European Food Safety Authority

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SCIENTIFIC OPINION

Safety of smoke flavour Primary Product – SmokEz Enviro 23[1]

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

(Question number EFSA-Q-2005-264)

Adopted on 14 May 2009

PANEL MEMBERS

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SUMMARY

The European Food Safety Authority has been asked to provide scientific opinions on the safety of smoke flavouring Primary Products used or intended for use in or on foods. This opinion concerns a Smoke Flavouring Primary Product, named SmokEz Enviro 23.

The Primary Product SmokEz Enviro 23 is obtained from mixed wood species. The average proportions reported by the applicant are as follows: maple (*Acer saccharum*) 47 % and oak (*Quercus alba*) 29 % as primary sources and hickory (*Carya ovata*), ash (*Fraxinus amaricana*), birch (*Betula papyrifera and Betula alleghaniensis*), wild black cherry (*Prunus serotina*), apple (*Malus domestica*), and beech (*Fagus grandifolia*) as secondary sources (2 %).

The production of SmokEz Enviro 23 comprises the following steps: (i) drying of the hardwood sawdust, (ii) heating of the dried sawdust in a specified reactor, (iii) condensing of the released smoke, and (iv) separation of the aqueous part of the smoke condensate from non-aqueous phases by settlement and filtering. Essential parameters of the manufacturing process have been provided by the applicant.

The water content of the Primary Product has been estimated as 67 wt. %. The amount of the volatile fraction determined by capillary gas chromatography amounted to 16 wt. % of the

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Primary Product. 14 wt. % (corresponding to 88 % of the volatile fraction) were identified which is in compliance with Commission Regulation (EC) 627/2006 (EC, 2006). The total identified mass (18 wt. % of the Primary Product) corresponds to 54 % of the solvent-free fraction which is in compliance with Commission Regulation (EC) 627/2006 (EC, 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 Enviro 23 by an external accredited laboratory using the US-Environmental Protection Agency (EPA) method 3510/8270-GC/MS. According to the applicant, for 5-methylchrysene, cyclopenta[*cd*]pyrene and dibenzo[*a,e*]pyrene no analyses were performed because the 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.

The reverse mutation test (Ames test) with bacteria was negative. The mouse lymphoma assay and the chromosome aberration assay *in vitro* both showed a positive response for the endpoint chromosome aberrations. The *in vivo* mouse micronucleus test and the *in vivo* UDS study were both negative.

Overall, it is concluded that SmokEz Enviro 23 is genotoxic *in vitro* in the mouse lymphoma and the chromosome 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 Enviro 23 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 1000 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.

Use levels of the Primary Product proposed by the applicant range between 1 g/kg food (ready-to-eat savouries, composite foods) and 5 g/kg food (dairy products, fish and meat). Dietary exposure to the primary product was not assessed by the applicant.

In order to estimate dietary exposure to the Primary Product SmokeEz Enviro 23, 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 20.8 to 33.3 mg/kg bw/day, when assuming that the Primary Product is present at the upper use levels, and from 8.7 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 34, when normal use levels are considered.

When assuming the use of Primary Product SmokeEz Enviro 23 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 for the Primary Product 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 the Primary Product SmokEz Enviro 23 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 Enviro 23 might be approved for traditionally smoked products, at use levels specified, to replace smoking.

Keywords: Smoke flavouring, Primary Product, SmokEz Enviro 23.

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BACKGROUND

Smoking is a process traditionally applied to certain perishable foods such as fish and meat. It was originally used for preservation purposes. In addition the process results in sensory changes (colour and flavour) which impart characteristic properties to smoked foods. With the development of other methods the preservative function of smoking decreased in importance over time and the sensory aspects prevailed.

Nowadays, liquid smoke flavourings are added to various foods to replace the smoking process or to impart smoke flavour to foods, which are not traditionally smoked.

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

The Regulation (EC) No 2065/2003 of the European Parliament and the Council (EC, 2003) established Community procedures for the safety assessment and the authorisation of smoke 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 required by to Article 8 of Regulation (EC) No. 2065/2003 of the European Parliament and of the Council on smoke flavourings used or intended for use in or on foods to carry out risk assessments and deliver a scientific opinion on the safety of Primary Products.

ACKNOWLEDGEMENTS

The European Food Safety Authority wishes to thank the members of the Working Group for the preparation of this opinion: D. Arcella, A. Carere, K.-H. Engel, D.M. Gott, J. Gry, R. Gürtler, D. Meier, I. Pratt, I.M.C.M. Rietjens[*], R. Simon and R. Walker.

[*] 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 Enviro23 manufactured strictly in conformity with the specified process and meeting the chemical specifications described in this opinion.

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 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 authorisations and evaluations of the Primary Product SmokEz Enviro23 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*) and oak (*Quercus alba*) are the primary wood genera used as source materials of the Primary Product. In addition, hickory (*Carya ovata*), 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 manufacturing process in varying amounts. On the basis of annual production data, the following average proportions and ranges have been provided by the applicant:

maple: 53 % (min. 25 % - max. 65 %)
oak: 45 % (min. 20 % - max. 75 %)
secondary woods: 2 % (min. 0% - max. 15 %).

According to the applicant, the lot R23-05083338 used for chemical tests was produced from 60% maple, 34% oak and 5% secondary woods (hickory, ash, birch, cherry, and/or beech).

The material used for all toxicological tests (lot R23-05054280), except for the *in vivo* rat liver unscheduled DNA synthesis test, chromosomal mutations in mammalian cells *in vitro* and the mouse micronucleus test *in vivo*, was produced from 49 % maple, 41 % oak, and 10% combined secondary woods (hickory, ash, birch, cherry, and/or beech).

The material for the *in vivo* rat liver unscheduled DNA synthesis (UDS) test (lot R23-06087119) was produced from 49 % maple, 48 % oak and 3 % secondary hardwoods.

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 hardwood sawdust with a defined moisture content is continuously pyrolysed in a reactor 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 an 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 Enviro 23. According to the applicant SmokEz Enviro 23 and CharSol Select 23 are identical Primary Products. SmokEz Enviro is Red Arrows international trade name and CharSol Select is the domestic trade name. The international trade name is used in this opinion.

2.2.2. Physical state of the Primary Product

SmokEz Enviro 23 is described as a clear brown liquid with "hardwood smoke aroma", an average specific gravity of 1.091 kg/l and a viscosity of 2.1 cP (25°C).

2.3. Chemical composition of the Primary Product

2.3.1. Overall characterisation

The overall characterisation of the Primary Product is as follows:

2.3.1.1. Solvent-free fraction

Water functions as the solvent of SmokeEz Enviro 23. 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 16 wt. % of the Primary Product. 14 wt. % (corresponding to 88 % 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. % - 16 wt. % = 17 wt. %. High performance liquid chromatography (HPLC) analysis of the Primary Product revealed a content of 2.1 wt. % levoglucosan and 1.9 wt. % cellobiosan. The unidentified volatile mass amounts to 2 wt. % (c.f. 2.3.1.2)

The total identified mass (18 wt. % of the Primary Product) corresponds to 54 % of the solvent-free fraction which is in compliance with Commission Regulation (EC) 627/2006 (EC, 2006).

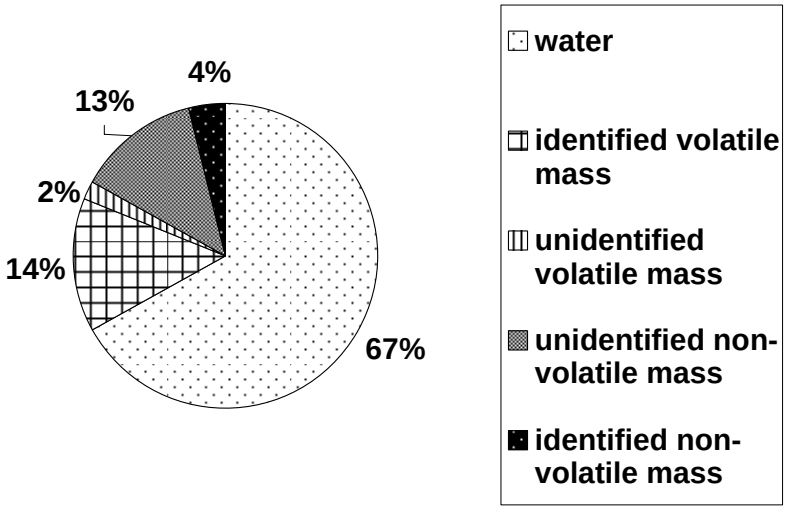

Figure 1. **Overall composition of SmokEz Enviro 23 (wt. % of Primary Product)**

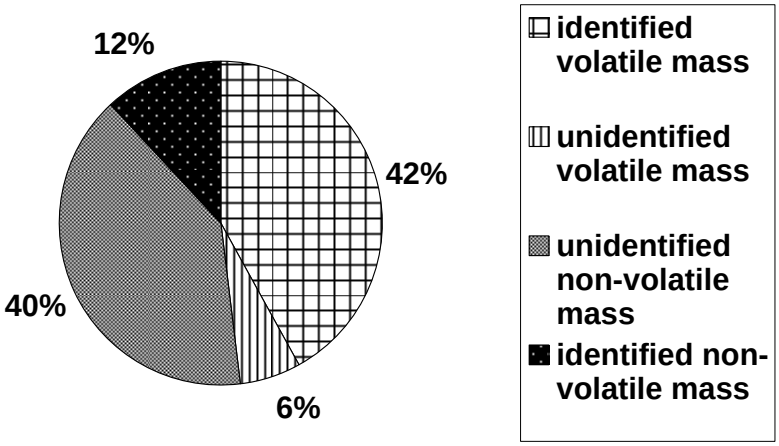

Figure 2. **Composition of the solvent-free fraction of SmokEz Enviro 23**

2.3.2. Chemical description of the Primary Product

Data have been provided on acidity, phenols, carbonyls, solids, and hydroxyacetaldehyde. Method descriptions for the analysis 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 Smoke Ez Enviro 23**

Parameter	R23-05083338 (used for chemical analysis)	R23-05054280 (used for toxicological studies)
Acids (wt.%)	5.9	5.9
Phenols (wt.%)	1.0	1.0
Carbonyls (wt.%)	12.5	13.8
Solids (°BRIX)	25.0	25.6
Hydroxyacetaldehyde (HA) (wt.%)	3.52	3.58

2.3.3. Identification and Quantification 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 14 wt. %. The 20 principal constituents are listed in Table 2.

Table 2. **Principal constituents of the Primary Product SmokEz Enviro 23 (batch R23-05083338)**

Constituent	g/kg
Acetic Acid	39
Hydroxyacetaldehyde	31
Acetol (Hydroxypropanone)	19
Glyceraldehyde	14
Formic Acid	7.2
Methanol	4.8
Formaldehyde	4.5
Catechol (2-Hydroxyphenol)	2.4
3-Buten-2-one	1.9
Propionic acid	1.8
Methylglyoxal	1.5
Glyoxal	1.3
2-Furaldehyde	1.3
Cyclotene (2-Hydroxy-3-methyl-2-cyclopenten-1-one)	1.2
Acetaldehyde	1.2
2,5-Dimethylphenol	1.0
Ethylene glycol	1.0
Methylacetate	1.0
5-Hydroxymethylfurfural	1.0
o-Cresol	0.8

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 Enviro 23 by an external accredited laboratory using

the EPA method 3510/8270-GC/MS (Table 3). According to the applicant, for 5-methylchrysene, cyclopenta[*cd*]pyrene and dibenzo[*a,e*]pyrene no analyses were performed because the calibration standards were not available at the time of the analysis.

The levels of benzo[*a*]pyrene and benzo[*a*]anthracene are below their resepective 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 Enviro 23**

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

n.a.: not analysed

2.3.4. Batch-to-batch variability

For 13 batches produced in 2004 GC-based data were presented for the contents of acetol, hydroxyacetaldehyde, acetic acid, cyclotene, guaiacol, phenol, 2,6-dimethylphenol and for three unidentified constituents. The relative standard deviations ranged from 5.5 to 33 %.

2.3.5. Stability

For one batch (R23-05054280) data obtained by analysis in May 2004 and in January 2006 were compared. The contents of cyclotene (0.14 %), guaiacol (0.03 %) and phenol (0.05 %) remained unchanged, those of acetol (from 1.2 to 1.3 %), acetic acid (from 3.9 to 5.3 %) and syringol (from 0.11 to 0.14%) were slightly increased and the content of glycoaldehyde was decreased (from 3.6 to 2.6 %).

2.3.6. Specifications

The specifications as provided by the applicant for the Primary Product SmokEz Enviro 23 are listed in Table 4.

Table 4. **Specifications of the Primary Product SmokEz Enviro 23**

pH	2.8 - 3.2
Total Acidity (as acetic acid)	6 .0 – 7.0 wt. %
Carbonyls	16.0 – 24.0 wt. %
Smoke flavour compounds*	10.0 – 16.0 mg/ml
Density	1.09 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	2.5	5

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	0
3	Edible ices, including sherbet and sorbet	0	0
4.1	Processed fruits	0	0
4.2	Processed vegetables (including mushrooms & fungi, roots & tubers, pulses & legumes) and nuts and seeds	0	0
5	Confectionery	0	0
6	Cereals and cereal products, including flours & starches from roots & tubers, pulses & legumes, excluding bakery	0	0
7	Bakery wares	0	0
8	Meat and meat products, including poultry and game	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
14.2	Alcoholic beverages, incl. alcohol-free and low-alcoholic counterparts	0	0
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	0.2	3

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) 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). 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 20.8 to 33.3 mg/kg bw/day, when assuming that the Primary Product is present at the upper use levels reported by the applicant and from 8.7 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 *	-	-

* 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	0.0
	Total dietary exposure	12.5	33.3
SMK-EPIC	Traditionally smoked food	6.8	14.5
	Other foods not traditionally smoked	2.0	6.3
	Beverages (alcoholic or non-alcoholic)	0.0	0.0
	Total dietary exposure	8.7	20.8
Applicant	Dietary exposure *	-	-

* not provided

5. Toxicological data

5.1. Identity of the test material

According to the applicant, the lot used for all toxicological tests (R23-05054280), except for the *in vivo* rat liver unscheduled DNA synthesis test, the chromosomal mutations in mammalian cells *in vitro* and the mouse Micronucleus test *in vivo*, consisted of 41% oak,

49% maple, 10% combined minor types (hickory, cherry, *etc.*). In the *in vivo* rat liver unscheduled DNA synthesis test, carried out in 2007, the batch tested was R23-06087119. It was produced from 49 % maple, 48 % oak and 3 % secondary hardwoods.

Deviating from this, lot no. R23-0316-6 of SmokEz Enviro 23 was used for the Micronucleus test *in vivo* study from 1996, the analyses results of which were reported by the applicant (without units) as: acids = 5.9; phenols = 13.7; solids (°BRIX) = 29.1; hydroxyacetaldehyde (HA) = 3.40.

According to the applicant, this lot was representative of the concentration of product produced at that time. The same reactor conditions were used today as those used to generate the lots of material used in the commissioned studies. One exception was in the concentration which was 29.1 °BRIX rather than the 24.0 - 24.5 °BRIX range used now. This reduction in concentration was to reduce dissolved tar content to improve the quality and in the opinion of the applicant should have no impact on the toxicological properties of the product. No composition of the source material was indicated by the applicant for the micronucleus assay (batch R23-0316-6).

5.2. Subchronic toxicity

A comprehensive 90-day subchronic toxicity study was conducted according to GLP guidelines on SmokEz Enviro 23 (TNO, 2005a).

Subchronic toxicity was assessed by dietary administration to groups of 10 animals per sex for 90 days at 0, 0.45, 1.5 and 4.5% (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). Two smoke condensates (SmokEz C-10 and SmokEz Enviro 23) were tested at each concentration in the study but only a single control (0%) group of 10 animals per sex was used. The results described below are for SmokEz Enviro 23.

No clinical signs were reported on observation (twice daily on working days, daily on weekends and holidays) or in motor activity assessment (clinical observations weekly until week 12, functional observational battery and motor activity assessment in week 13), thus SmokEz Enviro 23 showed no evidence of a neurotoxic potential. Ophthalmoscopic examinations were undertaken prior to dosing and final week in the control and high dose groups only, no adverse findings were observed.

Body weight decreases were observed in both sexes in the high-dose group and at some times points (days 42, 56 and 91) for mid-dose females; these body weight changes were accompanied by decreased food consumption but no changes in food conversion efficiency.. There was also decreased water consumption in high dose males.

In haematological testing, the mean corpuscular volume (MCV) increased in both sexes and mean corpuscular haemoglobin (MCH) in females at 4.5% SmokeEz Enviro 23, the thrombocyte count increased at 4.5% SmokeEz Enviro 23. The number of eosinophils decreased in males fed SmokEz Enviro 23 but these were considered incidental effects since these changes were marginal, not dose-related and not associated with significant effects on the relative distribution of eosinophils.

The following changes in clinical chemistry were observed:

- Decreases in aspartate aminotransferase (serum glutamic-oxaloacetic transaminase) in both sexes at 4.5% SmokeEz Enviro 23

- Decreases in alkaline phosphatase in females at 1.5 and 4.5% SmokeEz Enviro 23 (also decreased in mid but not high dose males considered fortuitous)
- Increases in albumin in males at 4.5% SmokeEz Enviro 23
- Increases in cholesterol and phospholipids in females at 4.5% SmokeEz Enviro 23
- Increases in triglycerides in females at 4.5% SmokeEz Enviro 23
- Decreases in creatinine in both sexes at 4.5% SmokeEz Enviro 23

Urinalysis showed increased urinary volume and decreased density in females at 4.5% SmokEz Enviro 23.

There were increases in relative kidney weight of 9% and 11% in the mid (1.5%) and high (4.5%) dose females and 1% in high dose males (4.5 %) for SmokEz Enviro 23. There were increases in relative liver weight of 8% and 16% in the mid (1.5%) and high (4.5%) dose females and 4% in high (4.5%) dose males on SmokEz Enviro 23. However there was no treatment related macroscopic or microscopic (histopathological) finding.

In the opinion of the Panel, the no-observed-adverse-effect level (NOAEL) in this study was therefore 290 mg/kg bw/day (0.45% in the diet) in female rats and 900 mg/kg bw/day in males based on kidney weights.

5.3. Genotoxicity

Three *in vitro* genotoxicity tests were evaluated (bacterial reverse mutation (TNO, 2005b), a chromosome aberration (Covance, 1997), and a gene mutation test with mouse lymphoma cells (TNO, 2005c)), together with an *in vivo* mouse micronucleus test in bone marrow (CHV, 1996) and an *in vivo* unscheduled DNA study in rat liver (TNO, 2007).

The bacterial reverse mutation assay was performed in accordance with OECD Guideline 471. SmokEz Enviro 23 was incubated in the presence and absence of S9 metabolic activation with *Salmonella typhimurium* TA98, TA100, TA1535, TA1537 and *Escherichia coli* WP2 uvrA at doses of 62 to 5000 µg/plate in the first assay and 313 to 5000 µg/plate in the second assay. No evidence of genotoxicity was seen in this assay.

A gene mutation test in L5178Y cells was performed in accordance with OECD Guideline 476. SmokEz Enviro 23 was incubated at 1.5 to 300 µg/ml in the absence of microsomal metabolic activation for 24 and 4 hours in the first and second assay, respectively, and 3.0 to 1250 µg/ml in the presence of microsomal metabolic activation for 4 hours in the first assay and 100 to 350 µg/ml for 4 hours in the second assay with mouse lymphoma L5178Y 3.7.2C cells.

The study protocol was a combined mutagenicity and cytogenetics (small and large colony) assay.

The study concludes that SmokEz Enviro 23 had mutagenic and clastogenic potential *in vitro*.

The *in vitro* mammalian chromosome aberration test was performed in accordance with OECD Guideline 473. SmokEz Enviro 23 was incubated in the presence and absence of metabolic activation with Sub-line (KI) of Chinese hamster ovary cell line.

A significant increase in cells with chromosomal aberrations was observed at 113 µg/ml in the absence of a microsomal metabolic activation system and at 200 and 300 µg/ml in the presence of microsomal metabolic activation system (endoreduplication observed at 200 µg/ml) in the first assay. A significant increase in cells with chromosomal aberrations was

observed at 75 and 113 µg/ml in the absence of microsomal metabolic activation system and 200 and 300 µg/ml at 20.1 hours and 200 µg/ml at 44 hours in the presence of microsomal metabolic activation system (endoreduplication observed at 150, 200 and 300 µg/ml at 22.1 hours) in confirmatory assay.

The report concludes positive for chromosomal aberrations and endoreduplication at the earlier sampling time only, but negative for polyploidy in the presence of microsomal metabolic activation system

The *in vivo* mouse micronucleus test (study reference 3E) was performed in accordance with OECD Guideline 474. SmokEz Enviro 23 was administered to 5 male and 5 female Crl:CD-1®(ICR) BR mice per dose and harvest time (24, 48 & 72 hours) as a single oral dose of 0, 1250, 2500 and 5000 mg/kg by oral gavage as a suspension in deionised water.

All animals in the control, 1250 and 2500 mg/kg groups appeared normal after dosing and remained healthy throughout. At 5000 mg/kg a number of clinical observations were reported, all animals appeared hypoactive at 1 hour, several were hypoactive or hypoactive and cold to the touch at 17 and 23 hours after dosing, 3 males and 1 female were found dead at 17 hours and an additional male at 41 hours.

There were no significant increases in micronucleated polychromatic erythrocytes in either sex at any time point. However the PCE/NCE ratio in all dosed males at 24 hours was statistically significantly lower than controls due to toxicity (0.54 ± 0.07, 0.57 ± 0.09, 0.35 ± 0.10 compared to 0.95 ± 0.13). Ratios were also lower in males at other harvest times but these were not statistically significant.

In the *in vivo* UDS study SmokEz Enviro 23 did not induce unscheduled DNA synthesis in liver cells of male rats, exposed to the test substances by gavage at the limit dose level (2000 mg/kg bw) under the conditions used in this study.

5.4. Other studies

No other studies on SmokEz Enviro 23 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 Enviro 23 by an external accredited laboratory using the EPA method 3510/8270-GC/MS. According to the applicant, for 5-methylchrysene, cyclopenta[*cd*]pyrene and dibenzo[*a,e*]pyrene no analyses were performed because the calibration standards were not available at the time the sample 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 TA98, TA100, TA1535, TA1537 and in *Escherichia coli* WP2 uvrA. Positive results were obtained in the mouse lymphoma L5178Y assay, showing both cytogenetic and mutagenic effects. In a test for chromosomal aberrations in Chinese Hamster Ovary (CHO) cells, SmokEz Enviro 23 resulted positive for chromosomal aberrations and endoreduplication at the earlier sampling time only, but negative for polyploidy in the presence of microsomal metabolic activation system. In the *in vivo* bone marrow micronucleus assay there were no significant increases in micronucleated polychromatic erythrocytes in either sex at any time point and an *in vivo* rat liver unscheduled DNA synthesis test was also negative.

Overall, it is concluded that SmokEz Enviro 23 is genotoxic *in vitro* in the mouse lymphoma and the chromosome 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 Enviro 23 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 findings on kidney weight and body weight 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 proposed by the applicant range between 1 g/kg food (ready-to-eat savouries and composite foods) and 5 g/kg food (dairy products, fish and meat). Dietary exposure to the primary product was not assessed by the applicant.

In order to estimate dietary exposure to the Primary Product SmokeEz Enviro 23, 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 20.8 to 33.3 mg/kg bw/day, when assuming that the Primary Product is present at the upper use levels, and from 8.7 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 Enviro 23 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 34, when normal use levels are considered (Table 7b).

When assuming the use of Primary Product SmokeEz Enviro 23 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 on 28 April 2009**

	Use level	Dietary exposure* (mg/kg bw/day)	NOAEL (mg/kg bw/day)	Margin of safety*
Total dietary exposure	Normal	8.7 / 12.5	300	24 / 34
	Upper	20.8 / 33.3	300	9 / 14
Traditionally	Normal	6.8 / 4.2	300	72 / 44

smoked food	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 the Primary Produt SmokeEz Enviro 23 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 for the Primary Product 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 the Primary Product SmokEz Enviro 23 at the proposed uses and use levels is of safety concern.

To decide whether despite the low margins of safety the use of Primary Product SmokeEz Enviro 23 might be approved for traditionally smoked products, at use levels specified, to replace smoking, is outside the remit of the Panel.

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.

Overall, it is concluded that SmokEz Enviro 23 is genotoxic *in vitro* in the mouse lymphoma and the chromosome aberration assay, whereas two *in vivo* genotoxicity tests are negative and sufficient to eliminate the concerns over the *in vitro* genotoxicity.

The NOAEL from a 90-day study is 300 mg/kg bw/day (0.45% in the diet) in female rats and 900 mg/kg bw/day in males.

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 34, when normal use levels are considered.

When assuming the use of Primary Product SmokeEz Enviro 23 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 for the Primary Product 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 Enviro 23 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 Enviro 23 might be approved for traditionally smoked products, at use levels specified, to replace smoking.

DOCUMENTATION PROVIDED TO EFSA

Dossier from Red Arrow Products Company LLC, June 2005
Responses from Red Arrow Products Company LLC to request for supplementary information.

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ABBREVIATIONS

2-AAF	2-Acetylaminofluorene
AFC	Scientific Panel on Additives, Flavourings, Processing aids and Materials in Contact with Food.
AST	Aspartate aminotransferase
AP	Alkaline phosphatase
bw	body weight
CEF	Scientific Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
CHO	Chinese Hamster Ovary cell line
EC	European Commission
EFSA	European Food Safety Authority
EPA	US-Environmental Protection Agency
EPIC	European Prospective Investigation into Cancer and Nutrition
FID	Flame Ionisation Detection
GC/MS	Gas Chromatography/Mass Spectrometry
GLP	Good Laboratory Practice
GS/FID	Gas Chromatography/Flame Ionisation Detection
HA	Hydroxyacetaldeyde
HPLC	High Performance Liquid Chromatography
mTAMDI	modified TAMDI
MCH	Mean Corpuscolar Haemoglobin
MCV	Mean Corpuscolar Volume
NDMA	N-nitroso-di-methylamine
NOAEL	No-Observed-Adverse-Effect Level
OECD	Organisation for Economic Cooperation and Development
PAHs	Polycyclic Aromatic Hydrocarbons
PCE/NCE	Polychromatic Erythrocytes/ Normochromatic Erythrocytes
SCF	Scientific Committee on Food
SMK-EPIC	Smoke flavouring EPIC model
SMK-TAMDI	Smoke Theoretical Added Maximum Daily Intake
TAMDI	Theoretical Added Maximum Daily Intake
UDS	Unscheduled DNA Synthesis