European Food Safety Authority

*The EFSA Journal* (2009) 982, 1-24

# SCIENTIFIC OPINION

## Safety of smoke flavour Primary Product - Zesti Smoke Code 10[1]

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

**(Question number EFSA-Q-2005-268)**

**Adopted on 29 January 2009**

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

**PANEL MEMBERS**

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**SUMMARY**

The European Food Safety Authority has been asked to provide scientific opinions on the safety of smoke flavouring Primary Products used or intended for use in or on foods. This Opinion concerns one such Smoke Flavouring Primary Product, named Zesti Smoke Code 10.

The Primary Product Zesti Smoke Code 10 is obtained from a specified mixture of the following woods: hickory (*Carya ovata*) and oak (*Quercus alba*). The production of Zesti Smoke Code 10 comprises the following steps: (i) drying and sieving 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 precipitated tar. Essential parameters of the manufacturing process have been provided by the applicant.

[1] For citation purposes: Scientific Opinion of the Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF) on the safety of smoke flavour Primary Product – Zesti Smoke Code 10. *The EFSA Journal* (2009) 982, 1-24.

[2] Ivonne Rietjens declared in her ADoI that she is advising FEMA on flavourings. In January 2009 she informed the Secretariat that FEMA also assesses smoke flavourings but also that she has never been involved in smoke flavourings evaluations there. According to EFSA Policy on DoI, that activity does not represent a conflict of interest. Upon request from the Secretariat, she updated accordingly her ADoI in May 2009. Therefore, an *addendum* to the minutes of the 4th CEF Plenary meeting (26-29 January 2009) and to the scientific opinions is introduced *a posteriori* to clarify this issue.

Identification and quantification of Primary Product constituents have been performed using different batches than were used for the toxicological studies. In both cases several batches covering a production period from 2004 to 2005 were used. Considering a relative standard deviation for the batch-to-batch reproducibility of 5 to 35% for the content of the individual identified constituents the material used in testing can be considered as representative for the Primary Product Zesti Smoke Code 10 in general.

The water content of the Primary Product is 64 wt. %. The volatile fraction identified by capillary gas chromatographic analysis (GC) accounts for 15 wt. %. The total amount of the unidentified constituents account for 33 wt. %. The concentrations of the polycyclic aromatic hydrocarbons (PAHs) listed in the EFSA guidance document on submission of a dossier on a Smoke Flavouring Primary Product have been provided. The concentrations of the individual substances were only above 1 µg/kg for benzo[*j*]fluoranthene (1.5 µg/kg in batch 0115738 in 2007) and cyclopenta[*c,d*]pyrene (1.3 g/kg in batch 0103638 and 1.5 µg/kg in batch 0320638 in 2006). The levels of benzo[*a*]anthracene and benzo[*a*]pyrene were below the limits as laid down in Regulation (EC) No 2065/2003. Analysis of ten batches revealed no significant batch-to-batch variability. No data were provided on the stability of the Primary Product.

Normal use levels of the Primary Product proposed by the applicant range between 1 g/kg food (ready-to-eat savouries, soups and broths) and 2.5 g/kg food (meat and meat products). Dietary exposure for the Primary Product, as estimated by the applicant, was 30 mg/kg body weight per day.

In order to estimate dietary exposure to the Primary Product Zesti Smoke Code 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 Zesti Smoke Code 10 is present at the normal or upper use levels provided by the applicant for the 18 food categories as outlined in Commission Regulation (EC). Dietary exposures from all sources range from 22.0 to 28.3 mg/kg bw/day, when assuming that the Primary Product Zesti Smoke Code 10 is present at the upper use levels, and from 9.3 to 11.7 mg/kg bw/day, when normal use levels are considered.

When dietary exposure estimates are based on use in only traditionally smoked foods dietary exposures range from 8.3 to 14.0 mg/kg bw/day, when assuming that the Primary Product Zesti Smoke Code 10 is present at the upper use levels, and from 4.2 to 6.5 mg/kg bw/day, when normal use levels are considered.

Genotoxicity studies conducted on Primary Product Zesti Smoke Code 10 included three *in vitro* studies (a bacterial reverse mutation test, a mammalian cell gene mutation assay and a chromosome aberration test) and two *in vivo* studies (a rat liver unscheduled DNA synthesis assay and a mouse bone marrow micronucleus test). The results obtained in these assays showed positive genotoxic responses in the *in vitro* assays but negative results *in vivo* in two well conducted studies.

Overall it is concluded that Primary Product Zesti Smoke Code 10 is genotoxic *in vitro*, whereas two *in vivo* genotoxicity tests were negative and sufficient to eliminate the concerns over the *in vitro* genotoxicity.

The Primary Product was investigated in two 90-day studies. The first one dates back to 1962 when Good Laboratory Practise (GLP) was not yet developed, the second one has been conducted recently according to GLP and OECD guidelines. The no-observed-adverse-effect level (NOAEL) was derived from the recent study based on the increased relative kidney weights and reduced body weight gain at the two highest dose groups and amounted to 2.5 g/kg diet equivalent to a minimum

dietary intake calculated by the applicant of 134 mg/kg bw/day in male rats and 178 mg/kg bw/day in female rats.

The NOAEL from this recent 90-day study appears to be similar to the NOAEL from the older 90-day study. Since for this older study the pathology report stated that "somewhat more distinct minor degenerative changes were observed in the liver and kidney" at 20 g/kg diet, and because slightly more marrow hyperplasia was also noted in this group, it was concluded that the lowest dose level of 2.5 g/kg diet was the NOAEL.

Based on these data it is concluded that when assuming that the Primary Product Zesti Smoke Code 10 is present at the normal or upper use levels provided by the applicant for the 18 food categories, the margins of safety as compared to the NOAEL of 134 mg/kg bw/day derived from the 90-day toxicity study in rats amount to 5 - 6 for the intake estimates based on the upper use levels and to 11 - 14 when normal use levels are considered.

When assuming the use of Primary Product Zesti Smoke Code 10 in traditionally smoked products only, the margins of safety would amount to 10 - 16 for the intake estimates based on the upper use levels and to 21 - 32 when normal use levels are considered.

Given i) the fact that these margins of safety are based on a 90-day toxicity study, ii) the absence of data on reproduction and developmental toxicity and iii) the absence of long term studies, it is concluded that the uses and use levels of Primary Product Zesti Smoke Code 10 would require a larger margin of safety. The Panel concludes that the margin of safety is insufficient and that the use of Primary Product Zesti Smoke Code 10 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 Zesti Smoke Code 10 might be approved for traditionally smoked products, at use levels specified, to replace smoking, is outside the remit of the Panel.

**Key words:** Smoke flavouring, Primary Product, Zesti Smoke Code 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 the preservative function of smoking decreased in importance over time and the sensory aspects prevailed. Nowadays, liquid smoke flavourings are added to various foods to replace the smoking process or to impart smoke flavour to foods which are not traditionally smoked.

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

The Regulation (EC) No 2065/2003 of the European Parliament and the Council (EC, 2003) established Community procedures for the safety assessment and the authorisation of smoke flavourings intended for use in or on foods. As stated herein the use of a Primary Product in or on foods shall only be authorised if it is sufficiently demonstrated that it does not present risks to human health. A list of Primary Products authorised to the exclusion of all others in the Community for use as such in or on food and/or for the production of derived smoke flavourings shall therefore be established after the European Food Safety Authority (EFSA) has issued an opinion on each Primary Product.

The Guidance on submission of a dossier on a smoke flavouring Primary Product for evaluation by EFSA (EFSA, 2005) lays down the administrative, technical and toxicological data required.

## TERMS OF REFERENCE

The EFSA is requested according to Article 8 of Regulation (EC) No. 2065/2003 of the European Parliament and of the Council on smoke flavourings used or intended for use in or on foods to carry out risk assessments and deliver a scientific opinion on the safety of Primary Products.

## ACKNOWLEDGEMENTS

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

## ASSESSMENT

The following evaluation applies only to the Primary Product Zesti Smoke Code 10 manufactured strictly in conformity with the specified process. In accordance with the guidance document (EFSA, 2005), data on the manufacturing process, the composition, intended use levels and toxicological tests have been submitted. The latter include two 90-day oral subchronic toxicity studies 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 Zesti Smoke Code 10 has been provided.

## 2. Technical data

### 2.1. Manufacturing process

#### 2.1.1. Source materials for the Primary Product

The Primary Product Zesti Smoke Code 10 is obtained from sawdust derived from a mixture of wood of the species hickory (*Carya ovata,* 50-60%) and oak (*Quercus alba,* 40-50%).

#### 2.1.2. Method of manufacture of the Primary Product

Sawdust is dried by moderate heating and cleaned by sieving. The method for determination of the moisture content is described. For the production of smoke the raw material is heated in a continuous process under exclusion of air. Mode of heating, reaction temperature range, and residence time of the sawdust are given. The smoke is transferred into a condensing tower. Temperature and mode of operation of the condensing tower are given. The raw product is stored for the separation of tar, which is not used in the further processing. A time for this settlement period is given. After settlement the smoke condensate is filtered to give Primary Product Zesti Smoke Code 10.

The process has been described in detail and a flow chart has been given by the applicant.

### 2.2. Identity of the Primary Product

#### 2.2.1. Trade names of the Primary Product

The trade name of the product is Zesti Smoke Code 10.

#### 2.2.2. Physical state of the Primary Product

The Primary Product is described as a brown clear liquid, soluble in water, ethanol, and other aqueous solvents.

## 2.3. Chemical composition

### 2.3.1. Overall characterisation

The overall characterisation of the Primary Product is as follows:

2.3.1.1. Solvent-free fraction

The water fraction (64 wt. %) of the Primary Product functions as solvent. Accordingly, the solvent-free mass amounts to 36 wt. % (Figure 1).

2.3.1.2. Volatile fraction

The mass of the volatile fraction has been calculated from the results of capillary gas chromatographic-mass spectrometric (GC-MS) analyses and totals to 17 wt. % of the Primary Product (Figure 1). This corresponds to 47 % of the solvent-free fraction (Figure 2). 15 wt. % (= 88 % of the volatile fraction) were identified which is in compliance with Regulation 627/2006 (EC, 2006).

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 volatile compounds determined by GC: 36 wt. % - 17 wt. % = 19 wt. %. Additionally, the Primary Product was analysed for the three sugars glucosan, xylose and glucose, the sum of which contributed to about 1 wt. %. The unidentified volatile mass amounts to 2 wt. % (c.f. 2.3.1.2).

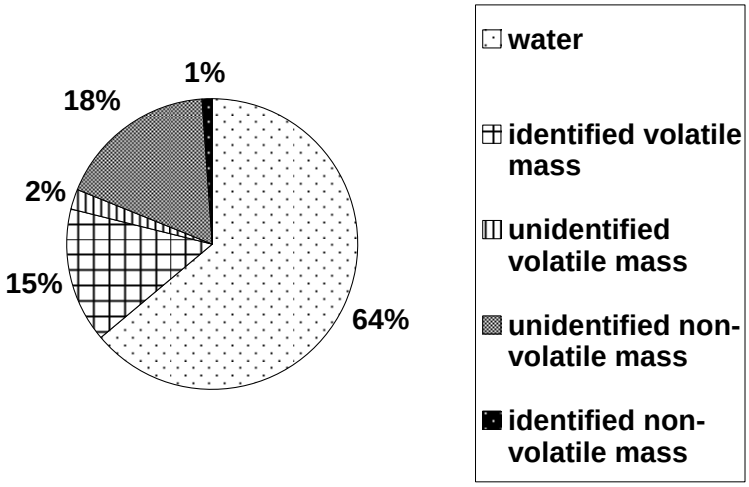


Figure 1. **Overall composition of Zesti Smoke Code 10 (wt. % of Primary Product)**

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

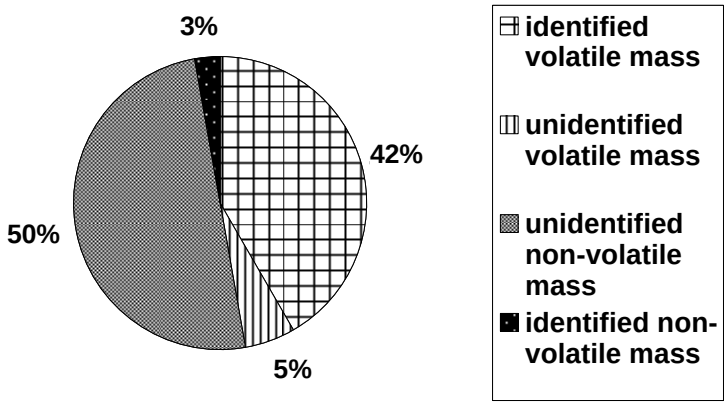


Figure 2. **Composition (%) of the solvent-free fraction of Zesti Smoke Code 10**

### 2.3.2. Chemical description of the Primary Product

Data have been given on staining index, acidity, carbonyls, phenols, specific gravity, pH, and the water content of the product. Method descriptions have been provided for all parameters. Except for the water content (average of 9 batches produced in 2004), data from 119 (2003) and 198 batches (2004) have been presented. Averages and standard deviations are listed in Table 1.

Table 1. **Description of major chemical parameters of the Primary Product Zesti Smoke Code 10**

| **Parameter** | **2003[(a)]** | | **2004[(b)]** | |
|---|---|---|---|---|
| | **Mean** | **RSD[(c)] [%]** | **Mean** | **RSD[(c)] [%]** |
| Staining index | 96 | 5.2 | 94 | 6.1 |
| Acidity (% acetic acid) | 10.8 | 1.2 | 10.8 | 1.7 |
| % Carbonyls | 20 | 13 | 21 | 7.6 |
| % Phenols | 1.8 | 5.6 | 1. 8 | 11 |
| Specific gravity | 1.081 | 1.9 | 1.080 | 0.4 |
| pH | 2.1 | 2.9 | 2.2 | 6.4 |
| % Water | | | 63.8[(d)] | 1.7 |

(a): Means of 119 batches; (b): Means of 198 batches ; (c): RSD = relative standard deviation ; (d): Mean of 9 batches

### 2.3.3. Identification and quantification of Primary Product constituents

2.3.3.1. Principal constituents

The volatile fraction of the Primary Product was analysed by capillary gas chromatographic (GC) analysis. Mass spectrometry (MS) was used for identification and flame ionisation detection (FID) for quantification. The compounds identified gave rise to signals corresponding to 86% of the total peak area in the FID-gas chromatogram. The mean contents and the standard deviations determined for the 12 principal constituents by analysis of 8 batches from 2007 are given in Table 2.

Table 2. **Principal constituents of the Primary Product Zesti Smoke Code 10**

| Identified Constituents | Mean g/l | SD g/l | RSD % |
|---|---|---|---|
| Acetic acid | 87.3 | 10.1 | 12 |
| Levoglucosan | 9.0 | 1.2 | 14 |
| 1-Hydroxy-2-propanone (acetol) | 5.6 | 0.4 | 7 |
| Formic acid | 4.8 | 0.6 | 12 |
| 2,6-Dimethoxyphenol | 4.2 | 0.3 | 7 |
| 2-Hydroxy-3-methyl-2-cyclopenten-1-one (Corylone) | 4.0 | 0.3 | 7 |
| Hydroxyacetaldehyde | 1.9 | 0.3 | 13 |
| 2-Methoxyphenol (Guaiacol) | 1.9 | 0.2 | 11 |
| 1-Hydroxy-2-butanone | 1.7 | 0.2 | 12 |
| Furfural | 1.4 | 0.4 | 31 |
| Propanal | 1.3 | 0.4 | 32 |
| 2(5H)-Furanone | 1.1 | 0.1 | 9 |
| 1,2,3-Trimethoxybenzene | 1.0 | 0.3 | 33 |
| Isopropyl hexanoate | 1.0 | 0.5 | 49 |
| 5-(Hydroxymethyl)-2-furfural | 0.9 | 0.2 | 27 |
| 2-Hydroxy-2-cyclopenten-1-one | 0.9 | 0.3 | 34 |
| 2-Butanone | 0.8 | 0.1 | 13 |
| Methanol | 0.8 | 0.3 | 36 |
| Tetrahydrofurfuryl alcohol | 0.8 | 0.05 | 7 |
| 1,4:3,6-Dianhydro-a-D-glucopyranose | 0.8 | 0.07 | 9 |

SD: standard deviation; RSD: relative standard deviation

2.3.3.2. Content of Polycyclic Aromatic Hydrocarbons (PAHs)

The contents of polycyclic aromatic hydrocarbons (PAHs) in the Primary Product Zesti Smoke Code 10 were determined using a method based on high performance liquid chromatography (HPLC) with UV-absorption and UV-fluorescence detection. The method has been validated and fulfills the performance criteria laid down by Commission Regulation No 627/2006 (EC, 2006).

The fifteen PAHs listed in Annex 2 of the EFSA guidance document (EFSA, 2005) were analysed in 55 batches of the Primary Product. Only for benzo[*j*]fluoranthene (1.5 µg/kg in batch 0115738 in 2007) and cyclopenta[*c,d*]pyrene (1.3 µg/kg in batch 0103638 and 1.5 µg/kg in batch 0320638 in 2006) values above 1 µg/kg were reported. In none of the batches the sum of the 15 PAHs exceeded 6 µg/kg. The levels of benzo[*a*]pyrene and benz[*a*]anthracene were below the respective maximum permitted values of 10 and 20 µg/kg, respectively, as laid down by Commission Regulation (EC) 2065/2003 (EC, 2003).

Exemplarily, data provided for the batch used in the toxicological studies are shown in Table 3.

Table 3. **Concentrations of PAHs in the batch of Primary Product used for the toxicological studies (Composite BJW 3225 Flavouring 37735 [Code 10], Item 1040100)**

| PAH | Mean (µg/kg)[a)] | RSD (%) |
|---|---|---|
| Chrysene | 0.1 | 69 |
| Benzo[*a*]anthracene | 0.1 | 7 |
| 5-Methylchrysene | < LOQ | - |
| Cyclopenta[*cd*]pyrene | 1.3 | 72 |
| Benzo[*b*]fluoranthene | < LOQ | - |
| Benzo[*j*]fluoranthene | 0.1 | 316 |
| Benzo[*k*]fluoranthene | < LOQ | - |
| Benzo[*a*]pyrene | 0.1 | 72 |
| Indeno[*1,2,3-cd*]pyrene | 0.5 | 5 |
| Dibenzo[*a,h*]anthracene | 0.1 | 95 |
| Benzo[*ghi*]perylene | 0.2 | 63 |
| Dibenzo[*a,e*]pyrene | 0.3 | 13 |
| Dibenzo[*a,h*]pyrene | 1.0 | 0.2 |
| Dibenzo[*a,i*]pyrene | 0.9 | 0.6 |
| Dibenzo[*a,l*]pyrene | < LOQ | - |
| **Total PAH** | **4.6** | |

[a)] data from ten replicate samples; LOQ: limit of quantification

### 2.3.4. Batch-to-batch variability

The batch-to-batch variability of the Primary Product was demonstrated as follows:

(a) Variability of the parameters listed in Table 1: 317 batches of the Primary Product Zesti Smoke Code 10 were analysed between 2003 and 2004. The batches showed no significant variability and all values were within the specifications given.

(b) Variability of the concentrations of unidentified chemical constituents in a dichloromethane extract by GC-FID: Eight batches were analysed and the variability was assessed on the basis of the peak areas of the chromatograms. The relative standard deviations ranged from 5 to 35 %.

(c) Variability of the main constituents in a dichloromethane extract by GC-MS: Four batches were analysed and 52 compounds was assessed. The relative standard deviations ranged from 9 % (for 5-methyl-2(5H)-furanone) to 71 % (for 1,2,3-trimethoxybenzene).

d) Variability of constituents after dilution of the Primary Product with acetone by GC-MS: The analysis of eight batches resulted in relative standard deviations (RSD) between 5 and 222 %. However, the high RSD values belonged to minor constituents; the values of the vast majority of constituents varied between 5 and 69 %.

(e) Variability of the concentrations of the 15 PAHs: The PAHs were determined in 49 batches from 2006. The values reported correspond to the pattern described in 2.3.3.2.

### 2.3.5. Stability

The stability of the product was checked by analysing a batch twice over a period of 24 months. On average the contents of the 22 major constituents decreased by 39 %; for individual constituents the

decreases varied between 11 % and 68 %. A second study on 300 constituents in 3 batches over a period of six months indicated losses up to 10 % for single volatiles.

### 2.3.6. Specifications

The specifications given for the Primary Product Zesti Smoke Code 10 are listed in Table 4.

Table 4. **Specifications of Zesti Smoke Code 10**

| | |
|---|---|
| Acidity, as acetic acid (%) | 10.5 – 11 |
| Staining index | 70 – 100 |
| Carbonyls (g/100 ml) | 15 – 25 |
| Phenols (mg/ml) | 12 – 22 |
| Specific gravity (at 25°C) | 1.070 – 1.088 |
| pH | 2.0 – 2.5 |

## 3. Proposed uses

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

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

| Food categories | | Use level (g/kg) | |
|---|---|---|---|
| | | Normal | Upper |
| 1 | Dairy products, excluding products of category 2 | 1.5 | 4 |
| 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 | 1.5 | 5 |
| 4.2 | Processed vegetables (including mushrooms & fungi, roots & tubers, pulses & legumes) and nuts and seeds | 1.5 | 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 | 1.5 | 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 | 4 |
| 16 | Composite foods (e.g. casseroles, meat pies, mincemeat) - foods that could not be placed in categories 1 – 15 | 1.5 | 4 |

## 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 flavouring substances (Scientific Committee for Food, 1995). As for the TAMDI, the SMK-TAMDI also assumes that the hypothetical consumer will daily consume a fixed amount of flavoured solid foods and liquids. However, in the SMK-TAMDI approach a single group "Beverages" is used for liquids whereas solid foods are divided in "traditionally smoked solid foods" and "other solid foods not traditionally smoked".

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

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

Normal and upper use levels for the breakdown of the food category 12 "Salts, spices, soups, sauces, salads, protein products etc." were requested to the applicant. This was considered necessary in order to reduce the overestimation of exposure due to the heterogeneity of this food category that contains products presenting significant differences in terms of consumption.

Dietary exposure estimates calculated by means of the above mentioned methods are reported in Tables 6. Dietary exposure from all sources range from 22.0 to 28.3 mg/kg bw/day, when assuming that the Primary Product is present at the upper use levels, and from 9.3 to 11.7 mg/kg bw/day, when normal use levels are considered.

The impact on exposure of authorizing the Primary Product only in traditionally smoked food products was also assessed. Out of the above mentioned 18 food categories, "Dairy products, excluding products of category 2", "Meat and meat products, including poultry and game" and "Fish and fish products, including molluscs, crustaceans and echinoderms" were considered as "Traditionally smoked solid foods". In this case, the SMK-EPIC model results in the highest exposure estimates of 6.5 and 14.0 mg/kg bw/day when using normal and upper use levels, respectively.

Dietary exposures to the Primary Product were also estimated by the applicant using food consumption data, per gender and age group, derived from the UK National Diet and Nutrition Survey (NDNS) in adults aged 16-64 (Henderson *et al*., 2002). The highest dietary exposure calculated by the applicant resulted in 30.0 mg/kg bw/day. This estimate is obtained by assuming that an individual would daily consume the maximum amount of all foods containing the Primary Product at the upper use levels.

Table 6. **Summary of the dietary exposure estimates to the Primary Product**

| Methodologies | | Dietary exposure (mg/kg bw/day) | |
|---|---|---|---|
| | | Normal use levels | Upper use levels |
| SMK-TAMDI | Traditionally smoked food | 4.2 | 8.3 |
| | Other foods not traditionally smoked | 7.5 | 20.0 |
| | Beverages (alcoholic or non-alcoholic) | 0.0 | 0.0 |
| | Total dietary exposure | 11.7 | 28.3 |
| SMK-EPIC | Traditionally smoked food | 6.5 | 14.0 |
| | Other foods not traditionally smoked | 2.8 | 8.0 |
| | Beverages (alcoholic or non-alcoholic) | 0.0 | 0.0 |
| | Total dietary exposure | 9.3 | 22.0 |
| Applicant | Dietary exposure | - | 30.0 |

## 5. 5. Toxicological data

### 5.1. Identity of the test material

The materials 37735 used for the toxicity studies were prepared as a composite of several (4 and 6) batches of the Primary Product Zesti Smoke Code 10 manufactured in the time period from 2002 to 2005. For the material SF-12 (used in 1962) no information has been given.

### 5.2. Subchronic toxicity

The Primary Product Zesti Smoke Code 10 was tested in two subchronic 90-day toxicity studies.

A 90-day oral (feeding) study in the rat has been conducted with liquid smoke SF-12 (an alternative designation for Primary Product Zesti Smoke Code 10) in 1962 (LAB, 1962).

Groups of 25 male and 25 female albino rats (strain not specified) were administered Primary Product Zesti Smoke Code 10 via the diet at levels of 0, 2.5 and 20 g/kg diet for 90 days. Animals were fed control diet for 1 week prior to initiation of the study. Body weight, food consumption and physical condition were assessed once weekly. General condition and incidence of mortality were noted daily. Haemograms consisting of haemoglobin, microhaematocrit and total and differential white cell counts were determined on five males and five females from the control groups and the high dose (20 mg/kg diet)-treated group at 6 and 13 weeks (90 days). At termination, all survivors were sacrificed and subjected to necropsy. Gross examination of tissues was performed on heart, liver, kidneys, ovaries and testes, and adrenal glands were weighed. Portions of these organs and the lung, stomach, small intestine, urinary bladder, bone marrow and thyroid from all animals were preserved in 10 % formalin. For five males and five females in each group the brain, pituitary, pancreas and thymus were also preserved in formalin. Histopathology was conducted on five males and five females from the negative and positive controls and high dose group. The decedent animal and those showing gross abnormalities at the time of necropsy were also examined histopathologically and included two females from the high dose group (20 g/kg diet), and one male and three females from the low dose group (2.5 g/kg diet).

One female in the low dose group (2.5 g/kg diet) died during the third week of the study. All other animals survived the duration of the experiment. There was no notable effect on bodyweight or food consumption following treatment with Primary Product Zesti Smoke Code 10. All animals showed apparently normal behaviour and general condition. There were no notable effects on haemoglobin, microhaematocrit or total and differential white cell counts which could be attributed to treatment with Primary Product Zesti Smoke Code 10. There were no grossly abnormal weight changes in organs from animals receiving Primary Product Zesti Smoke Code 10. Primary Product Zesti Smoke Code 10 at the levels used in this feeding study (2.5 and 20 mg/kg diet) was essentially devoid of any signs of toxicity. The histopathological changes seen in the experimental rats (comprising slight to moderate glycogen depletion, mild, moderate and marked congestion and evidence of parenchyma round cell foci in the liver; slight to moderate tubular degeneration and mild, moderate and marked congestion in the kidneys) were also seen in the negative controls. However, the frequency and extent (mild, moderate or marked) of these changes were generally greater in the 20 g/kg diet Primary Product Zesti Smoke Code 10-treated group, compared to the negative control. There was a tendency towards slight bone marrow hypoplasia in the rats given Primary Product Zesti Smoke Code 10 at the 20 g/kg diet level. However, the marrow appearances were reported to be within normal limits.

Since the pathology report stated that "somewhat more distinct minor degenerative changes were observed in the liver and kidney" at 20 g/kg diet test material, and because also slight bone marrow hypoplasia was noted in this group, the lowest dose level of 2.5 g/kg diet is assumed to be the no observed adverse effect level (NOAEL).

The applicant also provided the results of a recent 90-day study performed under Good Laboratory Practice (GLP) according to OECD guidelines (LAB, 2006d).

In this study the test article Primary Product Zesti Smoke Code 10 was administered by oral dietary administration to 10 male and 10 female Charles River Crl:WI(Han) rats at dose levels of 0 (control), 2.5, 25 and 50 g/kg in the diet.

The applicant provided the group mean compound consumption over the dosing period for the male and female rats. The minimum compound consumption for the 2.5, 25 and 50 g/kg diet groups amounted to 133.9, 1419.4 and 3280.2 mg/kg bw/day for male rats and to 177.7, 1889.0 and 4650.6 mg/kg bw/day for female rats.

Weight gains over week 1-13 in males given Primary Product Zesti Smoke Code 10 were 96 % (not significant), 87 % ($p<0.05$) and 74 % ($p< 0.001$) of that seen in controls, for the 2.5, 25 and 50 g/kg diet groups respectively. Corresponding gains in treated females over the same period were 102 % (not significant), 89 % (not significant), and 71 % ($p<0.001$) of controls. Since there was no treatment-related reduction in food consumption in either sex the Panel considers this reduction in weight gain an adverse effect.

There were no ophthalmoscopic abnormalities in any animal.

With respect to functional observations it was noted that at week 13 hind limb grip strength in females given the 50 g/kg diet was lower than controls by 15 %. The applicant indicates that in the absence of any other significant findings in the functional observation battery for this treatment group this difference was not considered to indicate an effect on neuromuscular function, and may instead correlate with the smaller size of the animals at week 13.

At week 13, males given 50 g/kg diet Primary Product Zesti Smoke Code 10 showed increased reticulocytes (24 % greater than controls), haemoglobin distribution width (12 % greater than controls) and lymphocytes (22 % greater than controls). The applicant indicates that based on

historical ranges for control animals, the magnitude of these changes was unlikely to be toxicologically important.

In clinical chemistry significant treatment-related reductions were noted in aspartate aminotransferase (AST), alanine aminotransferase (ALT) and creatinine for both sexes, urea in females and total cholesterol in males in the high and often also in the mid dose group. The significance of a decrease in AST and ALT was unclear, as toxic effects are usually associated with increases in these parameters. The applicant indicates that with the exception of lower creatinine in females given the 50 g/kg diet, these differences did not exceed normal limits (based on historical control data), and were therefore considered unlikely to be toxicologically relevant.

Treatment-related changes were observed in relative liver weights of both sexes and amounted to an increase by 6 % (not significant), 23 % ($p<0.001$) and 44 % ($p<0.001$) in relative liver weights in male and 5 % (not significant), 21 % ($p<0.01$) and 31 % ($p<0.001$) in female animals given respectively 2.5, 25 and 50 g/kg of Primary Product Zesti Smoke Code 10 in the diet (expressed as percentage differences from controls). Absolute liver weights were unaffected. After a 4 week treatment-free period for the highest dose group these increases appeared to be reversible, decreasing from 44 % to 11 % (not significant) for male and from 31 % to -12 % (not significant) for female rats.

Also treatment-related changes were observed in relative kidney weights of both sexes and these amounted to an increase by 2 % (not significant), 10 % ($p<0.05$) and 23 % ($p<0.001$) in male and -1 % (not significant), 11 % ($p<0.01$) and 18 % ($p<0.001$) in female animals given respectively 2.5, 25 and 50 g/kg of Primary Product Zesti Smoke Code 10 in the diet (expressed as percentage differences from controls). Absolute kidney weights were unaffected. After a 4 week treatment free period for the highest dose group these increases in relative kidney weight appeared to be only partially reversible, decreasing from 23 % to 17 % ($p<0.01$) for male and from 18 % to 13 % ($p<0.01$) for female rats.

The applicant indicated that at terminal kill of the test animals in the treatment and treatment-free groups, most tissues were macroscopically unremarkable and the findings seen were generally consistent with the usual pattern of findings in animals of this strain and age. Thus it was concluded that there were no macroscopic findings due to effects of Primary Product Zesti Smoke Code 10.

At terminal kill, microscopic findings in controls were generally infrequent, of a minor nature and consistent with the usual pattern of findings in animals of this strain and age.

Additionally, in treated animals, there were findings in the liver in males and females and the thyroid gland in males due to effects of Primary Product Zesti Smoke Code 10. In the liver, there was an increase in centrilobular hepatocellular hypertrophy in males and females dosed at 25 and 50 g/kg diet. This was characterised by hepatocytes with increased amounts of pale eosinophilic cytoplasm. The livers of animals dosed at 2.5 g/kg diet were comparable with the control animals.

In the thyroid, there was a minor increase in follicular cell hypertrophy recorded for males dosed at 50 g/kg diet. This was characterised by a diffuse increase in the height of the follicular epithelial cells. The thyroid glands of the males dosed at 2.5 and 25 g/kg diet and of the females dosed at 50 g/kg diet were comparable with control animals.

There were no other microscopic findings due to effects of Primary Product Zesti Smoke Code 10.

Based on the effects on the relative kidney weight which appeared not to be reversible upon a 4 week treatment-free period, the NOAEL of this study is 2.5 g/kg diet, amounting to minimum intake values calculated by the applicant of 133.9 mg/kg bw/day for males and 177.7 mg/kg bw/day for females.

The value of the NOAEL from this recent 90-day study of 2.5 g/kg diet appears to be the same as the NOAEL value of 2.5 g/kg diet in the older study.

### 5.3. Genotoxicity

The applicant provided the results of genotoxicity studies, which were all performed under GLP according to applicable OECD guidelines.

Primary Product Zesti Smoke Code 10 was assayed for mutagenicity in 5 histidine-requiring strains of *Salmonella typhimurium* (TA98, TA100, TA1535, TA1537 and TA102) in the absence and presence of metabolic activation up to the recommended limit concentration of 5000 µg/plate (LAB, 2004). Primary Product Zesti Smoke Code 10 showed no cytotoxicity or precipitation within the test system. A statistically significant ($p<0.005$) increase in the number of revertant colonies was seen at the top concentration tested (5000 µg/plate) in the range-finder experiment using TA100 (1.37-fold increase without S9 and 1.62-fold increase with S9-fold). Statistically significant (Dunnett's test to compare the counts of each dose with the control. and linear regression analysis to test for the presence of a dose response), dose-related and reproducible increases in revertant numbers were observed following treatment of strain TA100 at concentrations of 1000 µg/plate and above in the absence and presence of S9 up to 1.62-fold ($p<0.005$) and 1.40-fold ($p<0.005$) increase in the absence and presence of S9 at 5000 µg/plate. Statistically significant, dose-related and reproducible increases in revertant numbers were also observed following treatment of the closely related strain TA1535 at concentrations of 1000 µg/plate (1.21-fold increase)($p<0.05$) and at 5000 µg/plate (2.16 fold increase)($p<0.005$) in the absence of S9 and at the top concentration tested (5000 µg/plate) in the presence of S9 (1.51-fold increase)($p<0.005$). In TA102 a dose-dependent statistically significant increase up to a 1.45-fold increase ($p<0.005$) without S9 and a 1.39-fold increase ($p<0.01$) in the presence of S9 at 5000 µg/plate was observed (Batch no BJW 3842). The applicant concludes that Primary Product Zesti Smoke Code 10 showed weak mutagenic activity in this test system.

Primary Product Zesti Smoke Code 10 was assayed for its ability to induce mutation at the *tk* locus in mouse lymphoma cells (LAB, 2006c). The highest concentrations analysed were selected on the basis of cytotoxicity and amounted to 225 µg/ml in the absence of S9 and 450 µg/ml in the presence of S9 which yielded 24 % and 16 % relative survival, respectively. Statistically significant increases in mutant frequency were observed following treatment with Primary Product Zesti Smoke Code 10 at the top 3 concentrations analysed (150 to 225 µg/ml) in the absence of S9 and at the top four concentrations analysed (300 to 450 µg/ml) in the presence of S9. Highly significant linear trends were also obtained in both the absence and presence of S9. At concentrations where a statistically significant increase in mutant frequency was observed, increases in both small and large colony mutant frequencies were observed. The applicant concludes that Primary Product Zesti Smoke Code 10 was genotoxic in this test system.

Primary Product Zesti Smoke Code 10 (BJW 3842) was tested in an *in vitro* cytogenetic assay with cultured human peripheral blood lymphocytes in the absence and presence of metabolic activation (S9) up to the acceptable maximum concentration of 5000 µg/ml (LAB, 2006a). Primary Product Zesti Smoke Code 10 was tested using a three hour treatment period with a 17 hour recovery period (3+17 h) in both the absence and presence of S9 and using an extended treatment (20+0 h) in the absence of S9. The concentrations chosen for analysis for chromosome aberrations ranged from 219.9 to 429.5 µg/ml (-S9, 3+17h), 219.9 to 737.0 µg/ml (+S9, 3+17h) and 313.2 to 433.5 µg/ml (-S9, 20+0h) with the highest concentrations showing appropriate levels of toxicity (approximately 40 % to 62 % mitotic inhibition [reduction in mitotic index]). For some of the concentrations analysed less than the intended 200 cells (made up of 100 cells per replicate) were analysed.

However, at least 184 cells out of an intended 200 were analysed at each dose level. Treatment of cultures with Primary Product Zesti Smoke Code 10 in the absence and the presence of S9 resulted in frequencies of cells with structural aberrations, which were generally elevated compared to those observed in concurrent negative control cultures. For the majority of treatment conditions the increases in the frequencies of cells with aberrations were statistically significant with numbers of cells with aberrations up to 41/200 (41 out of 200 cells) at 429.5 µg/ml (-S9, 20+0h) versus 1/200 for the corresponding control ($p<0.0001$), 62/188 at 737.0 µg/ml (+S9, 3+17h) versus 2/200 for the corresponding control ($p<0.001$) and 65/190 at 433.5 µg/ml (-S9, 20+0h) versus 1/200 for the corresponding control ($p<0.001$). The applicant concludes that Primary Product Zesti Smoke Code 10 had the potential to induce chromosome aberrations in cultured human peripheral blood lymphocytes in both the absence and presence of S9.

Primary Product Zesti Smoke Code 10 (Batch BJW 0345) was tested for its ability to induce unscheduled DNA synthesis (UDS) in the livers of orally dosed male rats using an *in vivo/in vitro* procedure (LAB, 2005). The UDS assay is generally considered to show limited sensitivity to compounds which induce either point mutations or large deletions due to the nature of the endpoint (radio-labelling of sections of repaired DNA) used for detection of genotoxicity. However, there was evidence from both the mouse lymphoma assay (MLA) and the chromosome aberration test *in vitro* that Primary Product Zesti Smoke Code 10 induced clastogenic DNA damage in these studies, which would be detected by the UDS assay. Groups of four male Sprague Dawley Crl:CD®(SD) rats were treated once with the vehicle (15 % Tween 80 in purified water), Primary Product Zesti Smoke Code 10 (at the acceptable limit dose of 2000 mg/kg bw) or the required positive control, *via* oral gavage, Approximately 12-14 hours (Experiment 1) or 2-4 hours (Experiment 2) after dosing, animals were sacrificed and their livers perfused with collagenase to provide a primary culture of hepatocytes. Cultures were made from three animals in each dose group and were treated with [$^3$H] thymidine. There were no clinical signs or effects on body weight observed in any animal treated with Primary Product Zesti Smoke Code 10 in the main study. The applicant indicates that because Primary Product Zesti Smoke Code 10 is a complex mixture, confirmation of systemic exposure (*via* analysis of plasma) was not considered to be relevant, and that these assessments were not performed in this UDS study. Treatment with 2000 mg/kg flavouring Primary Product Zesti Smoke Code10 did not produce a group mean net nuclear grain (NNG) value greater than 0.5 nor were any more than 4.3 % cells found in repair at either harvest time point (2-4 hours and 12-14 hours post-dose). It was concluded that Primary Product Zesti Smoke Code 10 did not induce UDS detectable under the experimental conditions employed in this study.

Primary Product Zesti Smoke Code 10 (Batch no BJW 0345) was also assayed *in vivo* in a rat bone marrow micronucleus test at a dose level of 2000 mg/kg bw (the acceptable limit dose for a non-toxic material) (LAB, 2006b). The test article was administered *via* oral gavage once daily on two consecutive days to a group of 6 male Sprague Dawley Crl:CD®(SD) rats at a single dose of 2000 mg/kg bw/day. The animals were sacrificed 24 hours after the second administration. Male rats treated with Primary Product Zesti Smoke Code 10 exhibited a group mean % PCE (polychromatic erythrocytes) [42 %] that was lower than the value for the vehicle control group [50 %] and which fell below the lower limit of the historical 95 % confidence interval (48 %-67 %). The applicant indicates that this may be an indication of bone marrow toxicity and exposure, although there was some variability in %PCE between individual animals. The frequencies of micronucleated PCE (MN PCE) in the test article treated group were not significantly different from those observed in the concurrent vehicle control group and individual animal MN PCE frequencies were consistent with historical distribution data.

The applicant concludes that Primary Product Zesti Smoke Code 10 did not induce micronuclei in the polychromatic erythrocytes of the bone marrow of male rats treated at 2000 mg/kg bw/day.

The Panel notes that the bone marrow micronucleus test was a valid study as it was conducted using the acceptable limit dose of 2000 mg/kg bw. However in order to be confident about a negative result, evidence of adequate exposure of the target cells is required. No plasma analysis was conducted during the *in vivo* studies (due to practical constraints – Primary Product Zesti Smoke Code 10 is a complex mixture). However, a reduction in the PCE to NCE (normochromatic erythrocytes) ratio (42 % compared to 50 % for vehicle control) was observed and this may be an indication of bone marrow toxicity and hence exposure (although some variability in % PCE was noted between animals).

### 5.4. Other studies

There are no additional studies on Symrise Smoke Concentrate 809045 provided by the applicant.

## 6. Discussion

The Primary Product Zesti Smoke Code 10 is produced from sawdust originating from mixed hardwood species hickory (*Carya ovata,* 50-60%) and oak (*Quercus alba*, 40-50%). The toxicological studies have been conducted using material produced using the same mix of raw materials.

Altogether, the battery of genotoxicity studies conducted on Primary Product Zesti Smoke Code 10 consisted of three *in vitro* (bacterial reverse mutation test, mammalian cell gene mutation assay and chromosome aberration test) and two *in vivo* studies (unscheduled DNA synthesis assay and bone marrow micronucleus test).

The Panel noted that the genotoxicity of this product observed *in vitro* is not observed *in vivo* in two well conducted studies.

Overall it is concluded that Primary Product Zesti Smoke Code 10 is genotoxic *in vitro*, whereas two *in vivo* genotoxicity tests were negative and sufficient to eliminate the concerns over the *in vitro* genotoxicity.

The Primary Product was investigated in two 90-day studies. The first one dates back to 1962 when GLP was not yet developed, the second one has been conducted recently according to GLP and OECD guidelines. In this second study effects were limited to reduced body weight gain and a reduction in plasma creatinine levels. This latter change may have correlated with the reduction in body/muscle mass. Terminal investigations revealed treatment-related changes in the liver, thyroid and kidney. In the liver, increased relative weights at necropsy correlated with centrilobular hepatocyte hypertrophy. This finding is commonly seen as an adaptive response associated with the metabolism of xenobiotic compounds or metabolites (Greaves, 1990).

The level of hypertrophy was consistent with a physiological adaptive change and its presence, in association with a reduced bodyweight gain, was considered by the Panel not to be suggestive of specific target organ toxicity due to test article administration. This also because the increases in relative liver weight appeared to be reversible upon a four weeks treatment-free period.

In the rat, thyroid follicular cell hypertrophy is generally considered to be an adaptive change due to increased thyroid hormone metabolism in the liver and is commonly associated with liver cell hypertrophy (McClain, 1989, Montgomery, 1990). Therefore, the Panel also considers this effect not to be critical.

Relative kidney weights were significantly increased for both sexes given 25 and 50 g/kg diet Primary Product Zesti Smoke Code 10, by up to 23 %. Although this increase in relative kidney

weight was not accompanied by microscopically observable changes, it was considered by the Panel to be toxicologically significant especially because the increase in relative kidney weight appeared not be reversible upon a four week treatment-free period.

Therefore, the Panel concluded that the additional withdrawal study supported the adaptive effects on the liver, but not for the kidney. The NOAEL was derived based on the increased relative kidney weights at the two highest dose groups in both sexes and amounted to 2.5 g/kg diet equivalent to a minimum dietary intake calculated by the applicant of 134 mg/kg bw/day in male rats and 178 mg/kg bw/day in female rats.

Weight gains over week 1-13 in males given Primary Product Zesti Smoke Code 10 were significantly reduced at the two highest dose groups and in treated females in the highest dose group. Since there was no treatment-related reduction in food consumption in either sex the Panel considers this reduction in weight gain an adverse effect. This corroborates the NOAEL of 2.5 g/kg diet.

The Panel notes that the NOAEL of 2.5 g/kg diet from this recent 90-day study is similar to the NOAEL of 2.5 g/kg diet from the older 90-day study. Since for this study the pathology report stated that "somewhat more distinct minor degenerative changes were observed in the liver and kidney" at 20 g/kg diet test material, and because slightly more marrow hyperplasia was also noted in this group, the lowest dose level of 2.5 g/kg diet, is assumed to be the no observed adverse effect level (NOAEL).

The Panel noted the relatively large gap between the NOAEL and the next dose level tested in both 90-day studies.

In order to estimate dietary exposure to the Primary Product Zesti Smoke Code 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 Zesti Smoke Code 10 is present at the normal or upper use levels provided by the applicant for the 18 food categories as outlined in Commission Regulation (EC). Dietary exposure from all sources ranges from 22.0 to 28.3 mg/kg bw/day, when assuming that the Primary Product Zesti Smoke Code 10 is present at the upper use levels, and from 9.3 to 11.7 mg/kg bw/day, when normal use levels are considered.

When dietary exposure estimates are based on use in only traditionally smoked foods dietary exposure ranges from 8.3 to 14.0 mg/kg bw/day, when assuming that the Primary Product Zesti Smoke Code 10 is present at the upper use levels, and from 4.2 to 6.5 mg/kg bw/day, when normal use levels are considered.

Based on these data it is concluded that when assuming that the Primary Product Zesti Smoke Code 10 is present at the normal or upper use levels provided by the applicant for the 18 food categories, the margins of safety as compared to the NOAEL derived from the 90-day toxicity study in rats of 134 mg/kg bw/day amount to 5 - 6 for the intake estimates based on the upper use levels and to 11 - 14 when normal use levels are considered (Table 7).

When assuming the use of Primary Product Zesti Smoke Code 10 in traditionally smoked products only, the margins of safety would amount to 10 - 16 for the intake estimates based on the upper use levels and to 21 - 32 when normal use levels are considered (Table 7).

Given i) the fact that these margins of safety are based on a 90-day toxicity study, ii) the absence of data on reproduction and developmental toxicity and iii) the absence of long term studies, it is concluded that the uses and use levels of Primary Product Zesti Smoke Code 10 would require a

larger margin of safety. The Panel concluded that the proposed use of the primary product at the uses and use levels specified is of safety concern.

Table 7. **Margins of safety**

| | Use level | Dietary exposure (mg/kg bw/day) | NOAEL (mg/kg bw/day) | Margin of safety |
|---|---|---|---|---|
| Total dietary exposure | Normal | 9.3-11.7 | 134 | 11-14 |
| | Upper | 22.0-28.3 | 134 | 5-6 |
| Traditionally smoked food | Normal | 4.2 – 6.5 | 134 | 21-32 |
| | Upper | 8.3 – 14.0 | 134 | 10-16 |

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 Zesti Smoke Code 10 in such products was not assessed.

## CONCLUSIONS AND RECOMMENDATIONS

The technical data provided are complete and comply with the provisions given in the EFSA guidance document and in legislation.

Zesti Smoke Code 10 is genotoxic *in vitro*, but not *in vivo*. The NOAEL is derived from the most recent 90-day study and amounts to 134 mg/kg bw/day based on a non reversible increase in kidney weight and a decreased body weight gain at the higher dose levels.

Based on these data it is concluded that when assuming that the Primary Product Zesti Smoke Code 10 is present at the normal or upper use levels provided by the applicant for the 18 food categories, the margins of safety as compared to the NOAEL derived from the 90-day toxicity study in rats amount to 5 - 6 for the intake estimates based on the upper use levels and to 11 - 14 when normal use levels are considered.

When assuming the use of Primary Product Zesti Smoke Code 10 in traditionally smoked products only the margins of safety would amount to 10 - 16 for the intake estimates based on the upper use levels and to 21 - 32 when normal use levels are considered.

Given i) the fact that these margins of safety are based on a 90-day toxicity study, ii) the absence of data on reproduction and developmental toxicity and iii) the absence of long term studies, it is concluded that the uses and use levels of Primary Product Zesti Smoke Code 10 would require a larger margin of safety. The Panel concludes that the margin of safety is insufficient and that the use of Primary Product Zesti Smoke Code 10 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 Zesti Smoke Code 10 might be approved for traditionally smoked products, at use levels specified, to replace smoking, is outside the remit of the Panel.

## DOCUMENTATION PROVIDED TO EFSA

Dossier from Mastertaste, June 2005

Responses from Mastertaste to request for supplementary information.

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## GLOSSARY / ABBREVIATIONS

| | |
|---|---|
| AFC | Scientific Panel on Additives, Flavourings, Processing aids and Materials in Contact with Food. |
| bw | body weight |
| CEF | Scientific Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids |
| NDNS | National Diet and Nutrition Survey |
| EC | European Commission |
| EFSA | European Food Safety Authority |
| EPIC | European Prospective Investigation into Cancer and Nutrition |
| FID | Flame Ionisation Detection |
| GC/FID | Gas-chromatograms |
| GC/MS | Gas Chromatography/Mass Spectrometry |
| GLP | Good Laboratory Practice |
| mTAMDI | modified TAMDI |
| HPLC | High Performance Liquid Chromatography |
| NOAEL | No-Observed-Adverse-Effect Level |
| OECD | Organisation for Economic Cooperation and Development |
| PAH | Polycyclic Aromatic Hydrocarbons |
| PCE/NCE | Polychromatic Erythrocytes/ Normochromatic Erythrocytes |
| SCF | Scientific Committee on Food |
| SMK-EPIC | Smoke flavouring EPIC model |
| SMK-TAMDI | Smoke Theoretical Added Maximum Daily Intake |
| TAMDI | Theoretical Added Maximum Daily Intake |
| UDS | Unscheduled DNA synthesis |