

SCIENTIFIC OPINION

Safety of smoke flavour Primary Product – SmokEz C-10¹

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

(Question No EFSA-Q-2005-263)

Adopted on 14 May 2009

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SUMMARY

The European Food Safety Authority has been asked to provide scientific opinions on the safety of smoke flavouring Primary Products used or intended for use in or on foods. This opinion concerns a smoke flavouring Primary Product, named SmokEz C-10.

The Primary Product SmokEz C-10 is obtained from mixed wood species. The average proportions reported by the applicant are as follows: maple (*Acer saccharum*) 51 %, oak (*quercus alba*) 29 %, hickory (*Carya ovata*) 16 % as primary sources and ash (*Fraxinus americana*), birch (*Betula papyrifera* and *Betula alleghaniensis*), wild black cherry (*Prunus serotina*), and beech (*Fagus grandifolia*) as secondary sources (3 %).

The production of SmokEz C-10 comprises the following steps: (i) wood lots received are combined prior to pyrolysis and dried, (ii) pyrolysis of the saw dust in a rotary calciner reactor with continuous feeding in an inert atmosphere, (iii) condensing of the hot vapours, (iv) separation of tar, filtration and conditioning of the Primary Product. The applicant has provided essential parameters of the manufacturing process.

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The water content of the primary product is 67 wt. %. The volatile fraction identified by capillary gas chromatography analysis accounts for 22 wt. % of the Primary Product. 19 wt. % (corresponding to 86 % of the volatile fraction) were identified which is in compliance with Commission Regulation (EC) 627/2006. The total identified mass (21 wt. % of the Primary Product) corresponds to 64 % of the solvent-free fraction which is in compliance with Commission Regulation (EC) 627/2006.

The contents of 12 of the 15 PAHs listed in Annex 2 of the EFSA guidance document (EFSA, 2005) have been determined in SmokEz C-10 by an external accredited laboratory using the US-Environmental Protection Agency (EPA) method 3510/8270-GC/MS. According to the applicant, the analyses of 5-methylchrysene, cyclopenta[*cd*]pyrene and dibenzo[*a,e*]pyrene were not performed because the respective calibration standards were not available at the time of the analysis. 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 concentrations of 5-methylchrysene, cyclopenta[*cd*]pyrene and dibenzo[*a,e*]pyrene, PAHs known to be carcinogenic, were not provided, the Panel concluded that based on the reported levels of other carcinogenic PAHs, the levels for 5-methylchrysene, cyclopenta[*cd*]pyrene and dibenzo[*a,e*]pyrene would be expected to be similarly low.

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.

SmokeEz C-10 showed negative results in a *S. typhimurium* reverse mutation assay in strains TA1535, TA1537, TA 1538, TA98 and TA 100, both in the absence and presence of S9. The Panel noted that this non-GLP study, carried out in 1977, did not comply with current test guidelines, but did not consider that it was necessary to request the applicant to repeat the study, given that the other two *in vitro* studies submitted on SmokEz C-10 gave clearly positive results.

Positive results were obtained in the mouse lymphoma L5178Y *tk*+/- assay, primarily at cytotoxic concentrations of SmokEz C-10, with relatively more small than large colonies being formed. In a test for chromosomal aberrations in Chinese Hamster Ovary (CHO), cells SmokEz C-10 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 unscheduled DNA synthesis test was also negative.

Overall, it is concluded that SmokEz C-10 is genotoxic *in vitro* in the mouse lymphoma assay and the chromosomal aberration assay whereas two *in vivo* genotoxicity tests are negative and sufficient to eliminate the concerns over the *in vitro* genotoxicity.

In the 90-day toxicity study with SmokEz C-10 treatment-related effects were observed in both males and females at a dietary level of 4.5% (equivalent to a mean intake of 2600 mg/kg bw/day in males and 2800 mg/kg bw/day in females) and in female rats at a dietary level of 1.5% (equivalent to a mean intake of 900 mg/kg bw/day). The no-observed-adverse-effect level (NOAEL) was considered by the Panel to be 300 mg/kg bw/day, based on increased kidney weights in female rats at higher intake levels.

The applicant provided two data sets for use levels, one submitted originally in 2005, and the second in April 2009, after consulting with clients and seeking more detailed information on the

actual use levels. For transparency reasons both the initially provided data from 2005 and the updated data from 2009 were considered.

Use levels of the Primary Product provided by the applicant in 2009, based on finished food product weight, range from 0.2 g/kg (fats and oil) to 5 g/kg (dairy products, meat, fish). Dietary exposure to the Primary Product was not assessed by the applicant.

In order to estimate dietary exposure to the Primary Product SmokeEz C-10, 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).

Considering the initial data provided on use levels in 2005, the dietary exposure from all sources ranges from 23.9 to 26.0 mg/kg bw/day, when assuming that the Primary Product is present at the upper use levels, and from 10.9 to 13.0 mg/kg bw/day, when normal use levels are considered.

Considering the updated information on use levels from 28 April 2009, the dietary exposure from all sources ranges from 22.2 to 33.8 mg/kg bw/day, when assuming that the Primary Product is present at the upper use levels, and from 9.3 to 12.5 mg/kg bw/day, when normal use levels are considered.

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

Considering the initial data on use levels provided in 2005 the highest exposure estimates, resulting from the SMK-EPIC model, were 7.3 and 14.5 mg/kg bw/day when using normal and upper use levels, respectively. With the SMK-TAMDI model these figures were 4.2 and 8.3 mg/kg bw/day, respectively.

Considering the updated information on use levels from 28 April 2009 the highest exposure estimates, resulting from the SMK-EPIC model, were 6.8 and 14.5 mg/kg bw/day when using normal and upper use levels, respectively. With the SMK-TAMDI model these figures were 4.2 and 8.3 mg/kg bw/day, respectively.

Since the data on use levels originally provided in June 2005 have been updated by the applicant in April 2009, the Panel drew its conclusions based on the margins of safety calculated with these recent data.

Based on the intake data calculated with the new data provided by the applicant on 28 April 2009 for total dietary exposure (traditionally and non-traditionally smoked food), the margins of safety as compared to the NOAEL of 300 mg/kg bw/day in female rats derived from the 90-day toxicity study amount to 9 and 14 for the intake estimates based on the upper use levels and to 24 and 32, when normal use levels are considered.

When assuming the use of Primary Product SmokeEz C-10 in traditionally smoked products only the margins of safety would amount to 21 and 36 based on the upper use levels and to 44 and 72 when normal use levels are considered.

Considering that these margins of safety based on a 90-day toxicity study are inadequate, and that, in addition, data on reproduction and developmental toxicity as well as long term studies are absent, it is concluded that the uses and use levels of the Primary Product SmokEz C-10 in a wide range of product categories would require a larger margin of safety. The Panel concludes that the margins of safety are insufficient and that the use of Primary Product SmokEz C-10 at the proposed uses and use levels is of safety concern.

It is outside the remit of the Panel to decide whether, despite the low margins of safety, the use of Primary Product SmokEz C-10 might be approved for traditionally smoked products, at use levels specified, to replace smoking.

Key words: Smoke flavouring, Primary Product, SmokEz C-10.

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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 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 to replace the smoking process or to impart smoke flavour to foods, which are not traditionally smoked.

Smoke flavourings are produced by controlled thermal degradation of wood in a limited supply of oxygen (pyrolysis) and subsequent condensation of the vapours and fractionation of the resulting liquid products. The Primary Products (primary smoke condensates and primary tar fractions) may be further processed to produce smoke flavourings applied in 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 flavouring Primary Products 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 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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* 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

The following evaluation only applies to the Primary Product SmokEz C-10 manufactured strictly in conformity with the specified process and meeting the chemical specifications described in this opinion.

In accordance with the guidance document on submission of a dossier on a smoke flavouring Primary Product for evaluation by EFSA (EFSA, 2005), data on the manufacturing process, the composition, intended use levels and toxicological tests have been submitted. The latter include a 90-day oral subchronic 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 evaluation or authorisation of the Primary Product SmokEz C-10 has been provided.

2. Technical data

2.1. Manufacturing process

2.1.1. Source materials for the Primary Product

According to the applicant maple (*Acer saccharum*), oak (*Quercus alba*), and hickory (*Carya ovata*) are the primary wood genera used as source materials of the Primary Product. In addition, ash (*Fraxinus americana*), birch (*Betula papyrifera* and *Betula alleghaniensis*), wild black cherry (*Prunus serotina*), and beech (*Fagus grandifolia*) are employed as secondary wood genera in the production process. On the basis of annual production data, the following average proportions and ranges have been provided by the applicant:

maple:	51% (min. 25% - max. 60%)
oak:	29% (min. 10% - max. 40%)
hickory:	16% (min. 10% - max. 25%)
secondary woods:	3% (min. 0% - max. 15%)

The material used for chemical tests (lot C-10-05083301) was produced from 48% maple, 39% oak, 11% hickory, and 2% secondary hardwoods.

The material for the toxicological tests (lot C-10-05044209), except the bacterial gene mutation assay and the *in vivo* rat liver unscheduled DNA synthesis test, was produced from 45% maple, 39% oak, 13% hickory, and 3% secondary hardwoods.

The material for the *in vivo* rat liver unscheduled DNA synthesis test (lot C-10-01217120) was produced from 52 % maple, 31 % oak, 11 % hickory, and 6 % secondary hardwoods. The material used for the bacterial gene mutation assay was only identified as C-10 (batch not identified).

According to the applicant, pooled samples of the batches from one production year are routinely analysed for pesticides.

2.1.2. Method of manufacture of the Primary Product

Dried sawdust with a defined moisture content is continuously pyrolysed in a rotary kiln in an oxygen-restricted atmosphere. The smoke is drawn off to a condenser system. The condensate is cooled to room temperature and water is added. The resulting lower tar and the upper oil phase are separated from the aqueous phase, which constitutes the Primary Product. The latter is further filtered prior to storage. The process has been described in detail by the applicant.

2.2. Identity of the Primary Product

2.2.1. Trade names of the Primary Product

The trade name of the Primary Product is SmokEz C-10. It is produced from various proportions of genera listed in 2.1.1.

2.2.2. Physical state of the Primary Product

SmokEz C-10 is described as a clear brown liquid with an average density of 1.067 g/ml and a viscosity of 2.1 cP at 25 °C.

2.3. Chemical composition of the Primary Product

2.3.1. Overall characterisation

The overall characterization of the Primary Product is as follows:

2.3.1.1. Solvent-free fraction

Water functions as the solvent of SmokeEz-C10. A water content of 67 wt. % was determined by Karl Fischer titration, taking into account interferences of the method by the aldehydes present in the Primary Product. The solvent-free fraction of the Primary Product amounts to 33 wt. % (Figure 1).

2.3.1.2. 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 amount of the volatile fraction determined by GC was 22 wt. % of the Primary Product. 19 wt. % (corresponding to 86 % of the volatile fraction) were identified which is in compliance with Commission Regulation (EC) 627/2006 (EC, 2006) (Figure 2).

2.3.1.3. Unidentified constituents

The fraction of unidentified non-volatile mass can be estimated as the solvent-free mass minus the sum of all masses of volatiles compounds determined by GC: 33 wt. % - 22 wt. % = 11 wt. %. High performance liquid chromatography (HPLC) analysis of the Primary Product revealed a content of 2 wt. % levoglucosan. The unidentified volatile mass amounts to 3 wt. % (c.f. 2.3.1.2)

The total identified mass (21 wt. % of the Primary Product) corresponds to 64 % of the solvent-free fraction which is in compliance with Commission Regulation (EC) 627/2006 (EC, 2006).

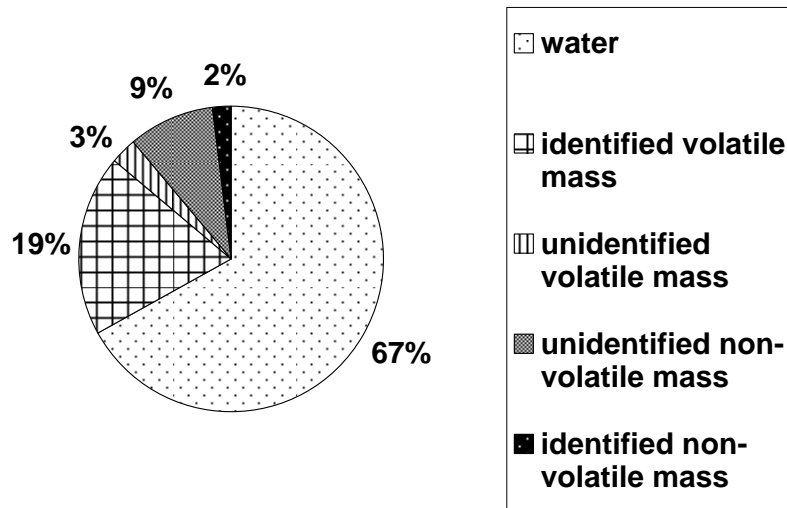


Figure 1. Overall composition of SmokEz C-10 (wt. % of Primary Product)

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

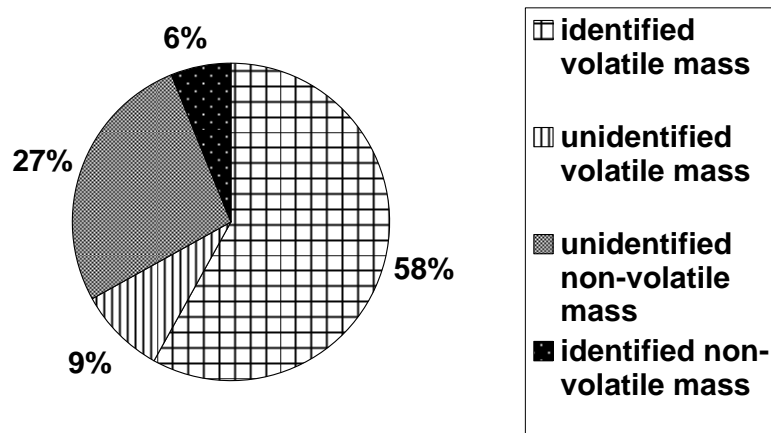


Figure 2. Composition (%) of the solvent-free fraction of SmokEz C-10

2.3.2. Chemical description of the Primary Product

Data have been given on acidity, phenols, carbonyls, solids, and hydroxyacetaldehyde. Method descriptions have been provided for all parameters. Parameters of the batches used for chemical and toxicological tests are presented in Table 1.

Table 1. Description of major chemical parameters of the Primary Product SmokEz C-10

Parameter	lot no. C-10-05083301 (used for chemical analysis)	lot no. C-10-05044209 (used for toxicological studies)
Acids (wt.%)	9.5	9.5
Phenols (wt.%)	1.3	1.1
Carbonyls (wt.%)	11.6	12.3
Solids (°BRIX)	25.0	24.6
Hydroxyacetaldehyde (HA) (wt.%)	2.07	2.19

2.3.3. Identification and quantitation of the Primary Product constituents

2.3.3.1. Principal constituents

GC and GC/MS analyses of the Primary Product resulted in the identification and quantification of 46 volatile constituents representing 19 wt. %. The 23 principal constituents are listed in Table 2.

Table 2. Principal constituents of the Primary Product SmokEz C-10 (lot C-10-05083301)

Constituent	g/kg
Acetic acid	67
Acetol (Hydroxypropanone)	26
Hydroxyacetaldehyde	18
Formic acid	14
Methanol	8.1
Glyceraldehyde	7.0
Formaldehyde	5.6
Propionic acid	4.0
Catechol (2-Hydroxyphenol)	3.5
Methylacetate	3.3
2,5-Dimethylphenol	2.9
2-Furaldehyde	2.4

Acetaldehyde	2.4
2,6-Dimethoxyphenol	2.2
Cyclotene (2-Hydroxy-3-methyl-2-cyclopenten-1-one)	2.0
Guaiacol (2-Methoxyphenol)	2.0
p-Cresol	1.8
3-Buten-2-one	1.4
Acrolein	1.3
Methylglyoxal	1.3
Ethylene glycol	1.2
5-Hydroxymethylfurfural	1.1
Glyoxal	1.1

2.3.3.2. Content of Polycyclic Aromatic Hydrocarbons (PAHs)

The contents of 12 of the 15 PAHs listed in Annex 2 of the EFSA guidance document (EFSA, 2005) have been determined in SmokEz C-10 by an external accredited laboratory using the EPA method 3510/8270-GC/MS (Table 3). According to the applicant, the analyses of 5-methylchrysene, cyclopenta[*cd*]pyrene and dibenzo[*a,e*]pyrene were not performed because the respective calibration standards were not available at the time the samples were analysed.

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).

Table 3. Concentrations of PAHs in the Primary Product SmokEz C-10 (lot C-10-05083301)

Compound	Content (µg/kg)
Chrysene	<10
Benzo[<i>a</i>]anthracene	<10
5-Methylchrysene	n.a.
Cyclopenta[<i>c,d</i>]pyrene	n.a.
Benzo[<i>b</i>]fluoranthene	<10
Benzo[<i>j</i>]fluoranthene	<10
Benzo[<i>k</i>]fluoranthene	<10
Benzo[<i>a</i>]pyrene	<10
Indeno[<i>1,2,3-cd</i>]pyrene	<10
Dibenzo[<i>a,h</i>]anthracene	<10

Benzo[<i>g,h,i</i>]perylene	<10
Dibenzo[<i>a,e</i>]pyrene	n.a.
Dibenzo[<i>a,h</i>]pyrene	<10
Dibenzo[<i>a,i</i>]pyrene	<10
Dibenzo[<i>a,l</i>]pyrene	<10

n.a. not analysed

2.3.4. Batch-to-batch variability

For 24 batches produced in 2005 GC-based data were presented for the contents of acetol, glycoaldehyde, acetic acid, cyclotene, guaiacol, phenol, 2,6-dimethylphenol and for three unidentified constituents. The relative standard deviations ranged from 11 to 24 %.

2.3.5. Stability

Storage stability was tested for 13 batches produced between October 2004 and December 2004. According to the data provided, sampling was “late 2004” and “late 2005”. GC-based data on the contents of acetol, hydroxyacetaldehyde, acetic acid, cyclotene, guaiacol, phenol, 2,6 dimethylphenol, and three unidentified constituents revealed an average decrease of 14% upon storage.

2.3.6. Specifications

The specifications as provided by the applicant for the Primary Product are presented in Table 4.

Table 4. Specifications of the Primary Product SmokeEz C-10

pH	2.1 - 2.6
Total Acidity (as acetic acid)	10.5 - 12.0 wt. %
Carbonyls	12.0 - 17.0 wt. %
Smoke Flavor Compounds*	10.0 - 15.0 mg/ml
Density	1.07 kg/l

* assumed to correspond to “phenols” as described in section 2.3.2.

The Panel noted that these figures are not in compliance with information given in Section 2.3.2.

3. Proposed uses

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

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

Food categories	Use level (g/kg)	
	Normal	Upper
1 Dairy products, excluding products of category 2	2.5	5
2 Fats and oils and fat emulsions (type water-in-oil)	2.5	5
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	1.2	2.5
5 Confectionery	1.2	2.5
6 Cereals and cereal products, including flours & starches from roots & tubers, pulses & legumes, excluding bakery	1.2	2.5
7 Bakery wares	2.5	5
8 Meat and meat products, including poultry and game	2.5	5
9 Fish and fish products, including molluscs, crustaceans and echinoderms	2.5	5
10 Egg and egg products	1.2	2.5
11 Sweeteners, including honey	0	0
12 Salts, spices, soups, sauces, salads, protein products etc.	2.5	5
13 Foodstuffs intended for particular nutritional uses	0	0
14.1 Non-alcoholic ("soft") beverages, excl. dairy products	0.1	0.2
14.2 Alcoholic beverages, incl. alcohol-free and low-alcoholic counterparts	0.05	0.1
15 Ready-to-eat savouries	2.5	5
16 Composite foods (e.g. casseroles, meat pies, mincemeat) - foods that could not be placed in categories 1 – 15	0.2	3

After consulting with the users and seeking more detailed information on the actual use the applicant provided updated use levels for the different food categories on 28 April 2009. These data are presented in Table 5b.

Table 5b. Normal and upper use levels for the Primary Product in food categories as outlined in Commission Regulation (EC) No 1565/2000 (Data provided on 28 April 2009)

Food categories	Use level (g/kg)	
	Normal	Upper
1 Dairy products, excluding products of category 2	1.5	5
2 Fats and oils and fat emulsions (type water-in-oil)	0.2	2
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	1.2	2.5
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	2.5	5
9 Fish and fish products, including molluscs, crustaceans and echinoderms	2.5	5
10 Egg and egg products	0	0
11 Sweeteners, including honey	0	0
12 Salts, spices, soups, sauces, salads, protein products etc.	1.5	5
13 Foodstuffs intended for particular nutritional uses	0	0
14.1 Non-alcoholic ("soft") beverages, excl. dairy products	0	0.1
14.2 Alcoholic beverages, incl. alcohol-free and low-alcoholic counterparts	0	0.1
15 Ready-to-eat savouries	1	5
16 Composite foods (e.g. casseroles, meat pies, mincemeat) - foods that could not be placed in categories 1 – 15	1	5

4. 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 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 and published for different European countries (Linseisen *et al.*, 2006). The CEF Panel used consumption data from the EPIC study to estimate the potential cumulative dietary exposure to smoke flavourings.

The Smoke flavouring EPIC model (SMK-EPIC) is based on a number of assumptions, in particular it assumes that a hypothetical high consumer of smoked meat is also an average consumer of the other traditionally smoked foods and an occasional consumer of smoked foods or beverages from each of the other categories.

Dietary exposure estimates were calculated by assuming that the Primary Product is present at the normal or upper use levels provided by the applicant for the 18 food categories as outlined in Commission Regulation (EC). When the normal use levels are used, the SMK-TAMDI can be considered as an adaptation of the modified TAMDI (mTAMDI), the method used by the AFC Panel (EFSA, 2004) to screen and prioritise flavouring substances.

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

The applicant provided two data sets for use levels, one submitted originally in 2005, and the second in April 2009, after consulting with clients and seeking more detailed information on the actual use levels.

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

Considering the initial data provided on use levels in 2005 the dietary exposure from all sources ranges from 23.9 to 26.0 mg/kg bw/day, when assuming that the Primary Product is present at the upper use levels, and from 10.9 to 13.0 mg/kg bw/day, when normal use levels are considered (Table 6a).

Considering the updated information on use levels from 28 April 2009 the dietary exposure from all sources ranges from 22.2 to 33.8 mg/kg bw/day, when assuming that the Primary Product is present at the upper use levels reported by the applicant and from 9.3 to 12.5 mg/kg bw/day, when normal use levels are considered (Table 6b).

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

Considering the initial data on use levels provided in 2005 the highest exposure estimates, resulting from the SMK-EPIC model, were 7.3 and 14.5 mg/kg bw/day when using normal and upper use levels, respectively. With the SMK-TAMDI model these figures were 4.2 and 8.3 mg/kg bw/day, respectively (Table 6a).

Considering the updated information on use levels from 28 April 2009 the highest exposure estimates, resulting from the SMK-EPIC model, were 6.8 and 14.5 mg/kg bw/day when using normal and upper use levels, respectively. With the SMK-TAMDI model these figures were 4.2 and 8.3 mg/kg bw/day, respectively (Table 6b).

Dietary exposure to the Primary Product was not assessed by the applicant.

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

Methodologies		Dietary exposure (mg/kg bw/day)	
		Normal use levels	Upper use levels
SMK-TAMDI	Traditionally smoked food	4.2	8.3
	Other foods not traditionally smoked	8.3	16.7
	Beverages (alcoholic or non-alcoholic)	0.5	1.0
	Total dietary exposure	13.0	26.0
SMK-EPIC	Traditionally smoked food	7.3	14.5
	Other foods not traditionally smoked	3.6	9.2
	Beverages (alcoholic or non-alcoholic)	0.1	0.2
	Total dietary exposure	10.9	23.9
Applicant	Dietary exposure	- ^a	- ^a

^a Not provided

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

Table 6b. Summary of the dietary exposure estimates to the Primary Product (based on use levels provided on 28 April 2009)

Methodologies		Dietary exposure (mg/kg bw/day)	
		Normal use levels	Upper use levels
SMK-TAMDI	Traditionally smoked food	4.2	8.3
	Other foods not traditionally smoked	8.3	25.0
	Beverages (alcoholic or non-alcoholic)	0.0	0.5
	Total dietary exposure	12.5	33.8
SMK-EPIC	Traditionally smoked food	6.8	14.5
	Other foods not traditionally smoked	2.6	7.6
	Beverages (alcoholic or non-alcoholic)	0.0	0.1
	Total dietary exposure	9.3	22.2
Applicant	Dietary exposure	- ^a	- ^a

^a Not provided

5. Toxicological data

5.1. Identity of the test material

The material used for all toxicological tests was batch number C-10-05044209, with the exception of the bacterial gene mutation assay and the *in vivo* rat liver unscheduled DNA synthesis (UDS) test. As indicated in Section 2.1.1, batch C-10-05044209 was not the same as that tested for chemical composition, however only minor differences were reported by the applicant in the overall chemical description of the two batches (Section 2.3.2). In the bacterial gene mutation assay, carried out in 1977, the material tested was described as C-10 (batch not identified), while in the *in vivo* rat liver unscheduled DNA synthesis test, carried out in 2007, the batch tested was C-10-01217120.

5.2. Subchronic toxicity

A comprehensive 90-day subchronic toxicity study was conducted according to GLP guidelines on SmokEz C-10, batch number 05044209 (TNO, 2005a).

The test material was administered to groups of 10 Wistar rats per sex at 0.45, 1.5 and 4.5% (w/w) in the diet. The intake of study substance per kg body weight per day was calculated from the nominal dietary concentration, the food consumption and the mean body weight in the relevant week, and was equivalent to overall mean intakes of 270, 900 and 2600 mg/kg bw/day in males and 300, 900 and 2800 mg/kg bw/day in females. 2,6-Dimethoxyphenol (2,6-DMP; present at relatively high levels in the test substance) was used as a tracer compound in the diets to calculate the actual content of SmokEz C-10, however analyses to determine the stability of the test material were not undertaken, given that it was a complex mixture.

There were no clinical signs of toxicity during the study, and none of the rats died. Neurobehavioural (Functional Observational Battery, FOB) and motor activity assessment showed no evidence of a neurotoxic potential, while ophthalmoscopic examination did not reveal any treatment-related effects. There was however a consistent and statistically significant decrease in body weight gain throughout the study in both males and females at the highest dose level of 4.5% in the diet (11% decrease in body weight gain overall compared with controls in males and 11.4% in females). Body weight was also significantly decreased at several of the assessment times in mid-dose females. Body weight changes were associated with decreased food consumption in the top dose animals and in affected mid-dose females, which was possibly due to palatability of the diet. Water consumption was decreased in high dose males.

Haematological examinations showed statistically significant increases in thrombocyte counts in female rats at both the 1.5 and 4.5% dietary level compared with controls (controls 946 ± 30 , 0.45% group 971 ± 23 , 1.5% group 1042 ± 18 ($p < 0.05$), 4.5% group 1065 ± 34 ($p < 0.01$), all results expressed as $\times 10^9/l$). Clinical chemistry investigations revealed significant decreases in aspartate aminotransferase in these same (female) groups (86% of control at 1.5% and 78% of control at 4.5%). Total plasma protein and also albumin was significantly increased in males at 4.5% (106% and 109% of control, respectively), while females at this level showed significant increases in plasma cholesterol (128% of control) and phospholipids (118% of control) and a significant decrease in plasma creatinine (76% of control). Urinalysis showed a non-significant trend towards increased urinary volumes in both male and female top dose rats.

Female rats at both the 1.5 and 4.5% dietary level showed significantly increased relative liver and kidney weight (relative liver weight 108% of control at 1.5% and 116% of control at 4.5%, relative kidney weight 109% of control at 1.5% and 111% of control at 4.5%). There were however no treatment-related macroscopic or microscopic (histopathological) findings in any group.

Overall in this study, females at both the 1.5% and the 4.5% dietary levels (equivalent to 900 or 2800 mg/kg bw/day, respectively) showed evidence of a treatment-related effect, comprising a consistent decrease in body weight gain (only in females receiving 2800 mg/kg bw/day), decreased aspartate aminotransferase and plasma creatinine, increases in plasma cholesterol and phospholipids (only in females receiving 2800 mg/kg bw/day) and increased relative liver and kidney weight. Males receiving 2600 mg/kg bw/day also showed decreased body weight gain and significant changes in some biochemical parameters.

In the opinion of the Panel, the no-observed-adverse-effect level (NOAEL) in this study was therefore 300 mg/kg bw/day (0.45% in the diet) in female rats and 900 mg/kg bw/day in males based on increased kidney weights.

5.3. Genotoxicity

SmokEz C-10 has been evaluated in tests for induction of gene mutations in bacteria (WARF Institute, 1977), gene mutations in mammalian cells *in vitro* (OECD 476) (TNO, 2005b), and chromosomal aberrations in mammalian cells *in vitro* (OECD 473) (TNO, 2005c). It has also been tested in an *in vivo* mouse micronucleus test (OECD 474) (TNO, 2005d) and in an *in vivo* rat liver unscheduled DNA synthesis test (TNO, 2007). With the exception of the gene mutations in the bacteria test, studies were performed in accordance with GLP.

The non-GLP assay for gene mutation in bacterial cells was carried out in 1977 on a material described as Carsol (Liquid Smoke), C-10. It used *Salmonella typhimurium* strains TA 1535, TA1537, TA 1538, TA98 and TA 100 at stated concentrations of 156, 313, 425 or 1250 µg/plate, in the presence or absence of a metabolic activating system and with appropriate positive controls. The material was toxic at levels of 2500 µg/plate and above in the absence of S9 and at 5000 µg/plate in the presence of S9. There was no evidence of an increased number of revertants at any of the non-toxic dose levels in any of the bacterial strains tested. Positive controls gave the expected responses. However, only summary tables of results were provided, containing no experimental details and the test is considered to be of limited validity.

SmokEz C-10 Liquid was tested for mutagenic potential in an *in vitro* mammalian cell mutation assay (OECD 476) using mouse lymphoma L5178Y *tk+/-* cells. Two independent assays were carried out. In the first assay, SmokEz C-10 was tested in the dose range 6.1 to 300 µg/ml in the absence of S9-mix and using a 24 hour exposure period, while in the presence of S9 the dose range used was 6.1 to 1250 µg/ml, with a 4 hour exposure period. Several lower test concentrations were also used but the cultures were discarded because they were not needed for analytical purposes. In the second assay SmokEz C-10 was tested in the dose range 3.0 to 300 µg/ml in the absence of S9-mix but using a 4 hour exposure period. In the presence of S9, a dose range of 100 to 300 µg/ml was used, with smaller intervals between the concentrations, duplicate cultures at each test concentration and an exposure time of 4 hours. A solvent control,

dimethylsulfoxid (DMSO), and positive controls (methylmethanesulphonate (MMS) and 3-methyl-cholanthrene (MCA) in the absence and presence of metabolic activation, respectively) were also included in both assays.

The highest concentrations evaluated for mutagenicity were 150 µg/ml in the absence of S9 and 350 µg/ml in the presence of S9, due to the cytotoxicity of SmokEz C-10. In the absence of S9-mix, the relative total growth (RTG) compared with vehicle control cultures was decreased at and above a concentration of 72 µg/ml (RTG 85% in first assay and 74% in second assay), while in the presence of S9-mix, the RTG was decreased at and above 300 µg/ml in the first assay (RTG 28%), and at and above 150 µg/ml in the second assay (RTG 79%). Concentration-related and reproducible positive responses in mutant frequency were observed at concentrations of and above 72 µg/ml in the absence of S9 and 210 µg/ml in the presence of S9. The criterion for a positive response was an increase in induced mutant frequency above concurrent control levels. At concentrations causing a positive response in mutant frequency, relatively more small than large colonies were formed.

It is concluded that SmokEz C-10 demonstrated both mutagenic and clastogenic potential in this *in vitro* mammalian cell mutation assay.

SmokEz C-10 was examined for its potential to induce structural chromosomal aberrations in Chinese Hamster Ovary (CHO) cells (OECD 473), in both the absence and presence of S9 mix. Two independent assays were conducted. The first assay used test concentrations of 39, 78 and 156 µg/ml in the absence of S9 and 156, 313 and 625 µg/ml in the presence of S9. In both cases cells were treated for 4 hours and harvested after 18h. In the second assay in the absence of S9 two treatment regimes were used involving (i) test concentrations of 150, 200 and 300 µg/ml, a 4 hour treatment period and harvesting at 18 hours, and (ii) test concentrations of 75, 100 and 150 µg/ml for a continuous 18 hour treatment period. In the presence of S9, test concentrations of 300, 400 and 500 µg/ml were used, cells were treated for 4 hours and harvested after 18h.

In the absence of S9, SmokEz C-10 caused a statistically significant ($p < 0.001$) increase in chromosomal aberrations when compared to the solvent control, at 156 µg/ml in the first assay (the highest concentration analysed, causing 50% inhibition of mitotic index and a mean incidence of cells showing chromosome aberrations of 14.5%), at 200 and 300 µg/ml following a 4 hour exposure in the second assay (74% and 82% inhibition of mitotic index and 12.5% and 15% aberrant cells, respectively), and in the continuous treatment group at all concentrations analysed (75, 100 and 150 µg/ml, showing 8%, 9.5% and 20.5% aberrant cells, respectively). In the presence of S9 there was also a statistically significant ($p < 0.001$) increase in the number of aberrant cells, occurring in the first assay at 625 µg/ml (the highest concentration analysed, causing 60% inhibition of mitotic index and 44% aberrant cells) and in the second assay at the two highest concentrations analysed (400 and 500 µg/ml, causing 47% and 62% inhibition of mitotic index and 3% and 10% aberrant cells, respectively). While SmokEz C-10 was cytotoxic in this test system, the clastogenic effect was evident within acceptable toxicity levels. SmokEz C-10 showed evidence of a positive, dose-related clastogenic effect activity in both, the absence and presence of S9.

SmokEz C-10 was examined for its genotoxic potential in an *in vivo* bone marrow mouse micronucleus test in male Charles River CD-1 mice (OECD 474). The test was carried out in

male mice only, since in the opinion of the applicant the subchronic toxicity study in rats carried out at approximate intakes of 2700 mg/kg had revealed no sex differences in toxicity. Group size was 5, and animals received 2 doses of 2000 mg/kg bw SmokEz C-10 in saline by gavage, the doses being given 24 hours apart. A positive control group received a single dose of 0.75 mg/kg bw mitomycin C intraperitoneally, negative control mice received saline alone. Animals were killed 24 hours after the second treatment. No evidence of clinical toxicity was seen in SmokEz C-10-treated animals, and there was no change in NCE/PCE ratio. There was no increase in the frequency of micronucleated polychromatic erythrocytes (MCPE) in male mice at 24 hours after treatment with SmokEz C-10 compared to the vehicle control, while the positive control showed the anticipated increases in MCPE.

An *in vivo* rat liver unscheduled DNA synthesis test was also performed with SmokEz C-10 (batch no. C-10-01217120). DNA repair in hepatocytes was measured following administration by gavage of 2000 mg/kg bw SmokEz C-10 in phosphate-buffered saline (PBS) to 6 male Wistar rats (CrI:[WI] WU BR). The negative control group were dosed with PBS alone, while positive control groups (n=2) received either 2-acetylaminofluorene (late sampling period) or N-nitrosodimethylamine (early sampling period). Hepatocytes were isolated at 2-4 h and 12-16 h after exposure, and unscheduled DNA synthesis was measured by autoradiography, following incubation of the hepatocyte cultures with [methyl-³H]-thymidine. SmokEz C-10 did not cause an increase in net nuclear grain count at either sampling time, while positive controls gave expected results. It can be concluded that under the conditions of this study, SmokEz C-10 does not induce unscheduled DNA synthesis in the rat liver.

5.4. Other studies

No other studies on SmokEz C-10 were provided by the applicant.

6. Discussion

The applicant provided information on the identity, composition, batch-to-batch variability and stability of the Primary Product as requested in the EFSA guidance document, which was considered acceptable.

The contents of 12 of the 15 PAHs listed in Annex 2 of the EFSA guidance document (EFSA, 2005) have been determined in SmokEz C-10 by an external accredited laboratory using the EPA method 3510/8270-GC/MS. According to the applicant, the analyses of 5-methylchrysene, cyclopenta[*cd*]pyrene and dibenzo[*a,e*]pyrene were not performed because the respective calibration standards were not available at the time the samples were analysed. 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 concentrations of 5-methylchrysene, cyclopenta[*cd*]pyrene and dibenzo[*a,e*]pyrene, PAHs known to be carcinogenic, were not provided, the Panel concluded that based on the reported levels of other carcinogenic PAHs, the levels for 5-methylchrysene, cyclopenta[*cd*]pyrene and dibenzo[*a,e*]pyrene would be expected to be similarly low.

SmokeEz C-10 showed negative results in a *S. typhimurium* reverse mutation assay in strains TA1535, TA1537, TA1538, TA98 and TA100, both in the absence and presence of S9. The Panel noted that this non-GLP study, carried out in 1977, did not comply with current test guidelines, but did not consider that it was necessary to request the applicant to repeat the study, given that the other two *in vitro* studies submitted on SmokEz C-10 gave clearly positive results.

Positive results were obtained in the mouse lymphoma L5178Y *tk*+/- assay, primarily at cytotoxic concentrations of SmokEz C-10, with relatively more small than large colonies being formed. In a test for chromosomal aberrations in Chinese Hamster Ovary (CHO) cells, SmokEz C-10 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 unscheduled DNA synthesis test was also negative.

Overall it is concluded that SmokEz C-10 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 90-day toxicity study with SmokEz C-10 treatment-related effects were observed in both males and females at a dietary level of 4.5% (equivalent to a mean intake of 2600 mg/kg bw/day in males and 2800 mg/kg bw/day in females) and in females at a dietary level of 1.5% (equivalent to a mean intake of 900 mg/kg bw/day). The no-observed-adverse-effect level (NOAEL) was considered by the Panel to be 300 mg/kg bw/day, based on the increased kidney weights in female rats.

The applicant provided two data sets for use levels, one submitted originally in 2005, and the second in April 2009, after consulting with clients and seeking more detailed information on the actual use levels. For transparency reasons both the initially provided data from 2005 and the updated data from 2009 were considered.

Use levels of the Primary Product by the applicant range based on finished food product weight range from 0.2 g/kg (fats and oil) to 5 g/kg (dairy products, meat, fish). Dietary exposure to the Primary Product was not assessed by the applicant.

In order to estimate dietary exposure to the Primary Product SmokeEz C-10, 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).

Considering the initial data provided on use levels in 2005 the dietary exposure from all sources ranges from 23.9 to 26.0 mg/kg bw/day, when assuming that the Primary Product is present at the upper use levels, and from 10.9 to 13.0 mg/kg bw/day, when normal use levels are considered (Table 6a).

Considering the updated information on use levels from 28 April 2009 the dietary exposure from all sources ranges from 22.2 to 33.8 mg/kg bw/day, when assuming that the Primary Product is present at the upper use levels, and from 9.3 to 12.5 mg/kg bw/day, when normal use levels are considered (Table 6b).

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

Considering the initial data on use levels provided in 2005 the highest exposure estimates, resulting from the SMK-EPIC model, were 7.3 and 14.5 mg/kg bw/day when using normal and upper use levels, respectively. With the SMK-TAMDI model these figures were 4.2 and 8.3 mg/kg bw/day, respectively (Table 6a).

Considering the updated information on use levels from 28 April 2009 the highest exposure estimates, resulting from the SMK-EPIC model, were 6.8 and 14.5 mg/kg bw/day when using normal and upper use levels, respectively. With the SMK-TAMDI model these figures were 4.2 and 8.3 mg/kg bw/day, respectively (Table 6b).

Based on the intake data originally provided by the applicant in June 2005 the margins of safety for total dietary exposure (traditionally and non-traditionally smoked food) as compared to the NOAEL of 300 mg/kg bw/day in female rats derived from the 90-day toxicity study amount to 12 and 13 for the intake estimates based on the upper use levels and to 23 and 28 when normal use levels are considered (Table 7a).

When assuming the use of Primary Product SmokeEz C-10 in traditionally smoked products only the margins of safety would amount to 21 and 36 based on the upper use levels and to 41 and 71 when normal use levels are considered. (Table 7a).

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

		Use level	Dietary exposure* (mg/kg bw/day)	NOAEL (mg/kg bw/day)	Margin of safety*
Total dietary exposure	Normal		10.9 / 13	300	23 / 28
	Upper		23.9 / 26	300	12 / 13
Traditionally smoked food	Normal		7.3 / 4.2	300	71 / 41
	Upper		14.5 / 8.3	300	36 / 21

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

Based on the intake data calculated with the new data provided by the applicant on 28 April 2009 for total dietary exposure (traditionally and non-traditionally smoked food), the margins of safety as compared to the NOAEL of 300 mg/kg bw/day in female rats derived from the 90-day toxicity study amount to 9 and 14 for the intake estimates based on the upper use levels and to 24 and 32, when normal use levels are considered (Table 7b).

When assuming the use of Primary Product SmokeEz C-10 in traditionally smoked products only the margins of safety would amount to 21 and 36 based on the upper use levels and to 44 and 72 when normal use levels are considered. (Table 7b).

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

		Use level	Dietary exposure* (mg/kg bw/day)	NOAEL (mg/kg bw/day)	Margin of safety*
Total exposure	dietary	Normal	9.3 / 12.5	300	24 / 32
		Upper	22.2 / 33.8	300	9 / 14
Traditionally smoked food		Normal	6.8 / 4.2	300	72 / 44
		Upper	14.5 / 8.3	300	36 / 21

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

The Panel did not anticipate that smoke flavourings would be used in food specifically designed for infants (0-12 months) and children (12-36 months). Therefore, the safety of use of Primary Product SmokEz C-10 in such products was not assessed

Considering that these margins of safety based on a 90-day toxicity study are inadequate, and that, in addition, data on reproduction and developmental toxicity as well as long term studies are absent, it is concluded that the uses and use levels of the Primary Product SmokEz C-10 in a wide range of product categories would require a larger margin of safety. The Panel concludes that the margin of safety is insufficient and that the use of Primary Product SmokEz C-10 at the proposed uses and use levels is of safety concern.

It is outside the remit of the Panel to decide whether, despite the low margins of safety, the use of Primary Product SmokEz C-10 might be approved for traditionally smoked products, at use levels specified, to replace smoking.

CONCLUSIONS AND RECOMMENDATIONS

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.

Primary Product SmokEz C-10 is genotoxic *in vitro* in the mouse lymphoma assay and the chromosomal aberration assay, whereas two *in vivo* genotoxicity tests are negative and sufficient to eliminate the concerns over the *in vitro* genotoxicity.

The NOAEL derived from a 90-day study is 300 mg/kg bw/day.

Since the data on use levels originally provided in June 2005 have been updated by the applicant in April 2009, the Panel drew its conclusions based on the margins of safety calculated with these recent data.

Based on the intake data calculated with the new data provided by the applicant on 28 April 2009 for total dietary exposure (traditionally and non-traditionally smoked food), the margins of safety as compared to the NOAEL of 300 mg/kg bw/day in female rats derived from the 90-day toxicity

study amount to 9 and 14 for the intake estimates based on the upper use levels and to 24 and 32, when normal use levels are considered (Table 7b).

When assuming the use of Primary Product SmokeEz C-10 in traditionally smoked products only the margins of safety would amount to 21 and 36 based on the upper use levels and to 44 and 72 when normal use levels are considered. (Table 7b).

Considering that these margins of safety based on a 90-day toxicity study are inadequate, and that, in addition, data on reproduction and developmental toxicity as well as long term studies are absent, it is concluded that the uses and use levels of the Primary Product SmokEz C-10 in a wide range of product categories would require a larger margin of safety. The Panel concludes that the margins of safety are insufficient and that the use of Primary Product SmokEz C-10 at the proposed uses and use levels is of safety concern.

It is outside the remit of the Panel to decide whether, despite the low margins of safety, the use of Primary Product SmokEz C-10 might be approved for traditionally smoked products, at use levels specified, to replace smoking.

DOCUMENTATION PROVIDED TO EFSA

1. Dossier from Red Arrow Products Company LLC, June 2005.
2. Response from Red Arrow Products Company LLC 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. Regulation 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.
- Linseisen, J., Rohrmann, S., Norat, T., Gonzalez, C.A., Dorransoro 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.
- TNO, 2005a. TNO Nutrition and Food Research Institute, The Netherlands. Sub-chronic (13-week) oral toxicity study with SmokeZ C-10 and SmokEz Enviro 23 in rats. Study Code No.5809/01 and 5809/02, March, 2005.
- TNO, 2005b. TNO Nutrition and Food Research Institute, The Netherlands. Gene mutation test at the TK-locus of L5178Y cells with SmokeZ C-10. Study Code No. 5603/13. May, 2005.
- TNO, 2005c. TNO Nutrition and Food Research Institute, The Netherlands. Chromosomal aberration test with SmokeZ C-10 in cultured Chinese Hamster Ovary (CHO) cells. Study Code No. 5602/16, January 2005.
- TNO, 2005d. TNO Nutrition and Food Research Institute, The Netherlands. Micronucleus test in bone marrow cells of mice treated with SmokeZ C-10. Study Code No. 6204/01, June 2005.
- TNO, 2007. TNO Nutrition and Food Research Institute, The Netherlands. Unscheduled DNA synthesis test with SmokEz C-10/CharSol C-10 and SmokEz Enviro 23/CharSol Select 23 in rat liver hepatocytes. Study Code No. 7633, December 2007.
- Scientific Committee for Food, 1995. First annual report on chemically defined flavouring substances. May 1995, 2nd draft prepared by the SCF Working Group on Flavouring 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.
- WARF Institute, 1977. Warf Institute, INC, Madison, Wisconsin. Ames Mutagenesis Test. Reference Number C-10 and Hyckory H-10. July, 1977.

ABBREVIATIONS

AFC	Scientific Panel on Additives, Flavourings, Processing aids and Materials in Contact with Food.
bw	body weight
CEF	Scientific Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
CHO	Chinese Hamster Ovary cell line
DMSO	Dimethyl sulfoxide
EC	European Commission
EFSA	European Food Safety Authority
EPA	Environmental Protection Agency
EPIC	European Prospective Investigation into Cancer and Nutrition
FID	Flame Ionisation Detection
FOB	Functional Observational Battery
GC-MS	Gas Chromatography/Mass Spectrometry
GLP	Good Laboratory Practice
HPLC	High Performance Liquid Chromatography
LOQ	Limit of quantification
mTAMDI	modified TAMDI
MCA	3-methyl-cholantrene
MCPE	Micronucleated polychromatic erythrocytes
MMS	methylmethansulphonate
MSD	Mass Selective Detection
NOAEL	No-Observed-Adverse-Effect Level
OECD	Organisation for Economic Cooperation and Development
PAH	Polycyclic Aromatic Hydrocarbons
PBS	Phosphate-Buffered Saline
PCE/NCE	Polychromatic Erythrocytes/ Normochromatic Erythrocytes
RTG	Relative Total Growth
SCF	Scientific Committee on Food
SMK-EPIC	Smoke flavouring EPIC model

SMK-TAMDI	Smoke Theoretical Added Maximum Daily Intake
SCF	Scientific Committee on Food
TAMDI	Theoretical Added Maximum Daily Intake
UDS	Unscheduled DNA Synthesis