Food and Chemical Toxicology 65 (2014) 147–154

Contents lists available at ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox

Chemical analysis of estragole in fennel based teas and associated safety assessment using the Margin of Exposure (MOE) approach ☆

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ARTICLE INFO

Article history:
Received 19 November 2013
Accepted 21 December 2013
Available online 30 December 2013

Keywords:
Estragole
Fennel tea
Margin of Exposure (MOE)
Safety assessment

ABSTRACT

This study describes the analysis of estragole in dry fennel preparations and in infusions prepared from them and an associated safety assessment. A wide range of estragole levels of 0.15–13.3 mg/g dry fennel preparation was found. The estragole content in infusions was considerably lower ranging between 0.4 and 133.4 μg/25 mL infusion prepared from 1 g dry material. Infusions prepared from whole fennel fruits contained about 3-fold less estragole compared to infusions prepared from fine cut fennel material. Safety assessment was performed using the Margin of Exposure (MOE) approach comparing available tumour data to the estimated daily estragole intakes from the consumption of 1–3 cups fennel tea. MOEs obtained for adults generally point at a low priority for risk management, especially when one cup of fennel tea is used daily during lifetime. MOEs for use of fennel teas by children were generally <10,000 indicating a priority for risk management. However, limiting such uses to 1–2 weeks, MOEs might be 3 orders of a magnitude higher and there would be no priority for risk management. These results indicate a low priority for risk management actions for use of fennel teas especially for short-term uses proposed for the symptomatic treatment of digestive disorders.

1. Introduction

For many years, botanicals have formed part of the regular diet as fruits or vegetables, wines, tea, food supplements or as flavours and fragrances added to food. Additionally, numerous botanicals and botanical preparations have a long history of use as traditional herbal medicine. An example of such a botanical used to maintain health or treat symptoms of disease is fennel (*Foeniculum vulgare* Mill.). Fennel based teas are traditionally used in many parts of Europe including France, Germany, Austria, Czech Republic and Poland were they are often used for the symptomatic treatment of digestive disorders alleviating mild spasmodic gastro-intestinal ailments and the relief of symptoms during inflammations of mucous membranes of the upper respiratory tract (EMA, 2008). Resulting from these proposed health-promoting effects, and the natural character of fennel, homemade fennel tea is often used as a remedy for gastrointestinal complaints in infants and young children (Crotteau et al., 2006; Perry et al., 2011). Although fennel based teas have a long history of use and are valued for their beneficial effects, fennel may contain active ingredients of concern, such as the alkenylbenzene estragole (Fig. 1) (EFSA, 2012a). Estragole was previously indicated to be genotoxic and carcinogenic (SCF, 2001). In response to these findings, regulatory actions were taken within the EU restricting the use of estragole (Regulation (EC) No. 1334/2008 of the European Parliament and of the Council, 16 December 2008). The potential risks for human health related to the use of estragole-containing fennel based teas to relief gastrointestinal disorders, especially in children, have not (yet) been thoroughly studied and the European Medicines Agency (EMA, former EMEA) concluded that "the use of fennel tea is not recommended in children under 4 years of age due to the lack of adequate data" (EMA, 2008).

A major issue in the safety assessment of estragole and estragole-containing food items is how to provide guidance on the potential risks for human health resulting from the exposure to (low levels of) food-borne compounds that are genotoxic and

Abbreviations: ADI, acceptable daily intake; BMDL10, lower confidence limit of the benchmark dose resulting in a 10% extra cancer incidence; EFSA, European Food Safety Authority; EMA, European Medicines Agency; ESCO, EFSA Scientific Cooperation; HPLC, high performance liquid chromatography; JECFA, Joint FAO/WHO Expert Committee on Food Additives; MOE, Margin of Exposure; ULC/MS, ultra liquid chromatography/mass spectrometry; UPLC, ultra performance liquid chromatography.

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http://dx.doi.org/10.1016/j.fct.2013.12.035

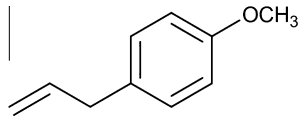

Fig. 1. Structural formula of estragole.

carcinogenic (EFSA, 2005). The European Food Safety Authority (EFSA) recently indicated that whenever genotoxic and carcinogenic compounds are present in a botanical or botanical preparation of interest, the Margin of Exposure (MOE) approach (EFSA, 2005) can be used to evaluate the potential risk for human health and the priority for risk management actions (EFSA, 2009b). The MOE is a dimensionless ratio that is obtained by comparing exposure levels causing malignant tumours in experimental animals (*e.g.* the $BMDL_{10}$ value, the lower confidence bound of the benchmark dose that gives 10% extra cancer incidence) with the estimated daily intake in humans (EFSA, 2005). In a previous evaluation of the EFSA Scientific Cooperation (ESCO) working group, the MOE was applied to the use of estragole-containing tea prepared from bitter fennel fruits (*F. Vulgare* Mill. var. vulgare) (EFSA, 2009a). The exposure to estragole was estimated to equal 1.9–15.8 mg per day corresponding to 33–263 μg estragole/kg bw/day for a person with a body weight of 60 kg (EFSA, 2009a). This theoretical exposure estimate was based on the assumption that 4.5–7.5 g of fennel fruits would be used on a daily basis for the preparation of homemade fennel tea, that fennel fruits contain 5% essential oil, that the essential oil contains 3.5–12% estragole and that the extraction efficiency of the essential oil into the infusion is equal to 25–35% (EFSA, 2009a). The ESCO working group calculated the MOE based on the exposure estimates obtained and $BMDL_{10}$ values derived from the incidence of malignant liver tumors in female CD-1 mice exposed to estragole (EFSA, 2009a; Miller et al., 1983). The MOEs thus obtained were found to be relatively low (*i.e.* MOEs equal to 34–1000) indicating that the daily use of homemade fennel tea prepared from bitter fennel fruits would be of high priority for risk management actions (EFSA, 2009a). Raffo et al. (2011) recently experimentally determined the estragole content in hot water extracts of commercial fennel teas providing a refinement of the exposure estimate made by the ESCO working group. It was demonstrated that the consumption of tea prepared from sweet fennel (*F. vulgare* Mill. Var. dulce), available on the Italian market, that was found to contain the highest estragole level amongst the preparations tested in that study, would result in 3–26-fold lower estragole exposures compared to those estimated by the ESCO working group (Raffo et al., 2011). Based on these lower exposure levels, Raffo et al. (2011) calculated the MOEs to range between 870 and 3210 which is thus higher than the MOEs of 34–1000 calculated by the ESCO working group (Raffo et al., 2011). However, by being lower than 10,000 these MOE values still point at a priority for risk management.

In general, the use of different exposure estimates (*e.g.* theoretical vs. experimental) may result in different daily intake levels for the same exposure scenario and thus also might provide different advice to risk managers on the potential risk to human health. In fact, limitations in exposure estimates are a major source of uncertainty in safety assessment. Thus, the use of refined intake estimates for estragole is required to facilitate an accurate outcome of the safety assessment of fennel based tea preparations. However, the concentration of estragole may show considerable variations depending on the variety, geographical origin, harvesting techniques and processing methods amongst others (Smith et al., 2002) and high variability of the estragole content in fennel fruits are reported in literature (Dadalioglu and Evrendilek, 2004; EFSA, 2012a; Miraldi, 1999; Ruberto et al., 2000). Although the work of Raffo et al. (2011) provided an important refinement of the exposure estimate of estragole resulting from the use of fennel tea, exposure estimates were only based on the use of sweet fennel obtained from the Italian market while in addition to sweet fennel also bitter fennel is widely used to prepare homemade fennel based teas. In this respect, a further refinement of exposure data of estragole resulting from the consumption of homemade fennel tea including different varieties and origins of fennel teas, but also the experimental determination of the extraction efficiency of estragole into the infusion could improve the accuracy of the safety assessment. Hence, the aims of the present study were (1) to make a chemical analysis of estragole in sweet and bitter fennel teas consisting of commercial preparations of fine cut fennel material or whole fruits derived from different geographical origins, and (2) to perform a safety assessment for estragole resulting from drinking fennel tea using the MOE approach, taking into account the previous work by the ESCO working group (EFSA, 2009a) and reported in literature (Raffo et al., 2011).

2. Materials and methods

2.1. Chemicals and materials

A total of 34 fennel tea preparations from different brands were purchased from the internet, obtained from local shops in the Netherlands, Belgium, Germany and Italy or provided by the European Herbal Infusion Association. Fennel tea preparations in the form of teabags, unpackaged fennel consisting of whole fruits or fine cut fennel material, and instant tea granules were included. 7 Fennel tea preparations consisted of sweet fennel material and 8 fennel tea preparations consisted of bitter fennel material. The variety of the remaining samples ($n = 18$) was not specified. Product information as indicated on the label of each product is summarized in Table 1. Estragole (purity 98%) was supplied by Acros organics (Geel, Belgium). Acetonitrile (ULC/MS gradient) and methanol (HPLC supra gradient) were acquired from Sigma Aldrich (Steinheim, Germany). Nanopure water was obtained from a Barnstead nanopure Type I ultra-pure water system.

2.2. Methanolic extracts

To allow quantification of the total estragole content in the fennel tea samples expressed per g dry fennel preparation, methanolic extracts were prepared based on the method previously described (Gursale et al., 2010; van den Berg et al., 2011). To correct for the amount of estragole lost in this extraction method, a recovery study was performed as described by van den Berg et al. (2011). The average percentage recovery was found to equal 90.6 ± 8.1%. This value was used to correct the total level of estragole in the selected dry fennel preparations for sample recovery.

2.3. Hot water extracts

In addition to the methanolic extracts, hot water extracts were made to reflect the preparation of homemade fennel tea. Extracts were prepared based on the method previously described by Raffo et al. (2011) with minor modifications. In short, tea extracts were prepared by adding 25 mL freshly boiled water to 1 g of fennel tea material. The mixture of hot water and fennel tea material was incubated for 7 min in a covered beaker and stirred three times with a spoon. After 7 min, all fennel material was removed from the infusion and the extract was cooled down to room temperature. Aliquots of the hot water extracts were centrifuged at 16,000*g* for 5 min and the supernatant was stored at −20 °C until ultra performance liquid chromatography (UPLC)-analysis.

2.4. UPLC analysis

Before UPLC analysis, aliquots of the methanolic extract solution were diluted in acetonitrile (1:10 v/v). Aliquots of the hot water extracts were analysed undiluted. For quantification of estragole, 3.5 μl of each sample was subjected to UPLC analysis ($n = 3$) as described previously (van den Berg et al., 2011).

2.5. Estimation of daily intakes of estragole resulting from the use of fennel based teas

The exposure estimate of estragole resulting from the use of fennel teas was based on the estragole content in the hot water extracts as determined in the present study (see results) together with the assumption that 1.5–2.5 g fennel would be used for the preparation of a cup of fennel tea as described by the ESCO working

Table 1
Characteristics of fennel based tea samples used, levels of estragole in dry fennel preparations, in water infusions prepared from the selected fennel samples and extraction efficiencies of estragole into the water infusion.

Sample No.	Variety[a]			Form			Origin	Average ± STDEV (μg estragole/g dry fennel preparation)[b]	Average ± STDEV (μg estragole/25 mL infusion extracted from 1 g fennel material)	Extraction efficiency of estragole into the infusion (%)
	Sweet	Bitter	Unknown	Fine cut material	Whole fruits	Granules for instant tea				
1	X			X			Turkey	654.3 ± 113.6	3.1 ± 0.1	0.5
2	X				X		Turkey	597.4 ± 19.3	ND	ND
3		X		X			China	929.8 ± 5.9	6.8 ± 0.6	0.7
4		X			X		China	1218.8 ± 212.5	1.1 ± 0.0	0.1
5		X		X			Bulgaria	1769.8 ± 83.2	16.8 ± 2.2	0.9
6		X			X		Bulgaria	1601.6 ± 129.1	2.8 ± 0.4	0.2
7	X			X			China	603.3 ± 65.4	4.9 ± 0.4	0.8
8	X				X		China	604.5 ± 34.5	1.5 ± 0.0	0.2
9			X	X			UK	3833.3 ± 196.6	26.6 ± 4.1	0.7
10			X	X			Netherlands	478.0 ± 37.4	6.8 ± 1.2	1.4
11			X	X			Netherlands	163.8 ± 17.1	3.7 ± 0.5	2.3
12			X	X			Netherlands	958.2 ± 122.3	5.2 ± 3.6	0.5
13			X	X			Germany	913.7 ± 49.5	3.8 ± 1.9	0.4
14			X		X		Austria	1686.9 ± 129.1	3.0 ± 0.7	0.2
15			X		X		Austria	1404.2 ± 87.4	9.3 ± 3.0	0.7
16			X		X		Germany	1453.1 ± 168.7	4.2 ± 1.6	0.3
17			X	X			Germany	627.8 ± 69.0	3.5 ± 1.1	0.6
18			X	X			Belgium	1443.8 ± 65.4	8.8 ± 4.9	0.6
19			X	X			Belgium	158.8 ± 30.4	3.7 ± 1.5	2.3
20			X			X	Belgium	ND	0.4 ± 0.1	ND
21			X	X			Italy	2227.8 ± 245.6	15.6 ± 5.4	0.7
22			X	X			India	150.2 ± 17.0	2.3 ± 0.6	1.5
23			X	X			India	7867.1 ± 1292.1	29.1 ± 8.4	0.4
24			X	X			Germany	281.9 ± 21.2	5.8 ± 1.2	2.0
25			X	X			Germany	914.2 ± 90.5	1.6 ± 0.4	0.2
26			X			X	Italy	ND	2.5 ± 0.7	ND
27	X			X	X		Unknown	791.0 ± 78.6	5.7 ± 0.6	0.7
28	X			X			Unknown	912.6 ± 31.8	12.8 ± 1.9	1.4
29	X			X			Unknown	638.6 ± 31.8	7.1 ± 0.4	1.1
30		X		X			Unknown	1499.0 ± 106.7	15.8 ± 5.2	1.1
31		X		X			Unknown	2383.5 ± 303.2	33.5 ± 2.2	1.4
32		X		X			Unknown	387.5 ± 18.1	3.3 ± 0.5	0.9
33		X		X			Unknown	871.9 ± 144.5	10.7 ± 1.4	1.2
34			X	X	X		Unknown	13,248.7 ± 1390.4	133.4 ± 18.5	1.0

ND: not determined.
[a] Sweet fennel, *Foeniculum Vulgare* Mill. Var. dulce; bitter fennel, *Foeniculum Vulgare* Mill. var. vulgare.
[b] Values represent the total level of estragole in the fennel samples and was determined with the methanolic extraction method applied and corrected for sample recovery (see materials and methods section for details).

group (EFSA, 2009a). Intake estimates of estragole were made for the daily consumption of one, two or three cups of fennel based tea assuming a body weight of 70 kg, the default value for adult body weight recently proposed by EFSA (2012b).

2.6. Calculation of the Margin of Exposure values

MOEs were calculated by comparing the previously calculated $BMDL_{10}$ values of 3.3–6.5 mg/kg bw/day (van den Berg et al., 2011) for the induction of hepatocellular carcinomas in female mice derived from the data of Miller et al. (1983), with the estimated daily intakes of estragole resulting from the use of fennel based teas as determined in the present study. MOEs were rounded to a single significant value.

3. Results

3.1. Chemical analysis of estragole in dry fennel preparations and in fennel based teas

Fig. 2 presents, as an example, part of the UPLC chromatogram of a methanolic as well as a hot water fennel extract detecting the presence and level of estragole. Table 1 shows that the total estragole content in the different dry fennel preparations was found to vary considerably ranging between 0.15 and 13.3 mg/g dry fennel preparation. Estragole could not be detected in two samples because the instant tea granules in those specific samples only partially dissolved in methanol. The analysis revealed that the content of estragole in the hot water extracts was considerably lower than the estragole content found in the methanolic extracts (Fig. 2 and Table 1). In fact, results for the hot water extracts from 1 g of fennel material, also presented in Table 1, revealed that the level of estragole was relatively low in these water infusions ranging between 0.4 and 133.4 μg/25 mL infusion (Table 1). In the hot water extract prepared from sample 2, estragole could even not be detected (Table 1). Comparing the concentration of estragole in the hot water extracts to the total concentration of estragole in the dry fennel preparations revealed an extraction efficiency of estragole from fennel into the infusion equal to 0.1–2.3% (Table 1).

Results obtained also show that the variation in the total estragole content as determined for the dry fennel preparations could not be attributed to the type of fennel (*i.e.* sweet or bitter) or the form (*i.e.* fine cut fennel material or whole fennel fruits). In fact, calculating the average estragole content showed the presence of 1.1 ± 0.4 and 1.3 ± 0.2 mg estragole/g dry weight in preparations of sweet ($n = 7$) and bitter ($n = 8$) fennel material, respectively. In addition, dry fennel preparations consisting of fine cut fennel material ($n = 23$) or whole fennel fruits ($n = 7$) were found to contain on average 1.3 ± 0.3 and 1.2 ± 0.2 mg estragole/g dry fennel preparation, respectively. In line with these findings, in water infusions prepared from 1 g sweet or bitter fennel material, the average estragole content was comparable (*i.e.* 9 ± 4 and 11 ± 4 μg

estragole/25 mL infusion, respectively) indicating that the estragole content in infusions cannot be attributed to the type of fennel. However, the average estragole content in infusions prepared from whole fennel fruits (*i.e.* 3 ± 1 μg estragole/25 mL infusion) was found to be about 3-fold lower compared to the average content of estragole in infusions prepared from fine cut fennel material (*i.e.* 10 ± 2 μg estragole/25 mL infusion) demonstrating that the extraction efficiency of whole fennel fruits is lower compared to that of fine cut fennel material.

Interestingly, it was shown that the average estragole content in water infusions prepared from fennel material (*i.e.* fine cut fennel material and whole fennel fruits) for which customary selections of the respective levels of estragole were done (samples 1–8, average estragole content of 5 ± 2 μg estragole/25 mL infusion prepared from 1 g dry material) was found to be about 3-fold lower compared to that of the remaining fennel tea preparations (samples 9–34, average estragole content of 14 ± 5 μg estragole/25 mL infusion). In addition, it was even shown that estragole could not be detected in a water infusion that was prepared from sample 2. These results demonstrate that the use of fennel material containing low levels of estragole can have a marked effect on the estragole content in fennel teas and thus also on the potential risk for human health resulting from the use of fennel based teas.

3.2. Intake estimates and safety assessment of estragole resulting from drinking fennel based tea by adults

Using the estragole content in hot water extracts as determined in the present study, intake estimates up to 4.8 or 14.3 μg estragole/kg bw/day were obtained for a 70 kg person assuming the daily consumption of one or three cups of fennel based tea (Table 2). Table 2 presents MOEs that are equal to 200–70,000 for the use of three cups of fennel based tea on a daily basis (*i.e.* 4.5–7.5 g fennel material) as used in the safety assessment made by the ESCO working group. For 10 of the 34 selected fennel preparations MOEs were calculated to be >10,000 when assuming the use of three cups of fennel based teas as presented in Table 2. Interestingly, the fennel tea preparations for which MOEs lower than 10,000 were found all consisted of fine cut fennel material. Assuming the daily consumption of one cup of fennel based tea throughout life, MOEs of 10,000 and higher were found for the vast majority (25 out of 34) of the selected fennel tea preparations (Table 2). In general, these fennel preparations consisted of whole fennel fruits or instant tea granules. It should be noted that also when assuming the daily use of one cup of fennel tea, 8 of the 9 samples for which MOEs lower than 10,000 were found consisted of fine cut fennel material and 1 of the 9 samples consisted of a mixture of fine cut fennel material and whole fennel fruits.

3.3. Intake estimates and safety assessment of estragole resulting from drinking fennel based tea by infants

Infants are the main consumer group of homemade fennel based tea for symptomatic treatment of mild, spasmodic gastrointestinal complaints. Therefore, a separate exposure estimate and subsequent safety assessment were made for this consumer group focussing on fennel based preparations that are specifically marketed for infants (*i.e.* sample 17, 18, 20 and 21).

Table 3 shows the exposure estimates for estragole resulting from the use of homemade fennel based teas that are specifically marketed for infants. Exposure estimates were made based on the estragole content in the fennel preparations as determined in the present study in hot water extracts together with the amount of fine cut fennel material present in the tea bag assuming one cup of fennel based tea would be consumed on a daily basis (samples 17, 18 and 21). For sample 20, consisting of instant tea granules, the weight of two whipped off tea spoons (the dose recommended by the respective manufacturer) was used to estimate the daily estragole intake. Exposure estimates reveal a daily intake of 0.5–6.5 μg/kg bw/day, 0.4–4.7 μg/kg bw/day, 0.3–3.6 μg/kg bw/day and 0.2–2.6 μg/kg bw/day for infants of 0–3 months, 3–6 months, 6–12 months and 12–36 months of age, respectively (Table 3). Comparing the $BMDL_{10}$ values for estragole with the exposure estimates obtained, for samples 17, 18 and 21 consisting

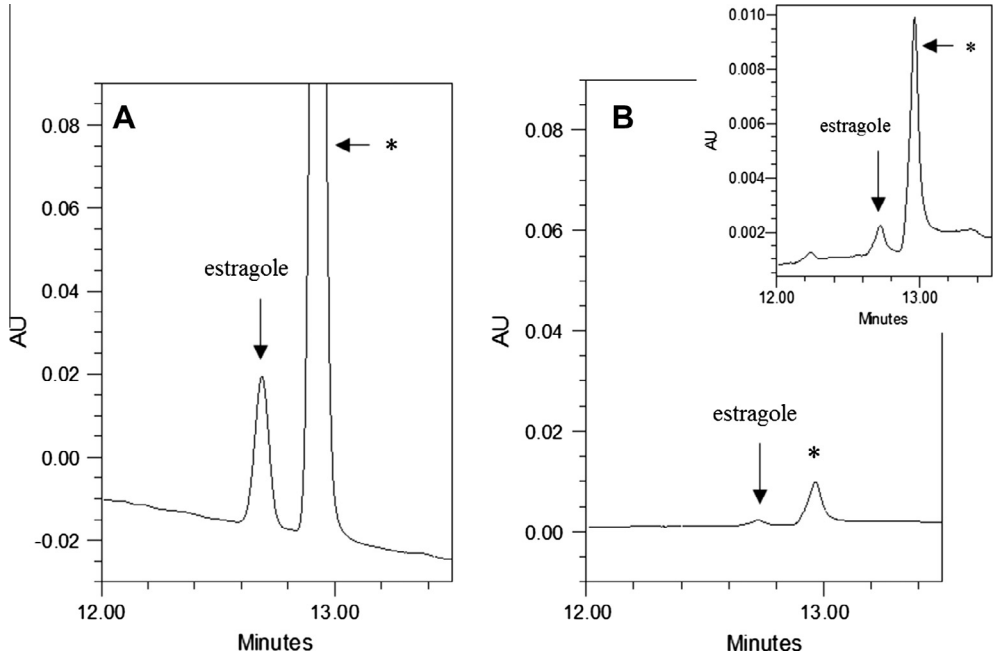

Fig. 2. Representative sections of UPLC chromatograms of a methanolic fennel extract (A) and a hot water extract (B). The insert presents the reduced scale of the same chromatogram. To allow chromatographic analysis, methanolic extracts were diluted in acetonitrile (1:10 v/v) whereas hot water extracts were analysed undiluted. Peaks marked with an asterisk (*) were not identified. Chromatograms were obtained at a wavelength of 225 nm.

Table 2
Daily estragole intake estimates resulting from the use of one, two or three cups of fennel tea and corresponding MOEs.

Sample no.	Daily intake of estragole based on the consumption of one cup fennel tea[a](μg/kg bw/day)	MOE[b,c]	Daily intake of estragole based on the consumption of two cupsof fennel tea[a] (μg/kg bw/day)	MOE[b,d]	Daily intake of estragole based on the consumption of three cups of fennel tea[a] (μg/kg bw/day)	MOE[b,e]
1	0.1	30,000–70,000	0.1–0.2	20,000–70,000	0.2–0.3	10,000–30,000
2	ND	>10,000	ND	>10,000	ND	>10,000
3	0.1–0.2	20,000–70,000	0.3–0.5	7000–20,000	0.4–0.7	5000–20,000
4	<0.1	>10,000	⩽0.1	>10,000	0.1	30,000–70,000
5	0.4–0.6	6000–20,000	0.7–1.2	3000–9000	1.1–1.8	2000–6000
6	0.1	30,000–70,000	0.1–0.2	20,000–70,000	0.2–0.3	10,000–30,000
7	0.1–0.2	20,000–70,000	0.2–0.4	8000–30,000	0.3–0.5	7000–20,000
8	⩽0.1	>10,000	0.1	30,000–70,000	0.1–0.2	20,000–70,000
9	0.6–0.9	4000–10,000	1.1–1.9	2000–6000	1.7–2.8	1000–4000
10	0.1–0.2	20,000–70,000	0.3–0.5	7000–20,000	0.4–0.7	5000–20,000
11	0.1	30,000–70,000	0.2–0.3	10,000–30,000	0.2–0.4	8000–30,000
12	0.1–0.2	20,000–70,000	0.2–0.4	8000–30,000	0.3–0.6	6000–20,000
13	0.1	30,000–70,000	0.2–0.3	10,000–30,000	0.2–0.4	8000–30,000
14	0.1	30,000–70,000	0.1–0.2	20,000–70,000	0.2–0.3	10,000–30,000
15	0.2–0.3	10,000–30,000	0.4–0.7	5000–20,000	0.6–1.0	3000–10,000
16	0.1–0.2	20,000–70,000	0.2–0.3	10,000–30,000	0.3–0.5	7000–20,000
17	0.1	30,000–70,000	0.2–0.3	10,000–30,000	0.2–0.4	8000–30,000
18	0.2–0.3	10,000–30,000	0.4–0.6	6000–20,000	0.6–0.9	4000–10,000
19	0.1	30,000–70,000	0.2–0.3	10,000–30,000	0.2–0.4	8000–30,000
20	<0.1	>10,000	<0.1	>10,000	<0.1	>10,000
21	0.3–0.6	6000–20,000	0.7–1.1	3000–9000	1.0–1.7	2000–7000
22	<0.1	>10,000	0.1–0.2	20,000–70,000	0.1–0.2	20,000–70,000
23	0.6–1.0	3,000–10,000	1.2–2.1	2000–5000	1.9–3.1	1000–3000
24	0.1–0.2	20,000–70,000	0.2–0.4	8000–30,000	0.4–0.6	6000–20,000
25	<0.1	>10,000	0.1	30,000–70,000	0.1–0.2	20,000–70,000
26	0.1	30,000–70,000	0.1–0.2	20,000–70,000	0.2–0.3	10,000–30,000
27	0.1–0.2	20,000–70,000	0.2–0.4	8000–30,000	0.4–0.6	6000–20,000
28	0.3–0.5	7000–20,000	0.5–0.9	4000–10,000	0.8–1.4	2000–8000
29	0.2–0.3	10,000–30,000	0.3–0.5	7000–20,000	0.5–0.8	4000–10,000
30	0.3–0.6	6000–20,000	0.7–1.1	3000–9000	1.0–1.7	2000–7000
31	0.7–1.2	3000–9000	1.4–2.4	1000–5000	2.2–3.6	900–3000
32	0.1	30,000–70,000	0.1–0.2	20,000–70,000	0.2–0.4	8000–30,000
33	0.2–0.4	8000–30,000	0.5–0.8	4000–10,000	0.7–1.1	3000–9000
34	2.9–4.8	700–2000	5.7–9.5	300–1000	8.6–14.3	200–800

ND: not determined.
[a] Daily intake estimates of estragole were obtained using the estragole content in water infusions prepared from 1 g fennel material as presented in Table 1, assuming the use of 1.5–2.5 g fennel material for the preparation of a cup of tea (EFSA, 2009a) and a body weight of 70 kg (EFSA, 2012b).
[b] MOE = $BMDL_{10}$ (mg/kg bw/day)/daily intake estragole (mg/kg bw/day).
[c] MOE assuming a daily intake of one cup of fennel tea.
[d] MOE assuming a daily intake of two cups of fennel tea.
[e] MOE assuming a daily intake of three cups of fennel tea.

of fine cut fennel material MOEs were found to generally be below 10,000 indicating a priority for risk management actions (Table 3). However, it should be noted that for sample 20 consisting of instant tea granules MOEs of 10,000 and higher were found when tea prepared from this product would be consumed by infants of 6 months and older.

4. Discussion

In the present study, a safety assessment for estragole resulting from drinking fennel based tea was made using the MOE approach taking into account the previous work in the field by the ESCO working group (EFSA, 2009a) and reported in literature (Raffo et al., 2011). The results presented show that the consumption of fennel teas generally presents a low priority for risk management actions, especially when one cup of fennel tea is consumed by adults on a daily basis.

Based on the chemical analysis performed in the present study, estragole was detected in the selected 34 fennel samples in quantities equal to 0.15–13.3 mg/g dry fennel preparation. This is comparable to the value resulting from the assumption made by the ESCO working group of 5% essential oil containing 3.5–12% estragole which would give rise to 1.75–6 mg/g fennel material (EFSA, 2009a). Assuming that 4.5–7.5 g fennel material per day would be used for the preparation of fennel tea as described by the ESCO working group (EFSA, 2009a) and that the extraction efficiency of the essential oil of fennel is 25–35% (EFSA, 2009a), the daily estragole intake would amount to 3–499 μg/kg bw/day for a person with a body weight of 70 kg, which is comparable to the estimate of 33–263 μg/kg bw/day reported by the ESCO working group. However, this indirect worst case approach using levels of estragole in fennel material might represent an overestimation of the actual risk for human health resulting from the use of fennel tea. Raffo et al. (2011) indicated that the extraction efficiency of fennel essential oil into the infusion of 25–35% as used by the ESCO working group (EFSA, 2009a) is relatively high leading to an overestimation of the estragole content in the infusion. In fact, Zeller and Rychlik (2006) experimentally determined the extraction efficiency for specific odorants occurring in fennel essential oil into an infusion demonstrating an extraction efficiency of 12% for estragole which is 2–3-fold lower than the extraction efficiency of 25–35% used in the theoretical worst case approach reported by the ESCO working group (EFSA, 2009a). Therefore, in the present study hot water extracts were used representing tea preparation by consumers. Results revealed that the levels of estragole were indeed found to be remarkably lower in the hot water extracts compared to the total estragole content in

Table 3
Level of estragole in hot water extracts of fennel based tea preparations specifically marketed for infants, corresponding daily intake estimates and MOEs.

Sample no.	Average ± STDEV (μg estragole/25 mL infusion)	Daily intake fennel (g)[a]	Daily Intake estragole (μg/kg bw/day)[b]	MOE[c]
Infants 0–3 months of age				
17	3.5 ± 1.1	1.5	1.1	3000–6000
18	8.8 ± 4.9	1.8	3.3	1000–2000
20	0.4 ± 0.1	6.0	0.5	6000–10,000
21	15.6 ± 5.4	2.0	6.5	500–1000
Infants 3–6 months of age				
17	3.5 ± 1.1	1.5	0.8	4000–8000
18	8.8 ± 4.9	1.8	2.3	1000–3000
20	0.4 ± 0.1	6.0	0.4	8000–20,000
21	15.6 ± 5.4	2.0	4.7	700–1000
Infants 6–12 months of age				
17	3.5 ± 1.1	1.5	0.6	6000–10,000
18	8.8 ± 4.9	1.8	1.8	2000–4000
20	0.4 ± 0.1	6.0	0.3	10,000–20,000
21	15.6 ± 5.4	2.0	3.6	900–2000
Toddlers 1–3 years of age				
17	3.5 ± 1.1	1.5	0.4	8000–20,000
18	8.8 ± 4.9	1.8	1.3	3000–5000
20	0.4 ± 0.1	6.0	0.2	20,000–30,000
21	15.6 ± 5.4	2.0	2.6	1000–3000

[a] Daily intake of fennel is based on the weight of the respective teabags and granules assuming one cup of homemade fennel tea will be used on a daily basis.
[b] Daily intake estimates of estragole were obtained assuming a body weight of 4.8 kg, 6.7 kg, 8.8 kg, and 11.9 kg for infants of 0–3 months of age, infants of 3–6 months of age, infants of 6–12 months of age and toddlers of 1–3 years of age respectively (EFSA, 2012b).
[c] MOE = $BMDL_{10}$ (mg/kg bw/day)/daily intake estragole (mg/kg bw/day).

dry fennel preparations (*i.e.* up to 1000-fold). The extraction efficiencies obtained were 0.1–2.3% and thus lower compared to the extraction efficiency determined by Zeller and Rychlik (2006). This difference might be explained by the fact that Zeller and Rychlik (2006) prepared tea from freshly comminuted fennel fruits (2.5 g in 150 mL hot water) while in the present study dried fine cut fennel material or intact fennel fruits were used. Raffo et al. (2011) previously indicated that estragole levels determined in water infusions prepared from intact fruits were 4–6 times lower compared to the levels in water infusions prepared from freshly comminuted fruits explaining the difference in extraction efficiency. Moreover, it is likely that fresh fennel fruits contain higher levels of volatile constituents including estragole compared to the packaged fennel materials used in the present study as a result of graduate losses of volatile constituents in crushed and/or powdered fennel material upon ageing (EMA, 2008). In fact, in teabags opened for a period of 30 days a 4–10% decrease in the level of essential oil was previously reported (EMA, 2008).

Calculation of the MOEs for the intake of estragole from fennel tea using the levels of estragole in the hot water extracts as determined in the present study resulted in MOE values that are generally above the default of 10,000. In spite of this, for some -though not all- fennel based teas MOEs are still below 10,000 indicating a potential risk for human health and a priority for risk management actions. However, it is important to note that while the daily estragole exposure resulting from the consumption of tea prepared from 1.5 to 2.5 g fennel material three times a day (*i.e.* 4.5–7.5 g fennel/day) might pose a potential risk for human health, the consumption of tea prepared from 1.5 to 2.5 g fennel seeds one time a day does not. This is because in the latter situation MOEs above the default of 10,000 are obtained for the vast majority of samples, *i.e.* 25 out of 34 samples. Important to note is that for water infusions prepared from fine cut fennel material generally relatively low MOEs were obtained. In contrast, MOEs for water infusions prepared from whole fennel fruits or instant tea granules were calculated to be generally higher than 10,000 indicating a low priority for risk management actions. In addition, the present study revealed that MOEs below 10,000 were obtained for infants of 0–3 years of age drinking one cup of fennel tea prepared from fine cut material on a daily basis indicating a priority for risk management. However, it should be noted that for infants of 6 months and older also MOEs above 10,000 were obtained for one sample consisting of instant tea granules. In fact, the label of the respective fennel based tea product recommends to use the product only for infants of 6–36 months of age which, based on the calculated MOEs of 10,000–30,000, would be a low risk. However, it must be emphasized that in the present paper MOEs are calculated assuming lifetime exposure while homemade fennel based teas are generally only used during periods of gastrointestinal complaints. For this reason, calculation of the MOEs using the intake estimates as daily intake estimates might overestimate the potential risk for human health. However, a general framework for taking intermittent and/or short-term instead of lifetime exposures to genotoxic carcinogens into account in the safety assessment is currently not in place. Felter et al. (2011) recently proposed a framework for assessing the risk from less-than lifetime exposures to carcinogens. They proposed to use the principle of Haber's Rule provided that chemical-specific carcinogenicity data are available and that data support a linear dose–response relationship (Felter et al., 2011). Haber's Rule assumes that the acceptable cumulative lifetime exposure can be averaged over the duration of short-term exposure, suggesting that higher daily intakes are acceptable when short-term exposure is considered (Felter et al., 2011). EMA previously indicated that fennel based teas should not be used for more than two weeks by adults and less than one week by children under the age of 12 (EMA, 2007). Applying Haber's Rule to assess the potential risk for short-term estragole exposure during a period of one week (children) and two weeks (adults) on an estimated life expectancy of 75 years might result in MOE values that are 3 orders of a magnitude higher than those obtained when assuming lifetime (75 years) daily use of fennel based tea. These results indicate that there may be no reason for risk management actions, when consuming three cups of fennel based tea on a daily basis for only two weeks.

Obviously, the calculation of MOEs is based on $BMDL_{10}$ data that are obtained from a long-term carcinogenicity study in which

pure estragole was administered to rodents (Miller et al., 1983). However, the presence of a natural botanical matrix might modulate the bioactivation of estragole thereby lowering the potential cancer risk. In fact, Alhusainy et al. (2012) previously demonstrated that methanolic extracts from different herbs and spices were able to inhibit the sulfotransferase-mediated bioactivation of estragole. The use of such carcinogenicity data in safety assessment of fennel based teas might thus overestimate the actual risk. However, in the study of Alhusainy et al. (2012) it was shown that among the tested alkenylbenzene-containing herbs and spices, fennel did not show any effect on the sulfotransferase enzyme activity. Moreover, it was previously shown that matrix-derived combination effects may be limited at lower dose levels which are relevant for the use of botanicals and preparations made thereof (van den Berg et al., 2013). On the basis of these findings it can be concluded that the used $BMDL_{10}$ values represent an adequate basis for the safety assessment of fennel based teas.

As shown in the present study, the level of estragole in water infusions prepared from fennel can vary considerably depending on the estragole content present in the fennel material used. In addition, the estragole content in the water infusion might also depend on the preparation method used. Raffo et al. (2011) demonstrated that the extent of dilution has only a limited effect on the level of estragole extracted in the infusion. In contrast, squeezing the teabag to remove any residual water following the preparation of a fennel infusion was found to result in a considerable increase in the estragole content and was previously experimentally determined by Raffo et al. (2011) to equal 15% and Zeller and Rychlik (2009) even demonstrated an increased extraction of estragole into the infusion equal to 45% (Zeller and Rychlik, 2009). In addition, the use of freshly comminuted fennel fruits was shown to increase the estragole content in the infusion by 4–6-fold compared to the use of intact fennel fruits (Raffo et al., 2011). It should be noted that reduction of the estragole content in fennel and fennel based teas is technically possible (Pank et al., 2003). This can be done by removing estragole from instant teas through fractionated distillation (Pank et al., 2003). Moreover, Zeller and Rychlik (2006) indicated that reduction of the estragole content in fennel tea would not influence the overall flavor of fennel tea. Reducing the estragole content is thus recommended to lower the potential risk to human health, if any, resulting from the use of fennel based teas. In fact, the estragole content of one of the analyzed fennel teas consisting of instant tea granules that was specifically marketed for infants of 6 months and older (*i.e.* sample 20) was relatively low indicating that such a technology might have been applied to reduce the estragole content. However, although fractionated distillation can markedly reduce the estragole content in instant teas, this procedure cannot be applied to the fennel fruits in their original form which is the form most commonly used in the preparation of fennel based teas (Pank et al., 2003). Breeding new cultivars without estragole is not (yet) possible (Pank et al., 2003). Moreover, breeding new fennel cultivars with reduced estragole levels was found to result in reduced total essential oil contents including trans-anethole, which gives the characteristic flavour to fennel tea, meaning that these cultivars are of lower quality. 8 Of the 34 selected samples were prepared from fennel material obtained by customary selections of the levels of estragole. The average estragole content in the water infusions prepared from the respective fennel tea preparations (samples 1–8) was found to be about 3-fold lower compared to that of the remaining fennel teas. Moreover, estragole could even not be detected in fennel based tea prepared from sample 2. This indicates that the use of fennel material with relatively low estragole levels can have a marked effect on the estragole intake resulting from the use of fennel based teas.

In addition to estragole, the EFSA compendium indicates trans-anethole as a substance of possible concern for human health present in *F. vulgare* Mill. (EFSA, 2012a). For trans-anethole the Joint FAO/WHO Expert Committee on Food Additives (JECFA) derived a temporary acceptable daily intake (ADI) of 0–2.0 mg/kg bw (JECFA, 1998), which can be used to define whether exposure resulting from proposed uses and use levels will be safe. Zeller and Rychlik (2006) previously demonstrated that trans-anethole is the most abundant odorant in fennel fruit and in fennel based tea (*i.e.* 3600 μg trans-anethole in an infusion extracted from 1 g fennel material). Based on the level of trans-anethole detected by Zeller and Rychlik (2006) and assuming the daily consumption of one cup of fennel tea prepared from 1.5 to 2.5 g fennel material the daily intake of trans-anethole would equal 77–129 μg/kg bw/day for a 70 kg person. Since this value is well below the ADI it can be concluded that the exposure to trans-anethole resulting from the use of fennel based tea is not of concern for human health.

Altogether, the present study showed that extraction efficiencies of estragole into the infusion were considerably lower than the 25–35% extraction efficiency used in the worst case exposure assessment performed by the ESCO working group. In general, extraction efficiencies were found to be lower for samples consisting of whole fennel fruits compared to that of samples consisting of fine cut fennel material indicating that exposure assessments should be done on a case-by case basis taking into account the physical characteristics of the product of interest. In fact, estragole levels were 3-fold lower in water infusions prepared from whole fennel fruits compared to that in water infusions prepared from fine cut fennel material. Also the use of fennel material with relatively low estragole levels obtained by customary selection to prepare fennel based teas resulted in reduced estragole levels of about 3-fold. The results obtained generally point at a low priority for risk management actions for the use of fennel teas, especially when consuming one cup of fennel tea on a daily basis prepared from 1.5 to 2.5 g fennel material. Moreover, assessing the risk from less-than lifetime exposures to estragole resulting from the use of fennel based teas during a period of one to two weeks resulted in MOE values that are 3 orders of a magnitude higher indicating low priorities for risk management actions. Taken together, these results indicate a low priority for risk management actions and a low risk for human health for the use of fennel based teas especially for the short-term proposed uses of fennel based teas for the symptomatic treatment of digestive disorders alleviating mild spasmodic gastro-intestinal ailments.

Conflict of Interest

The authors declare that there are no conflicts of interest.

Transparency documents associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.fct.2013.12.035.

Acknowledgements

The research leading to these results has received funding from the European Community's Seventh Framework Programme (FP7/2007–2013) under Grant agreement n° 245199. It has been carried out within the PlantLIBRA project (website: www.plantlibra.eu). This report does not necessarily reflect the Commission views or its future policy on this area.

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