Risk assessment of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in contact with Food (AFC) on the Smoke Flavouring Primary Product – FF-B

(Question number EFSA-Q-2005-260)

At its 22nd meeting the Panel concluded the risk assessment and agreed the final text on 7 June 2007 by written procedure

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 FF-B.

The Primary Product FF-B is obtained from a specified mixture of the following woods: red oak (*Quercus rubra)*, white oak (*Quercus alba*), maple (*Acer saccharum*, *Acer nigrum*), beech (*Fagus grandifolia*) and hickory (*Carya ovata*). The production of FF-B comprises the following steps: (i) screening and drying of the hardwood sawdust, (ii) heating of the dried sawdust in a rotary furnace, (iii) condensing of the released smoke and (iv) separation of the smoke condensate into an oil phase and an aqueous phase. Essential parameters of the manufacturing process have been provided by the applicant.

Identification and quantification of Primary Product constituents have been performed in the batch that was used for the toxicological studies. The water content of the Primary Product is 66 wt.%. The volatile fraction identified by capillary gas chromatographic analysis (GC) accounts for 16 wt.% and the unidentified constituents account for 18 wt.%. Except for benzo[*j*]fluoranthene, 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. The concentrations of dibenzo[*a,e*]pyrene, dibenzo[*a,h*]pyrene, dibenzo[*a,i*]pyrene, dibenzo[*a,l*]pyrene were less than 1 µg/kg, the concentrations of the other PAHs analysed were less than 0.5 µg/kg. The levels of benz[*a*]anthracene and benzo[*a*]pyrene were below the limits given in Regulation (EC) No 2065/2003. Analysis of five batches revealed no significant batch-to-batch variability. The stability of the Primary Product was demonstrated by GC analysis of a batch over a nine months period.

Use levels of the Primary Product proposed by the petitioner range between 0.1 g/kg food (alcoholic beverages) and 3.5 g/kg food (meat and meat products). Dietary exposure for the Primary Product, as estimated by the petitioner, was 3 mg/kg body weight per day.

The toxicological tests provided included a 90-day oral toxicity study and *in vitro* and *in vivo* genotoxicity studies. The genotoxicity studies showed that the Primary Product FF-B was positive in *in vitro* assays for mutagenicity in bacterial and mammalian cells and was clastogenic in CHO cells. *In vivo*, FF-B induced statistically significant increase in micronuclei in both sexes of mice when given in the highest recommended dose.

The Panel concludes that the Primary Smoke Flavouring Product FF-B can be regarded as weakly genotoxic *in vivo*. The Panel cannot establish its safety in use when added to food.

KEYWORDS

Smoke flavouring, Primary Product, FF-B, APS

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.[1]

ASSESSMENT

The following evaluation applies only to the Primary Product FF-B 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. An *in vivo* genotoxicity test has also been provided.

1 Information on existing authorisations and evaluations

No information on existing authorisations and evaluations of the Primary Product FF-B has been provided.

2 Technical data

2.1 Manufacturing process

2.1.1 Source materials for the Primary Product

The Primary Product FF-B is obtained from sawdust derived from a specified mixture of the following woods: red oak (*Quercus rubra*), white oak (*Quercus alba*), maple (*Acer saccharum*, *Acer nigrum*), beech (*Fagus grandifolia*) and hickory (*Carya ovata*).

2.1.2 Method of manufacture of the Primary Product

The production of FF-B comprises the following steps: (i) screening and drying of the hardwood sawdust, (ii) heating of the dried sawdust in a rotary furnace, (iii) condensing of the released smoke and (iv) separation of the smoke condensate into an oil phase and an aqueous phase.

The aqueous phase obtained after phase separation of the smoke condensate is indicated as the Primary Product FF-B. The manufacturing process is generally described in a patent (United States Patent Number 3,873,741).

Details were provided by the applicant on the following operational parameters of the manufacturing process:

- Drying of the hardwood dust
  The target and outlet temperatures of the dryer and the moisture content of the dried sawdust were given. The operation of the dryer is controlled and data are recorded on an hourly basis. An example of a dryer operator report including parameters such as

[1] On 23 April 2007, EFSA was informed that the manufacturer had stopped all activities shipping FF-B or derived products to the EU and therefore withdrew officially his application.

temperatures, feed rates, drum rotation speed and moistures was provided in the application.

- Heating of the sawdust
  External and internal temperatures of the rotary furnace were given. The operation is controlled and data are recorded on an hourly basis. An example of a furnace operator report was provided in the application.
- Condensing of the smoke
  The temperature of the heat exchanger employed as condensing system is given. The operation of the condensing system is controlled and data are recorded on an hourly basis. An example of an operator report was provided in the application.
- Phase separation
  The batches accumulated for phase separation are analysed for critical parameters including pH and levels of acids and phenols. The removal of the bottom heavy phase is done on a periodic basis and data are recorded.

2.2 Identity of Primary Product

2.2.1 Trade names of the Primary Product
The trade names FF-B and APS are used for the Primary Product.

2.2.2 Physical state of the Primary Product
The viscosity of the Primary Product averaged 4.4 ± 0.10mm^2/s. The density averaged 1.08 ± 0.002 g/ml. The values were obtained from five batches.

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 (66 wt.%) of the Primary Product functions as solvent. Accordingly, the solvent-free fraction of the Primary Product amounts to 34 wt.%.

2.3.1.2 Volatile fraction
48 % of the solvent-free fraction was determined by capillary gas chromatographic (GC) analysis. According to the petitioner, this accounts for 90 % of the number of peaks detected and results in an identified volatile fraction of 16 wt.%.

2.3.1.3 Unidentified constituents
The water content of the Primary Product is 66 wt.%. The identified volatile fraction accounts for 16 wt.% and the unidentified constituents account for 18 wt.%.

2.3.2 Chemical description of the Primary Product
Batch 021204BTK1 of the Primary Product (which was used for the toxicological studies) has been characterised according to the parameters listed in Table 1.

Table 1: Description of major chemical parameters of batch 021204BTK1 of the Primary Product

Water (wt.%)	66
Carbonyls (wt.%)	16
Phenols (wt.%)	0.7
Acids (millieq/g)	1.78
pH	2.0
Mercury (mg/kg)	< 0.01
Lead (mg/kg)	< 0.1
Arsenic (mg/kg)	0.3
Cadmium (mg/kg)	0.03

The Panel noted that the amount of phenols in batch 021204BTK1 was below the range given by the petitioner in the specifications (see Table 4).

Based on analysis of the solvent-free fractions of five batches, the constituents identified by (GC) analysis could be assigned to the following main chemical classes: acids (average proportion: 20 wt.%), aldehydes (2 wt.%), aromatics (0.2 wt. %), furans (3 wt.%), guaiacols (1 wt.%), ketones (6 wt.%), phenols (0.2 wt.%), sugars (9 wt.%), syringols (4 wt.%).

2.3.3 Identification and quantification of Primary Product constituents

2.3.3.1 Principal constituents

The proportions of 16 principal constituents (amounting to a total of 46 wt.%) determined by GC analysis of the solvent-free fraction of batch 021204BTK1 are listed in Table 2:

Table 2: Principal constituents of Primary Product batch 021204BTK1

Compound	(wt.%)
Acetic acid	21
Levoglucosan	7
Hydroxypropanone	4
Syringol	3
2-Furaldehyde	2
2-Hydroxy-3-methyl-2-cyclopentene-3-one	2
Propanoic acid	1
1-Hydroxy-2-butanone	1
Hydroxyacetaldehyde	1
(5H)-Furan-2-one	1
1,4:3,6-Dianhydro-α-D-glucopyranose	1
5-Hydroxymethyl-2-furaldehyde	1
Guaiacol	0.3
2-Hydroxy-2-cyclopenetene-1-one	0.3
Syringaldehyde	0.3
4-Methylsyringol	0.3

In addition to these major compounds, another 34 constituents were determined by GC, bringing the total to 48 wt.%.

2.3.3.2 Content of Polycyclic Aromatic Hydrocarbons

Except for benzo[*j*]fluoranthene, the concentrations of the polycyclic aromatic hydrocarbons (PAH) known to be potentially carcinogenic and/or genotoxic, listed in Annex 2 of the guidance document (EFSA, 2005) have been provided. In addition, the contents of fluorene, phenanthrene, anthracene, fluoranthene and pyrene have been determined (in contrast to the others, these five are not considered to be carcinogenic and/or genotoxic). The analyses were performed by an external accredited laboratory; the method used was equivalent to the method developed by the Joint Research Centre of the European Commission (Simon *et al.*, 2006a and b) and fulfilled the performance criteria of Commission Regulation No 627/2006 (EC, 2006), except for the analyte benzo[*j*]fluoranthene. The concentrations of the 19 PAH determined in batch 021204BTK1 of the Primary Product are listed in Table 3. The levels of benz[*a*]anthracene and benzo[*a*]pyrene are below their respective limits of 20 µg/kg and 10 µg/kg in Regulation (EC) No 2065/2003 (EC, 2003).

Table 3: Concentrations of PAH in batch 021204BTK1

PAH	(µg/kg)
Benz[*a*]anthracene	< 0.5[a]
Chrysene	< 0.5
Benzo[*b*]fluoranthene	< 0.5
Benzo[*k*]fluoranthene	< 0.5
Benzo[*a*]pyrene	< 0.5
Indeno[*1,2,3-cd*]pyrene	< 0.5
Dibenz[*a,h*]anthracene	< 0.5
Benzo[*ghi*]perylene	< 0.5
Cyclopenta[*cd*]pyrene	< 0.5
5-Methylchrysene	< 0.5
Dibenzo[*a,e*]pyrene	< 1
Dibenzo[*a,h*]pyrene	< 1
Dibenzo[*a,i*]pyrene	< 1
Dibenzo[*a,l*]pyrene	< 1
Fluorene	19
Phenanthrene	13
Anthracene	5
Fluoranthrene	2
Pyrene	3

[a] <: below limit of quantitation

2.3.4 Batch-to-batch variability

Five batches of the Primary Product were analyzed to demonstrate batch-to-batch variability. The following tests were performed:

(a) Variability of the parameters listed in Table 1: The five batches showed no significant variability. The levels of heavy metals were consistently below the values given in the specifications (Table 4).
(b) Variability of the proportions (wt.%) of the major chemical classes described in 2.3.2: The variability observed was considered acceptable. The relative standard deviations ranged from 0.3 % for the acids (20 wt.%) to 33 % for the aldehydes (2 wt.%) .
(c) Variability of the amounts (wt.%) of 59 constituents determined by GC-analysis: The standard deviations observed were considered acceptable. The average relative standard deviation was 12 %. The individual values ranged from 7 % for major constituents (e.g. acetic acid, 19 wt. %) to 44 % for minor constituents (e.g. 4-ethylsyringol, 0.1 wt.%).
(d) Variability of the PAH listed in Table 3.
The following average concentrations and standard deviations were determined: fluorene 17 ± 5.5 µg/kg, phenanthrene 14 ± 5.8 µg/kg, anthracene 3 ± 1.6 µg/kg, fluoranthene 1.6 ± 0.8 µg/kg, pyrene 1.5 ± 1.7 µg/kg. The relative standard deviations were considered acceptable. The concentrations of all other PAH were consistently below 1 µg/kg (with the exception of cyclopenta[*cd*]pyrene levels of 1.2 and 1.0 µg/kg in two batches).

2.3.5 Stability
The stability of the Primary Product was demonstrated by subjecting batch 021204BTK1 to GC analysis four times over a nine months period. No major changes were observed in the proportions of the 55 components measured in the solvent-free fraction. The average relative standard deviation was 21 %. The individual values ranged from 10 % for major constituents (e.g. acetic acid, 18 wt. %) to 59 % for minor constituents (e.g. 2-ethylbutanal, 0.1 wt. %). The batch investigated was the same as that used in the toxicological studies.

2.3.6 Specifications
The specifications given for the Primary Product are listed in Table 4:

Table 4: Specifications of the Primary Product

Density	1.070 – 1.088 g/ml
pH	2.0 – 3.0
Acids	10 – 11 %
Carbonyls	15 – 25 %
Phenols	1.4 – 2.4 %
Benzo[*a*]pyrene	< 10 µg/kg
Benz[*a*]anthracene	< 20 µg/kg
Mercury	< 0.01 mg/kg
Lead	< 0.1 mg/kg
Arsenic	< 1 mg/kg
Cadmium	< 0.1 mg/kg

3 Proposed uses

Upper use levels as described by the petitioner for the Primary Product FF-B 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 –Upper use levels of Primary product in food categories as described by the petitioner

Food categories		Upper use level (g/kg)
1	Dairy products, excluding products of category 2	0
2	Fats and oils, and fat emulsions (type water-in-oil)	0
3	Edible ices, including sherbet and sorbet	0
4.1	Processed fruits	0
4.2	Processed vegetables (incl. mushrooms & fungi, roots & tubers, pulses and legumes), and nuts & seeds	0
5	Confectionery	0
6	Cereals and cereal products, incl. flours & starches from roots & tubers, pulses & legumes, excluding bakery	0
7	Bakery wares	0
8	Meat and meat products, including poultry and game	3.5
9	Fish and fish products, including molluscs, crustaceans and echinoderms	3.5
10	Eggs and egg products	0
11	Sweeteners, including honey	0
12	Salts, spices, soups, sauces, salads, protein products etc.	1
13	Foodstuffs intended for particular nutritional uses	0
14.1	Non-alcoholic ("soft") beverages, excl. dairy products	0
14.2	Alcoholic beverages, incl. alcohol-free and low-alcoholic counterparts	0.1
15	Ready-to-eat savouries	2.75
16	Composite foods (e.g. casseroles, meat pies, mincemeat) - foods that could not be placed in categories 1 – 15	2.75

4 Dietary exposure assessment

A conservative assessment of dietary exposure to the Primary Product FF-B was performed by the petitioner by combining the upper use levels as reported in Table 5 with aggregated average consumption data from a food survey carried out in Great Britain in 2000 in adults aged 19 - 64 years (Henderson *et al.*, 2002). This gave a dietary exposure of 3 mg/kg body weight per day for adults.

5 Toxicological data

5.1 Identity and stability of the FF-B batch used for toxicological studies

The FF-B used for the toxicological studies was from the same batch (021204BTK1) as that

used for the chemical identification and quantification.
The data on batch-to-batch variability and stability confirm that the batch used for the toxicological studies is representative of the Primary Product.

5.2 Subchronic toxicity

A 90-day dietary toxicity study of FF-B was performed in male and female Wistar Crl: (WI)BR rats according to OECD Test Guideline 408 (OECD, 2000a) and Good Laboratory Practice (GLP) (LAB, 2005a). Four groups of rats (10/sex/group) were administered FF-B in the diet for 90 days at constant concentrations of 0, 1000, 3000 and 9000 mg/kg diet (according to the applicant equivalent to approximately 0, 70, 200 and 600 mg/kg bw/day in males and 0, 80, 300 and 1100 mg/kg bw/day in females). The Panel noted the large disparity in the achieved doses between male and female animals.

No mortalities occurred during the study. There were no changes in physical condition and behaviour or in the sensory reactivity to stimuli of different types, grip strength and motor activity. The daily food intake and body weight gain were similar in all experimental groups. There were no changes of toxicological relevance in ophthalmological, haematological, clinical chemistry or urine parameters. Organ weights were similar in all groups and no test item-related histopathological alterations were detected.

Overall, FF-B did not cause any test item-related adverse effects at any of the dietary concentrations tested. The no observed adverse effect level (NOAEL) was at least 9000 mg/kg diet (the highest dietary concentration tested), which was stated by the applicant to be approximately equivalent to 600 mg/kg bw in male and 1100 mg/kg bw in female rats.

5.3 Genotoxicity

Three *in vitro* and one *in vivo* genotoxicity studies were submitted. These were a bacterial reverse mutation assay, an *in vitro* mammalian chromosome aberration test, a mammalian cell mutation assay in mouse lymphoma cells and a mouse micronucleus test.

The bacterial reverse mutation assay was performed in accordance with OECD Test Guideline 471 (OECD, 2000b) (LAB, 2005b). FF-B was incubated in the presence and absence of metabolic activation with *Salmonella typhimurium* TA98, TA100, TA1535, TA1537 and *Escherichia coli* WP2 uvrA at doses ranging from 78 to 5000 µg/plate. A clear dose related increase in revertants was observed with TA100 strain in the presence and in the absence of metabolic activation (S9-mix). The highest fold-increases in mutations compared with controls were 2.45 (-S9-mix) in the plate incorporation assay and 2.34 (+S9-mix) using the pre-incubation method.
The *in vitro* mammalian chromosome aberration test was performed in accordance with OECD Test Guideline 473 (OECD, 2000c) (LAB, 2005c). FF-B was incubated in the presence and absence of metabolic activation with Sub-line (KI) of Chinese hamster ovary cell line at doses up to 200 µg/ml. Chromosome aberrations were increased up to 10-fold compared to control, both in the presence and absence of metabolic activation in a concentration-related manner. Relative survival was greater than 25 % at all concentrations.

The mammalian cell mutation assay in mouse lymphoma cells was performed in accordance with OECD Test Guideline 476 (OECD, 2000d) (LAB, 2005d). FF-B was incubated in the presence and absence of metabolic activation with mouse lymphoma cells at concentrations up to 400 µg/ml. A concentration-related, significant increase in mutation frequency up to 4-

fold higher than control, with relative survival greater than 20 %, was observed (both small and large colonies) both in the presence and absence of metabolic activation.

The mouse bone marrow micronucleus test was performed in accordance with OECD Test Guideline 474 (OECD, 2004) (LAB, 2005e). FF-B was administered to male and female CRL:NMRI BR mice as a single oral dose of 0, 500, 1000 and 2000 mg/kg bw with sampling at 24 and 48 hours. Compared with controls there were statistically significant ($p<0.01$) and biologically relevant increases in the frequency of micronucleated polychromatic erythrocytes (MPCEs) up to 2.7-fold in the female mice at the highest dose (2000 mg/kg bw) both at 24 and 48 hours after treatment. These were accompanied by a dose-related increase in MPCEs at the lower doses, which did not reach statistical significance. A similar trend was seen in male mice but with smaller increases. This was statistically significant ($p<0.05$) at 2000 mg/kg bw, both 24 and 48 hours after treatment. No biologically significant depression of the PCE/NCE ratio occurred.

5.4. Other studies

No other studies were provided by the petitioner.

DISCUSSION

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

The genotoxicity studies showed that the Primary Product FF-B was positive in *in vitro* assays for mutagenicity in bacterial and mammalian cells and was clastogenic in CHO cells. *In vivo*, FF-B induced statistically significant increase in micronuclei in both sexes of mice when given in the highest recommended dose. Based on these data the Panel considers that the Primary Product FF-B can be regarded as weakly genotoxic *in vivo*.

FF-B had consistently low levels of PAH in the batches tested. Based on these levels the amounts of the PAH potentially present at the concentrations of FF-B used in the genotoxicity studies were estimated. Although the concentration of benzo[*j*]fluoranthene, one of the PAHs known to be potentially carcinogenic, was not provided, the Panel concluded that based on the reported levels of other potentially carcinogenic PAHs benzo[*j*]fluoranthene levels would similarly be low. In the light of these estimates, the mutagenic activity of FF-B both in *in vitro* and *in vivo* test systems is unlikely to be explained by the presence of PAH. Thus the genotoxic component(s) remain(s) unidentified.

CONCLUSIONS

The Panel concludes that the Primary Smoke Flavouring Product FF-B can be regarded as weakly genotoxic *in vivo*. The Panel cannot establish its safety in use when added to food.

DOCUMENTATION PROVIDED TO EFSA

Dossier from Forest Flavors International Inc.

Response from Forest Flavors International Inc. to request for supplementary information

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