

## SCIENTIFIC OPINION

### Safety of smoke flavour Primary Product - Smoke Concentrate 809045<sup>1</sup>

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

(Question number EFSA-Q-2005-270)

Adopted on 29 January 2009

This opinion, published on 11 June 2009, replaces the earlier version published on 6 April 2009<sup>2,3</sup>.

#### PANEL MEMBERS

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#### 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 Smoke Concentrate 809045.

Smoke Concentrate 809045 is obtained from beech wood of the tree species *Fagus sylvatica* L.

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<sup>1</sup> 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 – Smoke Concentrate 809045. *The EFSA Journal* (2009) 981, 1-21.

<sup>2</sup> In the process of publication of this opinion, Table 3 had been deleted by mistake. In the current version, it is re-introduced, consistently with the opinion adopted by the Panel. The numbering of the following tables has been changed correspondingly. These changes do not affect the overall conclusions of the opinion.

<sup>3</sup> 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 minutes of the 4<sup>th</sup> CEF Plenary meeting (26-29 January 2009) and to the scientific opinions is introduced *a posteriori* to clarify this issue.

<sup>4</sup> Karl-Heinz Engel has declared an interest as a student in his laboratory is paid with a grant of Symrise. As the funding is going to the University and as the subject of the work is not related to Smoke Flavourings, this is not a conflict.

The production of Smoke Concentrate 809045 comprises the following steps: (i) smouldering pieces of wood under controlled conditions, (ii) condensing the released smoke, (iii) separation of the liquid phase from the tar, (iv) treatment with activated carbon, (v) distillation, and (vi) filtration. Essential process parameters have been provided by the applicant.

The water content of the Primary Product is estimated as 11 wt. %. The volatile fraction accounts for 40 wt. % of the Primary Product; 78 % of the volatile fraction have been identified. The total identified mass represents 52 wt. % of the Primary Product, corresponding to 59 % of the solvent-free mass. Considering the measurement uncertainties of the analytical methods and the variations in the batch-to-batch variability the Panel considered that these values could be regarded as to be in compliance with the requirements of Commission Regulation (EC) 627/2006.

The concentrations of the 15 polycyclic aromatic hydrocarbons (PAHs) listed in the EFSA guidance document on submission of a dossier on a smoke flavouring Primary Product have been provided; they were all below 10 µg/kg. The methods used and the resulting values are in compliance with Commission Regulations (EC) No 2065/2003 and (EC) 627/2006.

The analytical data provided demonstrated acceptable batch-to-batch variability of the Primary Product.

In order to estimate dietary exposure to the Primary Product Smoke Concentrate 809045, 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 (EC). Dietary exposures from all sources range from 0.29 to 0.50 mg/kg bw/day, when assuming that the Primary Product Smoke Concentrate 809045 is present at the upper use levels and from 0.06 to 0.13 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 0.15 to 0.21 mg/kg bw/day, when assuming that the Primary Product Smoke Concentrate 809045 is present at the upper use levels, and from 0.03 to 0.04 mg/kg bw/day, when normal use levels are considered.

Genotoxicity studies conducted on Primary Product Smoke Concentrate 809045 included three *in vitro* studies (a bacterial reverse mutation test, a mouse lymphoma gene mutation assay and a chromosome aberration test) and two *in vivo* studies (a mouse bone marrow micronucleus assay and a rat liver unscheduled DNA synthesis assay). The results obtained in these assays showed positive genotoxic responses in two of the *in vitro* assays but negative results *in vivo* in two well conducted studies.

Overall it is concluded that Primary Product Smoke Concentrate 809045 is genotoxic *in vitro*, whereas two *in vivo* genotoxicity tests were negative and sufficient to eliminate the concerns over the *in vitro* genotoxicity.

In the subchronic 90-day study in rats, the NOAEL was 1000 mg/kg bw/day, the highest dose level tested.

Based on these data it is concluded that when assuming that the Primary Product Smoke Concentrate 809045 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 1000 mg/kg bw/day derived from the 90-day toxicity study in rats amount to 2000 - 3400 for the intake estimates based on the upper use levels and to 7700 - 17000 when normal use levels are considered.

When assuming the use of Primary Product Smoke Concentrate 809045 in traditionally smoked products only, the margins of safety would amount to 4800 - 6700 for the intake estimate based on the upper use levels and to 25000 - 33000 when normal use levels are considered.

Given the margin of safety of at least 2000 and the fact that this margin of safety is based on a conservative exposure estimate and a NOAEL that is the highest dose level tested, the Panel concludes that the use of Smoke Concentrate 809045 at the proposed uses and use levels is not of safety concern.

**Key words:** Smoke flavouring, Primary Product, Smoke Concentrate 809045.

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## 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 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<sup>5</sup>, I. Pratt, I. Rietjens, R. Simon and R. Walker.

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<sup>5</sup> Dietrich Meier declared an interest because his Institute is doing analysis as contract work for Symrise. This was considered as a conflict of interest and he was excluded from the discussion on the Smoke Flavouring Primary Product Smoke Concentrate 809045.

## ASSESSMENT

The following evaluation applies only to the Primary Product Smoke Concentrate 809045 manufactured strictly in conformity with the specified process. 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

No information on existing authorisations and evaluations of the Primary Product Smoke Concentrate 809045 has been provided.

### 2. Technical data

#### 2.1. Manufacturing process

##### 2.1.1. Source materials for the Primary Product

The raw material consists of beech wood chips of the tree species *Fagus sylvatica L.* No other ingredients are used. The material is analysed once per year to confirm the absence of residual pesticides.

##### 2.1.2. Method of manufacture of the Primary Product

Pieces of wood are smouldered with a controlled amount of oxygen in a commercial smoke generator. Initial moisture content, oxygen content of the supply gas, maximum reaction temperature, and maximum temperature of the smoke are given. The produced smoke condensate is separated from the tar, which is discarded. The liquid part of the condensate is further processed by treatment with activated carbon, concentration by distillation and filtration steps to form the Primary Product. The process has been described in detail and a flow chart has been given by the applicant.

#### 2.2. Identity of the Primary Product

##### 2.2.1. Trade names of the Primary Product

The trade name of the Primary Product is Smoke Concentrate 809045.

##### 2.2.2. Physical state of the Primary Product

The Primary Product Smoke Concentrate 809045 is described as a viscous liquid of brown colour with a characteristic odour of freshly generated smoke.

#### 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

Smoke Concentrate 809045 contains 11 wt. % water as solvent as determined by Karl Fisher titration. Accordingly, the solvent-free fraction amounts to 89 wt. % (Figure 1).

### 2.3.1.2. Volatile fraction

The identification and quantification of the volatile fraction of the Primary Product was conducted using a gas chromatographic (GC) method similar to the one referred to in Commission Regulation (EC) No 627/2006 (EC, 2006). The volatile fraction totalled to 40 wt. % of the Primary Product (Figure 1). The mass of identified constituents amounted to 31 wt. % of the Primary Product (78 % of the volatile fraction) (Figure 2). The Panel noted that this is slightly lower than the proportion of 80 % of the volatile fraction required to be identified and quantified according to Commission Regulation (EC) 627/2006 (EC, 2006). Considering the measurement uncertainties of the analytical methods and the variations in the batch-to-batch variability the Panel considered that these values could be regarded as to be in compliance with the requirements of Commission Regulation (EC) 627/2006.

### 2.3.1.3. Unidentified constituents

Smoke Concentrate 809045 was subjected to precipitation analysis resulting in 21 wt. % of non-volatile pyrolytic lignin (corresponding to 24% of the solvent-free mass). The identity of the lignin fraction has been confirmed by pyrolysis gas chromatography-mass spectrometry (GC-MS).

The unidentified part of the non-volatile mass of the Primary Product amounted to 28 wt. %. Combined with the unidentified part of the volatile fraction (9 wt. % of the Primary Product), 37 wt. % of the Primary Product remained unidentified (41 % of the solvent-free fraction) (Figure 1).

The total identified mass represents 52 wt. % of the Primary Product, corresponding to 59 % of the solvent-free mass. This is in compliance with the requirements of Commission Regulation (EC) 627/2006 (EC, 2006).

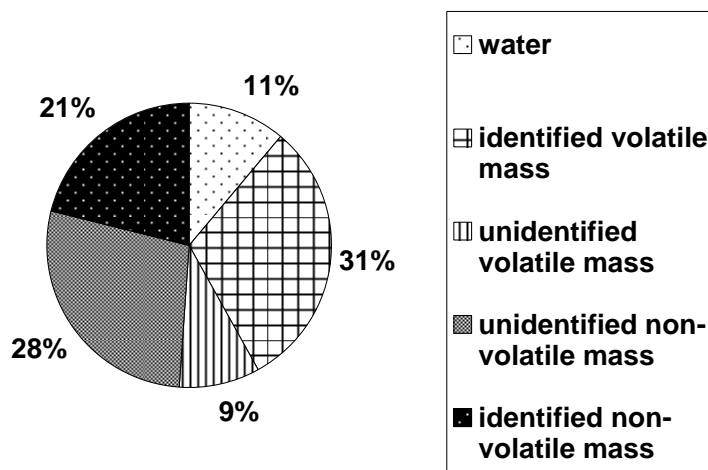


Figure 1. Overall composition of Smoke Concentrate 809045 (wt.% of Primary Product)

The overall composition of the solvent-free fraction is shown in Figure 2.

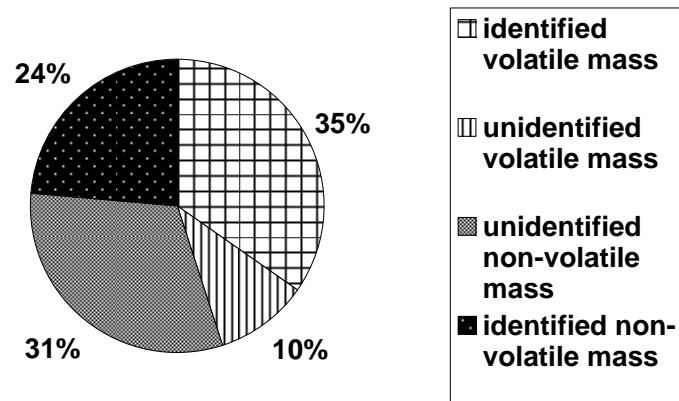


Figure 2. Composition (%) of the solvent-free fraction of Smoke Concentrate 809045

### 2.3.2. Chemical description of the Primary Product

The Primary Product has been characterised according to the parameters listed in Table 1 (average values from 18 batches). The applicant provided descriptions of the analytical methods.

Table 1. Description of major chemical parameters of the Primary Product Smoke Concentrate 809045 (average values of 18 batches)

Parameter	Value
Extinction (0.5 % dilution) [400 nm]	2.4
Benzo(a)pyrene-1,3 [ $\mu\text{g}/\text{kg}$ ]	2.6
Benz(a)anthracene [ $\mu\text{g}/\text{kg}$ ]	8.6
Refractive Index	1.5
Water [%]	11.2
Phenol(a) [%]	22
Phenol(b) [%]	0.5
Carbonyls [%]	14.1
Total Acid Value(c) [mg KOH/g]	12.6

<sup>(a)</sup> by titration as phenol, <sup>(b)</sup> by photometry after derivatisation as dimethoxyphenol, <sup>(c)</sup> by titration

The concentrations of the trace elements mercury, lead, arsenic, and cadmium have also been reported in Table 2.

Table 2. Contents of trace elements in the Primary Product

Element	mg/kg
Lead (Pb)	0.268
Cadmium (Cd)	0.132
Mercury (Hg)	< 0.01
Arsenic (As)	< 0.10



### 2.3.3. Identification and quantification of the Primary Product constituents

#### 2.3.3.1. Principal constituents

The volatile fraction (40 wt. % of the Primary Product) was determined by GC analysis. 59 peaks of the chromatogram representing 78 % of the volatile mass have been identified and quantified. The 20 most abundant constituents are listed in Table 3.

The constituents could be assigned to the following chemical classes: acids (6.2 wt. % of the Primary Product), alcohols (0.6 wt. %), aldehydes (1.3 wt. %), aromatic compounds (0.9 wt. %), furans (1.9 wt. %), guaiacols (1.6 wt. %), ketones (2.7 wt. %), phenols (0.4 wt. %), pyrans (0.3 wt. %), sugars (8.4 wt. %), syringols (6.7 wt. %), others (0.4 wt. %), and unknowns (9 wt. %).

An analysis of the unknown non-volatile fraction for carbohydrates showed the presence of 0.4% fructose and 0.35% glycerol.

Table 3. Principal constituents of the Primary Product

Compound	Sample 1 (Flr4r1) [wt. % ]	Sample 2 (Flr4r2) [wt. % ]
Acetic acid*	5.9	5.8
Levoglucozan*	5.2	5.5
2,6-Dimethoxyphenol* (syringol)	1.9	1.9
4-Methyl-2,6-dimethoxyphenol* (4-methylsyringol)	1.6	1.6
Hydroxypropanone*	1.2	1.2
Hydroxyacetaldehyde*	1.2	1.2
Anhydrosugar**	1.1	1.1
2-Hydroxy-3-methyl-2-cyclopentene-3-one*	1.0	1.0
5-Hydroxymethyl-2-furaldehyde*	0.8	0.9
1,2-Ethandiol*	0.6	0.7
Anhydrosugar**	0.6	0.7
1,2-Dihydroxyphenol** (catechol)	0.6	0.6
(E)-2,6-Dimethoxy-4-propenylphenol**	0.6	0.7
4-Ethyl-2,6-dimethoxyphenol* (4-Ethyl syringol)	0.6	0.6
1,4:3,6-Dianhydro- $\beta$ -D-glucopyranose**	0.6	0.6
Anhydrosugar**	0.6	0.7
4-Allyl- and 4-Propyl-2,6-dimethoxyphenol*	0.5	0.6
Maltol**	0.5	0.03
2-Methoxy-4-methylphenol* (4-methyl guaiacol)	0.4	0.4
(5H)-Furan-2-one*	0.4	0.4
Syringaldehyde*	0.4	0.4

\*: Quantified by GC-FID with reference compound and calculated response factor; \*\*: Quantification using assumed response factor

#### 2.3.3.2. Content of Polycyclic Aromatic Hydrocarbons (PAHs)

The concentrations of the 15 PAHs listed in the EFSA guidance document (EFSA, 2005) have been analysed in Smoke Concentrate 809045 using a validated method that fulfilled the performance criteria of Commission Regulation No 627/2006 (EC, 2006). The data were confirmed by measurements performed by a second laboratory. Table 4 shows contents determined by analysis of 11 batches. The average total content of quantified PAHs was 29.8  $\mu\text{g}/\text{kg}$ .

The contents of benzo[*a*]pyrene and benzo[*a*]anthracene are below their respective limits of 10 and 20 µg/kg as laid down in Regulation (EC) No. 2065/2003.

Table 4. Concentrations of PAHs (average of 11 batches)

Compound	Content [µg/kg]	SD [µg/kg]	RSD [%]
Chrysene	9.8	5.4	52
Benzo[ <i>a</i> ]anthracene	8.7	5.2	57
5-Methylchrysene	1.5	0.7	91
Cyclopenta[ <i>cd</i> ]pyrene	2.1	1.9	113
Benzo[ <i>b</i> ]fluoranthene	2.6	2.5	91
Benzo[ <i>j</i> ]fluoranthene	0.6	n a <sup>(*)</sup>	n a <sup>(*)</sup>
Benzo[ <i>k</i> ]fluoranthene	1.3	0.9	66
Benzo[ <i>a</i> ]pyrene	2.0	1.3	60
Indeno[ <i>1,2,3-cd</i> ]pyrene	0.6	0.2	59
Dibenzo[ <i>a,h</i> ]anthracene	<0.5	0.0	n a <sup>(*)</sup>
Benzo[ <i>ghi</i> ]perylene	0.6	0.3	62
Dibenzo[ <i>a,e</i> ]pyrene	<1.0	0.0	n a <sup>(*)</sup>
Dibenzo[ <i>a,h</i> ]pyrene	<1.0	0.0	n a <sup>(*)</sup>
Dibenzo[ <i>a,i</i> ]pyrene	<1.0	0.0	n a <sup>(*)</sup>
Dibenzo[ <i>a,l</i> ]pyrene	<1.0	0.0	n a <sup>(*)</sup>

(\*) n a : Not applicable (not sufficient number of data points); SD: standard deviation; RSD: relative standard deviation

#### 2.3.4. Batch-to-batch variability

19 batches of the Primary Product from different production dates covering a time span of approximately two years were used to demonstrate batch-to-batch variability. Average values and standard deviations provided for extinction and refractive index, for contents of water, phenols, carbonyls, acids and for the PAHs benzo[*a*]pyrene and benz[*a*]anthracene are listed in Table 5.

Table 5. Parameters determined in 19 batches of the Primary Product

Parameter		Average	± SD	% SD	
Extinction	[400 nm]	2.4	0.25	10.6	
Refractive Index		1.5	0.01	0.61	
Water	[%]	11.2	2.37	21.2	
Phenols	[%]	method 1	22	4.97	22.6
		method 2	0.5	0.06	11.6
Carbonyls	[%]	14.1	3.81	27.0	
Total Acid Value	[mg KOH/g]	method 3	12.6	0.68	5.4
		method 4	110	8.23	7.5
Benzo(a) pyrene	[µg/kg]	2.6	2.1	80.7	
Benz(a) anthracene	[µg/kg]	8.6	5.36	62.2	

method 1: By titration as phenol; method 2: By photometry after derivatisation as dimethoxyphenol; method 3: By titration; method 4: Method not defined

For 14 PAHs information has been provided on the concentrations in 11 batches (Table 4). The relative standard deviations ranged between 52 % (chrysene) and 113 % (cyclopenta[*cd*]pyrene).

Information has also been provided on the concentrations of lead, mercury, cadmium, and arsenic in 5 batches. The relative standard deviations were calculated by the Panel to range between 23 and 48 %.

The Panel considers the observed batch-to-batch variability of the Primary Product acceptable.

### 2.3.5. Stability

Gas chromatograms provided for 13 different batches looked visually similar. Although no numerical data on individual peaks and/or compounds were presented the Panel does not consider the stability of the Primary Product to be an issue.

### 2.3.6. Specifications

The specifications as provided by the applicant for Primary Product Smoke Concentrate 809045 are given in Table .

Table 6. Specifications of Smoke Concentrate 809045

<b>Flash point</b>	> 100°C	<b>Refractive index</b>	1.48 – 1.56
<b>Water</b>	5-15% w/w	<b>GC</b>	comparable to standard
<b>Benzo[a]pyrene</b>	< 10 µg/kg	<b>Benz[a]anthracene</b>	< 20 µg/kg
<b>Lead</b>	< 10 mg/kg	<b>Cadmium</b>	< 1 mg/kg
<b>Mercury</b>	< 1 mg/kg	<b>Arsenic</b>	< 1 mg/kg

## 3. Proposed uses

Normal and upper use levels as provided 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 Table 7.

Table 7. Normal and upper use levels of Primary Product 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	0.007	0.03
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	0	0
5 Confectionery	0	0
6 Cereals and cereal products, including 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	0.016	0.09
9	Fish and fish products, including molluscs, crustaceans and echinoderms	0	0
10	Egg and egg products	0	0
11	Sweeteners, including honey	0	0
12	Salts, spices, soups, sauces, salads, protein products etc.	0.018	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	0
15	Ready-to-eat savouries	0.09	0.45
16	Composite foods (e.g. casseroles, meat pies, mincemeat) - foods that could not be placed in categories 1 – 15	0	0

#### 4. Dietary exposure assessment

In order to estimate dietary exposure to the Primary Product, the Panel used two different methodologies, developed 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 for different European countries (Linseisen *et al.*, 2006). The CEF 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 (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.

Normal and upper use levels for the breakdown of the food category 12 "Salts, spices, soups, sauces, salads, protein products etc." were requested to the applicant. This was considered necessary in order to reduce the overestimation of exposure due to the heterogeneity of this food category that contains products presenting significant differences in terms of consumption.

Dietary exposure estimates calculated by means of the above mentioned methods are reported in Table 8. Dietary exposures from all sources range from 0.29 to 0.50 mg/kg bw/day, when assuming

that the Primary Product is present at the upper use levels, and from 0.06 to 0.13 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 by means of the two methods developed *ad hoc* by the CEF Panel for the specific case of smoke flavourings. 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 of 0.04 and 0.21 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 two different methods making use of food consumption data taken from the Dutch National Food Consumption Surveys (DNFCS) and poundage data, respectively. The dietary exposure calculated by the applicant by means of food consumption figures was 0.08 mg/kg bw/day. This estimate is obtained by assuming that all the foods in the food categories were smoke flavoured with the respective average recommended flavour dosage. Using the total amount of Primary Product sold in 2003, dietary exposure was estimated as 0.0006 mg/kg bw/day.

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	0.03	0.15
	Other foods not traditionally smoked	0.10	0.35
	Beverages (alcoholic or non-alcoholic)	0.00	0.00
	Total dietary exposure	0.13	0.50
SMK-EPIC	Traditionally smoked food	0.04	0.21
	Other foods not traditionally smoked	0.02	0.08
	Beverages (alcoholic or non-alcoholic)	0.00	0.00
	Total dietary exposure	0.06	0.29
Applicant	Dietary exposure	0.08	NA

## 5. Toxicological data

### 5.1. Identity of the test material

Smoke Concentrate 809045 was tested in a subchronic 90-days toxicity study performed according to OECD guidelines (OECD 408). The study was conducted on material designated as Flüssigrauch ZP-S-1011 809045, batch no. 38510552. The batch numbers are not provided for the batches used for the chemical characterisation. The applicant provided only specifications for the Smoke Concentrate 809045 and claimed that the batches used for testing chemical composition were

representative of the product used for the toxicity studies. The Panel notes that only limited experimental data to support this claim were provided.

## 5.2. Subchronic toxicity

In the subchronic toxicity study, groups of Wistar rats (10 animals of each sex per group) were given Smoke Concentrate 809045 in the diet for 90 days at nominal dietary concentrations of 0 (control), 1000, 3000 and 15000 mg/kg diet (TNO, 2000). The test material was mixed in the diet and high-performance liquid chromatographic (HPLC) analyses were conducted to monitor the stability, homogeneity and achieved concentration of the test substance, based on two principal marker peaks. Fresh batches of experimental diets were prepared at weekly intervals and achieved concentrations monitored on four batches of diets during the study. The test article was stable in the diet and was homogeneously distributed in the mid- and high-dose diets. The low-dose diet was up to 46% lower than the nominal level on three of four occasions that analysis was performed, which was attributed by the applicant to inhomogeneity. The content of the high dose diet was close to the nominal value for all but the last batch which was 17% low. The variability of the mid-dose diet ranged from -22% to +14% of the nominal value.

All animals were observed for clinical symptoms twice daily on week-days and once daily at week-ends. Body weights were recorded at the start and at weekly intervals thereafter. Ophthalmoscopic examinations were conducted prior to treatment and in the last week in control and high-dose groups only. During the last week, urinalysis was performed, and detailed haematology, clinical chemistry tests and gross pathological examinations were carried out at termination. At autopsy, organ weights were recorded (10 organs) and relative organ weights calculated. Complete histopathological examinations were performed on tissues from the control and high-dose groups and on a limited number of tissues (kidney, liver, lungs and gross lesions) from the mid-dose group.

As is normal, the food intake relative to body weight decreased with age throughout the test. The achieved intakes of the test material on a body weight basis were calculated by the applicant from the nominal dietary concentrations, mean weekly food consumption and mean body weights at start and end of each week. On this basis, the mean intakes were 0, 64, 197 and 971 mg/kg bw/day in males and 0, 74, 224 and 1089 mg/kg bw/day in females in the control, low-, mid- and high-dose groups, respectively.

No mortality was observed during the study and there was no treatment-related change in clinical signs. Food and water consumption, and body weight gain were unaffected by treatment in male rats. In females in the high-dose group only, body weight gain was significantly decreased by about 10% throughout the study associated with a reduced food intake, which might have been due to palatability problems; the low and intermediate groups were unaffected.

No ophthalmological changes were observed after 90-days administration of the test diets and there were no significant changes in haematological or clinical chemical parameters or in urine composition. Renal concentrating ability measured in the final week was unaffected by treatment.

The absolute and relative organ weights were not affected by treatment except for an increase in relative brain weight in high-dose females attributed to the lower terminal body weight.

When administered in the diet, Smoke Concentrate 809045 at a mean daily dose of 1089 mg/kg bw/day caused a decrement of body weight gain in females and no other adverse effects. The Panel notes that this decrease in body weight gain was accompanied by reduced food intake and not by any effects in the other parameters investigated, and that it was also not observed in males. Therefore the Panel concludes that the decrement of body weight gain in the females is not

toxicologically relevant. There were no treatment-related changes in males up to the highest dose tested of 971 mg/kg bw/day. The Panel derived an overall NOAEL of 1000 mg/kg bw/day.

### 5.3. Genotoxicity

Smoke Concentrate 809045 has been evaluated in tests for gene mutations in bacteria (OECD Guideline 471)(LAB, 1998a), gene mutations in mammalian cells *in vitro* (OECD Guideline 476) (TNO, 2005) and chromosomal aberrations in mammalian cells *in vitro* (OECD Guideline 473) (TNO, 2004). It was also tested for genotoxicity *in vivo* in the mouse micronucleus assay (OECD Guideline 474) (LAB, 1998b) and a rat liver Unscheduled DNA synthesis (UDS) assay (TNO, 2007). The studies were conducted in compliance with GLP requirements.

The bacterial gene mutation assay was conducted with *Salmonella typhimurium* strains TA1535, TA1537, TA1538, TA98 and TA100 using the standard plate incorporation assay with and without metabolic activation at concentrations of 50 to 5000 µg/plate. The experiment was repeated after a 3-day interval using the same protocol. No study was performed using a preincubation protocol.

In the absence of metabolic activation, cytotoxicity was observed towards TA1537 and TA1538 at 1500 µg/plate and towards the other strains at 5000 µg/plate. With metabolic activation, cytotoxicity was observed towards strains TA1537, TA1538 and TA98 at 5000 µg/plate. In the first experiment, Smoke Concentrate 809045 induced a significant concentration-dependent increase in mutation frequency in TA100, of up to 1.7 fold both with and without metabolic activation. These results were not reproduced in the second study in which the maximum mutation frequency was not increased in the presence of metabolic activation and increased only 1.3-fold in the absence of metabolic activation with no concentration dependence. Although the authors concluded from the first study that Smoke Concentrate 809045 was mutagenic to *Salmonella typhimurium* TA100 in the presence and absence of a metabolizing system, the Panel concluded that overall these results were equivocal.

In the test for gene mutations in eukaryotic cells, mouse lymphoma L5178Y cells were incubated with Smoke Concentrate 809045 for 4 and 24 hours in the absence and presence of metabolic activation. Cytotoxicity was observed at concentrations of 26 µg/ml in the absence of S9-mix and 49 µg/ml in the presence of S9. Relatively more small than large colonies were formed in the presence and absence of S9 after 4 hours which was considered to be indicative of clastogenic potential. Both in the absence and presence of S9-mix, under all conditions, positive responses were observed at dose levels causing less than 90% cell toxicity. The Panel concluded that Smoke Concentrate 809045 is mutagenic at the TK locus in L5178Y cells.

A test for clastogenicity (chromosomal aberrations) in Chinese Hamster Ovary (CHO) cells *in vitro* was conducted at concentrations of 25, 50 and 125 µg/ml in the absence of metabolic activation and 100, 125 and 250 µg/ml in the presence of S9-mix; dose levels were selected based on mitotic index. In a repeat experiment on a separate occasion, concentrations of 50, 75 and 100 µg/ml were used without metabolic activation and 150, 200 and 300 µg/ml in the presence of S9-mix. In the second study, the protocol used both pulse treatment (4h treatment harvested after 18h) and continuous treatment for 18h. In the first test, a statistically significant increase in aberrant cells was observed at the highest concentration tested without (but not with) metabolic activation. In the second experiment, an increase in aberrant cells was seen at the highest concentration tested in the pulse assay both in the absence and presence of metabolic activation and at the two highest concentrations in the continuous assay without metabolic activation. The Panel concluded that Smoke Concentrate 809045 was clastogenic to CHO cells.

In an *in vivo* mouse micronucleus assay, Smoke Concentrate 809045 was administered orally to groups of five male and five female NMRI mice at a dose level of 2000 mg/kg bw. Bone marrow smears were examined 24, 48 and 72 hours after treatment. The test material did not induce a significant increase in micronuclei; in some animals, the PCE:NCE ratio was decreased after 72 hours.

In an *in vivo* UDS assay conducted to GLP requirements, Smoke Concentrate 809045 was administered to male Wistar rats, strain CRL: [W1] WU BR rats by gavage in corn oil at a limit dose of 2000 mg/kg bw. Group sizes were 6 animals in the negative control and test groups and 2-3 animals respectively in positive control groups receiving 2-AAF or NDMA. After 2-4 hours and 12-16 hours, animals were sacrificed by perfusion and hepatocytes were isolated. Hepatocytes in monolayer culture were incubated with [methyl-<sup>3</sup>H]-thymidine and examined by autoradiography. Animals treated with NDMA or 2-AAF served as controls at the 2-4 hour and 12-16 hour time points, respectively.

Smoke Concentrate 809045 did not increase the mean net nuclear grain count in hepatocytes relative to untreated control rats. The positive controls induced the expected increase in UDS. It was concluded that the validity of the assay was confirmed and that Smoke Concentrate did not induce increased UDS under the conditions of the assay.

#### 5.4. Other studies

No other studies on Smoke Concentrate 809045 were provided by the applicant.

### 6. Discussion

The applicant provided adequate information on the identity, composition and variability of the Primary Product. The mass of identified constituents amounted to 31 wt. % of the Primary Product (78 % of the volatile fraction). The Panel noted that this is slightly lower than the proportion of 80 % of the volatile fraction required to be identified and quantified according to Commission Regulation (EC) 627/2006 (EC, 2006). Considering the measurement uncertainties of the analytical methods and the variations in the batch-to-batch variability the Panel considered that these values could be regarded as to be in compliance with the requirements of Commission Regulation (EC) 627/2006.

In order to estimate dietary exposure to the Primary Product Smoke Concentrate 809045, the CEF Panel used two different methodologies, developed 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 (EC).

Dietary exposures from all sources range from 0.29 to 0.50 mg/kg bw/day, when assuming that the Primary Product Smoke Concentrate 809045 is present at the upper use levels and from 0.06 to 0.13 mg/kg bw/day when normal use levels are considered.

When dietary exposure estimates are based on use in only in traditionally smoked foods dietary exposures range from 0.15 to 0.21 mg/kg bw/day when assuming that the Primary product Smoke Concentrate 809045 is present at the upper use levels, and from 0.03 to 0.04 mg/kg bw/day, when normal use levels are considered.

Two of the genotoxicity tests on Smoke Concentrate 809045 *in vitro*, the mammalian cell (mouse lymphoma) mutagenicity assay and a clastogenicity assay in CHO cells, were considered positive. Conversely, Smoke Concentrate 809045 was negative in an *in vivo* mouse micronucleus assay and



in a rat liver UDS assay, each conducted at a maximum tolerated dose. The results obtained in these assays showed positive genotoxic responses in the two *in vitro* assays but negative results *in vivo* in two well conducted studies.

Overall it is concluded that Primary Product Smoke Concentrate 809045 is genotoxic *in vitro*, whereas two *in vivo* genotoxicity tests were negative and sufficient to eliminate the concerns over the *in vitro* genotoxicity.

In the sub-chronic 90-day study in rats, the NOAEL was 1000 mg/kg bw/day the highest dose level tested.

Based on these data it is concluded that, when assuming that the Primary Product Smoke Concentrate 809045 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 1000 mg/kg bw/day derived from the 90-day toxicity study in rats amounts to 2000 - 3400 for the intake estimates based on the upper use levels and to 7700 - 17000 when normal use levels are considered.

When assuming the use of Primary Product Smoke Concentrate 809045 in traditionally smoked products only, the margins of safety would amount to 4800 - 6700 for the intake estimate based on the upper use levels and to 25000 - 33000 when normal use levels are considered (Table 9).

The Panel considered that given i) the conservative assumptions in its exposure estimates and ii) the fact that the NOAEL was the highest dose level tested, the use of the Primary Product Smoke Condensate 809045 at the proposed uses and use levels does not give rise to safety concern.

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	0.06 - 0.13	1000	7700 - 17000
	Upper	0.29 - 0.50	1000	2000 - 3400
Traditionally smoked food	Normal	0.03 - 0.04	1000	25000 - 33000
	Upper	0.15 - 0.21	1000	4800 - 6700

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 Smoke Concentrate 809045 in such products was not assessed.

## CONCLUSIONS AND RECOMMENDATIONS

The Panel concluded that the technical description of the manufacturing process is sufficient, and that the analytical methods used for the chemical characterisation of Smoke Concentrate 809045, and the content of PAHs are in compliance with legislation.

Smoke Concentrate 809045 is genotoxic *in vitro*, but not *in vivo*. The NOAEL is derived from a 90-day study and amounts to 1000 mg/kg bw/day, the highest dose level tested.

Given the margin of safety of at least 2000 and the fact that this margin of safety is based on a conservative exposure estimate and a NOAEL that is the highest dose level tested, the Panel

concludes that the use of Smoke Concentrate 809045 at the proposed uses and use levels is not of safety concern.

**DOCUMENTATION PROVIDED TO EFSA**

1. Dossier from Symrise, June 2005.
2. Responses from Symrise to request for supplementary information.

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## GLOSSARY / 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
CHO	Chinese Hamster Ovary cell line
DNFCS	Dutch National Food Consumption Surveys
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
HPLC	High Performance Liquid Chromatography
mTAMDI	modified TAMDI
NOAEL	No-Observed-Adverse-Effect Level
OECD	Organisation for Economic Cooperation and Development
PAH	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