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)

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).