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**EURL Evaluation Report on the Analytical Methods
submitted in connection with the Application for the
Authorisation of Feed Additives according to
Regulation (EC) No 1831/2003**

Dossier related to: FAD-2010-0400 - CRL/100331

Product Name: Smoke flavouring - Scansmoke SEF7525

Feed Additive (s): --

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EXECUTIVE SUMMARY

In the current application authorisation is sought under article 4(1) for the *smoke flavouring* product (*Scansmoke SEF7525*) as *feed additive* under the category 'sensory additives', functional group 2(b) 'flavouring compounds' according to the classification system of Annex I of Regulation (EC) No 1831/2003. The authorisation is sought to use the *feed additive* for pet cats and dogs.

Scansmoke SEF7525 is a liquid smoke flavouring product derived from a diethyl ether extraction of a tar. The tar is produced by the pyrolysis of a mixture of woods consisting of white oak *Quercus alba* (35 %), red oak *Quercus rubra* (35 %), Hickory *Carya ovata* (10 %), Beech *Fagus grandifolia* (10%) and Maple *Acer saccharum* (10 %). According to the Applicant the main chemical groups contained in *Scansmoke SEF7525* are: syringols (29 - 41 %), guiacols (9 - 17 %) and phenols (8 - 17 %). The Applicant states that the quality criteria of the product set in the Commission Regulation 2006/627/EC for the food additive could also apply to the *feed additive*. The *feed additive* is intended to be incorporated in *premixtures* and/or *feedingstuffs*. The Applicant proposes a maximum concentration of 80 mg *Scansmoke SEF7525*/kg *feedingstuffs*.

For the chemical analysis of *feed additive* (*Scansmoke SEF7525*) the Applicant proposed the internationally recognised FAO JECFA monograph for food additives, based on the determination of total acids, carbonyls and phenols. For the determination of water content and pH the Applicant submitted the ISO 6296:2000 method based on potentiometric Karl Fischer titration and the ISO 10523:2008 method based on ion selective electrode analysis, respectively. Furthermore, for the characterisation of volatile fraction of *Scansmoke SEF7525* the Applicant proposed two methods described in the internationally recognised FAO JECFA monograph for food additives, namely: gas chromatography-mass spectrometry (GC-MS) and gas chromatography coupled with flame ionization detection (GC-FID), using fluoranthene as internal standard. The Applicant analysed six batches of *Scansmoke SEF7525* applying all the above mentioned methods and reported results consistent with the specifications of the product.

Based on the experimental evidence provided, the EURL recommends all the above mentioned methods for official control of *Scansmoke SEF7525* in the *feed additive*.

The experimental evidence provided by the Applicant indicates that an accurate determination of *Scansmoke SEF7525* in *premixtures* and *feedingstuffs* is not achievable. Therefore the EURL cannot evaluate nor recommend any method for official control to determine *Scansmoke SEF7525* in *premixtures* and *feedingstuffs*.

Further testing or validation of the methods to be performed through the consortium of National Reference Laboratories as specified by Article 10 (Commission Regulation (EC) No 378/2005) is not considered necessary.

KEYWORDS

Smoke flavouring - Scansmoke SEF7525, sensory additives, flavouring compounds, pet cats and dogs

1. BACKGROUND

In the current application authorisation is sought under article 4(1) (new authorisation) for the *smoke flavouring* product (*Scansmoke SEF7525*) as *feed additive* under the category 'sensory additives', functional group 2(b) 'flavouring compounds' according to the classification system of Annex I of Regulation (EC) No 1831/2003 [1]. The authorisation is sought to use the *feed additive* for pet cats and dogs [2].

Scansmoke SEF7525 is a liquid smoke flavouring product derived from a diethyl ether extraction of a tar. The tar is produced by the pyrolysis of a mixture of woods consisting of white oak *Quercus alba* (35 %), red oak *Quercus rubra* (35 %), Hickory *Carya ovata* (10 %), Beech *Fagus grandifolia* (10%) and Maple *Acer saccharum* (10 %) [2]. According to the Applicant the main chemical groups contained in *Scansmoke SEF7525* are: syringols (29 - 41 %), guaiacols (9 - 17 %) and phenols (8 - 17 %) [2]. The Applicant states that the quality criteria of the product set in the Commission Regulation 2006/627/EC for the food additive could also apply to the *feed additive* [3].

The *feed additive* is intended to be incorporated in *premixtures* and/or *feedingstuffs* [3]. The Applicant proposes a maximum concentration of 80 mg *Scansmoke SEF7525*/kg *feedingstuffs* [2].

2. TERMS OF REFERENCE

In accordance with Article 5 of Regulation (EC) No 378/2005, as last amended by Regulation (EC) No 885/2009, on detailed rules for the implementation of Regulation (EC) No 1831/2003 of the European Parliament and of the Council as regards the duties and the tasks of the European Union Reference Laboratory concerning applications for authorisations of feed additives, the EURL is requested to submit a full evaluation report to the European Food Safety Authority for each application or group of applications. For this particular dossier, the methods of analysis submitted in connection with *Scansmoke SEF7525* and their suitability to be used for official controls in the frame of the authorisation were evaluated.

3. EVALUATION

Identification /Characterisation of the feed additive

Qualitative and quantitative composition of impurities in the additive

When required by EU legislation, analytical methods for official control of undesirable substances in the additive (e.g. arsenic, cadmium, lead, mercury, aflatoxin B1, dioxins and PAHs) are available from the respective European Union Reference Laboratories [4]. Residual diethyl ether in *Scansmoke SEF7525* can be determined by gas chromatography coupled with flame ionization detection (GC-FID) or/and gas chromatography-massspectrometry (GC-MS) described in internationally recognised FAO JECFA monograph for food additives [5].

Description of the analytical methods for the determination of the active substance in feed additive, premixtures, feedingstuffs and water

For the chemical analysis of *feed additive (Scansmoke SEF7525)* the Applicant proposed the internationally recognised FAO JECFA monograph for food additives [6], based on the determination of total acids, carbonyls and phenols, described hereafter:

- The determination of total acids is based on titration of the product with 0.1 N sodium hydroxide solution to the point of pH 8.15. The amount of total acids is expressed as percent by weight of acetic acid using the factor: 1 ml of 0.1 N sodium hydroxide is equivalent to 60.05 mg acetic acid;
- The determination of total carbonyls is based on the reaction of the product with 2,4-dinitrophenylhydrazine in acidic medium. The formed hydrazone derivatives are further detected by spectrophotometry at 430 nm. The amount of total carbonyls is expressed as amount of heptaldehyde;
- The determination of total phenols is based on the reaction of the product with 2,6-dibromo-*N*-chloro-*p*-benzoquinoneimine in alkaline medium. The formed indophenol derivatives are further detected by spectrophotometry at 610 nm. The amount of total phenols is expressed as amount of 2,6-dimethoxyphenol.

For the determination of water content the Applicant submitted the ISO 6296:2000 method based on potentiometric Karl Fischer titration. For the pH determination in *Scansmoke SEF7525* the Applicant submitted the ISO 10523:2008 method based on ion selective electrode analysis.

Furthermore, for the characterisation of volatile fraction of *Scansmoke SEF7525* the Applicant proposed two methods described in the internationally recognised FAO JECFA monograph for food additives [5]:

- gas chromatography-mass spectrometry (GC-MS); and
- gas chromatography coupled with flame ionization detection (GC-FID), using fluoranthene as internal standard.

An aliquot of the liquid *smoke flavouring* product is vigorously shaken and a 45 mg sample is then dissolved in 1 ml of acetone. A known amount of internal standard (fluoranthene) is added, at ca. 200 mg/ml. The sample is then filtered (if necessary) through 0.45 µm filter and further analysed by GC-MS/FID [3, 7, 8, 9].

The Applicant applied all the above mentioned methods for the characterisation of *Scansmoke SEF7525* and reported the results [3, 10] summarised in Table 1. The amounts (in weight percent) of main chemical groups and major components of volatile fraction of *Scansmoke SEF7525* were obtained from six different batches.

Table 1: The contents of the major constituents of *Scansmoke SEF7525*

Composition	Nominal value	Measured Amount	Unit	RSD _r
Phenols (total)	8 - 17	9.7 ⁽¹⁾ ; 8.1 ⁽²⁾	%	1.2 ⁽¹⁾ ; 0.6 ⁽²⁾
Carbonyls (total)	1.2 - 3.0	1.9 ⁽¹⁾	%	0.7 ⁽¹⁾
Acids (total)	0.06 - 0.25	0.15 ⁽¹⁾	meq/g	0.04 ⁽¹⁾
Water content	0.3 - 0.9	0.5 ⁽¹⁾	%	0.2 ⁽¹⁾
pH	1 - 4	1.7 ⁽¹⁾	-	0.5 ⁽¹⁾
Syringols (total)	29 - 41	38.0 ⁽²⁾	%	1.6 ⁽²⁾
Guaiacols (total)	9 - 17	11.7 ⁽²⁾	%	0.4 ⁽²⁾
Syringol	12.6 - 25.2	19.0 ⁽²⁾	%	2.8 ⁽²⁾
4-Methyl syringol	6.2 - 9.2	7.6 ⁽²⁾	%	0.8 ⁽²⁾
4-Propenyl syringol (trans)	0.8 - 3.6 ⁽³⁾	3.3 ⁽²⁾	%	0.6 ⁽²⁾
4-Ethyl syringol	2.7 - 3.1	2.9 ⁽²⁾	%	0.1 ⁽²⁾
4-Methyl guaiacol	2.0 - 2.6	2.3 ⁽²⁾	%	0.1 ⁽²⁾
4-Allyl syringol	1.8 - 2.3	2.0 ⁽²⁾	%	0.1 ⁽²⁾

Notes: ⁽¹⁾ The results obtained by chemical analysis; ⁽²⁾ The results obtained by GC-MS/FID; ⁽³⁾ Total amount of *-trans* and *-cis* isomers of 4-Propenyl syringol; RSD_r - relative standard deviation for repeatability.

Based on the experimental evidence provided the EURL recommends for official control of *Scansmoke SEF7525* in the *feed additive*, the various methods described in FAO JECFA monographs (No. 1, Vol. 4) and "smoke flavourings" monograph No. 1 (2006), Combined Compendium for Food Additive Specifications, together with the internationally recognised methods ISO 6296:2000 (for the determination of water content) and ISO 10523:2008 (for the pH measurements).

Furthermore, the Applicant applied the above mentioned GC-MS/FID method [3, 7, 8, 9] to analyse *feedingstuffs* samples spiked with *Scansmoke SEF7525* at three different concentrations (1000, 3000 and 9000 mg/kg). The grinded sample is extracted with acetone in Soxhlet apparatus for 8 h [11]. The solvent is carefully evaporated and the extract is dried over phosphorous pentoxide (P₂O₅). An exact amount of the dry extract is diluted with the internal standard solution (fluoranthene solution in acetone) for the further chromatographic analysis. The results are presented in Table 2.

While consistent experimental results are submitted in the frame of the characterisation of the *feed additive*, an unexpected inversion of main constituents is presented with no further explanation (the 4-methyl syringol content is higher than the syringol content in *feedingstuffs* (Table 2); it is inverted in the *feed additive* (Table 1). Furthermore, the *Scansmoke SEF7525* concentration range investigated in *feedingstuffs* (from 1000 to 9000 mg/kg) is significantly higher than the maximum dose of 80 mg/kg *feedingstuffs* proposed by the Applicant. Finally, a limit of quantification (LOQ) of 12 mg/kg *feedingstuffs* reported by the Applicant for the compounds of the volatile fraction [8] is equal or higher than the concentration of the volatile compounds in *feedingstuffs* at the maximum level proposed (one kilogram of *feedingstuffs* is equivalent to 15.2 mg of syringol, 6.1 mg of 4-methyl syringol and 2.3 mg of 4-ethyl syringol).

Table 2: The quantification of volatile components in *feedingstuffs* containing 1000, 3000 and 9000 mg *Scansmoke SEF7525*/kg (expressed as average \pm standard deviation)

Composition	1000	3000	9000
4-Methyl syringol	44.5 \pm 5.4	109.3 \pm 8.3	343.8 \pm 75.6
Syringol	28.4 \pm 5.8	90.9 \pm 7.4	311.2 \pm 70.5
4-Ethyl syringol	21.8 \pm 2.2	66.8 \pm 3.0	226.1 \pm 42.6
4-Methyl guaiacol	11.7 \pm 1.7	37.0 \pm 2.1	120.2 \pm 26.5

Note: All the values are expressed in mg/kg *feedingstuffs*.

Hence, the experimental evidence provided indicates that an accurate determination of *Scansmoke SEF7525* in *feedingstuffs* may not be achievable at the maximum levels proposed by the Applicant. Furthermore, the Applicant provided no data for the determination of *Scansmoke SEF7525* in *premixtures*. Therefore the EURL cannot evaluate nor recommend any method for official control to determine *Scansmoke SEF7525* in *premixtures* and *feedingstuffs*.

Further testing or validation of the methods to be performed through the consortium of National Reference Laboratories as specified by Article 10 (Commission Regulation (EC) No 378/2005) is not considered necessary.

4. CONCLUSIONS AND RECOMMENDATIONS

In the frame of this authorisation the EURL recommends for official control of *Scansmoke SEF7525* in the *feed additive*, the methods described in FAO JECFA “*smoke flavourings*” monograph No. 1 (2006) and monographs (No. 1, Vol. 4), Combined Compendium for Food Additive Specifications:

- titration with sodium hydroxide for determination of total acids;
- colour reaction with 2,4-dinitrophenylhydrazine in acidic medium and further spectrophotometry at 430 nm for determination of total carbonyls;
- colour reaction with 2,6-dibromo-*N*-chloro-*p*-benzoquinoneimine in alkaline medium and further spectrophotometry at 610 nm for determination of total phenols;
- gas chromatography-mass spectrometry (GC-MS); and
- gas chromatography coupled with flame ionization detection (GC-FID) for characterisation of volatile fraction of the product; together with
- the internationally recognised ISO 6296:2000 method based on potentiometric Karl Fischer titration for the determination of water content; and
- the internationally recognised ISO 10523:2008 method based on ion selective electrode analysis for the determination of pH.

Recommended text for the register entry (analytical method)

For the determination of *smoke flavouring - Scansmoke SEF7525* in *feed additive*:

- titration with sodium hydroxide for determination of total acids; and colour reactions with further spectrophotometry for determination of total carbonyls (at 430 nm) and total phenols (at 610 nm) (FAO JECFA Combined Compendium for Food Additive Specifications, "smoke flavourings", Monograph No. 1, 2006);
- gas chromatography-mass spectrometry (GC-MS); and gas chromatography coupled with flame ionization detection (GC-FID) for characterisation of the volatile fraction of the product (FAO JECFA Combined Compendium for Food Additive Specifications - Monographs No. 1, Vol. 4).

5. DOCUMENTATION AND SAMPLES PROVIDED TO EURL

In accordance with the requirements of Regulation (EC) No 1831/2003, reference samples of *smoke flavouring - Scansmoke SEF7525* have been sent to the European Union Reference Laboratory for Feed Additives. The dossier has been made available to the EURL by EFSA.

6. REFERENCES

- [1] *Application, Reference SANCO/D/2: Forw. Appl. 1831/(00205) (10291)-2010
 - [2] *Application, Proposal for Register Entry – Annex A
 - [3] *Technical dossier, Section II – Identity, characterisation and conditions of use of the additive; Methods of analysis
 - [4] Commission Regulation (EC) No 776/2006 amending Annex VII to Regulation (EC) No 882/2004 of the European Parliament and of the Council as regards to Community Reference Laboratories
 - [5] FAO JECFA Combined Compendium for Food Additive Specifications - *Analytical methods, test procedures and laboratory solutions used by and referenced in the food additive specifications*, Monographs No. 1, Vol. 4
<http://www.fao.org/docrep/009/a0691e/a0691e00.htm> (last visited on 08/05/2012)
 - [6] FAO JECFA Combined Compendium of Food Additive Specifications, "smoke flavourings", Monograph No. 1 (2006)
<http://www.fao.org/ag/agn/jecfa-additives/specs/Monograph1/Additive-386.pdf>
(last visited on 08/05/2012)
 - [7] *Supplementary information – Properties of fast pyrolysis liquids
 - [8] *Supplementary information – Method description
 - [9] *Supplementary information – J. Anal. Appl. Pyrolysis 85 (2009) 38 - 46
 - [10] *Supplementary information – SEF7525 GC report
 - [11] *Technical dossier, Section II – Annex II_5 - Feed analysis
- *Refers to Dossier No. FAD-2010-0400

7. RAPPORTEUR LABORATORY & NATIONAL REFERENCE LABORATORIES

The Rapporteur Laboratory for this evaluation was European Union Reference Laboratory for Feed Additives, IRMM, Geel, Belgium. This report is in accordance with the opinion of the consortium of National Reference Laboratories as referred to in Article 6(2) of Commission Regulation (EC) No 378/2005, as last amended by Regulation (EC) No 885/2009.

8. ACKNOWLEDGEMENTS

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- Thüringer Landesanstalt für Landwirtschaft (TLL), Abteilung Untersuchungswesen, Jena (DE)
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- Plantedirektoratet, Laboratorium for Foder og Gødning, Lyngby (DK)
- Österreichische Agentur für Gesundheit und Ernährungssicherheit (AGES), Wien (AT)