SCIENTIFIC OPINION

Safety of smoke flavour Primary Product - Fumokomp[1]

Scientific Opinion of the Panel on Food Contact Material, Enzymes, Flavourings and Processing Aids (CEF)[2]

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

This Opinion concerns the safety of the smoke flavouring Primary Product Fumokomp. The Panel considered the technical and analytical data provided acceptable to characterise the Primary Product and to demonstrate its batch-to-batch variability and stability. The product has been tested in *in vitro* and *in vivo* genotoxicity studies and in a 90-day feeding study in rats. While a positive result was obtained in one out of three *in vitro* genotoxicity tests, two *in vivo* genotoxicity tests were negative and sufficient to eliminate the concerns over the *in vitro* genotoxicity. The 90-day study was conducted before the advent of GLP, and only a summary report was available. In addition the identity of the material tested in this study is unknown. Therefore the Panel was not able to confirm the validity of the study and concluded that the toxicological data do not enable the safety of Fumokomp to be established.

KEY WORDS

Smoke flavouring, Primary Product, Fumokomp.

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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 one such smoke flavouring Primary Product, named Fumokomp.

The raw material consists of at least 85 % beech (*Fagus sylvatica* L.) wood, the remaining portion is hornbeam (*Carpinus betulus* L.). The dried material is pyrolysed. The raw wood tar is subjected to a series of fractionated vacuum distillations. Information on key operational parameters has been provided. The Primary Product is obtained by combining appropriate distillate fractions.

The Primary Product is a solvent-free distillate. The total identified mass (92 %) is in compliance with Commission Regulation (EC) 627/2006. Except for benzo[*j*]fluoranthene and cyclopenta[*cd*]pyrene, data on the concentrations of the polycyclic aromatic hydrocarbons (PAHs) listed in Annex 2 of the EFSA Guidance document have been provided. The analyses were performed by an external laboratory using a GC/MS-based method; experimental conditions but no validation or performance data were provided. Additional analyses using a validated method revealed the levels of benzo[*a*]pyrene and benzo[*a*]anthracene to be below their respective limits of 10 and 20 ug/kg given in Regulation (EC) No. 2065/2003 (EC, 2003).

The data provided on batch-to-batch variability and stability of the Primary Product were limited but considered acceptable by the Panel.

Fumokomp gave negative results in a bacterial reverse mutation assay performed in accordance with OECD Guideline 471, using *Salmonella typhimurium* TA98, TA100, TA1535, TA1537 and *Escherichia coli* WP2 uvrA, both in the absence and presence of S9. Negative results were also obtained in an i*n vitro* mammalian chromosome aberration test carried out in Chinese hamster ovary cells in accordance with OECD Guideline 471. Positive results were obtained in the mouse lymphoma L5178Y tk+/- assay, with and without S9, with increases of both large and small colonies, thus indicating the ability of the test material to induce genotoxic effects both at gene and chromosome level. However, an *in vivo* bone marrow micronucleus assay was negative and an *in vivo* rat liver unscheduled DNA synthesis test was also negative.

Overall it was concluded that Fumokomp is genotoxic *in vitro*, based on the results of a mammalian cell mutation assay, whereas two *in vivo* genotoxicity tests were negative and sufficient to eliminate the concerns over the *in vitro* genotoxicity.

In the 90-day rat dietary feeding study carried out at levels of 200 mg/kg and 400 mg/kg diet, equivalent to intakes of 10 and 20 mg/kg bw/day, the study authors concluded that the KHV smoke flavour preparation did not have any toxic effects at either dietary level. On this basis, the level of 400 mg Primary Product/kg diet appeared to be a NOAEL, equivalent to 20 mg/kg bw/day.

The Panel was unable to confirm this conclusion. The 90-day study in rats was carried out before GLP standards were introduced, the number of parameters investigated was limited, and the study report was only available as a summary. The Panel also noted that the identity of the material tested in the study is unknown. For these reasons, the Panel considered that the validity of the study could not be confirmed and hence the study was not used in the safety evaluation of Fumokomp.

In order to estimate dietary exposure to the Primary Product Fumokomp, 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) No 1565/2000.

Dietary exposure from all sources ranges from 0.13 to 0.20 mg/kg bw/day, when assuming that the Primary Product is present at the upper use levels, and from 0.08 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 ranges from 0.05 to 0.09 mg/kg bw/day when assuming that the Primary Product Fumokomp is present at the upper use levels, and from 0.03 to 0.06 mg/kg bw/day, when normal use levels are considered.

The Panel was unable to derive a NOAEL for Fumokomp and consequently, no margins of safety have been calculated.

The Panel concluded that the toxicological data do not enable the safety of Fumokomp to be established.

TABLE OF CONTENTS

Abstract ... 1
Summary ... 2
Table of contents ... 4
Background ... 5
Terms of reference ... 5
Acknowledgements ... 5
Assessment ... 6
1. Introduction ... 6
2. Information on existing authorisations and evaluations ... 6
3. Technical data ... 6
3.1. Manufacturing process ... 6
3.1.1. Source materials for the Primary Product ... 6
3.1.2. Method of manufacture of the Primary Product ... 6
3.2. Identity of the Primary Product ... 7
3.2.1. Trade names of the Primary Product ... 7
3.2.2. Physical state of the Primary Product ... 7
3.3. Chemical composition ... 7
3.3.1. Overall characterisation ... 7
3.3.2. Identification and quantification of Primary Product constituents ... 8
3.3.3. Batch-to-batch variability ... 9
3.3.4. Stability ... 10
3.3.5. Specifications ... 10
4. Proposed uses ... 10
5. Dietary exposure assessment ... 11
6. Toxicology ... 12
6.1. Identity of the test material ... 12
6.2. Subchronic toxicity ... 13
6.3. Genotoxicity ... 13
6.4. Other studies ... 15
7. Discussion ... 15
Conclusions ... 16
Documentation provided to EFSA ... 16
Abbreviations ... 19

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

ACKNOWLEDGEMENTS

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

ASSESSMENT

1. Introduction

The following evaluation applies only to the Primary Product Fumokomp 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 summary of a 90-day oral subchronic toxicity study and three *in vitro* genotoxicity tests. Two *in vivo* genotoxicity tests have also been provided.

2. Information on existing authorisations and evaluations

- Production, distribution and usage licence issued by the Hungarian authority for 1975 (Ministry of Health licence 52.990/1975.V.3.)
- Registration as a food additive issued by the Hungarian authority for 1976 (3223/1975. OÉTI)
- Recommendation for granting a manufacturing licence for 1992 (1457/1991. OÉTI)
- Manufacturing licence issued by the Hungarian authority for 1992 (OTfH 12.191.1992.7.24.ÁNTSZ)

3. Technical data

3.1. Manufacturing process

3.1.1. Source materials for the Primary Product

The raw material consists of at least 85 % beech (*Fagus sylvatica* L.) wood, the remaining portion is hornbeam (*Carpinus betulus* L.).

3.1.2. Method of manufacture of the Primary Product

The dried material is pyrolysed in a Lambiotte retort with automatic gas-rinsing. The condensable part of the gas in the retort is continuously separated; the raw wood tar is obtained by sedimentation. The wood tar is subjected to a series of fractionated vacuum distillations. Information on key operational parameters has been provided.

The Primary Product obtained by manually combining appropriate distillate fractions is first evaluated on the basis of its sensory properties. Primary Products passing this step are subsequently subjected to tests regarding physical/chemical properties (density, refraction index) and to analyses of benzo[*a*]anthracene and benzo[*a*]pyrene. Batches not complying with the specifications are returned to the intermediates and subjected to re-distillation.

According to the applicant, an internal quality control and traceability system (not specified in the application) has been established.

3.2. Identity of the Primary Product

3.2.1. Trade names of the Primary Product

The trade name of the Primary Product is Fumokomp. This name may be supplemented by "Conc".

3.2.2. Physical state of the Primary Product

The Primary Product is a viscous, oily, yellow-brown liquid with a density of 1.070 – 1.130 g/cm^3 and a refractive index (20 °C) of 1.485 – 1.525.

3.3. Chemical composition

3.3.1. Overall characterisation

The overall characterisation of the Primary Product is as follows:

3.3.1.1. Solvent-free fraction

The Primary Product is a solvent-free distillate.

3.3.1.2. Volatile fraction

The Primary Product was analysed by capillary gas chromatography (GC). Mass spectrometry (MS) was used for identification and flame ionisation detection (FID) for quantification. The identified fraction of the Primary Product amounts to 92 %.

3.3.1.3. Unidentified constituents

The fraction of unidentified constituents amounts to 8 % of the Primary Product. The total identified mass (92 % of the solvent-free fraction) is in compliance with Commission Regulation (EC) 627/2006.

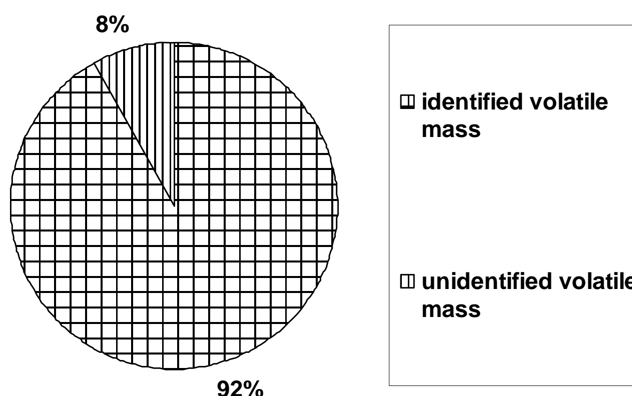

Figure 1: **Overall composition of the Primary Product Fumokomp**

3.3.2. Identification and quantification of Primary Product constituents

3.3.2.1. Principal constituents

The Primary Product was subjected to capillary gas chromatography/mass spectrometry (GC/MS). GC retention data and percentage distributions were provided for 47 constituents. Structures were assigned to 38 of the substances (91.7 %). It remained unclear which compounds were identified by comparison to authentic references and which were assigned solely on the basis of MS spectra from libraries. No data have been provided on the procedure applied to quantify the compounds. Table 1 shows the twenty principal constituents identified and quantified in the Primary Product.

Table 1: **Principal constituents of the Primary Product Fumokomp ("KHV")**

Constituent	(%)
Syringol	11.1
3-Methyl-1,2-cyclopentadione	6.6
4-Ethyl-2-methoxyphenol	5.4
m/p-Cresol	5.1
Phenol	4.9
4-Methyl-2-methoxyphenol	4.6
4-Methoxyphenol	4.6
4-Hydroxy-3-methoxybenzoic acid	4.1
2,3-Dimethylphenol	2.7
2,5-Dimethylphenol	2.6
5-Methyl-4-heptene-3-one	2.5
Butyrolactone	2.4
3-Methyl-1,2-cyclopentadiene (isomer)	2.4
2,6-Dihydroxy-4-methoxyacetophenone	2.3
3,4,5-Trimethylphenol	1.8
3-Methyl-2-cyclopentene-1-one	1.8
2,6-Dimethylphenol	1.7
Acetic acid	1.6
Hexanal	1.6
1,2,3-trimethoxy-5-methylbenzene	1.6

According to the applicant, the mass spectral data of the remaining unidentified compounds (8.3 %) indicate that they are alkoxyphenol derivatives.

3.3.2.2. Content of Polycyclic Aromatic Hydrocarbons (PAHs)

Except for benzo[*j*]fluoranthene and cyclopenta[*cd*]pyrene, the concentrations of the polycyclic aromatic hydrocarbons (PAHs) listed in Annex 2 of the EFSA Guidance document (EFSA, 2005) have been provided. Concentrations of the PAHs reported for the sample "KHV" of the Primary Product are listed in Table 2.

Table 2. **Concentrations of PAHs in the Primary Product Fumokomp (sample "KHV")**

PAH	**[µg/kg]**
Chrysene	0.005-0.05
Benzo[*a*]anthracene	0.007-0.05
5-Methylchrysene	n.d. [a]
Cyclopenta[*cd*]pyrene	- [b]
Benzo[*b*]fluoranthene	0.004-0.05
Benzo[*k*]fluoranthene	0.005-0.05
Benzo[*a*]pyrene	0.008-0.05
Indeno[*1,2,3-cd*]pyrene	0.01-0.05
Dibenzo[*a,h*]anthracene	0.04-0.06
Benzo[*ghi*]perylene	n.d.
Dibenzo[*a,e*]pyrene	n.d.
Dibenzo[*a,h*]pyrene	n.d
Dibenzo[*a,i*]pyrene	n.d.
Dibenzo[*a,l*]pyrene	n.d.
Dibenzo-perylene	0.01-0.03

[a] "not detected" according to the applicant; no data on limit of detection provided.
[b] PHA listed by the applicant but no data provided

The data given by the applicant in Table 2 had been based on analyses performed by an external laboratory using a GC/MS-method; experimental conditions but no validation or performance data had been provided. Considering the extremely low levels of PAHs reported, the Panel asked for additional confirmation. In response, the applicant reported contents of 0.5 µg/kg benzo[*a*]anthracene and 0.8 µg/kg benzo[*a*]pyrene in a batch (6363) of the Primary Product produced in May 2008, determined using a validated GC/MS-based method. These levels are below their respective limits of 10 and 20 µg/kg given in Regulation (EC) No. 2065/2003 (EC, 2003).

3.3.3. Batch-to-batch variability

For five batches of the Primary Product GC-based data on the contents of twelve selected constituents were provided (Table 3). The average relative standard deviation (RSD) was 20 %. In addition, the applicant provided ranges for these constituents in the Primary Product (Table 3). The ranges are rather broad; the basis for their determination has not been described.

Table 3. **Variability of constituents in the Primary Product Fumokomp**

Batch	**KHV**	**6080** [a]	**6080** [b]	**6183**	**6203**	**6300**	**Mean** [c]	**RSD**	**Range** [d]
Constituent					**(%)**				
Syringol	11.1	15.2	14.7	13.4	14.0	13.0	13.9	6.9	7 - 20
3-Methyl-1,2-cyclopentadione	6.6	2.8	3.4	3.4	3.1	3.8	3.3	13.0	3 - 13
4-Ethyl-2-methoxyphenol	5.4	2.7	2.6	3.3	2.4	2.8	2.8	13.4	1 - 9
m/p-Cresol	5.1	3.9	4.2	4.3	4.2	4.5	4.2	5.9	4 - 10
Phenol	4.9	1,6	1.6	3.1	1.2	2.5	2.3	42.8	1 - 8
4-Methyl-2-methoxyphenol	4.6	4.6	4.8	3.8	6.1	3.6	4.5	25.1	0.5 - 8
4-Methoxyphenol	4.6	2.2	2.3	4.2	2.1	3.9	3.1	35.6	1 - 8

4-Hydroxy-3-methoxybenzoic acid	4.1	8.0	8.0	7.9	8.1	6.5	7.6	9.9	2 - 8
Butyrolactone	2.4	1.0	1.1	1.0	1.0	1.4	1.1	18.2	1 - 5
2,6-Dihydroxy-4-methoxyacetophenone	2.3	5.9	5.5	4.0	7.3	5.1	5.6	24.9	1 - 8
2,6-Dihydroxy-4-ethoxyacetophenone	1.5	2.9	4.2	1.8	4.4	3.4	3.1	34.6	1 - 5
Acetic acid	1.6	1.0	1.3	1.3	1.2	1.2	1.2	10.7	1 - 8

[a] analysis in June 2005; [b] analysis in February 2008; [c] average of data from batches KHV, 6080a, 6183, 6203 and 6300; [d] basis for determination not described by the applicant

3.3.4. Stability

Batch 6080 of the Primary Product was analysed in June 2005, subsequently stored in the refrigerator and re-analysed in February 2008. As shown in Table 3, the variability of the twelve constituents determined by GC was quite low.

3.3.5. Specifications

The specification of the Primary Product provided by the applicant is as follows:

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

Sensory properties	Typically a viscous and oily, pale or intense yellow-brown liquid, not diluted by water, which is free of all extraneous smells and flavours and has a fragrance reminiscent of the smoke of deciduous trees
Density (20°C)	$\rho = 1.070 – 1.130\ g/cm^3$
Refractive index (20°C)	$\alpha = 1.485 – 1.525$
Benz[*a*]anthracene	max. 20 µg/kg
Benzo[*a*]pyrene	max. 10 µg/kg

The product should be stored in a cool location protected from light and damp. The product retains its properties for at least two years under such conditions.

4. Proposed uses

The Primary Product is mixed with food-quality salt, resulting in a final content of 3% of the Primary Product in the derived product.

The trade name of the derived product is FUMOKOMP S-30.

Normal and upper use levels as described originally 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 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	0.015	0.03

2	Fats and oils and fat emulsions (type water-in-oil)	0.02	0.03
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	0.02	0.03
9	Fish and fish products, including molluscs, crustaceans and echinoderms	0.02	0.03
10	Egg and egg products	0	0
11	Sweeteners, including honey	0	0
12	Salts, spices, soups, sauces, salads, protein products etc.	0.015	0.03
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	0.02	0.03
16	Composite foods (e.g. casseroles, meat pies, mincemeat) - foods that could not be placed in categories 1 – 15	0.02	0.03

5. Dietary exposure assessment

In order to estimate dietary exposure to the Primary Product, 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 (EC) No. 1565/2000. 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).

Dietary exposure estimates calculated by means of the above mentioned methods are reported in Tables 6. Dietary exposure from all sources ranges from 0.13 to 0.20 mg/kg bw/day, when assuming that the Primary Product is present at the upper use levels, and from 0.08 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. 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: 0.06 and 0.09 mg/kg bw/day when using normal and upper use levels, respectively.

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	0.03	0.05
	Other foods not traditionally smoked	0.10	0.15
	Beverages (alcoholic or non-alcoholic)	0.00	0.00
	Total dietary exposure	0.13	0.20
SMK-EPIC	Traditionally smoked food	0.06	0.09
	Other foods not traditionally smoked	0.02	0.04
	Beverages (alcoholic or non-alcoholic)	0.00	0.00
	Total dietary exposure	0.08	0.13
Applicant	Dietary exposure	-[a]	-[a]

[a] Not provided

6. Toxicology

6.1. Identity of the test material

The toxicology studies provided by the applicant in support of this submission consisted of a 90-day feeding study carried out in 1974, and three *in vitro* genotoxicity studies carried out with representative samples taken from the commercial batches. Two *in vivo* genotoxicity tests have also been provided.

The material used in the studies was referred to as KHV, the general descriptor used for “Fumokomp” smoke flavour. No further information was provided on the specific batch or batches used in the tests. The applicant provided data on physical parameters of batches produced from 1982 to 2005, and concluded that the batch quality was virtually constant, based on deviations of these physical parameters from the average. In relation to the material used in the 90-day feeding study, the applicant states that the Primary Product tested in 1974 as ‘KHV smoke flavour product’ is comparable to the Primary Product to be introduced onto the market.

6.2. Subchronic toxicity

Only a summary report is available on the 90-day feeding study carried out in 1974 by OÉTI, the Hungarian National Institute for Food Safety and Nutrition (OÉTI, 1974). A full study report could not be located and experimental design and results cannot therefore be verified. The study was carried out before the introduction of the GLP standards, but the applicant stated that the test procedure and the test laboratory complied with the highest international standards pertaining at that time.

The smoke flavouring samples used in the study were reported by the applicant to represent different fractions of the primary product produced at that time (1974). The qualitative analysis of these fractions, obtained by repeated fractioned distillation, was performed by means of organoleptic tests. Approved fractions and combinations thereof were submitted for toxicological tests. It was not clear which of these samples was used in the 90-day toxicology study. The Panel thus could not confirm the identity of the material that was tested, and had to take on trust the statement of the applicant that the product tested in 1974 as ‘KHV smoke flavour product’ was comparable to the Primary Product to be introduced onto the market.

The test material was administered in the diet at levels of 200 mg/kg diet and 400 mg/kg diet to groups of 20 male and 20 female rats from the OÉTI (Hungarian National Institute for Food Safety and Nutrition) random stock. These dietary levels represent intakes of 10 and 20 mg/kg bw/day Fumokomp assuming an intake level of 1/20th of the level in feed. The control group comprised 10 male and 10 female rats. Test parameters investigated included food consumption, body weight, clinical condition, limited haematological examination (white and red blood cell count and haemoglobin) during the 2nd month of study and at termination and histopathological examination of selected organs (not specified). No information on statistical analysis of any of the parameters evaluated was available in the summary report of the study.

The information provided in the summary report indicated that food consumption was initially reduced in the test groups due to palatability problems but returned to normal after a few days. Haemoglobin was reported to be slightly reduced in animals receiving 400 mg/kg in the diet at the time of the first blood sample, but had returned to normal by the end of the study. Alveolar wall thickening and eosinophilia in the lung and small intestine was reported to be more frequent in the 400 mg/kg group. Overall, the study authors concluded that the KHV smoke flavour preparation did not have any toxic effects in this study, at either dietary level. On this basis, the level of 400 mg Primary Product/kg diet appeared to be a NOAEL, equivalent to 20 mg/kg bw/day.

However, since comprehensive haematological, biochemical and histopathological investigations according to current guidelines apparently were not carried out, the Panel could not make an independent assessment of any toxic effects including target organ toxicity produced by Fumokomp in this study. The Panel also noted that the identity of the material tested in the study is unknown. Given this fact and the absence of a full study report the Panel was unable to confirm the validity of the study and thus could not derive a NOAEL for Fumokomp. For these reasons the study was not used in the safety evaluation of Fumokomp.

6.3. Genotoxicity

Fumokomp has been evaluated in three recent (2005*)* *in vitro* genotoxicity studies. The studies provided were (i) a test for gene mutations in bacteria (Lab International Research Centre, 2005a), (ii) a test for induction of gene mutations in mammalian cells *in vitro* (Lab International Research Centre, 2005b), and (iii) a test for induction of chromosomal aberrations in mammalian cells in vitro (Lab International Research Centre, 2005c). It has also been tested in the *in vivo* mouse micronucleus test (Toxi-Coop Kkt, 2008a) and, at the request of the Panel, in an *in vivo* rat liver unscheduled DNA synthesis test (Toxi-Coop Kkt, 2008b). All studies were performed under GLP according to OECD guidelines.

The bacterial reverse mutation assay was performed in accordance with OECD Guideline 471, using *Salmonella typhimurium* TA98, TA100, TA1535, TA1537 and *Escherichia coli* WP2 uvrA and dose levels of Fumokomp of 5000, 4000, 2000, 800, 320 and 128 µg/plate and in the presence and absence of S9 metabolic activation, using both a plate incorporation method and a pre-incubation method. Cytotoxicity was primarily evident in the plate incorporation method at dose levels of 4000 – 5000 µg, and also in the pre-incubation method in the presence of S9. In the absence of S9 in the pre-incubation method toxicity was apparent at dose levels down to 800 µg/plate. No evidence of increased revertants was seen with any of the bacterial strains, either with or without S9, under the conditions of this test. Positive control substances gave the expected responses.

Fumokomp was tested in an *in vitro* mammalian chromosome aberration test in Chinese hamster ovary cells (line CHO-KI) in accordance with OECD Guideline 473. Two independent assays were carried out. In study 1, concentrations of 75, 25 and 5 µg/ml were incubated for 4 hours in the presence and absence of metabolic activation, whilst in study 2, concentrations of 50, 25 and 5 µg/ml were incubated for 20 hours in the absence of metabolic activation and concentrations of 75, 25 and 5 µg/ml were incubated for 4 hours in the presence of metabolic activation. Dose levels were selected based on a preliminary cytotoxicity assay, in which mitotic index was reduced to approximately 50% of control at 25 - 50 µg/ml. There was no significant increase in cells showing chromosomal aberrations or polyploid or endoreplicated metaphases in either study, in the presence or absence of metabolic activation, and it was concluded that the Primary Product did not show evidence of clastogenic activity in this test.

A mammalian cell mutation assay in mouse lymphoma cells (L5478Y TK+/- 3.7.2C cells) was performed with Fumokomp in accordance with OECD Guideline 476. Cells were treated with concentrations of 100, 75, 50, 25 and 10 µg/ml test material for 3 hours in the presence and absence of S9 metabolic activation, and with concentrations of 40, 30, 20 and 10 µg/ml test material for 24 hours in the absence of S9. Statistically significant increases in mutation frequencies were seen at concentrations of 100, 75 and 50 µg/ml after the 3-hours treatment, both in the presence and absence of S9 metabolic activation. Both large and small colonies were increased. After the 24-hours treatment without S9, statistically significant increases in mutation frequencies were seen at concentrations of 40, 30 and 20 µg/ml. The test material was cytotoxic at concentrations of 50 µg/ml in the presence of S9 and at 20 – 25 µg/ml in its absence It is concluded that the Primary Product gave a clearly positive result in the mouse lymphoma tk-assay, with and without S9, with increases of both large and small colonies, thus indicating the ability of the test material to induce genotoxic effects both at gene and chromosome level.

Fumokomp was examined for its genotoxic potential in an *in vivo* bone marrow mouse micronucleus test in NMRI BR mice in accordance with OECD Guideline 474. Following a dose-range study, in the main study groups of 5 male and 5 female mice received a single dose of 500, 1000 or 2000 mg/kg bw test substance in polyethylene glycol 400 by gavage, while a positive control group (5 males and 5 females) received a single dose of 60 mg/kg bw cyclophosphamide intraperitoneally. The study included both untreated and vehicle controls. Test animals and positive and untreated and vehicle controls were killed 24 hours after dosing for sampling of bone marrow, and additional groups of 5 male and 5 female mice receiving either 0 or 2000 mg/kg bw Fumokomp were included to allow a second sampling period at 48 hours after treatment. In both the dose-range study and the main study, evidence of clinical toxicity was seen in Fumokomp-treated animals at levels of 1000 and 2000 mg/kg bw. These consisted of decreased activity, hunchbacked posture, piloerection and (at 2000 mg/kg bw only) loss of coordination and increased respiration persisting for up to 4 hours after dosing. The PCE/NCE ratio was reduced at 48 hours (with a slight reduction also at 24 hours) in both males and females receiving 2000 mg/kg bw Fumokomp compared with vehicle controls. There was no significant increase in the frequency of micronucleated polychromatic erythrocytes (MCPE) in male or female mice at either 24 hours or 48 hours after treatment with Fumokomp compared to the vehicle or untreated controls, while the positive control showed the anticipated increases in MPCE.

An *in vivo* rat liver unscheduled DNA synthesis (UDS) test was also performed with Fumokomp. DNA repair in hepatocytes was measured following administration by gavage of 500, 1000 or 2000 mg/kg bw Fumokomp in polyethylene glycol 400 (PEG) to groups of male Wistar rats (Crl:[WI]BR). The negative control group were dosed with PEG alone, while positive control groups (n=3 for each substance) received either 2-acetylaminofluorene (late sampling period) or N, N'-dimethylhydrazine dihydrochloride (DMH) (early sampling period). Hepatocytes were isolated at 4 or 12 hours after treatment of the rats with 0, 500, 1000 or 2000 mg/kg bw Fumokomp (n=3 per group) or with the positive control DMH (at 4 hours) or 2-AAF (at 12 hours). Unscheduled DNA synthesis was measured by autoradiography, following a 4 hour incubation of the hepatocyte cultures with [methyl-^{3}H]-thymidine. Fumokomp did not cause an increase in net nuclear grain count at either sampling time, while positive controls gave expected increases. It can be concluded that under the conditions of this study, Fumokomp did not induce unscheduled DNA synthesis in the rat liver.

Overall it is concluded that Fumokomp is genotoxic *in vitro*, based on the results of a mammalian cell mutation assay, whereas two *in vivo* genotoxicity tests were negative and sufficient to eliminate the concerns over the *in vitro* genotoxicity.

6.4. Other studies

No other studies were presented by the applicant.

7. Discussion

The applicant provided information on the identity, composition, variability and stability of the Primary Product as requested in the EFSA guidance document. This information was limited but considered acceptable by the Panel.

The Primary Product is a solvent-free distillate. The total identified mass (92 %) is in compliance with Commission Regulation (EC) 627/2006. Except for benzo[*j*]fluoranthene and cyclopenta[*cd*]pyrene, data on the concentrations of the polycyclic aromatic hydrocarbons (PAHs) listed in Annex 2 of the EFSA Guidance document have been provided. The analyses were performed by an external laboratory using a GC/MS-based method; experimental conditions but no validation or performance data were provided. The levels of benzo[*a*]pyrene and benzo[*a*]anthracene are below their respective limits of 10 and 20 µg/kg given in Regulation (EC) No. 2065/2003 (EC, 2003). Although the concentration of benzo[*j*]fluoranthene and cyclopenta[*cd*]pyrene, two of the PAHs known to be potentially carcinogenic, was not provided, the Panel concluded that, based on the reported levels of other potentially carcinogenic PAHs, benzo[*j*]fluoranthene and cyclopenta[*cd*]pyrene levels would be expected to be similarly low.

The *in vitro* genotoxicity studies on the Primary Product indicated that it is not mutagenic in bacterial cells and did not have a clastogenic effect in CHO cells, but was genotoxic in the mammalian cell mutation assay, with increases of both large and small colonies, thus indicating the ability of the test material to induce genotoxic effects both at gene and chromosome level. However, an *in vivo* bone marrow micronucleus assay was negative and an *in vivo* rat liver unscheduled DNA synthesis test was also negative.

Overall it is concluded that Fumokomp is genotoxic *in vitro*, based on the results of a mammalian cell mutation assay, whereas two *in vivo* genotoxicity tests were negative and sufficient to eliminate the concerns over the *in vitro* genotoxicity.

In the 90-day rat dietary feeding study carried out at dietary levels of 200 mg/kg and 400 mg/kg diet, equivalent to intakes of 10 and 20 mg bw/kg/day, the study authors concluded that the KHV smoke flavour preparation did not have any toxic effects at either dietary level. On this basis, the level of 400 mg Primary Product/kg diet appeared to be a NOAEL, equivalent to 20 mg/kg bw/day.

However, since comprehensive haematological, biochemical and histopathological investigations according to current guidelines apparently were not carried out, the Panel could not make an independent assessment of any toxic effects including target organ toxicity produced by Fumokomp in this study. The Panel also noted that the identity of the material tested in the study is unknown. Given this fact and the absence of a full study report the Panel was unable to confirm the validity of the study and thus could not derive a NOAEL for Fumokomp. For these reasons the study was not used in the safety evaluation of Fumokomp.

In order to estimate dietary exposure to the Primary Product Fumokomp, 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) No 1565/2000. Dietary exposure from all sources ranges from 0.13 to 0.20 mg/kg bw/day, when assuming that the Primary Product is present at the upper use levels, and from 0.08 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 ranges from 0.05 to 0.09 mg/kg bw/day when assuming that the Primary Product Fumokomp is present at the upper use levels, and from 0.03 to 0.06 mg/kg bw/day, when normal use levels are considered.

The Panel considered that the validity of the 90-day feeding study in the rat could not be confirmed and that no NOAEL from this study could be derived. Thus, the Panel did not derive margins of safety based on the dietary exposure estimates. The safety of Fumokomp cannot therefore be established.

CONCLUSIONS

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

Fumokomp is genotoxic *in vitro*, based on the results of a mammalian cell mutation assay, whereas two *in vivo* genotoxicity tests were negative and sufficient to eliminate the concerns over the *in vitro* genotoxicity.

The Panel considered that there were major deficiencies in the summary of the 90-day study in rats submitted as part of the application dossier for Fumokomp, and that since the detailed report of this study was not provided, the validity of the study could not be confirmed. Furthermore the identity of the material tested in the study is unknown. For these reasons the study could not be used in the safety evaluation of Fumokomp.

The Panel concluded that the toxicological data do not enable the safety of Fumokomp to be established.

DOCUMENTATION PROVIDED TO EFSA

1. Dossier from KOMPOZÍCIÓ KFT., May 2005.

2. Response from KOMPOZÍCIÓ KFT to request for supplementary information.

REFERENCES

EC, 2000. Commission Regulation (EC) No 1565/2000 of 18 July 2000 laying down the measures necessary for the adoption of an evaluation programme in application of Regulation (EC) No 2232/96 of the European Parliament and of the Council. Official Journal of the European Union L 180 p. 8-16. http://eur-lex.europa.eu/LexUriServ/site/en/oj/2000/l_180/l_18020000719en00080016.pdf Visited on 10 April 2007

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

EC, 2006. Commission Regulation (EC) No 627/2006 of 21 April 2006 implementing Regulation (EC) No 2065/2003 of the European Parliament and of the Council as regards quality criteria for validated analytical methods for sampling, identification and characterisation of primary smoke products. Official Journal of the European Union L 109 p.3-6. http://eur-lex.europa.eu/LexUriServ/site/en/oj/2006/l_109/l_10920060422en00030006.pdf Visited on 10 April 2007

EFSA, 2004. 7th plenary meeting of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food (AFC). Brussels, 12-13 July 2004.

EFSA, 2005. Guidance from the Scientific Panel on Food Additives, Flavourings, Processing aids and Materials in Contact with Food. Guidance on submission of a dossier on a smoke flavouring primary product for evaluation by EFSA, Adopted on 7 October 2004; Revised on 27 April 2005. http://www.efsa.europa.eu/etc/medialib/efsa/science/afc/afc_guidance/680.Par.0001.File.dat/guidancedocument1.pdf visited on 8 September 2006

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

Lab International Research Centre, 2005a. The Testing of "Fumokomp" Smoke Flavour with Bacterial Reverse Mutation Test. Lab International Research Centre Hungary Ltd. Study Code 05/069-007M. November 2005.

Lab International Research Centre,2005b. The Testing of "Fumokomp" Smoke Flavour by Mouse Lymphoma Assay. Lab International Research Centre Hungary Ltd. Study Code 05/069-033EL December 2005.

Lab International Research Centre, 2005c. The Testing of "Fumokomp" Smoke Flavour with in vitro Chromosomal Aberration Test. Lab International Research Centre Hungary Ltd. Study Code 05/069-020C December 2005.

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

OÉTI, 1974. Summary report on the short-term (90-day) rat experiment conducted to examine any possible toxic effects of the KHV smoke flavour product.

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

Toxi-Coop Kkt, 2008a. Testing of Mutagenic Effect of "Fumokomp" Smoke Flavour by Mouse Micronucleus Test. Study Code 07/030-008E January 2008

Toxi-Coop Kkt, 2008b. Test of "Fumokomp" in Unscheduled DNA Synthesis (UDS) Assay with Rat Liver Cells in Vivo. Study Number 421.738.1525 November 2008

ABBREVIATIONS

2-AAF	2-Acetylaminofluorene
AFC	Scientific Panel on Additives, Flavourings, Processing aids and Materials in Contact with Food
bw	body weight
CEF	Scientific Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
CHO	Chinese Hamster Ovary cell line
DMH	N, N'-dimethylhydrazine dihydrochloride
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
GS/FID	Gas Chromatography/Flame Ionisation Detection
FID	Flame Ionisation Detection
mTAMDI	modified TAMDI
MCPE	Micronucleated Polychromated Erythrocytes
NOAEL	No-Observed-Adverse-Effect Level
OECD	Organisation for Economic Cooperation and Development
OÉTI	Hungarian National Institute for Food Safety and Nutrition
PAHs	Polycyclic Aromatic Hydrocarbons
PEG	Polyethylene Glycol
PCE/NCE	Polychromatic Erythrocytes/ Normochromatic Erythrocytes
RSD	Relative Standard Deviation
SCF	Scientific Committee on Food
SMK-EPIC	Smoke flavouring EPIC model
SMK-TAMDI	Smoke Theoretical Added Maximum Daily Intake
TAMDI	Theoretical Added Maximum Daily Intake
UDS	Unscheduled DNA Synthesis