DNA and protein adduct formation (Section 3.5)

In this guide

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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.