SCIENTIFIC OPINION

Scientific Opinion on Safety of smoke flavour Primary Product – AM 01[1]

EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF) [2,3]

European Food Safety Authority (EFSA), Parma, Italy

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ABSTRACT

This opinion concerns the safety of the smoke flavouring Primary Product AM 01. The Panel noted the shortcomings in the provided analytical data for the characterisation. The product has been tested toxicologically in *in vitro* and *in vivo* genotoxicity studies and in a 90-day feeding study in rats. A positive result was obtained in one of three *in vitro* genotoxicity tests, and another test was inconclusive. *In vivo*, no indication of DNA damage was obtained in a Comet assay of limited validity. In the light of the reduction of WBC in both sexes and a reduction of lung weight in male at the highest dose level of 500 mg/kg bw/day, the Panel concluded that the NOAEL in the 90-day study was 250 mg/kg bw/day. Given the limitations in the data set, the Panel concluded that the genotoxic potential in vivo of the Primary Product AM 01 can not be ruled out. Furthermore, the Panel noted that there were low margins of safety based on the NOAEL in the 90-day study. Therefore, the use of the substance at the intended uses and use levels would be of safety concern.

KEY WORDS

Smoke flavouring, Primary Product, AM 01

1 On request from the European Commission, Question No EFSA-Q-2005-269, adopted on 26 November 2009.

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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 AM 01.

The Primary Product AM 01 is obtained from beech wood (*Fagus sylvatica L.*). The production of AM 01 comprises the following steps: (i) pyrolysis of wood particles in a smoke generator under controlled conditions, (ii) condensation of the hot vapors, (iii) dissolution of the raw product in a solvent with subsequent cleaning with active charcoal, (iv) distillation of the solution to the desired concentration of AM 01.

The water content of the Primary Product is estimated as 91 wt.%. The identified volatile fraction as determined by gas chromatographic analysis accounts for 3.1 wt.% of the Primary Product, corresponding to 75 wt.% of the volatile fraction, which is not in compliance with Commission Regulation (EC) 627/2006. The total identified mass represents 95 wt.% of the Primary Product, corresponding to 35 wt.% of the solvent-free fraction. This is not in compliance with 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 smoke flavouring Primary Product have been provided; they were all below 10 µg/kg. The measurements were performed by a non accredited laboratory using a not validated method. In addition, 8 PAHs were determined by another accredited laboratory using a validated method.

The analytical characterisation of the product showed a large degree of batch-to-batch variability along with evidence of significant compositional changes during the shelf life of the product. This variability in the composition gives rise to an extra degree of uncertainty in this assessment since it is unclear to what extent the batch(es) tested toxicologically is representative of to the material of commerce.

The genotoxicity studies indicated that the Primary Product AM 01 was positive in an *in vitro* assay for gene mutations at the hprt locus in V79 cells, only in the absence of metabolic activation. Negative results were obtained in a bacterial mutation test, while an *in vitro* micronucleus assay was considered inconclusive. *In vivo*, no indication of DNA damage in lymphocytes and hepatocytes of rats treated orally with Primary Product AM 01 for 14 days was obtained in a Comet assay of limited validity. No information was available on the possible induction of genotoxic effects at the site of first contact.

In a subchronic 90-day study in rats with Primary Product AM 01, the no-observed-adverse-effect level (NOAEL) was 250 mg/kg bw/day by gavage, based on a reduction in white blood cell count in both sexes and of lung weight in male at the highest dose level of 500 mg/kg bw/day.

The applicant provided two data sets for use levels in each of the 18 food categories as outlined in Commission Regulation (EC) No 1565/2000 (EC, 2000), one submitted originally in 2005, and the second in June 2009, after consulting with customers. For transparency reasons both the initially provided data from 2005 and the updated data from 2009 were considered.

In order to estimate dietary exposure to the Primary Product AM 01, 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 AM 01 is present at the normal or upper use levels provided by the applicant for the 18 food categories as outlined in Commission Regulation (EC).

Considering the initial data provided on use levels in 2005, the dietary exposures from all sources were 16.7 and 35.0 mg/kg bw/day, when assuming that the Primary Product AM 01 is present at the upper use levels, 11.6 and 25.8 mg/kg bw/day, when normal use levels are considered.

Considering the updated information on use levels from 22 June 2009, the dietary exposures from all sources were 12.9 and 15.5 mg/kg bw/day, when assuming that the Primary Product AM 01 is present at the upper use levels, 8.3 and 11.9 mg/kg bw/day, when normal use levels are considered.

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

Considering both, the initial data on use levels provided in 2005 and the new ones, the highest dietary exposures estimates, resulting from the SMK-EPIC model, were 6.1 and 8.7 mg/kg bw/day when using normal and upper use levels, respectively. With the SMK-TAMDI model these figures were 3.3 and 5.0 mg/kg bw/day, respectively.

Based on the data provided by the applicant on 22 June 2009 for total dietary exposure (traditionally and non-traditionally smoked food), the margins of safety, as compared to the NOAEL of 250 mg/kg bw/day derived from the 90-day toxicity study with Primary Product AM 01 in rats, amount to 16 and 19 for the intake estimates based on the upper use levels and to 21 and 30 when normal use levels are considered.

When assuming the use of Primary Product AM 01 in traditionally smoked products only, the margins of safety would amount to 29 and 50 for the intake estimates based on the upper use levels and to 41 and 76 when normal use levels are considered.

Given the limitations in the data set, the Panel concluded that the genotoxic potential of the Primary Product AM 01 *in vivo* can not be ruled out. Furthermore, the Panel noted that there were low margins of safety based on the NOAEL in the 90-day study. Therefore, the use of the substance at the intended uses and use levels would be of safety concern.

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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 such foods. With the development of other methods of preservation this function of smoking decreased in importance over time and the sensory aspects prevailed.

Nowadays liquid smoke flavourings are added to various foods either 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) and 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 and 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 and 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.

ASSESSMENT

1. Introduction

The following evaluation only applies to the Primary Product AM 01 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 on the manufacturing process, the composition, intended use levels and toxicological tests have been submitted. The latter include a 90-day oral subchronic study and three *in vitro* genotoxicity tests. One *in vivo* genotoxicity test has also been provided. Though not required, an acute oral toxicity study had been performed on Primary Product AM 01 in rats.

2. Information on existing authorisations and evaluations

No information on existing evaluation of the Primary Product AM 01 has been provided.

3. Technical data

3.1. Manufacturing Process

3.1.1. Source materials for the Primary Product

The Primary Product is obtained exclusively from chemically untreated beech wood (*Fagus sylvatica L.)* particles named "Räuchergold", type KL 2-16 with a particle size between 4-12 mm.

3.1.2. Method of manufacture of the Primary Product

Sawdust with an adjusted moisture content is pyrolysed in a temperature-controlled smoke generator. The hot volatiles are cooled and purified with water in a spray tower. The condensate then passes through scrubbing towers operated with ethanol/water mixtures. This solution is further purified by active charcoal and represents the Primary Product. Operational details on temperatures of pyrolysis and condensation, residence times and purification were provided by the applicant.

3.2. Identity of the Primary Product

3.2.1. Trade names of the Primary Product

The trade name of the Primary Product is AM 01.

3.2.2. Physical state of the Primary Product

The Primary Product is described by the applicant as a dark brown oily liquid with a density of 0.885 kg/l and a refractive index of 1.3832. The Panel noted the low density. It can be explained, as the solvent of AM 01 is an ethanol/water mixture. At the given density the ethanol concentration is estimated to be about 60 wt.%

3.3. Chemical Composition of the Primary Product

The overall characterisation of the Primary Product is as follows:

3.3.1. Overall characterisation

3.3.1.1. Solvent free-fraction

According to the production process, an ethanol/water mixture (approx. 60/40 wt.%) functions as the solvent of the Primary Product AM 01. A solvent content of 91.4 wt.% was determined by heating the sample to 105 °C. Accordingly, the solvent-free fraction of the Primary Product amounts to 8.6 wt.% (Figure 1).

3.3.1.2. Volatile fraction

The amount of the volatile fraction was determined by two methods:

a) Gravimetric method: The Primary Product was heated to 200 °C. The residual, non-volatile fraction amounts to 4.2 wt %. Accordingly, the volatile fraction amounts to 4.4 wt.%, calculated as solvent-free fraction minus non-volatile fraction (8.6 wt.% – 4.2 wt.%). This represents 51 wt. % of the solvent-free fraction (Figure 2). The Panel noted that this method would result in a considerable loss of organic compounds.

b) GC/FID method: The Primary Product was analysed by capillary gas chromatography (GC). Mass spectrometry was used for identification and flame ionisation detection (FID) for quantification, employing external standard calibration. 1 wt.% of the GC chromatogram remained unidentified. The identified constituents determined by GC/MS analysis amount to a total of 3.1 wt.% of the Primary Product, representing 75 wt.% of the volatile fraction (as determined indirect gravimetrically to be 4.4 wt.%). This is not in compliance with Commission Regulation (EC) 627/2006 (EC, 2006), which requires 80%. However, the Panel noted the uncertainties resulting from the use of the external standard calibration for quantification.

3.3.1.3. Unidentified constituents

The unidentified constituents amount to 5.6 wt. % of the Primary Product. The total identified mass (3.1 wt. %) corresponds to 35 % of the solvent-free fraction. This is not in compliance with Commission Regulation (EC) 627/2006 (EC, 2006), which requires 50 %. However, the Panel noted the drawbacks of gravimetric method described in section 3.3.1.2 a).

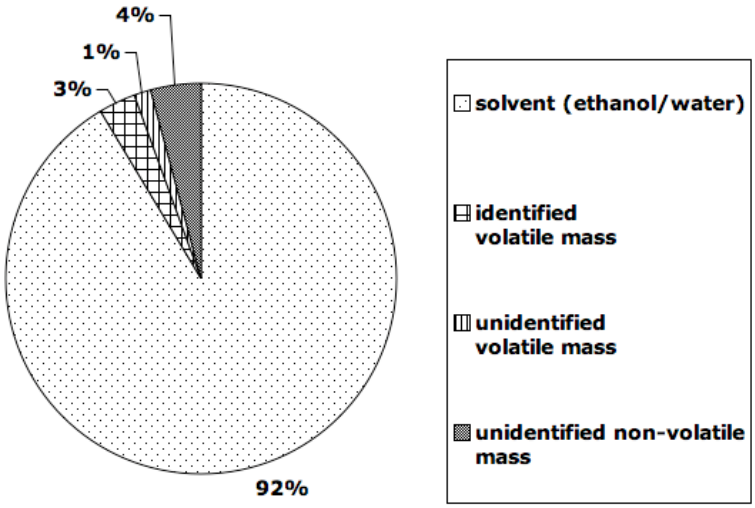

Figure 1. **Overall composition of AM 01 (wt.% of Primary Product)**

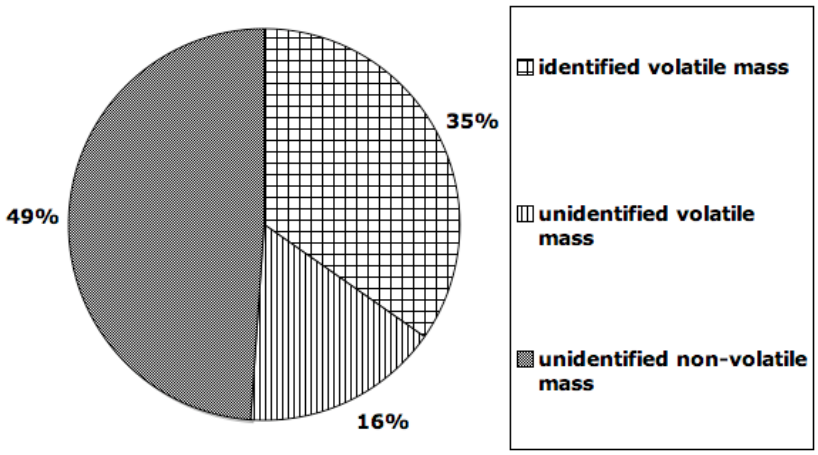

Figure 2. **Composition (wt.%) of the solvent-free fraction of AM 01**

3.3.2. Chemical description of the Primary Product

The constituents of the Primary Product have been assigned to the following chemical classes: carboxylic acids (4.1 g/kg), carbonyls (7.6 g/kg), phenols (10.4 g/kg) and others (7.8 g/kg).
The content of selected heavy metals has been reported as follows: arsenic < 0.1 mg/kg, cadmium: < 0.03 mg/kg, mercury: < 0.005 mg/kg, lead: < 0.17 mg/kg.

3.3.3. Identification and quantification of the Primary Product constituents

3.3.3.1. Principal constituents

The 23 principal constituents of the Primary Product are listed in Table 1. In addition, 19 constituents identified and quantified below 0.5 g/kg have been reported. Determination of the constituents with gas chromatography/flame ionisation detection (GC/FID) resulted in a total of 30.8 g/kg. For quantification, the external standard calibration was applied using eugenol as reference.

Table 1. **Principal constituents of the Primary Product AM 01**

Component	g/kg
Acetic acid	3.9
Syringol	2.7
Levoglucosan	2.0
4-methylsyringol	1.6
2,5 diethoxytetrahydrofuran	1.5
4-methylguaiacol	1.2
Guaiacol	1.1
2-furaldehyde	1.0
4-hydroxy-4-methyl-2-pentanone	1.0
2-hydroxy-3-methyl-2-cyclopenten-1-one	0.9
5-hydroxymethyl-2-furfural	0.8
2,6-dihydroxy-4-methoxyacetophenone	0.8
1-hydroxy-2-butanone	0.7
2,2-diethoxyethanol	0.6

Ethyl-4-acetoxybutanoate	0.6
2-(5H)-furanone	0.5
3-methyl-2-cyclopentene	0.5
4-methylphenol (p-Cresol)	0.5
1,1-diethoxyhexane (valeraldehyde-diethylacetal)	0.5
3-methoxy-1,2-benzenediol (methoxycatechol)	0.5
4-ethylguaiacol	0.5
1,1-diethoxyheptane	0.5
1,2,3,6-penta-O-acetyl-D-glucose	0.5

3.3.3.2 Content of the Polycyclic Aromatic Hydrocarbons (PAHs)

A total of 15 PAHs were determined in the Primary Product AM 01, lot no. 10/05. The sample was measured at an external laboratory (Laboratory I). Details of the method were provided by the applicant. However, the method has not been validated. In addition, the applicant provided data obtained using a validated method by another external laboratory (Laboratory II accredited), which analysed 8 of the PAHs. The combined results are presented in Table 2.

Table 2. **Concentrations of PAHs in the Primary Product AM 01 (lot no. 10/05)**

Compound	**Laboratory I** **µg/kg**	**Laboratory II (accredited)** **µg/kg**
Chrysene	5.0	<5.0
Benzo[*a*]anthracene	7.0	8.46
5-Methylchrysene	<1.0	n/a
Cyclopenta[*c*,*d*]pyrene	2.5	n/a
Benzo[*b*]fluoranthene	2.0	<5.0
Benzo[*j*]fluoranthene	1.5	n/a
Benzo[*k*]fluoranthene	1.5	<5.0
Benzo[*a*]pyrene	2.5	3.09
Indeno[1,2,3-*cd*]pyrene	2.0	<5.0
Dibenzo[*a*,*h*]pyrene	<0.3	n/a
Benzo[*g*,*h*,*i*]perylene	2.0	<5.0
Dibenzo[*a*,*e*]pyrene	<0.2	n/a
Dibenzo[*a*,*h*]anthracene	<0.5	<5.0
Dibenzo[*a*,*i*]pyrene	<0.2	n/a
Dibenzo[*a*,*l*]pyrene	<0.2	n/a

n/a not analysed

3.3.4 Batch-to-batch variability

Batch-to-batch variability was demonstrated by GC/MS/FID data for a batch produced in 2005 (#10/05) and two batches produced within the same week in 2007 (#02/07 and #03/07) (Table 3). The average relative standard deviation for the samples produced within 2 weeks was 9.2 %, ranging from

0.0 to 25 %. When comparing to the sample from 2005, the average relative standard deviation was 39.6 %, ranging from 9 to 88 %.

Table 3. **Concentrations of main components in various lots of the Primary Product AM 01**

No.	Compound	Lot #: 10/05 (g/kg)	Lot #: 10/05S (g/kg)	Lot #: 02/07 (g/kg)	Lot #: 03/07 (g/kg)
1	Acetic acid	3.90	2.33	1.77	1.35
5	1-hydroxy-2-butanone	0.70	0.15	0.18	0.16
7	2–furancarboxaldehyde	1.00	0.46	0.44	0.39
8	4–hydroxy-4-methyl-2-pentanone	1.00	1.54	1.51	1.52
12	2(5H)-furanone	0.50	0.25	0.23	0.23
14	2,2-diethoxy ethanol	0.60	0.19	0.22	0.17
15	3-methyl-2-cyclopentene	0.50	0.18	0.21	0.16
18	Ethyl 4-acetoxybutanoate	0.60	0.20	0.21	0.29
19	2,5-diethoxy tetrahydrofuran	1.50	0.31	0.42	0.5
20	2-hydroxy-3-methyl-2-cyclopenten-1-one	0.90	0.63	0.64	0.62
23	4-methylphenol (p-cresol)	0.50	0.24	0.3	0.21
26	2-methoxy phenol (guaiacol)	1.10	0.61	0.67	0.63
29	2-methoxy-4-methyl phenol	1.20	0.88	0.89	0.84
33	5-(hydroxymethyl)-2-furancarboxaldehyde	0.80	0.50	0.46	0.39
34	3-methoxy-1,2-benzenediol	0.50	0.95	0.9	1.04
35	4-ethyl-2-methoxy phenol	0.50	0.80	0.79	0.8
37	2,6-dimethoxy phenol (syringol)	2.70	3.06	3.02	2.98
38	1,1-diethoxy heptane	0.50	0.25	0.28	0.26
40	2,6-dimethoxy-4-methyl phenol (methyleugenol)	1.60	2.35	2.3	2.21
42	Levoglucosan	2.00	2.28	1.9	1.38
43	1-(2,6-dihydroxy-4-methoxyphenyl) ethanone	0.80	0.98	0.94	0.94
45	1,2,3,4,6-penta-O-acetyl-D-glucose	0.5	0.36	0.34	0.32

3.3.5. Stability

Data on the stability of the Primary Product were limited to a comparison of GC/MS/FID data for a batch produced in 2005 (#10/05) to those of the batch stored for 2.5 years (#10/05S). The average relative standard deviation was 42.5 %, ranging from 9.3 to 93 %.
The applicant recommends a maximum storage time of 12 months in airtight containers at a dark and cold place.

3.3.6. Specifications

Specifications as provided by the applicant are presented in Table 4.

Table 4. **Specifications of the Primary Product AM 01**

Carbonyls	min. 6 g/kg
Phenols	min. 8 g/kg
Benzo[*a*]pyrene	max. 8 µg/kg
Benz[*a*]anthracene	max. 16 µg/kg
Specific gravity	885.5 kg/m^3 @ 20 °C
Refractive index	1.3832 @ 20 °C
Stability	12 months

4. Proposed uses

Normal and upper use levels as described originally by the applicant in June 2005 for the Primary Product in each of the 18 food categories as outlined in Commission Regulation (EC) No 1565/2000 (EC, 2000) are reported in Table 5a.

Table 5a. **Normal and upper use levels for the Primary Product in food categories as outlined in Commission Regulation (EC) No 1565/2000 (Data provided in June 2005)**

Food categories		Use level (g/kg)	
		Normal	Upper
1	Dairy products, excluding products of category 2	2.5	3
2	Fats and oils and fat emulsions (type water-in-oil)	1.5	3
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	1.5	3
7	Bakery wares	1.5	3
8	Meat and meat products, including poultry and game	2	3
9	Fish and fish products, including molluscs, crustaceans and echinoderms	2	3
10	Egg and egg products	0	0
11	Sweeteners, including honey	0	0
12	Salts, spices, soups, sauces, salads, protein products etc.	2.5	3
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	2.5	3
15	Ready-to-eat savouries	1.5	3
16	Composite foods (eg. casseroles, meat pies, mincemeat) - foods that could not be placed in categories 1 - 15	2	3

After consulting with the customers the applicant provided updated use levels for the different food categories on 22 June 2009. These data are presented in Table 5b.

Table 5b. **Normal and upper use levels for the Primary Product in food categories as outlined in Commission Regulation (EC) No 1565/2000 (Data provided on 22 June 2009)**

Food categories		Use level (g/kg)	
		Normal	Upper
1	Dairy products, excluding products of category 2	2.5	3
2	Fats and oils and fat emulsions (type water-in-oil)	1.5	3
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	1.0	1.5
7	Bakery wares	1.5	3
8	Meat and meat products, including poultry and game	2	3
9	Fish and fish products, including molluscs, crustaceans and echinoderms	2	3
10	Egg and egg products	0	0
11	Sweeteners, including honey	0	0
12	Salts, spices, soups, sauces, salads, protein products etc.	≤ 2.5§	3
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	0.05	0.1
15	Ready-to-eat savouries	1.5	3
16	Composite foods (eg. casseroles, meat pies, mincemeat) - foods that could not be placed in categories 1 - 15	0.2	1.5

§The following normal use levels for the breakdown of the food category 12 "Salts, spices, soups, sauces, salads, protein products etc." were provided by the applicant and used to assess the exposure: 1.5 g/kg for Salt and salt substitutes (food category12.1), 1 g/kg for Herbs, spices, seasonings and condiments (food category 12.2), 2.5 g/kg for Soups and broths (food category 12.5) and 0 for the remaining subgroups.

5. Dietary exposure assessment

In order to estimate dietary exposure to the Primary Product AM 01, 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 approach 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 1565/2000 (EC, 2000). When the normal use levels are used, the SMK-TAMDI can be considered as an adaptation of the modified TAMDI (mTAMDI), the method used by the AFC Panel (EFSA, 2004) to screen and prioritise flavouring substances.

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

The applicant provided two data sets for use levels, one submitted originally in 2005, and the second in June 2009. After consulting with the customers the applicant provided updated use levels for the different food categories on 22 June 2009.

Dietary exposure estimates calculated by means of the above mentioned methods are reported in Table 6a and b. For transparency reasons both the initially provided data from 2005 and the updated data from 2009 were considered.

Considering the initial data provided on use levels in 2005 the dietary exposures from all sources were 16.7 and 35.0 mg/kg bw/day, when assuming that the Primary Product is present at the upper use levels, 11.6 and 25.8 mg/kg bw/day, when normal use levels are considered (Table 6a).

Considering the updated information on use levels from 22 June 2009, the dietary exposures from all sources were 12.9 and 15.5 mg/kg bw/day when assuming that the Primary Product is present at the upper use levels, 8.3 and 11.9 mg/kg bw/day when normal use levels are considered (Table 6b).

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

Considering both the data provided by the applicant in 2005 and in 2009, the SMK-EPIC model results in the highest exposure estimates: 6.1 and 8.7 mg/kg bw/day when using normal and upper use levels, respectively (Table 6a and b). With the SMK-TAMDI model these figures were 3.3 and 5.0 mg/kg bw/day, respectively (Table 6a and b).

Table 6a. **Summary of the dietary exposure estimates to the Primary Product (based on use levels provided in June 2005)**

Methodologies		Dietary exposure (mg/kg bw/day)	
		Normal use levels	Upper use levels
SMK-TAMDI	Traditionally smoked food	3.3	5.0
	Other foods not traditionally smoked	10.0	15.0
	Beverages (alcoholic or non-alcoholic)	12.5	15.0
	Total dietary exposure	25.8	35.0
SMK-EPIC	Traditionally smoked food	6.1	8.7
	Other foods not traditionally smoked	3.8	5.9
	Beverages (alcoholic or non-alcoholic)	1.8	2.1
	Total dietary exposure	11.6	16.7
Applicant	Dietary exposure	-a	-a

[a] Not provided

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

Table 6b. **Summary of the dietary exposure estimates to the Primary Product (based on use levels provided on 22 June 2009)**

Methodologies		Dietary exposure (mg/kg bw/day)	
		Normal use levels	Upper use levels
SMK-TAMDI	Traditionally smoked food	3.3	5.0
	Other foods not traditionally smoked	8.3	10.0
	Beverages (alcoholic or non-alcoholic)	0.3	0.5
	Total dietary exposure	11.9	15.5
SMK-EPIC	Traditionally smoked food	6.1	8.7
	Other foods not traditionally smoked	2.3	4.1
	Beverages (alcoholic or non-alcoholic)	0.0	0.1
	Total dietary exposure	8.3	12.9
Applicant	Dietary exposure	-[a]	-[a]

[a]: Not provided

6. Toxicological data

6.1. Identity of the test material

The material used for the genotoxicity studies was described as smoke flavouring Primary Product AM 01, batch 10/05. The material used for the subchronic study was similarly described as Lot No. 10/05. The composition of this material was described in respect of the content of arsenic, cadmium,

mercury, lead and nine polycyclic aromatic hydrocarbons. No information was given concerning the material tested in the *in vivo* Comet assay.

6.2. Subchronic toxicity

A 90-day oral toxicity study (SMU, 2006) of smoke flavouring Primary Product AM 01 was performed in Wistar SPS strain rats of both sexes. The study was conducted according to the current OECD Guideline 408 and in compliance with GLP. The Primary Product was administered to groups of 10 males and 10 females daily by gavage in water vehicle at doses of 0, 25, 250 or 500 mg/kg bw/day in a total volume of 1ml/100g bw.

No mortality occurred during the study and no clinical signs or behavioural changes were observed throughout. There were no treatment-related changes in body weight and growth rate.
At termination, no treatment-related changes were seen in gross pathology. No changes in organ weights were noted in females but in males there was a decrease in lung weight of approximately 15% at the highest dose but this effect was not accompanied by histological changes and was not seen in females. There was a treatment- but not dose-related increase in liver weight, maximally 19%, in the intermediate dose group. This change was not considered adverse. A small but statistically significant increase in heart weight was seen in the low and middle dose groups but not in the high dose group males and this change was not considered to be due to treatment.
There were no treatment-related histological changes reported in any of the tissues examined. With the exception of the reduced lung weight in males in the top dose group, the Panel considered these reported changes to be incidental and not toxicologically relevant since they were not accompanied by any histological changes. However, the Panel determined that the reduced lung weight should be considered as an adverse consequence of treatment.
Statistically significant differences from controls were reported in several haematological parameters. These generally were small or not dose-related or both. However, a significant reduction in white blood cells (WBC) of 25% and 45% in males and females respectively was observed at the highest dose and this was considered by the Panel to be treatment- and dose-related. Some statistically significant changes in clinical chemistry parameters were reported but these were small, sporadic and not considered of toxicological significance.

The authors of the report do not derive a NOAEL from this study but conclude that the substance is "non-toxic". In the light of the reduction of WBC in both sexes and a reduction of lung weight in male at the highest dose level of 500 mg/kg bw/day, the Panel concluded that the NOAEL was 250 mg/kg bw/day.

6.3. Genotoxicity

The genotoxic potential of smoke flavouring Primary Product AM 01 (Batch 10/05) was tested in three *in vitro* genotoxicity assays. All genotoxicity studies were stated to have been conducted according to current OECD Guidelines and in compliance with GLP.

The Primary Product did not induce toxicity nor gene mutations in a bacterial assay (Slovnaft Vùrup, 2005a) in *Salmonella typhimurium* strains TA97a, TA98, TA100 and TA102 at concentrations up to 80µl per plate, with and without metabolic activation. The top dose applied corresponded to approximately 7 mg of solvent free material, which is above the maximum recommended for non-toxic compounds in this assay.

Smoke flavouring Primary Product AM 01 was tested for mutagenic potential at the hprt locus in Chinese hamster V79 cells at concentrations of up to 0.8 µl/ml with and without metabolic activation, using an incubation time of 3 hours. In the absence S9 3- to 10-fold increased incidences of mutant clones were observed in cultures treated with the Primary Product. Such effect, although not dose-

related, was observed in two independent duplicate studies. No increase in mutation frequency was observed in the presence of S9. It is concluded that the Primary Product AM 01 is mutagenic without metabolic activation in this forward mutation assay (VULM, 2006).

The Primary Product was tested in a cytogenetic study using human peripheral lymphocytes *in vitro* (Slovnaft Vùrup, 2005b) at concentrations of up to 1µl/ml, without and with metabolic activation. In the dose range assayed (0.25 to 1 µl/ml) the Primary Product AM 01 did not induce a statistically significant increase in micronuclei nor any evidence of cytotoxicity (decreased mitotic index), while cytotoxic effects were observed at 2 and 4 µl/ml, but these dose levels were not analysed. Given the absence of treatment related cytotoxicity, this study has to be considered as inconclusive. Moreover, it is noted that in the absence of metabolic activation a dose related increase in micronucleated cells was observed, which was not adequately addressed by the limited statistical analysis performed.

An *in vivo* Comet assay (SMU, 2008) was performed on lymphocytes and hepatocytes from groups of six male Wistar rats (groups of three in the hepatocyte assay) given the Primary Product by gavage at daily doses of 0, 8.4, 84 and 850 mg/kg bw for 14 days [vehicle not indicated]. The doses selected were stated to be based on 1, 10 and 100 times the estimated human exposure. In the lymphocyte assay, no differences from control values were observed for strand breaks or oxidative damage at purine or pyrimidine sites in any of the dose groups. The small number of animals used in the hepatocyte assay precluded statistical analysis but the report claimed that there were no differences from controls in DNA damage or sensitivity to hydrogen peroxide. However, the Panel noted that this assay should be considered of limited validity because of some shortcomings with respect to the study design and reporting (*e.g.* no positive control group used, number of animals used for hepatocyte analysis was too small, time of sacrifice was not reported, number of gels per animals not reported, distribution of damaged cells not reported). The Panel also observed that the lymphocytes in this assay were frozen prior to examination and considered that this would increase baseline cell damage and the lower sensitivity. Furthermore, the Panel noted that although the authors of the study report claimed that the study had been performed according to GLP, there was no signed GLP compliance statement demonstrating that the experimental and non-experimental phases of the study had been audited. Moreover, the Panel noted that the genotoxicity of Primary Product AM 01 at the site of contact (*i.e.* the upper GI tract) was not investigated in this study which was considered to be important because the Primary Product was mutagenic *in vitro* only without metabolic activation.

6.4. Other studies

Though not required, an acute oral toxicity study had been performed on Primary Product AM 01 in rats according to OECD 423. The LD50 was reported to be >2000 mg/kg bw/day.

7. DISCUSSION

The analytical characterisation of the product showed a large degree of batch-to-batch variability along with evidence of significant compositional changes during the shelf life of the product. This variability in the composition gives rise to an extra degree of uncertainty in this assessment since it is unclear to what extent the batch(es) tested toxicologically is representative of the material of commerce.

The genotoxicity studies indicated that the Primary Product was positive in an *in vitro* assay for gene mutations at the hprt locus in V79 cells in the absence of metabolic activation. In accordance with the Panel's guidelines, an *in vivo* Comet assay was performed which was reported by the study authors to be negative. However, the Panel noted that this assay cannot be considered as being valid since there are some shortcomings with respect to the study design and reporting of methods and results (*e.g.* no positive control group used, number of animals used for hepatocyte analysis was too small, time of

sacrifice was not reported, number of gels per animals not reported, distribution of damaged cells not reported). The response of the applicant to a request of EFSA addressing these issues was not convincing. In addition, the Panel was informed that the lymphocytes were frozen prior to conducting gel electrophoresis, which would be expected to increase background damage and reduce the sensitivity. Furthermore, the Panel noted that although the authors of the study report claimed that the study had been performed according to GLP, there was no signed GLP compliance statement demonstrating that the experimental and non-experimental phases of the study had been audited. Moreover, the Panel noted that the genotoxicity of Primary Product AM 01 at the site of contact (*i.e.* the upper GI tract) was not investigated in this study which was considered to be important because the Primary Product was mutagenic *in vitro* only without metabolic activation.

Accordingly, the genotoxic potential *in vivo* of the Primary Product AM 01 can not be ruled out.

The Panel noted that changes in a number of parameters were observed in the 90-day rat study. The increase in liver weight seen in male rats was not clearly dose-dependent and was not accompanied by any histopathological changes and a similar effect was not seen in females. Therefore this change was not considered adverse. In the light of the reduction of WBC in both sexes and a reduction of lung weight in male at the highest dose level of 500 mg/kg bw/day, the Panel concluded that the NOAEL was 250 mg/kg bw/day.

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

In order to estimate dietary exposure to the Primary Product AM 01, the CEF Panel used two different methodologies, developed by the Panel specifically for smoke flavourings (EFSA, 2009). Dietary exposure estimates were calculated by assuming that the Primary Product AM 01 is present at the normal or upper use levels provided by the applicant for the 18 food categories as outlined in Commission Regulation 1565/2000.

Considering the initial data provided on use levels in 2005 the dietary exposures from all sources were 16.7 and 35.0 mg/kg bw/day, when assuming that the Primary Product is present at the upper use levels, 11.6 and 25.8 mg/kg bw/day, when normal use levels are considered.

Considering the updated information on use levels from 22 June 2009 the dietary exposures from all sources were 12.9 and 15.5 mg/kg bw/day, when assuming that the Primary Product is present at the upper use levels, 8.3 and 11.9 mg/kg bw/day, when normal use levels are considered.

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

Considering both the data provided by the applicant in 2005 and in 2009, the highest exposure estimates, resulting from the SMK-EPIC model, were 6.1 and 8.7 mg/kg bw/day when using normal and upper use levels, respectively. With the SMK-TAMDI model these figures were 3.3 and 5.0 mg/kg bw/day, respectively.

Based on the intake data originally provided by the applicant in June 2005, the margins of safety, as compared to the NOAEL of 250 mg/kg bw/day derived from the 90-day toxicity study with Product AM 01 in rats, amount to 7 and 15 for the intake estimates based on the upper use levels and to 10 and 22 when normal use levels are considered (Table 7a).

Table 7a. **Margins of safety based on the intake estimated with the data provided in June 2005**

	Use level	Dietary exposure* (mg/kg bw/day)	NOAEL (mg/kg bw/day)	Margin of safety*
Total dietary exposure	Normal	11.6 / 25.8	250	22 / 10
	Upper	16.7 / 35.0	250	15 / 7
Traditionally smoked food	Normal	6.1 / 3.3	250	41 / 76
	Upper	8.7 / 5.0	250	29 / 50

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

Based on the new data provided by the applicant on 22 June 2009 for total dietary exposure (traditionally and non-traditionally smoked food), the margins of safety as compared to the NOAEL of 250 mg/kg bw/day derived from the 90-day toxicity study with Primary Product AM 01 in rats amount to 16 and 19 for the intake estimates based on the upper use levels and to 21 and 30 when normal use levels are considered (Table 7b).

Table 7b. **Margins of safety based on the intake estimated with the data provided in April 2009**

	Use level	Dietary exposure* (mg/kg bw/day)	NOAEL (mg/kg bw/day)	Margin of safety*
Total dietary exposure	Normal	8.3/ 11.9	250	30 / 21
	Upper	12.9 / 15.5	250	19 / 16
Traditionally smoked food	Normal	6.1 / 3.3	250	41 / 76
	Upper	8.7 / 5.0	250	29 / 50

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

When assuming the use of Primary Product AM 01 in traditionally smoked products only, considering both the set of the original and updated levels provided by the applicant in 2009, the margins of safety would amount to 29 and 50 for the intake estimates based on the upper use levels and to 41 and 76 when normal use levels are considered (Table 7a and b).

The Panel did not anticipate that smoke flavourings would be used in food specifically designed for infants (0-12 months) and small children (12-36 months). Therefore, the safety of use of Primary Product AM 01 in such products was not assessed.

CONCLUSIONS

The analytical characterisation of the product showed a large degree of batch-to-batch variability along with evidence of significant compositional changes during the shelf life of the product. This variability in the composition gives rise to an extra degree of uncertainty in this assessment since it is unclear to what extent the batch(es) tested toxicologically is(are) representative of the material of commerce.

The genotoxicity studies indicated that the Primary Product AM 01 was positive in an *in vitro* assay for gene mutations, only in the absence of metabolic activation. An *in vivo* Comet assay was performed and reported to be negative. However, the Panel noted that this assay is of limited validity since there are some shortcomings with respect to the study design and reporting of methods and results. Therefore the Panel concluded that the genotoxicity *in vivo* of the Primary Product can not be ruled out. Furthermore, the Panel noted that the genotoxicity of Primary Product AM 01 at the site of contact (i.e. the upper GI tract) was not investigated in this study which was considered to be important because the Primary Product was mutagenic *in vitro* only without metabolic activation.

With regard to the 90-day study in rats, the Panel concluded that a depression in white blood cells (WBC) in both the sexes and of the lung weight in male seen at the highest dose tested should be considered adverse and established a NOAEL of 250 mg/kg bw/day on the basis of these findings.

Based on the intake data calculated with the data provided by the applicant on 22 June 2009 for total dietary exposure (traditionally and non-traditionally smoked food), the margins of safety as compared to the NOAEL of 250 mg/kg bw/day derived from the 90-day toxicity study in rats with Primary Product AM 01, amount to 16 and 19 for the intake estimates based on the upper use levels and to 21 and 30 when normal use levels are considered.
When assuming the use of Primary Product AM 01 in traditionally smoked products only the margins of safety would amount to 29 and 50 for the intake estimates based on the upper use levels and to 41 and 76 when normal use levels are considered.

Given the limitations in the data set, the Panel concluded that the genotoxic potential *in vivo* of the Primary Product AM 01 can not be ruled out. Furthermore, the Panel noted that there were low margins of safety based on the NOAEL in the 90-day study. Therefore, the use of the substance at the intended uses and use levels would be of safety concern.

DOCUMENTATION PROVIDED TO EFSA

1. Dossier submitted by AROMARCO s.r.o., June 2005.
2. Response from AROMARCO s.r.o. to request for supplementary information.

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ABBREVIATIONS

AFC	Scientific Panel on Additives, Flavourings, Processing aids and Materials in Contact with Food
bw	body weight
CEF	Scientific Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
EC	European Commission
EFSA	European Food Safety Authority
EPIC	European Prospective Investigation into Cancer and Nutrition
GC/MS	Gas Chromatography/Mass Spectrometry
GI	Gastro Intestinal
GLP	Good Laboratory Practice
GS/FID	Gas Chromatography/Flame Ionisation Detection
mTAMDI	modified TAMDI
NOAEL	No-Observed-Adverse-Effect Level
OECD	Organisation for Economic Cooperation and Development
PAHs	Polycyclic Aromatic Hydrocarbons
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
WBC	White Blood Cells