EFSA CEF Penal (EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids), 2014. Scientific Opinion on Flavouring Group Evaluation 212, Revision 2 (FGE.212Rev2): ,-Unsaturated alicyclic ketones and precursors from chemical subgroup 2.6 of FGE.19

EFSA Publication; Beltoft, Vibe Meister; Binderup, Mona-Lise; Lund, Pia; Nørby, Karin Kristiane

Link to article, DOI:
10.2903/j.efsa.2014.3584

Publication date:
2014

Document Version
Publisher's PDF, also known as Version of record

European Food Safety Authority

EFSA Journal 2014;12(2):3584

SCIENTIFIC OPINION

Scientific Opinion on Flavouring Group Evaluation 212, Revision 2 (FGE.212Rev2): α,β-Unsaturated alicyclic ketones and precursors from chemical subgroup 2.6 of FGE.19[1]

EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF)[2, 3]

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

The Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids of the European Food Safety Authority was requested to evaluate the genotoxic potential of 24 flavouring substances from subgroup 2.6 of FGE.19 in the Flavouring Group Evaluation 212, Revision 2. The Panel concluded in FGE.212, that the genotoxic potential could be ruled out for *d*-carvone [FL-no: 07.146] together with the structurally related *l*-carvone [FL-no: 07.147] as well as carveol and the carvyl derivatives [FL-no: 02.062, 09.143, 09.215 and 09.870]. Based on available genotoxicity data and new submitted genotoxicity data from the Industry, the Panel concluded that the genotoxic potential could be ruled out for the 11 isophorone derivatives [FL-no: 02.083, 02.101, 07.035, 07.098, 07.126, 07.129, 07.172, 07.175, 07.196, 07.202 and 07.255] and the two vetiveryl derivatives [FL-no: 02.214 and 09.821] in FGE.212Rev1 and FGE.212Rev2, respectively. For the remaining five substances [FL-no: 07.033, 07.094, 07.112, 07.140 and 07.219] from subgroup 2.6 there is still a genotoxicity concern and additional data are required.

KEY WORDS

α,β-unsaturated ketones, FGE.212Rev2, FGE.19 subgroup 2.6, flavouring substances, safety evaluation

---

[1] On request from the European Commission, Question No EFSA-Q-2013-00168 and EFSA-Q-2013-00169, adopted on 29 January 2014.

[2] Panel members: Ulla Beckman Sundh, Mona-Lise Binderup, Claudia Bolognesi, Leon Brimer, Laurence Castle, Alessandro Di Domenico, Karl-Heinz Engel, Roland Franz, Nathalie Gontard, Rainer Gürtler, Trine Husøy, Klaus-Dieter Jany, Martine Kolf-Clauw, Wim Mennes, Maria Rosaria Milana, Iona Pratt, Kettil Svensson, Maria de Fatima Tavares Poças, Fidel Toldra and Detlef Wölfle. Correspondence: cef@efsa.europa.eu

[3] The Panel wishes to thank the members of the Genotoxicity Working Group on Flavourings : Mona-Lise Binderup, Claudia Bolognesi, Angelo Carere, Riccardo Crebelli, Rainer Gürtler, Natália Kovalkovičová, Daniel Marzin and Pasquale Mosesso for the preparatory work on this scientific opinion and, the hearing experts: Vibe Beltoft, Pia Lund and Karin Nørby and, EFSA staff: Annamaria Rossi, Maria Carfi, Moretti Silvia and Kim Rygaard Nielsen for the support provided to this scientific opinion.

Suggested citation: EFSA CEF Panel (EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids), 2014. Scientific Opinion on Flavouring Group Evaluation 212, Revision 2 (FGE.212Rev2): α,β-Unsaturated alicyclic ketones and precursors from chemical subgroup 2.6 of FGE.19. EFSA Journal 2014;12(2):3584, 43 pp. doi:10.2903/j.efsa.2014.3584

Available online: www.efsa.europa.eu/efsajournal

SUMMARY

The European Food Safety Authority (EFSA) asked the Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (the Panel) to provide scientific advice to the Commission on the implications for human health of chemically defined flavouring substances used in or on foodstuffs in the Member States. In particular, the Panel was asked to evaluate flavouring substances using the Procedure as referred to in the Commission Regulation (EC) No 1565/2000.

Flavouring Group Evaluation 212 (FGE.212) concerned 23 substances. The 23 substances correspond to subgroup 2.6 of FGE.19. Fifteen of these substances are α,β-unsaturated alicyclic ketones [FL-no: 07.033, 07.035, 07.094, 07.098, 07.112, 07.126, 07.129, 07.140, 07.146, 07.147, 07.172, 07.175, 07.196, 07.202 and 07.255] and eight are precursors for such ketones [FL-no: 02.062, 02.083, 02.101, 02.214, 09.143, 09.215, 09.821 and 09.870].

In the first version of this Opinion, FGE.212, the Panel expressed the following view.

d-Carvone [FL-no: 07.146] was found genotoxic *in vitro*. However, *d*-carvone was not carcinogenic in mice. Therefore, the Panel concluded that this substance together with the structurally related *l*-carvone [FL-no: 07.147] as well as carveol and the carvyl derivatives [FL-no: 02.062, 09.143, 09.215 and 09.870] could be evaluated through the Procedure.

3,5,5-Trimethylcyclohex-2-en-1-one (isophorone) [FL-no: 07.126] is genotoxic *in vitro*. There is also some evidence of carcinogenicity in male rats and equivocal evidence of carcinogenicity in male mice. Since a non-threshold mechanism could not be excluded based on the data currently available, the Panel concluded that additional data were required for isophorone in order to clarify whether genotoxicity occurs *in vivo* and whether there is a threshold for the effects observed in the target organs in the long-term bioassays. Therefore, an *in vivo* Comet assay in F344/N rats covering these target organs was required in addition to an *in vivo* bone marrow assay with oral application. Due to structural similarities to isophorone and lack of data that addressed concerns regarding genotoxicity, the remaining substances could not be evaluated through the Procedure [FL-no: 02.083, 02.101, 02.214, 07.033, 07.035, 07.094, 07.098, 07.112, 07.129, 07.140, 07.172, 07.175, 07.196, 07.202, 07.255 and 09.821]. Additional data on genotoxicity were requested for representative substances of this subgroup, according to the opinion of the Panel on Genotoxicity Test Strategy for Substances Belonging to Subgroups of FGE.19.

In FGE.212Rev1, new data on genotoxicity submitted by Industry on the representative substance 3,5,5-Trimethylcyclohex-2-en-1-one [FL-no: 07.126] were evaluated. Based on these data, the Panel could rule out the genotoxicity concern for isophorone and the substances structurally related to isophorone [FL-no: 02.083, 02.101, 07.035, 07.098, 07.129, 07.172, 07.175, 07.196, 07.202 and 07.255]. On the other hand, the Panel could not agree with the Industry argument that isophorone could be representative for not only the six-carbon ring substances in subgroup 2.6 but also the five-carbon ring substances [FL-no: 07.033, 07.094, 07.112, and 07.140]. For these substances additional data were still requested as well as for the seven-carbon ring substances [FL-no: 02.214 and 09.821].

The present revision of FGE.212, FGE.212Rev2, deals with the evaluation of additional genotoxicity data submitted by the Industry on the seven-carbon ring substance, vetiveryl acetate [FL-no: 09.821]. These data are also intended to cover the corresponding alcohol moiety, vetiveryl alcohol [FL-no: 02.214] for which there was a request for genotoxicity data in FGE.212 and FGE.212Rev1. Additionally, since the last revision of FGE.212, one additional five-carbon ring substance, trans-3-methyl-2-(2-pentenyl)-2-cyclopenten-1-one [FL-no: 07.219], has been included in the FGE.

Based on these data the Panel concluded in the present opinion that the genotoxicity concern for vetiveryl acetate [FL-no: 09.821] and the structurally related vetiverol [FL-no: 02.214] could be ruled out and that these two substances can be evaluated using the Procedure.

For the remaining five-carbon ring substances [FL-no: 07.033, 07.094, 07.112, 07.140 and 07.219] from subgroup 2.6 there is still a genotoxicity concern and additional data are required.

TABLE OF CONTENTS

BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

The use of flavourings is regulated under Regulation (EC) No 1334/2008[4] of the European Parliament and Council of 16 December 2008 on flavourings and certain food ingredients with flavouring properties for use in and on foods. On the basis of article 9(a) of this Regulation an evaluation and approval are required for flavouring substances.

The Union List of flavourings and source materials was established by Commission Implementing Regulation (EC) No 872/2012[5]. The list contains flavouring substances for which the scientific evaluation should be completed in accordance with Commission Regulation (EC) No 1565/2000[6].

EFSA has evaluated 23 flavouring substances, which correspond to subgroup 2.6 of FGE.19, in its evaluation of the flavouring group 212 (FGE.212 and FGE.212Rev1). The opinions were adopted on 27 November 2008 and on 25 November 2010.

EFSA concluded that a genotoxic potential of seven α,β-unsaturated alicyclic ketones and precursors in the present FGE.212 could not be ruled out.

Information on one representative material, vetiveryl acetate [FL-no: 09.821], has now been submitted by the European Flavour Association. This information is intended to cover the re-evaluation of this substance and of vetiverol [FL-no: 02.214].

The commission asks EFSA to evaluate this new information and depending on the outcome proceed to the full evaluation of the flavouring substances.

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

The European Commission requests the European Food Safety Authority to carry out a safety assessment on the following two flavouring substances: vetiveryl acetate [FL-no: 09.821] and vetiverol [FL-no: 02.214] in accordance with Commission Regulation (EC) No 1565/2000[6].

[4] Regulation (EC) No 1334/2008 of the European Parliament and of the Council of 16 December 2008 on flavourings and certain food ingredients with flavouring properties for use in and on foods and amending Council Regulation (EEC) No 1601/91, Regulations (EC) No 2232/96 and (EC) No 110/2008 and Directive 2000/13/EC. OJ L 354, 31.12.2008, p. 34-50.

[5] EC (European Commission), 2012. Commission implementing Regulation (EU) No 872/2012 of 1 October 2012 adopting the list of flavouring substances provided for by Regulation (EC) No 2232/96 of the European Parliament and of the Council, introducing it in Annex I to Regulation (EC) No 1334/2008 of the European Parliament and of the Council and repealing Commission Regulation (EC) No 1565/2000 and Commission Decision 1999/217/EC. OJ L 267, 2.10.2012, p. 1-161.

[6] Commission Regulation (EC) No 1565/2000 of 18 July 2000 laying down the measures necessary for the adoption of an evaluation programme in application of Regulation (EC) No 2232/96 of the European Parliament and of the Council. OJ L 180, 19.7.2000, p. 8-16.

HISTORY OF THE EVALUATION OF FGE.19 SUBSTANCES

Flavouring Group Evaluation 19 (FGE.19) contains 360 flavouring substances from the EU Register being α,β-unsaturated aldehydes or ketones and precursors which could give rise to such carbonyl substances via hydrolysis and / or oxidation (EFSA, 2008a).

The α,β-unsaturated aldehyde and ketone structures are structural alerts for genotoxicity (EFSA, 2008a). The Panel noted that there were limited genotoxicity data on these flavouring substances but that positive genotoxicity studies were identified for some substances in the group.

The α,β-unsaturated carbonyls were subdivided into subgroups on the basis of structural similarity (EFSA, 2008a). In an attempt to decide which of the substances could go through the Procedure, a (quantitative) structure-activity relationship (Q)SAR prediction of the genotoxicity of these substances was undertaken considering a number of models (DEREKfW, TOPKAT, DTU-NFI-MultiCASE Models and ISS-Local Models, (Gry et al., 2007)).

The Panel noted that for most of these models internal and external validation has been performed, but considered that the outcome of these validations was not always extensive enough to appreciate the validity of the predictions of these models for these alpha, beta- unsaturated carbonyls. Therefore, the Panel considered it inappropriate to totally rely on (Q)SAR predictions at this point in time and decided not to take substances through the procedure based on negative (Q)SAR predictions only.

The Panel took note of the (Q)SAR predictions by using two ISS Local Models (Benigni and Netzeva, 2007a; Benigni and Netzeva, 2007b) and four DTU-NFI MultiCASE Models (Gry et al., 2007; Nikolov et al., 2007) and the fact that there are available data on genotoxicity, *in vitro* and *in vivo*, as well as data on carcinogenicity for several substances. Based on these data the Panel decided that 15 subgroups (1.1.1, 1.2.1, 1.2.2, 1.2.3, 2.1, 2.2, 2.3, 2.5, 3.2, 4.3, 4.5, 4.6, 5.1, 5.2 and 5.3) (EFSA, 2008a) could not be evaluated through the Procedure due to concern with respect to genotoxicity. Corresponding to these subgroups, 15 Flavouring Group Evaluations (FGEs) were established: FGE.200, 204, 205, 206, 207, 208, 209, 211, 215, 219, 221, 222, 223, 224 and 225.

For 11 subgroups the Panel decided, based on the available genotoxicity data and (Q)SAR predictions, that a further scrutiny of the data should take place before requesting additional data from the Flavouring Industry on genotoxicity. These subgroups were evaluated in FGE.201, 202, 203, 210, 212, 213, 214, 216, 217, 218 and 220. For the substances in FGE.202, 214 and 218 it was concluded that a genotoxic potential could be ruled out and accordingly these substances will be evaluated using the Procedure. For all or some of the substances in the remaining FGEs, FGE.201, 203, 210, 212, 213, 216, 217 and 220, the genotoxic potential could not be ruled out.

To ease the data retrieval of the large number of structurally related α,β-unsaturated substances in the different subgroups for which additional data are requested, EFSA worked out a list of representative substances for each subgroup (EFSA, 2008c). Likewise an EFSA genotoxicity expert group has worked out a test strategy to be followed in the data retrieval for these substances (EFSA, 2008b).

The Flavouring Industry has been requested to submit additional genotoxicity data according to the list of representative substances and test strategy for each subgroup.

The Flavouring industry has now submitted additional data and the present FGE concerns the evaluation of these data requested on genotoxicity.

ASSESSMENT

1. History of the Evaluation of the Substances in the Present FGE

Flavouring Group Evaluation 212 (FGE.212) concerned 23 substances. The 23 substances, corresponding to subgroup 2.6 of FGE.19. Fifteen of these substances are α,β-unsaturated alicyclic ketones [FL-no: 07.033, 07.035, 07.094, 07.098, 07.112, 07.126, 07.129, 07.140, 7.146, 07.147, 07.172, 07.175, 07.196, 07.202 and 07.255] and eight are precursors for such ketones [FL-no: 02.062, 02.083, 02.101, 02.214, 09.143, 09.215, 09.821 and 09.870].

In FGE.212 the Panel concluded that based on available data the concern for genotoxicity could be ruled out for *d*-carvone [FL-no: 07.146], *l*-carvone [FL-no: 07.147], as well as carveol and carvyl derivatives in subgroup 2.6 [FL-no: 02.062, 09.143, 09.215 and 09.870]. Therefore these substances could be evaluated through the Procedure. For isophorone [FL-no: 07.126] and the structurally-related substances [FL-no: 02.083, 02.101, 02.214, 07.033, 07.035, 07.094, 07.098, 07.112, 07.129, 07.140, 07.172, 07.175, 07.196, 07.202, 07.255 and 09.821] additional genotoxicity data were requested for representative substances according to the Test Strategy (EFSA, 2008b). In the EFSA Opinion "List of α,β-unsaturated aldehydes and ketones representative of FGE.19 substances for genotoxicity testing" (EFSA, 2008c), representative flavouring substances have been selected for subgroup 2.6 (Table 1), corresponding to FGE.212, for which additional data on genotoxicity were requested.

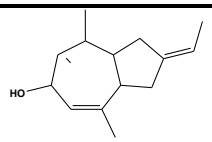
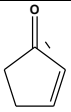
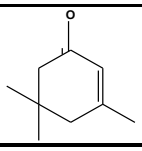
In FGE.212Rev1, new data on genotoxicity were submitted by Industry on the representative substance 3,5,5-Trimethylcyclohex-2-en-1-one [FL-no: 07.126]. Based on these data the Panel concluded that the concern for genotoxicity could be ruled out for [FL-no: 07.126] and for the 10 six-carbon ring substances of subgroup 2.6 [FL-no: 02.083, 02.101, 07.035, 07.098, 07.129, 07.172, 07.175, 07.196, 07.202 and 07.255]. For the six remaining substances in FGE.212 [FL-no: 02.214, 07.033, 07.094, 07.112, 07.140 and 09.821] additional genotoxicity data were still requested.

FGE	Adopted by EFSA	Link	No. of Substances
FGE.212	27 November 2008	http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1211902780085.htm	23
FGE.212Rev1	November 2010	http://www.efsa.europa.eu/en/efsajournal/pub/1923.htm	23
FGE.212Rev2	January 2014		24

The present revision of FGE.212, revision 2 (FGE.212Rev2) concerns the evaluation of additional genotoxicity data submitted by the Industry (IOFI, 2012) for one of the seven-carbon ring substances of subgroup 2.6, namely vetiveryl acetate [FL-no: 09.821] which is structurally related to vetiverol [FL-no: 02.214][7]. For the five-membered ring substances of subgroup 2.6 [FL-no: 07.033, 07.094, 07.112 and 07.140] additional genotoxicity data are requested. Additionally, since the last revision of FGE.212 one additional five-carbon ring substance, trans-3-methyl-2-(2-pentenyl)-2-cyclopenten-1-one [FL-no: 07.219], has been included in the FGE.

[7] Vetiverol [FL-no: 02.214], representing the 7-carbon ring substances, are not in common use in the flavour industry. The acetyl ester of [FL-no: 02.214], vetiveryl acetate [FL-no: 09.821] is in more common use, and on that basis was available for testing. Therefore, this report presents data for the vetiveryl acetate (REF:7521).

Table 1: Representative substances for subgroup 2.6 of FGE.19 (EFSA, 2008c)

FL-no JECFA-no	Subgroup	EU Register name	Structural formula	Comments
02.214 1866	2.6	Vetiverol		Representative: 2,6-Dimethyl-9-(1-methylethylidene)-bicyclo[5.3.0]dec-2-en-4-one (not in register) or its precursor [02.214].
07.112 1105	2.6	3-Methyl-2-cyclopenten-1-one		
07.126 1112	2.6	3,5,5-Trimethylcyclohex-2-en-1-one (isophorone)		

Sections 2, 3 and 4 report the same information that was presented in FGE.212 and FGE.212Rev1.

Section 5 describes additional data submitted by the Industry in response to the data requested in FGE.212Rev1.

2. Presentation of the Substances in FGE.212Rev2

2.1. Description

The present Flavouring Group Evaluation 212 Revision 2 (FGE.212Rev2) concerns 24 substances, which are presented in Table 2. The 24 substances correspond to subgroup 2.6 of FGE.19 (EFSA, 2008a). Sixteen of these substances are α,β-unsaturated alicyclic ketones (α,β-unsaturation in the side chain) [FL-no: 07.033, 07.035, 07.094, 07.098, 07.112, 07.126, 07.129, 07.140, 7.146, 07.147, 07.172, 07.175, 07.196, 07.202, 07.219 and 07.255] and eight are precursors for such ketones [FL-no: 02.062, 02.083, 02.101, 02.214, 09.143, 09.215, 09.821 and 09.870].

Twenty-one of the substances have previously been evaluated by the JECFA at their 51st, 59th and 69th meetings (JECFA, 1999; JECFA, 2003; JECFA, 2009a). A summary of their evaluation status by the JECFA is given in Table 3.

As the α,β-unsaturated ketone structure is considered to be a structural alert for genotoxicity (EFSA, 2008a) the available data on genotoxic or carcinogenic activity for the 16 ketones in FGE.212 [FL-no: 07.033, 07.035, 07.094, 07.098, 07.112, 07.126, 07.129, 07.140, 7.146, 07.147, 07.172, 07.175, 07.196, 07.202, 07.219 and 07.255] and one non-Register ketone [2,6-dimethyl-9-(1-methylethylidene)-bicyclo[5.3.0]dec-2-en-4-one] corresponding to the 24 substances in FGE.212, will be considered in this FGE.

The Panel also noted that for one substance [FL-no: 07.033], the CAS No, name and chemical structure were not consistent (Table 2). Therefore a clarification is needed.

The Panel has also taken into consideration the outcome of the predictions from five selected (Q)SAR models (Benigni and Netzeva, 2007a; Gry et al., 2007; Nikolov et al., 2007) on 15 ketones [FL-no: 07.033, 07.035, 07.094, 07.098, 07.112, 07.126, 07.129, 07.140, 7.146, 07.147, 07.172, 07.175,

07.196, 07.202 and 07.255] and one non-Register ketone [2,6-dimethyl-9-(1-methylethylidene)-bicyclo[5.3.0]dec-2-en-4-one] in the original version of FGE.212. These 15 ketones and the one non-Register ketone as well as their (Q)SAR predictions are shown in Table 4.

3. Toxicity[8]

3.1. (Q)SAR Predictions

In Table 4 the outcomes of the (Q)SAR predictions for possible genotoxic activity in five *in vitro* (Q)SAR models (ISS Local Model-Ames test, DTU-NFI MultiCASE-Ames test, Chromosomal aberration test in Chinese hamster ovary cells (CHO), Chromosomal aberration test in Chinese hamster lung cells (CHL) and Mouse lymphoma test) are presented.

Positive predictions have been obtained for six substances with the MultiCASE Mouse lymphoma model and for one of these substances also with the MultiCASE model on chromosomal aberrations. For the other substances, the predictions of the MultiCASE models were negative, equivocal or the substances were out of domain. All substances were out of domain in the ISS model.

3.2. Carcinogenicity Studies

Groups of 50 male and 50 female F344/N rats were administered isophorone (3,5,5-trimethylcyclohex-2-en-1-one [FL-no: 07.126]) in corn oil by gavage at dose levels of 0 (controls), 250 or 500 mg/kg body weight (bw)/day, five times per week for 103 weeks. During the study the body weights of the high-dose male and female rats were slightly lower than those of the vehicle controls. The survival of high-dose male rats was significantly lower than that of the vehicle controls after week 96. Dosed male rats showed a variety of proliferative lesions of the kidney (tubular cell hyperplasia, 0/50, 1/50, 4/50; tubular cell adenoma, 0/50, 0/50, 2/50; tubular cell adenocarcinoma, 0/50, 3/50, 0/50; epithelial hyperplasia of the renal pelvis, 0/50, 5/50, 5/50). Dosed male rats also exhibited increased mineralisation of the medullary collecting ducts (1/50, 31/50, 20/50) and low-dose male rats showed a more severe nephropathy than is commonly seen in aging F344/N rats. Carcinomas of the preputial gland were significantly increased ($P<0.03$) in high-dose male rats (0/50, 0/50, 5/50). With the exception of a moderate increase in nephropathy (21/50, 39/50, 32/50), female rats did not show chemically related increased incidences of neoplastic or non-neoplastic lesions (NTP, 1986).

Groups of 50 male and 50 female $B6C3F_1$ mice were administered isophorone (3,5,5-trimethylcyclohex-2-en-1-one [FL-no: 07.126]) in corn oil by gavage at dose levels of 0 (controls), 250 or 500 mg/kg bw/day, five times per week for 103 weeks. During the study the body weights of the high-dose female mice were slightly lower than those of the vehicle controls. The survival of male mice was low, whereas there was a significant trend toward increased survival of dosed female mice relative to that of the vehicle controls. In high-dose male mice, isophorone exposure was associated with an increased incidence of hepatocellular adenomas and carcinomas (18/48, 18/50, 29/50) and of mesenchymal tumors of the integumentary system (fibroma, fibrosarcoma, neurofibrosarcoma or sarcoma, 6/48, 7/50, 14/50). An increased incidence of lymphomas or leukemias was noted in low-dose male mice (8/48, 18/50, 5/50). Coagulative necrosis (3/48, 10/50, 11/50) and hepatocytomegaly (23/48, 39/50, 37/50) were observed more frequently in the livers of dosed male mice than in vehicle controls. No compound-related neoplastic or non-neoplastic lesions associated with isophorone exposure were seen in female mice (NTP, 1986).

The Panel concluded that isophorone increased the incidences of renal tubular cell adenomas and adenocarcinomas and of carcinomas of the preputial gland in male rats but not in female rats. In male mice, but not in females, it produced increased incidences of hepatocellular adenomas and carcinomas, mesenchymal tumors in the integumentary system, and malignant lymphomas.

[8] The data presented in Section 3 is cited from the first version of the present FGE.212. These data are the basis for the conclusions in FGE.212 requesting additional genotoxicity data.

The Panel agrees with the authors of the NTP report who concluded that "under the conditions of these 2-year gavage studies, there was some evidence of carcinogenicity of isophorone in male F344/N rats as shown by the occurrence of renal tubular cell adenomas and adenocarcinomas in animals given 250 or 500 mg/kg bw per day; carcinomas of the preputial gland were also observed at increased incidence in male rats given 500 mg/kg bw. There was no evidence of carcinogenicity in female F344/N rats given 250 or 500 mg/kg bw per day. For male $B6C3F_1$ mice, there was equivocal evidence of carcinogenicity of isophorone as shown by an increased incidence of hepatocellular adenomas or carcinomas (combined) and of mesenchymal tumors in the integumentary system in animals given 500 mg/kg bw per day and by an increase in malignant lymphomas in animals given 250 mg/kg bw per day. There was no evidence of carcinogenicity of isophorone in female $B6C3F_1$ mice given 250 or 500 mg/kg bw per day."

Groups of 50 male and 50 female $B6C3F_1$ mice (7-week old) were administered 0, 375 or 750 mg/kg bw *d*-carvone [FL-no: 07.146] in corn oil by gavage, five days per week for 103 weeks. The mean body weights of dosed and control male and female mice were similar throughout most of the study. The survival of both the low-dose and the high-dose females were significantly greater than that of the controls. No differences in survival were observed between any groups of male mice. Atrophy of the olfactory epithelium and hyperplasia of the underlying Bowman's glands occurred together with high incidence in either sex in both dosed groups. This effect was found due to a local effect of *d*-carvone caused by reflux of the gavage material when the gavage needle was withdrawn. No increases in tumour incidences were seen in mice administered *d*-carvone. The incidences of male mice with primary neoplasms and the total numbers of primary neoplasms were significantly lower in the dosed groups than in the vehicle controls (NTP, 1990).

The Panel concluded that *d*-carvone was not carcinogenic in mice under the study conditions. It agrees with the authors of the NTP report who concluded that "under the conditions of these 2-year gavage studies, there was no evidence of carcinogenic activity of *d*-carvone for male or female $B6C3F_1$ mice administered 375 or 750 mg/kg, 5 days per week for 2 years."

Study validation and results are presented in Table 5.

3.3. Genotoxicity Studies

In subgroup 2.6 there are studies available for four substances: tetramethyl ethylcyclohexenone (mixture of isomers) [FL-no: 07.035], 3,5,5 trimethylcyclohex-2-en-1-one [FL-no: 07.126] (isophorone), *d*-carvone [FL-no: 07.146] and *l*-carvone [FL-no: 07.147].

Study validation and results are presented in Tables 6 and 7.

3,5,5 Trimethylcyclohex-2-en-1-one [FL-no: 07.126] (isophorone) did not induce gene mutations in bacteria but it induced mutations in mammalian cells in a mouse lymphoma TK assay in the absence of metabolic activation (it was not tested in the presence of metabolic activation) (NTP, 1986). No mutations in the MLTK assay were observed in a study of O'Donoghue et al. (O'Donoghue et al., 1988) at comparable concentrations. Isophorone induced chromosomal aberrations in Chinese hamster lung fibroblasts with and without metabolic activation (Matsuoka et al., 1996) and sister chromatid exchanges (SCE) in CHO cells without metabolic activation (Gulati et al., 1989). Chromosomal aberrations have not been observed in two other studies (Gulati et al., 1989; NTP, 1986); however, the validity of the results was limited because the types of aberrations were not reported. Isophorone did not induce unscheduled DNA synthesis (UDS) in rat hepatocytes *in vitro*. *In vivo*, isophorone was tested negative in a sex-linked recessive lethal mutation assay in *Drosophila* (Foureman et al., 1994) and in two micronucleus assays in mice (McKee et al., 1987; O'Donoghue et al., 1988). However, the *Drosophila* assay has only limited relevance and the micronucleus assays were of limited validity.

Negative results were also observed with tetramethyl ethylcyclohexenone [FL-no: 07.035] in bacteria, in a sex-linked recessive lethal mutation assay in *Drosophila* (Wild et al., 1983) and in a mouse

micronucleus assay (Wild et al., 1983); however, there was a mixture of isomers tested and the studies were only of limited validity.

d-Carvone [FL-no: 07.146] was not mutagenic in bacteria but induced SCE and chromosomal aberrations in CHO cells in the presence and absence of metabolic activation, respectively (NTP, 1990).

3.4. Conclusion on Genotoxicity and Carcinogenicity

The Panel concluded that 3,5,5 trimethylcyclohex-2-en-1-one [FL-no: 07.126] (isophorone) is genotoxic *in vitro* while a final conclusion on the genotoxicity *in vivo* could not be drawn based on the data available. It is carcinogenic in male rats and male mice. It was also predicted to be genotoxic in one of the four MultiCASE models (while it was out of domain in the ISS model).

d-Carvone [FL-no: 07.146] is genotoxic *in vitro* while no *in vivo* data were available. *d*-Carvone, was not carcinogenic in mice and was predicted to be non-genotoxic in the four MultiCASE models (while it was out of domain in the ISS model). No data are available on *l*-carvone. However, *in vivo* studies in humans show that the metabolism of ingestion-correlated amounts of *d*- or *l*-carvone occurs via a major oxidative pathway of the isopropylene side chain yielding diol and two carboxylic acids, irrespective of the stereochemical difference between the two parent isomers of carvone (Engel, 2001). Accordingly, the results for *d*-carvone can be used for *l*-carvone as well.

The negative results reported from *in vivo* studies on the genotoxicity of tetramethyl ethylcyclohexenone [FL-no: 07.035] were only of limited validity.

3.5. Conclusion

The present Flavouring Group Evaluation 212 (FGE.212) concerns 23 substances. The 23 substances correspond to subgroup 2.6 of FGE.19. Fifteen of these substances are alpha,beta-unsaturated alicyclic ketones [FL-no: 07.033, 07.035, 07.094, 07.098, 07.112, 07.126, 07.129, 07.140, 7.146, 07.147, 07.172, 07.175, 07.196, 07.202 and 07.255] and eight are precursors for such ketones [FL-no: 02.062, 02.083, 02.101, 02.214, 09.143, 09.215, 09.821 and 09.870].

d-Carvone [FL-no: 07.146] was found genotoxic *in vitro*. However, *d*-carvone was not carcinogenic in mice. Therefore, the Panel concluded that this substance together with the structurally related *l*-carvone [FL-no: 07.147] as well as carveol and the carvyl derivatives [FL-no: 02.062, 09.143, 09.215 and 09.870] could be evaluated through the Procedure.

Isophorone [FL-no: 07.126 (3,5,5-trimethylcyclohex-2-en-1-one)] is genotoxic *in vitro* and since there is some evidence of carcinogenicity in male rats and equivocal evidence of carcinogenicity in male mice and since a non-threshold mechanism could not be excluded based on the data currently available, the Panel concluded that additional data are required for isophorone in order to clarify whether genotoxicity occurs *in vivo* and whether there is a threshold for the effects observed in the target organs in the long-term bioassays. Therefore, an *in vivo* Comet assay in F344/N rats covering these target organs is required in addition to an *in vivo* bone marrow assay with oral application.

Due to structural similarities and lack of data, the remaining substances cannot presently be evaluated through the Procedure [FL-no: 02.083, 02.101, 02.214, 07.033, 07.035, 07.094, 07.098, 07.112, 07.129, 07.140, 07.172, 07.175, 07.196, 07.202, 07.255 and 09.821]. Additional data on genotoxicity are requested for representative substances of this subgroup according to the opinion of the Panel on the Genotoxicity Test Strategy for Substances Belonging to Subgroups of FGE.19 (EFSA, 2008b).

4. Industry Response to Data Requested in FGE.212[9]

4.1. Presentation of the Additional Data

Honma et al. (Honma et al., 1999a; Honma et al., 1999b) found that isophorone did not clearly induce mutations in the mouse lymphoma assay (MLA) following 3 hours treatments, but observed that it was mutagenic after 24 hours treatments in the absence of S9. Although only graphs are plotted, it seems that increases in mutation frequency (MF) that exceeded the Global Evaluation Factor (GEF) occurred at around 1250-1500 µg/ml where toxicity (by relative survival) reached 70-90 %.

The NTP conducted a mouse bone marrow chromosomal aberration (CA) study on isophorone. Groups of 8 male B6C3F1 mice (larger group sizes than required by OECD) were dosed i.p. with isophorone at 125, 250 and 500 mg/kg bw. The standard protocol for *in vivo* CA is not given on the NTP website. However, based on Shelby and Witt (Shelby and Witt, 1995), animals should have been sampled at 17 hours and, if negative, also at 36 hours. The data on the NTP website are only for bone marrow sampled at 36 hours. It is therefore possible that a 17 hours sample was also taken, and found to be negative, but the data have not been posted. Fifty cells per animal were scored for CA and no increases in CA were seen. No measures of toxicity were recorded, but i.p. dosing should have guaranteed systemic exposure. The control CA frequency was normal (2.75 %) and the positive control (dimethylbenzanthracene) produced a significant response in CA frequency.

A DNA binding study was conducted in which F344-rats and $B6C3F_1$-mice (the strains used in the NTP carcinogenicity study) were exposed to isophorone (Thier et al., 1990). Animals of both sexes were dosed once or five times by gavage with 500 mg/kg bw of unlabelled isophorone spiked with [1,3,5-^{14}C]-isophorone (specific activity: 52 mCi per mmol, 1.92 GBq per mmol). An additional group of acute dosed male rats received undiluted ^{14}C-isophorone for increased sensitivity. Rats and mice were maintained for 24 hours in closed metabolic cages. Twenty four hours after exposure, livers and kidneys (the tumour target tissues) were removed from the animals. DNA was isolated through hydroxyapatite chromatography and radioactivity was measured by liquid scintillation counting. No positive controls were included. Also no untreated controls were included, but, except for the liver sample of one mouse in the five times dose group, radioactivity values were within 2σ of background (6 dpm). Radioactivity values therefore did not indicate significant attachment of radioactivity to DNA. From these results it can be concluded that neither isophorone nor its metabolites bind covalently to DNA.

In addition, a report by Morishita et al. (Morishita et al., 1997) submitted to EPA (EPA, 1997), is relevant and appears to have been previously submitted only as an abstract. This study was designed to investigate whether isophorone and/or α2µ-globulin[10] might be involved in the induction of preputial gland tumours in F-344 rats (10/sex/dose group). A series of experiments was performed in order to study several parameters including:

- binding of isophorone to DNA of kidney and preputial gland. Groups of 10 male rats were dosed by gavage with 500 mg/kg of [^{14}C]-isophorone (specific activity 14.65 mCi/mmol; 100 µCi/animal). Positive control animals were dosed with ^{3}H-labeled methyl nitrosourea.

- DNA adduct detection by ^{32}P-postlabeling in young adult male and female rats (7 per group) dosed by gavage with 0, 250 or 500 mg/kg isophorone for five days.

Extraction of preputial gland and kidney DNA from rats treated with single 500 mg/kg labeled doses yielded no evidence of isophorone binding to DNA, whereas the positive control showed significant

[9] The data presented in Section 4 is cited from revision 1 of FGE.212 (FGE.212Rev1). These data are the basis for the conclusions in FGE.212Rev1 requesting additional genotoxicity data.

[10] Since interaction with α2µ-glubulin is not of direct relevance for the evaluation of genotoxic potential, this information is omitted from this study summary.

binding to DNA of preputial gland and kidney. These negative results with isophorone were confirmed in the ^{32}P -postlabeling assays.

In addition Industry has also asked whether the information submitted for isophorone, (cyclohexenyl derivative), could also be applied to evaluate the genotoxic potential of the five-carbon membered ring substances (i.e. cyclopentenyl derivatives) in subgroup 2.6 (letter of EFFA to EFSA, dated 14/4-2010). This request was supported by the argumentation that there is structural resemblance with respect to steric hindrance around the alpha,beta-unsaturated double bond. In addition, Industry argued that the π-conjugation systems in these molecules is very nearly planar and that therefore the reactivity and genotoxic potentials of the five- and six-membered ring systems would be similar. No further data were provided to substantiate this argumentation.

4.2. Discussion of the Additional Data

Conflicting results were reported in two valid studies with the mouse lymphoma assay (MLA): one negative (O'Donoghue et al., 1988) and one positive (NTP, 1986) at comparable concentrations. Mixed results were also reported in two studies of limited validity: one negative (Honma et al., 1999a) and one positive (Honma et al., 1999b). Another negative result was reported in a study (McKee et al., 1987), the validity of which cannot be evaluated. In the light of the clearly negative results in two valid bacterial gene mutation tests (Ames test) and in a valid Sex Linked Recessive Lethal Mutations test (SLRL) in *Drosophila*, and taking into account the lack of specificity and high sensitivity of the MLA, overall the results presently available are considered of questionable relevance. The Panel agrees that isophorone demonstrates some genotoxic activity *in vitro* but that the new data demonstrate lack of clastogenicity *in vivo*. In addition, the new DNA-binding data from two separate studies provide convincing evidence that isophorone does not induce tumours via a genotoxic mechanism. On the basis of these data it may be argued that there is no need to perform further *in vivo* genotoxicity studies such as the Comet assay or bone marrow micronucleus test. Thus, based on the data available the Panel concluded that there is no concern with respect to genotoxicity of isophorone.

4.3. Conclusion on Additional Data

Since based on the additional information the concern for the genotoxic potential for isophorone [FL-no: 07.126] has been alleviated, a genotoxic potential can also be ruled out for the other structurally related six-carbon members of FGE.19 subgroup 2.6 related to isophorone [FL-no: 02.083, 02.101, 07.035, 07.098, 07.129, 07.172, 07.175, 07.196, 07.202 and 07.255].

The Panel also concluded that isophorone can only be considered as representative for the six-carbon ring members of FGE.19 subgroup 2.6. The argumentation of Industry to expand this conclusion also to the cyclopentenyl derivatives in this subgroup [FL-no: 07.033, 07.094, 07.112 and 07.140] was considered too limited, given the lack of support from experimental data. Therefore, additional genotoxicity tests are still required for the representative substance [FL-no: 07.112] already chosen by the Panel. Alternatively, a more thorough explanation (physico-chemical parameters; experimental underpinning) of the proposed similar reactivity of six- and five-membered ring substances should be provided by Industry. Also for the seven-ring carbon substance [FL-no: 02.214] (also covering [FL-no: 09.821]) additional data on genotoxicity are still required.

5. Industry Response to Data Requested in FGE.212Rev1

In response to the EFSA request in FGE.212 and FGE.212Rev1 for additonal genotoxicity data for FGE.19 subgroup 2.6, the Flavour Industry (IOFI, 2012) has submitted *in vitro* genotoxicity data on: vetiveryl acetate [09.821].

5.1. In vitro Genotoxicity Studies

Bacterial Reverse Mutation Tests

Vetiveryl acetate containing 1 % alpha-tocopherol (a common stabiliser present in the large majority of commercially available solutions of vetiveryl acetate to increase shelf-life typically for up to 18 months) was tested in *Salmonella typhimurium* strains TA97, TA98, TA100, TA102 and TA1535 in two independent experiments in the absence and presence of metabolic activation (by liver S9-mix fraction from phenobarbitone/β-naphthoflavone-induced rats) (Gocke, 2000), the results are summarised in Table 8. The study complies with GLP and current guidelines (OECD Guideline 471, 1997). The first experiment used the plate incorporation method and the second used the pre-incubation method. Treatments were performed at concentrations of 0, 20, 63.2, 200, 632 and 2000 µg/plate of stabilised vetiveryl acetate (dissolved in DMSO) with triplicate plates per test concentration. Some precipitation (milky appearance) was seen at the higher concentrations (> 200 µg/plate for the plate incorporation assay and > 20 µg/plate for the pre-incubation test) and evidence of toxicity was observed in some strains in the pre-incubation experiments. On this basis, 2000 µg/plate was the highest concentration that could be practically tested. Negative results were obtained with all five bacterial strains in the presence and absence of S9-mix up to the maximum test concentration of 2000 µg/plate.

In another study, vetiveryl acetate extra (stabilisation not stated) (dissolved in DMSO) was tested in *S. typhimurium* strains TA100, TA97a, TA98, TA1535, and TA102 at concentrations ranging from 5 - 5000 µg/plate with and without S9-mix metabolic activation (Scheerbaum, 2001). Different concentrations from this range were used for different strains or within the same strain in the presence or absence of S9-mix. Cytotoxicity, in the form of background bacteria lawn reduction, in the absence of S9-mix was noted at 500 µg/plate and above in strain TA100, 1600 µg/plate and above in strain TA1535, and 5000 µg/plate in strain TA97a. In presence of S9-mix, cytotoxicity was noted at 500 µg/plate and above in strain TA100 and TA1535, 1600 µg/plate and above in strain TA97a and TA102, and 5000 µg/plate in strain TA98. No mutagenic potential was observed in any strain under any condition or concentration. This study design complies with published recommendations (OECD Guideline 471, 1997).

A series of Ames studies were conducted with stabilised vetiveryl acetate (stabiliser identity unknown) that had been stored for 18 - 24 months. In one Ames study, stabilised vetiveryl acetate (stored for 18 months) was tested for mutagenicity in *S. typhimurium* strains TA98, TA100, TA102, TA1535 and TA1537, in two separate experiments (Poth, 2003) (Table 8). Experiment 1 was conducted in all five tester strains at vetiveryl acetate (dissolved in DMSO) concentrations of 33, 100, 333, 1000, 2500 and 5000 µg/plate in the absence and presence of metabolic activation system (S9-mix prepared from phenobarbital/β-naphthoflavone induced male Wistar rat liver). Precipitation of test material occurred at the highest concentration. Cytotoxicity was noted in the presence of S9-mix, at 5000 µg/plate in strain TA1537 and at 1000 µg/plate and above in strains TA100 and TA102. In experiment 2, using a modified protocol including a pre-incubation method, vetiveryl acetate was tested at the same concentrations up to 5000 µg/plate in the absence and presence of S9-mix in all five strains, and from a starting concentration of 10 µg/plate in strains TA100 and TA102 in the presence of S9-mix. Cytotoxicity was noted in the presence of S9-mix, at 2500 µg/plate and above in strains TA1535 and TA102, and at 1000 µg/plate and above in strains TA1537 and TA100. No increase in revertant frequencies was observed between treated and control cultures at any concentration, either in the

absence or in the presence of metabolic activation. This study design complies with OECD Guideline 471 (OECD, 1997a).

In a second study, *S. typhimurium* strains TA98, TA100, TA102, TA1535 and TA1537 were incubated with stabilised vetiveryl acetate that had been stored for 24 months (Sokolowski, 2003a). Two independent experiments were performed in the absence and presence of S9-mix metabolic activation. The first experiment used the plate incorporation method and the second used the pre-incubation method. Concentrations of 0, 33, 100, 333, 1000, 2500 and 5000 µg/plate of stabilised vetiveryl acetate were included in each part of the study. Cytotoxicity, in the form of background bacteria lawn reduction, was noted in the presence of S9-mix, at 5000 µg/plate in strains TA98 (both experiments) and TA102 (first experiment only), at 2500 µg/plate and above in strain TA1535 (both experiments), and at 1000 µg/plate and above (first experiment) or 2500 µg/plate and above (second experiment) in strain TA1537. No increase in mutagenicity was observed in any bacterial strain, either in the presence or absence of S9-mix, up to the maximum test concentration of 5000 µg/plate. No precipitation of the test material was noted up the highest concentration tested. This study design complies with published recommendations (OECD Guideline 471, 1997).

In a third study, *S. typhimurium* strains TA98, TA100, TA102, TA1535 and TA1537 were incubated with a formulation (112 extra) of stabilized vetiveryl acetate that had been stored for 24 months (Sokolowski, 2003b). The exact differences between the formulations of the stabilised vetiveryl acetate have not been determined. Two independent experiments were performed in the absence and presence of S9-mix metabolic activation. The first experiment used the plate incorporation method and the second used the pre-incubation method. The same concentrations of 0, 33, 100, 333, 1000, 2500 and 5000 µg/plate of stabilised vetiveryl acetate were included in each experiment. Precipitation of test material occurred at the highest concentration. Cytotoxicity, in the form of background bacteria lawn reduction, in the absence of S9-mix, was noted only at the highest concentration and only in strain TA1535. In presence of S9-mix, cytotoxicity was noted at 5000 µg/plate in strains TA98 (second experiment) and TA102 (first experiment), at 2500 µg/plate and above in strain TA1535 (first experiment), and at 5000 µg/plate (first experiment) or 2500 µg/plate and above (second experiment) in strain TA1537. No increase in mutagenicity was observed in any bacterial strain, either in the presence or absence of S9-mix, up to the maximum test concentration of 5000 µg/plate. This study design complies with published recommendations (OECD Guideline 471, 1997).

In conclusion, different formulations of vetiveryl acetate with purity and stabilisation not reported were tested in a number of studies with Ames assay in five strains of *S. typhimurium* in presence and absence of S9-mix. No increase of revertants was detected in any of these studies up to a concentration of 5000 µg/plate.

Tests in Mammalian Cells

A study was conducted *in vitro* in human lymphocytes to assess the ability of vetiveryl acetate [FL-no: 09.821] (purity 98 % and stabilised with 1 % alpha-tocopherol) to induce structural chromosomal aberrations (CA), both in the absence and in the presence of metabolic activation by S9-mix, in two separate experiments (Morris, 2011). The study complies with GLP and OECD Guideline 473 (OECD, 1997b).

The initial dose levels chosen for experiment 1 and experiment 2 were based on a previous chromosomal aberration study conducted in Chinese Hamster Ovary (CHO) cells by Harlan Laboratories Ltd (Morris and Durward, 2010). While it produced negative results, the study in CHO cells was not considered valid due to a high percentage of cells with chromosomal aberrations in the vehicle controls.

In experiment 1, duplicate lymphocytes cultures were exposed to concentrations of freshly prepared vetiveryl acetate of 10, 20, 30, 40, 50 and 60 µg/ml for 4 hours in the absence of S9-mix along with vehicle and positive (mitomycin C (MMC) 0.4 µg/ml) controls and for 4 hours in the presence of S9-

mix (2 % final concentration) at concentrations of 40, 60, 80, 100, 110, 120, 130 and 140 µg/ml along with vehicle and positive (cyclophosphamide (CP) 4.5 µg/ml) controls, followed by 20 hours in treatment-free media. Mitosis was arrested by the addition of demecolcine (colcemid 0.1 µg/ml) two hours before cell harvest. The cells were processed, coded and scored for number of cells in metaphase and polyploidy cell frequency. Concentrations of 0, 20, 30, 40 and 45 µg/ml in the absence of S9-mix, and 0, 40, 60, 80 and 100 µg/ml in the presence of S9-mix were selected for quantitative analysis, based on toxicity seen at higher concentrations. The results indicated that vetiveryl acetate did not induce statistically significant increases in the frequency of cells with chromosomal aberrations at any concentration, either in the absence or presence of S9-mix. Also, vetiveryl acetate did not result in a statistically significant increase in the polyploid cell frequency at any concentration, either in the absence or presence of S9-mix.

In experiment 2, duplicate cultures were exposed to concentrations of freshly prepared vetiveryl acetate of 10, 20, 40, 60, 80, 100, 110 and 120 µg/ml for 24 hours continuous exposure in the absence of S9-mix along with vehicle and positive (MMC 0.2 µg/ml) controls. Cultures were also exposed to vetiveryl acetate concentrations of 20, 40, 60, 80, 100, 110, 120 and 140 µg/ml for 4 hours in the presence of S9-mix (1 % final concentration), along with vehicle and positive (CP) controls followed by 20 hours in treatment-free media. Mitosis was arrested by the addition of demecolcine (colcemid 0.1 µg/ml) two hours before cell harvest. The cells were processed, coded and scored for number of cells in metaphase and for polyploidy frequency. Concentrations of 0, 20, 40, 80 and 100 µg/ml vetiveryl acetate for 24 hour treatment and concentrations of 0, 20, 40, 60 and 80 µg/ml for 4 hours treatment in the presence of S9-mix were selected for quantitative analysis, based on toxicity seen at higher concentrations. The results indicated that vetiveryl acetate did not induce statistically significant increases in the frequency of cells with aberrations or polyploid cell frequency at any concentration, either in the absence or presence of S9-mix.

In conclusion, vetiveryl acetate did not induce chromosomal aberrations in cultured human peripheral blood lymphocytes when tested for 4 + 20 hours up to 45 µg/ml or 100 µg/ml, in the absence or in the presence of rat liver metabolic activation (S9-mix), respectively. In the same test system, vetiveryl acetate did not induce chromosomal aberrations when tested for 24 hours of continuous exposure up to 100 µg/ml in the absence of S9-mix.

CONCLUSION

Industry submitted additional genotoxicity data for vetiveryl acetate [FL-no: 09.821].

The overall conclusion for the *in vitro* genotoxicity data indicate that the FGE.19 subgroup 2.6 substance, vetiveryl acetate, does not give rise to a safety concern with respect to genotoxicity, and accordingly, vetiveryl acetate [FL-no: 09.821] and the corresponding alcohol moiety, vetiverol [FL-no: 02.214] can be evaluated using the Procedure.

For the remaining five substances [FL-no: 07.033, 07.094, 07.112, 07.140 and 07.219] from subgroup 2.6, there is still a genotoxicity concern and additional data are required.

SUMMARY OF SPECIFICATION FOR SUBSTANCES IN FGE.212REV2 (JECFA, 1998; JECFA, 2002; JECFA, 2009B)

Table 2: Specification Summary of the Substances in the Flavouring Group Evaluation 212Rev2

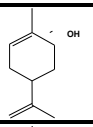
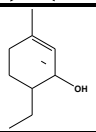
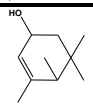
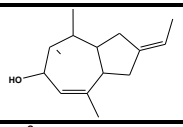
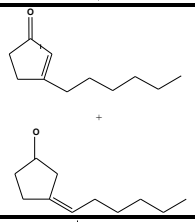
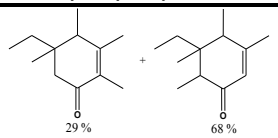
FL-no JECFA-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)
02.062 381	Carveol		2247 2027 99-48-9	Liquid $C_{10}H_{16}O$ 152.24	Freely soluble	226-227 IR 96 %	1.493-1.497 0.947-0.953
02.083 434	p-Menth-1-en-3-ol		3179 10248 491-04-3	Liquid $C_{10}H_{18}O$ 154.25		232 NMR 97 %	1.4762 (25C) 0.930-0.936
02.101 1404	Pin-2-en-4-ol 6)		3594 10304 473-67-6	Solid $C_{10}H_{16}O$ 152.24	Very slightly soluble Soluble	n.a. 63-67 NMR 95 %	n.a. n.a.
02.214 1866	Vetiverol		4217 10321 89-88-3	Solid $C_{15}H_{24}O$ 220.35	Practically insoluble or insoluble Freely soluble	n.a. 69 NMR 95 %	n.a. n.a.
07.033 1115	Isojasmone 6)		3552 167 11050-62-7	Liquid $C_{11}H_{18}O$ 166.26		144 (13 hPa) NMR 95 %	1.472-1.477 0.917-0.924
07.035 1111	Tetramethyl ethylcyclohexenone (mixture of isomers)		3061 168 17369-60-7	Liquid $C_{12}H_{20}O$ 180.29	Slightly soluble Miscible	113-115 NMR 97 %	1.485-1.490 0.927-0.934

Table 2: Specification Summary of the Substances in the Flavouring Group Evaluation 212Rev2

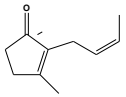
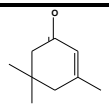
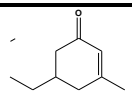
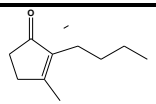
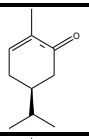
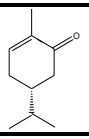
FL-no JECFA-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)
07.094 1114	3-Methyl-2-(pent-2(cis)-enyl)cyclopent-2-en-1-one		3196 11786 488-10-8	Liquid $C_{11}H_{16}O$ 164.25		248 NMR 98 %	1.495-1.501 0.942-0.948
07.098 1107	3-Methylcyclohex-2-en-1-one		3360 11134 1193-18-6	Liquid $C_7H_{10}O$ 110.16	Miscible Miscible	199-200 NMR 98 %	1.490-1.498 0.967-0.972
07.112 1105	3-Methyl-2-cyclopenten-1-one		3435 11137 2758-18-1	Liquid C_6H_8O 96.12		74 (20 hPa) NMR 98 %	1.485-1.491 0.968-0.975
07.126 1112	3,5,5-Trimethylcyclohex-2-en-1-one		3553 11918 78-59-1	Liquid $C_9H_{14}O$ 138.21	Slightly soluble Miscible	213-215 NMR 97 %	1.474-1.481 0.919-0.927
07.129 1113	3-Methyl-5-propylcyclohex-2-en-1-one		3577 3720-16-9	Liquid $C_{10}H_{16}O$ 152.23	Insoluble Miscible	242-244 NMR 95 %	1.481-1.486 0.924-0.928
07.140 1406	3-Methyl-2-pentylcyclopent-2-en-1-one		3763 1128-08-1	Liquid $C_{11}H_{18}O$ 166.26	Very slightly soluble Soluble	79 (0.2 hPa) NMR 99 %	1.676-1.682 0.911-0.917
07.146 380.1	*d*-Carvone		 2244-16-8				
07.147 380.2	*l*-Carvone		 6485-40-1				

Table 2: Specification Summary of the Substances in the Flavouring Group Evaluation 212Rev2

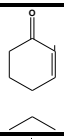
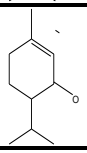
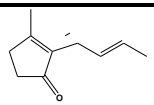
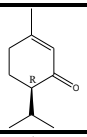
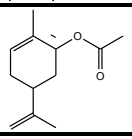
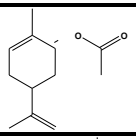
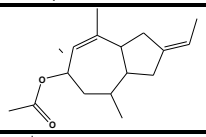
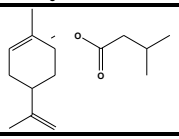
FL-no JECFA-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)
07.172 1110	4-Isopropylcyclohex-2-en-1-one		3939 11127 500-02-7	Liquid $C_9H_{14}O$ 138.21	Insoluble Miscible	198 NMR 97 %	1.481-1.490 0.930-0.950
07.175 435	p-Menth-1-en-3-one (+/- piperitone)		2910 2052 89-81-6	Liquid $C_{10}H_{16}O$ 152.24	Insoluble	233-235 IR 94 %	1.483-1.487 0.929-0.934
07.196 1870	Pin-2-en-4-one 6)		4216 11186 80-57-9	Liquid $C_{10}H_{14}O$ 150.22	Insoluble Freely soluble	90 (16 hPa) NMR MS 95 %	1.492-1.498 0.975-0.981
07.202	2,6,6-Trimethylcyclohex-2-en-1-one		20013-73-4	Liquid $C_9H_{14}O$ 138.21	Slightly soluble Freely soluble	63 (16 hPa) MS 95 %	1.470-1.476 0.924-0.930
07.219	trans-3-Methyl-2-(2-pentenyl)-2-cyclopenten-1-one		3196 11786 6261-18-3	Liquid $C_{11}H_{16}O$ 164.25	Soluble Soluble	248 MS 98 %	1.495-1.501 0.942-0.948
07.255 1856	*l*-Piperitone		4200 4573-50-6	Liquid $C_{10}H_{16}O$ 152.24	Slightly soluble Freely soluble	246 MS 99 %	1.482-1.488 0.929-0.935
09.143 383	Carvyl propionate		2251 424 97-45-0	Liquid $C_{13}H_{20}O_2$ 208.30	Insoluble	239 IR 98 %	1.469-1.479 0.942-0.962

Table 2: Specification Summary of the Substances in the Flavouring Group Evaluation 212Rev2

FL-no JECFA-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)
09.215 382	Carvyl acetate		2250 2063 97-42-7	Liquid $C_{12}H_{18}O_2$ 194.27	Slightly soluble	229 IR 98 %	1.473-1.479 0.964-0.970
09.821 1867	Vetiveryl acetate		4218 11887 117-98-6	Solid $C_{17}H_{26}O_2$ 262.39	Freely soluble	406 73 95 %	n.a. n.a.
09.870	Carvyl-3-methylbutyrate		94386-39-7	Liquid $C_{15}H_{24}O_2$ 236.37	Practically insoluble or insoluble Freely soluble	343 MS 95 %	1.462-1.468 0.932-0.938

1) Solubility in water, if not otherwise stated.
2) Solubility in 95 % ethanol, if not otherwise stated.
3) At 1013.25 hPa, if not otherwise stated.
4) At 20°C, if not otherwise stated.
5) At 25°C, if not otherwise stated.

SUMMARY OF SAFETY EVALUATION APPLYING THE PROCEDURE (JECFA, 1999; JECFA, 2003; JECFA, 2009A)

Table 3: Summary of Safety Evaluation Applying the Procedure (based on intakes calculated by the MSDI approach)

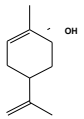
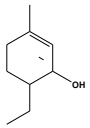
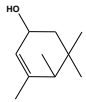
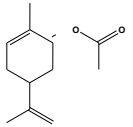
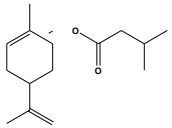
FL-no JECFA-no	EU Register name	Structural formula	MSDI 1) (μg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	Outcome on the material of commerce [6), 7), or 8)]
02.062 381	Carveol		9.5 140	Class I A3: Intake below threshold	4)	Evaluated in FGE.212, genotoxic concern could be ruled out. Evaluated by JECFA before 2000. No further EFSA consideration required
02.083 434	p-Menth-1-en-3-ol		0.012 0.02	Class I A3: Intake below threshold	4)	Evaluated in FGE.212Rev1, genotoxic concern could be ruled out. Evaluated by JECFA before 2000. No further EFSA consideration required
02.101 1404	Pin-2-en-4-ol		0.012 0.2	Class I A3: Intake below threshold	4)	Evaluated in FGE.212Rev1, genotoxicity concern could be ruled out. Evaluated through the Procedure in FGE.87Rev1. No safety concern at the estimated level of intake based on the MSDI approach.
09.143 383	Carvyl propionate		ND 0.04	Class I A3: Intake below threshold	4)	Evaluated in FGE.212, genotoxic concern could be ruled out. Evaluated by JECFA before 2000. No further EFSA consideration required
09.215 382	Carvyl acetate		4.0 36	Class I A3: Intake below threshold	4)	Evaluated in FGE.212, genotoxic concern could be ruled out. Evaluated by JECFA before 2000. No further EFSA consideration required
09.870	Carvyl-3-methylbutyrate		0.0012	Class I A3: Intake below threshold	4)	Evaluated in FGE.212, genotoxic concern could be ruled out. Evaluated through the Procedure in FGE.09Rev2. No safety concern at the estimated level of intake based on the MSDI approach.

Table 3: Summary of Safety Evaluation Applying the Procedure (based on intakes calculated by the MSDI approach)

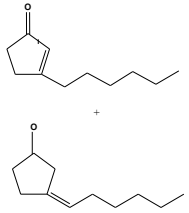
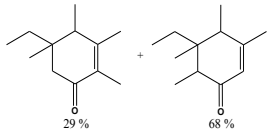
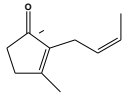
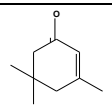
FL-no JECFA-no	EU Register name	Structural formula	MSDI 1) (μg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	Outcome on the material of commerce [6), 7), or 8)]
07.033 1115	Isojasmone		0.37 0.01	Class II A3: Intake below threshold	4)	Evaluated in FGE.212Rev1, genotoxic concern could not be ruled out. Additional genotoxicity data required for the representative [FL-no: 07.112]
07.035 1111	Tetramethyl ethylcyclohexenone (mixture of isomers)		7.8 0.2	Class II A3: Intake below threshold	4)	Evaluated in FGE.212Rev1, genotoxic concern could be ruled out. Evaluated through the Procedure in FGE.51Rev1. No safety concern at the estimated level of intake based on the MSDI approach.
07.094 1114	3-Methyl-2-(pent-2(cis)-enyl)cyclopent-2-en-1-one		13 7.2	Class II A3: Intake below threshold	4)	Evaluated in FGE.212Rev1, genotoxic concern could not be ruled out. Additional genotoxicity data required for the representative [FL-no: 07.112]
07.098 1107	3-Methylcyclohex-2-en-1-one		0.012 0.1	Class II A3: Intake below threshold	4)	Evaluated in FGE.212Rev1, genotoxic concern could be ruled out. Evaluated through the Procedure in FGE.51Rev1. No safety concern at the estimated level of intake based on the MSDI approach.
07.112 1105	3-Methyl-2-cyclopenten-1-one		0.06 ND	Class II A3: Intake below threshold	4)	Evaluated in FGE.212Rev1, genotoxic concern could not be ruled out. Additional genotoxicity data required for the representative [FL-no: 07.112]
07.126 1112	3,5,5-Trimethylcyclohex-2-en-1-one		4.6 0.1	Class II A3: Intake below threshold	4)	Evaluated in FGE.212Rev1, genotoxic concern could be ruled out. Evaluated through the Procedure in FGE.51Rev1. No safety concern at

Table 3: Summary of Safety Evaluation Applying the Procedure (based on intakes calculated by the MSDI approach)

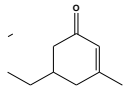
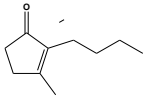
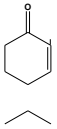
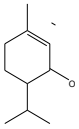
FL-no JECFA-no	EU Register name	Structural formula	MSDI 1) (µg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	Outcome on the material of commerce [6), 7), or 8)]
						the estimated level of intake based on the MSDI approach.
07.129 1113	3-Methyl-5-propylcyclohex-2-en-1-one		0.097 4.1	Class II A3: Intake below threshold	4)	Evaluated in FGE.212Rev1, genotoxic concern could be ruled out. Evaluated through the Procedure in FGE.51Rev1. No safety concern at the estimated level of intake based on the MSDI approach.
07.140 1406	3-Methyl-2-pentylcyclopent-2-en-1-one		0.34 0.2	Class II A3: Intake below threshold	4)	Evaluated in FGE.212Rev1, genotoxic concern could not be ruled out. Additional genotoxicity data required for the representative [FL-no: 07.112]
07.172 1110	4-Isopropylcyclohex-2-en-1-one		0.0012 0.001	Class II A3: Intake below threshold	4)	Evaluated in FGE.212Rev1, genotoxic concern could be ruled out. Evaluated through the Procedure in FGE.51Rev1. No safety concern at the estimated level of intake based on the MSDI approach.
07.175 435	p-Menth-1-en-3-one (+/- piperitone)		44 10	Class II A3: Intake below threshold	4)	Evaluated in FGE.212Rev1, genotoxicity concern could be ruled out. Evaluated by JECFA before 2000. No further EFSA consideration required.
07.196 1870	Pin-2-en-4-one		15	Class II A3: Intake below threshold	4)	Evaluated in FGE.212Rev1, genotoxicity concern could be ruled out. Evaluated through the Procedure in FGE.47Rev1. No safety concern at the estimated level of intake based on the MSDI approach.

Table 3: Summary of Safety Evaluation Applying the Procedure (based on intakes calculated by the MSDI approach)

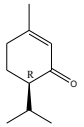
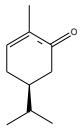
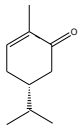
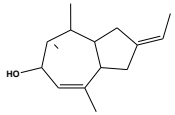
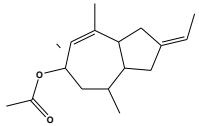
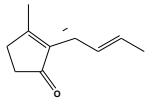
FL-no JECFA-no	EU Register name	Structural formula	MSDI 1) (μg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	Outcome on the material of commerce [6), 7), or 8)]
07.202	2,6,6-Trimethylcyclohex-2-en-1-one		0.12	Class II A3: Intake below threshold	4)	Evaluated in FGE.212Rev1, genotoxic concern could be ruled out. Evaluated through the Procedure in FGE.09Rev4. No safety concern at the estimated level of intake based on the MSDI approach.
07.255 1856	*l*-Piperitone		12	Class II A3: Intake below threshold	4)	Evaluated in FGE.212Rev1, genotoxic concern could be ruled out. Evaluated through the Procedure in FGE.09Rev4. No safety concern at the estimated level of intake based on the MSDI approach.
07.146 380.1	*d*-Carvone		2390 9900	Class II A3: Intake above threshold, A4: Not endogenous, A5: Adequate NOAEL exists	4)	Evaluated in FGE.212, genotoxicity concern could be ruled out. Evaluated by JECFA before 2000. No further EFSA consideration required.
07.147 380.2	*l*-Carvone		2390 9900	Class II A3: Intake above threshold, A4: Not endogenous, A5: Adequate NOAEL exists	4)	Evaluated in FGE.212, genotoxic concern could be ruled out. Evaluated by JECFA before 2000. No further EFSA consideration required.
02.214 1866	Vetiverol		0.011	Class II A3: Intake below threshold	4)	Evaluated in FGE.212Rev2, genotoxic concern can be ruled out. Can be evaluated through the Procedure in FGE.47Rev2.
09.821 1867	Vetiveryl acetate		0.011	Class II A3: Intake below threshold	4)	Evaluated in FGE.212 Rev2, genotoxic concern can be ruled out. Can be evaluated using the Procedure in FGE.47Rev2.
07.219	trans-3-Methyl-2-(2-pentenyl)-2-cyclopenten-1-one		4.7	No evaluation	Not evaluated by the JECFA	Evaluated in FGE.212Rev2, genotoxic concern could not be ruled out. Additional genotoxicity data required for the representative [FL-

Table 3: Summary of Safety Evaluation Applying the Procedure (based on intakes calculated by the MSDI approach)

FL-no JECFA-no	EU Register name	Structural formula	MSDI 1) (µg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	Outcome on the material of commerce [6), 7), or 8)]
						no: 07.112]

1) EU MSDI: Amount added to food as flavour in (kg / year) x 10E9 / (0.1 x population in Europe (= 375 x 10E6) x 0.6 x 365) = µg/capita/day.
2) Thresholds of concern: Class I = 1800 µg/person/day, Class II = 540 µg/person/day, Class III = 90 µg/person/day.
3) Procedure path A substances can be predicted to be metabolised to innocuous products. Procedure path B substances cannot.
4) No safety concern based on intake calculated by the MSDI approach of the named compound.
5) Data must be available on the substance or closely related substances to perform a safety evaluation.

QSAR PREDICTIONS ON MUTAGENICITY IN FIVE MODELS FOR 16 KETONES FROM SUBGROUP 2.6

Table 4: QSAR Predictions on Mutagenicity in Five Models for 16 Ketones from Subgroup 2.6

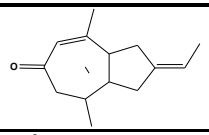
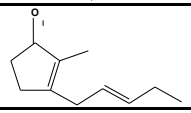
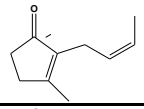
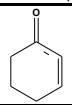
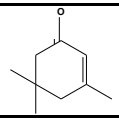
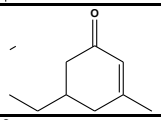
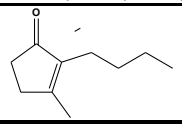
FL-no JECFA-no	EU Register name	Structural formula	ISS Local Model Ames Test TA100	MultiCASE Ames test	MultiCASE Mouse lymphoma test	MultiCASE Chromosomal aberration test in CHO	MultiCASE Chromosomal aberration test in CHL
Not in Register	2,6-Dimethyl-9-(1-methylethylidene)-bicyclo[5.3.0]dec-2-en-4-one		OD	NEG	NEG	NEG	NEG
07.033 1115	Isojasmone		OD	NEG	NEG	NEG	NEG
07.094 1114	3-Methyl-2-(pent-2(cis)-enyl)cyclopent-2-en-1-one		OD	NEG	OD	NEG	NEG
07.098 1107	3-Methylcyclohex-2-en-1-one		OD	NEG	POS	NEG	EQU
07.112 1105	3-Methyl-2-cyclopenten-1-one		OD	NEG	POS	NEG	EQU
07.126 1112	3,5,5-Trimethylcyclohex-2-en-1-one		OD	NEG	POS	NEG	EQU
07.129 1113	3-Methyl-5-propylcyclohex-2-en-1-one		OD	NEG	POS	NEG	EQU
07.140 1406	3-Methyl-2-pentylcyclopent-2-en-1-one		OD	NEG	OD	NEG	NEG

Table 4: QSAR Predictions on Mutagenicity in Five Models for 16 Ketones from Subgroup 2.6

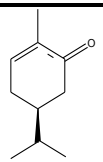
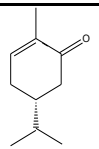
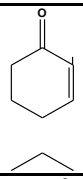
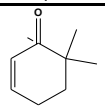
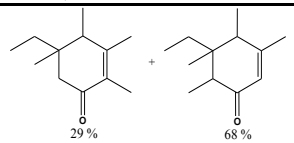
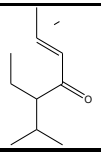
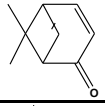
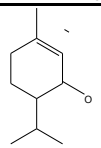
FL-no JECFA-no	EU Register name	Structural formula	ISS Local Model Ames Test TA100	MultiCASE Ames test	MultiCASE Mouse lymphoma test	MultiCASE Chromosomal aberration test in CHO	MultiCASE Chromosomal aberration test in CHL
07.146 380.1	*d*-Carvone		OD	NEG	NEG	NEG	NEG
07.147 380.2	*l*-Carvone		OD	NEG	NEG	NEG	NEG
07.172 1110	4-Isopropylcyclohex-2-en-1-one		OD	NEG	NEG	NEG	EQU
07.202	2,6,6-Trimethylcyclohex-2-en-1-one		OD	NEG	OD	NEG	NEG
07.035 1111	Tetramethyl ethylcyclohexenone (mixture of isomers)		OD	NEG	NEG	NEG	NEG
07.255	*l*-Piperitone		OD	NEG	OD	NEG	EQU

Table 4: QSAR Predictions on Mutagenicity in Five Models for 16 Ketones from Subgroup 2.6

FL-no JECFA-no	EU Register name	Structural formula	ISS Local Model Ames Test TA100	MultiCASE Ames test	MultiCASE Mouse lymphoma test	MultiCASE Chromosomal aberration test in CHO	MultiCASE Chromosomal aberration test in CHL
07.196 -	Pin-2-en-4-one		OD	NEG	POS	NEG	POS
07.175	p-Menth-1-en-3-one (+/- piperitone)		OD	NEG	POS	NEG	OD

Column 3: Structure group 2.6: α,β-unsaturated ketones.
Column 4: Local model on aldehydes and ketones, Ames TA100. (NEG: Negative; POS: Positive; OD*: out of domain).
Column 5: MultiCase Ames test (OD*: Out of domain; POS: Positive; NEG: Negative; EQU: Equivocal).
Column 6: MultiCase Mouse Lymphona test (OD*: Out of domain; POS: Positive; NEG: Negative; EQU: Equivocal).
Column 7: MultiCase Chromosomal aberration in CHO (OD*: Out of domain; POS: Positive; NEG: Negative; EQU: Equivocal).
Column 8: MultiCase Chromosomal aberration in CHL (OD*: Out of domain; POS: Positive; NEG: Negative; EQU: Equivocal).
* OD, out of applicability domain: not matching the range of conditions where a reliable prediction can be obtained in this model. These conditions may be physicochemical, structural, biological etc.

CARCINOGENICITY STUDIES CONSIDERED BY THE PANEL IN FGE.212

Table 5: Carcinogenicity Studies

Chemical Name [FL-no]	Species; Sex No./Group	Route	Dose levels	Duration	Results	Reference	Comments*
3,5,5-Trimethylcyclohex-2-en-1-one [07.126]	Rats; Male, Female 50/sex/group	Gavage in corn oil	0 (controls), 250 or 500 mg/kg bw/day, five times per week	103 weeks	Males: Increased incidences of renal tubular cell adenomas and adenocarcinomas and of carcinomas of the preputial gland Females: No carcinogenic effect	(NTP, 1986)	Valid
	Mice; Male, Female 50/sex/group	Gavage in corn oil	0 (controls), 250 or 500 mg/kg bw/day, five times per week	103 weeks	Males: Increased incidences of hepatocellular adenomas and carcinomas, mesenchymal tumors in the integumentary system, and malignant lymphomas Females: No carcinogenic effect	(NTP, 1986)	Valid
d-Carvone [07.146]	Mice; Male, Female 50/sex/group	Gavage	0, 375 or 750 mg/kg bw/day, five times per week	103 weeks	Males and females: No increases in tumour incidences	(NTP, 1990)	Valid

* Validity of genotoxicity studies:
Valid.
Limited validity (e.g. if certain aspects are not in accordance with OECD guidelines or current standards and / or limited documentation).
Insufficient validity (e.g. if main aspects are not in accordance with any recognised guidelines (e.g. OECD) or current standards and/or inappropriate test system).
Validity cannot be evaluated (e.g. insufficient documentation, short abstract only, too little experimental details provided).

GENOTOXICITY DATA (*IN VITRO*) CONSIDERED BY THE PANEL IN FGE.212 AND FGE.212REV1

Table 6: Summary of Genotoxicity data (*in vitro*)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Reported Result	Reference	Comments [e]
Tetramethyl ethylcyclohexenone (mixture of isomers) [07.035]	Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538	5 concentrations up to cytotoxicity or max. 3600 µg/plate	Negative[a]	(Wild et al., 1983)	Limited validity (no TA102 or *E. Coli*); possibly slightly low maximal concentration tested.
3,5,5-Trimethylcyclohex-2-en-1-one [07.126]	Reverse mutation	*S. typhimurium* TA97, TA98, TA100, TA1535, TA1537	33 – 10000 µg/plate	Negative[a]	(Mortelmans et al., 1986)	Valid
	Mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537	33 – 10000 µg/plate	Negative[a]	(NTP, 1986)	NTP study carried out according to standard US-EPA Guideline; result is considered as valid.
	Mutation	L5178YTk+/– mouse lymphoma cells	67 – 810 µg/ml	Negative[c]	(McKee et al., 1987)	Validity cannot be evaluated (tested with S9; abstract only with very limitred information).
	Mutation	L5178YTk+/– mouse lymphoma cells	130 – 1300 µg/ml	Negative[b]	(McKee et al., 1987)	Validity cannot be evaluted (tested without S9; abstract only with very limitred information).
	Mutation	L5178YTk+/– mouse lymphoma cells	0.089 – 0.89 µl/ml	Negative[c]	(O'Donoghue et al., 1988)	Valid according to current guidelines
	Mutation	L5178YTk+/– mouse lymphoma cells	0.13 – 1.3 µl/ml	Negative[b]	(O'Donoghue et al., 1988)	Valid according to current guidelines
	Mutation	L5178YTk+/– mouse lymphoma cells	1200 µg/ml	Positive[b]	(NTP, 1986)	NTP study carried out according to standard US-EPA Guideline. Not tested with S9. Result is considered as valid.
	Mutation	L5178YTk+/– mouse lymphoma cells	Not reported (however, up to cytotoxic concentrations) for 3 hours exposure.	Negative[a]	(Honma et al., 1999a)	Limited validity since data were presented in a summarised table format only (as a result of an international

Table 6: Summary of Genotoxicity data (*in vitro*)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Reported Result	Reference	Comments [e]
						collaborative study).
	Mutation	L5178YTk+/– mouse lymphoma cells	Up to 1500 µg/ml	Positive[b]	(Honma et al., 1999b)	Limited validity since mutation frequencies were not reported in table format. Tested only in the absence of S9. Isophorone was mutagenic after 24-hour treatments in the absence of S9. Although only graphs are plotted, it seems that increases in MF that exceeded the GEF occurred at around 1250-1500 µg/ml where toxicity (by relative survival) reached 70-90 %.
	Chromosomal aberration	Chinese hamster ovary cells	5 – 1600 µg/ml	Negative[a]	(Gulati et al., 1989)	Limited validity (not clear if gaps were included in the scores).
	Chromosomal aberration	Chinese hamster ovary cells	250 – 1600 µg/ml	Negative[a]	(NTP, 1986)	NTP study carried out according to standard US-EPA Guideline; result is considered as valid.
	Chromosomal aberration	Chinese hamster lung fibroblasts	0 - 1250[b] µg/ml 0 – 1500[c] µg/ml	Positive[a]	(Matsuoka et al., 1996)	Valid.
	Chromosomal aberration	Chinese hamster lung fibroblasts	250 – 1000 mg/ml	Negative[a]	(Matsuoka et al., 1996)	Valid. Exposed to isophorone without metabolic activation for 24 or 48 hours, cytotoxic at highest concentrations.
	Sister chromatid exchange	Chinese hamster ovary cells	5 – 1600 mg/ml	Positive[b,d]	(Gulati et al., 1989)	Valid (pos – S9; neg + S9).
	Sister chromatid exchange	Chinese hamster ovary cells	160 – 1000 mg/ml	Negative[a]	(NTP, 1986)	NTP study carried out according to Standard US-EPA Guideline; result is

Table 6: Summary of Genotoxicity data (*in vitro*)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Reported Result	Reference	Comments [e]
						considered as valid.
	Unscheduled DNA synthesis	Rat hepatocytes	0.005 – 0.4 µl/ml	Negative	(O'Donoghue et al., 1988)	Valid according to current guidelines
	Unscheduled DNA synthesis	Rat hepatocytes	5 - 200 µl/ml	Negative[a]	(McKee et al., 1987)	Validity cannot be evaluated (abstract only with very limited information)
Carvone (isomer not specified)	Gene mutation	*S. typhimurium* TA1535, TA1537, TA98, TA100	3 µmol/plate	Negative	(Florin et al., 1980)	Insufficient validity (spot test, not according to OECD Guideline, methods and results insufficiently reported). Isomer (*d* or *l*) not reported.
	Rec assay	*B. subtilis* H17 (rec+) and M45 (rec-)	0.6 ml/disc	Negative	(Matsui et al., 1989)	The test system used is considered inappropriate,
d-Carvone [07.0146]	Gene mutation	*S. typhimurium* TA1535, TA98, TA100, TA1537	333 µg/plate	Negative[a]	(NTP, 1990)	Valid
	Gene mutation (pre-incubation)	*S. typhimurium* TA1535, TA98, TA100, TA1537	560 µg/plate	Negative	(Mortelmans et al., 1986)	Valid
	Sister chromatid exchange	Chinese hamster ovary cells	502 µg/ml	Positive[a]	(NTP, 1990)	Valid
	Chromosomal aberration	Chinese hamster ovary cells	400 µg/ml	Positive[a]	(NTP, 1990)	Valid

a: With and without metabolic activation.
b: Without metabolic activation.
c: With metabolic activation.
d: Cytotoxic at next highest dose tested (1600 mg/ml).
e: Validity of genotoxicity studies:
Valid.
Limited validity (e.g. if certain aspects are not in accordance with OECD guidelines or current standards and / or limited documentation).
Insufficient validity (e.g. if main aspects are not in accordance with any recognised guidelines (e.g. OECD) or current standards and/or inappropriate test system).
Validity cannot be evaluated (e.g. insufficient documentation, short abstract only, too little experimental details provided).

GENOTOXICITY DATA (*IN VIVO*) CONSIDERED BY THE PANEL IN FGE.212 AND IN FGE.212REV1

Table 7: Summary of Genotoxicity data (*in vivo*)

Chemical Name [FL-no]	Test System	Test Object	Route	Dose	Result	Reference	Comments [a]
Tetramethyl ethylcyclohexenone (mixture of isomers [07.035]	Sex-linked recessive lethal mutation	*D. melanogaster*	Feed	10 mM	Negative	(Wild et al., 1983)	Limited validity (low nr of chromosomes, limited reporting).
	Micronucleus formation	Mouse bone marrow	i.p.	180, 307 and 450 mg/kg bw	Negative	(Wild et al., 1983)	Limited validity. Only analysis at one time point; no PCE/NCE ratio reported.
3,5,5-Trimethylcyclohex-2-en-1-one [07.126]	Sex-linked recessive lethal mutation	*D. melanogaster*		2000[f] and 12500[g] ppm	Negative	(Foureman et al., 1994)	Valid, however, only limited relevance.
	Micronucleus formation	CD-1 mice	i.p.	540 mg/kg bw (MTD)	Negative	(McKee et al., 1987)	Validity cannot be evaluated. Abstract only; very limited information no data on PCE/NCE ratio.
		CD-1 mice	i.p.	0.54 ml/kg bw	Negative	(O'Donoghue et al., 1988)	Limited validity. Only one dose level tested, this dose level corresponded to the LD20; sample schedule inadequate
	Chromosomal aberration	B6C3F1 mice	i.p.	125, 250 and 500 mg/kg bw	Negative	NTP-Website	Valid. Submitted by Industry in 2009. The standard protocol for *in vivo* CA is not given on the NTP website. However, based on Shelby and Witt (1995), animals should have been sampled at 17 hours and, if negative, also at 36 hours. The data on the NTP website are only for bone marrow sampled at 36 hours. It is therefore possible that a 17 hours sample was also taken, and found to be negative, but the data not posted. Fifty cells per animal were scored for CA and no increases in CA were seen. No measures of toxicity were recorded, but i.p. dosing should have guaranteed systemic exposure.
	DNA binding	F344 rats	Gavage	500 mg unlabelled isophorone / kg bw	Negative	Thier et al., 1990	Limited validity. Submitted by Industry in 2009. No positive controls and no

				spiked with C14 isophorone (0.4 mCi/rat)			untreated controls used. Liver and kidney were analysed.
	DNA binding	B6C3F1 mice	Gavage	500 mg unlabelled isophorone / kg bw spiked with C14 isophorone (0.08 mCi/mouse)	Negative	Thier et al., 1990	Limited validity. Submitted by Industry in 2009. No positive controls and no untreated controls used. Liver and kidney were analysed.
	DNA binding	F344 rats (10 males)	Gavage	500 mg/kg bw ^{14}C-isophorone (0.1 mCi/rat)	Negative	Morishita et al., 1997	Valid. Preputial glands and kidneys were analysed.
	DNA adducts (^{32}P-Postlabelling)	F344 rats (7 males and 7 females per dose group)	Gavage	0, 250 and 500 mg/kg/day for 5 days.	Negative	Morishita et al., 1997	Valid. Preputial glands were analysed.

a: Validity of genotoxicity studies:
Valid.
Limited validity (e.g. if certain aspects are not in accordance with OECD guidelines or current standards and / or limited documentation).
Insufficient validity (e.g. if main aspects are not in accordance with any recognised guidelines (e.g. OECD) or current standards and/or inappropriate test system).
Validity cannot be evaluated (e.g. insufficient documentation, short abstract only, too little experimental details provided).

GENOTOXICITY DATA (*IN VITRO*) CONSIDERED BY THE PANEL IN FGE.212REV2

Table 8: Summary of Additionally Genotoxicity Data on [FL-no: 09.821] of Subgroup 2.6

Chemical Name [FL-no:]	Test System *in vitro*	Test Object	Concentrations of Substance and Test Conditions	Result	Reference	Comments
Vetiveryl acetate [09.821]	Reverse Mutation	*S. typhimurium* TA97, TA98, TA100, TA102, and TA1535	20, 63.2, 200, 632 and 2000 µg/plate [6,7] 20, 63.2, 200, 632 and 2000 µg/plate [6,8]	Negative Negative	(Gocke, 2000)	1 % alpha-tocopherol was present in the solution of vetiveryl acetate as a stabiliser. Some precipitation (milky appearance) was seen at the higher concentrations and there was some evidence of toxicity in the pre-incubation experiments. Study design complies with current recommendations (OECD, Guideline 471).
		S. typhimurium TA100, TA97a, TA98, TA1535, and TA102	5 - 5000 µg/plate [6,7] 5 - 5000 µg/plate [6,8]	Negative Negative	(Scheerbaum, 2001)	Limited toxicity was noted at 500, 1600 or 5000 µg/plate. No precipitation was seen. Study design complies with current recommendations (OECD, Guideline 471).
		S. typhimurium TA98, TA100, TA102, TA1535 and TA1537 *S. typhimurium* TA98, TA1535 and TA1537 *S. typhimurium* TA100, TA102	33, 100, 333, 1000, 2500 and 5000 µg/plate [6,7] 33, 100, 333, 1000, 2500 and 5000 µg/plate [6,8] 10 [9, 8] 33, 100, 333, 1000, 2500 and 5000 µg/plate [6,8]	Negative Negative Negative	(Poth, 2003)	Stabilized material (18 months). Precipitation occurred at the highest concentration. In the first experiment, cytotoxicity was noted in the presence of S9-mix at 1000 µg/plate and above (TA100 and TA102) or at 5000 µg/plate (TA1537). In the second experiment, cytotoxicity was noted in the presence of S9-mix, at 1000 µg/plate and above (TA1537 and TA100) and at 2500 µg/plate and above (TA1535 and TA102). Study design complies with current recommendations (OECD, Guideline 471).
		S. typhimurium TA98, TA100, TA102, TA1535 and TA1537	33, 100, 333, 1000, 2500 and 5000 µg/plate [6,7] 33, 100, 333, 1000, 2500 and 5000 µg/plate [6,8]	Negative Negative	(Sokolowski, 2003a)	Stabilized material (24 months). No precipitation was observed up to the highest concentration. Cytotoxicity was noted in the presence of S9-mix, at 1000 µg/plate and above. Study design complies with current recommendations (OECD, Guideline 471).

Table 8: Summary of Additionally Genotoxicity Data on [FL-no: 09.821] of Subgroup 2.6

Chemical Name [FL-no:]	Test System *in vitro*	Test Object	Concentrations of Substance and Test Conditions	Result	Reference	Comments
		S. typhimurium TA98, TA100, TA102, TA1535 and TA1537	33, 100, 333, 1000, 2500 and 5000 µg/plate [6,7] 33, 100, 333, 1000, 2500 and 5000 µg/plate [6,8]	Negative Negative	(Sokolowski, 2003b)	Stabilised material 112 extra (24 months). No precipitation was observed up to the highest concentration. Cytotoxicity was noted in the presence of S9-mix, at 1000 µg/plate and above. Study design complies with current recommendations (OECD, Guideline 471).
	Chromosomal aberrations	Human Lymphocytes	10 - 60 µg/ml [1,4]; 40 - 140 µg/ml [2,4]; 20 - 140 µg/ml [3,4]; 10 - 120 µg/ml [1,5]	Negative	(Morris, 2011)	The dose selection was based on a preliminary toxicity test performed in CHO cells, in a previous chromosome aberration study (Morris and Durward, 2010). Hemolysis was observed at 20 µg/ml and 80 µg/ml in the absence and presence of S9-mix, respectively. No precipitation was observed. Study design complies with current recommendations (OECD, Guideline 473).
		Chinese Hamster Ovary Cells	2.5 - 40 µg/ml [1,4]; 10 - 80 µg/ml [2,4]; 10 - 70 µg/ml [3,4]; 5 - 35 µg/ml [1,5]	Negative	(Morris and Durward, 2010)	Small increases in chromosomal aberrations and polyploidy were not dose-dependent and not consistent, and therefore they were considered of no biological significance. A marked toxicity was observed at concentrations higher than 82 µg/ml in the absence of S9-mix and higher than 164 µg/ml in the presence of S9-mix (4+20 hours). A marked toxicity was observed at concentrations higher than 41 µg/ml in the 24 hour continuous exposure group. In the first experiment, the maximum concentrations selected for metaphase analysis were 30 µg/ml and 60 µg/ml in the absence and presence of S9-mix respectively. In the second experiment, the maximum concentrations selected for analysis were 20 µg/ml and 50 µg/ml in the absence and presence of S9-mix respectively. Precipitation was seen at 30 µg/ml and above or 50 µg/ml and above, in the absence or presence of S9-mix, respectively. Study design complies with current recommendations (OECD, Guideline 473).

[1] Without S9-mix metabolic activation.
[2] With S9-mix metabolic activation (2%).
[3] With S9-mix metabolic activation (1%).
[4] 4-hour incubation with 20-hour recovery period.
[5] 24-hour incubation with no recovery period.
[6] With and without S9-mix metabolic activation.
[7] Standard plate incorporation method.
[8] Modified pre-incubation method.
[9]With S9-mix metabolic activation.

REFERENCES

Benigni R and Netzeva T, 2007a. Report on a QSAR model for prediction of genotoxicity of α,ß-unsaturated aldehydes in *S. typhimurium* TA100 and its application for predictions on α,ß-unsaturated aldehydes in Flavouring Group Evaluation 19 (FGE.19). Unpublished report submitted by FLAVIS Secretariat to EFSA.

Benigni R and Netzeva T, 2007b. Report on a QSAR model for prediction of genotoxicity of α,ß-unsaturated ketones in S. typhimurium TA100 and its application for predictions on α,ß-unsaturated aldehydes in Flavouring Group Evaluation 19 (FGE.19). Unpublished report submitted by FLAVIS Secretariat to EFSA.

EFFA (European Flavour Association), 2010. EFFA Letters to EFSA for clarification of specifications and isomerism for which data were requested in published FGEs.

EFFA (European Flavour Association), 2012. Private Communication forwarded to FLAVIS Secretariat, Danish Food Institute, Technical University of Denmark, dated 17 January 2012, 14, 23 and 24 February 2012 and 19 March 2012. Specification data related to substances in FGE.29.Rev1: [FL-no: 01.015]; FGE.09Rev4 [FL-no: 07.059, 09.843 and 09.920] and FGE.51Rev1 [FL-no: 07.034, 07.035, 07.095, 07.129, 07.172, 07.257 and 09.930]. FLAVIS/8.143.

EFSA (European Food Safety Authority), 2008a. Minutes of the 26th Plenary meeting of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food, Held in Parma on 27 - 29 November 2007. Parma, 7 January 2008. Available online: http://www.efsa.europa.eu/EFSA/Event_Meeting/afc_minutes_26thplen_en.pdf

EFSA (European Food Safety Authority), 2008b. Statement of the Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF) on Genotoxicity Test Strategy for Substances belonging to Subgroups of FGE.19. The EFSA Journal 2008, 854, 1-5.

EFSA (European Food Safety Authority), 2008c. Statement of the Panel on food contact materials, enzymes, flavourings and processing aids (CEF) on List of alpha, beta-Unsaturated Aldehydes and Ketones representative of FGE.19 substances for Genotoxicity Testing. The EFSA Journal 2008, 910, 1-7.

Engel W, 2001. *In vivo* studies on the metabolism of the monoterpenes S-(+) - and R-(-)-carvone in humans using the metabolism of ingestion-correlated amounts (MICA) approach. Journal of Agricultural and Food Chemistry, 49, 4069-4075.

EPA, 1997. Mechanistic studies on isophorone and preputial gland carcinomas (Morishita K, Schoonhoven R and Swenberg J), with cover letter dated 4/15/1997. EPA/OTS; Doc #86970000764.

Florin I, Rutberg L, Curvall M and Enzell CR, 1980. Screening of tobacco smoke constituents for mutagenicity using the Ames' test. Toxicology, 18, 219-232.

Foureman P, Mason JM, Valencia R and Zimmering S, 1994. Chemical mutagenesis testing in Drosophila. X. Results of 70 coded chemicals tested for the National Toxicology Program. Environmental and Molecular Mutagenesis, 23, 208-227.

Gocke E, 2000. Evaluation of vetiveryl acetate + 1% tocopherol alpha for mutagenic activity in the ames test. F. Hoffmann La-Roche Ltd. Study no. 373M00. October 16, 2000. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Gry J, Beltoft V, Benigni R, Binderup M-L, Carere A, Engel K-H, Gürtler R, Jensen GE, Hulzebos E, Larsen JC, Mennes W, Netzeva T, Niemelä J, Nikolov N, Nørby KK and Wedebye EB, 2007. Description and validation of QSAR genotoxicity models for use in evaluation of flavouring substances in Flavouring Group Evaluation 19 (FGE.19) on 360 α,ß-unsaturated aldehydes and ketones and precursors for these. Unpublished report submitted by FLAVIS Secretariat to EFSA.

Gulati DK, Witt K, Anderson B, Zeiger E and Shelby M, 1989. Chromosome aberration and sister-chromatid exchange test in Chinese hamster ovary cells *in vitro* III: Results with 27 chemicals. Environmental and Molecular Mutagenesis, 13, 133-193.

Honma M, Hayashi M, Shimada H, Tanaka N, Wakuri S, Awogi T, Yamamoto KI, Kodani N-U, Nishi Y, Nakadate M and Sofuni T, 1999a. Evaluation of the mouse lymphoma tk assay (microwell method) as an alternative to the *in vitro* chromosomal aberration test. Mutagenesis, 14(1), 5-22.

Honma M, Zhang L-S, Sakamoto H, Ozaki M, Takeshita K, Momose M, Hayashi M and Sufuni T, 1999b. The need for long-term treatment in the mouse lymphoma assay. Mutagenesis, 14(1), 23-29.

IOFI (International Organization of the Flavor Industry), 2012. Flavouring Group Evaluation 19 Subgroup 2.6 Part A - Flavouring Substances of the Chemical Group 3 (Annex I of 1565/2000/EC) Alicyclic α,ß-unsaturated aldehydes, ketones and related substances with the a,ß-conjugation in the ring or in the side chain. 17/12-2012. FLAVIS/8.183.

JECFA (Joint FAO/WHO Expert Committee on Food Additives), 1998. Compendium of food additive specifications. Addendum 6. Joint FAO/WHO Expert Committee of Food Additives 51st session. Geneva, 9-18 June 1998. FAO Food and Nutrition paper 52 Add. 6.

JECFA (Joint FAO/WHO Expert Committee on Food Additives), 1999. Safety evaluation of certain food additives. Fifty-first Meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA). WHO Food Additives Series: 42. IPCS, WHO, Geneva.

JECFA (Joint FAO/WHO Expert Committee on Food Additives), 2002. Compendium of food additive specifications. Addendum 10. Joint FAO/WHO Expert Committee of Food Additives 59th session. Geneva, 4-13 June 2002. FAO Food and Nutrition paper 52 Addition 10.

JECFA (Joint FAO/WHO Expert Committee on Food Additives), 2003. Safety evaluation of certain food additives. Fifty-ninth Meeting of the Joint FAO/WHO Expert Committee on Food Additives, WHO Food Additives Series: 50. IPCS, WHO, Geneva.

JECFA (Joint FAO/WHO Expert Committee on Food Additives), 2009a. Safety evaluation of certain food additives and contaminants. Sixty-ninth Meeting of the Joint FAO/WHO Expert Committee on Food Additives, WHO Food Additives Series: 60. IPCS, WHO, Geneva 2009. Available online: http://whqlibdoc.who.int/publications/2009/9789241660600_eng.pdf.

JECFA (Joint FAO/WHO Expert Committee on Food Additives), 2009b. JECFA Online Edition ”Specification for Flavourings”. Available online: http://www.fao.org/ag/agn/jecfa-flav/search.html.

Matsui S, Yamamoto R and Yamada H, 1989. The Bacillus Subtilis/Microsome rec-assay for the detection of DNA damaging substances which may occur in chlorinated and ozonated waters. Water Science & Technology, 21, 875-887.

Matsuoka A, Yamakage K, Kusakabe H, Wakuri S, Asakura M, Noguchi T, Sugiyama T, Shimada H, Nakayama S, Kasahara Y, Takahashi Y, Miura KF, Hatanaka M, Ishidate M, Morita T, Watanabe K, Hara M, Odawara K, Tanaka N, Hayashi M and Sofuni T, 1996. Re-evaluation of chromosomal aberration induction on nine mouse lymphoma assay 'unique positive' NTP carcinogens. Mutation Research, 369, 243-252.

McKee RH, Phillips RD, Lerman SA, Slesinski RS, Rogers-Back AM, Curren RD and Putman DL, 1987. The genotoxic potential of isophorone. Environmental Mutagenesis, 9(8), 71.

Morishita K, Schoonhoven R and Swenberg JA, 1997. Mechanistic studies on isophorone and preputial gland carcinomas. Department of Environmental Sciences and Engineering, University of North Carolina, Chapel Hill, N.C. March 25, 1997. Unpublished report submitted by EFFA to FLAVIS secretariat.

Morris A and Durward R, 2010. Chromosome aberation test in cho cell: *in vitro*. Vetiveryl acetate. Harlan Laboratories Ltd. Project no. 2153/0007. 16 April 2010. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Morris A, 2011. Chromosome aberration test in human lymphocytes *in vitro*. Vetiveryl acetate. Harlan Laboratories Ltd. Project no. 41101037. 22 November 2011. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Mortelmans K, Haworth S, Lawlor T, Speck W, Tainer B and Zeiger E, 1986. Salmonella mutagenicity tests II. Results from the testing of 270 chemicals. Environmental and Molecular Mutagenesis, 8(7), 1-119.

Nikolov N, Jensen GE, Wedebye EB and Niemelä J, 2007. Report on QSAR predictions of 222 α,ß-unsaturated aldehydes and ketones from Flavouring Group Evaluation 19 (FGE.19) on 360 α,ß-unsaturated aldehydes and ketones and precursors for these. Unpublished report submitted by FLAVIS Secretariat to EFSA.

NTP (National Toxicology Program), 1986. Toxicology and carcinogenesis studies of isophorone (CAS No. 78-59-1) in F344/N rats and B6C3F1 mice (gavage studies). NTP-TR 291. NIH Publication no. 86-2547.

NTP (National Toxicology Program), 1990. Toxicology and carcinogenesis studies of d-carvone (CAS No. 2244-16-8) in B6C3F1 mice (gavage studies). February 1990. NTP-TR 381. NIH Publication no. 90-2836.

O'Donoghue JL, Haworth SR, Curren RD, Kirby PE, Lawlor T, Moran EJ, Phillips RD, Putnam DL, Rogers-Back AM, Slesinski RS and Thilagar A, 1988. Mutagenicity studies on ketone solvents: methyl ethyl ketone, methyl isobutyl ketone, and isophorone. Mutation Research, 206, 149-161.

OECD (Organisation for Economic Co-operation and Development), 1997a. Test No. 471: Bacterial Reverse Mutation Test. OECD Guidelines for the Testing of Chemicals, Section 4.

OECD (Organisation for Economic Co-operation and Development), 1997b. Test No. 473: *In vitro* Mammalian Chromosome Aberration Test. OECD Guidelines for the Testing of Chemicals, Section 4.

Poth A, 2003. Salmonella typhimurium reverse mutation assay with vetiveryl acetate stab. (18 months). R C C Cytotest Cell Research GMBH. Study no. 846892. March 14, 2003. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Scheerbaum D, 2001. Reverse Mutation Assay (Ames test) with *Salmonella typhimurium*. Vetiverylacetate extra. Dr.U.Noack-Laboratorium für Angewandte Biologie. 5 March 2001. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Shelby MD and Witt KL, 1995. Comparision of results from mouse bone marrow chromosome aberration and micronucleus test. Environmental and Molecular Mutagenesis, 25, 302-313.

Sokolowski A, 2003a. *Salmonella typhimurium* reverse mutation assay with vetiveryl acetate stab. (24 months). R C C Cytotest Cell Research GMBH. Study no. 849668. June 24, 2003. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Sokolowski A, 2003b. *Salmonella typhimurium* reverse mutation assay with vetiveryl acetate 112 extra stab. (24 months). R C C Cytotest Cell Research GMBH. Study no. 849669. June 30, 2003. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Thier R, Peter H, Wiegland HJ and Bolt HM, 1990. Letter to the Editors. DNA binding study of isophorone in rats and mice. Archives of Toxicology 64, 684-685.

Wild D, King MT, Gocke E and Eckhard K, 1983. Study of artificial flavouring substances for mutagenicity in the Salmonella/microsome, BASC and micronucleus tests. Food and Chemical Toxicology 21(6), 707-719.

ABBREVIATIONS

bw	Body Weight
CA	Chromosomal Aberrations
CAS	Chemical Abstract Service
CEF	Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
CHO	Chinese hamster ovary (cells)
CoE	Council of Europe
CP	Cyclophosphamide
DMSO	Dimethylsulfoxyd
DNA	Deoxyribonucleic acid
dpm	Disintegrations Per Minute
EC	European Commission
EFFA	European Flavour and Fragrance Association
EFSA	European Food Safety Authority
EU	European Union
FAO	Food and Agriculture Organization of the United Nations
FEMA	Flavor and Extract Manufacturers Association
FGE	Flavouring Group Evaluation
FLAVIS (FL)	Flavour Information System (database)
GEF	Global Evaluation Factor
ID	Identity
IOFI	International Organization of the Flavour Industry
i.p.	intraperitoneal
IR	Infrared spectroscopy
JECFA	Joint FAO/WHO Expert Committee on Food Additives
MF	Mutation Frequency
MLA	Mouse Lymphoma Assay
MLTK	Mouse Lymphoma Thymidine Kinase (gene mutation assay)
MMC	Mitomycin C
MS	Mass spectrometry
NCE	Normochromatic Erythrocytes
No	Number
NTP	National Toxicology Program
OECD	Organisation for Economic Co-operation and Development
PCE	Polychromatic Erythrocytes
QSAR	Quantitative Structure-Activity Relationship

SCE	Sister Chromatid Exchange
SLRL	Sex Linked Recessive Lethal Mutations test
UDS	Unscheduled DNA Synthesis
WHO	World Health Organisation