European Food Safety Authority

The EFSA Journal (2009) 1056, 1-23

SCIENTIFIC OPINION

Safety of smoke flavour Primary Product - Scansmoke PB 1110 [1]

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

(Question No EFSA-Q-2005-261)

Adopted on 26 March 2009

This opinion, published on 11 June 2009, replaces the earlier version published on 22 April 2009[2].

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 Scansmoke PB 1110.

The Primary Product Scansmoke PB 1110 is obtained from mixed wood species: 90 % beech (*Fagus sylvatica*) and 10 % oak (*Quercus alba).*

[1] For citation purposes: Scientific Opinion of the Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF) on the safety of smoke flavour Primary Product – Scansmoke PB 1110. *The EFSA Journal* (2009) 1056, 1-23.

[2] Ivonne Rietjens declared in her ADoI that she is advising FEMA on flavourings. In January 2009 she informed the Secretariat that FEMA also assesses smoke flavourings but also that she has never been involved in smoke flavourings evaluations there. According to EFSA Policy on DoI, that activity does not represent a conflict of interest. Upon request from the Secretariat, she updated accordingly her ADoI in May 2009. Therefore, an *addendum* to the scientific opinion is introduced *a posteriori* to clarify this issue.

The production of Scansmoke PB 1110 comprises the following steps: (i) adjustment of the moisture content, (ii) smouldering of the wood under controlled conditions, (iii) separation of the primary tar, (vi) condensing of the released smoke, (v) separation of the liquid phase from residual tar, (vi) distillation and extraction of the liquid smoke condensate and the primary tar, (vi) mixing of the two fractions. Essential parameters of the process have been provided by the applicant.

Scansmoke PB 1110 contains 50 wt. % of water as solvent. The mass of the volatile fraction amounts to 25 wt. %; 88 % of the volatile fraction has been identified. The mass of identified constituents (22 wt. %) corresponds to 44 % of the solvent-free mass. The Panel noted that this is lower than the proportion of at least 50 % of the solvent-free mass required to be identified and quantified according to Commission Regulation (EC) 627/2006 (EC, 2006). However, taking into account the uncertainties in GC/FID quantitation and that a large proportion of the volatile fraction was identified, the Panel considered the value acceptable. Except for benzo[*j*]fluoranthene, the concentrations of the polycyclic aromatic hydrocarbons (PAHs) listed in Annex 2 of the EFSA Guidance document 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). Scansmoke PB 1110 had consistently low levels of PAHs in the batches tested. The Panel considered the data provided on the batch-to-batch variability and on the stability of the Primary Product as sufficient.

Normal use levels of the Primary Product proposed by the applicant range between 1 g/kg food (ready-to-eat savouries, composite foods) and 5 g/kg food (dairy products, processed vegetables, meat and meat products, salts, spices, soups, salads, protein products and composite foods). Dietary exposure for the Primary Product, as estimated by the applicant, was 14 mg/kg bw/per day.

In order to estimate dietary exposure to the Primary Product Scansmoke PB 1110, 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 PB 1110 is present at the normal or upper use levels provided by the applicant for the 18 food categories as outlined in Commission Regulation (EC). Dietary exposure from all sources range from 21.8 to 30.0 mg/kg bw/day, when assuming that the Primary Product Scansmoke PB 1110 is present at the upper use levels, and from 16.2 to 28.3 mg/kg bw/day, when normal use levels are considered.

When dietary exposure estimates are based on use in only traditionally smoked foods dietary exposures range from 8.3 to 14.5 mg/kg bw/day, when assuming that the Primary Product Scansmoke PB 1110 is present at the upper use levels, and from 6.7 to 12.1 mg/kg bw/day, when normal use levels are considered.

Genotoxicity studies conducted on Scansmoke PB 1110 included 3 *in vitro* studies (bacterial reverse mutation test, mammalian cell gene mutation assay and chromosome aberration test) and two *in vivo* studies (mouse bone marrow micronucleus test and a UDS test).

In vitro genotoxicity tests with Scansmoke PB 1110 in bacteria showed essentially negative results in the *S. typhimurium* reverse mutation assay.

Positive results were obtained *in vitro* in the mouse lymphoma assay, indicating the ability of Scansmoke PB 1110 to induce genotoxic effects at gene and chromosome level.

Negative results were obtained in the *in vitro* chromosome aberration test in CHO cells treated with Scansmoke PB 1110, however at very low concentrations.

The *in vivo* mouse bone marrow micronucleus assay was negative, without significant depression of the PCE:NCE ratio.

Scansmoke PB 1110 has not shown any evidence of causing unscheduled DNA synthesis (UDS) in hepatocytes of Crl:CD^{TM}(SD)IGS BR (Sprague Dawley) rats following oral administration.
Overall, it is concluded that Scanmoke PB 1110 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 Wistar rats performed according to OECD guidelines. Scansmoke PB 1110 was given at levels of 0 (control), 1000, 3000 and 9000 mg/kg diet. The NOAEL was 9000 mg/kg diet, the highest dose level tested, which, according to calculations made by the applicant, amounted to 689 mg/kg bw/day in male and 975 mg/kg bw/day in female rats.

Based on these data it is concluded that when assuming that the Primary Product Scansmoke PB 1110 is present at the normal or upper use levels provided by the applicant for the 18 food categories, the margins of safety as compared to the NOAEL of 700 mg/kg bw/day, derived from the 90-day toxicity study with Scansmoke PB 1110 in rats, amounts to 23 - 32 for the intake estimates based on the upper use levels and to 25 - 43 when normal use levels are considered.

When assuming the use of Primary Product Scansmoke PB 1110 in traditionally smoked products only, the margins of safety would amount to 48 - 84 for the intake estimates based on the upper use levels and to 58 - 104 when normal use levels are considered.

Given i) the fact that these margins of safety are based on a 90-day toxicity study, ii) the absence of data on reproduction and developmental toxicity and iii) the absence of long term studies, it is concluded that the uses and use levels of Primary Product Scansmoke PB 1110 would require a larger margin of safety. The Panel concludes that the margin of safety is insufficient and that the use of Primary Product Scansmoke PB 1110 at the proposed uses and use levels is of safety concern.

To decide whether despite the low margins of safety the use of Primary Product Scansmoke PB 1110 might be approved for traditionally smoked products, at use levels specified, to replace smoking, is outside the remit of the Panel.

Key words: Smoke flavouring, Primary Product, Scansmoke PB 1110.

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, 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 flavourings 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 requested according to 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[3], I. Pratt, I.M.C.M. Rietjens, R. Simon and R. Walker.

[3] Dietrich Meier declared an interest because his Institute is doing analysis as contract work for Broste and he is doing information management for Broste. 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 PB 1110.

ASSESSMENT

The following evaluation only applies to the Primary Product Scansmoke PB 1110 manufactured strictly in conformity with the specified process and meeting the chemical specifications described in this opinion. In accordance with the guidance document on submission of a dossier on a smoke flavouring Primary Product for evaluation by EFSA (EFSA, 2005), data are required 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

No information on an existing evaluation or authorisation has been provided.

2. Technical data

2.1. Manufacturing process

2.1.1. Source materials for the Primary Product

The raw material consists of 90 % beech (*Fagus sylvatica*) and 10 % oak (*Quercus alba*) wood. Wood of other species might be present in quantities less than 1%. No other ingredients are used. According to a certificate provided by the supplier the wood used is not subjected to chemical treatment.

2.1.2. Method of manufacture of the Primary Product

Pieces of dried wood are pyrolysed in a series of connected retorts. Initial moisture content, oxygen content of the supply gas, and temperatures of the exhaust gases have been given. The produced smoke condensate and wood tar are further processed separately in several distillation and extraction steps and combined at a fixed ratio to form the Primary Product.

The process has been described in detail and a flow chart has been provided by the applicant. Information on the drying step, the range of temperature profiles of the pyrolysis process, and the distillation conditions has been given.

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 PB 1110.

2.2.2. Physical state of the Primary Product

Scansmoke PB 1110 is a liquid with a density between 1.090 and 1.150 g/ml.

2.3. Chemical composition

2.3.1. Overall characterisation

The overall characterisation of the Primary Product is as follows:

2.3.1.1. Solvent-free fraction

Water functions as the solvent of the Primary Product Scansmoke PB 1110. In the batch used for the toxicological studies (06a/04) a water content of 50.4 wt. % was determined by Karl Fischer titration. Thus the solvent-free fraction of the Primary Product amounts to 49.6 wt. % (Figure 1).

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 25 wt. % of the Primary Product. 22 wt. % (corresponding to 88 % of the volatile fraction) were identified which is in compliance with Commission Regulation (EC) 627/2006.

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: 50 wt. % - 22 wt. % = 28 wt %. The mass of identified constituents (22 wt. %) corresponds to 44 % of the solvent-free mass (Figure 2). The Panel noted that this is lower than the proportion of at least 50 % of the solvent-free mass required to be identified and quantified according to Commission Regulation (EC) 627/2006 (EC, 2006). However, taking into account the uncertainties in GC/FID quantitation and that a large proportion of volatiles was identified, the Panel considered the value acceptable.

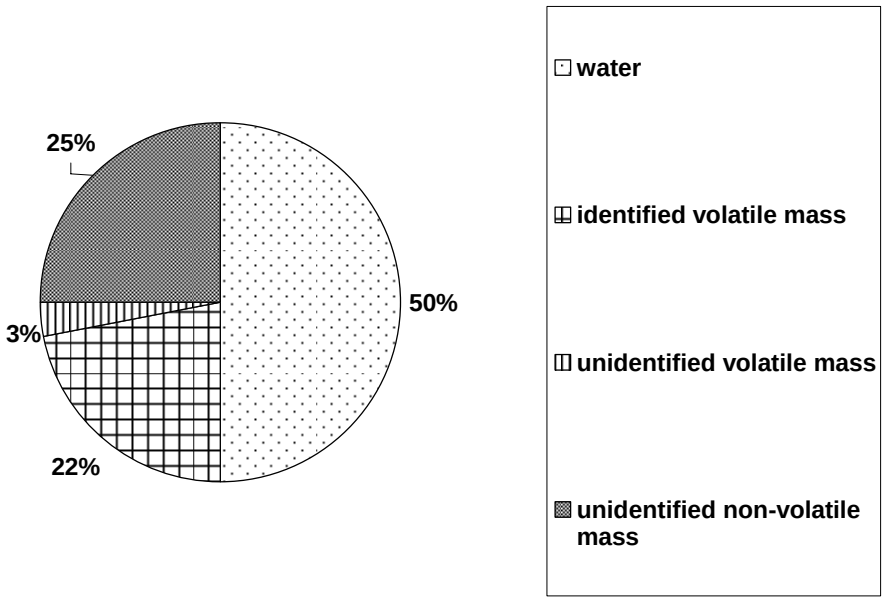

Figure 1. **Overall composition of Scansmoke PB 1110 (wt. % of Primary Product)**

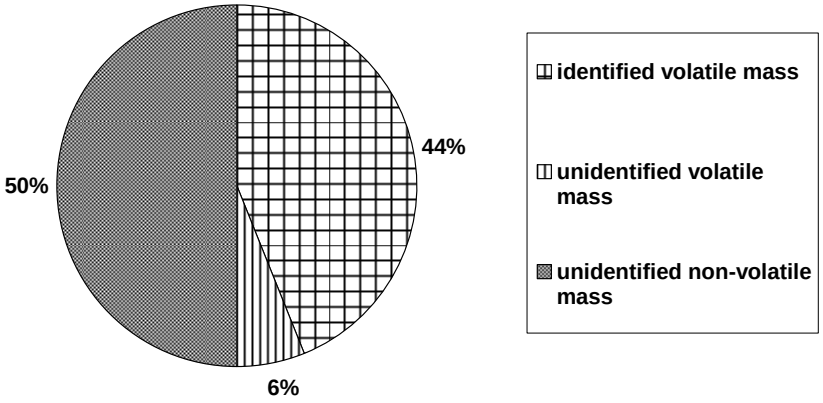

Figure 2. **Composition (%) of the solvent-free fraction of Scansmoke PB 1110**

2.3.2. Chemical description of the Primary Product

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

Table 1. **Description of major chemical parameters of the Primary Product Scansmoke PB 1110**

	Batch								
	32a/04	**34/04**	**41b/04**	**47/04**	**51b/04**	**02a/05**	**03a/05**	**Mean**	**SD**
Phenols, as syringol [mg/g]	10.8	15.5	16.4	15.1	14.3	16.4	18.5	15.3	2.4
Acids, as acetic acid [%]	9.9	10.4	11.1	12.6	9.1	9.7	9.7	10.4	1.2
Carbonyls [%]	20.3	19	18.9	19.5	19.5	18.8	20.1	19.4	0.6
Water [%]	49.3	49.6	51.1	49.8	54.9	54.5	51.5	51.5	2.3
Non volatile residues [wt.%]	29.8	29.4	29.9	30.8	26	28.6	- [a]	29.1	1.7
pH	2.6	2.6	2.6	2.6	2.4	2.5	2.4	2.5	0.1
Staining-Index at 440 nm	109	108	110	114	114	109	119	111.9	4.0
Staining-Index at 495 nm	83.7	87.2	- [a]	89.3	93	92	98.5	90.6	5.1

[a] data not provided

For the batch used for the toxicological studies (06a/04) the heavy metal contents listed in Table 2 have been reported.

Table 2. **Heavy metal contents of Scansmoke PB 1110 (batch 06a/04)**

Mercury (Hg) [mg/kg]	< 0.04
Lead (Pb) [mg/kg]	0.2
Arsenic (As) [mg/kg]	0.4
Cadmium (Cd) [mg/kg]	0.6

2.3.3. Identification and Quantification of Primary Product constituents

2.3.3.1. Principal constituents

Table 3 shows the twenty principal constituents (expressed as wt. % of the dry matter) determined by GC and GC/MS analyses in five batches of the Primary Product Scansmoke PB 1110.

Table 3. **Principal constituents (wt. % of the dry matter) of the Primary Product Scansmoke PB 1110**

Compound	Batch 1 47/04 [wt. %]	Batch 2 51b/04 [wt. %]	Batch 3 01/05 [wt. %]	Batch 4 02a/05 [wt. %]	Batch 5 03a/05 [wt. %]	Mean [wt. %]	SD [wt. %]
Acetic acid	11.8	9.0	10.4	10.8	8.5	10	1.4
Anhydrosugar I	5.1	5.6	5.1	5.6	5.8	5.4	0.31
Hydroxyacetaldehyde	4.5	6.2	5.1	5.1	5.2	5.2	0.6
Levoglucosan	5.1	5.2	4.8	5.2	5.2	5.1	0.19
Syringol	3.1	3.5	3.5	3.7	3.8	3.5	0.25
Hydroxypropanone	2.6	2.8	2.6	2.7	2.6	2.6	0.08
2-Hydroxy-3-methyl-2-cyclopentene-3-one	1.2	1.4	1.3	1.4	1.4	1.3	0.09
3-Hydroxypropanal	1.3	1.2	1.3	1.3	1.4	1.3	0.1
1,4:3,6-Dianhydro-α-D-glucopyranose	1	1.1	1.1	1.2	1.2	1.1	0.07
Anhydrosugar II	1.0	1.0	0.9	0.9	1.0	0.9	0.04
4-Methyl syringol	0.6	0.6	0.6	0.7	0.7	0.6	0.04
4-Ethyl syringol	0.6	0.6	0.6	0.6	0.7	0.6	0.05
3-Ethyl-2-hydroxy-2-cyclopentene-1-one	0.4	0.5	0.4	0.6	0.6	0.5	0.12
Propanoic acid	0.7	0.6	0.1	0.1	0.5	0.4	0.3
1-Hydroxy-2-butanone	0.4	0.4	0.4	0.4	0.4	0.4	0.01
4-Methyl guaiacol	0.4	0.4	0.3	0.4	0.4	0.4	0.02
Maltol	0.4	0.4	0.4	0.1	0.1	0.3	0.17
(5H)-Furan-2-one	0.2	0.3	0.3	0.3	0.3	0.3	0.02
Syringyl acetone	0.2	0.3	0.3	0.3	0.3	0.3	0.02
5-Hydroxymethyl-2-furaldehyde	0.2	0.3	0.2	0.3	0.3	0.2	0.03

2.3.3.2. Content of Polycyclic Aromatic Hydrocarbons (PAHs)

Except for benzo[*j*]fluoranthene, the concentrations of the polycyclic aromatic hydrocarbons (PAHs) known to be potentially 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 (06a/04) of the Primary Product used for the toxicological studies are listed in Table 4. 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 4. **Concentrations of PAHs in the Scansmoke PB1110 batch (06a/04) used for the toxicological studies**

PAH	**[µg/kg]**
Chrysene	1.5
Benzo[*a*]anthracene	<0.5
5-Methylchrysene	<0.5
Cyclopenta[*cd*]pyrene	<0.5
Benzo[*b*]fluoranthene	<0.5
Benzo[*k*]fluoranthene	<0.5
Benzo[*a*]pyrene	<0.5
Indeno[*1,2,3-cd*]pyrene	<0.5
Dibenzo[*a,h*]anthracene	<0.5
Benzo[*ghi*]perylene	<0.5
Dibenzo[*a,e*]pyrene	<1
Dibenzo[*a,h*]pyrene	<1
Dibenzo[*a,i*]pyrene	<1
Dibenzo[*a,l*]pyrene	<1
Fluorene	17
Phenanthrene	21
Anthracene	7.2
Fluroanthene	6.0
Pyrene	9.7

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 chemical parameters used to describe the Primary Product in seven batches (Table 1).

(b) Variability of the twenty principal constituents determined by GC and GC/MS analyses of five batches of the Primary Product (Table 3).

(c) Variability of the PAHs listed in Table 4 in five batches. For the 14 measured PAHs listed in Annex 2 of the EFSA guidance document the concentrations were consistently <1 μg/kg (except for chrysene detected at levels of 1, 1.2 and 1.6 μg/kg in three of the batches).

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

2.3.5. Stability

For demonstration of the stability of Scansmoke PB 1110 the concentrations of the principal constituents were measured at five time points over a period of almost a year (11 months) by GC-MS. The data of the twenty most abundant constituents are displayed in Table 5. The average relative standard deviation (RSD) was 35 %. The individual values ranged from 9 % for major constituents (e.g. hydroxypropanone, 3.2 wt. %) to 152 % for minor constituents (e.g. anhydrosugar III, 0.4 wt. %). For some constituents (*e.g* 1,2-ethanediol and the anhydrosugar III) the concentration determined over the storage period declined strongly. However, overall the Panel considered the data provided as sufficient to demonstrate the stability of the Primary Product.

Table 5. **Variability of principal constituents (wt. % of the dry matter) of the Primary Product Scansmoke PB 1110 over a period of 11 months**

Date of analysis	**06/04** [wt.%]	**08/04** [wt.%]	**12/05** [wt.%]	**02/05** [wt.%]	**04/05** [wt.%]	**mean** [wt.%]	**SD** [wt.%]	**RSD** [%]
Acetic acid	7.7	9.8	8.7	10.4	9.1	9.1	1.1	12
Levoglucosan	8.1	8.6	7.0	7.1	6.3	7.4	0.94	13
Anhydrosugar I	7.5	8.3	6.3	7.0	6.2	7.0	0.86	12
Hydroxyacetaldehyde	4.7	5.0	3.8	4.4	4.4	4.5	0.50	10
Hydroxypropanone	2.9	3.3	3.0	3.6	3.3	3.2	0.29	9
Syringol	1.2	3.5	2.7	3.4	3.1	2.8	0.93	34
1,4:3,6-Dianhydro-α-D-glucopyranose	1.3	1.2	1.0	1.2	1.1	1.2	0.10	9
2-Hydroxy-3-methyl-2-cyclopentene-3-one	1.0	1.3	0.9	1.3	1.2	1.1	0.17	15
Anhydrosugar II	1.0	0.6	1.3	1.5	1.2	1.1	0.34	30
1,2 Ethanediol	1.1	1.0	0.80	0.50	0.05	0.7	0.40	63
Propanoic acid	0.40	0.70	0.50	0.70	0.70	0.6	0.10	22
4-Methyl syringol	0.59	0.62	0.50	0.59	0.55	0.6	0.05	9
Anhydrosugar III	1.48	0.66	0.00	0.00	0.00	0.4	0.65	152
Maltol	0.00	0.63	0.69	0.23	0.53	0.4	0.29	70
4-Ethyl syringol	0.34	0.61	0.24	0.21	0.63	0.4	0.20	49
4-Methyl guaiacol	0.25	0.07	0.47	0.47	0.40	0.3	0.17	51
1-Hydroxy-2-butanone	0.24	0.22	0.24	0.38	0.41	0.3	0.09	30
3-Ethyl-2-hydroxy-2-cyclopentene-1-one	0.00	0.37	0.13	0.54	0.31	0.3	0.21	78
(5H)-Furan-2-one	0.30	0.20	0.20	0.20	0.20	0.2	0.03	13
Syringyl acetone	0.22	0.21	0.19	0.24	0.22	0.2	0.02	9

2.3.6. Specifications

The specifications for the Primary Product Scansmoke PB 1110 as proposed by the applicant are given in Table 6.

Table 6. **Specifications of the Primary Product as proposed by the applicant**

Staining Index at 440 nm	105-125	Acids, as acetic acid	8-12 %
Carbonyl compounds	17-25 %	Acetic acid	< 9.9 %
Specific gravity	1.090 – 1.150 g/mL	pH	2.1-2.9
Benzo[*a*]pyrene	< 10 µg/kg	Benz[*a*]anthracene	< 20 µg/kg
Mercury	< 0.01 mg/kg	Lead	< 0.1 mg/kg
Arsenic	< 0.03 mg/kg	Cadmium	< 0.01 mg/kg

The Panel noted that these figures are not fully consistent with the data provided in Table 2.

3. Proposed uses

Normal and upper use levels as described by the applicant 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 7.

Table 7. **Normal and upper use levels of Primary Product as proposed by the applicant in food categories as outlined in Commission Regulation (EC) No 1565/2000**

Food categories		Use level (g/kg)	
		Normal	Upper
1	Dairy products, excluding products of category 2	5*	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 (including mushrooms & fungi, roots & tubers, pulses & legumes) and nuts and seeds	5*	5
5	Confectionery	1*	1
6	Cereals and cereal products, including flours & starches from roots & tubers, pulses & legumes, excluding bakery	0	0
7	Bakery wares	1	1
8	Meat and meat products, including poultry and game	4§	5
9	Fish and fish products, including molluscs, crustaceans and echinoderms	4§	5
10	Egg and egg products	0	0
11	Sweeteners, including honey	0	0
12	Salts, spices, soups, sauces, salads, protein products etc.	5*	5
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	1*	1
15	Ready-to-eat savouries	1	5

16	Composite foods (e.g. casseroles, meat pies, mincemeat) - foods that could not be placed in categories 1 – 15	1	5

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

§ For food categories 8 and 9 the applicant did not provide a unique value for Normal Use levels as requested. Instead a range of values was provided. The highest value was used in the exposure assessment.

4. Dietary exposure assessment

In order to estimate dietary exposure to the Primary Product Scansmoke PB1110, 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 flavouring substances (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 for different European countries (Linseisen *et al.*, 2006). The Panel used consumption data from the EPIC study to estimate the overall potential 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 (EC). 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).

Dietary exposure estimates calculated by means of the above mentioned methods are reported in Tables 8. Dietary exposure from all sources range from 21.8 to 30.0 mg/kg bw/day, when assuming that the Primary Product is present at the upper use levels, and from 16.2 to 28.3 mg/kg bw/day, when normal use levels are considered.

The impact on exposure of authorising 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". In this case the SMK-EPIC model results in the highest exposure estimates: 12.1 and 14.5 mg/kg bw/day when using normal and upper use levels, respectively.

Dietary exposures to the Primary Product were 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 14.0 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 8. **Summary of the dietary exposure estimates to the Primary Product**

Methodologies		**Dietary exposure (mg/kg bw/day)**	
		Normal use levels	**Upper use levels**
SMK-TAMDI	Traditionally smoked food	6.7	8.3
	Other foods not traditionally smoked	16.7	16.7
	Beverages (alcoholic or non-alcoholic)	5.0	5.0
	Total dietary exposure	28.3	30.0
SMK-EPIC	Traditionally smoked food	12.1	14.5
	Other foods not traditionally smoked	3.4	6.6
	Beverages (alcoholic or non-alcoholic)	0.7	0.7
	Total dietary exposure	16.2	21.8
Applicant	Dietary exposure	-	14.0

5. Toxicological data

5.1. Identity of the test material

According to the applicant, the material (batch 06a/04 of the Primary Product) used for the analysis of the chemical composition has been used for all toxicological tests.

5.2. Subchronic toxicity

Scansmoke PB 1110 was tested in a subchronic 90-days toxicity study performed according to OECD guidelines (LAB, 2005a). The applicant provided specifications for the Scansmoke PB 1110 batch used for the toxicity studies and these were according to those given for the product.

For the subchronic toxicity study groups of Wistar rats (10/sex/group) were administered Scansmoke PB 1110 in the diet for up to 90 days at levels of 0 (control), 1000, 3000 and 9000 mg/kg diet. The applicant calculated the nominal dose levels on the basis of the daily mean food consumption and the mean body weight of the animals and these values amounted to 0, 71, 225 and 689 mg Scansmoke PB 1110/kg bw/day for male rats and to 0, 100, 299 and 975 mg Scansmoke PB 1110/kg bw/day for female rats.

The test item was mixed in the diet on weight basis in three concentrations (1000, 3000 and 9000 mg/kg). Mixing was performed once during the study. A premix was prepared and then appropriate weights of the premix were added to the basal diet.

The mixing and pellet formation were performed in a GMP facility, the quality of mixing and homogeneity of the process used was validated, and the repeat samples which were assayed for pellets on this study and on similar studies were found to have adequate homogeneity.

The data were reported to show a good proportionality between the nominal concentration of each smoke product, and the measured components. Hence the achieved dose level concentrations were considered to be valid.

All animals were observed once daily for general appearance, behaviour, signs of toxicity and mortality. A functional battery (modified Irwin test) was conducted before the first treatment, and repeated on week 13. Body weight and food consumption were measured weekly. Ophthalmological examinations were conducted on all animals before treatment and in the control and high dose groups on week 12. Haematology tests, clinical chemistry, urinalysis and gross pathology were performed on all animals at the end of the study. Histopathological examinations were performed on organs collected from all animals in the high dose group and control animals. In addition, histological examinations were made on organs showing abnormalities in the low and middle dose groups as well.

No mortality occurred during the study. General physical condition and behaviour of animals were normal in all experimental groups. No difference was found between the experimental groups in the sensory reactivity to stimuli of different types, grip strength and motor activity. The body weight gain was similar in all experimental groups except for the male medium dose group and the male and female high dose groups where the body weight gain in week 6 was significantly lower than in the control group, but this did not result in a difference in body weight at the end of the 90-day study. The daily mean food consumption was similar for all experimental groups.

Haematological, clinical chemistry and urine parameters changed within the historical control ranges specific for this species and strain with some statistically significant effects that were however without clear dose dependency and the applicant therefore concluded that these changes were not considered to be of toxicological significance.

In ophthalmology studies no eye alterations were found after the 90-day oral administration of Scansmoke PB 1110.

The organ weights (absolute and relative to the body and brain weight) of male animals were similar in all experimental groups. The alterations of female animals (reduced kidney, brain and spleen weight in the medium and high dose groups and reduced kidney, spleen and uterus weight in the low dose group), were claimed by the applicant to be within the normal ranges of biological variations. No dose-relationships and supporting histopathological findings were found, so the applicant concludes that statistically significant changes in organ weights are not of toxicological relevance.

Macroscopic alterations of organs were related by the applicant to the sacrificing procedure (emphysema and pinprick-sized hemorrhages in the lungs) or were common findings in experimental rats (hydrometra in uterus and bilateral pyelectasis).

Histopathological examinations revealed no test item-related histopathological changes in the organs subjected to microscopic examination.

The applicant concludes that this repeated dose sub-chronic 90-day oral study in rats shows that Scansmoke PB 1110 did not cause any toxic or other test-related alterations in male and female CRL:(WI)BR rats at levels of 1000, 3000 and 9000 mg/kg diet.

The no-observed-adverse-effect level (NOAEL) from this study was 9000 mg/kg in the diet, which, according to the calculations made by the applicant amounted to 689 mg/kg bw/day in male rats and 975 mg/kg bw/day in female rats. The Panel derives a NOAEL of 700 mg/kg bw/day, being the highest dose level tested.

In another subchronic toxicity study Scansmoke PB 1060, being a Scansmoke PB 1110 preparation 50% diluted in water, was tested in a 90-day oral toxicity study in Wistar rats (Scantox, 1992). This repeated dose 90-day dietary study in rats with Scansmoke PB 1060 was conducted in 1992 according to OECD guidelines and GLP standards at that time. It could not be established whether the specifications of the material tested actually matched those presented for Scansmoke PB 1110 in the present opinion. Therefore the Panel decided to base the evaluation on the recent 90-day study conducted with Scansmoke PB 1110 performed in 2005 and described above.

5.3. Genotoxicity

Scansmoke PB 1110 has been evaluated in tests for induction of gene mutations in bacteria (OECD 471) (LAB, 2005b), gene mutations in mammalian cells *in vitro* (OECD 476) (LAB, 2005c) and chromosomal aberrations in mammalian cells *in vitro* (OECD 473) (LAB, 2005d). It has also been tested in the *in vivo* mouse bone marrow micronucleus test (OECD 474) (LAB, 2005e) and in an *in vivo* UDS test (HLS, 2007).

The assay for gene mutation in bacterial cells for Scansmoke PB 1110 was negative in TA100 and TA1535, negative in TA98 and TA1537 at concentrations up to 5000 µg/plate. The levels of revertants in all dose groups were generally similar to the control levels. The few sporadic cases of statistically significant increases (TA 100 with S9-mix and TA 1535 without S9-mix) were well below a doubling of the concurrent spontaneous level, and no such effects were seen in the duplicate tests.

In the test for gene mutations in mammalian cells, 3-hours treatment of the L5478Y $TK^{+/-}$ 3.7.2 C cells with Scansmoke PB 1110 in the presence and absence of S9 metabolic activation, as well as the 24-hours treatment in the absence of S9 resulted in statistically significant increases in mutation frequencies at concentrations of 100, 150, 175, 200, 250, 300 µg/ml after the 3-hours treatment, and 50 and 100 µg/ml after the 24-hours treatment of the L5478Y $TK^{+/-}$ 3.7.2 C cells. It is concluded that the mouse lymphoma *tk* assay was clearly positive with and without S9, with increases of both large and small colonies, thus indicating the ability of Scansmoke PB 1110 to induce genotoxic effects both at gene and chromosome level; the cytotoxicity did not affect these results.

A test for chromosomal aberrations in mammalian cells *in vitro* was also performed. The concentrations used were relatively low and amounted to 5, 10 and 30 µg/ml. Scansmoke PB 1110 tested both with and without metabolic activation did not induce chromosomal aberrations in Chinese hamster ovary cells (CHO). It is concluded that the CHO chromosomal aberrations test was negative; however, the concentrations used were much lower than those used for the mouse lymphoma cells, due to the much higher sensitivity of CHO cells to the cytotoxic effects of Scansmoke PB 1110. In practice this means that the results of these two tests are not comparable for the clastogenic potential of Scansmoke PB 1110.

In the *in vivo* mouse bone marrow micronucleus test dose levels of 500, 1000, 2000 mg Scansmoke PB 1110 /kg bw/day given by single oral administration were tested. Five males and females per dose group were used for sampling on each occasion. Cyclophosphamide was used as the positive control and administered intraperitoneally whereas control vehicle was

administered by oral route. Bone marrow was obtained from two femurs of the mice from every dose-sex-time point. Two thousand PCEs were scored per animal. There was no change in PCE:NCE ratio and there were no increases in the frequency of micronucleated polychromated erythrocytes (MCPE) in male and female mice at either 24 or 48 hours after treatment compared to the vehicle control.

Unscheduled DNA Synthesis (UDS) was assessed in hepatocytes of Crl:CD™(SD)IGS BR (Sprague Dawley) rats following oral gavage administration of Scansmoke PB 1110 on two separate occasions (the second dose being administered 14 hours after the first dose and 2 hours before perfusion). Scansmoke PB 1110 was administered at dosages of 600 and 2000 mg/kg bw. A preliminary toxicity test had previously confirmed that 2000 mg/kg bw/day, the standard limit dose for the UDS test, was tolerated in male rats. This dose level was therefore selected as the limit dose in this test system.
All vehicle control and treatment group animals were dosed orally by gavage. The vehicle control group received the vehicle, purified water. A positive control group was treated orally by gavage with 2-acetylaminofluorene (2-AAF) at 75 mg/kg on one occasion, 16 hours before perfusion.
Hepatocytes were isolated by enzymatic dissociation after exposure of the animals to the test substance. Four animals were assessed at each experimental point with the exception that only two animals from the positive control group were assessed.
DNA repair was assessed by comparing the grain count of hepatocyte nuclei with the accompanying cytoplasmic grain count. The gross nuclear grain count and the net nuclear grain count from Scansmoke PB 1110 treated cultures were compared with vehicle control values. Scansmoke PB 1110 did not cause any significant increases in the mean (gross) nuclear grain count or the mean net nuclear grain count at any dose level compared to vehicle control values.
Positive control group animals showed a statistically significant increase in the mean net nuclear grain count, accompanied by an increase in the mean (gross) nuclear grain count.
It is concluded that Scansmoke PB 1110 has not shown any evidence of causing unscheduled DNA synthesis (UDS) in hepatocytes of Crl:CD™(SD)IGS BR (Sprague Dawley) rats following oral administration in this *in vivo* test system.

Scansmoke PB 1060, a 50% diluted Scansmoke PB 1110 preparation, has also been tested in an assay for gene mutations in bacteria (*S. typhimurium* TA98, TA100, TA1535 and TA1537) and for chromosomal aberrations in human lymphocytes *in vitro*. Dose levels of Scansmoke PB1060 up to 1345 µg/plate without metabolic activation and up to 4037 µg/plate with metabolic activation did not induce an increase in the number of revertants. Human lymphocytes were exposed to 63, 125 and 250 µg/ml without S-9 mix and 250, 500 and 1000 µg/ml in the presence of S-9 mix. No chromosomal aberrations were detected. It could not be established whether the specifications of the material tested actually matched those presented for Scansmoke PB 1110 in the present opinion.

5.4. Other studies

No other studies on Scansmoke PB 1110 were provided by the applicant

6. Discussion

The applicant provided information on the identity, composition, variability and stability of the Primary Product as requested in the EFSA guidance document.

The Panel noted that the mass of identified constituents (44 % of the solvent-free mass) is lower than the proportion of at least 50 % of the solvent-free mass required to be identified and quantified according to Commission Regulation (EC) 627/2006 (EC, 2006). However, taking into account the uncertainties in GC/FID quantitation and that a large proportion of the volatile fraction was identified, the Panel considered the value acceptable.

Scansmoke PB 1110 had consistently low levels of PAHs in the batches tested. Although the concentration of benzo[*j*]fluoranthene, one of the PAHs known to be potentially carcinogenic, was not provided, the Panel concluded that based on the reported levels of other potentially carcinogenic PAHs, benzo[*j*]fluoranthene levels would similarly be low.

Normal use levels of the Primary Product proposed by the applicant range between 1 g/kg food (ready-to-eat savouries, composite foods) and 5 g/kg food (dairy products, processed vegetables, meat and meat products, salts, spices, soups, salads, protein products and composite foods). Dietary exposure for the Primary Product, as estimated by the applicant, was 14 mg/kg bw/day.

In order to estimate dietary exposure to the Primary Product Scansmoke PB 1110, 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 PB 1110 is present at the normal or upper use levels provided by the applicant for the 18 food categories as outlined in Commission Regulation (EC). Dietary exposure from all sources range from 21.8 to 30.0 mg/kg bw/day, when assuming that the Primary Product Scansmoke PB 1110 is present at the upper use levels, and from 16.2 to 28.3 mg/kg bw/day, when normal use levels are considered.

When dietary exposure estimates are based on use in only traditionally smoked foods dietary exposures range from 8.3 to 14.5 mg/kg bw/day, when assuming that the Primary Product Scansmoke PB 1110 is present at the upper use levels, and from 6.7 to 12.1 mg/kg bw/day, when normal use levels are considered.

In vitro genotoxicity tests with Scansmoke PB 1110 in bacteria showed essentially negative results in the *S. typhimurium* reverse mutation assay. Positive results were obtained in the *in vitro* mouse lymphoma assay, indicating the ability of Scansmoke PB 1110 to induce genotoxic effects at gene and chromosome level.

Negative results were obtained in the *in vitro* chromosome aberration test in CHO cells treated with Scansmoke PB 1110, however at very low concentrations.

The *in vivo* mouse bone marrow micronucleus assay was negative, without significant depression of the PCE:NCE ratio. Scansmoke PB 1110 has not shown any evidence of causing unscheduled DNA synthesis (UDS) in hepatocytes of Crl:CD™(SD)IGS BR (Sprague Dawley) rats following oral administration.

Overall, it is concluded that Scansmoke PB 1110 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 Wistar rats performed according to OECD guidelines. Scansmoke PB 1110 was given at levels of 0 (control), 1000, 3000 and 9000 mg/kg diet. The NOAEL was 9000 mg/kg diet, the highest dose level tested, which, according to calculations made by the applicant amounted to 689 mg/kg bw/day in male and 975 mg/kg bw/day in female rats. The Panel derives a NOAEL of 700 mg/kg bw/day, the highest dose level tested.

Based on these data it is concluded that when assuming that the Primary Product Scansmoke PB 1110 is present at the normal or upper use levels provided by the applicant for the 18 food categories, the margins of safety as compared to the NOAEL of 700 mg/kg bw/day derived from the 90-day toxicity study with Scansmoke PB 1110 in rats amounts to 23 - 32 for the intake estimates based on the upper use levels and to 25 - 43 when normal use levels are considered.

When assuming the use of Primary Product Scansmoke PB 1110 in traditionally smoked products only the margins of safety would amount to 48 - 84 for the intake estimates based on the upper use levels and to 58 - 104 when normal use levels are considered.

Table 9. **Margins of safety**

	Use level	**Dietary exposure (mg/kg bw/day)**	**NOAEL (mg/kg bw/day)**	**Margin of safety**
Total dietary exposure	Normal	16.2 – 28.3	700	25-43
	Upper	21.8 – 30.0	700	23-32
Traditionally smoked food	Normal	6.7 – 12.1	700	58-104
	Upper	8.3 – 14.5	700	48-84

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 PB 1110 in such products was not assessed.

Given i) the fact that these margins of safety are based on a 90-day toxicity study, ii) the absence of data on reproduction and developmental toxicity and iii) the absence of long term studies it is concluded that the uses and use levels of Primary Product Scansmoke PB 1110 would require a larger margin of safety. The Panel concludes that the margin of safety is insufficient and that the use of Primary Product Scansmoke PB 1110 at the proposed uses and use levels is of safety concern.

To decide whether despite the low margins of safety the use of Primary Product Scansmoke PB 1110 might be approved for traditionally smoked products, at use levels specified, to replace smoking, is outside the remit of the Panel.

CONCLUSIONS AND RECOMMENDATIONS

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

Primary Product Scansmoke PB 1110 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 is 700 mg/kg bw/day, the highest dose level tested.

Based on these data it is concluded that when assuming that the Primary Product Scansmoke PB 1110 is present at the normal or upper use levels provided by the applicant for the 18 food categories, the margin of safety as compared to the NOAEL of 700 mg/kg bw/day derived

from the 90-day toxicity study with Scansmoke PB 1110 in rats amounts to 23 - 32 for the intake estimates based on the upper use levels and to 25 - 43 when normal use levels are considered.

When assuming the use of Primary Product Scansmoke PB 1110 in traditionally smoked products only the margins of safety would amount to 48 - 84 for the intake estimates based on the upper use levels and to 58 - 104 when normal use levels are considered.

Given i) the fact that these margins of safety are based on a 90-day toxicity study, ii) the absence of data on reproduction and developmental toxicity and iii) the absence of long term studies, it is concluded that the uses and use levels of Primary Product Scansmoke PB 1110 would require a larger margin of safety. The Panel concludes that the margin of safety is insufficient and that the use of Primary Product Scansmoke PB 1110 at the proposed uses and use levels is of safety concern.

To decide whether despite the low margins of safety the use of Primary Product Scansmoke PB 1110 might be approved for traditionally smoked products, at use levels specified, to replace smoking, is outside the remit of the Panel.

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. 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 (EC) 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/site/en/oj/2006/l_109/l_10920060422en00030006.pdf Visited on 10 April 2007

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.

HLS (2007). Huntingdon Life Sciences Ltd. Scansmoke PB 1110 *in vivo* DNA repair (UDS) test using rat hepatocytes, Study Code: BTO 0002/073746, November 2007.

JECFA, 2006. Sixty-seventh meeting of the Joint FAO/WHO Expert Committee on Food Additives. Summary and conclusions. Rome, 20-29 June 2006.

LAB (2005a). LAB International Research Centre Hungary Ltd, 90-day Oral/Dietary Toxicity Study of Scansmoke PB 1110 in Rats, Study Code: 04/829-101P, June 2005.

LAB (2005b). LAB International Research Centre Hungary Ltd, Testing of Liquid Smoke PB1110 with Bacterial Reverse Mutation Assay, Study Code: 04/829-007M, June 2005.

LAB (2005c). LAB International Research Centre Hungary Ltd, Testing of Scansmoke PB1110 with *In vitro* Mammalian Chromosome Aberration Test, Study Code: 04/829-020C, June 2005.

LAB (2005d). LAB International Research Centre Hungary Ltd, Testing of Mutagenic Effect of Scansmoke PB1110 by Mouse Lymphoma Assay, Study Code: 04/829-033E, June 2005.

LAB (2005e). LAB International Research Centre Hungary Ltd, Testing of Mutagenic Effect of Test Item Scansmoke PB1110 by Mouse Micronucleus Test, Study Code: 04/829-013E, June 2005.

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.

Scantox Lab (1992). Scantox Lab No.13300. Scansmoke PB1060 Three Months Oral Toxicity Study in the rat, Study Number: 13300, July 1992.

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.

GLOSSARY / ABBREVIATIONS

2-AAF	2-Acetylaminofluorene
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
CHO	Chinese Hamster Ovary cell line
EC	European Commission
EFSA	European Food Safety Authority
EPIC	European Prospective Investigation into Cancer and Nutrition
GC/MS	Gas Chromatography/Mass Spectrometry
GLP	Good Laboratory Practice
GMP	Good Manufacturing Practice
GS/FID	Gas Chromatography/Flame Ionisation Detection
JECFA	Joint FAO/WHO Expert Committee on Food Additives
mTAMDI	modified TAMDI
MCPE	Micronucleated Polychromated Erythrocytes
NOAEL	No-Observed-Adverse-Effect Level
OECD	Organisation for Economic Cooperation and Development
PAH	Polycyclic Aromatic Hydrocarbons
PCE:NCE	Polychromatic Erythrocytes/ Normochromatic Erythrocytes
RSD	Relative Standard Deviation
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