European Food Safety Authority

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SCIENTIFIC OPINION

Safety of smoke flavour Primary Product - Unismoke [1]

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

(Question No EFSA-Q-2005-267)

Adopted on 29 January 2009

This opinion, published on 11 June 2009, replaces the earlier version published on 6 April 2009[2].

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 Unismoke.

The Primary Product Unismoke is obtained from a specified mixture of oak (*Quercus alba*) and beech wood (*Fagus sylvatica*). The production of Unismoke comprises the following steps: (i) preparation of the correct oak/beech ratio and drying, (ii) pyrolysis of the wood mixture in a rotating drum kiln in an inert atmosphere, (iii) cleaning and condensing of the pyrolysis vapours, (iv) separation of tar and conditioning of the supernatant phase to the Primary Product. The applicant has provided essential parameters of the manufacturing process.

[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 – Unismoke. *The EFSA Journal* (2009) 983, 1-20.

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

Identification and quantification of Primary Product constituents have been performed in a typical batch. The water content of the Primary Product is 94 wt. %. The volatile fraction identified by capillary gas chromatographic analysis (GC) accounts for 6.7 wt. %; no unidentified constituents were reported. The concentrations of the polycyclic aromatic hydrocarbons (PAH) listed in the EFSA guidance document on submission of a dossier on a smoke flavouring Primary Product have been provided. They were all below the respective limits of detection. The levels of benzo[*a*]anthracene and benzo[*a*]pyrene were below the limits given in Regulation (EC) No 2065/2003. Analysis of ten batches demonstrated acceptable batch-to-batch variability. No data on the stability of the Primary Product were provided.

Maximum use levels of the Primary Product proposed by the applicant are given for meat and meat products (5 g/kg) and salts, spices, soups, sauces, salads, protein products etc. (4 g/kg). Dietary exposure for the Primary Product, as estimated by the applicant, was 17 mg/kg bw/day.

In order to estimate dietary exposure to the Primary Product Unismoke, 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 Unismoke 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 12.7 to 21.7 mg/kg bw/day, when assuming that the Primary Product Unismoke is present at the upper use levels, and from 10.1 to 16.7 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 10.8 mg/kg bw/day, when assuming that the Primary Product Unismoke is present at the upper use levels, and from 6.7 to 8.7 mg/kg bw/day, when normal use levels are considered.

Unismoke showed negative results in the *S. typhimurium* reverse mutation assay in strains TA1535, TA1537 and TA98, but positive results in TA100 both in the absence and presence of S9. Therefore, the Panel concluded that it is positive in this bacterial mutagenicity tests.

Positive results were also obtained in the mouse lymphoma assay, both in the absence and presence of S9. In a test for chromosomal aberrations in human lymphocytes Unismoke showed evidence of clastogenic activity in both the absence and presence of S9.

The *in vivo* bone marrow micronucleus assay was negative without significant depression of the PCE:NCE ratio and an *in vivo* rat liver DNA repair test was also negative.

Overall it is concluded that Unismoke 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 toxicity study with Unismoke the no-observed-adverse-effect level (NOAEL) was the lowest dose level tested which amounted to 600 and 700 mg test material/kg bw/day for males and females, respectively, based on an increase in relative kidney weight and related changes in blood biochemistry and haematology at the higher dose levels.

Taking into account that this relates to doses of the two-fold diluted Primary Product these NOAELs amount to doses of 300 and 350 mg Primary Product/kg bw/day for males and females, respectively. The Panel derived an overall NOAEL of 300 mg/kg bw/day.

Based on these data it is concluded that when assuming that the Primary Product Unismoke 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 derived from the 90-day toxicity study in rats amount to 14 - 24 for the intake estimates based on the upper use levels and to 18 - 30 when normal use levels are considered.

When assuming the use of Primary Product Unismoke in traditionally smoked products only the margins of safety would amount to 28 - 36 for the intake estimates based on the upper use levels and to 34 - 45 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 Unismoke would require a larger margin of safety. The Panel concludes that the margin of safety is insufficient and that the use of Primary Product Unismoke 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 Unismoke 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, Unismoke.

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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: Davide Arcella, Angelo Carere, Karl-Heinz Engel, David Gott, Jørn Gry, Rainer Gürtler, Ionna Pratt, Ivonne Rietjens, Rupert Simon and Ron Walker. The valuable contribution of Dietrich Meier is also acknowledged.

ASSESSMENT

The following evaluation applies only to the Primary Product Unismoke 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 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 Unismoke has been provided.

2. Technical data

2.1 Manufacturing process

2.1.1 Source material for the Primary Product

The raw material is a 50:50 mixture of oak (*Quercus alba*) and beech wood (*Fagus sylvatica*). The wood ratio is verified by adding ammonia as staining reactant. The wood mixture is dried and each batch (approx. 7-8 t/batch) is further tested for pentachlorophenol (PCP) (reporting limit 5 mg/kg).

2.1.2 Method of manufacture of the Primary Product

Details of the manufacturing parameters were provided by the applicant comprising:

Pyrolysis step. Wood chips are pyrolysed in an inert atmosphere in the presence of steam using a rotating kiln. Wall temperatures and product gas temperatures are controlled over the whole period. Feeding rates, residence times, and pyrolysis temperatures have been provided by the applicant.

Cleaning and condensing of the pyrolysis gases. The pyrolysis vapours are cleaned from solids and rapidly cooled in a condensing system. Operational principles and temperatures have been given in the application.

Phase separation. Phase separation proceeds by pumping off the heavy fraction from the bottom of the tank and by decanting the light fraction. After eight hours of storage time, additionally formed heavy tar is discarded, and air is mixed with the light condensed smoke fraction. The crude Primary Product is analysed for carbonyl and total acid content for later standardisation through water and lye addition. The standardised Primary Product is filtered and stored.

2.2 Identity of the Primary Product

2.2.1 Trade names of the Primary Product

The trade name of the Primary Product is Unismoke.

2.2.2 Physical state of the Primary Product

Unismoke is a brown liquid at room temperature. The applicant submitted no other data on the physical state.

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

The water fraction (94 wt. %; determined by drying) of the Primary Product functions as solvent. Accordingly, the solvent-free fraction of the Primary Product amounts to 6 wt. % (Figure 1).

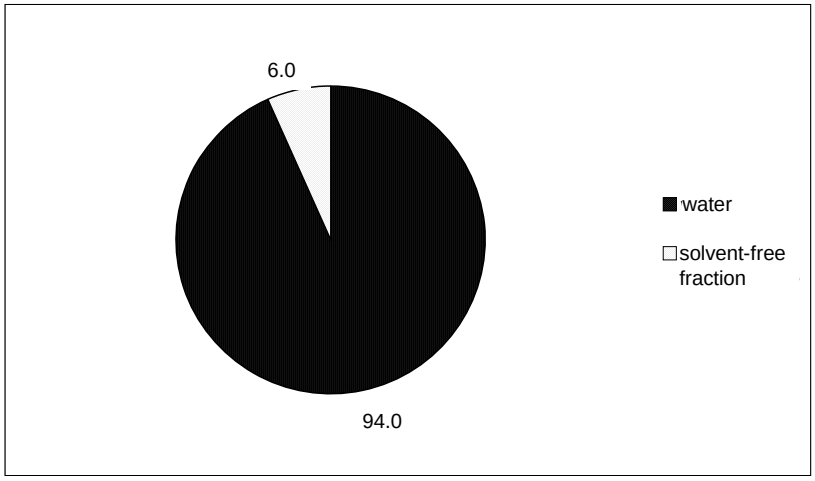

Figure 1. **Overall composition of Unismoke (wt. % of Primary Product)**

2.3.1.2 Volatile fraction

The Primary Product was analysed by capillary gas chromatographic (GC) analysis. Mass spectrometry (MS) was used for identification and flame ionisation detection (FID) for quantification. The volatile fraction determined by the applicant using response factors for single constituents amounted to 6.7 wt. %.

2.3.1.3 Unidentified constituents

No unidentified constituents were reported by the applicant.

2.3.2 Chemical description of the Primary Product

A total of 10 batches (5 batches: article code 4945, 5 batches: article code 4949) of the Primary Product have been characterised according to the parameters listed in Table 1. The applicant provided detailed descriptions of the analytical methods.

Table 1. **Description of major parameters of the Primary Product Unismoke**

	Water wt.%	pH	Acids (as acetic acid) g/l	Carbonyls (g/l)	Phenols (g/l)	Colour index	Dry matter (wt.%)
Min.	93.3	2.1	54.3	54.6	2.8	15.6	5.6
Max.	94.4	2.4	70.0	69.1	4.4	18.8	6.6
Mean	93.8	2.2	59.8	66.1	3.8	17.1	6.2
No. of batches	2	8	7	8	8	7	2
STD	n/a	0.08	5.5	5.0	0.6	1.1	n/a
Rel. STD (%)	n/a	3.6	9.2	7.6	15.8	6.4	n/a

n/a: not applicable

2.3.3 Identification and quantification of Primary Product constituents

2.3.3.1 Principal constituents

GC analysis resulted in the determination of 23 components listed in Table 2. No further components were reported by the applicant.

Table 2. **Principal constituents of the Primary Product Unismoke (article code 4949)**

Compound	g/kg
Hydroxypropanone (Acetol)	29.7
Propanoic acid	17.6
Levoglucosan	5.3
5-Hydroxymethyl-2-furaldehyde	2.2
2-Hydroxy-3-methyl-2-cyclopentene-1-one	1.9
2-Ethylbutanal	1.4
Furfural	1.3
Eugenol	1.3
2-[5H]-Furanone	1.3
1-Hydroxy-2-butanone	0.9
4-Ethylsyringol	0.6
2-Methoxy-4-ethylphenol (4-Ethylguaiacol)	0.5
Dihydromethylfuranone	0.4
1,2-Cyclopentanedione	0.4
2-Methoxyphenol (Guaiacol)	0.4
Guaiacylacetone	0.3
γ -Hydroxymethyl-γ-butyrolactone	0.3
2,4-Pentanedione (Acetylacetone)	0.2
2-Methoxy-4-methylphenol (4-Methylguaiacol)	0.2
Hydroxypropanal	0.2
2-Methoxy-4-propylphenol (4-Propylguaiacol)	0.2
Propiosyringone	0.2
Syringaldehyde	0.1
TOTAL	67.1

2.3.3.2 Content of Polycyclic Aromatic Hydrocarbons (PAHs)

The contents of polyaromatic hydrocarbons (PAHs) were determined using a method developed by the Joint Research Centre of the European Commission (Simon *et al.*, 2006a; Simon *et a*l., 2006b). The method fulfilled the performance criteria of Commission Regulation No 627/2006 (EC, 2006).

The concentrations of the 15 PAHs determined in the Primary Product (article code 4949; sample S2645503) are listed in Table 3. They are all below the respective limits of detection. The levels of benzo[*a*]anthracene and benzo[*a*]pyrene are significantly below the limits (20 µg/kg and 10 µg/kg, respectively) as laid down in Commission Regulation (EC) No 2065/2003 (EC, 2003).

Table 3. **Concentrations of PAHs in the Primary Product (article code 4949; sample S2645503)**

Compound	**(µg/kg)**
Chrysene	<0.02
Benzo[*a*]anthracene	<0.02
5-Methylchrysene	<0.03
Cyclopenta[*c,d*]pyrene	<0.01
Benzo[*b*]fluoranthene	<0.09
Benzo[*j*]fluoranthene	<0.07
Benzo[*k*]fluoranthene	<0.1
Benzo[*a*]pyrene	<0.11
Indeno[*1,2,3-cd*]pyrene	<0.21
Dibenzo[*a,h*]anthracene	<0.25
Benzo[*g h i*]perylene	<0.25
Dibenzo[*a,e*]pyrene	<0.35
Dibenzo[*a,h*]pyrene	<0.54
Dibenzo[*a,i*]pyrene	<0.55
Dibenzo[*a,l*]pyrene	<0.28

2.3.4 Batch-to-batch variability

Ten batches of the Primary Product from different production dates covering a time period of 7 years were used to demonstrate batch-to-batch variability. Minimum, maximum and mean values determined for water, pH, acetic acid, carbonyls, phenols, colour, and dry matter are shown in Table 1. The Panel considers the observed variability as acceptable.

2.3.5 Stability

The applicant did not present analytical data on storage effects. He only stated that the Primary Product has a shelf life of twelve months and that beyond this time precipitation takes place.

2.3.6 Specifications

The specifications provided for the Primary Product are shown in Table 4.

Table 4. **Specifications of the Primary Product Unismoke**

Acids, as acetic acid (g/l)	54-60
Carbonyls, as butanone (g/l)	60-74
Phenols, as dimethoxyphenol (g/l)	3.6-4.2
pH	2.2-2.6
Colour index	16-20

The Panel noted that these figures are not fully consistent with the data provided for the 10 batches in Table 1.

The Panel also noted that according to the data given in Table 4 the solvent-free (organic) fraction would be higher than the amounts determined by drying (6%) and GC analysis (6.7 %), respectively. However, it has to be considered that the sum parameters shown in Table 4 were obtained using different analytical methods and are therefore not directly comparable.

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 5.

Table 5. **Normal and upper use levels of the Primary Product in food categories as outlines in Commission Regulation (EC) No 1565/2000**

Food categories		Use level (g/kg)	
		Normal	Upper
1	Dairy products, excluding products of category 2	0	0
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	4	5
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.	3	4
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	0
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 Unismoke, the Panel used two different methodologies developed by the Panel specifically for smoke flavourings (EFSA, 2009).

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

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

Dietary exposure estimates were calculated by assuming that the Primary Product is present at the normal or upper use levels provided by the applicant for the 18 food categories as outlined in Commission Regulation (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 from 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 6. Dietary exposures from all sources range from 12.7 to 21.7 mg/kg bw/day, when assuming that the Primary Product is present at the upper use levels, and from 10.1 to 16.7 mg/kg bw/day, when normal use levels are considered.

The impact on exposure of authorizing 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: 8.7 and 10.8 mg/kg bw/day when using normal and upper use levels, respectively.

It is noteworthy to highlight that exposure estimates are influenced by the fact that the applicant requested the use of the Primary Product in only two food categories: "Meat and meat products, including poultry and game" and "Salts, spices, soups, sauces, salads, protein products etc" (expressly in "Soups and broths").

Dietary exposure to the Primary Product was also estimated to be equal to 17.0 mg/kg bw/day by the applicant. This estimate was obtained by combining upper use levels for each of the above-mentioned two food categories with food consumption data (high percentiles among consumers only) obtained from the UK National Diet and Nutrition Survey (NDNS) in adults aged 16-64, 1986-87 Only the food category resulting in the highest potential dietary exposure was then considered.

Table 6. **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	10.0	13.3
	Beverages (alcoholic or non-alcoholic)	0.0	0.0
	Total dietary exposure	16.7	21.7
SMK-EPIC	Traditionally smoked food	8.7	10.8
	Other foods not traditionally smoked	1.4	1.9
	Beverages (alcoholic or non-alcoholic)	0.0	0.0
	Total dietary exposure	10.1	12.7
Applicant	Dietary exposure	-	17.0

5. Toxicological data

5.1 Identity and stability of the batch of Unismoke used for toxicological studies

The material used for the genotoxicity studies was Unismoke (Liquid Smoke Unilever) with the article code 4933.

The article code of the material used for the 90-days toxicity study was 4960 (internal laboratory codes S14271T5, S14274T2 and S14274 T1). The applicant indicates that it is assumed that a pH adjustment was made to ensure there were no palatability issues in the 13-week study. The applicant confirmed that the 90-day study was conducted on a diluted material (i.e. a secondary product from) of Unismoke primary material. This implies that the dose given as a percentage in the feed represents a percentage of the adjusted (diluted) Primary Product.

The applicant indicates that a number of batches of Unismoke were used as the test material for the 90-day study. Samples which contained carbonyl compounds in excess of 30g/liter were diluted with water to standardize them to 30g/liter. Given that the carbonyl content of the Primary Product is 60 -74 g/l, it is concluded that the material tested in the 90-day study was a 2-fold dilution of the Primary Product.

The batch of material used for all genotoxicity studies had the article code number 4933. Quality control analysis of this material was conducted by the applicant and found to be equivalent to those having the article code numbers 4960, 4926 and 4949.

5.2 Subchronic toxicity

A 90-day subchronic toxicity study was conducted according to GLP guidelines as applied at that time (ESL, 1986). There is no study director signature page in the final report. Legislation

for GLP was introduced in Europe in 1988, which was two years after the study was reported and 4 years after the study was conducted. Up until that time the OECD Principles of 1981 were advisory only.

The applicant indicates that there is significant evidence in the raw data package to show GLP compliance, for example a Quality Assurance Statement, Study Protocol, Standard Operating Procedures and Records of Quality Assurance audits of all relevant study phases and activities.

Groups of rats (20 male and 20 female rats per dose group) were fed for 90-days on diets containing article code 4960 (internal batch numbers S14274 T5, S14274 T2 and S14274 T1) at levels amounting to 0% (control), 0.5-1.0%, 1.5-3.0% and 3.0-6.0% (increasing with increasing time of exposure to match the decreasing feed intake on a body weight basis) in their diet resulting in mean daily intakes for males and females of respectively 0.6 and 0.7 g/kg bw/day, 1.9 and 2.2 g/kg bw/day and 3.8 and 4.4 g/kg bw/day. Taking into account that this relates to doses of the two-fold diluted Primary Product these dose levels amount to doses of 0.30 and 0.35 g Primary Product/kg bw/day, 0.85 and 1.10 g Primary Product/kg bw/day, and 1.9 and 2.2 g Primary Product/kg bw/day.

No rats died during the study and there were no overall treatment-related effects on growth.

Plasma and serum levels of various analytes were significantly different from control after 90-days feeding with Unismoke. For the males these included increased sodium and serum albumin, decreased lactate dehydrogenase, alkaline phosphatase and creatinine all in the 3.0-6.0% diet group, decreased magnesium in the 1.5-3.0% diet group, increases in inorganic phosphate for all dose groups and a decrease in aspartate transaminase in the two highest dose groups. For the females these changes included increased sodium and decreased alanine transaminase in the 3.0-6.0% diet group, decreased aspartate transaminase and creatinine in the two highest dose groups, decreased creatine kinase in the mid dose group and increased serum albumin, albumin to globulin ratio, and decreased alpha-1-globulin and beta-globulin in the lowest and highest dose group.

The following haematological parameters were significantly different from the control groups after 90-days feeding with the highest dose group: increased packed cell volume, haemoglobin and red cell count for the males and increased number of neutrophils for the females. For the females significantly increased mean cell volume was detected in the two highest dose groups.

The applicant indicates that the biochemical effects are not considered to be toxicologically relevant, because: i) Unismoke treatment-related increases were small and within the normal range for rats of this age, ii) plasma levels of certain enzymes were significantly decreased whereas they would be expected to increase if the treatment was toxic, iii) no associated morphological changes were detected histologically, and iv) the absence of changes in blood biochemistry in the 90-days recovery groups.

Furthermore, the applicant indicates that the significant differences in haematological measurements were not considered to be toxicologically important because of the very small size of the changes detected. Based on these considerations the applicant concluded that the no observed adverse effect level (NOAEL) is 3.0-6.0 % in the diet, the highest dose tested, which resulted in intakes of 3.8 and 4.4 g test material/kg bw/day for males and females, respectively. Taking into account that this relates to doses of the two-fold diluted Primary Product these NOAELs amount to 1.9 and 2.2 g Primary Product/kg bw/day for males and females respectively.

The Panel considered that the haematology at high dose shows increased packed cell volume, indicative of hypovolaemia, and renal involvement. The clinical chemistry demonstrates a

variety of changes, including decreased creatinine (implying higher glomerular filtration), and altered ionic homeostasis; this indicates renal involvement, and is consistent with the hypovolaemia. Small though statistically significant increases (up to at most 14%) in the absolute and relative weights of liver and kidneys were seen in both male and female rats fed Unismoke-containing diets in the two highest dose groups. No histopathological changes were detected for these organs. Furthermore, the liver and kidney weight returned to control values after a 90-day recovery period. In spite of the fact that the kidney weight increases were not accompanied by histological changes and were reversible upon a 90-day recovery period, the Panel considers these effects toxicologically relevant because 1) they were dose-dependent, 2) the recovery period was considered too long to take the results into account and 3) the kidney effects likely result from the perturbation of ionic homeostasis as indicated by the changes in blood biochemistry and haematology.

Therefore the Panel concluded that the no observed adverse effect level (NOAEL) is 0.5-1.0% in the diet, the lowest dose tested, which resulted in intakes of 600 and 700 mg test material/kg bw/day for males and females, respectively. Taking into account that this relates to doses of the two-fold diluted Primary Product these NOAELs amount to doses of 300 and 350 mg Primary Product/kg bw/day for males and females respectively. The Panel derived an overall NOAEL of 300 mg/kg bw/day.

5.3 Genotoxicity

Unismoke with article code 4933 has been evaluated in tests for induction of gene mutations in bacteria (HRC, 1995a), gene mutations in mammalian cells *in vitro* (HRC, 1995d) and chromosomal aberrations in mammalian cells *in vitro* (HRC, 1995b). It has also been tested in the *in vivo* mouse micronucleus test (HRC, 1995c) and an *in vivo* rat liver DNA repair test (HRC, 1995e). Since all tests were performed in 1994/1995 they were not performed according to the most recent OECD guidelines, but they did conform to the OECD based GLP guidelines at that time.

The assay for gene mutation in bacterial cells for Unismoke article code 4933 (sample no S2091401) was negative in *Salmonella typhimurium* strains TA 1535, TA1537 and TA98 at concentration up to 5000 μg/plate. A dose-dependent increase was observed with strain TA100 in both the absence and presence of S9 mix amounting to increases up to 1.9- and 3.1- fold at 5000 μg/plate respectively in the absence and presence of S9 and up to 2.6- and 3.9-fold at 7500 μg/plate in the absence and presence of S9. It was concluded by the applicant that Unismoke with article 4933 was mutagenic in this bacterial system.

Unismoke with article code 4933 (Unilever sample no S2063201) was tested for mutagenic potential in an *in vitro* mammalian cell mutation assay using mouse lymphoma L5178YTK+/- cells. Increases in mutant frequencies were observed in all of the tests, both in the absence and presence of S9 also at concentrations that showed moderate cytotoxicity (for example: without S9 a non significant 1.3-fold increase at 100 μg/ml with 82% survival, a significant 2.5-fold increase at 250 μg/ml with 66% survival and a significant 15-fold increase at 500 μg/ml with 1% survival, and with S9 a non significant 1.3-fold increase at 100 μg/ml with 82% survival, a significant 1.9-fold increase at 250 μg/ml with 55% survival and a significant 4.2-fold increase at 500 μg/ml with 20% survival). It was concluded by the applicant that Unismoke with article code 4933 demonstrates mutagenic potential in this *in vitro* mammalian cell mutation assay.

A test for chromosomal aberrations in human lymphocytes *in vitro* was also performed. In the absence of S9, Unismoke with article code 4933 (sample no S2063201) caused a statistically

significant 7.1-fold increase in chromosomal aberrations when compared to the solvent control, at the highest concentration tested of 468.8 µg/ml. In the presence of S9 there was a statistically significant 7.1-fold increase in the number of aberrant cells at 1250 µg/ml in the first test and a dose-dependent significant increase at all concentrations tested (625, 937.5 and 1250 µg/ml giving rise to respectively a 3.5-, 5.3- and 5.8-fold increase) in the second test. All these increases were reported to be outside the historical control range. It was concluded by the applicant that in this test Unismoke with article code 4933 shows evidence of clastogenic activity in both the absence and presence of S9.

In the *in vivo* mouse micronucleus test dose levels of 500, 1000 and 2000 mg/kg bw of Unismoke with article code 4933 (sample no S2091401) given by single intragastrical gavage were tested. Mice treated with Unismoke 4933 did not show any significant increase in the frequency of micronucleated polychromatic erythrocytes. There was no significant decrease in the ratio of polychromatic to normochromatic erythrocytes (PCE:NCE ratio) after treatment of the animals with Unismoke 4933.

An *in vivo* rat liver DNA repair test was also performed with Unismoke with article code 4933 (sample no S2091401 (sub.001B)). Unismoke was assessed for induction of DNA repair in hepatocytes following acute oral administration of 600 and 2000 mg/kg bw to albino Sprague-Dawley male rats (n=4 per dose group). Hepatocytes were isolated at 2 and 14 hours after exposure. Unismoke did not cause an increase in either the gross nuclear grain count or the net nuclear grain count (*i.e.* the gross nuclear grain count minus the cytoplasmatic grain count) at any dose level at either sampling time. The applicant concludes that Unismoke does not induce unscheduled DNA synthesis in the rat liver.

5.4 Other studies

No other studies on Unismoke were provided by the applicant.

6. Discussion

The applicant provided information on the identity, composition and variability of the Primary Product. Data on the stability of the product are missing.

Unismoke showed negative results in the *S. typhimurium* reverse mutation assay in strains TA1535, TA1537 and TA98, but positive results in TA100 both in the absence and presence of S9. Therefore, the Panel concludes that it is positive in this bacterial mutagenicity test.

Positive results were also obtained in the mouse lymphoma assay, both in the absence and presence of S9. In a test for chromosomal aberrations in human lymphocytes Unismoke showed evidence of clastogenic activity in both the absence and presence of S9.

The *in vivo* bone marrow micronucleus assay was negative without significant depression of the PCE:NCE ratio and an *in vivo* rat liver DNA repair test was also negative.

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

The Panel concluded that the no observed adverse effect level (NOAEL) in the subchronic toxicity study with Unismoke is 0.5-1.0% in the diet, the lowest dose tested, which resulted in intakes of 600 and 700 mg test material/kg bw/day for males and females, respectively. Taking into account that this relates to doses of the two-fold diluted Primary Product these NOAELs

amount to doses of 300 and 350 mg Primary Product/kg bw/day for males and females respectively. The Panel derived an overall NOAEL of 300 mg/kg bw/day.

In order to estimate dietary exposure to the Primary Product Unismoke the Panel used two different methodologies developed by the Panel specifically for smoke flavourings. Dietary exposure estimates were calculated by assuming that the Primary Product Unismoke 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 ranges from 12.7 to 21.7 mg/kg bw/day, when assuming that the Primary Product Unismoke is present at the upper use levels, and from 10.1 to 16.7 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 10.8 mg/kg bw/day, when assuming that the Primary Product Unismoke is present at the upper use levels, and from 6.7 to 8.7 mg/kg bw/day, when normal use levels are considered.

Based on these data it is concluded that when assuming that the Primary Product Unismoke 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 derived from the 90-day toxicity study in rats amount to 14 to 24 for the intake estimates based on the upper use levels and to 18 to 30 when normal use levels are considered (Table 7).

When assuming the use of Primary Product Unismoke in traditionally smoked products only the margins of safety would amount to 28 to 36 for the intake estimates based on the upper use levels and to 34 to 45 when normal use levels are considered (Table 7).

Table 7. **Margins of safety**

	Use level	Dietary exposure (mg/kg bw/day)	NOAEL (mg/kg bw/day)	Margin of safety
Total dietary exposure	Normal	10.1 – 16.7	300	18-30
	Upper	12.7 – 21.7	300	14-24
Traditionally smoked food	Normal	6.7 – 8.7	300	34-45
	Upper	8.3 – 10.8	300	28-36

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 Produt Unismoke 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 Unismoke would require a larger margin of safety. The Panel concludes that the margin of safety is insufficient and that the use of Primary Product Unismoke 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 Unismoke might be approved for traditionally smoked products, at use levels specified, to replace smoking, is outside the remit of the Panel.

CONCLUSIONS AND RECOMMENDATIONS

Except for the missing data on the stability of the product, the technical data provided comply with the provisions given in the EFSA guidance document and in the legislation.

Unismoke is genotoxic *in vitro*, but not *in vivo*. The NOAEL derived from a 90-day study is 300 mg/kg bw/day, based on an increase in relative kidney weight and related changes in blood biochemistry and haematology at the higher dose levels.

Based on these data it is concluded that when assuming that the Primary Product Unismoke 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 derived from the 90-day toxicity study in rats amount to 14 - 24 for the intake estimates based on the upper use levels and to 18 - 30 when normal use levels are considered.

When assuming the use of the Primary Product Unismoke in traditionally smoked products only the margins of safety would amount to 28 - 36 if the intake estimates are based on the upper use levels and to 34 - 45 if 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 Unismoke would require a larger margin of safety. The Panel concludes that the margin of safety is insufficient and that the use of Primary Product Unismoke 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 Unismoke 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 from Unilever, June 2005.
2. Response from Unilever 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
NDNS	National Diet and Nutrition Survey
EC	European Commission
EFSA	European Food Safety Authority
EPIC	European Prospective Investigation into Cancer and Nutrition
FID	Flame Ionisation Detection
CHO	Chinese Hamster Ovary cell line
GC/MS	Gas Chromatography/Mass Spectrometry
GLP	Good Laboratory Practice
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