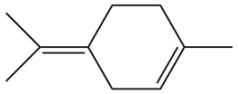
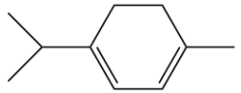
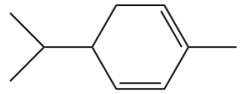
- Standard approach: The reference value for the main components of tea tree oil is based on **read-across** from terpineol (4-terpinenol and α-terpineol in CG 6) and *d*-limonene (γ-terpinene, α-terpinene and terpinolene in CG 31,III)
- Terpineol [02.230] was tested as a **mixture of isomers**, composition not available (from commercial website: α-terpineol 60-85%, γ-terpineol 10-25%, β-terpineol 0.5-13%, 4-terpinenol ?)
- **Large difference (up to 25-fold)** in the NOAEL(s) for the individual components (250 mg/kg bw per day for terpineol and *d*-limonene) and the NOAELs for the whole mixture (between 10 and 75 mg/kg bw per day)
- Possible reasons:
  - High uncertainty in read-across from terpineol to 4-terpinenol (mainly)
  - Considering the similarity between CG 6 and CG 31,III compounds, grouping is adequate?
  - Presence of degradation products (ascaridole, 1,2,4-trihydroxymenthane)
- Whole mixture approach preferred to component-based approach: the lowest NOAEL of 10 mg/kg bw per day was selected

AND NEXT? CBA *vs* WMA

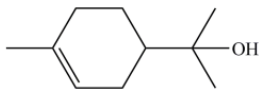
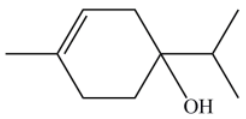
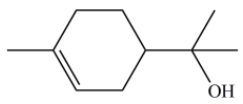
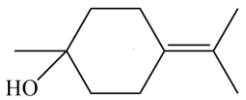
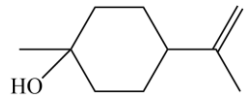
- In the case reference values are missing for **minor components**
  - The WG/Panel still applies read-across, e.g. from terpineol to 4-terpinenol, α-terpineol, γ-terpineol, etc. applying an UF of 2 to the NOAEL of 250 mg/kg bw per day (BDG 01)
- In the case reference values are missing for **major components**
- If toxicological data are available for the whole mixture (for an additive considered sufficiently similar and representative)
  - The WG/Panel may consider it as less uncertain to rely on a whole mixture approach rather than on a component-based approach based on read-across (for studies of short duration, an additional UF is applied)
- If toxicological data for the whole mixture are not available
  - an additional UF may be needed for uncertainty in read-across (magnitude established on a case-by-case basis)
  - or the threshold of toxicological concern (TTC) may be applied

GROUPING OF COMPOUNDS NEEDS REFINEMENT?

- The initial definition of assessment groups (corresponding to the chemical groups as defined in Annex I of Regulation (EC) No 1565/2000) may be refined based on the experience and the application of computational tools (foreseen by the GD on mixtures)
- Refinement of CG 31,III: two subgroups *d*-limonene and α-terpinene

d-limonene [01.045]	γ-terpinene [01.020]	terpinolene [01.005]	α-terpinene [01.019]	α-phellandrene [01.006]
				

- The groups under scrutiny are CG 31,III (terpenes) and CG 6 (related alcohols)

Terpineol [02.230]	4-terpineol [02.072]	α-terpineol [02.014]	γ-terpineol	β-terpineol
				

- Specific expertise added to the WG on feed flavourings
- EFSA GD on read-across under development

RESULTS OF TOLERANCE TRIALS

- When experimental results in the target animals are available for a component of an essential oil (i.e., tested in tolerance trials with a mixture of flavourings)
- Case 1: Experimental data in laboratory animals are not available for that component
  - The preferred approach is to rely on the results of the tolerance trials rather than on a component-based approach based on read-across
  - Example 1: *d,l*-isomenthone and menthone
- Case 2: Experimental data in laboratory animals are available for a component structurally related to that tested in tolerance trials
  - No read-across applied from the results of tolerance studies with a structurally related compound, when reliable compound-specific toxicological data are available
  - Example 2: *d*-carvone and *l*-carvone

EXAMPLE 1: *d,l*-ISOMENTHONE AND MENTHONE

- Example 1: *d,l*-isomentone [07.078] and menthone [07.176]
- **Geranium rose oil** (ON-8186, 2023)
  - read-across from menthol [02.015] applied to *d,l*-isomentone (up to 8%) menthone (1.16%).
  - the NOAEL of 375 mg/kg bw per day for menthol [02.015] divided by a factor of 2 to account for uncertainty in read-across
  - wide MOET for CG 8

CG 8								
d,l-Isomenthone	07.078	5.99	0.031	0.0027	(II)	187[(d)]	6,953	
Menthone	07.176	1.16	0.023	0.0020	(II)	187[(d)]	35,822	
MOET CG 8								5,686

EXAMPLE 1: *d,l*-ISOMENTHONE AND MENTHONE

- Subsequently, *d,l*-isomentone [07.078] tested in 'Herbal mixture' (ON-8340, 2023): safe at 5 mg/kg complete feed. Conclusions extended to menthone [07.176].
- **Peppermint oil** (ON-9076)
  - results of the tolerance used trials to assess to *d,l*-isomentone (up to 10%) and menthone (up to 32%), both included in specifications
  - safe concentrations of peppermint oil in feed reduced to 12 mg/kg complete feed based on the presence of these two compounds (assessed together)
- Any contradiction between the two assessments?
  - The results of 'Herbal' mixture were not available for geranium rose oil
  - Refinement of the assessment always possible, if new data become available
  - No impact on the conclusions on geranium rose oil
    - the highest feed concentration for the sum of *d,l*-isomentone and menthone was 0.044 mg/kg << 5 mg/kg complete feed, the safe level based on tolerance

RESULTS OF TOLERANCE TRIALS: *l*- AND *d*-CARVONE

- Example 2: *d*-carvone [07.146] and *l*-carvone [07.147]
- For *d*-carvone a complete toxicological dataset is available, including short-term, subchronic, carcinogenicity, developmental and reproductive toxicity
- Evaluation by the EFSA scientific Committee (EFSA SC, 2014)
- For *d*-carvone a the $BMDL_{10}$ of 60 mg/kg bw per day derived from two 90-day studies, based on toxicological effects (increased liver weight and increased kidney weight)
- Subsequently, *l*-carvone [07.147] was tested in tolerance trials with 'Herbal mixture' (ON-8340, 2023): safe at 10 mg/kg complete feed
- Tolerance trials: limited endpoints
- No read-across is applied from the results of tolerance studies with a structurally related compound, when compound-specific toxicological data are available

RESULTS OF TOLERANCE TRIALS: *l*- AND *d*-CARVONE

- **Caraway oil** (ON-8906, 2024)
- *d*-Carvone [07.146] present up to 65% by specification
- Assessed based on the $BMDL_{10}$ of 60 mg/kg bw per day, read-across applied to structurally related compounds (resulting in MOET of 36)

CG 8							
d-Carvone	07.012	57.03	14.258	1.2799	(II)	**60**	47
trans-Dihydrocarvone	–	0.69	0.172	0.0154	(II)	60	3891
cis-Dihydrocarvone	–	0.72	0.181	0.0162	(II)	60	3703
Neodihydrocarveol	–	0.55	0.138	0.0123	(I)	60	4861
Carveol	02.062	0.52	0.131	0.0117	(I)	60	5121
Dihydrocarveol	02.061	0.25	0.063	0.0056	(II)	60	10,694
6-Camphenone	–	0.09	0.023	0.0020	I	*3*	1485
Isocarveol	–	0.11	0.028	0.0025	I	*3*	1183

- Proposal to apply the results of tolerance studies with *l*-carvone to *d*-carvone (more favourable)
- Not possible to read-across based on tolerance studies with *l*-carvone, when toxicological data are available for the candidate substance *d*-carvone
- Conclusions supported by the results of a 28-day study with the whole mixture

UNCERTAINTY FACTOR FOR CATS

- As the general rule, a MOET of 500 is needed for cats, considering that cats have an unusually low capacity for glucuronidation, particularly of aromatic compounds
- Few exceptions were made, on a case-by-case basis, where a MOET of 100 was considered acceptable for cats
- **Omicha tincture** (ON-8731)
  - limiting group: dibenzocyclooctadiene lignans
  - no evidence of toxicity of lignans from the literature (focusing on beneficial effects), but no toxicity studies available to identify a NOAEL
  - lignans allocated to CC III (very conservative)
  - dose addition, highest value of the sum (conservative exposure estimate), MOET of 5 for cats, resulting in 20-fold reduction of the safe levels in feed
  - for all other assessment groups, the MOET was considerably higher (> 80-fold)
  - intake of 10-20 µg/kg bw: reduced glucuronidation capacity of cats is not an issue
  - Special case, not generalisable

UNCERTAINTY FACTOR FOR CATS

- Exception not applied in other assessments

Opinion	Limiting group, Cramer class	MOET	Considerations on other groups
Juniper oil (ON-7977)	CG 31,VI, CC I	39	MOET > 500 for cats in all groups
Coriander oil (ON-8349)	CG 13, CC II	59	MOET > 500 for cats in all groups
Lavender oil (ON-9017)	CG 13, CC II	22	MOET < 500 for cats in other groups (CG 05, CG 08, CG 31,II)
Clary sage oil (ON-9016)	CG 31,VI, CC I	52	MOET > 500 for cats in all groups

BOTANICALS AND NEW ASSESSMENT METHODOLOGIES

EFSA NAMs case study on essential oils as feed additives and interspecies metabolic differences (OC/EFSA/SCER/2021/14),

Contractor: Wageningen Food Safety Research, The Netherlands,

WFSR: Annelies Noorlander, Leonie Lautz, Wendy Jansen Holleboom, Patrick P.J. Mulder, Geert Stoopen, Ans Punt

EFSA Staff: Maria Chiara Astuto, Irene Cattaneo, Jean Lou CM Dorne, Paola Manini and Adriana Scattareggia Marchese

Experts of the FEEDAP WG on feed flavourings: Maria Bastos, Birgit Dusemund, Dieter Schrenk, and Johannes Westendorf

Kick-off meeting: 24 January 2022

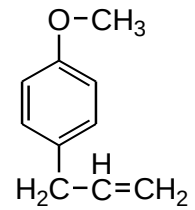
Final meeting: 9 February 2024

Report available:

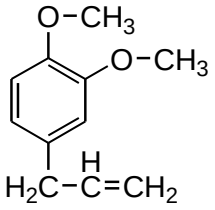
https://efsa.onlinelibrary.wiley.com/doi/10.2903/sp.efsa.2024.EN-8820

p-ALLYLALKOXYBENZENES

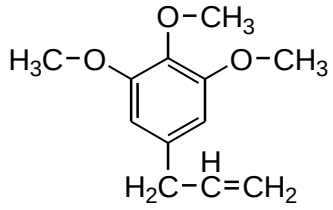
- Present in herbs and spices: fennel, basil, nutmeg, mace, star anise and foods, like pesto
- Genotoxic (via direct DNA reactive mode of action) and carcinogenic
- Estragole, safrole and methyleugenol induce liver cancer in rodents at high doses



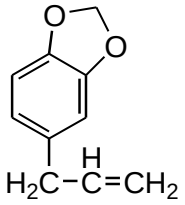
estragole



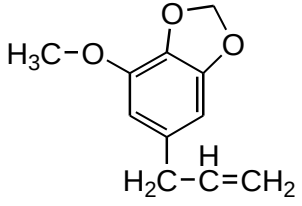
methyleugenol



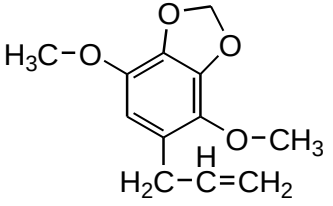
elemicin



safrole



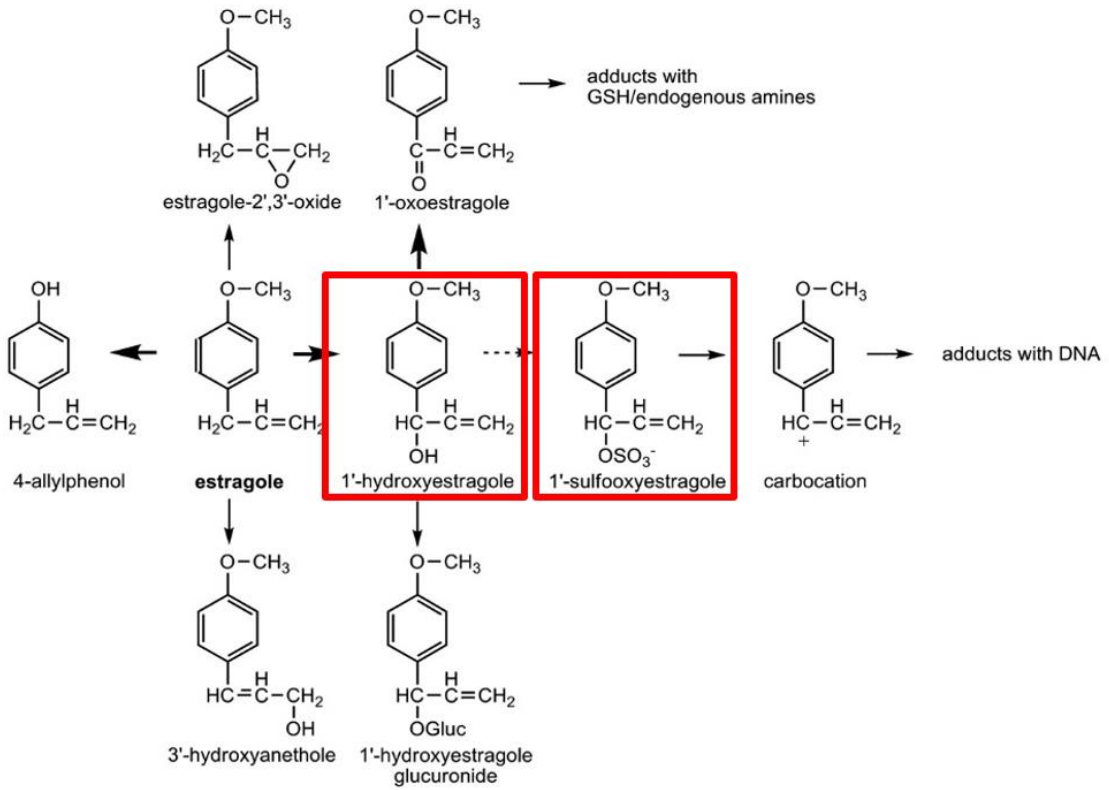
myristicin



apiole

METABOLIC ACTIVATION OF *P*-ALLYLALKOXYBENZENES

- Bioactivation pathway
  - Hydroxylation by CYP450 enzymes
  - Conjugation with sulphate catalysed by SULTs
- Remaining metabolic pathways represent detoxification

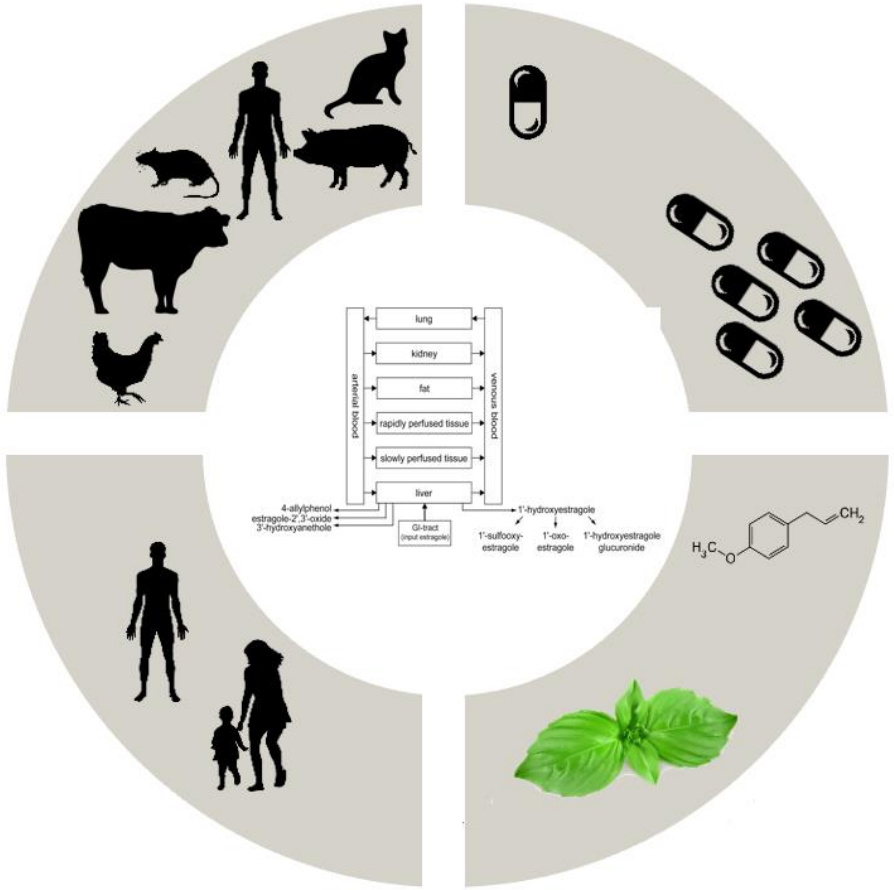


CONSUMER SAFETY: NEED FOR NEW CONCEPTS

- Essential oils are added to a wide range of food categories for flavouring purposes
- No residues data in products of animal origin are available
- **Assumption:** the individual components are extensively metabolised and are not expected to accumulate in tissues and products
- No increase of human background exposure
- This assumption is also applied to **methyleugenol, safrole and estragole**, based on the evidence available on the absorption, distribution, metabolism and excretion in laboratory animals
- **New assessment methodologies (NAMs)** to fill the data gaps and inform the risk assessment of combined exposure to multiple chemicals

NAMS TO FILL DATA GAPS

- Data gaps related to the **sensitivity of different species** and the potential effects of other constituents in herbs and spices on the bioactivation
- New approach methodologies (NAMs) can contribute to fill these data gaps
- In vitro metabolism data, combined with physiologically based kinetic (PBK) modelling allows for example to predict:
  - Species differences in bioactivation
  - Human variability
  - Matrix effects
  - Dose-dependent effects



GOAL OF THE PROJECT

- Design and conduct a set of NAMs-based experimental studies for the *p*-allylalkoxybenzenes, using an IATA (Integrated Approach to Testing and Assessment)
  - assess qualitative and quantitative differences and similarities in metabolic competences across different species
  - *in vitro* to *in vivo* extrapolation of the results obtained with PBK models and making the comparison among species

BOTANICALS AND NEW ASSESSMENT METHODOLOGIES

- *In vitro* metabolism data are generated for ***p*-allylalkoxybenzenes** using liver S9 incubation of prioritised target species
  - ***Priositised items***: estragole, methyleugenol, safrole, myristicin and elemicin
  - ***Prioritised species***: pig, chicken, bovine and cat, and compared with available data in rodents and humans
- Interaction with other components of the mixture (e.g. terpenes) are investigated: ***matrix effects***
- Quantitative *in vitro* to *in v*ivo extrapolations made using physiologically based kinetic models (PBK): ***transfer to tissues and products***
- The results will be used to **inform/refine** the risk assessment with respect to (i) species differences in kinetics, (ii) differences in the bioactivation between different *p*-allylalkoxybenzenes, (iii) the transfer of the compounds from feed to food, (iv) the effect of other constituents of the mixture

TEST SYSTEM AND TEST ITEM

- **Prioritised species** liver S9 incubations: human, rat, chicken, cow, pig and cat
- **Prioritised compounds**: estragole, methyleugenol, safrole, elemicin and myristicin
- Prioritised compounds to study **combination effects**: (limonene, α-pinene, 1,8-cineole)

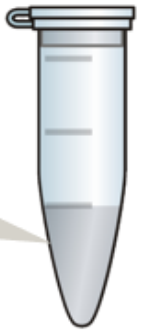
- **Preliminary experiments** to test the metabolic competence of the test system for Phase I (CYPs) and Phase II metabolism (UDPGA, PAPS)

Species (No. of liver samples)	Protein concentration (mg/ml)	Source
human (n=10)	20	corning lot#1166002
rat (n=24)	20	corning lot#9217001
pig (n=3)	45	Wageningen University (ASG) and Agrifirm
bovine (n=3)	50	slachthuis Veenendaal b.v., Veenendaal
chicken (n=3)	44	poultry slaughterhouse Remkes, Epe
cat (n=2)	34	Jasja Dekker, Dierecologie b.v., Arnhem

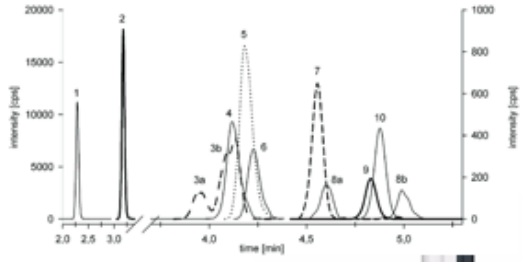
EXPERIMENTAL SET UP

- **Liver S9**
- Phosphate buffer
- Alamethicin
- Compound
- *Cofactor*

Incubation



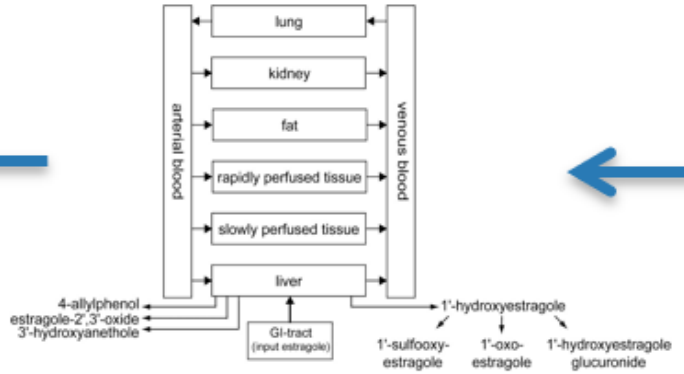
37°C, 300 rpm



LC-MS **GC-MS**

For quantification

Clearance rates (Clint) for all relevant metabolic conversions



PBK model

Predictions:

- Formation reactive metabolite(s) in rats and humans
- Transfer to feed

MAIN OUTCOMES: DIFFERENCES IN BIOACTIVATION (*IN VITRO*)

- Overall, *in vitro* studies using liver S9 fractions showed to be useful to fill data gaps regarding species differences in metabolism
- As expected, species differences regarding the bioactivation of *p*-allylalkoxybenzenes in both phase I and phase II metabolism were observed:

On species level

- For phase I the order of bioactivation in liver S9 (for 1'-hydroxy metabolites) is:
  bovine > pig > rat > human > cat > chicken
- For phase II the order of bioactivation (for 1'-sulfooxy metabolites) is :
  cat > chicken > pig > bovine > human > rat

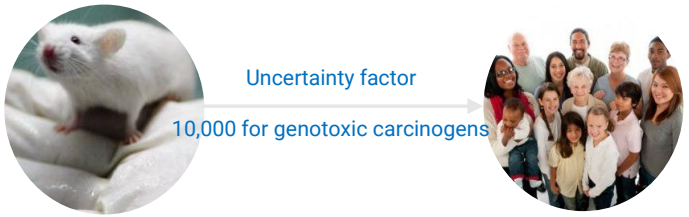
On compound level

- Phase I bioactivation in liver S9 is:
  methyleugenol > elemicin > estragole > safrole > myristicin
- Phase II bioactivation the order is:
  estragole > safrole > elemicin > myristicin > methyleugenol

No combination effects

- The potential influence of three terpenoids (limonene, α-pinene and 1,8-cineole) on phase I and phase II bioactivation revealed to be **negligible** and inconclusive

SPECIES DIFFERENCES IN KINETICS (PBK)



4 for species differences in **kinetics**
2.5 for species differences in dynamics

3.16 for *human* variation in **kinetics**
3.16 for *human* variation in dynamics
100 for additional uncertainties

PBK-model predictions > 4

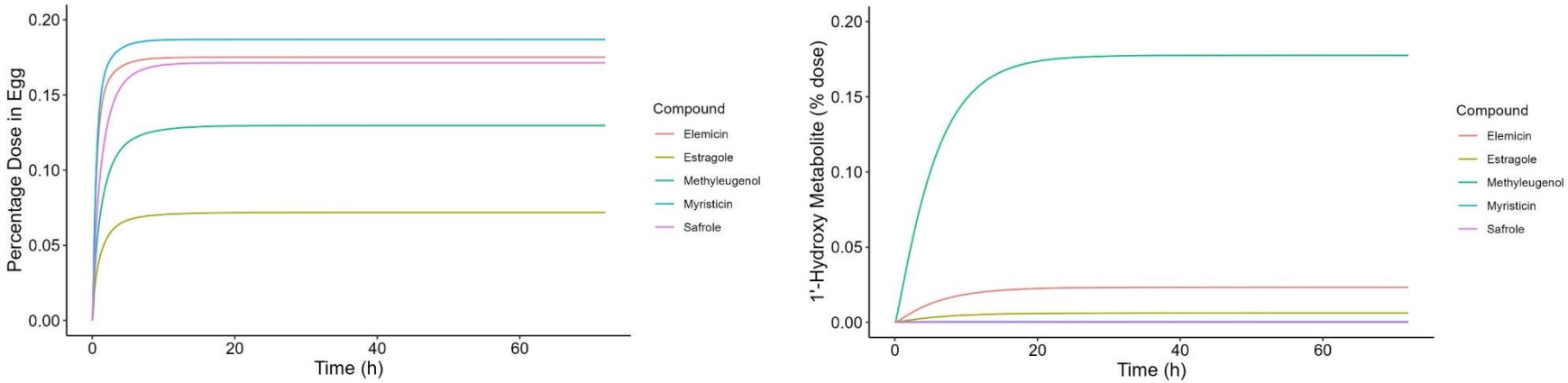
Table J1. Comparison of the phase II bioactivation (1'-sulfooxy metabolite) of the different species to rat 1'-sulfooxy metabolite

Compound	Cat	Chicken	Cow	Human	Pig
Elemicin	139	2	9	49	46
Estragole	18	14	9	9	28
Methyleugenol	940	67	30	10	64
Myristicin	108	6	11	117	10
Safrole	97	0	2	13	12

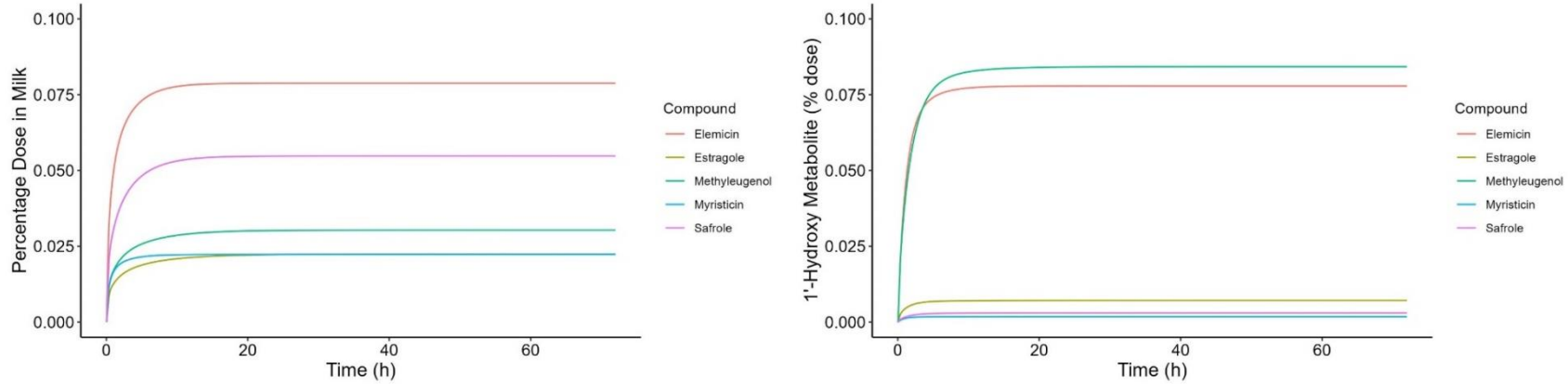
- For *methyleugenol* and *elemicin* all species revealed to have a relatively **higher** formation of the 1'-sulfonate metabolite than the rat
- The differences are **higher** than the default uncertainty factor of 4 that is used in the MOE approach. Human: 10-fold, Cat: 940-fold
- The simulated species differences can therefore be used to provide an argument to increase the default factor of 4
- In particular, **for cats** the results show that a **large factor** for species differences in kinetics may need to be taken into account

MAIN OUTCOMES: TRANSFER TO ANIMAL PRODUCTS (PBK)

- Transfer of parent compounds (limited, <0.02-0.18%) and 1'-hydroxy metabolites to eggs (negligible)



- Transfer of parent compounds (limited, 0.025-0.075%) and 1'-hydroxy metabolites to milk (negligible)

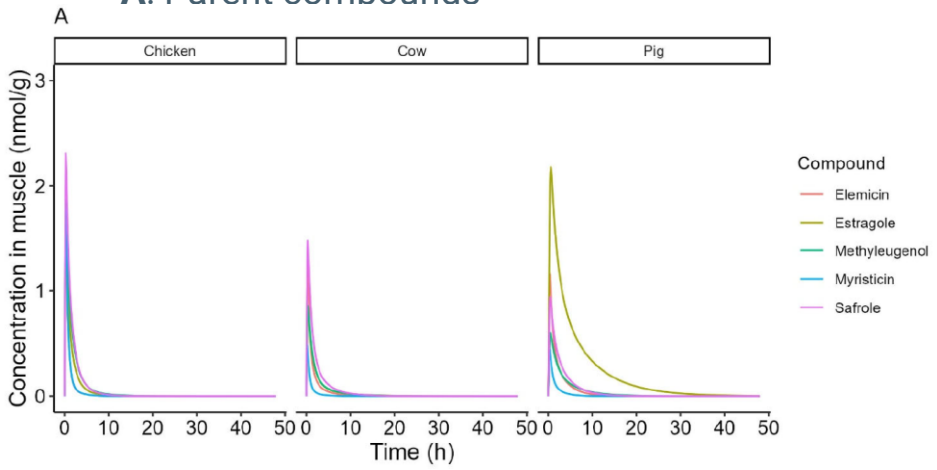


MAIN OUTCOMES: TRANSFER TO ANIMAL TISSUES (PBK)

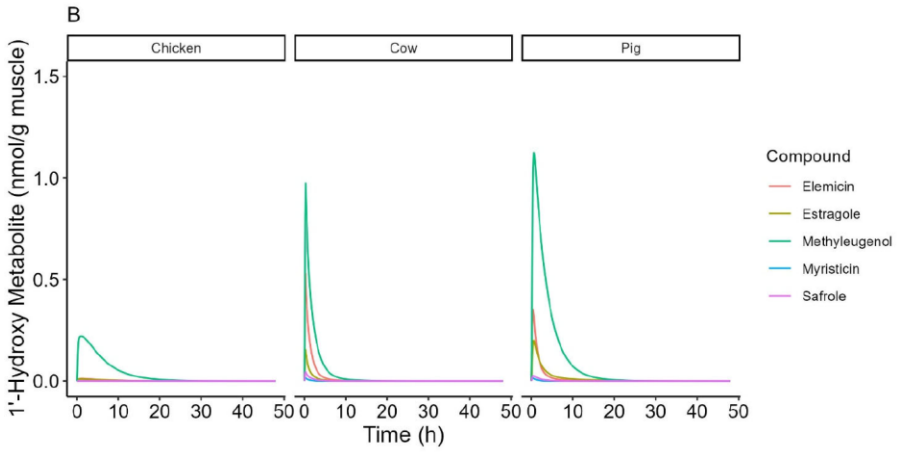
Transfer to tissues: kidney, liver, adipose tissue and muscle

- Overall, the deposition of *p*-allylalkoxybenzenes (elemicin, estragole, methyleugenol, myristicin and safrole) and their related 1'-hydroxy metabolites in the edible tissues is predicted to be **negligible**
- *p*-Allylalkoxybenzenes and related 1'-hydroxy metabolites are cleared from the tissues after 24 hours

A: Parent compounds



B: 1'-Hydroxymetabolites



UNCERTAINTIES

- Quality differences in liver S9
- A limited number of individuals per S9 samples (n = 2-3)
- Liver S9 is prepared from feral cats (differences in preparation)
- dGuo is used as trapping agent for the reactive 1’-sulfooxy metabolite
- Limited *in vivo* data are available to compare the PBK model predictions
- …

CONCLUSIONS

- Overall, *in vitro* studies using liver S9 fractions showed to be **useful** to fill data gaps regarding species differences in metabolism
- The use of liver S9 fractions as the *in vitro* metabolic system resulted in an improved insight into the metabolism of the chosen *p*-allylalkoxybenzenes: in a set of representative model species: *human, rat, pig, cow, chicken and cat*
- As expected, **species differences** regarding the bioactivation of *p*-allylalkoxybenzenes in both phase I and phase II metabolism were observed. Differences also observed among the different *p*-allylalkoxybenzenes
- The potential influence of three terpenoids (limonene, α-pinene and 1,8-cineole) on phase I and phase II bioactivation revealed to be negligible or inconclusive
- The PBK model predictions indicate a **limited transfer** into milk and eggs, and negligible transfer to tissues
- Further discussion on how to integrate such results in the risk assessment, in particular for cats
- Results presented to risk managers (November 2024)

INTERPRETATION OF THE WORDING LOW CONCERN

- Minutes of the 179th FEEDAP Plenary 26-28 November 2024
- (LINK to minutes under 9.2 (a))
- **Discussion on the use of the Margin of Exposure (MoE) guidance and how it translates into conclusions for the FEEDAP Panel.**

  The MoE approach has been applied by the FEEDAP Panel to assess the safety for the target species of botanical feed additives containing substances that are genotoxic and carcinogenic. In particular, the MoE has been applied to perform a quantitative risk assessment of compounds belonging to the class of *p*-allylalkoxybenzenes (e.g. methyleugenol, estragole), present in a large number of additives. In line with the principles of the EFSA Scientific Committee documents (2005, 2012), the wording 'low concern' has been used by the FEEDAP Panel, as of 2023, in the conclusions on the safety for the target species, when a MoE ≥ 10,000 is obtained comparing the exposure of long-living and reproductive animals with the $BMDL_{10}$ from an animal carcinogenicity study.

INTERPRETATION OF THE WORDING LOW CONCERN

The aim of the discussion was to provide clarification to risk managers on how the use of the MoE approach is translated into conclusions in FEEDAP opinions. The discussion focused on the possibility to explain the wording 'low concern' (associated in FEEDAP assessments with a MoE ≥ 10,000) in terms of the likelihood that the use of an additive **would not induce adverse effects** in the target species.

The Panel clarified that, following the terminology of the EFSA Statement (2012) the use of an additive in feed is considered of low concern for long-living target species if the MoE is ≥ 10,000. Consequently, the FEEDAP Panel considers it **very unlikely** that the use of that feed additive will induce adverse effects during the lifetime of the long-living target species.

This clarification applies to the opinions adopted in 2023 and 2024 on: laurel leaf oil (EFSA-Q-2022-00107), nutmeg oil (EFSA-Q-2010-01296), anise oil and anise tincture (EFSA-Q-2023-00180), star anise oil (EFSA-Q-2023-00398), bitter fennel oil and sweet fennel oil (EFSA-Q-2023-00587), clove bud oil and clove leaf oils (EFSA-Q-2023-00397), and citronella oil (EFSA-Q-2024-00190).

RE-EVALUATION OF BOTANICALS: WHERE WE ARE

- No. 163 additives to be assessed (initially 268, 105 withdrawn)

Where we are

- No. 71 additives already assessed (44%), No. 62 opinions adopted
- No. 16 additives under assessment (53%, assessed + under assessment)
- Missing data for ~ 76 additive (47%)

What's next in the next 2.5 years

- Submission of new datasets: 30 additives/year
- Working Group meetings (2025): 10 x 2 days (0.4 hrs) = 10 days
- Plenary: 4-5 additives/Plenary (25-30 additives/year)
- End of the exercise: expected in 2028

RESTART OF BOTANICALLY DEFINED GROUPS

- Seven botanically defined groups (BDGs) on hold at the end of 2024:
- BDG 03, 04, 05, 10, 11, 15 and 20
- FEFANA requested to restart the assessment of all BDGs
- Six BDGs validated on 22/01/2025, BDG 20 restarted on the same date
- Request of supplementary information will be sent after the WG meeting
  - this will be the last request of supplementary information, a question on efficacy added to the letter
  - additional data will not be requested, request of clarifications still possible
  - a DL of 9 months granted to generate the data
  - **Action needed:** In case of need to extend the deadline, the applicants are invited to send a request to EFSA and provide a justification

EFFICACY

- For a number of additives, there is no evidence of the efficacy as flavourings (e.g. ginkgo tinctures)
- For additives, which are not included in the standard lists of flavourings (e.g. Fenaroli’s Handbook of Flavour Ingredients), there is the need to provide evidence of the efficacy

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