European Food Safety Authority

The EFSA Journal (2009) 1224, 1-26

SCIENTIFIC OPINION

Safety of smoke flavour primary product - Scansmoke SEF7525 [1]

Opinion of the Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF)

(Question No EFSA-Q-2005-262)

Adopted on 14 May 2009

PANEL MEMBERS

Arturo Anadón, David Bell, Mona-Lise Binderup, Wilfried Bursch, Laurence Castle, Riccardo Crebelli, Karl-Heinz Engel, Roland Franz, Nathalie Gontard, Thomas Haertlé, Trine Husøy, Klaus-Dieter Jany, Catherine Leclercq, Jean-Claude Lhuguenot, Wim C. Mennes, Maria Rosaria Milana, Karla Pfaff, Kettil Svensson, Fidel Toldrá, Rosemary Waring, Detlef Wölfle.

SUMMARY

The European Food Safety Authority has been asked to provide scientific opinions on the safety of smoke flavouring Primary Products used or intended for use in or on foods. This opinion concerns a smoke flavouring Primary Product, named SEF7525.

The source material for the Primary Product is tar produced from a mixture of 35 % red oak (*Quercus rubra*), 35 % white oak (*Quercus alba*), 10 % maple (*Acer saccharum*), 10 % beech (*Fagus grandifolia*) and 10 % hickory (*Carya ovata*). A mixture of the tar with water and sodium hydroxide is subjected to two consecutive extractions with organic solvent at different pH-values. The extracts are washed with water, the solvent is removed by distillation and the resulting concentrate is filtrated with active charcoal. The final Primary Product is obtained by combining the two extracts at a defined ratio.

The Primary Product SEF7525 is solvent-free. The water-free fraction amounts to 99.1 wt. %. The amount of the volatile fraction determined by GC was 58.7 wt. % of the Primary Product. 55.8 wt.

[1] For citation purposes: Scientific Opinion of the Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF) on a request from the Commission on Safety of smoke flavour Primary Product – Scansmoke SEF7525. *The EFSA Journal* (2009) 1224, 1-24

% (corresponding to 95 % of the volatile fraction) were identified which is in compliance with Commission Regulation (EC) 627/2006. The mass of identified constituents (55.8 wt. %) corresponds to 56 % of the water-free mass. This is in compliance with Commission Regulation (EC) 627/2006 (EC, 2006).

Except for benzo[*j*]fluoranthene, the concentrations of the polycyclic aromatic hydrocarbons (PAHs) listed in Annex 2 of the EFSA Guidance document (EFSA, 2005) have been provided. In addition, the contents of fluorene, phenanthrene, anthracene, fluoranthene and pyrene have been determined. The levels of benzo[*a*]pyrene and benzo[*a*]anthracene are below their respective limits of 10 and 20 μg/kg given in Regulation (EC) No. 2065/2003 (EC, 2003). Although the concentration of benzo[*j*]fluoranthene, one of the PAHs known to be carcinogenic, was not provided, the Panel concluded that based on the reported levels of other carcinogenic PAHs, benzo[*j*]fluoranthene levels would be expected to be similarly low.

The Panel considered the data provided on the batch-to-batch variability and on the stability of the Primary Product as sufficient.

The genotoxic potential of the Scansmoke SEF7525 was tested in three *in vitro* studies (a bacterial reverse mutation test, a mouse lymphoma gene mutation assay and a chromosome aberration test) and two *in vivo* genotoxicity assays (a mouse bone marrow micronucleus assay and a rat liver unscheduled DNA synthesis assay). These studies were performed according to current OECD guidelines and in compliance with GLP, respectively. The Primary Product did not induce gene mutations in the bacterial assay and it did not induce chromosomal aberrations in Chinese Hamster Ovary cells *in vitro*. Statistically significant and dose-related increases in the mutant frequency were observed in mouse lymphoma cells in the MLTK assay both in the absence and presence of metabolic activation with relatively more large than small colonies being formed. However, no induction of genotoxicity was observed in the two *in vivo* studies.

Overall, it is concluded that Scansmoke SEF7525 is genotoxic *in vitro* in the mouse lymphoma assay, whereas two *in vivo* genotoxicity tests are negative and sufficient to eliminate the concerns over the *in vitro* genotoxicity.

The Primary Product was investigated in a 90-day study in rats performed according to the current OECD guideline 408 (1998) and in compliance with GLP. Scansmoke SEF7525 was administered in the diet at concentrations of 1000, 3000 and 9000 mg/kg resulting in 67, 211 and 650 mg/kg bw/day in males and 96, 270 and 823 mg/kg bw/day in females. The Panel considered the mid dose of 3000 mg/kg diet (equal to 211 and 270 mg/kg bw/day in males and females, respectively) as No-Observed-Adverse-Effect-Level (NOAEL) based on a statistically significant decrement in body weight gain of more than 10 % at the 9000 mg/kg dose in males and females. The lower NOAEL from these two figures (rounded to 210 mg/kg bw/day) was taken for the calculation of the margin of safety.

The applicant provided two data sets for use and use levels, one submitted originally in 2005, and the second in April 2009 after consulting with the users and seeking more detailed information. For transparency reasons both the initially provided data from 2005 and the updated data from 2009 were considered.

In order to estimate dietary exposure to the Primary Product SEF7525, the CEF Panel used two different methodologies, developed by the Panel specifically for smoke flavourings. Dietary exposure estimates were calculated by assuming that the Primary Product is present at the normal or upper use levels provided by the applicant for the 18 food categories as outlined in Commission Regulation 1565/2000 (EC, 2000).

Considering the initial data provided on use levels in 2005 dietary exposure from all sources ranges from 5.2 to 6.3 mg/kg bw/day, when assuming that the Primary Product is present at the upper use levels. When normal use levels are considered, dietary exposures range from 0.8 to 2.5 mg/kg bw/day.

Considering the updated information on use levels from 24 April 2009 dietary exposure from all sources ranges from 0.2 to 0.6 mg/kg bw/day, when assuming that the Primary Product is present at the upper use levels and from < 0.1 to 0.1 mg/kg bw/day when normal use levels are considered.

The impact on exposure of using the Primary Product only in traditionally smoked food products was also assessed.

Considering the initial data on use levels provided in 2005 the highest exposure estimates, resulting from the SMK-EPIC model, were 0.4 and 3.9 mg/kg bw/day when using normal and upper use levels, respectively. With the SMK-TAMDI model these figures were 0.3 and 2.5 mg/kg bw/day, respectively.

Considering the updated information on use levels from 24 April 2009 the highest exposure estimates, resulting from the SMK-EPIC and the SMK-TAMDI model, were < 0.1 and 0.1 mg/kg bw/day when using normal and upper use levels, respectively.

Since the data on use levels originally provided in June 2005 have been updated in 2009 and according to the applicant, these reflecting better the actual uses und use levels, the Panel drew its conclusions based on the margins of safety calculated with these recent data.

Based on the intake data calculated with the new data provided by the applicant on 24 April 2009 the margins of safety for total dietary exposure (traditionally and non-traditionally smoked food) as compared to the NOAEL of 210 mg/kg bw/day derived from the 90-day toxicity study in rats amount to 350 and 1050 for the intake estimates based on the upper use levels and to at least 2100 when normal use levels are considered.

When dietary exposure estimates are based on use in only traditionally smoked foods the margins of safety using the two smoke flavour exposure estimates, SMK-EPIC and SMK-TAMDI, respectively would amount to 2100 based on the upper use levels and to more than 2100 when normal use levels are considered.

The Panel noted that the margin of safety of at least 350 is based on a conservative exposure estimate. Therefore, even though i) these margins of safety are based on a 90-day toxicity study only, ii) data on reproduction and developmental toxicity are absent and iii) long term studies are absent, the Panel concluded that the uses and use levels specified are not of safety concern.

Key words: Smoke flavouring, Primary Product, Scansmoke SEF7525.

TABLE OF CONTENTS

BACKGROUND

Smoking is a process traditionally applied to certain perishable foods such as fish and meat. It was originally used for preservation purposes. In addition the process results in sensory changes (colour and flavour) which impart characteristic properties to smoked foods. With the development of other methods the preservative function of smoking decreased in importance over time and the sensory aspects prevailed. Nowadays, liquid smoke flavourings are added to various foods to replace the smoking process or to impart smoke flavour to foods, which are not traditionally smoked.

Smoke flavourings are produced by controlled thermal degradation of wood in a limited supply of oxygen (pyrolysis), subsequent condensation of the vapours and fractionation of the resulting liquid products. The Primary Products (primary smoke condensates and primary tar fractions) may be further processed to produce smoke flavourings applied in or on foods.

The Regulation (EC) No 2065/2003 of the European Parliament and the Council (EC, 2003) established Community procedures for the safety assessment and the authorisation of smoke flavouring Primary Products intended for use in or on foods. As stated herein the use of a Primary Product in or on foods shall only be authorised if it is sufficiently demonstrated that it does not present risks to human health. A list of Primary Products authorised to the exclusion of all others in the Community for use as such in or on food and/or for the production of derived smoke flavourings shall therefore be established after the European Food Safety Authority (EFSA) has issued an opinion on each Primary Product.

The Guidance on submission of a dossier on a smoke flavouring Primary Product for evaluation by EFSA (EFSA, 2005) lays down the administrative, technical and toxicological data required.

TERMS OF REFERENCE

The EFSA is required by Article 8 of Regulation (EC) No. 2065/2003 of the European Parliament and of the Council on smoke flavourings used or intended for use in or on foods to carry out risk assessments and deliver a scientific opinion on the safety of Primary Products.

ACKNOWLEDGEMENTS

The European Food Safety Authority wishes to thank the members of the Working Group for the preparation of this opinion: D. Arcella, A. Carere, K.-H. Engel, D.M. Gott, J. Gry, R. Gürtler, D. Meier[2], I. Pratt, I.M.C.M.Rietjens[3], R. Simon and R. Walker.

[2] Dietrich Meier declared an interest because his Institute is doing analysis as contract work for Brøste and he is doing information management for Brøste. This was considered as a conflict of interest and he was excluded from the discussion in the Working Group on the smoke flavouring Primary Product Scansmoke SEF7525.

[3] Ivonne Rietjens declared that she is advising FEMA on flavourings but that she has never been involved in smoke flavourings evaluations there. According to EFSA Policy on DoI, this activity does not represent a conflict of interest.

ASSESSMENT

The following evaluation only applies to the Primary Product Scansmoke SEF7525 manufactured strictly in conformity with the specified process and meeting the chemical specifications described in this opinion.

In accordance with the guidance document (EFSA, 2005), data on the manufacturing process, the composition, intended use levels and toxicological tests have been submitted. The latter include a 90-day oral subchronic toxicity study and three *in vitro* genotoxicity tests. Two *in vivo* genotoxicity tests have also been provided.

1. Information on existing authorisations and evaluations

According to the applicant, two intermediate products obtained by extraction of the tar with organic solvent at different pH-values have been accepted for use by the Danish Veterinary and Food Administration in solid foods including cereals in amounts up to 0.02 g/kg (Scansmoke Extract Flavour I) and 0.06 g/kg (Scansmoke Extract Flavour II).

2. Technical data

2.1. Manufacturing process

2.1.1. Source materials for the Primary Product

The source material for the Primary Product is tar produced from a mixture of 35 % red oak (*Quercus rubra*), 35 % white oak (*Quercus alba*), 10 % maple (*Acer saccharum*), 10 % beech (*Fagus grandifolia*) and 10 % hickory (*Carya ovata*).

According to the applicant, the wood used in the production is not treated with any chemicals approximately 7 months before the manufacturing process begins.

2.1.2. Method of manufacture of the Primary Product

The Primary Product is a purified tar fraction which is produced as follows: A mixture of the tar with water and sodium hydroxide is subjected to two consecutive extractions with organic solvent at different pH-values. The extracts are washed with water, the solvent is removed by distillation and the resulting concentrate is filtrated with active charcoal. The final Primary Product is obtained by combining the two extracts at a defined ratio.

2.2. Identity of the Primary Product

2.2.1. Trade names of the Primary Product

The trade name of the Primary Product is Scansmoke SEF7525.

2.2.2. Physical state of the Primary Product

Based on the analysis of five batches, an average viscosity of 211.7 mm^2/s (standard deviation: 67.3) and a density of 1.1 g/mL have been reported for the Primary Product.

2.3. Chemical composition of the Primary Product

2.3.1. Overall characterisation

The overall characterisation of the Primary Product is as follows:

2.3.1.1. Solvent-free fraction

The Primary Product Scansmoke SEF7525 is solvent-free. In the batch used for the toxicological studies (12236) a water content of 0.9 wt. % was determined by Karl-Fischer titration (thus, the water-free fraction amounts to 99.1 wt. %).

2.3.1.2. Volatile fraction

The Primary Product was analysed by capillary gas chromatography (GC). Mass spectrometry (MS) was used for identification and flame ionisation detection (FID) for quantification. The amount of the volatile fraction determined by GC was 58.7 wt. % of the Primary Product. 55.8 wt. % (corresponding to 95 % of the volatile fraction) were identified which is in compliance with Commission Regulation (EC) 627/2006 (EC, 2006).

2.3.1.3. Unidentified constituents

The fraction of unidentified mass has been estimated as the water-free mass minus the mass of the identified volatile compounds: 99.1 wt. % - 55.8 wt. % = 43.3 wt. %. The mass of identified constituents (55.8 wt. %) corresponds to 56 % of the water-free mass (Figure 2). This is in compliance with Commission Regulation (EC) 627/2006 (EC, 2006).

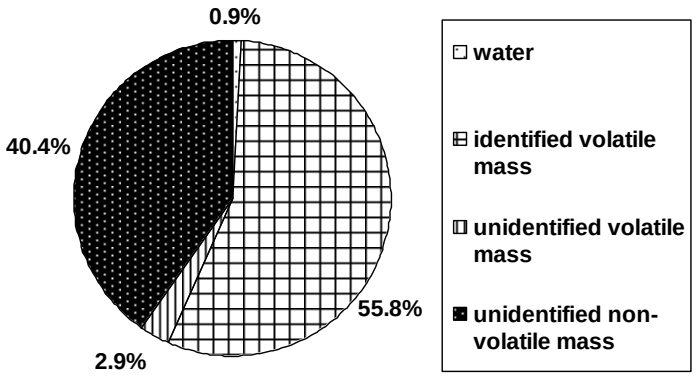

Figure 1. **Overall composition of Scansmoke SEF7525 (wt. % of Primary Product)**

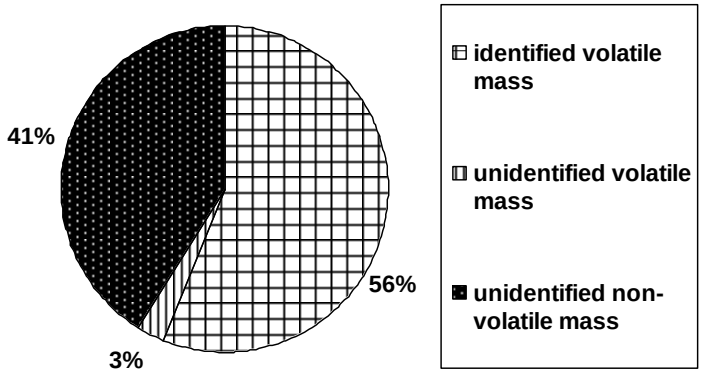

Figure 2. **Composition (%) of the solvent-free fraction of Scansmoke SEF7525**

2.3.2. Chemical description of the Primary Product

The Primary Product has been characterised by the parameters listed in Table 1.

Table 1**. Description of chemical parameters of the Primary Product Scansmoke SEF7525**

batch	12652-1	13193-1	12752-1	12867-1	12945-1
Phenols [wt.%]	8.1	11.4	10.0	9.9	9.1
Carbonyls [wt.%]	1.8	1.3	2.9	1.4	2.1
Acids [meq./g]	0.168	0.154	0.110	0.097	0.202
Water [wt. %]	0.32	0.45	0.84	0.55	0.52
pH	1.1	2.4	1.4	1.9	1.6
Mercury [mg/kg]	<0.01	<0.01	<0.01	<0.01	<0.01
Lead [mg/kg]	<0.1	<0.1	<0.1	<0.1	<0.1
Arsenic [mg/kg]	0.35	<0.1	0.14	0.11	0.10
Cadmium [mg/kg]	<0.025	<0.025	<0.025	<0.025	<0.025

2.3.3. Identification and quantification of the Primary Product constituents

2.3.3.1. Principal constituents

Table 2 shows the 20 principal constituents (expressed as wt. % of the dry matter) determined by GC and GC/MS in the Primary Product.

Table 2. **Principal constituents determined by GC analysis of batch 12236 of the Primary Product Scansmoke SEF7525**

	(wt.%)
Syringol	19.6
4-Methylsyringol	6.9
4-Propenylsyringol (trans)	3.6
4-Ethylsyringol	2.8

4-Methylguaiacol	2.3
4-Allylsyringol	2.0
4-Ethylguaiacol	1.9
4-Propylsyringol	1.5
Guaiacol	1.4
2,4-Dimethylphenol	1.4
Eugenol	1.2
Isoeugenol (trans)	1.2
4-Propenylsyringol (cis)	1.1
o-Cresol	1.1
Phenol	0.9
p-Cresol	0.9
4-Propylguaiacol	0.8
Isoeugenol (cis)	0.6
m-Cresol	0.6
3-Ethyl phenol	0.6

In total 60 compounds have been determined by GC. They amount to 59.2 wt. % of the dry matter, corresponding to 58.7 wt. % of the Primary Product.

2.3.3.2. Contents of Polycyclic Aromatic Hydrocarbons (PAHs)

Except for benzo[*j*]fluoranthene, the concentrations of the polycyclic aromatic hydrocarbons (PAHs) known to be carcinogenic and/or genotoxic, listed in Annex 2 of the EFSA Guidance document (EFSA, 2005) have been provided. In addition, the contents of fluorene, phenanthrene, anthracene, fluoranthene and pyrene have been determined (in contrast to the others, these five are not considered to be carcinogenic and/or genotoxic). The analyses were performed by an external accredited laboratory; the method used was equivalent to the method developed by the Joint Research Center of the European Commission (Simon *et al.*, 2006a and b) and fulfilled the performance criteria of Commission Regulation (EC) No 627/2006 (EC, 2006), except for the analyte benzo[*j*]fluoranthene. The concentrations of the 19 PAHs determined in the batch 12236 of the Primary Product are listed in Table 3. The levels of benzo[*a*]pyrene and benzo[*a*]anthracene are below their respective limits of 10 and 20 μg/kg given in Regulation (EC) No. 2065/2003 (EC, 2003).

Table 3. **Concentration of PAHs in the Primary Product Scansmoke SEF7525 (batch 12236)**

PAH	**Concentration (μg/kg)**
Chrysene	12
Benzo[*a*]anthracene	12
5-Methylchrysene	< 0.5
Cyclopenta[*cd*]pyrene	16
Benzo[*b*]fluoranthene	3.3

Benzo[*k*]fluoranthene	2.3
Benzo[*a*]pyrene	2.5
Indeno[*1,2,3-cd*]pyrene	0.5
Dibenzo[*a,h*]anthracene	< 0.5
Benzo[*ghi*]perylene	0.7
Dibenzo[*a,e*]pyrene	< 1
Dibenzo[*a,h*]pyrene	< 1
Dibenzo[*a,i*]pyrene	< 1
Dibenzo[*a,l*]pyrene	< 1
Fluorene	9600
Phenanthrene	2900
Anthracene	830
Fluoranthrene	230
Pyrene	270

2.3.4. Batch-to-batch variability

The applicant demonstrated the batch-to-batch variability of the Primary Product as follows:

(a) Variability of the parameters listed in Table 1 for five batches.
(b) Variability of 53 individual components determined by GC-analysis including the principal constituents listed in Table 2 in five batches. The average relative standard deviation was 21 %. For major constituents such as syringol (average content 18.9 wt. % of the dry matter) and 4-methyl syringol (7.7 wt. %) relative standard deviations of 17 % and 10 %, respectively, were determined. For minor constituents higher relative standard deviations up to 167 % (dimethyl-2-cyclopentene-1-one, 0.01 wt. %) were observed.
(c) Variability of the PAHs listed in five batches (Table 4).

Table 4. **Batch-to-batch variability of PAHs in the Primary Product SEF7525**

PAH	**Batch 12458 [µg/kg]**	**Batch 12588 [µg/kg]**	**Batch 12518 [µg/kg]**	**Batch 11389 [µg/kg]**	**Batch 10810 [µg/kg]**
Chrysene	8.4	3.4	2.9	17	7.4
Benzo[*a*]anthracene	9.8	3.6	3.6	17	6.5
5-Methylchrysene	<0.5	<0.5	<0.5	<0.5	<0.5
Cyclopenta[*cd*]pyrene	8.0	3.0	3.4	21	4.6
Benzo[*b*]fluoranthene	3.6	1.7	1.4	4.1	1.3
Benzo[*k*]fluoranthene	3.2	1.2	1.2	3.3	0.6
Benzo[*a*]pyrene	5.1	2.2	2.2	3.2	0.6
Indeno[*1,2,3-cd*]pyrene	1.3	0.6	0.5	0.7	<0.5
Dibenzo[*a,h*]anthracene	<0.5	<0.5	<0.5	<0.5	<0.5
Benzo[*ghi*]perylene	1.3	0.7	0.5	1.2	<0.5
Dibenzo[*a,e*]pyrene	<1	<1	<1	<1	<1
Dibenzo[*a,h*]pyrene	<1	<1	<1	<1	<1
Dibenzo[*a,i*]pyrene	<1	<1	<1	<1	<1
Dibenzo[*a,l*]pyrene	<1	<1	<1	<1	<1

PAH	Batch 12458 [µg/kg]	Batch 12588 [µg/kg]	Batch 12518 [µg/kg]	Batch 11389 [µg/kg]	Batch 10810 [µg/kg]
Fluorene	2200	2600	3000	5600	3100
Phenanthrene	1700	460	360	3900	1200
Anthracene	530	120	65	1200	280
Fluroanthene	92	39	37	410	96
Pyrene	110	46	42	460	120

The Panel considered the data provided on the batch-to-batch-variability of the Primary Product as sufficient.

2.3.5 Stability

Batch 12236 of the Primary Product was subjected to GC analysis four times from August 2004 to April 2005 and the variability of the contents of 59 constituents, including those listed in Table 2, was determined. The average relative standard deviation was 45 %. Individual values ranged from 4 and 3 %, respectively, for the major constituents syringol (average content 20.3 wt. % of dry matter) and 4-methyl syringol (7.0 wt. %) to 222 % for minor constituents, such as 4-propyl phenol (0.02 wt. %).

2.3.6 Specifications

The specifications of the Primary Product as provided by the applicant are given in Table 5.

Table 5. **Specifications of the Primary Product SEF7525**

Water	0.3 – 0.9 wt. %
Acid	0.09 – 0.25 meq/g
Carbonyls	1.2 – 3.0 wt. %
Phenols	8 -12 wt. %
Benz(a)anthracene	< 20 µg/kg
Benzo(a)pyrene	< 10 µg/kg
Mercury	< 0.01 mg/kg
Lead	< 0.1 mg/kg
Arsenic	< 1 mg/kg
Cadmium	< 0.025 mg/kg

3. Proposed uses

Normal and upper use levels as described originally by the applicant in June 2005 for the Primary Product in each of the 18 food categories as outlined in Commission Regulation (EC) No 1565/2000 (EC, 2000) are reported in Table 6a.

Table 6a. **Normal and upper use levels of the Primary Product in food categories as outlined in Commission Regulation (EC) No 1565/2000 (Data provided in June 2005)**

Food categories	Use levels (g/kg)

		Normal	Upper
1	Dairy products, excluding products of category 2	0.5*	0.5
2	Fats and oils, and fat emulsions (type water-in-oil)	0	0
3	Edible ices, including sherbet and sorbet	0	0
4.1	Processed fruits	0	0
4.2	Processed vegetables (incl. mushrooms & fungi, roots & tubers, pulses and legumes), and nuts & seeds	0.5*	0.5
5	Confectionery	0.5*	0.5
6	Cereals and cereal products, incl. flours & starches from roots & tubers, pulses & legumes, excluding bakery	0	0
7	Bakery wares	0.5*	0.5
8	Meat and meat products, including poultry and game	0.05§	1.5
9	Fish and fish products, including molluscs, crustaceans and echinoderms	0.05§	1.5
10	Eggs and egg products	0	0
11	Sweeteners, including honey	0	0
12	Salts, spices, soups, sauces, salads, protein products etc.	1*	1
13	Foodstuffs intended for particular nutritional uses	0	0
14.1	Non-alcoholic ("soft") beverages, excl. dairy products	0	0
14.2	Alcoholic beverages, incl. alcohol-free and low-alcoholic counterparts	0.1*	0.1
15	Ready-to-eat savouries	0.05§	1.5
16	Composite foods (e.g. casseroles, meat pies, mincemeat) - foods that could not be placed in categories 1 – 15	0.003	0.5

* The Upper use level is here used because the applicant declared to be unable to provide a Normal use level.

§ The applicant provided a range for the Normal use level instead of a single value, the highest figure in the range is here used.

After consulting with the users and seeking more detailed information the applicant provided updated uses and use levels for the different food categories on 24 April 2009. According to the applicant these reflect better the actual uses and use levels.

Table 6b. **Normal and upper use levels of the Primary Product in food categories as outlined in Commission Regulation (EC) No 1565/2000 (Data provided on 24 April 2009)**

Food categories		Use levels (g/kg)	
		Normal	Upper
1	Dairy products, excluding products of category 2	0.001	0.05
2	Fats and oils, and fat emulsions (type water-in-oil)	0	0
3	Edible ices, including sherbet and sorbet	0	0
4.1	Processed fruits	0	0
4.2	Processed vegetables (incl. mushrooms & fungi, roots & tubers, pulses and legumes), and nuts & seeds	0	0
5	Confectionery	0	0
6	Cereals and cereal products, incl. flours & starches from roots & tubers, pulses & legumes, excluding bakery	0	0
7	Bakery wares	0	0

8	Meat and meat products, including poultry and game	0.01	0.05
9	Fish and fish products, including molluscs, crustaceans and echinoderms	0.01	0.05
10	Eggs and egg products	0	0
11	Sweeteners, including honey	0	0
12	Salts, spices, soups, sauces, salads, protein products etc.	$\leq 0.02^{\S}$	$\leq 0.173^{\wedge}$
13	Foodstuffs intended for particular nutritional uses	0	0
14.1	Non-alcoholic ("soft") beverages, excl. dairy products	0	0
14.2	Alcoholic beverages, incl. alcohol-free and low-alcoholic counterparts	0.005	0.05
15	Ready-to-eat savouries	0.01	0.05
16	Composite foods (e.g. casseroles, meat pies, mincemeat) - foods that could not be placed in categories 1 – 15	0.003	0.05

§ The following normal use levels for the breakdown of the food category 12 "Salts, spices, soups, sauces, salads, protein products etc." were provided by the applicant and used to assess the exposure: 0.02 g/kg for Herbs, spices, seasonings and condiments (12.2), 0.015 g/kg for Soups and broths (12.5) and Sauces and like products (12.6) and 0 g/kg for the remaining subgroups

^ The following upper use levels for the breakdown of the food category 12 "Salts, spices, soups, sauces, salads, protein products etc." were provided by the applicant and used to assess the exposure: 0.173 g/kg for Herbs, spices, seasonings and condiments (12.2), 0.05 g/kg for Soups and broths (12.5) and Sauces and like products (12.6) and 0 g/kg for the remaining subgroups

4. Dietary exposure assessment

In order to estimate dietary exposure to the Primary Product Scansmoke SEF7525, the CEF Panel used two different methodologies developed by the Panel specifically for smoke flavourings (EFSA, 2009).

The Smoke Theoretical Added Maximum Daily Intake (SMK-TAMDI) is an adaptation of the Theoretical Added Maximum Daily Intake (TAMDI) method used by the Scientific Committee on Food (SCF) to assess exposure to single flavourings (Scientific Committee for Food, 1995). As for the TAMDI, the SMK-TAMDI also assumes that the hypothetical consumer will daily consume a fixed amount of flavoured solid foods and liquids. However, in the SMK-TAMDI a single group "Beverages" is used for liquids whereas solid foods are divided in "traditionally smoked solid foods" and "other solid foods not traditionally smoked".

The European Prospective Investigation into Cancer and Nutrition (EPIC) study is one of the few cases in which the consumption levels of "smoked meat" were assessed and published for different European countries (Linseisen *et al.*, 2006). The Panel used consumption data from the EPIC study to estimate the potential cumulative dietary exposure to smoke flavourings. The Smoke flavouring EPIC model (SMK-EPIC) is based on a number of assumptions, in particular it assumes that a hypothetical high consumer of smoked meat is also an average consumer of the other traditionally smoked foods and an occasional consumer of smoked foods or beverages from each of the other categories.

Dietary exposure estimates were calculated by assuming that the Primary Product is present at the normal or upper use levels provided by the applicant for the 18 food categories as outlined in Commission Regulation 1565/2000 (EC, 2000). When the normal use levels are used, the SMK-TAMDI can be considered as an adaptation of the modified TAMDI (mTAMDI), the method used by the AFC Panel (EFSA, 2004) to screen and prioritise flavouring substances.

Details of the methodologies are described in the dietary exposure document (EFSA, 2009).

The applicant provided two data sets for use levels, one submitted originally in 2005, and the second in April 2009. After consulting with the users and seeking more detailed information the applicant provided updated uses and use levels for the different food categories on 24 April 2009. According to the applicant these reflect better the actual uses und use levels. The Panel therefore used them in the safety evaluation.

Dietary exposure estimates calculated by means of the above mentioned methods are reported in Table 7a and 7b. For transparency reasons both the initially provided data from 2005 and the updated data from 2009 were considered.

Considering the initial data provided on use levels in 2005 dietary exposure from all sources ranges from 5.2 to 6.3 mg/kg bw/day, when assuming that the Primary Product is present at the upper use levels and from 0.8 to 2.5 mg/kg bw/day, when normal use levels are considered (Table 7a).

Considering the updated information on use levels from 24 April 2009 dietary exposure from all sources ranges from 0.2 to 0.6 mg/kg bw/day, when assuming that the Primary Product is present at the upper use levels and from < 0.1 to 0.1 mg/kg bw/day when normal use levels are considered (Table 7b).

The impact on exposure of using the Primary Product only in traditionally smoked food products was also assessed. Out of the above mentioned 18 food categories, "Dairy products, excluding products of category 2", "Meat and meat products, including poultry and game" and "Fish and fish products, including molluscs, crustaceans and echinoderms" were considered as "Traditionally smoked solid foods"

Considering the initial data on use levels provided in 2005 the highest exposure estimates, resulting from the SMK-EPIC model, were 0.4 and 3.9 mg/kg bw/day when using normal and upper use levels, respectively. With the SMK-TAMDI model these figures were 0.3 and 2.5 mg/kg bw/day, respectively (Table 7a).

Considering the updated information on use levels from 24 April 2009 the highest exposure estimates, resulting from the SMK-EPIC and the SMK-TAMDI model, were < 0.1 and 0.1 mg/kg bw/day when using normal and upper use levels, respectively (Table 7b).

Dietary exposure to the Primary Product was also estimated by the applicant using average food consumption data derived from the UK National Diet and Nutrition Survey (NDNS) in adults aged 16-64 (Henderson *et al.*, 2002). The dietary exposure calculated by the applicant resulted to be equal to 2.6 mg/kg bw/day. This estimate is obtained by assuming that only 25% of food consumed is flavoured with the Primary Product at the upper use levels.

Table 7a. **Summary of the dietary exposure estimates to the Primary Product (Data provided in June 2005)**

Methodologies		**Dietary exposure (mg/kg bw/day)**	
		Normal use levels	**Upper use levels**
SMK-TAMDI	Traditionally smoked food	0.3	2.5
	Other foods not traditionally smoked	1.7	3.3

	Beverages (alcoholic or non-alcoholic)	0.5	0.5
	Total dietary exposure	2.5	6.3
SMK-EPIC	Traditionally smoked food	0.4	3.9
	Other foods not traditionally smoked	0.4	1.3
	Beverages (alcoholic or non-alcoholic)	0.1	0.1
	Total dietary exposure	0.8	5.2
Applicant	Dietary exposure	-[a]	2.6

[a] Not provided

The new data provided by the applicant led to the following figures for dietary exposure.

Table 7b. **Summary of the dietary exposure estimates to the Primary Product (Data provide on 24 April 2009)**

Methodologies		**Dietary exposure (mg/kg bw/day)**	
		Normal use levels	**Upper use levels**
SMK-TAMDI	Traditionally smoked food	< 0.1	0.1
	Other foods not traditionally smoked	0.1	0.3
	Beverages (alcoholic or non-alcoholic)	< 0.1	0.3
	Total dietary exposure	0.1	0.6
SMK-EPIC	Traditionally smoked food	< 0.1	0.1
	Other foods not traditionally smoked	< 0.1	0.1
	Beverages (alcoholic or non-alcoholic)	< 0.1	< 0.1
	Total dietary exposure	< 0.1	0.2
Applicant	Dietary exposure	-	-

5. Toxicological data

5.1. Identity of the test material

The same batch (no. 12236) of Scansmoke SEF7525, that was tested chemically, was used for the subchronic toxicity study as well as for the genotoxicity studies except for the *in vivo* UDS assay in which batch 17410 was used.

5.2. Subchronic toxicity

A 90-day oral toxicity study of Scansmoke SEF7525 was performed in CRL (WI) BR rats. The study was conducted according to the current OECD guideline 408 (1998) and in compliance with GLP. The Primary Product was administered in the diet at concentrations of 1000, 3000 and 9000

mg/kg resulting in 67, 211 and 650 mg/kg bw/day in males and 96, 270 and 823 mg/kg bw/day in females.

No mortality occurred during the study. No effects attributable to the test item were detected by clinical observation. No eye alterations were found nor were any test item-related effects observed in clinical chemistry and urinalysis.

The body weight was measured weekly. The mean body weight was statistically significantly reduced compared to the control in males (between -7 and -14%) and in females (between -1 and -15%) of the 9000 mg/kg groups from day 7 and 14, respectively, up to the end of the study. In the 3000 mg/kg groups the body weight was only slightly reduced in males and females without statistical significance, respectively, and in the 1000 mg/kg groups only minor changes in the body weight were observed in males and females without statistical significance, respectively. The body weight gain up to the end of the study was depressed in the low, medium and high dose groups compared to controls in males (-4%, -14% and -30%, respectively,) and in the medium and high dose groups in females (+12%, -9% and -44%). However, the decrement in body weight gain was statistically significant ($p<0.01$) only in the high dose groups of males and females.

Haematological investigation revealed statistically significant alteration of some parameters in all treated groups. Most haematological changes observed were found only sporadically and were not dose-related. However, white blood cell counts (WBC) were statistically significantly increased at all dose levels ($p < 0.05$ at low and medium dose and $p < 0.001$ at high dose) of males. The effects were 46, 45 and 61 % above control in the low, medium and high dose group, respectively. The WBC were also increased in females (23, 33 and 6 % above control), but not statistically significantly. No test item-related effects were observed by macroscopic examination of organs nor were any substance-related effects detected in organs subjected to histopathologic examination.

Since statistically significant haematological effects were found in all dose groups in males, a No-Observed-Effect Level (NOEL) could not be derived from this study. However, since WBC were not statistically significantly changed in females and the effects observed in males were not accompanied by any histopathological or macroscopic changes, the Panel considered the haematological effects as being not adverse. The Panel considered that the only test item related alteration observed was significant body weight gain depression. As there was no change in food intake the body weight changes cannot be attributed to palatability problems. The Panel considered the mid dose of 3000 mg/kg diet (equal to 211 and 270 mg/kg bw/day in males and females, respectively) as No-Observed-Adverse-Effect-Level (NOEL) based on a statistically significant decrement in body weight gain of more than 10% at the high dose in males and females.

5.3. Genotoxicity

The genotoxic potential of the Scansmoke SEF7525 was tested in three *in vitro* and two *in vivo* genotoxicity assays. All genotoxicity studies were conducted according to current OECD guidelines and in compliance with GLP. This is in line with the requirements of Regulation (EC) 2065/2003 (EC, 2002) and of the EFSA Guidance document (EFSA, 2005).

The Primary Product did not induce gene mutations in a bacterial assay which was performed using *Salmonella typhimurium* strains TA98, TA100, TA1535 and TA1537 and the *Escherichia coli* strain WP2 uvrA. The experiments have been carried out in the presence and absence of a metabolic activation system prepared from enzyme-induced rat liver. Phenobarbitone and ß-naphthoflavone

were used for enzyme-induction. The assay was performed in accordance with OECD guideline 471 (1997).

The Primary Product induced statistically significant and dose-related increases in the mutant frequency in mouse lymphoma cells in the MLTK assay (performed in accordance with OECD guideline 476 (1997)) both in the absence (up to 3.3-fold at 0.04 mg/ml resulting in a Relative Survival of 25%) and presence of metabolic activation (up to 3.9-fold at 0.1 mg/ml resulting in a Relative Survival of 20%). The metabolic activation system was prepared from rat liver. It was not reported whether the liver enzymes have been induced, however, since the assay has been performed in the same laboratory as the bacterial assay it could be assumed that the metabolic activation system used was prepared from enzyme-induced rat liver likewise. Both large and small colonies were induced with large colonies predominating at all concentrations in the absence of a metabolising system and at all, except the highest, concentrations in the presence of S9. This suggests that the genotoxic effects observed may be due to both clastogenicity and the induction of gene mutations in this assay.

The Primary Product did not induce chromosomal aberrations *in vitro* in Chinese Hamster Ovary cells in an assay performed in accordance with OECD guideline 473 (1997). The experiments have been carried out in the presence and absence of a metabolic activation system. The type of S9-fraction was not reported, however, since the assay has been performed in the same laboratory as the bacterial assay it could be assumed that the metabolic activation system used was prepared from enzyme-induced rat liver likewise.

Based on the results of a cytotoxicity assay the cells were exposed to the Primary Product at 1, 15, and 30 µg/mL with and without S9 mix during 4 hours in a first experiment and at 1, 5 and 15 µg/mL without S9 mix for 20 hours and at 1, 5 and 30 µg/mL with S9 mix for 4 hours in a second experiment.

In experiment 1, chromosomal aberrations were induced at the highest concentration up to 2-fold compared to solvent control in the test without S9 and up to 2.4-fold with S9. No clear dose-response and no statistically significant differences between treated cells and solvent controls were observed. In experiment 2, chromosomal aberrations were induced up to 1.8-fold with and without S9 mix, but the result was not dose-related and not statistically significant. Although the mean aberration frequency of 6 % observed at 30 µg/mL in the presence of S9 mix in the first experiment was above the maximum value of the historical control data for the solvent control (4 %), this result was not reproducible in the second experiment where the mean percent aberrant cells was 3.5 % which was within the range of the historical control data obtained with the solvent control. Thus, the Panel agreed with the authors of the study report and considered the result negative.

In vivo, the Primary Product was tested in a mouse micronucleus assay performed in accordance with OECD guideline 474 (1997). It was administered orally by gavage to male and female mice at dose levels of 500, 1000 and 2000 mg/kg. The Primary Product induced a dose-related slight increase in the percentage of micronucleated polychromatic erythrocytes (MCPE) in bone marrow of male mice at 24 hours (up to 1.5-fold compared to control) which was, however, not statistically significant. At 24 hours in females, there was no statistically significant increase nor were the results dose-related. At 72 hours, the Primary Product induced a slight increase in females (1.4-fold) which was not clearly dose-related but statistically significant. However, in males there was only a marginal increase up to 1.2-fold at 72 hours which was not statistically significant. Statistically significant effects were only observed at the later sampling time (72 hours) and not consistent with the lack of

cytotoxicity indicated by the unchanged PCE/NCE ratio. Therefore the Panel concluded that this study is negative.

Scansmoke SEF7525 was also tested *in vivo* in an unscheduled DNA synthesis (UDS) assay which was performed in compliance with GLP. UDS was assessed in hepatocytes of Sprague Dawley rats following oral gavage administration of Scansmoke SEF7525 on two separate occations (the second dose being administered 14 hours after the first dose and 2 hours before perfusion). This study design deviated slightly from the OECD guideline 486 (1997) with respect to dosing and sampling. However, the protocol was considered acceptable. Scansmoke SEF7525 was administered at dosages of 500, 1000 and 2000 mg/kg bw. Under the conditions of this study, Scansmoke SEF7525 did not induce UDS *in vivo*.

In summary, Scansmoke SEF7525 is considered mutagenic *in vitro* in mouse lymphoma cells in the MLTK assay while *in vivo* negative results were obtained from a mouse micronucleus assay and an unscheduled DNA synthesis assay.

5.4. Other studies

No other studies on Scansmoke SEF7525 were provided by the applicant.

6. DISCUSSION

The applicant provided information on the identity, composition, batch-to-batch variability and stability of the Primary Product as requested in the EFSA guidance document. The Panel considered the data provided on the batch-to-batch variability and on the stability of the Primary Product as sufficient.

Except for benzo[*j*]fluoranthene, the concentrations of the polycyclic aromatic hydrocarbons (PAHs) listed in Annex 2 of the EFSA Guidance document (EFSA, 2005) have been provided. In addition, the contents of fluorene, phenanthrene, anthracene, fluoranthene and pyrene have been determined. The levels of benzo[*a*]pyrene and benzo[*a*]anthracene are below their respective limits of 10 and 20 μg/kg given in Regulation (EC) No. 2065/2003 (EC, 2003). Although the concentration of benzo[*j*]fluoranthene, one of the PAHs known to be carcinogenic, was not provided, the Panel concluded that based on the reported levels of other carcinogenic PAHs, benzo[*j*]fluoranthene levels would be expected to be similarly low.

The genotoxic potential of the Primary Product was investigated in three *in vitro* and two *in vivo* assays which were performed according to the current OECD guidelines and in compliance with GLP. Scansmoke SEF7525 did not induce gene mutations in bacteria. It induced statistically significant and dose-related increases in the mutant frequency in mouse lymphoma cells in the MLTK assay both in the absence and presence of metabolic activation. Both large and small colonies were induced at similar rates with and without metabolic activation (with large colonies predominating at all concentrations in the absence of a metabolising system and at all except the highest concentrations in the presence of S9). This suggests that the genotoxic effects observed may be due to both clastogenicity and the induction of gene mutations in this assay. The Primary Product Scansmoke

SEF7525 did not induce chromosomal aberrations in Chinese Hamster Ovary cells in *vitro* and gave negative results in the *in vivo* bone marrow micronucleus assay and the *in vivo* UDS assay.

Overall, it is concluded that Scansmoke SEF7525 is genotoxic *in vitro* in the mouse lymphoma assay, whereas two *in vivo* genotoxicity tests are negative and sufficient to eliminate the concerns over the *in vitro* genotoxicity.

The Primary Product was investigated in a 90-day study in CRL (WI) BR rats performed according to the current OECD guideline 408 (1998) and in compliance with GLP. Scansmoke SEF7525 was administered in the diet at concentrations of 1000, 3000 and 9000 mg/kg resulting in 67, 211 and 650 mg/kg bw/day in males and 96, 270 and 823 mg/kg bw/day in females. Haematological investigation revealed statistically significant alteration of some parameters in all treated groups. However, most haematological changes observed were found only sporadically and were not dose-related and white blood cell counts (WBC) were statistically significant increased in males but not in females and the effects observed in males were not accompanied by any histopathological or macroscopic changes. Therefore the Panel considered the haematological effects as being not adverse. The Panel considered that the only test item related alteration observed was significant body weight gain depression. As there was no change in food intake the body weight changes cannot be attributed to palatability problems. The Panel considered the mid dose of 3000 mg/kg diet (equal to 211 and 270 mg/kg bw/day in males and females, respectively) as NOAEL based on a statistically significant decrement in body weight gain of more than 10% at the high dose in males and females. The lower NOAEL from these two figures (rounded to 210 mg/kg bw/day) was taken for the calculation of the margin of safety (Table 8a and b).

The applicant provided two data sets for use levels, one submitted originally in 2005, and the second in April 2009. After consulting with the users and seeking more detailed information the applicant provided updated uses and use levels for the different food categories on 24 April 2009. According to the applicant these reflect better the actual uses und use levels. The Panel therefore used them in the safety evaluation.

In order to estimate dietary exposure to Scansmoke SEF7525, the CEF Panel used two different methodologies, developed by the Panel specifically for smoke flavourings. Dietary exposure estimates were calculated by assuming that the Primary Product Scansmoke SEF7525 is present at the normal or upper use levels provided by the applicant for the 18 food categories as outlined in Commission Regulation 1565/2000 (EC, 2000).

Considering the initial data provided on use levels in 2005 dietary exposure from all sources ranges from 5.2 to 6.3 mg/kg bw/day, when assuming that the Primary Product is present at the upper use levels. When normal use levels are considered, dietary exposures range from 0.8 to 2.5 mg/kg bw/day (Table 7a).

Considering the updated information on use levels from 24 April 2009 dietary exposure from all sources ranges from 0.2 to 0.6 mg/kg bw/day, when assuming that the Primary Product is present at the upper use levels and from < 0.1 to 0.1 mg/kg bw/day when normal use levels are considered (Table 7b).

The impact on exposure of using the Primary Product only in traditionally smoked food products was also assessed.

Considering the initial data on use levels provided in 2005 the highest exposure estimates, provided by the SMK-EPIC model, were 0.4 and 3.9 mg/kg bw/day when using normal and upper use levels, respectively. With the SMK-TAMDI model these figures were 0.3 and 2.5 mg/kg bw/day, respectively (Table 7a).

Considering the updated information on use levels from 24 April 2009 the highest exposure estimates, provided both by the SMK-EPIC and SMK-TAMDI model, were < 0.1 and 0.1 mg/kg bw/day when using normal and upper use levels, respectively (Table 7b).

Based on the intake data originally provided by the applicant in June 2005, the margins of safety for total dietary exposure (traditionally and non-traditionally smoked food) as compared to the NOAEL of 210 mg/kg bw/day derived from the 90-day toxicity study in rats amount to 33 and 40 for the intake estimates based on the upper use levels and to 84 and 263, when normal use levels are considered (Table 8a).

When dietary exposure estimates are based on use in only traditionally smoked foods the margins of safety using the two smoke flavour exposure estimates, SMK-EPIC and SMK-TAMDI, respectively would amount to 54 and 84 based on the upper use levels and to 525 and 700 when normal use levels are considered (Table 8a).

Table 8a. **Margins of safety based on the intake estimated with the data provided originally in June 2005**

	Use level	**Dietary exposure* (mg/kg bw/day)**	**NOAEL (mg/kg bw/day)**	**Margin of safety***
Total dietary exposure	Normal	0.8 / 2.5	210	84 / 263
	Upper	5.2 / 6.3	210	33 / 40
Traditionally smoked food	Normal	0.4 / 0.3	210	700 / 525
	Upper	3.9 / 2.5	210	84 / 54

* The first figure refers to dietary exposure estimated on the basis of the Smoke-EPIC model; the second one refers to dietary exposure estimated on the basis of the Smoke-TAMDI model.

Based on the intake data calculated with the new data provided by the applicant on 24 April 2009 for total dietary exposure (traditionally and non-traditionally smoked food), the margin of safety as compared to the NOAEL of 210 mg/kg bw/day derived from the 90-day toxicity study in rats amounts to 350 and 1050 for the intake estimates based on the upper use levels and to at least 2100, when normal use levels are considered (Table 8b).

When dietary exposure estimates are based on use in only traditionally smoked foods, the margins of safety using the two smoke flavour exposure estimates, SMK-EPIC and SMK-TAMDI, respectively would amount to 2100 based on the upper use levels and to more than 2100 when normal use levels are considered (Table 8b).

Table 8b. Margins of safety based on the intake estimated with the new data provided in April 2009

	Use level	Dietary exposure* (mg/kg bw/day)	NOAEL (mg/kg bw/day)	Margin of safety*
Total dietary exposure	Normal	< 0.1 / 0.1	210	≥ 2100
	Upper	0.2 / 0.6	210	350 / 1050
Traditionally smoked food	Normal	< 0.1[&]	210	> 2100[&]
	Upper	0.1[&]	210	2100[&]

* The first figure refers to dietary exposure estimated on the basis of the Smoke-EPIC model; the second one refers to dietary exposure estimated on the basis of the Smoke-TAMDI model..

[&] Dietary exposure level is the same from the two methods.

The Panel did not anticipate that smoke flavourings would be used in food specifically designed for infants (0-12 months) and children (12-36 months). Therefore the safety of use of Primary Product Scansmoke SEF7525 in such products was not assessed.

CONCLUSIONS AND RECOMMENDATIONS

The Panel considered the technical and analytical data provided acceptable to characterise the Primary Product and to demonstrate its batch-to-batch variability and stability.

Scansmoke SEF7525 is genotoxic *in vitro* in the mouse lymphoma assay, whereas two *in vivo* genotoxicity tests are negative and sufficient to eliminate the concerns over the *in vitro* genotoxicity.

The NOAEL derived from a 90-day study in rats is 210 mg/kg bw/day, the mid dose level tested.

The data on use levels originally provided in June 2005 have been updated by the applicant in April 2009. The Panel drew its conclusions based on the margins of safety calculated with these recent data as according to the applicant they reflect better the actual uses and use levels.

Based on the intake data calculated with the new data provided by the applicant on 24 April 2009 the margin of safety for total dietary exposure (traditionally and non-traditionally smoked food) as compared to the NOAEL of 210 mg/kg bw/day derived from the 90-day toxicity study in rats amounts to 350 and 1050 for the intake estimates based on the upper use levels and to at least 2100 when normal use levels are considered.

When dietary exposure estimates are based on use in only traditionally smoked foods, the margins of safety using the two smoke flavour exposure estimates, SMK-EPIC and SMK-TAMDI, respectively would amount to 2100 based on the upper use levels and to more than 2100 when normal use levels are considered.

The Panel noted that the margin of safety of at least 350 is based on a conservative exposure estimate. Therefore, even though i) these margins of safety are based on a 90-day toxicity study only, ii) data on reproduction and developmental toxicity are absent and iii) long term studies are absent, the Panel concluded that the uses and use levels specified are not of safety concern.

DOCUMENTATION PROVIDED TO EFSA

1. Dossier submitted by Brøste A/S.

2. Response from Brøste A/S to request for supplementary information.

REFERENCES

EC, 2000. Commission Regulation (EC) No 1565/2000 of 18 July 2000 laying down the measures necessary for the adoption of an evaluation programme in application of Regulation (EC) No 2232/96 of the European Parliament and of the Council. Official Journal of the European Union L 180 p. 8-16.

http://eur-lex.europa.eu/LexUriServ/site/en/oj/2000/l_180/l_18020000719en00080016.pdf Visited on 10 April 2007

EC, 2003. Commission Regulation No 2065/2003 of the European Parliament and of the Council of 10 November 2003 on smoke flavourings used or intended for use in or on foods, Official Journal of the European Union L 309 p.1-8.
http://eur-lex.europa.eu/LexUriServ/site/en/oj/2003/l_309/l_30920031126en00010008.pdf visited on 8 September 2006

EC, 2006. Commission Regulation No 627/2006 of 21 April 2006 implementing Regulation (EC) No 2065/2003 of the European Parliament and of the Council as regards quality criteria for validated analytical methods for sampling, identification and characterisation of primary smoke products, Official Journal of the European Union L 109, p.3-6.
http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2006:109:0003:0006:EN:PDF

EFSA, 2004. 7th plenary meeting of the scientific Panel on food additives, flavourings, processing aids and materials in contact with food (AFC). Brussels, 12-13 July 2004.

EFSA, 2005. Guidance from the Scientific Panel on Food Additives flavourings, processing aids and materials in Contact with Food. Guidance on submission of a dossier on a smoke flavouring primary product for evaluation by EFSA, Adopted on 7 October 2004; Revised on 27 April 2005.

http://www.efsa.europa.eu/etc/medialib/efsa/science/afc/afc_guidance/680.Par.0001.File.dat/guidancedocument1.pdf visited on 8 September 2006

EFSA, 2009. EFSA opinion on Dietary Exposure Assessment methods for Smoke Flavouring Primary Products. Adopted on 29 January 2009.

Henderson L, Gregory J and Swan G., 2002. The National Diet & Nutrition Survey: adults aged 19 to 64 years. Types and quantities of foods consumed. Volume 1. ISBN 0 11 621566 6.

Linseisen, J., Rohrmann, S., Norat, T., Gonzalez, C.A., Dorronsoro Iraeta, M., Morote Gomez P., Chirlaque, M.D., Pozo, B.G., Ardanaz, E., Mattisson, I., Pettersson, U., Palmqvist, R., Van Guelpen, B., Bingham, S.A., McTaggart, A., Spencer, E.A., Overvad, K., Tjonneland, A., Stripp, C., Clavel-Chapelon, F., Kesse, E., Boeing, H., Klipstein-Grobusch K., Trichopoulou, A., Vasilopoulou, E., Bellos, G., Pala, V., Masala, G., Tumino, R., Sacerdote, C., Del Pezzo, M., Bueno-de-Mesquita, H.B., Ocke, M.C., Peeters, P.H., Engeset, D., Skeie, G., Slimani, N., Riboli, E., 2006. Dietary intake of different types and characteristics of processed meat which might be associated with cancer risk--results from the 24-hour diet recalls in the European Prospective Investigation into Cancer and Nutrition (EPIC). *Public Health Nutr*. 9(4):449-64.

Scientific Committee for Food, 1995. First annual report on chemically defined flavouring substances. May 1995, 2nd draft prepared by the SCF Working Group on Flavoruing Substances (Submitted by the SCF Secretariat, 17 May 1995). CS/FLAV/FL/140-Rev.2. Annex 6 to Document III/5611/95, European Commission, Directorate-General III, Industry.

Simon, R., Palme, S., and Anklam, E., 2006a. Single-laboratory validation of a gas chromatography-mass spectrometry method for quantitation of 15 European priority polycyclic aromatic hydrocarbons in spiked smoke flavourings. Journal of Chromatography A 1103, 307-313.

Simon, R., Palme, S., Anklam, E., Delaire, L., Hanot, V., M.W. Holzer, H., Jira, W., Levasseur, C., Monteau, F., Obiedzinski, M.W., Ojala, M., Peschke, D., Ramakrishnan, S., Ranta, C., Ruthenschror, A., Schirmacher, M., Seidel, A., Sopelana, P., Wolz, G. and Wright, C., 2006b. Method validation for determination of the 15 European-priority polycyclic aromatic hydrocarbons in primary smoke condensates by gas chromatography/mass spectrometry: Interlaboratory study. Journal of AOAC International 89:772-781.

ABBREVIATIONS

AFC	Scientific Panel on Additives, Flavourings, Processing aids and Materials in Contact with Food.
bw	body weight
CEF	Scientific Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
EC	European Commission
EFSA	European Food Safety Authority
EPA	Environmental Protection Agency
EPIC	European Prospective Investigation into Cancer and Nutrition
FID	Flame Ionisation Detection
GC-MS	Gas Chromatography/Mass Spectrometry
GLP	Good Laboratory Practice
mTAMDI	modified TAMDI
MCPE	Micronucleated polychromatic erythrocytes
MLTK	Mouse Lymphoma Tyrosine Kinase
NOAEL	No-Observed-Adverse-Effect Level
NOEL	No-Observed-Effect Level
OECD	Organisation for Economic Cooperation and Development
PAHs	Polycyclic Aromatic Hydrocarbons
PCE/NCE	Polychromatic Erythrocytes/ Normochromatic Erythrocytes
SCF	Scientific Committee on Food
SMK-EPIC	Smoke flavouring EPIC model
SMK-TAMDI	Smoke Theoretical Added Maximum Daily Intake
TAMDI	Theoretical Added Maximum Daily Intake
UDS	Unscheduled DNA Synthesis
UK NDNS	United Kingdom National Diet and Nutrition Survey
WBC	White Blood Cells Counts