Draft EFSA Scientific Opinion on the evaluation of the safety of preparations from the fruits of sweet and bitter fennel (Foeniculum vulgare Mill. and Foeniculum piperitum (Ucria) C.Presl)

Draft EFSA Scientific Opinion on the evaluation of the safety of preparations from the fruits of sweet and bitter fennel (Foeniculum vulgare Mill. and Foeniculum piperitum (Ucria) C.Presl)

Introduction and Background

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Introduction

1. EFSA published a public consultation on a draft Opinion on the safety of preparations from the fruits of sweet and bitter fennel on 16th of July 2025 (see Annex A for link). The COT are being asked to review the draft opinion and provide any comments they may have; the Secretariat will then submit the Committee's comments to EFSA.

2. A document has been provided in the Members Area Teams folder, for Members to add comments before or after the COT Meeting but can also send any additional comments directly to the Secretariat. The closing date for the public consultation is the 17th of September 2025. Please provide any comments latest by **Friday the 12th of September** giving the relevant line and section number where possible.

3. The following paper provides a brief overview of the draft EFSA Opinion.

Background

4. Following a requested by the European Commission (EC) EFSA provided an assessment on the intake of preparations from the fruits of *Foeniculum vulgare* Miller subsp. *vulgare* var. *vulgare* (bitter fennel) and *Foeniculum vulgare* Miller subsp. *vulgare* var. *dulce* (Miller) Thellung (sweet fennel). The EC request followed safety concerns raised by the German Federal Institute for Risk Assessment (BfR) in relation to possible adverse effects

associated with the consumption of fennel fruit preparations by infants and young children due to the presence of estragole, a known genotoxic carcinogen.

5. Estragole belongs to the group of alkoxy-substituted allylbenzenes (*p*-allylalkoxybenzenes), and while estragole and other *p*-allylalkoxybenzenes are present in a variety of foods of the diet, estragole is the only member of this group present in fennel fruits and preparations thereof. *p*-Allylalkoxybenzenes all share similar structures, fates in the body and modes of action where their sulfooxy metabolites can lead to deoxyribonucleic acid (DNA) adduct formation (Smith et al., 2002; Hartwig et al., 2020; Eisenreich et al., 2021). Two *p*-allylalkoxybenzenes, methyleugenol and safrole, have also been classified as genotoxic carcinogens like estragole (Alajlouni et al., 2016; Götz et al., 2022). Furthermore, though no more *p*-allylalkoxybenzenes have been classified as genotoxic carcinogens most members of this group have been reported to lead to DNA adduct formation (EFSA., 2022a). Two *in vivo* studies (Phillips et al., 1984; Randerath et al., 1984) suggested the following order of potencies for six *p*-allylalkoxybenzenes: methyleugenol > safrole > estragole > myristicin > elemicin > dillapiole. Estragole was demonstrated to be the most potent in an *in vitro* study (Zhou et al., 2007).

6. EFSA has not previously evaluated the safety of fennel fruit preparations, however, they have performed an assessment on the safety and efficacy of a feed additive consisting of an extract of olibanum from *Boswellia serrata* Roxb. ex Colebr. for use in dogs and horses which contains both estragole and methyleugenol (EFSA, 2022b). EFSA concluded that the additive is considered safe for consumers when used at the proposed conditions of use in horses. Furthermore, when individuals handle the additive unprotected, exposure to estragole and methyleugenol could not be excluded, therefore, to reduce the risk EFSA recommended that measures should be taken to minimise exposure.

7. The European Medicines Agency (EMA) Committee on Herbal Medicinal Products (HMPC) published a statement on the safety of human consumption of herbal medicinal products containing estragole (EMA HMPC, 2023) and recommended that exposure to estragole from medicinal products should be kept as low as practically achievable. An acceptable daily intake (ADI) could not be set but a guidance value of 0.05 mg estragole per day for adults and adolescents and 1 µg estragole per kg bw per day for children was suggested. The EMA did not recommend the use of fennel fruit infusions in children up to the age of 4 years or the use of fennel fruit preparations in pregnant or lactating women due to limited data on the extent of potential adverse effects in these sub-populations (EMA

HMPC, 2024b, c).

8. In the draft opinion EFSA highlighted “Regulation (EC) No 1334/2008 on flavourings and certain food ingredients with flavouring properties for use in and on foods prohibits the addition of estragole to foods and sets maximum levels of certain substances, naturally present in flavourings and food ingredients with flavouring properties, in compound foods. Estragole originating from food ingredients with flavouring properties may be present in dairy products, processed fruits and vegetables, nuts and seeds and fish products at a maximum concentration of 50 mg/kg and in non-alcoholic beverages at a maximum concentration of 10 mg/kg. These maximum levels do not apply where a compound food contains no added flavourings and the only food ingredients with flavouring properties which have been added are fresh, dried or frozen herbs and spices.”

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EFSA Draft Opinion

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9. The risk assessment by the EFSA Panel on Nutrition, Novel Foods and Food Allergens (NDA) considered:

- "Is there a link between the consumption of preparations from the fruits of sweet and bitter fennel and harmful effects on health?"
- "What is the maximum level of total chronic daily intake (i.e. daily intake over a substantial part of the lifespan) of preparations from the fruits of sweet and bitter fennel, which is unlikely to pose a risk of adverse health effects to humans?"

10. These overarching questions were split into sub-questions (sQs) alongside methods to address each sQ (please see Table 1 of the draft opinion).

11. For detail on data collection and methodologies please see Sections 2.1 and 2.2, respectively. Please note that EFSA decided to calculate the margin of exposure (MoE) for total *p*-allylalkoxybenzenes and then establish the extent of the contribution of fennel fruit preparations to the total *p*-allylalkoxybenzenes exposure. This approach was taken because *p*-allylalkoxybenzenes are also present in other foods in the diet, not only fennel fruit preparations, and therefore exposure to *p*-allylalkoxybenzenes was unavoidable.

12. EFSA carried out two dietary exposure scenarios, one was a general chronic dietary exposure scenario while the other scenario considered fennel fruit infusion consumers only. To better estimate the higher percentiles of exposure, consumption of unspecified herbal blends were assumed to contain dried fennel fruits (for detail see Section 2.2.2).

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ADME of estragole (Section 3.3)

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13. “Estragole is lipophilic and readily absorbed in the intestinal tract, with a pronounced first pass effect catalysed by cytochrome P450 (CYP450) enzymes (Jeurissen et al., 2007b).” Please note, EFSA does not provide any further data on the absorption and distribution of estragole (see Section 3.3.1.1).

14. Figure 2 of the EFSA opinion provides a schematic of the known metabolic pathways of estragole. The metabolism of estragole is complex and only limited in vivo rodent and human data is available. However, the evidence available suggests estragole is metabolised primarily through phase I enzymes (O-demethylation, epoxidation, and/or hydroxylation) and phase II enzymes (glucuronidation, sulfation, conjugation with glycine). O-demethylation has been suggested as the predominant pathway for metabolism of estragole, with studies in rats demonstrating at least 34-53 % of ingested estragole being metabolised via this pathway (Zangouras et al., 1981; Anthony et al., 1987). Furthermore, in rats, 10 % of ingested estragole appears to undergo 3’-hydroxylation (Anthony et al.,1987; Solheim and Scheline, 1973), 6-10 % epoxidation (Solheim and Scheline, 1973) and 26-50 % 1-hydroxylation, however data on this last pathway is more limited (Solheim and Scheline, 1973; Anthony et al., 1987; Zangouras et al., 1981) (see Section 3.3.1.2 for detail).

15. Most data identified by EFSA on the metabolism and excretion of estragole in humans originated from two studies (Sangster et al., 1987; Zeller et al., 2009). Zeller et al. (2009) dosed seven human volunteers of both sexes with a single 500 mL fennel fruit infusion containing 0.02-0.03 mg/kg bw estragole and found that around 20 % of the ingested estragole was excreted as conjugated 4-allylphenol, an estragole metabolite formed via O-demethylation. Sangster et al. (1987) administered two human volunteers a dose of 0.001 mg/kg bw of [methoxy 14C]-estragole and found that at least 12 % of ingested estragole was excreted via the lungs as CO2, also produced via O-demethylation. Overall, 12-20 % of ingested estragole was demonstrated to have been metabolised via O-demethylation in humans, suggesting this pathway is of less importance in humans than in rats. Sangster et al. (1987) also reported that around 4 % and 1.3 % of ingested estragole (around 5 % total) was excreted as estragole metabolites 4-methoxyphenyllactic acid and 4-methoxyphenylacetylglycine formed via epoxidation. Furthermore, this study reported that 12% of ingested estragole was excreted as the metabolite 4-methoxyhippuric acid in urine, formed via 3’-hydroxylation, a similar proportion to the O-demethylation pathway.

16. EFSA concluded that the remaining 60-70 % of ingested estragole (after excluding O-demethylation, epoxidation and 3’-hydroxylation metabolism) was

metabolised via 1’-hydroxylation and that 1’-hydroxylation therefore was the major pathway for estragole metabolism in humans. CYP450 enzymes hydroxylate the 1’-carbon atom of the allyl side chain (1’-hydroxylation) of estragole forming 1’-hydroxyestragole. Following this, sulfonation of 1’-hydroxyestragole via sulfotransferases (SULTs; Suzuki et al., 2012) to unstable 1’-sulfooxyestragole can then result in the formation of reactive electrophilic intermediates (carbocations) which can form protein and DNA adducts (Phillips et al., 1981); however, 1’-sulfooxyestragole can be detoxified by reacting with water and glutathione to form mercapturic acid which is excreted in urine (Monien et al., 2019). Physiologically based biokinetic (PBBK) models estimated that around 0.20 % of the originally ingested dose of estragole was metabolised to 1’-sulfooxyestragole (Punt et al., 2009b); however, there is no in vivo data to support this estimate nor to establish a dose-response curve for the formation of genotoxic intermediates in humans.

17. Instead of sulfonation 1’-hydroxyestragole can also be detoxified by glucuronidation having been found in the urine of rats and humans as the metabolite 1’-hydroxyestragole glucuronide (Zangouras et al., 1981; Anthony et al., 1987; Sangster et al., 1987; Zeller et al., 2009). 1’-hydroxyestragole can also be oxidised to 1’-oxoestragole (Solheim and Scheline, 1973) which has been proved capable of forming DNA adducts, however, one study found that 1’-oxoestragole caused less hepatomas following intraperitoneal administration in mice than 1’-hydroxyestragol (Wiseman et al., 1987). Detoxification is thought to occur via conjugation with glutathione or N-acetylcysteine followed by excretion in urine and bile as shown for 1’-oxosafrole in mice and rats (Fennell et al.,1984).

18. Studies in rodents demonstrated that between 26 % and 60 % of ingested estragole was excreted in urine within 48 hours, the percentage increasing with higher ingested doses (Solheim and Scheline, 1973; Anthony et al., 1987). Only 0.4 to 1.3 % of ingested estragole was found excreted in rat faeces 48 hours after administration (Anthony et al., 1987). Whilst in humans Sangster et al. (1987) failed to detect any radioactivity in faeces collected up to 4 days after a single dose of 100 µg [methoxy 14C]estragole, 54-62% of the dose was eliminated in urine within 48 hours of administration. Zeller et al. (2009) also demonstrated that urinary excretion of free and conjugated 1′-hydroxyestragole in human volunteers of both sexes following ingestion of a single 500 mL fennel fruit infusion was mostly complete within 6-8 hours.

19. Excretion of estragole via the lungs as CO2 has been demonstrated as a terminal product of O-demethylation in humans (Sangster et al., 1987). In rats administered [methoxy 14C]estragole, exhaled 14C accounted for between 30

and 50 % (Zangouras et al., 1981; Anthony et al., 1987) of the ingested estragole dose while in humans it was at least 12 % (Sangster et al., 1987).

ADME of other p-allylalkoxybenzenes (Section 3.4)

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20. The draft EFSA opinion did not discuss the ADME of other *p*-allylalkoxybenzenes. Only the metabolism of safrole and methyleugenol were briefly discussed (lines 792-802).

21. Safrole and methyleugenol are metabolised by the same pathways as estragole; however, PBBK models have demonstrated that the relative importance of each pathway differs (Al-Subeihi et al., 2012; Martati et al., 2012). *O*-demethylation of methyleugenol has been suggested to be less efficient than for estragole due to steric hindrance created by the two methoxy groups present in methyleugenol. The National Toxicology Programme (NTP) (2000) study found that only 0.1% of [^{14}C] could be recovered in breath of rats (as CO2 via *O*-demethylation). Metabolites of safrole were found to take much longer to be excreted than estragole and methyleugenol taking 120 hours instead of 24 hours, indicating safrole was metabolised slower than other *p*-allylalkoxybenzenes (Martati et al., 2012).

22. Please note the Al-Subeihi et al. (2012) reference is missing from the draft EFSA opinion but has been found and referenced within this summary document. This will be noted in the comments for EFSA.

23. EFSA also identified some evidence that *p*-allylalkoxybenzenes can cross the placenta, however, this was limited to a single study where DNA adducts were found in the foetus of pregnant ICR mice orally dosed with safrole at 97 mg/kg bw on day 18 of gestation (Lu et al., 1986).

24. EFSA also highlighted two studies that provided evidence of transfer of *p*-allylalkoxybenzenes into breast milk. Denzer et al. (2015) reported transfer of estragole from an ingested infusion into breast milk of lactating women, however, there was wide variation within the measured levels in breast milk ranging from 1 to 21 % of the ingested estragole dose. Vesselinovitch et al. (1979) demonstrated transfer of safrole into breast milk of B6C3F1 mice which was found to be cancerogenic in the male offspring. The lactating females were intragastrically exposed to 120 mg/kg bw safrole 12 times every second day after parturition.

25. The draft opinion also discusses tissue retention (section 3.3.1.4); however, no data was identified for estragole, only a single study with

methyleugenol studying tissue distribution in Fischer 344 rats after a single oral dose of 118 mg/kg bw [^{14}C]methyleugenol (NTP, 2000). After 72 hours, 3.8 % of the ingested labelled methyleugenol were still detectable in tissues with the highest concentrations found in the liver (mean 0.104 %), muscle (0.073 %), blood (0.068 %), skin (0.064 %) and fat (0.049 %). In other tissues the concentrations were <0.01 %.

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DNA and protein adduct formation (Section 3.5)

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26. Most *p*-allylalkoxybenzenes have been reported to lead to DNA adduct formation, while estragole, methyleugenol and safrole having classified as genotoxic carcinogens. This is discussed in detail in Section 3.5 of the draft EFSA opinion, the following paragraphs provide a brief overview.

DNA and protein adduct formation (Section 3.5.1 and 3.5.2)

27. A study by Herrmann et al. (2013) investigated DNA adducts in non-tumorous tissue samples from 30 Caucasian individuals undergoing surgery for liver tumours or metastases and reported the number of DNA adducts formed by 1'-sulfoxymethyleugenol originating from the diet as 13 adducts per 10^8 nucleosides (median) and 37 adducts per 10^8 nucleotides (maximum). A study by Monien et al. (2015) examined ten non-tumorous lung tissue samples of lung tumour patients and reported between 7.5 and 21.5 adducts per 10^8 nucleotides. Tremmel et al. (2017) reported an interindividual variation of 122-fold between the lowest and highest levels of adduct formation originating from methyleugenol exposure. Their findings also revealed a linear correlation between adduct levels and the copy number of the SULT1A1 gene, which encodes a sulfotransferase enzyme. An increased SULT1A1 gene copy number may enhance susceptibility to DNA damage induced by *p*-allylalkoxybenzenes, due to elevated adduct formation. Daniels and Kadlubar (2014) reported that the SULT1A1 four-copy genotype occurs at around 1-5% in European populations.

28. EFSA also identified four rodent studies that investigated the formation of DNA adducts after exposure by oral gavage to either estragole, methyleugenol or safrole (Lu et al., 1986; Paini et al., 2012; Suzuki et al., 2012; Herrmann et al., 2014). Overall, these studies reported DNA adduct formation to increase linearly with increasing doses.

29. An *in vitro* study by Schulte-Hubbert et al. (2020) found that increasing doses of estragole resulted in the dose-dependent formation of DNA adduct in primary rat hepatocytes. Additionally, Ackermann et al. (2025) used a range of human and rat liver cell models to demonstrate that a threshold of DNA adducts existed below which clastogenic effects were not triggered. However, EFSA noted it is uncertain how this would relate to an *in vivo* situation with chronic low dose exposures.

30. EFSA did not identify any evidence available in humans which would allow the derivation of a dose-response curve for DNA adduct formation.

DNA and protein adduct formation estimated by PBBK modelling (Section 3.5.3)

31. EFSA considered four studies, in detail, which used PBBK and physiologically based biodynamic (PBBD) models to estimate the extent of DNA and protein adduct formation by *p*-allylalkoxybenzenes in humans and rodents (Paini et al., 2010; Rietjens et al., 2011; Punt et al., 2016; Yang et al., 2022). In summary, assuming an estimated daily intake of estragole of 0.01 mg/kg bw the PBBK and PBBD models simulated that DNA adducts were formed at levels below the levels of methyleugenol-derived adducts reported by Herrmann et al., (2013) of 13 adducts per 10^8 nucleosides (median) and 37 adducts per 10^8 nucleotides (maximum).

Repair of *p*-allylalkoxybenzenes adduct formation (Section 3.5.4)

32. Evidence from *in vivo* rodent studies and *in vitro* studies using rat and human cells suggests that DNA adducts formed from *p*-allylalkoxybenzenes can accumulate following repeated exposure, and that at least one type of adduct was not being recognised by the excision repair mechanism (Phillips et al., 1984; Randerath et al., 1984; Herrmann et al., 2014; Yang et al., 2020; Yang, 2021). EFSA concluded that this may account for the persistence of DNA adducts in the liver, despite an initial decline in adduct levels observed within the first few days after exposure to estragole. EFSA also noted that the repair efficiency of these adducts was limited in both humans and rats.

Interindividual difference in humans (Section 3.5.5)

33. EFSA identified multiple PBBK models that showed large interindividual differences in humans in the formation of the 1’-sulfooxy metabolite and DNA adduct formation. Ning et al. (2017) reported that at an estimated daily intake of estragole of 0.01 mg/kg bw 0.02 % of the ingested dose was converted to 1’-sulfooxyestragole in Chinese populations compared to 0.09% in Caucasians (4.5-fold difference). At the same exposure level Martati et al. (2012) reported variation up to 12-fold in 1’-sulfooxyestragole formation, while Punt et al. (2016) estimated up to 21-fold variation in 1’-sulfooxyestragole formation and DNA adduct formation between 1.6 adducts per 10^8 nucleotides at the 50^{th} percentile and 8.8 adducts per 10^8 nucleotides at the 99^{th} percentile.

34. EFSA highlighted that the accuracy and reliability of PBBK and PBBD models can vary substantially depending on the quality of the model and experimental data they rely upon. How accurately these models reproduce *in vivo* situations is uncertain.

Influence of the food matrix (Section 3.5.6)

35. EFSA considered in its opinion how the food matrix affects the bioactivation of *p*-allylalkoxybenzenes, in particular the role of SULT inhibitors in reducing the formation of 1’-sulfooxyestragole and subsequent DNA adducts.

36. In a study by Monien et al. (2019) one individual was exposed to pure estragole and estragole from a fennel fruit infusion and results showed that the pure compound was metabolised slightly faster than the infusion. The excretion of N-acetyl-S-[3′-(4-methoxyphenyl)allyl]-L-cysteine (AMPAC), a potential marker for conjugation of 1’-sulfooxyestragole with glutathione, was measured following consumption of the fennel fruit infusion and 106 ng AMPAC were excreted in urine compared to 133 ng following consumption of pure estragole.

37. EFSA further highlighted two rodent studies. Alhusainy et al. (2013) reported oral coadministration of nevadensin, a SULT inhibitor, with estragole , which resulted in a significant reduction in the levels of estragole-derived DNA adducts in the liver of rats. Boberg et al. (1983) found that in female CD-1 mice the presence of pentachlorophenol, another SULT inhibitor, reduced the proportion of mice with hepatomas when administered safrole or 1’-

hydroxysafrole.

38. Two *in vitro* studies found that a methanolic basil extract containing nevadensin inhibited the sulfoconjugation of 1’-hydroxyestragole by SULTs (Jeurissen et al., 2008; Alhusainy et al., 2010). Alhusainy et al. (2010) also reported a reduction in DNA adduct formation in primary rat hepatocytes and HepG2 cells after co-exposure to nevadensin and 1’-hydroxyestragole. Another *in vitro* study by Alhusainy et al. (2012) used pooled male rat liver S9 fractions to explore the inhibitory effects of different herb and spice methanolic extracts, containing *p*-allylalkoxybenzenes, on SULT activity. Results suggested that a basil extract had the greatest inhibitory effects while a fennel extract had no effect on SULT activity. The major SULT inhibitors identified in *p*-allylalkoxybenzene containing herbs and spices were quercetin, kaempferol, myricetin, apigenin, luteolin and nevadensin. The authors also reported combinations of kaempferol, myricetin, apigenin and luteolin as well as quercetin and kaempferol alone reduced DNA adduct formation in human HepG2 cells following exposure to 1’-hydroxyestragole.

39. PBBK modelling further suggested that co-ingestion of SULT inhibitors with *p*-allylalkoxybenzenes could significantly reduce the formation of associated 1′-sulfooxyestragole in the human liver (Rietjens et al., 2011; Alhusainy et al., 2012).

40. Based on the available data, EFSA concluded that there was no evidence to suggest that fennel fruit preparations contain SULT inhibitors at levels sufficient to suppress the formation of 1′-sulfooxyestragole.

Hazard characterisation (Section 3.6)

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41. Estragole, methyleugenol and safrole are considered genotoxic carcinogens (SCF, 2001b, a, 2002; EMA HMPC, 2005, 2023, 2024a, d) and EFSA considered only a single 2-year carcinogenicity study for methyleugenol sufficient to derive a dose response. The study exposed, rats and mice to methyleugenol by oral gavage at 0, 37, 75 or 150 mg/kg bw for 5 days per week (equivalent to 0, 26.4, 53.6 or 107.1 mg/kg bw per day) and rats to a second higher dose of 300 mg/kg bw per day (NTP, 2000).

42. EFSA had previously identified a lower confidence limit for a benchmark response of 10 % (BMDL10) for methyleugenol of 22.2 mg/kg bw per day as reference point for the entire *p*-allylalkoxybenzene group for the safety

assessment of a feed additive (EFSA, 2022b). The BMDL10 had been derived by Suparmi et al. (2019) based on the incidence of liver tumours in male rats in the NTP study.

43. For this draft EFSA opinion on the safety of fennel fruit preparations EFSA decided to repeat the Suparmi et al. (2019) benchmark dose (BMD) analysis using Bayesian approaches as implemented in EFSA's Benchmark Dose modelling software and using sex as a covariate to increase power. To identify the BMDL10, data from male rats was used as they were more sensitive to hepatocarcinoma than the females. After model averaging the BMD identified was 32.4 mg/kg bw per day with a 90 % credible interval of 21.0 to 48.2 mg/kg bw per day. Therefore, the revised BMDL10 for methyleugenol was 21.0 mg/kg bw per day and in line with the EFSA approach this BMDL10 was applied to the whole *p*-allylalkoxybenzene group.

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Exposure assessment (Section 3.7)

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44. Occurrence data on *p*-allylalkoxybenzenes in food were collected by systematic review and a call for data as described under Section 2 'Data and Methodologies' of the draft EFSA opinion.

45. Please see Subsection 2.2.2 for more detail on the methodology of the exposure assessment, however in summary, two exposure scenarios were considered, a) a general chronic dietary exposure scenario in the whole population and b) a scenario to estimate the exposure in fennel fruit infusion consumers only. To better estimate the higher percentiles of exposure, consumption of unspecified herbal blends were assumed to contain dried fennel fruits.

46. The chronic dietary exposure scenario for the whole population showed that the highest average exposure and highest 95th percentile (P95) exposure to *p*-allylalkoxybenzenes were in toddlers (4.1 and 21.9 µg/kg bw, respectively), followed by other children (3.9 and 14.7 µg/kg bw, respectively) and infants (3.7 and 13.8 µg/kg bw, respectively). A summary of these exposures is provided in Table 8 of the draft EFSA opinion.

47. In the whole population exposure scenario most, estimated mean exposures were greater than a margin of exposure (MoE) of 10,000, with the exception of infants (<12 months), toddlers (≥1 to <3 years) and other children (≥3 to <10 years) in a few Member States. At the higher percentile exposures (P90 and P95) the MoE was more often <10,000 (range: 712-9,901 at the P95, median: 4,013) especially in younger age groups. A summary of the mean, P90 and P95 MoEs is presented in Table 9 of the draft EFSA opinion.

48. In the whole population scenario, the most frequently consumed food groups across dietary surveys and age groups contributing to the exposure were aromatic herbs, spices, fruits and vegetables, and cola-type drinks. In this scenario, the consumption of fennel fruit infusions also had a relevant contribution to *p*-allylalkoxybenzene in certain countries, i.e., Germany and Poland for the young age groups (<10 and <3 years respectively).

49. In the fennel fruit infusions consumers scenario, the highest average exposures were in other children (16.1 µg/kg bw), followed by toddlers (5.86 µg/kg bw) and infants (4.75 µg/kg bw). At P95 the highest exposure was reported for toddlers (17.4 µg/kg bw), followed by infants (17.3 µg/kg bw) and other children (14.4 µg/kg bw). A summary of these exposures is provided in Table 10 of the draft EFSA opinion.

50. For the fennel fruit consumers scenario EFSA draws attention to Figures 6 and 7 of the draft opinion which illustrate how consumption of fennel fruit infusions impacts exposure to *p*-allylalkoxybenzenes. These figures showed that in infants fennel fruit infusions may contribute to >75 % of the total average exposure to *p*-allylalkoxybenzenes. Furthermore, in toddlers and other children, infusions may contribute to >50 % of the total average exposure to *p*-allylalkoxybenzenes. In general, as age increase the relative contribution of fennel fruit infusions to average *p*-allylalkoxybenzene exposure decreases with an exception for the elderly where contribution may also exceed 50 %. A summary of the calculated MoEs for the fennel infusion consumer scenario is provided in Table 10 of the draft EFSA opinion. Appendix A.2 of the draft EFSA opinion provides lists of the MoEs for total *p*-allylalkoxybenzenes exposure in the fennel fruit infusion consumer scenario for each EU Member State. Please note, there are two tables named “Table 10” in the draft opinion in Section 3.7.2.2.

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Risk characterisation and Conclusions (Sections 3.8 and 5)

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51. In the current draft opinion, EFSA followed its own guidance documents for the assessment of genotoxic carcinogens, specifically, the risk assessment acknowledged that chemical substances which were genotoxic carcinogens should not purposefully be added to foods or the food chain but that if the substance was unavoidable i.e., part of the typical diet, and if data was available, it was possible to qualify the safety concern based on an MoE approach.

52. Based on the whole population exposure assessment scenario, high consumption (P90 and P95) resulted predominantly in MoEs of <10,000 (range: 712-9,901 at the P95, median: 4,013) for *p*-allylalkoxybenzenes, whilst average

consumption resulted in MoE generally >10,000, except for infants, toddlers and other children in some Member States including Cyprus, Germany, France, Italy and Portugal. Please see Appendix A.1 of the draft EFSA opinion for a list of MoEs for total *p*-allylalkoxybenzenes exposure in the whole population in EU Member States.

53. In the whole population scenario, consumption of fennel fruit infusions in Germany and Poland were significant contributors to total *p*-allylalkoxybenzene in infants, toddlers, and in addition in other children for Germany only. These exposures are in line with exposures of scenario 2, demonstrating that fennel fruit infusion could contribute significantly to total *p*-allylalkoxybenzene exposure in children up to 10 years of age and the elderly (see paragraph 50). EFSA noted there were insufficient data within EFSA's food consumption database to create exposure scenarios for other fennel fruit preparations; thus, it is uncertain how much they could potentially contribute to total dietary *p*-allylalkoxybenzene exposure.

54. EFSA noted that in children aged ≥3 to <10 years (other children), removing exposure to *p*-allylalkoxybenzenes from fennel fruit infusions generally led to increased MOEs at the P90 and P95 intake distribution. In this group, MOEs for P90 values ranged from 2,776 to 16,653, while MOEs for P95 values ranged from 1,462 to 7,710.

55. EFSA concluded that "the exposure to fennel fruit infusions in infants and young children and to food supplements with fennel fruit preparations in all population groups containing detectable amounts of estragole assessed through advanced and validated analytical procedures falls under the consideration that substances that are both genotoxic and carcinogenic should not be deliberately added to foods or used in the food chain (EFSA Scientific Committee, 2005, 2012)."

56. EFSA noted that *p*-allylalkoxybenzenes have been reported to cross the placenta and form DNA adducts in the foetus. They have also been detected in breast milk following maternal consumption and to have subsequently been carcinogenic in the offspring of mice (Vesselinovitch et al., 1979). Therefore, EFSA concluded that intake of foods containing genotoxic and carcinogenic compounds including estragole-containing fennel fruit preparations during pregnancy and lactation may pose health risks to both the unborn child and the newborn.

57. In adolescents and adults, fennel fruit infusions containing estragole generally contributed only a small proportion to the overall exposure to *p*-allylalkoxybenzenes. However, EFSA noted that regardless of total *p*-

allylalkoxybenzenes intake, reducing estragole exposure still helps to mitigate the health risks associated with genotoxic carcinogens.

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Uncertainties (Section 4)

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58. Section 4 of the draft EFSA Opinion describes the uncertainties relating to: ADME of *p*-allylalkoxybenzenes, DNA adduct formation and repair, matrix effects and the exposure assessment (occurrence data, food consumption data and exposure scenarios). The key points have been summarised below, but please refer to the draft opinion for more detail.

ADME of *p*-allylalkoxybenzenes

59. The BMDL10 value used for risk assessment of *p*-allylalkoxybenzenes was derived for methyleugenol from a 2-year carcinogenicity study and EFSA applied this BMDL10 to the entire *p*-allylalkoxybenzene group because they possess similar structures, fates in the body and modes of action. This approach assumed the effects from combined exposure to single *p*-allylalkoxybenzenes to be additive with equal potency. The assumption was due to the absence of data on other *p*-allylalkoxybenzenes, preventing the identification of BMDLs10 for each single compound. Therefore, this method does not account for the likely possibility that each individual *p*-allylalkoxybenzene may have different carcinogenic potencies.

DNA adduct formation and repair

60. The estimation of DNA adduct formation in humans at background exposure levels (around 40-50 adducts per 10^8 nucleotides derived from methyleugenol) was based on studies on non-tumorous tissue samples from tumour patients or humanised mice (Herrmann et al., 2013; Herrmann et al., 2014). However, these findings may not be representative of tissues of healthy humans.

61. This section also describes uncertainties regarding the relationship between the amount of DNA adducts and tumorigenesis, the mechanism of formation of adducts and how they introduce mutations, interindividual variation in metabolism between humans and the risk of acute versus low level chronic exposure to *p*-allylalkoxybenzenes.

Matrix effects

62. There has been evidence that some compounds naturally present in herbs and spices were capable of inhibiting SULT enzymes and may therefore lead to the reduction of the formation of sulfooxy metabolites and DNA adducts (Boberg et al., 1983; Alhusainy et al., 2013; Marabini et al., 2019). However, currently the findings have been limited by the use of a cell model incapable of replicating whole-body metabolism. Therefore, extrapolating these results to *in vivo* conditions was not possible.

Exposure assessment

63. Please see lines 1734-1799 of the draft EFSA opinion for the detailed discussion on uncertainties of the exposure assessment. Key points for each subsection are bulleted below.

Occurrence data

- EFSA used pooled values for the concentration of estragole in dried fennel fruits, fennel fruit infusions and infusions made from herbal blends containing fennel fruits for the exposure assessment.
- In several cases, the content in the food had to be estimated from concentrations of the *p*-allylalkoxybenzenes in the essential oil combined with the essential oil yield.
- Concentrations in some of the dried spices/herbs were estimated from concentrations in fresh spices/herbs or vice versa depending on the case.
- Non-EU food data were used in cases when EU data was absent. The non-EU food samples generally had higher levels of *p*-allylalkoxybenzenes than food sampled in EU countries, suggesting a potential under- or overestimation.
- Occurrence data of *p*-allylalkoxybenzenes for some commonly consumed foods were not available and were therefore not considered for the exposure assessment, leading to a possible underestimation of exposure.
- Exposure from food supplements was not included in the assessment and may lead to underestimation of the exposure to *p*-allylalkoxybenzenes.

Food consumption data

- Consumption of herbs and spices, such as dried fennel fruits, may have been underreported in the EFSA Comprehensive Food Consumption Database as they are often not accurately captured in surveys.
- Difficulties to accurately quantify amounts used/portions of fennel fruits and other herbs and spices may lead to under- or overestimation, based on the

assumptions made. There is also uncertainty due to a wide variability in cultural and individual preferences.
- It was unknown how well the exposure assessment performed in capturing exposure from *p*-allylalkoxybenzene-containing flavourings and food ingredients that have been added to compound foods.

Exposure scenarios

- Unspecified herbal blends were assumed to contain fennel fruits within the fennel fruit infusion exposure scenario. This assumption was made to obtain more reliable, and conservative estimated of exposure to *p*-allylalkoxybenzenes. Estimates shown for the whole population have been produced without this assumption and hence were likely to underestimate the importance of the contribution of fennel fruits to total *p*-allylalkoxybenzene exposure.

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6. Hazard characterisation (Section 3.6) - EFSA Opinion on safety of preparations from fruits of sweet and bitter fennel
7. Exposure assessment (Section 3.7) - EFSA Opinion on safety of preparations from fruits of sweet and bitter fennel
8. Risk characterisation and Conclusions (Sections 3.8 and 5) - EFSA Opinion on safety of preparations from fruits of sweet and bitter fennel
9. Uncertainties (Section 4) EFSA Opinion on safety of preparations from fruits of sweet and bitter fennel
10. Questions for the Committee - EFSA Opinion on safety of preparations from fruits of sweet and bitter fennel
11. Abbreviations - EFSA Opinion on safety of preparations from fruits of sweet and bitter fennel
12. References - EFSA Opinion on safety of preparations from fruits of sweet and bitter fennel

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i. Do Members have any comments on the specific sections of the draft EFSA Opinion?

ii. Does the Committee agree with the approach taken by EFSA?

Secretariat

September 2025

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Abbreviations

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Acronym	Definition
ADI	Acceptable daily intake
ADME	Absorption, Distribution, Metabolism and Excretion
AMPAC	N-acetyl-S-[3′-(4-methoxyphenyl)allyl]-L-cysteine

BfR	German Federal Institute for Risk Assessment
BMD	Benchmark dose
BMDL10	Lower confidence limit for a benchmark response of 10%
COT	Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment
CYP450	Cytochrome P450
DNA	Deoxyribonucleic acid
EC	European Commission
EFSA	European Food Safety Authority
EMA	European Medicines Agency
EU	European Union
HMPC	Committee on Herbal Medicinal Products
MoE	Margin of exposure
NDA	Panel on Nutrition, Novel Foods and Food Allergens
NTP	National Toxicology Programme
P90	90th percentile

P95	95th percentile
PBBD	Physiologically based biodynamic
PBBK	Physiologically based biokinetic
sQ	Subquestion
SULT	Sulfotransferase

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8. Risk characterisation and Conclusions (Sections 3.8 and 5) - EFSA Opinion on safety of preparations from fruits of sweet and bitter fennel
9. Uncertainties (Section 4) EFSA Opinion on safety of preparations from fruits of sweet and bitter fennel
10. Questions for the Committee - EFSA Opinion on safety of preparations from fruits of sweet and bitter fennel
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This is a paper for discussion. This does not represent the views of the Committee and should not be cited.

Ackermann G, Peil M, Quarz C, Schmidt A, Halaczkiewicz M, Thomas AD, Stegmüller S, Richling E, Manolikakes G, Christmann M, Küpper JH, Schrenk D and Fahrer J, 2025. Molecular dosimetry of estragole and 1'-hydroxyestragole-induced DNA adduct formation, clastogenicity and cytotoxicity in human liver cell models. Archives of Toxicology. https://doi.org/10.1007/s00204-025-04084-2

Alajlouni AM, Al_Malahmeh AJ, Kiwamoto R, Wesseling S, Soffers AEMF, Al-Subeihi AAA, Vervoort J and Rietjens IMCM, 2016. Mode of action based risk assessment of the botanical food-borne alkenylbenzene apiol from parsley using physiologically based kinetic (PBK) modelling and read-across from safrole. Food and Chemical Toxicology, 89:138-150. https://doi.org/10.1016/j.fct.2016.01.018

Alhusainy W, Paini A, Punt A, Louisse J, Spenkelink A, Vervoort J, Delatour T, Scholz G, Schilter B, Adams T, van Bladeren PJ and Rietjens IM, 2010. Identification of nevadensin as an important herb-based constituent inhibiting estragole bioactivation and physiology-based biokinetic modeling of its possible in vivo effect. Toxicology and Applied Pharmacology, 245:179-190. https://doi.org/10.1016/j.taap.2010.02.017

Alhusainy W, Paini A, van den Berg JH, Punt A, Scholz G, Schilter B, van Bladeren PJ, Taylor S, Adams TB and Rietjens IM, 2013. In vivo validation and physiologically based biokinetic modeling of the inhibition of SULT-mediated estragole DNA adduct formation in the liver of male Sprague-Dawley rats by the basil flavonoid nevadensin. Molecular Nutrition & Food Research, 57:1969-1978. https://doi.org/10.1002/mnfr.201300144

Alhusainy W, van den Berg SJ, Paini A, Campana A, Asselman M, Spenkelink A, Punt A, Scholz G, Schilter B, Adams TB, van Bladeren PJ and Rietjens IM, 2012. Matrix modulation of the bioactivation of estragole by constituents of different alkenylbenzene-containing herbs and spices and physiologically based biokinetic modeling of possible in vivo effects. Toxicological Sciences, 129:174-187. https://doi.org/10.1093/toxsci/kfs196

Al-Subeihi, A. A., Spenkelink, B., Punt, A., Boersma, M. G., van Bladeren, P. J., & Rietjens, I. M. 2012. Physiologically based kinetic modeling of bioactivation and detoxification of the alkenylbenzene methyleugenol in human as compared with rat. Toxicology and applied pharmacology, 260(3), 271–284. https://doi.org/10.1016/j.taap.2012.03.005

Anthony A, Caldwell J, Hutt AJ and Smith RL, 1987. Metabolism of estragole in rat and mouse and influence of dose size on excretion of the proximate carcinogen 1′- hydroxyestragole. Food and Chemical Toxicology, 25:799-806. https://doi.org/10.1016/0278-6915(87)90257-2

Boberg EW, Miller EC, Miller JA, Poland A and Liem A, 1983. Strong evidence from studies with brachymorphic mice and pentachlorophenol that 1'-sulfoöxysafrole is the major ultimate electrophilic and carcinogenic metabolite of 1'-hydroxysafrole in mouse liver. Cancer Research, 43:5163-5173.

Daniels J and and Kadlubar S, 2014. Pharmacogenetics of Sult1A1. Pharmacogenomics, 15:1823-1838. https://doi.org/10.2217/pgs.14.134

Denzer MY, Kirsch F and Buettner A, 2015. Are odorant constituents of herbal tea transferred into human milk? Journal of Agricultural and Food Chemistry, 63:104-111. https://doi.org/10.1021/jf504073d

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2022a. Scientific Opinion on the safety and efficacy of a feed additive consisting of an essential oil from the fruit of Cuminum cyminum L. (cumin oil) for use in all animal species (FEFANAasbl). EFSA Journal 2022;20(12):7690, 26 pp. https://doi.org/10.2903/j.efsa.2022.769

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2022b. Scientific Opinion on the safety and efficacy of a feed additive consisting of an extract of olibanum from Boswellia serrata Roxb. ex Colebr. for use in dogs and horses (FEFANA asbl). EFSA Journal 2022;20(3):7158, 24 pp. https://doi.org/10.2903/j.efsa.2022.7158

Eisenreich A, Götz ME, Sachse B, Monien BH, Herrmann K and Schäfer B, 2021. Alkenylbenzenes in foods: Aspects impeding the evaluation of adverse health effects. Foods, 10:2139. https://doi.org/10.3390/foods10092139

EMA HMPC (European Medicines Agency, Committee on Herbal Medicinal Products), 2005. Public statement on the use of herbal medicinal products containing methyleugenol. EMEA/HMPC/138363/2005. HMPC Public Statement on the use of HMP cont methyleugenol

EMA HMPC (European Medicines Agency, Committee on Herbal Medicinal Products), 2023. Public statement on the use of herbal medicinal products containing estragole. EMA/HMPC/137212/2005 Rev 1 Corr 1*. HMPC Public Statement on the use of HMP containing estragole- draft Corr 1

EMA HMPC (European Medicines Agency, Committee on Herbal Medicinal Products), 2024a. Assessment report on Foeniculum vulgare Miller subsp. vulgare var. vulgare, aetheroleum Final – Revision 1. EMA/HMPC/271394/2022. Foeniculi amari fructus aetheroleum - AR

EMA HMPC (European Medicines Agency, Committee on Herbal Medicinal Products), 2024b. European Union herbal monograph on Foeniculum vulgare Miller subsp. vulgare var. dulce (Mill.) Batt. & Trab., fructus Final – Revision 1. EMEA/HMPC/372839/2016, 10 pp. Foeniculi dulcis fructus - MO

EMA HMPC (European Medicines Agency, Committee on Herbal Medicinal Products), 2024c. European Union herbal monograph on Foeniculum vulgare Miller subsp. vulgare var. vulgare, fructus Final – Revision 1. EMA/HMPC/372841/2016, 9 pp. Foeniculi amari fructus - MO

EMA HMPC (European Medicines Agency, Committee on Herbal Medicinal Products), 2024d. Public statement on Foeniculum vulgare Miller subsp. vulgare var. vulgare, aetheroleum. EMA/HMPC/522456/2021. Foeniculi amari fructus aetheroleum - Public statement

Fennell TR, Miller JA and Miller EC, 1984. Characterization of the biliary and urinary glutathione and N-acetylcysteine metabolites of the hepatic carcinogen 1'-hydroxysafrole and its 1'-oxo metabolite in rats and mice. Cancer Research, 44:3231-3240. Characterization of the biliary and urinary glutathione and N-acetylcysteine metabolites of the hepatic carcinogen 1'-hydroxysafrole and its 1'-oxo metabolite in rats and mice - PubMed

Götz ME, Sachse B, Schäfer B and Eisenreich A, 2022. Myristicin and elemicin: Potentially toxic alkenylbenzenes in food. Foods, 11. https://doi.org/10.3390/foods11131988

Hartwig A, Arand M, Epe B, Guth S, Jahnke G, Lampen A, Martus H-J, Monien B, Rietjens IMCM, Schmitz-Spanke S, Schriever-Schwemmer G, Steinberg P and Eisenbrand G, 2020. Mode of action-based risk assessment of genotoxic carcinogens. Archives of Toxicology, 94:1787-1877. https://doi.org/10.1007/s00204-020-02733-2

Herrmann K, Engst W, Meinl W, Florian S, Cartus AT, Schrenk D, Appel KE, Nolden T, Himmelbauer H and Glatt H, 2014. Formation of hepatic DNA adducts by methyleugenol in mouse models: drastic decrease by Sult1a1 knockout and strong increase by transgenic human SULT1A1/2. Carcinogenesis, 35:935-941. https://doi.org/10.1093/carcin/bgt408

Herrmann K, Schumacher F, Engst W, Appel KE, Klein K, Zanger UM and Glatt H, 2013. Abundance of DNA adducts of methyleugenol, a rodent hepatocarcinogen, in human liver samples. Carcinogenesis, 34:1025-1030. https://doi.org/10.1093/carcin/bgt013

Jeurissen SM, Punt A, Delatour T and Rietjens IM, 2008. Basil extract inhibits the sulfotransferase mediated formation of DNA adducts of the procarcinogen 1'-hydroxyestragole by rat and human liver S9 homogenates and in HepG2 human hepatoma cells. Food and Chemical Toxicology, 46:2296-2302. https://doi.org/10.1016/j.fct.2008.03.010

Jeurissen SMF, Punt A, Boersma MG, Bogaards JJP, Fiamegos YC, Schilter B, van Bladeren PJ, Cnubben NHP and Rietjens IMCM, 2007b. Human cytochrome P450 enzyme specificity for the bioactivation of estragole and related alkenylbenzenes. Chemical Research in Toxicology, 20:798-806. https://doi.org/10.1021/tx700012d

Lu L-JW, Disher RM, Reddy MR and Randerath K, 1986. 32P-Postlabeling Assay in Mice of Transplacental DNA Damage Induced by the Environmental Carcinogens Safrole, 4-Aminobiphenyl, and Benzo(a)pyrene1 | Cancer Research | American Association for Cancer Research, 46:3046-3054.

Marabini L, Galli CL, Fauci PL and Marinovich M, 2019. Effect of plant extracts on the genotoxicity of 1'-hydroxy alkenylbenzenes. Regulatory toxicology and pharmacology: RTP, 105. https://doi.org/10.1016/j.yrtph.2019.03.017

Martati E, Boersma MG, Spenkelink A, Khadka DB, van Bladeren PJ, Rietjens IMCM and Punt A, 2012. Physiologically Based Biokinetic (PBBK) Modeling of safrole bioactivation and detoxification in humans as compared with rats. Toxicological Sciences, 128:301-316. https://doi.org/10.1093/toxsci/kfs174

Monien BH, Sachse B, Niederwieser B and Abraham K, 2019. Detection of N-Acetyl-S-[3'-(4-methoxyphenyl)allyl]-l-Cys (AMPAC) in human urine samples after controlled exposure to fennel tea: A new metabolite of estragole and trans-anethole. Chemical Research in Toxicology, 32:2260-2267. https://doi.org/10.1021/acs.chemrestox.9b00287

Monien BH, Schumacher F, Herrmann K, Glatt H, Turesky RJ and Chesné C, 2015. Simultaneous detection of multiple DNA adducts in human lung samples by isotope dilution UPLC-MS/MS. Analytical Chemistry, 87:641-648. https://doi.org/10.1021/ac503803m

Ning J, Louisse J, Spenkelink B, Wesseling S and Rietjens IMCM, 2017. Study on inter-ethnic human differences in bioactivation and detoxification of estragole using physiologically based kinetic modeling. Archives of Toxicology, 91:3093-3108. https://doi.org/10.1007/s00204-017-1941-x

NTP (National Toxicology Programme), 2000. Technical report on the toxicology and carcinogenesis studies of methyleugenol (CAS No. 93-15-2) in F344/N rats and B6C3F1 mice. https://ntp.niehs.nih.gov/go/tr491abs

Paini A, Punt A, Scholz G, Gremaud E, Spenkelink B, Alink G, Schilter B, Bladeren PJv and Rietjens IM, 2012. In vivo validation of DNA adduct formation by estragole in rats predicted by physiologically based biodynamic modelling. Mutagenesis, 27. https://doi.org/10.1093/mutage/ges031

Paini A, Punt A, Viton F, Scholz G, Delatour T, Marin-Kuan M, Schilter B, van Bladeren PJ and Rietjens IM, 2010. A physiologically based biodynamic (PBBD) model for estragole DNA binding in rat liver based on in vitro kinetic data and estragole DNA adduct formation in primary hepatocytes. Toxicology and Applied Pharmacology, 245:57-66. https://doi.org/10.1016/j.taap.2010.01.016

Phillips DH, Miller JA, Miller EC and Adams B, 1981. Structures of the DNA Adducts Formed in Mouse Liver after Administration of the Proximate Hepatocarcinogen 1′-Hydroxyestragole1 | Cancer Research | American Association for Cancer Research , 41:176-186.

Phillips DH, Reddy MV and Randerath K, 1984. 32 P-Post-labelling analysis of DNA adducts formed in the livers of animals treated with safrole, estragole and other naturally occurring alkenylbenzenes. II. Newborn male B6C3F 1 mice. Carcinogenesis, 5:1623-1628. https://doi.org/10.1093/carcin/5.12.1623

Punt A, Paini A, Boersma MG, Freidig AP, Delatour T, Scholz G, Schilter B, Bladeren PJv and Rietjens IMCM, 2009b. Use of physiologically based biokinetic (PBBK) modeling to study estragole bioactivation and detoxification in humans as compared with male rats. Toxicological Sciences, 110:255-269. https://doi.org/10.1093/toxsci/kfp102

Punt A, Paini A, Spenkelink A, Scholz G, Schilter B, van Bladeren PJ and Rietjens IMCM, 2016. Evaluation of interindividual human variation in bioactivation and DNA adduct formation of estragole in liver predicted by physiologically based kinetic/dynamic and Monte Carlo modeling. Chemical Research in Toxicology, 29:659-668. https://doi.org/10.1021/acs.chemrestox.5b00493

Randerath K, Haglund RE, Phillips DH and Reddy MV, 1984. 32 P-Post-labelling analysis of DNA adducts formed in the livers of animals treated with safrole, estragole and other naturally-occurring alkenylbenzenes. I. Adult female CD-1 mice. Carcinogenesis, 5:1613-1622. https://doi.org/10.1093/carcin/5.12.1613

Rietjens IMCM, Huseiny WA and Boersma MG, 2011. Flavonoids and alkenylbenzenes: New concepts in bioactivation studies. Chemico-Biological Interactions, 192:87-95. https://doi.org/10.1016/j.cbi.2010.09.016

Sangster SA, Caldwell J, Hutt AJ, Anthony A and Smith RL, 1987. The metabolic disposition of [methoxy-14C]-labelled trans-anethole, estragole and p-propylanisole in human volunteers. Xenobiotica, 17:1223-1232. https://doi.org/10.3109/00498258709167414

SCF (Scientific Committee on Food), 2001a. Opinion on estragole (1-allyl-4-methoxybenzene). SCF/CS/FLAV/FLAVOUR/6 ADD2 FINAL, Opinion on Estragole (1-Allyl-4-methoxybenzene)

SCF (Scientific Committee on Food), 2001b. Opinion on methyleugenol (4-allyl-1,2- dimethoxybenzene). SCF/CS/FLAV/FLAVOUR/4 ADD1 FINAL, Opinion on Methyleugenol (4-Allyl-1,2-dimethoxybenzene)

SCF (Scientific Committee on Food), 2002. Opinion on the safety of the presence of safrole (1-allyl-3,4-methylene dioxy benzene) in flavourings and other food ingredients with flavouring properties. SCF/CS/FLAV/FLAVOUR/6 ADD3 Final,

Opinion of the Scientific Committee on Food on the safety of the presence of safrole (1-allyl-3,4-methylene dioxy benzene...

Schulte-Hubbert R, Küpper J-H, Thomas AD and Schrenk D, 2020. Estragole: DNA adduct formation in primary rat hepatocytes and genotoxic potential in HepG2-CYP1A2 cells. Toxicology, 444:152566. https://doi.org/10.1016/j.tox.2020.152566

Smith RL, Adams TB, Doull J, Feron VJ, Goodman JI, Marnett LJ, Portoghese PS, Waddell WJ, Wagner BM, Rogers AE, Caldwell J and Sipes IG, 2002. Safety assessment of allylalkoxybenzene derivatives used as flavouring substances — methyl eugenol and estragole. Food and Chemical Toxicology, 40:851-870. https://doi.org/10.1016/s0278-6915(02)00012-1

Solheim E and Scheline RR, 1973. Metabolism of alkenebenzene derivatives in the rat. I. p-Methoxyallylbenzene (Estragole) and p-methoxypropenylbenzene (Anethole). Xenobiotica: The Fate of Foreign Compounds in Biological Systems, 3. https://doi.org/10.3109/00498257309151538

Suparmi S, Ginting AJ, Mariyam S, Wesseling S and Rietjens I, 2019. Levels of methyleugenol and eugenol in instant herbal beverages available on the Indonesian market and related risk assessment. Food and Chemical Toxicology, 125:467-478. https://doi.org/10.1016/j.fct.2019.02.001

Suzuki Y, Umemura T, Ishii Y, Hibi D, Inoue T, Jin M, Sakai H, Kodama Y, Nohmi T, Yanai T, Nishikawa A and Ogawa K, 2012. Possible involvement of sulfotransferase 1A1 in estragole-induced DNA modification and carcinogenesis in the livers of female mice. Mutation Research, 749:23-28. https://doi.org/10.1016/j.mrgentox.2012.07.002

Tremmel R, Herrmann K, Engst W, Meinl W, Klein K, Glatt H and Zanger UM, 2017. Methyleugenol DNA adducts in human liver are associated with SULT1A1 copy number variations and expression levels. Archives of Toxicology, 91:3329-3339. https://doi.org/10.1007/s00204-017-1955-4

Vesselinovitch SD, Rao KV and Mihailovich N, 1979. Transplacental and Lactational Carcinogenesis by Safrole1 | Cancer Research | American Association for Cancer Research, 39:4378-4380.

Wiseman RW, Miller EC, Miller JA and Liem A, 1987. Structure-Activity Studies of the Hepatocarcinogenicities of Alkenylbenzene Derivatives Related to Estragole and Safrole on Administration to Preweanling Male C57BL/6J × C3H/HeJ F1 Mice1 | Cancer Research | American Association for Cancer Research, 47:2275-2283

Yang S, Diem M, Liu JDH, Wesseling S, Vervoort J, Oostenbrink C and Rietjens I, 2020. Cellular levels and molecular dynamics simulations of estragole DNA adducts point at inefficient repair resulting from limited distortion of the double-stranded DNA helix. Archives of Toxicology, 94:1349-1365. https://doi.org/10.1007/s00204-020-02695-5

Yang S, Kawai T, Wesseling S and Rietjens IMCM, 2022. In vitro and in silico study on consequences of combined exposure to the food-borne alkenylbenzenes estragole and safrole. Toxicology In Vitro, 79:105290. https://doi.org/10.1016/j.tiv.2021.105290

Yang S, Meng Z, Li Y, Chen R, Yang Y and Zhao Z, 2021. Evaluation of physiological characteristics, soluble sugars, organic acids and volatile compounds in ‘Orin’ apples (Malus domestica) at different ripening stages. Molecules, 26:807. https://doi.org/10.3390/molecules26040807

Zangouras A, Caldwell J, Hutt AJ and Smith RL, 1981. Dose dependent conversion of estragole in the rat and mouse to the carcinogenic metabolite, 1'-hydroxyestragole. Biochemical Pharmacology, 30. https://doi.org/10.1016/0006-2952(81)90329-4

Zeller A, Horst K and Rychlik M, 2009. Study of the metabolism of estragole in humans consuming fennel tea. Chemical Research in Toxicology, 22:1929-1937. https://doi.org/10.1021/tx900236g

Zhou G-D, Moorthy B, Bi J, Donnelly KC and Randerath K, 2007. DNA adducts from alkoxyallylbenzene herb and spice constituents in cultured human (HepG2) cells. Environmental and Molecular Mutagenesis, 48:715-721. https://doi.org/10.1002/em.20348