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Scientific Opinion on Flavouring Group Evaluation 7, Revision 5 (FGE.07Rev5): saturated and unsaturated aliphatic secondary alcohols, ketones and esters of secondary alcohols and saturated linear or branched-chain carboxylic acids from chemical group 5

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Abstract

The EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids was requested to evaluate 53 flavouring substances attributed to the Flavouring Group Evaluation 07, including four new substances but-3-en-2-ol, non-1-en-e-ol, hex-1-en-3-one and 1-nonene-3-one [FL-nos: 02.131, 02.187, 07.161 and 07.210] in this Revision 5, using the Procedure in Commission Regulation (EC) No 1565/2000. None of the 53 substances was considered to have genotoxic potential. The substances were evaluated through a stepwise approach that integrates information on the structure–activity relationships, intake from current uses, toxicological threshold of concern (TTC), and available data on metabolism and toxicity. The Panel concluded that all 53 substances do not give rise to safety concerns at their levels of dietary intake, estimated on the basis of the 'Maximised Survey-derived Daily Intake' (MSDI) approach. Besides the safety assessment of the flavouring substances, the specifications for the materials of commerce have also been considered and found adequate. For 50 substances, further information is required based on comparison of the 'modified Theoretical Added Maximum Daily Intakes' (mTAMDIs) with the TTCs. This would include more reliable intake data and then, if required, additional toxicological data.

Keywords: flavouring substances, aliphatic secondary alcohols, ketones, esters, FGE.07, FGE.63

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1. Introduction

1.1. Background and Terms of Reference as provided by the requestor

1.1.1. Background

The use of flavourings is regulated under Regulation (EC) No 1334/2008 of the European Parliament and Council of 16 December 2008[1] 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[2]. The list contains flavouring substances for which the scientific evaluation should be completed in accordance with Commission Regulation (EC) No 1565/2000[3].

On 27 September 2012, the EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids adopted an opinion on Flavouring Group Evaluation 205 (FGE.205): consideration of genotoxic potential on α,β-unsaturated aliphatic ketones with terminal double bonds and precursors from chemical subgroup 1.2.2 of FGE.19.

The Panel concluded that for the two representative substances, oct-1-en-3-one [FL-no: 07.081] and pent-1-en-3-one [FL-no: 07.102], the positive effects in the bacterial mutagenicity assays cannot be overruled by one negative and one equivocal gene mutation test in mammalian cells. Accordingly, an *in vivo* Comet assay on the first site of contact (e.g. the stomach or duodenum) and on the liver is requested for the most potent substance, pent-1-en-3-one. As an alternative, a transgenic animal assay would also be acceptable.

On 10 March 2015, the applicant submitted additional studies on the representative substances [FL-no: 07.102] and [FL-no: 07.081]. These studies are intended to cover the substances in this group, namely: FL-nos: 02.023, 02.099, 02.104, 02.131, 02.136, 02.155, 02.187, 07.161, 07.210, 09.281 and 09.282.

1.1.2. Terms of Reference

The European Commission requests the European Food Safety Authority (EFSA) to evaluate the new information and, depending on the outcome, proceed to the full evaluation on the flavouring substances in accordance with Commission Regulation (EC) No 1565/2000[3].

1.1.3. Interpretation of the terms of reference

In FGE.205 Revision 1, EFSA evaluated the additional data on genotoxicity submitted for the substances, oct-1-en-3-one [FL-no: 07.081] and pent-1-en-3-one [FL-no: 07.102], by the flavour industry. For these substances, being the representative of four of the flavouring substances in FGE.07, the Panel concluded that the concern for a genotoxic potential could be ruled out. As a follow up to this conclusion, the substances, but-3-en-2-ol [FL-no: 02.131], non-1-en-e-ol [FL-no: 02.187], hex-1-en-3-one [FL-no: 07.161] and 1-nonene-3-one [FL-no: 07.210], were evaluated by the Panel in accordance with the Procedure described in Commission Regulation (EC) No 1565/2000[3], in line with the background and terms of references as provided by the European Commission.

1.1.4. History of the evaluation

The first version of the Flavouring Group Evaluation 07, FGE.07, dealt with 35 saturated and unsaturated aliphatic secondary alcohols, ketones and esters with secondary alcohol moiety.

The first revision of FGE.07, FGE.07Rev1, included the assessment of six additional flavouring substances [FL-nos: 02.190, 07.162, 07.201, 07.236, 07.676 and 09.926]. No new data on toxicity

[1] 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.

[2] 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.

[3] 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. Official Journal of the European Communities 19.7.2000, L 180, p. 8–16.

were provided. For two of the new substances, [FL-nos: 07.162 and 07.201], data on metabolism were provided. Additional information on 20 flavouring substances [FL-nos: 02.124, 02.142, 02.148, 02.177, 02.182, 02.183, 07.156, 07.157, 07.182, 07.185, 07.205, 09.304, 09.323, 09.325, 09.328, 09.332, 09.386, 09.388, 09.391 and 09.880] was made available since the FGE.07 was published.

The second revision of FGE.07, FGE.07Rev2, included the assessment of two additional flavouring substances [FL-nos: 02.255 and 07.239]. No new data on toxicity and metabolism were provided.

The third revision of FGE.07, FGE.07Rev3, included the assessment of one additional candidate substance [FL-nos: 07.262]. Toxicity data (acute toxicity, 28-days study and an Ames test) were submitted. No metabolism data were provided for this substance. A search in open literature did not provide any further data on toxicity or metabolism for this substance. Furthermore additional information on the specifications for eight candidate substances requested in FGE.07Rev2 had been submitted by industry and included in this FGE.

The fourth revision of FGE.07, FGE.07Rev4, included the assessment of five additional candidate substances [FL-nos: 02.145, 02.194, 02.211, 07.198 and 07.204]. These substances had been considered with respect to genotoxicity in FGE.206 (EFSA CEF Panel, 2011) and the Panel concluded that the data available ruled out the concern for genotoxicity and accordingly the substances could be evaluated through the Procedure.

FGE	Adopted	Link	Substances
FGE.07	9.12.2004	http://www.efsa.europa.eu/en/scdocs/scdoc/164.htm	35
FGE.07Rev1	26.9.2007	http://www.efsa.europa.eu/en/scdocs/scdoc/722.htm	41
FGE.07Rev2	26.3.2009	http://www.efsa.europa.eu/en/scdocs/scdoc/1020.htm	43
FGE.07Rev3	30.9.2010	http://www.efsa.europa.eu/en/efsajournal/pub/1845.htm	44
FGE.07Rev4	27.9.2012	http://onlinelibrary.wiley.com/doi/10.2903/j.efsa.2012.2899/full	49
FGE.07Rev5	1.2.2017	http://onlinelibrary.wiley.com/doi/10.2903/j.efsa.2017.4725/full	53

The present revision of FGE.07, FGE.07Rev5 includes the assessment of four additional candidate substances [FL-nos: 02.131, 02.187, 07.161 and 07.210]. These substances had been considered with respect to genotoxicity in FGE.205Rev1 (EFSA CEF Panel, 2016). Based on new genotoxicity data submitted by the flavour industry, the Panel concluded that the concern for genotoxicity could be ruled out, and therefore, the four substances could be evaluated through the Procedure in FGE.07Rev5.

A search in open literature for these four new substances conducted for metabolism and toxicity did not reveal any pertinent new information.

2. Assessment

2.1. Presentation of the substances in FGE.07Rev5

2.1.1. Identity of the substances

The present Flavouring Group Evaluation 7, Revision 5 (FGE.07Rev5), using the Procedure as referred to in the Commission Regulation (EC) 1565/2000 (the Procedure – shown in schematic form in Appendix A), deals with 53 saturated and unsaturated aliphatic acyclic secondary alcohols, ketones and esters with a secondary alcohol moiety. These 53 flavouring substances belong to the chemical group 5 of Annex I of Commission Regulation (EC) No 1565/2000[3].

The 53 flavouring substances (candidate substances) are closely related to 67 flavouring substances (supporting substances) evaluated at the 51st, 59th and 69th meetings of the Joint FAO/WHO Expert Committee on Food Additives (JEFCA) in the group 'Saturated Aliphatic Acyclic Secondary Alcohols, Ketones, and Related Saturated and Unsaturated Esters' (JECFA, 2000a, 2002a, 2003, 2009b).

The 53 candidate substances under consideration in the present evaluation are listed in Table 1, as well as their chemical Register names, FLAVIS- (FL-), Chemical Abstract Service- (CAS-), Council of Europe- (CoE-) and Flavor and Extract Manufacturers Association- (FEMA-) numbers, and structures.

Seven flavouring substances are saturated aliphatic acyclic secondary alcohols [FL-nos: 02.077, 02.142, 02.148, 02.177, 02.182, 02.183 and 02.190]; seven are unsaturated aliphatic secondary alcohols [FL-nos: 02.124, 02.131, 02.145, 02.187, 02.194, 02.211 and 02.255] of which five contain a terminal double bond [FL-nos: 02.131, 02.145, 02.187, 02.194 and 02.211]; 13 are saturated aliphatic ketones [FL-nos: 07.072, 07.084, 07.150, 07.157, 07.158, 07.160, 07.178, 07.181, 07.182, 07.185,

07.189, 07.199 and 07.205]; 10 are unsaturated aliphatic ketones [FL-nos: 07.156, 07.161, 07.162, 07.198, 07.201, 07.204, 07.210, 07.236, 07.239 and 07.262] of which seven contain a terminal double bond [FL-nos: 07.161, 07.162, 07.201, 07.204, 07.210, 07.239 and 07.262] and 16 are esters of aliphatic acyclic secondary alcohols and linear or branched-chain aliphatic carboxylic acids [FL-nos: 09.304, 09.323, 09.325, 09.328, 09.332, 09.386, 09.388, 09.391, 09.604, 09.605, 09.606, 09.608, 09.609, 09.676, 09.880 and 09.926].

The hydrolysis products of the candidate esters are listed in Appendix B, Table B.2.

The names and structures of the 67 supporting substances are listed in Appendix B, Table B.3, together with their evaluation status (CoE, 1992; SCF, 1995; JECFA, 1999a, 2002a, 2003, 2009b).

2.1.2. Stereoisomers

It is recognised that geometrical and optical isomers of substances may have different properties. Their flavour may be different; they may have different chemical properties resulting in possible variability in their absorption, distribution, metabolism, elimination and toxicity. Thus, information must be provided on the configuration of the flavouring substance, i.e. whether it is one of the geometrical/optical isomers, or a defined mixture of stereoisomers. The available specifications of purity will be considered in order to determine whether the safety evaluation carried out for candidate substances for which stereoisomers may exist can be applied to the materials of commerce. Flavouring substances with different configurations should have individual chemical names and codes (CAS number, FLAVIS number, etc.).

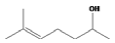
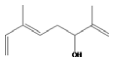
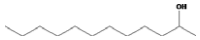
Twenty-seven candidate substances possess a chiral centre [FL-nos: 02.124, 02.131, 02.142, 02.145, 02.148, 02.177, 02.183, 02.187, 02.190, 02.194, 02.211, 02.255, 07.157, 07.182, 07.185, 07.239, 09.304, 09.323, 09.325, 09.328, 09.332, 09.386, 09.388, 09.391, 09.676, 09.880 and 09.926] and two of the candidate substances possess two chiral centres [FL-nos: 02.182 and 07.205] (see Table 1).

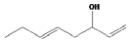
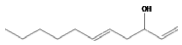
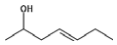
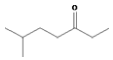
Due to the presence and the position of double bonds, 10 candidate substances can exist as geometrical isomers [FL-nos: 02.145, 02.194, 02.211, 02.255, 07.156, 07.198, 07.236, 07.239, 09.386 and 09.880]. (EFFA, 2010; EFFA, 2012) (see Table 1).

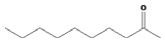
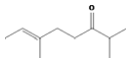
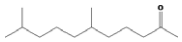
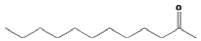
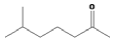
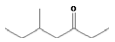
2.1.3. Specifications

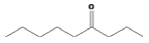
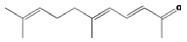
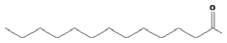
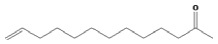
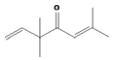
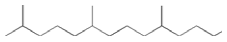
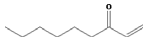
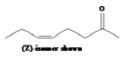
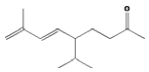
Purity criteria for all 53 candidate substances have been provided by the flavour industry (EFFA, 2002a,c, 2007c; Flavour Industry, 2006,2009). Judged against the requirements in Annex II of Commission Regulation (EC) No 1565/2000[3], the information is adequate for all candidate substances (EFFA, 2010, 2012, 2016, 2017) (see Section 2.1.2 and Table 1). Adequate specifications including purity and identity for the materials of commerce have been provided for all the candidate substances.

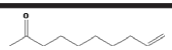
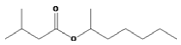
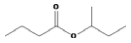
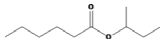
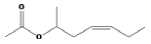
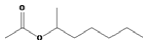
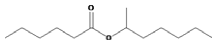
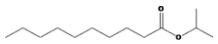
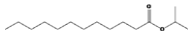
Table 1: Specification summary of the substances in the flavouring group evaluation 7, Revision 5

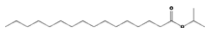
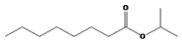
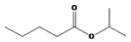
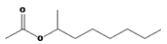
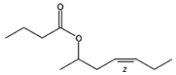
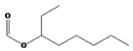
FL-no	EU register name	Structural formula	FEMA no CoE no CAS no	Phys. form Mol. formula Mol. weight	Solubility[a] Solubility in ethanol[b]	Boiling point, °C[c] Melting point, °C ID test Assay minimum	Refrac. Index[d] Spec. gravity[e]	Specification comments
02.077	Pentan-3-ol		2349 584-02-1	Liquid $C_5H_{12}O$ 88.15	Slightly soluble Freely soluble	115 MS 98%	1.407–1.413 0.815–0.822	
02.124	6-Methylhept-5-en-2-ol		10264 1569-60-4	Liquid $C_8H_{16}O$ 128.21	Slightly soluble Freely soluble	77 (20 hPa) MS 95%	1.447–1.453 0.848–0.854	Racemate (EFFA, 2002a)
02.131	But-3-en-2-ol		598-32-3	Liquid C_4H_8O 72.11	Slightly soluble Freely soluble	90 MS 95%	1.409–1.415 0.831–0.837	Racemate (EFFA, 2016)
02.142	3,3-Dimethylbutan-2-ol		464-07-3	Liquid $C_6H_{14}O$ 102.18	Slightly soluble Freely soluble	120 MS 95%	1.410–1.416 0.814–0.820	Racemate (EFFA, 2002a)
02.145	2,6-Dimethylocta-1,5,7-trien-3-ol		29414-56-0	Liquid $C_{10}H_{16}O$ 152.24	Slightly soluble Freely soluble	240 MS 95%	1.484–1.490 0.895–0.901	Racemate Mixture of *E/Z* stereoisomers: 50–80% (E) (EFFA, 2012)
02.148	Dodecan-2-ol		11760 10203-28-8	Liquid $C_{12}H_{26}O$ 186.34	Insoluble Freely soluble	129 (15 hPa) 19 MS 95%	1.438–1.444 0.829–0.835	Racemate (EFFA, 2002a)
02.177	2-Methylhexan-3-ol		10266 617-29-8	Liquid $C_7H_{16}O$ 116.20	Slightly soluble Freely soluble	144 MS 95%	1.418–1.424 0.820–0.826	Racemate (EFFA, 2002a)
02.182	3-Methylpentan-2-ol		10276 565-60-6	Liquid $C_6H_{14}O$ 102.18	Insoluble Freely soluble	134 MS 95%	1.415–1.421 0.827–0.833	Racemate (EFFA, 2010)
02.183	4-Methylpentan-2-ol		10279 108-11-2	Liquid $C_6H_{14}O$ 102.18	Slightly soluble Freely soluble	132 MS 99%	1.407–1.414 0.802–0.808	Racemate (EFFA, 2002a)
02.187	Non-1-en-3-ol		10291 21964-44-3	Liquid $C_9H_{18}O$ 142.24	Practically insoluble or insoluble Freely soluble	195 MS 98%	1.438–1.444 0.835–0.845	Racemate (EFFA, 2016)

FL-no	EU register name	Structural formula	FEMA no CoE no CAS no	Phys. form Mol. formula Mol. weight	Solubility[a] Solubility in ethanol[b]	Boiling point, °C[c] Melting point, °C ID test Assay minimum	Refrac. Index[d] Spec. gravity[e]	Specification comments
02.190	Nonan-3-ol		10290 624-51-1	Liquid $C_9H_{20}O$ 144.26	Practically insoluble or insoluble Freely soluble	195 MS 95%	1.425–1.431 0.818–0.824	Racemate (EFFA, 2010)
02.194	Octa-1,5-dien-3-ol		83861-74-9	Liquid $C_8H_{14}O$ 126.20	Practically insoluble or insoluble Freely soluble	187 MS 95%	1.441–1.447 0.832–0.838	Racemate (EFFA, 2017) Mixture of *E*/*Z* stereoisomers: 60–90% (E) (EFFA, 2012)
02.211	Undeca-1,5-dien-3-ol		56722-23-7	Liquid $C_{11}H_{20}O$ 168.28	Practically insoluble or insoluble Freely soluble	244 NMR 95%	1.456–1.462 0.872–0.878	Racemate (EFFA, 2017) Mixture of *E*/*Z* stereoisomers: 60–90% (E) (EFFA, 2012) CASrn refers to the (*Z*)-isomer only. CASrn in the Union List to be changed to 319497-21-7
02.255	(*Z*)-4-Hepten-2-ol		66642-85-1	Liquid $C_7H_{14}O$ 114.19	Insoluble Freely soluble	154 MS 92%	1.433–1.453 0.832–0.852	Racemate (EFFA, 2017) Mixture of *E*/*Z* stereoisomers: (*Z*)-isomer (approx. 92%), (E)-isomer (approx. 4%). Minor constituents 2-heptanol (< 1), trans-3-hepten-2-ol (< 1%), *cis*-3-hepten-2-ol (< 1%) (EFFA, 2010) CAS nr does not specify the geometrical isomer Name in the Union List to be changed to 4-Hepten-2-ol
07.072	6-Methylheptan-3-one		2143 624-42-0	Liquid $C_8H_{16}O$ 128.21	Insoluble Freely soluble	162 MS 95%	1.412–1.418 0.813–0.819	
07.084	Pentan-3-one		2350 96-22-0	Liquid $C_5H_{10}O$ 86.13	Partly soluble Freely soluble	102 MS 99%	1.389–1.395 0.812–0.818	

FL-no	EU register name	Structural formula	FEMA no CoE no CAS no	Phys. form Mol. formula Mol. weight	Solubility[a] Solubility in ethanol[b]	Boiling point, °C[c] Melting point, °C ID test Assay minimum	Refrac. Index[d] Spec. gravity[e]	Specification comments
07.150 2074	Decan-2-one		4271 11055 693-54-9	Liquid $C_{10}H_{20}O$ 156.27	Insoluble Freely soluble	210 MS 98%	1.423–1.429 0.821–0.827	
07.156	2,6-Dimethyloct-6-en-3-one (mixture of *E* and *Z*)		90975-15-8	Liquid $C_{10}H_{18}O$ 154.25	Insoluble Freely soluble	80 (13 hPa) NMR 95%	1.442–1.448 0.823–0.829	Mixture of *E*/*Z* isomers: 50–80% (E) (EFFA, 2017)
07.157	6,10-Dimethylundecan-2-one		11068 1604-34-8	Liquid $C_{13}H_{26}O$ 198.35	Insoluble Freely soluble	121 (16 hPa) MS 95%	1.433–1.439 0.828–0.834	Racemate (EFFA, 2002a)
07.158	Dodecan-2-one		11069 6175-49-1	Liquid $C_{12}H_{24}O$ 184.32	Insoluble Freely soluble	119 (13 hPa) 20 MS 99%	1.431–1.437 0.825–0.835	
07.160	Heptadecan-2-one		11089 2922-51-2	Solid $C_{17}H_{34}O$ 254.46	Insoluble Freely soluble	144 (1 hPa) 48 MS 95%	n.a. n.a.	
07.161	Hex-1-en-3-one		1629-60-3	Liquid $C_6H_{10}O$ 98.14	Practically insoluble or insoluble Freely soluble	128 MS 95%	1.420–1.426 0.849–0.855	
07.162	Hex-5-en-2-one		109-49-9	Liquid $C_6H_{10}O$ 98.14	Slightly soluble Freely soluble	128 MS 95%	1.418–1.424 0.839–0.845	
07.178	3-Methylbutan-2-one		11131 563-80-4	Liquid $C_5H_{10}O$ 86.13	Slightly soluble Freely soluble	94 MS 95%	1.387–1.393 0.801–0.807	
07.181	6-Methylheptan-2-one		11146 928-68-7	Liquid $C_8H_{16}O$ 128.21	Insoluble Freely soluble	167 MS 95%	1.412–1.418 0.813–0.819	
07.182	5-Methylheptan-3-one		541-85-5	Liquid $C_8H_{16}O$ 128.21	Insoluble Freely soluble	158 MS 95%	1.418–1.424 0.816–0.824	Racemate (EFFA, 2002a)

FL-no	EU register name	Structural formula	FEMA no CoE no CAS no	Phys. form Mol. formula Mol. weight	Solubility[a] Solubility in ethanol[b]	Boiling point, °C[c] Melting point, °C ID test Assay minimum	Refrac. Index[d] Spec. gravity[e]	Specification comments
07.185	3-Methylpentan-2-one		11157 565-61-7	Liquid $C_6H_{12}O$ 100.16	Insoluble Freely soluble	117 MS 95%	1.398–1.404 0.810–0.816	Racemate (EFFA, 2002a)
07.189	Nonan-4-one		11161 4485-09-0	Liquid $C_9H_{18}O$ 142.24	Insoluble Freely soluble	188 MS 95%	1.416–1.422 0.821–0.827	
07.198	Pseudo-ionone		4299 11191 141-10-6	Liquid $C_{13}H_{20}O$ 192.30	Insoluble Freely soluble	144 (16 hPa) MS 95%	1.529–1.535 0.894–0.903	Mixture of *E/Z* stereoisomers: > 50% EE (EFFA, 2012)
07.199	Tetradecan-2-one		11192 2345-27-9	Solid $C_{14}H_{28}O$ 212.37	Insoluble Freely soluble	146 (16 hPa) 33 MS 95%	n.a. n.a.	
07.201	Tridec-12-en-2-one		60437-21-0	Liquid $C_{13}H_{24}O$ 196.33	Insoluble Freely soluble	129 (13 hPa) NMR 95%	1.441–1.447 0.815–0.821	
07.204	3,3,6-Trimethylhepta-1,5-dien-4-one		546-49-6	Liquid $C_{10}H_{16}O$ 152.24	Practically insoluble or insoluble Freely soluble	181 MS 95%	1.462–1.468 0.867–0.873	
07.205	6,10,14-Trimethylpentadecan-2-one		11205 502-69-2	Liquid $C_{18}H_{36}O$ 268.48	Insoluble Freely soluble	174 (13 hPa) MS 95%	1.445–1.451 0.834–0.840	Racemate (EFFA, 2002a)
07.210	1-Nonene-3-one		24415-26-7	Liquid $C_9H_{16}O$ 140.22	Insoluble Freely soluble	80 (16 hPa) MS 95%	1.436–1.442 0.826–0.830	
07.236	(*Z*)-5-Octen-2-one		11171 22610-86-2	Liquid $C_8H_{14}O$ 126.20	Practically insoluble or insoluble Freely soluble	115 NMR 95%	1.431–1.437 0.842–0.848	
07.239 1840	[*R*-(*E*)]-5-Isopropyl-8-methylnona-6,8-dien-2-one		4331 2278-53-7	Liquid $C_{13}H_{22}O$ 194.31	Practically insoluble or insoluble Freely soluble	238 MS 95%	1.471–1.477 0.846–0.852	

FL-no	EU register name	Structural formula	FEMA no CoE no CAS no	Phys. form Mol. formula Mol. weight	Solubility[a] Solubility in ethanol[b]	Boiling point, °C[c] Melting point, °C ID test Assay minimum	Refrac. Index[d] Spec. gravity[e]	Specification comments
07.262	9-Decen-2-one		4706 35194-30-0	Liquid $C_{10}H_{18}O$ 154	Slightly soluble Soluble	206.3 IR NMR MS 99%	1.426–1.446 0.834–0.854	
09.304	*sec*-Heptyl isovalerate		10806 238757-71-6	Liquid $C_{12}H_{24}O_2$ 200.32	Insoluble Freely soluble	235 NMR 95%	1.423–1.429 0.867–0.873	Racemate (EFFA, 2002a)
09.323	*sec*-Butyl acetate		10527 105-46-4	Liquid $C_6H_{12}O_2$ 116.16	Slightly soluble Freely soluble	111 MS 95%	1.385–1.391 0.867–0.873	Racemate (EFFA, 2002a)
09.325	*sec*-Butyl butyrate		10528 819-97-6	Liquid $C_8H_{16}O_2$ 144.21	Slightly soluble Freely soluble	152 MS 95%	1.399–1.405 0.858–0.864	Racemate (EFFA, 2002a)
09.328	*sec*-Butyl formate		10532 589-40-2	Liquid $C_5H_{10}O_2$ 102.13	Slightly soluble Freely soluble	94 MS 95%	1.386–1.392 0.877–0.883	Racemate (EFFA, 2002a)
09.332	*sec*-Butyl hexanoate		10533 820-00-8	Liquid $C_{10}H_{20}O_2$ 172.27	Insoluble Freely soluble	82 (21 hPa) NMR 95%	1.408–1.414 0.861–0.867	Racemate (EFFA, 2002a)
09.386	*sec*-Hept-4(*cis*)-enyl acetate		94088-33-2	Liquid $C_9H_{16}O_2$ 156.22	Insoluble Freely soluble	185 MS 95%	1.412–1.418 0.854–0.860	Racemate (EFFA, 2002a)
09.388	*sec*-Heptyl acetate		10802 5921-82-4	Liquid $C_9H_{18}O_2$ 158.24	Insoluble Freely soluble	172 MS 95%	1.406–1.412 0.862–0.868	Racemate (EFFA, 2002a)
09.391	*sec*-Heptyl hexanoate		10805 6624-58-4	Liquid $C_{13}H_{26}O_2$ 214.35	Insoluble Freely soluble	126 (20 hPa) MS 95%	1.421–1.427 0.851–0.857	Racemate (EFFA, 2002a)
09.604	Isopropyl decanoate		10730 2311-59-3	Liquid $C_{13}H_{26}O_2$ 214.35	Insoluble Freely soluble	88 (3 hPa) MS 95%	1.421–1.427 0.851–0.857	
09.605	Isopropyl dodecanoate		10233-13-3	Liquid $C_{15}H_{30}O_2$ 242.40	Insoluble Freely soluble	105 (1 hPa) MS 95%	1.427–1.433 0.851–0.857	

FL-no	EU register name	Structural formula	FEMA no CoE no CAS no	Phys. form Mol. formula Mol. weight	Solubility[a] Solubility in ethanol[b]	Boiling point, °C[c] Melting point, °C ID test Assay minimum	Refrac. Index[d] Spec. gravity[e]	Specification comments
09.606	Isopropyl hexadecanoate		10732 142-91-6	Liquid $C_{19}H_{38}O_2$ 298.51	Insoluble Freely soluble	342 13 MS 95%	1.433–1.439 0.852–0.858	
09.608	Isopropyl octanoate		10731 5458-59-3	Liquid $C_{11}H_{22}O_2$ 186.29	Insoluble Freely soluble	124 (53 hPa) MS 95%	1.414–1.420 0.853–0.859	
09.609	Isopropyl valerate		18362-97-5	Liquid $C_8H_{16}O_2$ 144.21	Insoluble Freely soluble	165 MS 95%	1.398–1.404 0.855–0.861	
09.676	*sec*-Octyl acetate		10799 2051-50-5	Liquid $C_{10}H_{20}O_2$ 172.27	Practically insoluble or insoluble Freely soluble	193 MS 95%	1.409–1.415 0.857–0.863	Racemate (EFFA, 2010)
09.880	(*Z*)-Hept-4-enyl-2 butyrate		94088-12-7	Liquid $C_{11}H_{20}O_2$ 184.28	Practically insoluble or insoluble Freely soluble	224 MS 95%	1.414–1.420 0.852–0.858	Racemate (EFFA, 2010)
09.926 2070	Octan-3-yl formate		4009 84434-65-1	Liquid $C_9H_{18}O_2$ 158.24	Practically insoluble or insoluble Freely soluble	71 (9 hPa) IR NMR MS 98%	1.413–1.417 0.865–0.875	Racemate (EFFA, 2010)

FL-no: FLAVIS number; FEMA: Flavor and Extract Manufacturers Association; CoE: Council of Europe; CAS: Chemical Abstract Service; ID: identity; MS: mass spectrometry; NMR: nuclear magnetic resonance; IR: infrared spectroscopy.
(a): Solubility in water, if not otherwise stated.
(b): Solubility in 95% ethanol, if not otherwise stated.
(c): At 1 atm (1,013.25 hPa), if not otherwise stated.
(d): At 20°C, if not otherwise stated.
(e): At 25°C, if not otherwise stated.

2.1.4. Natural occurrence in food

Forty-five of the candidate substances have been reported to occur naturally. These occurrences include among others: milk and milk products as cheese of various types, beef, chicken, guinea hen, lamb and mutton, fish, oysters, scallops and shrimps, passion fruit, plum, papaya, strawberry, citrus fruits, apples, hop oil, camomile, tomatoes and potatoes, cocoa and tea, maize, nuts and different alcoholic beverages. The highest quantified natural occurrences in foods are presented in Table 2 (full data set are available in Appendix F).

Table 2: Candidate substances reported to occur naturally in food (VCF online 2016)

FL-no	Name	Quantitative data reported
02.077	Pentan-3-ol	Up to 34 mg/kg in tea
02.124	6-Methylhept-5-en-2-ol	Up to 50 mg/kg in citrus fruits
02.145	2,6-Dimethylocta-1,5,7-trien-3-ol	Up to 100 mg/kg in sage
07.084	Pentan-3-one	Up to 14 mg/kg in mushroom
09.323	*sec*-Butyl acetate	Up to 67 mg/kg in vinegar
09.391	*sec*-Heptyl hexanoate	Up to 6,634 mg/kg in passion fruit

For eight candidate substances listed in Table 3 no natural occurrence data have been identified (VCF online, 2016).

Table 3: Candidate substances for which no natural occurrence data in food are available (VCF online, 2016)

FL-no	Name
07.162	Hex-5-en-2-one
07.201	Tridec-12-en-2-one
07.210	1-Nonene-3-one
07.239	[*R*-(*E*)]-5-Isopropyl-8-methylnona-6,8-dien-2-one
07.262	9-Decen-2-one
09.332	*sec*-Butyl hexanoate
09.880	(*Z*)-Hept-4-enyl-2 butyrate
09.926	Octan-3-yl formate

2.2. Intake data

Annual production volumes of the flavouring substances as surveyed by industry can be used to calculate the 'Maximised Survey-derived Daily Intake' (MSDI) by assuming that the production figure only represents 60% of the use in food due to underreporting and that 10% of the total European Union (EU) population are consumers (SCF, 1999).

However, the Panel noted that due to year-to-year variability in production volumes, to uncertainties in the underreporting correction factor and to uncertainties in the percentage of consumers, the reliability of intake estimates on the basis of the MSDI approach is difficult to assess.

The Panel also noted that in contrast to the generally low per capita intake figures estimated on the basis of this MSDI approach, in some cases, the regular consumption of products flavoured at use levels reported by the flavour industry in the submissions would result in much higher intakes. In such cases, the human exposure thresholds below which exposures are not considered to present a safety concern might be exceeded.

Considering that the MSDI model may underestimate the intake of flavouring substances by certain groups of consumers, the Scientific Committee on Food (SCF) recommended also taking into account the results of other intake assessments (SCF, 1999).

One of the alternatives is the 'Theoretical Added Maximum Daily Intake' (TAMDI) approach, which is calculated on the basis of standard portions and upper use levels (SCF, 1995) for flavourable beverages and foods in general, with exceptional levels for particular foods. This method is regarded as a conservative estimate of the actual intake by most consumers because it is based on the assumption that the consumer regularly eats and drinks several food products containing the same flavouring substance at the upper use level.

One option to modify the TAMDI approach is to base the calculation on normal rather than upper use levels of the flavouring substances. This modified approach is less conservative (e.g. it may underestimate the intake of consumers being loyal to products flavoured at the maximum use levels reported). However, it is considered as a suitable tool to screen and prioritise the flavouring substances according to the need for refined intake data (EFSA, 2004).

2.2.1. Estimated daily per capita intake (MSDI approach)

The intake estimation is based on the MSDI approach, which involves the acquisition of data on the amounts used in food as flavourings (SCF, 1999). These data are derived from surveys on annual production volumes in Europe. These surveys were conducted in 1995 by the International Organization of the Flavour Industry (IOFI), in which flavour manufacturers reported the total amount of each flavouring substance incorporated into food sold in the EU during the previous year (IOFI, 1995). The intake approach does not consider the possible natural occurrence in food.

Average per capita intake (MSDI) is estimated on the assumption that the amount added to food is consumed by 10% of the population[4] (Eurostat, 1998). This is derived for candidate substances from estimates of annual volume of production provided by industry and incorporates a correction factor of 0.6 to allow for incomplete reporting (60%) in the industry surveys (SCF, 1999).

In the present (FGE.07Rev5, the total annual volume of production of the 53 candidate substances for use as flavouring substances in Europe has been reported to be approximately 690 kg (EFFA, 2002b,c, 2007c; Flavour industry, 2009) and for 64 of the 67 supporting substances approximately 750,000 kg (isopropyl alcohol accounts for 690,000 kg and acetone for 50,000 kg) (cited by the JECFA (1999a). For three supporting substances, no EU annual volumes of production are available (JECFA, 2003; IOFI, 1995) (Tables 4 and B.3).

Table 4: Tonnage data and MSDI for candidate and supporting substances

	Tonnage (kg/year)		MSDI (μg/capita per day)	
	Class I	**Class II**	**Class I**	**Class II**
FGE.07Rev5	49.7 (28 substances)	639.0 (25 substances)	5.9 (28 substances)	77.7 (25 substances)
FGE.07Rev5supp	742,832 (34 substances)	11,096 (30 substances)	90,441 (34 substances)	1,351 (30 substances)
Total	742,882	11,735	90,450	1,430

MSDI: Maximised Survey-derived Daily Intake.

On the basis of the annual volumes of production reported for the 53 candidate substances, the daily per capita intakes for each of these flavourings have been estimated (Table B.1). Approximately 90% of the total annual volume of production for the candidate substances (EFFA, 2002b, 2007c) is accounted for by one candidate substance, 9-decen-2-one [FL-no: 07.262]. The estimated daily per capita intake of this candidate substance from use as a flavouring substance is 73 μg/capita per day. The daily per capita intakes for each of the remaining substances is less than 2 μg/capita per day (Table B.1).

2.2.2. Intake estimated on the basis of the modified TAMDI (mTAMDI)

The method for calculation of modified Theoretical Added Maximum Daily Intake (mTAMDI) values is based on the approach used by SCF up to 1995 (SCF, 1995). The assumption is that a person may consume a certain amount of flavourable foods and beverages per day.

For the present evaluation of the 53 candidate substances, information on food categories and normal and maximum use levels,[5,6,7] were submitted by the flavour industry (EFFA, 2002a,c, 2007a,b,c;

[4] EU figure 375 million. This figure relates to EU population at the time for which production data are available, and is consistent (comparable) with evaluations conducted prior to the enlargement of the EU. No production data are available for the enlarged EU (Eurostat, 1998).

[5] 'Normal use' is defined as the average of reported usages and'maximum us' is defined as the 95th percentile of reported usages (EFFA, 2002d).

[6] The normal and maximum use levels in different food categories have been extrapolated from figures derived from 12 model flavouring substances (EFFA, 2004).

[7] The use levels from food category 5'Confectionery' have been inserted as default values for food category 14.2'Alcoholic beverages' for substances for which no data have been given for food category 14.2 (EFFA, 2007a).

Flavour Industry, 2006, 2009, EFFA, 2016). The 53 candidate substances are used in flavoured food products divided into the food categories, outlined in Annex III of the Commission Regulation (EC) No 1565/2000[3], as summarised in Table 5. For the present calculation of mTAMDI, the reported normal use levels were used. In the case where different use levels were reported for different food categories, the highest reported normal use level was used.

Table 5: Use in various food categories for 53 candidate substances for which data on use have been provided

Food category	Description	Flavourings used
01.0	Dairy products, excluding products of category 2	All
02.0	Fats and oils, and fat emulsions (type water-in-oil)	All except [FL-no: 07.262]
03.0	Edible ices, including sherbet and sorbet	All
04.1	Processed fruits	All
04.2	Processed vegetables (including mushrooms & fungi, roots & tubers, pulses and legumes), and nuts & seeds	Only [FL-no: 07.262]
05.0	Confectionery	All except [FL-no: 07.205]
06.0	Cereals and cereal products, including flours & starches from roots & tubers, pulses & legumes, excluding bakery	All except [FL-nos: 02.255 & 07.262]
07.0	Bakery wares	All except [FL-no: 07.262]
08.0	Meat and meat products, including poultry and game	All except [FL-nos: 02.255 & 07.262]
09.0	Fish and fish products, including molluscs, crustaceans and echinoderms	All except [FL-nos: 09.608, 02.255 & 07.262]
10.0	Eggs and egg products	None
11.0	Sweeteners, including honey	None
12.0	Salts, spices, soups, sauces, salads, protein products etc.	All except [FL-nos: 07.156, 02.255 & 07.262]
13.0	Foodstuffs intended for particular nutritional uses	All
14.1	Non-alcoholic ('soft') beverages, excl. dairy products	All
14.2	Alcoholic beverages, incl. alcohol-free and low-alcoholic counterparts	All except [FL-no: 07.205]
15.0	Ready-to-eat savouries	All except [FL-nos: 02.255, 07.157, 09.609 & 07.262]
16.0	Composite foods (e.g. casseroles, meat pies, mincemeat) – foods that could not be placed in categories 1–15	All

According to the flavour industry, the normal use levels for the 53 candidate substances are in the range of 1–30 mg/kg food, and the maximum use levels are in the range of 5–150 mg/kg (EFFA, 2002a,b,c,d, 2007a,b,c, 2016; Flavour Industry, 2006, 2009).

The mTAMDI values for the 28 candidate substances from structural class I (see Section 2.4) range from 1,600 to 3,900 μg/person per day. For the 25 candidate substance from structural class II, the mTAMDI range from 1,500 to 6,600 μg/person per day.

For detailed information on use levels and intake estimations based on the mTAMDI approach, see Appendix C.

2.3. Absorption, distribution, metabolism and excretion

In general, aliphatic secondary alcohols and ketones are expected to be rapidly absorbed in the gastrointestinal tract. The candidate aliphatic esters are expected to be hydrolysed enzymatically to their component secondary alcohols and carboxylic acids. The carboxylic acids are completely oxidised in the fatty acid pathway and the tricarboxylic acid pathway (see Appendix D).

Secondary alcohols may undergo oxidation to the corresponding ketone; however, in the *in vivo* situation, the alcohol is removed from the equilibrium by conjugation to glucuronic acid, which represents the major pathway of metabolism for secondary alcohols. The glucuronides of the candidate secondary alcohols are expected to be eliminated via the urine (Felsted and Bachur, 1980; Kasper and Henton, 1980; JECFA, 1999a).

In general, the major metabolic pathway for aliphatic ketones is reduction of the ketone to the corresponding secondary alcohol and subsequent excretion as glucuronic acid conjugate (Felsted and Bachur, 1980; JECFA, 1999a).

Short-chain ketones ($C < 5$) that contain a carbonyl function at the C2 position may undergo oxidation to yield an alpha-keto carboxylic acid, which through decarboxylation will be oxidised to carbon dioxide and a simple aliphatic carboxylic acid that will enter the fatty acid pathway and citric acid cycle (Dietz et al., 1981). Ketones may also be metabolised by omega- or omega-1-oxidation yielding a hydroxy-ketone that may be further reduced to a diol and excreted in the urine as glucuronic acid conjugate. Longer chain aliphatic ketones ($C \geq 5$) are primarily metabolised via reduction, but omega- and omega-1-oxidation are competing pathways at high concentrations (Dietz et al., 1981; Topping et al., 1994).

Omega-1-oxidation of certain aliphatic ketones may yield gamma-diketones, which may give rise to neuropathy of giant-axonal type. The metabolic pathway includes oxidation of the omega-1-carbon, first to a hydroxy-ketone and then to a diketone. The gamma-spacing of the carbonyl functions has been shown to be a prerequisite for neurotoxic effects, thus, only ketones with this structural feature may yield the neurotoxic metabolites. Neurotoxic effects are, however, only observed at relatively high dosages (Topping et al., 1994). One of the candidate substances, 5-methylheptan-3-one [FL-no: 07.182], may potentially be oxidised to a gamma-diketone.

Twelve of the candidate substances, but-3-en-2-ol, 2,6-dimethylocta-1,5,7-triene-3-ol, non-1-en-3-ol, octa-1,5-dien-3-ol, undeca-1,5-dien-3-ol, hex-1-en-3-one, hex-5-en-2-one, tridec-12-en-2-one, 3,3,6-trimethylhepta-1,5-dien-4-one, 1-nonene-3-one, ([*R*-(*E*)]-5-isopropyl-8-methylnona-6,8-dien-2-one and 9-decen-2-one [FL-nos: 02.131, 02.145, 02.187, 02.194, 02.211, 07.161, 07.162, 07.201, 07.204, 07.210, 07.239 and 07.262] have terminal double bonds. These double bonds may be oxidised to the corresponding epoxides. Epoxides are highly reactive molecules, due to the large strain associated with this three-membered ring structure, and they react easily with nucleophilic sites of cellular macromolecules. However, epoxides will be conjugated with glutathione by glutathione *S*-transferases or hydrolysed to diols by epoxide hydrolases. These two reactions can be considered to be detoxications (Sanchez and Kauffman, 2010). 1-Alkenes are metabolised by P450 through both double bond oxidation to the corresponding epoxide and allylic oxidation (Chiappe et al., 1998). The rates of the two reactions measured with different P450 isoforms indicate that epoxide formation is generally favoured (Chiappe et al., 1998).

Based on the low levels of intake of alkenones and alkenols characterised by a carbonyl or an alcohol group in addition to the terminal double bond, it is expected that the detoxication reactions of the formed epoxides (conjugation with glutathione or epoxide hydrolase mediated hydrolysis) would not be saturated and would outweigh the rate of epoxide formation. The presence of the terminal double bond is therefore not considered of concern under the intended conditions of use.

In addition to reduction and oxidation pathways, low molecular weight alcohols and ketones may be excreted unchanged in expired air (Brown et al., 1987).

Concluding remarks on metabolism

Fifty-two of the candidate substances, seven saturated aliphatic acyclic secondary alcohols, seven unsaturated aliphatic secondary alcohols, 12 saturated aliphatic ketones, 10 unsaturated aliphatic ketones and 16 esters of aliphatic acyclic secondary alcohols and linear and branched-chain aliphatic carboxylic acids, may be expected to be metabolised to innocuous substances at the estimated levels of intake, based on the MSDI approach, as flavouring substances.

One candidate substance, 5-methylheptan-3-one [FL-no: 07.182], may be oxidised to a potentially neurotoxic gamma-diketone. Therefore, this substance will be evaluated via the B-side of the Procedure (see section 2.4).

More detailed information on the metabolism of candidate substances is given in Appendix D.

2.4. Application of the procedure for the safety evaluation of flavouring substances

The application of the Procedure is based on intakes estimated on the basis of the MSDI approach. Where the mTAMDI approach indicates that the intake of a flavouring substance might exceed its corresponding threshold of concern, a formal safety assessment is not carried out using the Procedure. In these cases, the Panel requires more precise data on use and use levels. For comparison of the intake estimations based on the MSDI approach and the mTAMDI approach, see Section 2.5.

For the safety evaluation of the 53 candidate substances the Procedure as outlined in Appendix A was applied, based on the MSDI approach. The stepwise evaluations of the substances are summarised in Table B.1.

Step 1

Twenty-eight of the candidate substances [FL-nos: 02.077, 02.124, 02.142, 02.148, 02.177, 02.182, 02.183, 02.190, 02.255**,** 07.084, 07.178, 07.239, 09.304, 09.323, 09.325, 09.328, 09.332, 09.386, 09.388, 09.391, 09.604, 09.605, 09.606, 09.608, 09.609, 09.676, 09.880 and 09.926] are classified in structural class I, according to the decision tree approach presented by Cramer et al. (Cramer et al., 1978). The remaining 25 candidate substances [FL-nos: 02.131, 02.145, 02.187, 02.194, 02.211, 07.072, 07.150, 07.156, 07.157, 07.158, 07.160, 07.161, 07.162, 07.181, 07.182, 07.185, 07.189, 07.198, 07.199, 07.201, 07.204, 07.205, 07.210, 07.236 and 07.262], which are unsaturated aliphatic secondary alcohols or acyclic aliphatic saturated or unsaturated ketones, are in structural class II.

Step 2

Fifty-two candidate substances were considered to be metabolised to innocuous products and would not be expected to saturate available detoxification pathways at estimated levels of intake, based on the MSDI approach, from use as flavouring substances. Therefore, these 52 substances proceed via the A-side of the Procedure scheme (Appendix A). One candidate substance, 5-methylheptan-3-one [FL-no: 07.182], cannot be predicted to be metabolised to innocuous products and therefore, proceeds to step B3.

Step A3

The 28 candidate substances assigned to structural class I, have estimated European daily per capita intakes ranging from 0.0012 to 1.3 μg (Table 6). These intakes are below the threshold of concern of 1,800 μg/person per day for structural class I. The 24 unsaturated aliphatic secondary alcohols and ketones, which have been assigned to structural class II, have estimated European daily per capita intakes ranging from 0.0012 to 73 μg (Table 6). These intakes are below the threshold of concern of 540 μg/person per day for structural class II. Based on results of the safety evaluation sequence, the 52 candidate substances proceeding via the A-side of the Procedure do not pose a safety concern when used as flavouring substances at the estimated levels of intake, based on the MSDI approach.

Step B3

The estimated per capita intake of 5-methylheptan-3-one [FL-no: 07.182] of 0.32 μg/capita per day does not exceed the threshold of concern for structural class II of 540 μg/person per day. Accordingly, the candidate substance proceeds to step B4 of the Procedure.

Step B4

On the basis of a study on the neurotoxic effects of orally administered 5-methylheptan-3-one [FL-no: 07.182] to male rats, a no observed adverse effect level (NOAEL) of 82 mg/kg body weight (bw) per day was established (IBM Corp., 1989). This NOAEL provides a margin of safety of 1.5×10^7 based on the estimated intake of the candidate substance of 0.32 μg/capita per day. Based on results of the safety evaluation sequence, this candidate substance does not pose a safety concern when used as flavouring substance at the estimated level of intake, based on the MSDI approach.

2.5. Comparison of the intake estimations based on the MSDI and the mTAMDI approach

The estimated intakes for the 28 candidate substances in structural class I based on the mTAMDI approach range from 1,600 to 3,900 μg/person per day. For three [FL-nos: 07.084, 07.178 and 07.239] of these 28 substances, the mTAMDI is below the threshold of concern of 1,800 μg/person per day.

The estimated intake for the 21 candidate substances assigned to structural class II based on the mTAMDI range from 1,500 to 6,600 μg/person per day, which are all above the threshold of concern for structural class II substances of 540 μg/person per day.

Therefore, for 50 candidate substances, further information is required. This would include more reliable intake data and then, if required, additional toxicological data.

For comparison of the MSDI and mTAMDI values, see Table 6.

Table 6: Estimated intakes based on the MSDI approach and the mTAMDI approach

FL-no	EU register name	MSDI (µg/capita per day)	mTAMDI (µg/person per day)	Structural class	TTC (µg/person per day)
02.077	Pentan-3-ol	0.19	3,900	Class I	1,800
02.124	6-Methylhept-5-en-2-ol	0.0061	3,900	Class I	1,800
02.142	3,3-Dimethylbutan-2-ol	0.24	3,900	Class I	1,800
02.148	Dodecan-2-ol	0.35	3,900	Class I	1,800
02.177	2-Methylhexan-3-ol	0.12	3,900	Class I	1,800
02.182	3-Methylpentan-2-ol	0.12	3,900	Class I	1,800
02.183	4-Methylpentan-2-ol	0.0012	3,900	Class I	1,800
02.190	Nonan-3-ol	0.011	3,900	Class I	1,800
02.255	(*Z*)-4-Hepten-2-ol	0.03	2,500	Class I	1,800
07.084	Pentan-3-one	0.24	1,600	Class I	1,800
07.178	3-Methylbutan-2-one	0.073	1,600	Class I	1,800
07.239	[*R*-(*E*)]-5-Isopropyl-8-methylnona-6,8-dien-2-one	0.24	1,600	Class I	1,800
09.304	*sec*-Heptyl isovalerate	0.0012	3,900	Class I	1,800
09.323	*sec*-Butyl acetate	0.0012	3,900	Class I	1,800
09.325	*sec*-Butyl butyrate	1.3	3,900	Class I	1,800
09.328	*sec*-Butyl formate	0.12	3,900	Class I	1,800
09.332	*sec*-Butyl hexanoate	0.024	3,900	Class I	1,800
09.386	*sec*-Hept-4(*cis*)-enyl acetate	0.024	3,900	Class I	1,800
09.388	*sec*-Heptyl acetate	0.12	3,900	Class I	1,800
09.391	*sec*-Heptyl hexanoate	0.12	3,900	Class I	1,800
09.604	Isopropyl decanoate	0.12	3,900	Class I	1,800
09.605	Isopropyl dodecanoate	0.12	3,900	Class I	1,800
09.606	Isopropyl hexadecanoate	0.012	3,900	Class I	1,800
09.608	Isopropyl octanoate	1.3	3,900	Class I	1,800
09.609	Isopropyl valerate	0.012	3,500	Class I	1,800
09.676	*sec*-Octyl acetate	0.011	3,900	Class I	1,800
09.880	(*Z*)-Hept-4-enyl-2 butyrate	0.79	3,900	Class I	1,800
09.926	Octan-3-yl formate	0.24	3,900	Class I	1,800
02.131	But-3-en-2-ol	0.0012	3,900	Class II	540
02.145	2,6-Dimethylocta-1,5,7-trien-3-ol	0.0085	3,900	Class II	540
02.187	Non-1-en-3-ol	0.58	3,900	Class II	540
02.194	Octa-1,5-dien-3-ol	0.061	3,900	Class II	540
02.211	Undeca-1,5-dien-3-ol	0.061	3,900	Class II	540
07.072	6-Methylheptan-3-one	0.19	1,600	Class II	540
07.150	Decan-2-one	0.52	1,600	Class II	540
07.156	2,6-Dimethyloct-6-en-3-one (mixture of *E* and *Z*)	0.0012	1,600	Class II	540
07.157	6,10-Dimethylundecan-2-one	0.085	1,500	Class II	540
07.158	Dodecan-2-one	0.73	1,600	Class II	540
07.160	Heptadecan-2-one	0.12	1,600	Class II	540
07.161	Hex-1-en-3-one	0.012	1,600	Class II	540
07.162	Hex-5-en-2-one	0.049	1,600	Class II	540
07.181	6-Methylheptan-2-one	0.0012	1,600	Class II	540
07.182	5-Methylheptan-3-one	0.32	1,600	Class II	540
07.185	3-Methylpentan-2-one	1.2	1,600	Class II	540

FL-no	EU register name	MSDI (μg/capita per day)	mTAMDI (μg/person per day)	Structural class	TTC (μg/person per day)
07.189	Nonan-4-one	0.52	1,600	Class II	540
07.198	Pseudo-ionone	0.12	1,600	Class II	540
07.199	Tetradecan-2-one	0.073	1,600	Class II	540
07.201	Tridec-12-en-2-one	0.024	1,600	Class II	540
07.204	3,3,6-Trimethylhepta-1,5-dien-4-one	0.012	1,600	Class II	540
07.210	1-Nonene-3-one	0.0012	1,600	Class II	540
07.205	6,10,14-Trimethylpentadecan-2-one	0.0073	1,500	Class II	540
07.236	(*Z*)-5-Octen-2-one	0.0097	1,600	Class II	540
07.262	9-Decen-2-one	73	6,600	Class II	540

MSDI: Maximised Survey-derived Daily Intake; mTAMDI: modified Theoretical Added Maximum Daily Intake; TTC: toxicological threshold of concern.

2.6. Considerations of combined intakes from use as flavouring substances

Because of structural similarities of candidate and supporting substances, it can be anticipated that many of the flavourings are metabolised through the same metabolic pathways and that the metabolites may affect the same target organs. Furthermore, in case of combined exposure to structurally related flavourings, the pathways could be overloaded. Therefore, combined intake should be considered. As flavourings not included in this FGE may also be metabolised through the same pathways, the combined intake estimates presented here are only preliminary. Currently, the combined intake estimates are only based on MSDI exposure estimates, although it is recognised that this may lead to underestimation of exposure. After completion of all FGEs, this issue should be readdressed. The total estimated combined daily per capita intake of structurally related flavourings is estimated by summing the MSDI for individual substances.

On the basis of the reported annual production volumes in Europe (EFFA, 2002b, c, 2007c; Flavour Industry, 2009), the total estimated daily per capita intake as flavourings of the 28 candidate flavouring substances assigned to structural class I is 6 μg, which does not exceed the threshold of concern for a substance belonging to structural class I of 1,800 μg/person per day. For the combined intake of the 25 candidate flavouring substances assigned to structural class II is 78 μg, which does not exceed the threshold of concern for a substance belonging to structural class II of 540 μg/person per day.

The 53 candidate substances are structurally related to 67 supporting substances evaluated by the JEFCA at its 51st, 59th and 69th meetings in the groups 'Saturated aliphatic acyclic secondary alcohols, ketones, and related saturated and unsaturated esters' (JECFA, 2000a, 2002a, 2003, 2009b). The total combined intake of candidate and supporting substances of structural class I and II would be 90,450 μg/capita per day and 1,430 μg/capita per day, respectively. Both intakes exceed the threshold of their structural class of 1,800 and 540 μg/person per day. However, the major contribution (> 99%) was provided by two supporting substances, namely acetone [FL-no: 07.050] (6,100 μg/capita per day) and isopropanol [FL-no: 02.079] (84,000 μg/capita per day). These are present in the body as endogenous compounds, which are easily eliminated, either by excretion into the urine and exhaled air or after enzymatic metabolism (Morgott, 1993). Therefore, they would not be expected to give rise to perturbations outside the physiological range (JECFA, 1999a). Excluding the two major contributors, the estimated total combined intake (in Europe) for the candidate (Table 6) and supporting substances (Table B.3) belonging to structural class I would be 350 μg/capita per day, which does not exceed the threshold of concern for the corresponding structural class (1,800 μg person per day); the estimated total combined intake (in Europe) for the candidate (Table 6) and supporting substances (Table B.3) belonging to structural class II would be 1,430 μg/capita per day, which is 2.6 fold higher than the threshold of concern for the corresponding structural class (540 μg/person per day). Five of the supporting substances from structural class II, oct-1-en-3-ol, heptan-2-one, undecan-2-one, nonan-2-one and tridecan-2-one [FL-nos: 02.023, 07.002, 07.016, 07.020 and 07.103], contribute with 1,050 μg/capita per day to the combined MSDI of 1,430 μg/capita per day (Table B.3). A 90-day study for

nonan-2-one [FL-no: 07.020] (O'Donoghue and Krasavage, 1980) provides a NOAEL of 2,000 mg/kg bw per day. Based on this NOAEL, a margin of safety of 11.5×10^4 can be derived for the combined intake of [FL-nos: 02.023, 07.002, 07.016, 07.020 and 07.103]. For the remaining substances from structural class II, the estimated combined intake of 380 μg/capita per day is below the threshold of structural class II of 540 μg/capita per day.

If the candidate substance 5-methylheptan-3-one [FL-no: 07.182] and the two supporting substances heptan-3-ol [FL-no: 02.044] and 3-heptanone [FL-no: 07.003], which can all be metabolised to neurotoxic gamma-diketones, were consumed concomitantly on a daily basis, the estimated combined intake (in Europe) would be 3.7 μg/capita per day, corresponding to 0.06 μg/kg bw per day. This value does not exceed the threshold of concern for the corresponding structural class II (540 μg/person per day) and is also much lower than the NOAEL for 5-methylheptan-3-one [FL-no: 07.182] of 82 mg/kg bw per day for neurotoxicity in the rat. Therefore, it can be concluded that there is no safety concern for human health for the combined exposure to these three neurotoxic substances at the estimated level of intake as flavourings.

2.7. Genotoxicity

In vitro

In vitro genotoxicity data have been reported for nine candidate substances. Negative results were obtained in bacterial systems (+/− metabolic activation) with six candidate substances, one saturated aliphatic acyclic secondary alcohol [FL-no: 02.183], two saturated ketones [FL-nos: 07.181 and 07.205], two unsaturated ketones [FL-nos: 07.198 and 07.262] and the ester isopropyl hexadecanoate [FL-no: 09.606]. Negative results were also obtained for the candidate substances pseudo-ionone [FL-no: 07.198], pentan-3-ol [FL-no: 02.077] and methyl-3-butan-2-one [FL-no: 07.178], the two-first mentioned being tested for chromosomal aberrations in mammalian cells and the latter for induction of aneuploidy in yeast cells, respectively.

Induction of aneuploidy in yeast cells has been demonstrated for pentan-3-one [FL-no: 07.084]. The effect, measured only at high concentrations, approaching cytotoxic levels, can be considered to be a threshold effect, not mediated by direct interaction with DNA. In addition, induction of aneuploidy described in the paper is strongly potentiated by ice treatments included in the experimental protocol, consistently with tubulin dissociation at low temperature *in vitro*; in the absence of this passage the effect is very weak. Therefore, the effect could be considered as an effect occurring only under unrealistic experimental conditions and the extrapolation of this result to the *in vivo* situation in humans is questionable. Furthermore, it is well recognised that the relevance of fungal systems is limited when induction of aneuploidy in mammalian systems has to be evaluated.

Pseudo-ionone [FL-no: 07.198] was considered with respect to genotoxicity in FGE.206 (EFSA CEF Panel, 2011) where the Panel concluded that the data available ruled out the concern for genotoxicity. Pseudo-ionone was tested in *Salmonella* Typhimurium strains TA98, TA100, TA1535, TA1537 and TA102 in the presence or absence of S9 and it is concluded that under the test conditions applied pseudo-ionone is not mutagenic in bacteria. Pseudo-ionone was also evaluated in an *in vitro* micronucleus assay in human peripheral blood lymphocytes for its ability to induce chromosomal damage or aneuploidy in the presence and absence of rat S9 fraction as an *in vitro* metabolising system. Under the conditions of this study, pseudo-ionone was not clastogenic and/or aneugenic in cultured human lymphocytes. As pseudo-ionone [FL-no: 07.198] is a representative with respect to genotoxicity evaluation for [FL-nos: 02.145, 02.194, 02.211 and 07.204] in FGE.206 the safety concern for genotoxicity can also be ruled out for these four substances and they can be evaluated using the Procedure in the present FGE.

In vitro genotoxicity data are also available for 10 supporting substances.

No evidence of mutagenicity obtained with either bacterial or mammalian cells systems was reported for one saturated aliphatic acyclic secondary alcohol [FL-no: 02.079], five saturated [FL-nos: 07.002, 07.050, 07.017, 07.053 and 07.122], two unsaturated [FL-nos: 07.015 and 07.099] aliphatic acyclic ketones and two esters of an aliphatic acyclic secondary alcohol with linear aliphatic carboxylic acids [FL-nos: 09.003 and 09.105]. 4-Methyl-2-pentanone [FL-no: 07.017] gave negative results also when tested for chromosomal aberration activity.

Besides the negative results in *in vitro* bacterial point mutation tests, acetone [FL-no: 07.050] showed no evidence of increased sister chromatid exchanges in several cytogenetic assays on different mammalian cells, as well as no induction of chromosomal aberrations in Chinese hamster ovary cells up to very high concentrations. Only one test on hamster lung fibroblasts (conducted at an unspecified

acetone concentration) and an aneuploidy induction test on *Saccharomyces cerevisiae* (about 7% acetone) gave positive results. However, these two studies were considered not relevant on the basis of their poor quality and taking into account all the other negative genotoxicity results obtained with acetone, including results *in vivo* (see below).

6-Methylhepta-3,5-dien-2-one [FL-no: 07.099] was considered with respect to genotoxicity in FGE.206 (EFSA CEF Panel, 2011) where the Panel concluded that the data available ruled out the concern for genotoxicity. 6-Methylhepta-3,5-dien-2-one was tested in *S.* Typhimurium strains TA98, TA100, TA1535, TA1537 and TA102 in the presence or absence of S9 and it was concluded that under the test conditions applied 6-methylhepta-3,5-dien-2-one is not mutagenic in bacteria. 6-Methylhepta-3,5-dien-2-one was also evaluated in an *in vitro* micronucleus assay in human peripheral blood lymphocytes for its ability to induce chromosomal damage or aneuploidy in the presence and absence of rat S9 fraction as an *in vitro* metabolising system. Under the conditions of this study, 6-methylhepta-3,5-dien-2-one was not clastogenic and/or aneugenic in cultured human lymphocytes. As 6-methylhepta-3,5-dien-2-one [FL-no: 07.099] is a representative with respect to genotoxicity evaluation for [FL-nos: 02.145, 02.194, 02.211 and 07.204] in FGE.206, the safety concern for genotoxicity can also be ruled out for these four substances and they can be evaluated using the Procedure in the present FGE.

In vivo

In vivo data are available for four supporting substances: one saturated aliphatic secondary alcohol [FL-no: 02.079] and three saturated aliphatic ketones [FL-nos: 07.017, 07.050 and 07.053], which exhibited no genotoxic potential in the micronucleus cytogenetic assay at doses approaching the LD_{20} and the LD_{50} of the tested substances.

Candidate substances with alpha-beta-unsaturated carbonyl structural alert

Oct-1-en-3-one [FL-no: 07.081] and pent-1-en-3-one [FL-no: 07.102] were evaluated with respect to genotoxicity in FGE.205 (EFSA CEF Panel, 2012a) and FGE.205Rev1 (EFSA CEF Panel, 2016). Due to positive effects in the bacterial mutagenicity assays of the two representative substances pent-1-en-3-one [FL-no: 07.102] and oct-1-en-3-one [FL-no: 07.081], an *in vivo* Comet assay on the first site of contact (e.g. the stomach or duodenum) and on the liver was requested on the most potent substance, pent-1-en-3-one (EFSA CEF Panel, 2012a). In response to the data request in FGE.205, the industry submitted *in vivo* data on both pent-1-en-3-one and oct-1-en-3-one. Pent-1-en-3-one [FL-no: 07.102] was tested for its potential to induce micronuclei in the polychromatic erythrocytes of the bone marrow of treated rats and to induce DNA damage in the liver and duodenum of the same animals (Keig-Shevlin, 2015b,c). Oct-1-en-3-one [FL-no: 07.081] was tested in a Comet assay for its potential to induce DNA damage in the liver of rats (Keig-Shevlin, 2015a). Furthermore, to investigate the mechanism of action of the mutagenic activity observed in the bacterial reverse mutation tests of previous studies, a new Ames test with oct-1-en-3-one was performed with strain TA100 (Bowen, 2013). Pent-1-en-3-one [FL-no: 07.102] tested *in vivo* in a combined micronucleus and comet assay did not show genotoxic effects in either the liver or duodenum of treated rats. The negative results of the bone marrow micronucleus assay are considered inconclusive because there is no evidence of bone marrow exposure to the tested substance. However, as results of the *in vitro* micronucleus assay were negative, no additional *in vivo* follow-up studies on clastogenicity and aneugenicity were needed. The bacterial mutation assay provided for oct-1-en-3-one [FL-no: 07.081] confirms the weak mutagenic effect in bacteria shown in previous studies, but does not clarify the mechanism of action. The liver comet assay is considered of limited validity due to low values of mean tail intensity and tail moment. However, based on the data available on the most potent of the two representative substances for the other substances of FGE.205, pent-1-en-3-one [FL-no: 07.102], the Panel concluded that there is no concern for genotoxicity. As pent-1-en-3-one [FL-no: 07.102] is a representative with respect to genotoxicity evaluation for [FL-nos: 02.131, 02.187, 07.161 and 07.210] in FGE.205, the safety concern for genotoxicity can also be ruled out for these four substances and they will be evaluated using the Procedure in the present FGE.

Overall conclusion on genotoxicity

From the available *in vitro* and *in vivo* tests on candidate and supporting substances and on the basis of the results for substances evaluated in FGE.205, FGE.206 and FGE.205rev1 (EFSA CEF Panel, 2011, 2012a, 2016), no concern is raised with respect to genotoxicity. Consequently the candidate substances can be evaluated using the Procedure.

The genotoxicity data are summarised in Appendix E, Tables E.4–E.7.

2.8. Toxicity

2.8.1. Acute toxicity

Data are available for 12 candidate substances under consideration and for 23 supporting substances. Most of the candidate and supporting substances have rat and/or mouse oral LD50 values exceeding 2,000 mg/kg bw indicating that their oral acute toxicity is low.

The acute toxicity data are summarised in Appendix E, Table E.1.

2.8.2. Short-term and subchronic toxicity

Data on oral subchronic toxicity are available for three candidate substances, pentan-3-one [FL-no: 07.084], 5-methylheptan-3-one [FL-no: 07.182] and 9-decen-2-one [FL-no: 07.262] with identification of a NOAEL. Data on subacute and subchronic oral toxicity are also available for ten supporting substances, one saturated aliphatic secondary alcohol [FL-no: 02.079], seven saturated [FL-nos: 07.002, 07.003, 07.017, 07.020, 07.050, 07.058, 07.122] and two unsaturated [FL-nos: 07.100 and 07.114] aliphatic ketones evaluated by JEFCA (JECFA, 1999a, 2003).

During the application of the Procedure (Appendix A), the following study on 5-methylheptan-3-one [FL-no: 07.182], which possesses structural alerts for neurotoxicity, has been used to calculate the NOAEL: 5-Methylheptan-3-one [FL-no: 07.182] (purity 98.9%) dissolved in distilled water was administered by gavage to groups of five adult male Sprague–Dawley rats at dose levels 0, 82, 410 and 820 mg/kg bw per day, five days/week for 13 weeks. In the high-dose group, clinical signs, including depression of activity, gait disturbances, reductions in food consumption and body weight gain were observed; moreover, results of the Functional Observational Battery (FOB) indicated peripheral neuropathy. Similar clinical signs and functional deficits were observed less frequently and with reduced severity in the mid-dose group. No functional deficits were observed in the low-dose group animals. Microscopic histopathological examinations of the sciatic and tibial nerves from high-dose animals revealed lesions typical of the 'giant' axonal neuropathy produced by gamma-diketones. Changes observed in the mid-dose group animals reflected the occurrence of reparative processes in the nerves. Nerves from the low-dose group animals did not show any evidence of pathology attributable to treatment. Based on behavioural effects and microscopic changes occurring at 410 and 820 mg/kg bw per day, the NOAEL for 5- methylheptan-3-one induced neurotoxicity was 82 mg/kg bw per day (IBM Corp., 1989).

The short-term and subchronic toxicity data are summarised in Appendix E, Table E.2.

2.8.3. Reproductive and developmental toxicity

Data on reproductive toxicity are available for pentan-3-one [FL-no: 07.084] and data on developmental toxicity are available for pseudo-ionone [FL-no: 07.198]. For one supporting substance, isopropyl alcohol [FL-no: 02.079], data are available on both developmental and reproductive toxicity. With a NOAEL of 50 mg/kg bw per day for intraperitoneal administration in mice for [FL-no: 07.084] and of 960 mg/kg bw per day for oral administration of [FL-no: 07.198], it was concluded that the developmental/reproductive toxicity was low after oral exposure.

The developmental/reproductive toxicity data are summarised in Appendix E, Table E.3.

2.8.4. Other studies

Pseudo-ionone [FL-no: 07.198] has been subjected to investigations concerning its potential as a dermal sensitiser as follows:

a) A guinea pig study (Csato and Chubb, 1996) performed as a GLP OECD 406 maximisation test. There were some problems with reading the result after challenge because of intense red-brown skin staining. Therefore a rechallenge was performed 7 days later, when skin staining was much reduced and did not prevent assessment of the skin reaction. Test agent concentrations were 3.125% and 1.563% in water, scoring was performed after 24 and 48 h. None of the animals in the control (n = 10) or test (n = 20) groups showed a reaction. Based on this guinea pig maximisation test performed under GLP conditions according to OECD guidelines, pseudo-ionone is not a dermal sensitiser. However, the problems with skin staining and delayed challenge possibly may bring in some uncertainty (contribution toward false negative results).

b) Four maximisation test series with pseudo-ionone were carried out on a total of 108 human volunteers by Kligman (1976) [unpublished] and Epstein (1978) [unpublished]. Test concentration was 8% in petrolatum. The outcome was '2/25 (Kligman, 1976), 4/25 (Epstein, 1978), 2/25 (Kligman, 1976), and 1/33 (Epstein, 1978) sensitisation reactions', as reported by Ford et al. (1988). Thus, there were altogether nine positive out of 108 subjects (8.3%). No further details are given by Ford and the original reports never were published. The fact that pseudo-ionone is an irritant still may bring in some uncertainty (contribution towards false positive results).

Based on the human studies, there is evidence that pseudo-ionone may be a weak dermal sensitiser. In accordance with this and as based on the report by Ford et al. (Ford et al., 1988), both the International Fragrance Association (IFRA, 2002) and subsequently the European Union Scientific Committee on Cosmetic Products and Non-Food Products (SCCNFP, 2000) recommended a ban on the use of pseudo-ionone as a fragrance ingredient but tolerated it as an impurity at $\leq$ 2% in various ionones.

Considering that allergic contact sensitisation in the mouth to components in ingested food is extremely rare (EFSA CEF Panel, 2012b), that worsening of skin manifestations of contact dermatitis after ingestion of foods with relatively high levels of the allergen appears to be an uncommon occurrence, and that contact allergic manifestations in the gut although claimed in rare cases have not been well described, it is unlikely that pseudo-ionone used as a flavouring substance will cause allergic reactions.

3. Conclusions

Following a request from the European Commission, the EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF) was asked to deliver a scientific opinion 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 requested to evaluate 53 flavouring substances allocated to the FGE.07Rev5, using the Procedure as referred to in the Commission Regulation (EC) No 1565/2000[3]. These flavouring substances are listed in the Union List, which was adopted by Commission Regulation (EU) No 872/2012[2] and its consecutive amendments.

The present Revision of FGE.07, FGE.07Rev5, includes the assessment of four additional candidate substances [FL-nos: 02.131, 02.187, 07.161 and 07.210]. These substances possess an α,β-unsaturated structure, which is considered a structural alert for genotoxicity. They have been evaluated by EFSA in FGE.205Rev1, and the genotoxicity concern could be ruled out. The Panel concluded that the substances with [FL-nos: 02.131, 02.187, 07.161 and 07.210] can be evaluated through the Procedure.

The 53 candidate substances are saturated and unsaturated aliphatic secondary alcohols, ketones and esters of secondary alcohols and saturated linear or branched-chain saturated carboxylic acids from chemical group 5.

Twenty-seven candidate substances possess one chiral centre [FL-nos: 02.124, 02.131, 02.142, 02.145, 02.148, 02.177, 02.183, 02.187, 02.190, 02.194, 02.211, 02.255, 07.157, 07.182, 07.185, 07.239, 09.304, 09.323, 09.325, 09.328, 09.332, 09.386, 09.388, 09.391, 09.676, 09.880 and 09.926], and two of the candidate substances possess two chiral centres [FL-nos: 02.182 and 07.205].

Due to the presence and the position of double bonds, 10 candidate substances can exist as geometrical isomers [FL-nos: 02.145, 02.194, 02.211, 02.255, 07.156, 07.198, 07.236, 07.239, 09.386, and 09.880].

Twenty-eight candidate substances belong to structural class I, and 25 candidate substances belong to structural class II.

Forty-five of the flavouring substances in the present group of 53 flavouring substances have been reported to occur naturally in a wide range of food items.

According to the default MSDI approach, 53 candidate substances have European daily per capita intakes ranging from 0.0012 to 73 μg, which are below the threshold of concern for structural class I and class II substances (1,800 and 540 μg/person per day, respectively). On the basis of the reported annual production in Europe (MSDI approach), the combined intakes of the 28 of the candidate substances belonging to structural class I and of the 25 candidate substances belonging to structural class II would result in total intakes of 6 and 78 μg/capita per day, respectively.

These values are lower than the thresholds of concern for structural class I or class II substances. The total combined estimated levels of intake of the candidate and supporting substances is approximately 350 μg/capita per day (without acetone and isopropanol) for structural class I substances and 1,430 μg/capita per day for structural class II substances. This latter value does exceed the threshold of concern for the structural class. For the structural class II substances, ca 70%

of the combined exposure estimate is represented by five supporting substances [FL-nos: 02.023, 07.002, 07.016, 07.020 and 07.103]. For [FL no: 07.020], a NOAEL of 2,000 mg/kg bw per day has been reported, which provides an adequate margin of safety of 11.5×10^4. For the remaining 20 structural class II substances, the combined exposure estimate (380 μg/capita per day) remains below the TTC for this structural class.

From the available *in vitro* and *in vivo* tests on candidate and supporting substances, no concern is raised with respect to genotoxicity. Fifty-two candidate substances would be expected to be metabolised to innocuous substances at the estimated levels of intake as flavouring substances. One candidate substance, 5-methylheptan-3-one [FL-no: 07.182], may be oxidised to a potential neurotoxic gamma-diketone. However, this metabolic path does not pose a safety concern at the estimated level of intake as a flavouring substance. Indeed, for this substance, a NOAEL for neurotoxicity of 82 mg/kg bw per day was established in a subchronic study on adult male rats dosed with 0, 82, 410 and 820 mg/kg bw per day for 13 weeks. This NOAEL provides a margin of safety of 1.5×10^7 based on the estimated intake (MSDI) of the candidate substance of 0.32 μg/capita per day.

Otherwise, it was noted that where toxicity data were available on single flavouring substances they were consistent with the conclusions in the present FGE using the Procedure.

It is considered that on the basis of the default MSDI approach none of the 53 candidate substances would give rise to safety concerns at the estimated levels of intake arising from their use as flavouring substances.

In order to determine whether the conclusion for the 53 candidate substances evaluated through the Procedure can be applied to the materials of commerce, it is necessary to consider the available specifications. Adequate specifications including purity and identity for the materials of commerce have been provided for all the candidate substances.

The Panel concluded that all 53 flavouring substances [FL-nos: 02.077, 02.124, 02.131, 02.142, 02.145, 02.148, 02.177, 02.182, 02.183, 02.187, 02.190, 02.194, 02.211, 02.255, 07.072, 07.084, 07.150, 07.156, 07.157, 07.158, 07.160, 07.161, 07.162, 07.178, 07.181, 07.182, 07.185, 07.189, 07.198, 07.199, 07.201, 07.204, 07.205, 07.210, 07.236, 07.239, 07.262, 09.304, 09.323, 09.325, 09.328, 09.332, 09.386, 09.388, 09.391, 09.604, 09.605, 09.606, 09.608, 09.609, 09.676, 09.880 and 09.926] evaluated in this FGE would not be expected to present a safety concern at their estimated levels of intake based on the MSDI approach.

When the estimated intakes were based on the mTAMDI, they ranged from 1,600 to 3,900 μg/person per day for the 28 candidate substances from structural class I. The intakes were all above the threshold of concern for structural class I of 1,800 μg/person per day, except for three flavouring substances [FL-nos: 07.084, 07.178 and 07.239]. These three substances have mTAMDI intake estimates below the threshold of concern for the structural class, and are also expected to be metabolised to innocuous products. The estimated intakes of the 25 candidate substances assigned to structural class II, based on the mTAMDI, range from 1,500 to 6,600 μg/person per day, which are all above the threshold of concern for structural class II of 540 μg/person per day.

In conclusion, for all candidate substances except [FL-nos: 07.084, 07.178 and 07.239] further information is required. This would include more reliable intake data and then, if required, additional toxicological data.

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Abbreviations

AUC	area under curve
BW	body weight
CAS	Chemical Abstract Service
CEF	EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
CoE	Council of Europe
EFFA	European Flavour and Fragrance Association
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)
FOB	Functional Observational Battery
HGPRT	hypoxanthine-guanine phosphoribosyltransferase
ID	identity
IFRA	International Fragrance Association
IOFI	International Organization of the Flavour Industry
IP	intraperitoneal
IR	infrared spectroscopy
JECFA	The Joint FAO/WHO Expert Committee on Food Additives
LD_{50}	lethal dose, 50%; Median lethal dose
MS	mass spectrometry
MSDI	Maximised Survey-derived Daily Intake
mTAMDI	Modified Theoretical Added Maximum Daily Intake
NAD	nicotinamide adenine dinucleotide
NADH	nicotinamide adenine dinucleotide – reduced form
NADP	nicotinamide adenine dinucleotide phosphate
NADPH	nicotinamide adenine dinucleotide phosphate – reduced form
NOAEL	no observed adverse effect level
NMR	nuclear magnetic resonance
NTP	National Toxicology Program
SCCNFP	European Union Scientific Committee on Cosmetic Products and Non-Food Products
SCE	sister chromatid exchange
SCF	Scientific Committee on Food
TAMDI	Theoretical Added Maximum Daily Intake
TTC	toxicological threshold of concern
UGAC	average urinary output of glucuronide
VCF	Volatile Compounds in Food
WHO	World Health Organisation

Appendix A – Procedure for the safety evaluation

The approach for a safety evaluation of chemically defined flavouring substances as referred to in Commission Regulation (EC) No 1565/2000[3], named the 'Procedure', is shown in schematic form in Figure A.1. The Procedure is based on the Opinion of the Scientific Committee on Food expressed on 2 December 1999 (SCF, 1999), which is derived from the evaluation Procedure developed by the Joint FAO/WHO Expert Committee on Food Additives at its 44th, 46th and 49th meetings (JECFA, 1995, 1996, 1997, 1999b).

The Procedure is a stepwise approach that integrates information on intake from current uses, structure–activity relationships, metabolism and, when needed, toxicity. One of the key elements in the Procedure is the subdivision of flavourings into three structural classes (I, II and III) for which thresholds of concern (human exposure thresholds) have been specified. Exposures below these thresholds are not considered to present a safety concern.

Class I contains flavourings that have simple chemical structures and efficient modes of metabolism, which would suggest a low order of oral toxicity. Class II contains flavourings that have structural features that are less innocuous, but are not suggestive of toxicity. Class III comprises flavourings that have structural features that permit no strong initial presumption of safety, or may even suggest significant toxicity (Cramer et al., 1978). The thresholds of concern for these structural classes of 1,800, 540 or 90 μg/person per day, respectively, are derived from a large database containing data on subchronic and chronic animal studies (JECFA, 1996).

In Step 1 of the Procedure, the flavourings are assigned to one of the structural classes. The further steps address the following questions:

- Can the flavourings be predicted to be metabolised to innocuous products[8] (Step 2)?
- Do their exposures exceed the threshold of concern for the structural class (Steps A3 and B3)?
- Are the flavourings or their metabolites endogenous[9] (Step A4)?
- Does a NOAEL exist on the flavourings or on structurally related substances (Steps A5 and B4)?

In addition to the data provided for the flavouring substances to be evaluated (candidate substances), toxicological background information available for compounds structurally related to the candidate substances is considered (supporting substances), in order to assure that these data are consistent with the results obtained after application of the Procedure.

The Procedure is not to be applied to flavourings with existing unresolved problems of toxicity. Therefore, the right is reserved to use alternative approaches if data on specific flavourings warranted such actions.

[8] 'Innocuous metabolic products': Products that are known or readily predicted to be harmless to humans at the estimated intakes of the flavouring agent (JECFA, 1997).

[9] 'Endogenous substances': Intermediary metabolites normally present in human tissues and fluids, whether free or conjugated; hormones and other substances with biochemical or physiological regulatory functions are not included (JECFA, 1997).

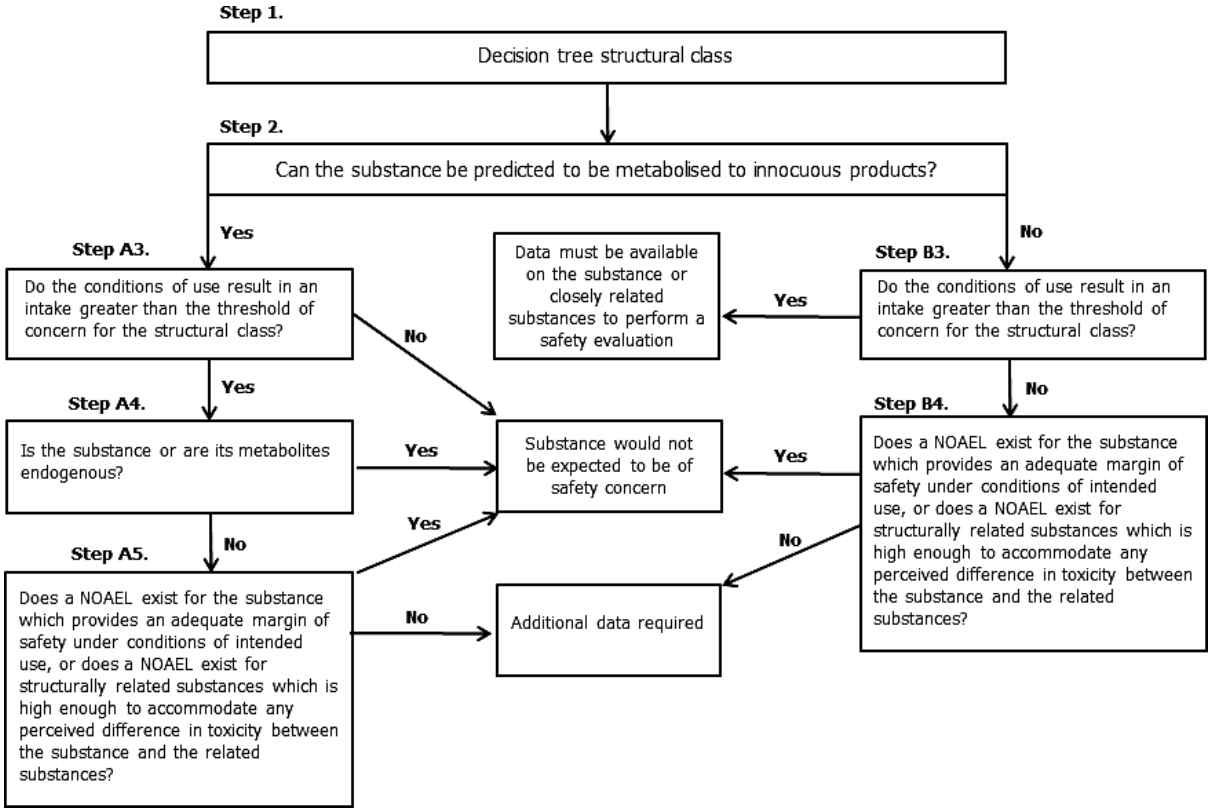
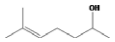
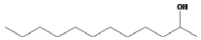
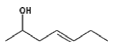
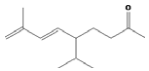
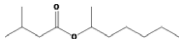
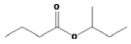
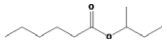
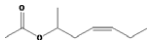
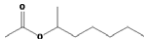
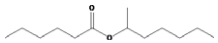
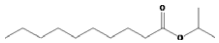


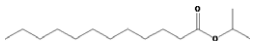
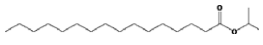
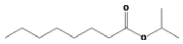
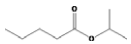
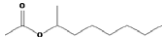
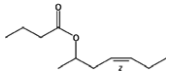
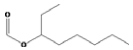
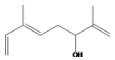
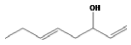
Figure A.1: Procedure for safety evaluation of chemically defined flavouring substances

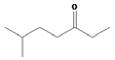
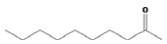
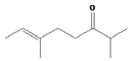
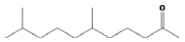
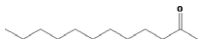
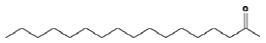
Appendix B – Summary of safety evaluations

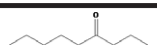
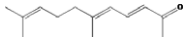
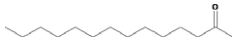
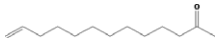
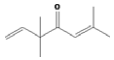
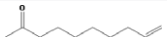
Table B.1: Summary of safety evaluation applying the Procedure (based on intakes calculated by the MSDI approach)

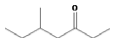
FL-no	EU register name	Structural formula	MSDI[(a)] (μg/capita per day)	Class[(b)] Evaluation procedure path[(c)]	Outcome on the named compound[(d),(e)]	Outcome on the material of commerce[(f),(g),(h)]	Evaluation remarks
02.077	Pentan-3-ol		0.19	Class I A3: intake below threshold	d	f	
02.124	6-Methylhept-5-en-2-ol		0.0061	Class I A3: intake below threshold	d	f	
02.142	3,3-Dimethylbutan-2-ol		0.24	Class I A3: intake below threshold	d	f	
02.148	Dodecan-2-ol		0.35	Class I A3: Intake below threshold	d	f	
02.177	2-Methylhexan-3-ol		0.12	Class I A3: Intake below threshold	d	f	
02.182	3-Methylpentan-2-ol		0.12	Class I A3: Intake below threshold	d	f	
02.183	4-Methylpentan-2-ol		0.0012	Class I A3: Intake below threshold	d	f	
02.190	Nonan-3-ol		0.011	Class I A3: Intake below threshold	d	f	
02.255	(*Z*)-4-Hepten-2-ol		0.03	Class I A3: Intake below threshold	d	f	
07.084	Pentan-3-one		0.24	Class I A3: Intake below threshold	d	f	

FL-no	EU register name	Structural formula	MSDI[a] (μg/capita per day)	Class[b] Evaluation procedure path[c]	Outcome on the named compound[d],[e]	Outcome on the material of commerce[f],[g],[h]	Evaluation remarks
07.178	3-Methylbutan-2-one		0.073	Class I A3: Intake below threshold	d	f	
07.239	[*R*-(*E*)]-5-Isopropyl-8-methylnona-6,8-dien-2-one		0.24	Class I A3: Intake below threshold	d	f	
09.304	*sec*-Heptyl isovalerate		0.0012	Class I A3: Intake below threshold	d	f	
09.323	*sec*-Butyl acetate		0.0012	Class I A3: Intake below threshold	d	f	
09.325	*sec*-Butyl butyrate		1.3	Class I A3: Intake below threshold	d	f	
09.328	*sec*-Butyl formate		0.12	Class I A3: Intake below threshold	d	f	
09.332	*sec*-Butyl hexanoate		0.024	Class I A3: Intake below threshold	d	f	
09.386	*sec*-Hept-4(*cis*)-enyl acetate		0.024	Class I A3: Intake below threshold	d	f	
09.388	*sec*-Heptyl acetate		0.12	Class I A3: Intake below threshold	d	f	
09.391	*sec*-Heptyl hexanoate		0.12	Class I A3: Intake below threshold	d	f	
09.604	Isopropyl decanoate		0.12	Class I A3: Intake below threshold	d	f	

FL-no	EU register name	Structural formula	MSDI[a] (μg/capita per day)	Class[b] Evaluation procedure path[c]	Outcome on the named compound[d],[e]	Outcome on the material of commerce[f],[g],[h]	Evaluation remarks
09.605	Isopropyl dodecanoate		0.12	Class I A3: Intake below threshold	d	f	
09.606	Isopropyl hexadecanoate		0.012	Class I A3: Intake below threshold	d	f	
09.608	Isopropyl octanoate		1.3	Class I A3: Intake below threshold	d	f	
09.609	Isopropyl valerate		0.012	Class I A3: Intake below threshold	d	f	
09.676	*sec*-Octyl acetate		0.011	Class I A3: Intake below threshold	d	f	
09.880	(*Z*)-Hept-4-enyl-2		0.79	Class I A3: Intake below threshold	d	f	
09.926	Octan-3-yl formate		0.24	Class I A3: Intake below threshold	d	f	
02.131	But-3-en-2-ol		0.0012	Class II A3: Intake below threshold	d	f	
02.145	2,6-Dimethylocta-1,5, 7-trien-3-ol		0.0085	Class II A3: Intake below threshold	d	f	
02.187	Non-1-en-3-ol		0.58	Class II A3: Intake below threshold	d	f	
02.194	Octa-1,5-dien-3-ol		0.061	Class II A3: Intake below threshold	d	f	

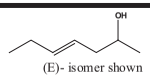
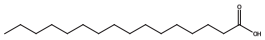
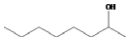
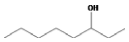
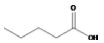
FL-no	EU register name	Structural formula	MSDI[a] (μg/capita per day)	Class[b] Evaluation procedure path[c]	Outcome on the named compound[d],[e]	Outcome on the material of commerce[f],[g],[h]	Evaluation remarks
02.211	Undeca-1,5-dien-3-ol		0.061	Class II A3: Intake below threshold	d	f	
07.072	6-Methylheptan-3-one		0.19	Class II A3: Intake below threshold	d	f	
07.150	Decan-2-one		0.52	Class II A3: Intake below threshold	d	f	
07.156	2,6-Dimethyloct-6-en-3-one (mixture of *E* and *Z*)		0.0012	Class II A3: Intake below threshold	d	f	
07.157	6,10-Dimethylundecan-2-one		0.085	Class II A3: Intake below threshold	d	f	
07.158	Dodecan-2-one		0.73	Class II A3: Intake below threshold	d	f	
07.160	Heptadecan-2-one		0.12	Class II A3: Intake below threshold	d	f	
07.161	Hex-1-en-3-one		0.012	Class II A3: Intake below threshold	d	f	
07.162	Hex-5-en-2-one		0.049	Class II A3: Intake below threshold	d	f	
07.181	6-Methylheptan-2-one		0.0012	Class II A3: Intake below threshold	d	f	
07.185	3-Methylpentan-2-one		1.2	Class II A3: Intake below threshold	d	f	

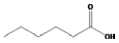
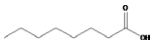
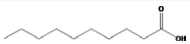
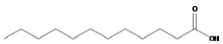
FL-no	EU register name	Structural formula	MSDI[a] (μg/capita per day)	Class[b] Evaluation procedure path[c]	Outcome on the named compound[d],[e]	Outcome on the material of commerce[f],[g],[h]	Evaluation remarks
07.189	Nonan-4-one		0.52	Class II A3: Intake below threshold	d	f	
07.198	Pseudo-ionone		0.12	Class II A3: Intake below threshold	d	f	
07.199	Tetradecan-2-one		0.073	Class II A3: Intake below threshold	d	f	
07.201	Tridec-12-en-2-one		0.024	Class II A3: Intake below threshold	d	f	
07.204	3,3,6-Trimethylhepta-1,5-dien-4-one		0.012	Class II A3: Intake below threshold	d	f	
07.205	6,10,14-Trimethylpentadecan-2-one		0.0073	Class II A3: Intake below threshold	d	f	
07.210	1-Nonene-3-one		0.0012	Class II A3: Intake below threshold	d	f	
07.236	(*Z*)-5-Octen-2-one		0.0097	Class II A3: Intake below threshold	d	f	
07.262	9-Decen-2-one		73	Class II A3: Intake below threshold	d	f	

FL-no	EU register name	Structural formula	MSDI[(a)] (μg/capita per day)	Class[(b)] Evaluation procedure path[(c)]	Outcome on the named compound[(d),(e)]	Outcome on the material of commerce[(f),(g),(h)]	Evaluation remarks
07.182	5-Methylheptan-3-one		0.32	Class II B3: Intake below threshold B4: Adequate NOAEL exists	d	f	NOAEL for neurotoxicity: 82 mg/kg bw per day

MSDI: Maximised Survey-derived Daily Intake; NOAEL: no observed adverse effect level; bw: body weight.
(a): EU MSDI: Amount added to food as flavour in (kg/year) × 10E9/(0.1 × population in Europe (= 375 × 10E6) × 0.6 × 365) = μg/capita per day.
(b): Thresholds of concern: Class I = 1,800 μg/person per day, Class II = 540 μg/person per day, Class III = 90 μg/person per day.
(c): Procedure path A substances can be predicted to be metabolised to innocuous products. Procedure path B substances cannot.
(d): No safety concern based on intake calculated by the MSDI approach of the named compound.
(e): Data must be available on the substance or closely related substances to perform a safety evaluation.
(f): No safety concern at the estimated level of intake of the material of commerce meeting the specification requirement (based on intake calculated by the MSDI approach).
(g): Tentatively regarded as presenting no safety concern (based on intake calculated by the MSDI approach) pending further information on the purity of the material of commerce and/or information on stereoisomerism.
(h): No conclusion can be drawn due to lack of information on the purity of the material of commerce.

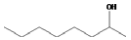
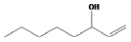
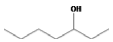
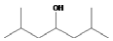
Table B.2: Evaluation status of hydrolysis products of candidate esters

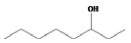
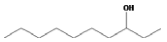
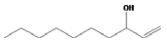
FL-no	EU register name JECFA no	Structural formula	SCF status[a] JECFA status[b] CoE status[c] EFSA status	Structural class[d] Procedure path (JECFA)[e]	Comments
	4-Hepten-2-ol	 (E)- isomer shown	Not evaluated as flavouring substance		Not in EU-Register
	Hexadecanoic acid		Not evaluated as flavouring substance		Not in EU-Register
02.022	Octan-2-ol 289		Category 1 (SCF, 1995) No safety concern (JECFA, 2000a) Category B (CoE, 1992)	Class I A3: Intake below threshold	
02.045	Heptan-2-ol 284		Category 1 (SCF, 1995) No safety concern (JECFA, 2000a) Category B (CoE, 1992)	Class I A3: Intake below threshold	
02.079	Isopropanol 277		Category 1 (SCF, 1995) No safety concern (JECFA, 2000a)	Class I A3: Intake above threshold, A4: Endogenous	
02.098	Octan-3-ol 291		Category 2 (SCF, 1995) No safety concern (JECFA, 2000a)	Class I A3: Intake below threshold	
02.121	Butan-2-ol		Category 1 (SCF, 1995)	Class I No evaluation	
08.001	Formic acid 79		Category 1 (SCF, 1995) No safety concern (JECFA, 1999b) Deleted (CoE, 1992)	Class I A3: Intake below threshold	
08.002	Acetic acid 81		Category 1 (SCF, 1995) No safety concern (JECFA, 1999b) Category A (CoE, 1992)	Class I A3: Intake above threshold, A4: Endogenous	
08.005	Butyric acid 87		Category 1 (SCF, 1995) No safety concern (JECFA, 1999b) Category A (CoE, 1992)	Class I A3: Intake above threshold, A4: Endogenous	
08.007	Valeric acid 90		Category 1 (SCF, 1995) No safety concern (JECFA, 1999b) Category A (CoE, 1992)	Class I A3: Intake below threshold	

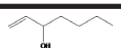
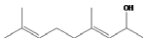
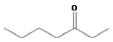
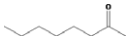
FL-no	EU register name JECFA no	Structural formula	SCF status[(a)] JECFA status[(b)] CoE status[(c)] EFSA status	Structural class[(d)] Procedure path (JECFA)[(e)]	Comments
08.008	3-Methylbutyric acid 259		Category 1 (SCF, 1995) No safety concern (JECFA, 1999b) Category A (CoE, 1992)	Class I A3: Intake below threshold	
08.009	Hexanoic acid 93		Category 1 (SCF, 1995) No safety concern (JECFA, 1999b) Category A (CoE, 1992)	Class I A3: Intake above threshold, A4: Endogenous	
08.010	Octanoic acid 99		Category 1 (SCF, 1995) No safety concern (JECFA, 1999b) Category A (CoE, 1992)	Class I A3: Intake above threshold, A4: Endogenous	
08.011	Decanoic acid 105		Category 1 (SCF, 1995) No safety concern (JECFA, 1999b) Category A (CoE, 1992)	Class I A3: Intake below threshold	
08.012	Dodecanoic acid 111		Category 1 (SCF, 1995) No safety concern (JECFA, 1999b) Category A (CoE, 1992)	Class I A3: Intake below threshold	

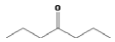
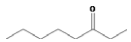
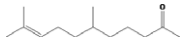
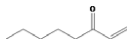
SCF: Scientific Committee on Food; JECFA: The Joint FAO/WHO Expert Committee on Food Additives; CoE: Council of Europe.
(a): Category 1: Considered safe in use, Category 2: Temporarily considered safe in use, Category 3: Insufficient data to provide assurance of safety in use, Category 4: Not acceptable due to evidence of toxicity.
(b): No safety concern at estimated levels of intake.
(c): Category A: Flavouring substance, which may be used in foodstuffs, Category B: Flavouring substance which can be used provisionally in foodstuffs.
(d): Threshold of concern: Class I = 1,800 μg/person per day, Class II = 540 μg/person per day, Class III = 90 μg/person per day.
(e): Procedure path A substances can be predicted to be metabolised to innocuous products. Procedure path B substances cannot.

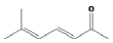
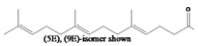
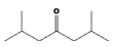
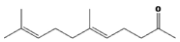
Table B.3: Supporting substances summary

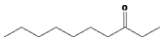
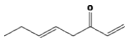
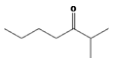
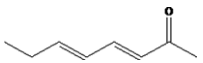
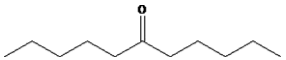
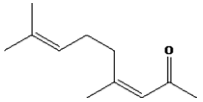
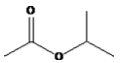
FL-no	EU register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU)[a] (μg/capita per day)	SCF status[b] JECFA status[c] CoE status[d]	Comments
02.022	Octan-2-ol		2801 71 123-96-6	289 JECFA specification (JECFA, 1998)	11	Category 1 (SCF, 1995) No safety concern (JECFA, 2000a) Category B (CoE, 1992)	JECFA evaluated 2-octanol (CASrn as in Register). (*R*)- or (*S*)-enantiomer not specified by CASrn in Register
02.023	Oct-1-en-3-ol		2805 72 3391-86-4	1152 JECFA specification (JECFA, 2002b)	240	No safety concern (JECFA, 2002a) Category A (CoE, 1992)	
02.044	Heptan-3-ol		3547 544 589-82-2	286 JECFA specification (JECFA, 1998)	0.12	Category 2 (SCF, 1995) No safety concern (JECFA, 2000a) Category B (CoE, 1992)	JECFA evaluated 3-heptanol (CASrn as in Register). (*R*)- or (*S*)-enantiomer not specified by CASrn in Register
02.045	Heptan-2-ol		3288 554 543-49-7	284 JECFA specification (JECFA, 1998)	6.8	Category 1 (SCF, 1995) No safety concern (JECFA, 2000a) Category B (CoE, 1992)	JECFA evaluated 2-heptanol (CASrn as in Register). (*R*)- or (*S*)-enantiomer not specified by CASrn in Register
02.079	Isopropanol		2929 67-63-0	277 JECFA specification (JECFA, 1998)	84,000	Category 1 (SCF, 1995) No safety concern (JECFA, 2000a)	
02.081	2,6-Dimethylheptan-4-ol		3140 11719 108-82-7	303 JECFA specification (JECFA, 1998)	ND	Category 2 (SCF, 1995) No safety concern (JECFA, 2000a)	
02.086	Undecan-2-ol		3246 11826 1653-30-1	297 JECFA specification (JECFA, 1998)	0.24	Category 1 (SCF, 1995) No safety concern (JECFA, 2000a)	JECFA evaluated 2-undecanol (CASrn as in Register). (*R*)- or (*S*)-enantiomer not specified by CASrn in Register
02.087	Nonan-2-ol		3315 11803 628-99-9	293 JECFA specification (JECFA, 1998)	0.61	Category 1 (SCF, 1995) No safety concern (JECFA, 2000a)	JECFA evaluated 2-nonanol (CASrn as in Register). (*R*)- or (*S*)-enantiomer not specified by CASrn in Register

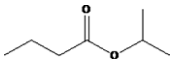
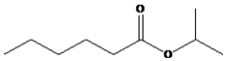
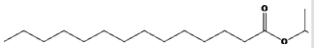
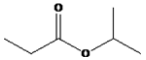
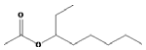
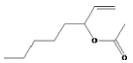
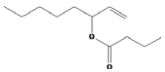
FL-no	EU register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU)[a] (μg/capita per day)	SCF status[b] JECFA status[c] CoE status[d]	Comments
02.088	Pentan-2-ol		3316 11696 6032-29-7	280 JECFA specification (JECFA, 1998)	5.4	Category 1 (SCF, 1995) No safety concern (JECFA, 2000a)	JECFA evaluated 2-pentanol (CASrn as in Register). (*R*)- or (*S*)-enantiomer not specified by CASrn in Register
02.089	Hexan-3-ol		3351 11775 623-37-0	282 JECFA specification (JECFA, 1998)	11	Category 2 (SCF, 1995) No safety concern (JECFA, 2000a)	JECFA evaluated 3-hexanol (CASrn as in Register). (*R*)- or (*S*)-enantiomer not specified by CASrn in Register
02.098	Octan-3-ol		3581 11715 589-98-0	291 JECFA specification (JECFA, 1998)	4.7	Category 2 (SCF, 1995) No safety concern (JECFA, 2000a)	JECFA evaluated 3-octanol (CASrn as in Register). (*R*)- or (*S*)-enantiomer not specified by CASrn in Register
02.099	Pent-1-en-3-ol		3584 11717 616-25-1	1150 JECFA specification (JECFA, 2002b)	2.1	No safety concern (JECFA, 2002a)	
02.103	Decan-3-ol		3605 10194 1565-81-7	295 JECFA specification (JECFA, 1998)	ND	Category 2 (SCF, 1995) No safety concern (JECFA, 2000a)	JECFA evaluated 3-decanol (CASrn as in Register). (*R*)- or (*S*)-enantiomer not specified by CASrn in Register
02.104	Hex-1-en-3-ol		3608 10220 4798-44-1	1151 JECFA specification (JECFA, 2002b)	0.55	No safety concern (JECFA, 2002a)	
02.111	3-Methylbutan-2-ol		3703 598-75-4	300 JECFA specification (JECFA, 2000b)	0.49	Category 2 (SCF, 1995) No safety concern (JECFA, 2000a)	JECFA evaluated 3-methyl-2-butanol (CASrn as in Register). (*R*)- or (*S*)-enantiomer not specified by CASrn in Register
02.136	Dec-1-en-3-ol		3824 51100-54-0	1153 JECFA specification (JECFA, 2002b)	ND	No safety concern (JECFA, 2002a)	

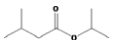
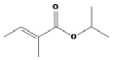
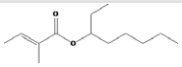
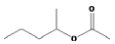
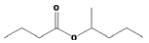
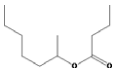
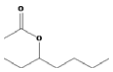
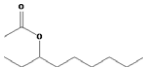
FL-no	EU register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU)[a] (μg/capita per day)	SCF status[b] JECFA status[c] CoE status[d]	Comments
02.155	1-Hepten-3-ol		4129 10218 4938-52-7	1842	0.13	No safety concern (JECFA, 2009b)	
02.252	4,8-Dimethyl-3,7-nonadien-2-ol		4102 67845-50-5	1841 JECFA specification (JECFA, 2009a).	3	No safety concern (JECFA, 2009b)	
07.002	Heptan-2-one		2544 136 110-43-0	283 JECFA specification (JECFA, 1998)	96	Category 1 (SCF, 1995) No safety concern (JECFA, 2000a) Category A (CoE, 1992)	
07.003	Heptan-3-one		2545 137 106-35-4	285 JECFA specification (JECFA, 1998)	3.3	Category 2 (SCF, 1995) No safety concern (JECFA, 2000a) Category B (CoE, 1992)	
07.015	6-Methylhept-5-en-2-one		2707 149 110-93-0	1120 JECFA specification (JECFA, 2002b).	100	No safety concern (JECFA, 2002a)Category B (CoE, 1992)	
07.016	Undecan-2-one		3093 150 112-12-9	296 JECFA specification (JECFA, 1998)	330	Category 1 (SCF, 1995) No safety concern (JECFA, 2000a) Category A (CoE, 1992)	
07.017	4-Methylpentan-2-one		2731 151 108-10-1	301 JECFA specification (JECFA, 1998)	6.1	No safety concern (JECFA, 2000a) Category B (CoE, 1992)	
07.019	Octan-2-one		2802 153 111-13-7	288 JECFA specification (JECFA, 1998)	93	Category 1 (SCF, 1995) No safety concern (JECFA, 2000a) Category A (CoE, 1992)	
07.020	Nonan-2-one		2785 154 821-55-6	292 JECFA specification (JECFA, 1998)	320	Category 1 (SCF, 1995) No safety concern (JECFA, 2000a) Category A (CoE, 1992)	

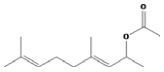
FL-no	EU register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU)[a] (μg/capita per day)	SCF status[b] JECFA status[c] CoE status[d]	Comments
07.050	Acetone		3326 737 67-64-1	139 JECFA specification (JECFA, 1998)	6,100	Category 1 (SCF, 1995) No safety concern (JECFA, 2000a) Category A (CoE, 1992)	
07.053	Butan-2-one		2170 753 78-93-3	278 JECFA specification (JECFA, 1998)	96	Category 1 (SCF, 1995) No safety concern (JECFA, 2000a) Category A (CoE, 1992)	
07.054	Pentan-2-one		2842 754 107-87-9	279 JECFA specification (JECFA, 1998)	120	Category 1 (SCF, 1995) No safety concern (JECFA, 2000a) Category A (CoE, 1992)	
07.058	Heptan-4-one		2546 2034 123-19-3	287 JECFA specification (JECFA, 1998)	1.9	Category 2 (SCF, 1995) No safety concern (JECFA, 2000a) Category B (CoE, 1992)	
07.062	Octan-3-one		2803 2042 106-68-3	290 JECFA specification (JECFA, 1998)	2.8	Category 2 (SCF, 1995) No safety concern (JECFA, 2000a) Category B (CoE, 1992)	
07.069	Tetrahydro-pseudo-ionone		3059 2053 4433-36-7	1121 JECFA specification (JECFA, 2002b)	0.012	No safety concern (JECFA, 2002a) Category B (CoE, 1992)	JECFA evaluated 3,4,5,6-tetrahydropseudoionone (CASrn as in Register). CASrn refers to the racemate
07.081	Oct-1-en-3-one		3515 2312 4312-99-6	1148 JECFA specification (JECFA, 2002b)	1.2	No safety concern (JECFA, 2002a) Category B (CoE, 1992)	
07.096	Hexan-3-one		3290 11097 589-38-8	281 JECFA specification (JECFA, 1998)	0.37	Category 2 (SCF, 1995) No safety concern (JECFA, 2000a)	

FL-no	EU register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU)[a] (μg/capita per day)	SCF status[b] JECFA status[c] CoE status[d]	Comments
07.099	6-Methylhepta-3,5-dien-2-one		3363 11143 1604-28-0	1134 JECFA specification (JECFA, 2002b)	13	No safety concern (JECFA, 2002a)	
07.100	5-Methylhex-5-en-2-one		3365 11150 3240-09-3	1119 JECFA specification (JECFA, 2002b).	0.24	No safety concern (JECFA, 2002a)	
07.102	Pent-1-en-3-one		3382 11179 1629-58-9	1147 JECFA specification (JECFA, 2002b)	0.29	No safety concern (JECFA, 2002a)	
07.103	Tridecan-2-one		3388 11194 593-08-8	298 JECFA specification (JECFA, 2000b)	62	Category 1 (SCF, 1995) No safety concern (JECFA, 2000a)	
07.113	Nonan-3-one		3440 11160 925-78-0	294 JECFA specification (JECFA, 1998)	0.12	Category 2 (SCF, 1995) No safety concern (JECFA, 2000a)	
07.114	6,10,14-Trimethylpentadeca-5,9,13-trien-2-one		3442 11206 762-29-8	1123 JECFA specification (JECFA, 2002b).	0.085	No safety concern (JECFA, 2002a)	JECFA evaluated 2,6,10-trimethyl-2,6,10-pentadecatrien-14-one (CASrn as in Register). (*R*)- or (*S*)-enantiomer not specified by CASrn in Register
07.122	2,6-Dimethylheptan-4-one		3537 11914 108-83-8	302 JECFA specification (JECFA, 1998)	0.18	No safety concern (JECFA, 2000a)	
07.123	Geranylacetone		3542 11088 3796-70-1	1122 JECFA specification (JECFA, 2002b).	41	No safety concern (JECFA, 2002a)	JECFA evaluated 6,10-dimethyl-5,9-undecadien-2-one (CASrn as in Register). (*R*)- or (*S*)-enantiomer not specified by CASrn in Register

FL-no	EU register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU)[a] (μg/capita per day)	SCF status[b] JECFA status[c] CoE status[d]	Comments
07.137	Pentadecan-2-one		3724 11808 2345-28-0	299 JECFA specification (JECFA, 2000b)	18	Category 1 (SCF, 1995) No safety concern (JECFA, 2000a)	
07.151	Decan-3-one		3966 11056 928-80-3	1118 JECFA specification (JECFA, 2002b).	3	No safety concern (JECFA, 2002a)	
07.190	Octa-1,5-dien-3-one		4405 65213-86-7	1848 JECFA specification (JECFA, 2009a).	0.061	No safety concern (JECFA, 2009b)	
07.240	2-Methylheptan-3-one		4000 13019-20-0	1156 JECFA specification (JECFA, 2002b).	3	No safety concern (JECFA, 2002a)	
07.247	(*E*,*E*)-3,5-Octadien-2-one		4008 30086-02-3	1139 JECFA specification (JECFA, 2002b).	3	No safety concern (JECFA, 2002a)	JECFA evaluated (*E*,*E*)-3,5-Octadien-2-one (CASrn as in Register). CASrn in Register to be verified
07.249	Undecan-6-one		4022 927-49-1	1155 JECFA specification (JECFA, 2002b).	3	No safety concern (JECFA, 2002a)	
07.256	(*E*) & (*Z*)-4,8-Dimethyl-3,7-nonadiene-2- one		3969 817-88-9	1137 JECFA specification (JECFA, 2002b).	6.1	No safety concern (JECFA, 2002a)	
09.003	Isopropyl acetate		2926 193 108-21-4	305 JECFA specification (JECFA, 1998)	35	No safety concern (JECFA, 2000a) Category A (CoE, 1992)	No ADI allocated (JECFA, 1980)

FL-no	EU register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU)[a] (μg/capita per day)	SCF status[b] JECFA status[c] CoE status[d]	Comments
09.041	Isopropyl butyrate		2935 267 638-11-9	307 JECFA specification (JECFA, 1998)	6	No safety concern (JECFA, 2000a) Category A (CoE, 1992)	
09.062	Isopropyl hexanoate		2950 312 2311-46-8	308 JECFA specification (JECFA, 2001)	3.2	No safety concern (JECFA, 2000a) Category A (CoE, 1992)	
09.105	Isopropyl tetradecanoate		3556 386 110-27-0	311 JECFA specification (JECFA, 2000b)	19	No safety concern (JECFA, 2000a) Category B (CoE, 1992)	
09.123	Isopropyl propionate		2959 404 637-78-5	306 JECFA specification (JECFA, 2001)	0.012	No safety concern (JECFA, 2000a)Category A (CoE, 1992)	
09.165	Isopropyl formate		2944 503 625-55-8	304 JECFA specification (JECFA, 2001)	0.45	No safety concern (JECFA, 2000a) Category A (CoE, 1992)	
09.254	3-Octyl acetate		3583 2347 4864-61-3	313 JECFA specification (JECFA, 1998)	0.61	No safety concern (JECFA, 2000a) Category B (CoE, 1992)	JECFA evaluated 3-octyl acetate (CASrn as in Register). (*R*)- or (*S*)-enantiomer not specified by CASrn in Register
09.281	Oct-1-en-3-yl acetate		3582 11716 2442-10-6	1836	2.1	No safety concern (JECFA, 2009b)	
09.282	Oct-1-en-3-yl butyrate		3612 16491-54-6	1837	0.0012	No safety concern (JECFA, 2009b)	
09.415	Isopropyl isobutyrate		2937 290 617-50-5	309 JECFA specification (JECFA, 1998)	0.49	No safety concern (JECFA, 2000a) Category A (CoE, 1992)	

FL-no	EU register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU)[a] (μg/capita per day)	SCF status[b] JECFA status[c] CoE status[d]	Comments
09.450	Isopropyl isovalerate		2961 445 32665-23-9	310 JECFA specification (JECFA, 2002b)	0.24	No safety concern (JECFA, 2000a) Category B (CoE, 1992)	
09.513	Isopropyl 2-methylcrotonate		3229 10733 1733-25-1	312 JECFA specification (JECFA, 1998)	0.012	No safety concern (JECFA, 2000a)	JECFA evaluated isopropyl tiglate (CASrn 6284-46-4). CASrn in Register refers to (*E*)-isomer
09.539	Oct-3-yl 2-methylcrotonate		3676 94133-92-3	448 JECFA specification (JECFA, 2001)	0.012	No safety concern (JECFA, 2000a)	JECFA evaluated 1-ethylhexyl tiglate (CASrn as in Register). (*R*)- or (*S*)-enantiomer not specified by CASrn in Register
09.657	1-Methylbutyl acetate		4012 10761 626-38-0	1146 JECFA specification (JECFA, 2002b)	2.9	No safety concern (JECFA, 2002a)	JECFA evaluated 2-pentyl acetate (CASrn as in Register). (*R*)- or (*S*)-enantiomer not specified by CASrn in Register
09.658	1-Methylbutyl butyrate		3893 10763 60415-61-4	1142 JECFA specification (JECFA, 2002b)	0.47	No safety concern (JECFA, 2002a)	JECFA evaluated 2-pentyl butyrate (CASrn as in Register). CASrn refers to the racemate
09.923	Hept-2-yl butyrate		3981 39026-94-3	1144 JECFA specification (JECFA, 2002b)	3	No safety concern (JECFA, 2002a)	
09.924	3-Heptyl acetate (mixture of *R* and *S*)		3980 5921-83-5	1143 JECFA specification (JECFA, 2002b)	3	No safety concern (JECFA, 2002a)	
09.925	Nonan-3-yl acetate		4007 60826-15-5	1145 JECFA specification (JECFA, 2002b)	3	No safety concern (JECFA, 2002a)	

FL-no	EU register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU)[a] (μg/capita per day)	SCF status[b] JECFA status[c] CoE status[d]	Comments
09.936	4,8-Dimethyl-3,7-nonadien-2-yl acetate		4103 91418-25-6	1847 JECFA specification (JECFA, 2009a).	3	No safety concern (JECFA, 2009b)	

FEMA: Flavor and Extract Manufacturers Association; CoE: Council of Europe; CAS: Chemical Abstract Service; JECFA: The Joint FAO/WHO Expert Committee on Food Additives; MSDI: Maximised Survey-derived Daily Intake; SCF: Scientific Committee on Food.
(a): EU MSDI: Amount added to food as flavouring substance in (kg/year) × 10E9/(0.1 × population in Europe (= 375 × 10E6) × 0.6 × 365) = μg/capita per day.
(b): Category 1: Considered safe in use, Category 2: Temporarily considered safe in use, Category 3: Insufficient data to provide assurance of safety in use, Category 4: Not acceptable due to evidence of toxicity.
(c): No safety concern at estimated levels of intake.
(d): Category A: Flavouring substance, which may be used in foodstuffs, Category B: Flavouring substance which can be used provisionally in foodstuffs.
ND: no intake data reported.

Appendix C – Use levels/mTAMDI

Normal and maximum use levels

For each of the 18 food categories (Table C.1) in which the candidate substances are used, flavour industry reports a 'normal use level' and a 'maximum use level'.[1] According to the flavour industry, the 'normal use' is defined as the average of reported usages and 'maximum use' is defined as the 95th percentile of reported usages (EFFA, 2002d). The normal and maximum use levels in different food categories have been extrapolated from figures derived from 12 model flavouring substances (EFFA, 2004).

Table C.1: Food categories according to Commission Regulation (EC) No 1565/2000[3]

Food category	Description
01.0	Dairy products, excluding products of category 02.0
02.0	Fats and oils, and fat emulsions (type water-in-oil)
03.0	Edible ices, including sherbet and sorbet
04.1	Processed fruit
04.2	Processed vegetables (including mushrooms & fungi, roots & tubers, pulses and legumes), and nuts & seeds
05.0	Confectionery
06.0	Cereals and cereal products, including flours & starches from roots & tubers, pulses & legumes, excluding bakery
07.0	Bakery wares
08.0	Meat and meat products, including poultry and game
09.0	Fish and fish products, including molluscs, crustaceans and echinoderms
10.0	Eggs and egg products
11.0	Sweeteners, including honey
12.0	Salts, spices, soups, sauces, salads, protein products, etc.
13.0	Foodstuffs intended for particular nutritional uses
14.1	Non-alcoholic ('soft') beverages, excl. dairy products
14.2	Alcoholic beverages, including alcohol-free and low-alcoholic counterparts
15.0	Ready-to-eat savouries
16.0	Composite foods (e.g. casseroles, meat pies, mincemeat) - foods that could not be placed in categories 01.0–15.0

The 'normal and maximum use levels' are provided by industry for all 49 candidate substances in the present flavouring group (Table C.3).

mTAMDI calculations

The method for calculation of modified Theoretical Added Maximum Daily Intake (mTAMDI) values is based on the approach used by SCF up to 1995 (SCF, 1995). The assumption is that a person may consume the amount of flavourable foods and beverages listed in Table C.2. These consumption estimates are then multiplied by the reported use levels in the different food categories and summed up.

Table C.2: Estimated amount of flavourable foods, beverages, and exceptions assumed to be consumed per person per day (SCF, 1995)

Class of product category	Intake estimate (g/day)
Beverages (non-alcoholic)	324.0
Foods	133.4
Exception a: Candy, confectionery	27.0
Exception b: Condiments, seasonings	20.0
Exception c: Alcoholic beverages	20.0
Exception d: Soups, savouries	20.0
Exception e: Others, e.g. chewing gum	e.g. 2.0 (chewing gum)

The mTAMDI calculations are based on the normal use levels reported by industry. The seven food categories used in the SCF TAMDI approach (SCF, 1995) correspond to the 18 food categories as outlined in Commission Regulation (EC) No 1565/2000[3] and reported by the flavour industry in the following way (see Table C.3):

- Beverages (SCF, 1995) correspond to food category 14.1
- Foods (SCF, 1995) correspond to the food categories 1, 2, 3, 4.1, 4.2, 6, 7, 8, 9, 10, 13, and/or 16
- Exception a (SCF, 1995) corresponds to food category 5 and 11[3]
- Exception b (SCF, 1995) corresponds to food category 15[3]
- Exception c (SCF, 1995) corresponds to food category 14.2[3]
- Exception d (SCF, 1995) corresponds to food category 12[3]
- Exception e (SCF, 1995) corresponds to others, e.g. chewing gum.

Table C.3: Distribution of the 18 food categories listed in Commission Regulation (EC) No 1565/2000[3] into the seven SCF food categories used for TAMDI calculation (SCF, 1995)

	Food categories according to Commission Regulation (EC) No 1565/2000[3]	Distribution of the seven SCF food categories		
Key	**Food category**	**Food**	**Beverages**	**Exceptions**
01.0	Dairy products, excluding products of category 02.0	Food		
02.0	Fats and oils, and fat emulsions (type water-in-oil)	Food		
03.0	Edible ices, including sherbet and sorbet	Food		
04.1	Processed fruit	Food		
04.2	Processed vegetables (including mushrooms & fungi, roots & tubers, pulses and legumes), and nuts & seeds	Food		
05.0	Confectionery			Exception a
06.0	Cereals and cereal products, including flours & starches from roots & tubers, pulses & legumes, excluding bakery	Food		
07.0	Bakery wares	Food		
08.0	Meat and meat products, including poultry and game	Food		
09.0	Fish and fish products, including molluscs, crustaceans and echinoderms	Food		
10.0	Eggs and egg products	Food		
11.0	Sweeteners, including honey			Exception a
12.0	Salts, spices, soups, sauces, salads, protein products, etc.			Exception d
13.0	Foodstuffs intended for particular nutritional uses	Food		
14.1	Non-alcoholic ('soft') beverages, excl. dairy products		Beverages	
14.2	Alcoholic beverages, including alcohol-free and low-alcoholic counterparts			Exception c
15.0	Ready-to-eat savouries			Exception b
16.0	Composite foods (e.g. casseroles, meat pies, mincemeat) - foods that could not be placed in categories 01.0–15.0	Food		

Table C.4: Normal and maximum use levels (mg/kg) for the candidate substances in FGE.07Rev4 (EFFA, 2002a,c, 2007a,b,c, 2016; Flavour Industry, 2006, 2009)

FL-no	Food categories Normal use levels (mg/kg) Maximum use levels (mg/kg)																
	01.0	**02.0**	**03.0**	**04.1**	**04.2**	**05.0**	**06.0**	**07.0**	**08.0**	**09.0**	**10.0**	**11.0**	**12.0**	**13.0**	**14.1**	**14.2**	**15.0**	**16.0**
02.077	7 35	5 25	10 50	7 35	– –	10 50	5 25	10 50	2 10	2 10	– –	– –	5 25	10 50	5 25	10 50	20 100	5 25
02.124	7 35	5 25	10 50	7 35	– –	10 50	5 25	10 50	2 10	2 10	– –	– –	5 25	10 50	5 25	10 50	20 100	5 25
02.131	7 35	5 25	10 50	7 35	– –	10 50	5 25	10 50	2 10	2 10	– –	– –	5 25	10 50	5 25	10 50	20 100	5 25
02.142	7 35	5 25	10 50	7 35	– –	10 50	5 25	10 50	2 10	2 10	– –	– –	5 25	10 50	5 25	10 50	20 100	5 25
02.145	7 35	8 25	10 50	7 35	– –	10 50	5 25	10 50	2 10	2 10	– –	– –	5 25	10 50	5 25	10 50	20 100	5 25
02.148	7 35	5 25	10 50	7 35	– –	10 50	5 25	10 50	2 10	2 10	– –	– –	5 25	10 50	5 25	10 50	20 100	5 25
02.177	7 35	5 25	10 50	7 35	– –	10 50	5 25	10 50	2 10	2 10	– –	– –	5 25	10 50	5 25	10 50	20 100	5 25
02.182	7 35	5 25	10 50	7 35	– –	10 50	5 25	10 50	2 10	2 10	– –	– –	5 25	10 50	5 25	10 50	20 100	5 25
02.183	7 35	5 25	10 50	7 35	– –	10 50	5 25	10 50	2 10	2 10	– –	– –	5 25	10 50	5 25	10 50	20 100	5 25
02.187	7 35	5 25	10 50	7 35	– –	10 50	5 25	10 50	2 10	2 10	– –	– –	5 25	10 50	5 25	10 50	20 100	5 25
02.190	7 35	5 25	10 50	7 35	– –	10 50	5 25	10 50	2 10	2 10	– –	– –	5 25	10 50	5 25	10 50	20 100	5 25
02.194	7 35	5 25	10 50	7 35	– –	10 50	5 25	10 50	2 10	2 10	– –	– –	5 25	10 50	5 25	10 50	20 100	5 25
02.211	7 35	5 25	10 50	7 35	– –	10 50	5 25	10 50	2 10	2 10	– –	– –	5 25	10 50	5 25	10 50	20 100	5 25
02.255	5 20	– –	10 50	– –	– –	10 60	– –	10 60	– –	– –	– –	– –	– –	5 20	2 10	10 40	– –	– –

FL-no	Food categories Normal use levels (mg/kg) Maximum use levels (mg/kg)																
	01.0	02.0	03.0	04.1	04.2	05.0	06.0	07.0	08.0	09.0	10.0	11.0	12.0	13.0	14.1	14.2	15.0	16.0
07.072	3 15	2 10	3 15	2 10	– –	4 20	2 10	5 25	1 5	1 5	– –	– –	2 10	3 15	2 10	4 20	5 25	2 10
07.084	3 15	2 10	3 15	2 10	– –	4 20	2 10	5 25	1 5	1 5	– –	– –	2 10	3 15	2 10	4 20	5 25	2 10
07.150	3 15	2 10	3 15	2 10	– –	4 20	2 10	5 25	1 5	1 5	– –	– –	2 10	3 15	2 10	4 20	5 25	2 10
07.156	3 15	2 10	3 15	2 10	– –	4 20	2 10	5 25	1 5	1 5	– –	– –	– –	3 15	2 10	4 20	5 25	2 10
07.157	3 15	2 10	3 15	2 10	– –	4 20	2 10	5 25	1 5	1 5	– –	– –	2 10	5 25	2 10	4 20	– –	2 10
07.158	3 15	2 10	3 15	2 10	– –	4 20	2 10	5 25	1 5	1 5	– –	– –	2 10	3 15	2 10	4 20	5 25	2 10
07.160	3 15	2 10	3 15	2 10	– –	4 20	2 10	5 25	1 5	1 5	– –	– –	2 10	3 15	2 10	4 20	5 25	2 10
07.161	3 15	2 10	3 15	2 10	– –	4 20	2 10	5 25	1 5	1 5	– –	– –	2 10	3 15	2 10	4 20	5 25	2 10
07.162	3 15	2 10	3 15	2 10	– –	4 20	2 10	5 25	1 5	1 5	– –	– –	2 10	3 15	2 10	4 20	5 25	2 10
07.178	3 15	2 10	3 15	2 10	– –	4 20	2 10	5 25	1 5	1 5	– –	– –	2 10	3 15	2 10	4 20	5 25	2 10
07.181	3 15	2 10	3 15	2 10	– –	4 20	2 10	5 25	1 5	1 5	– –	– –	2 10	3 15	2 10	4 20	5 25	2 10
07.182	3 15	2 10	3 15	2 10	– –	4 20	2 10	5 25	1 5	1 5	– –	– –	2 10	3 15	2 10	4 20	5 25	2 10
07.185	3 15	2 10	3 15	2 10	– –	4 20	2 10	5 25	1 5	1 5	– –	– –	2 10	3 15	2 10	4 20	5 25	2 10
07.189	3 15	2 10	3 15	2 10	– –	4 20	2 10	5 25	1 5	1 5	– –	– –	2 10	3 15	2 10	4 20	5 25	2 10
07.198	3 15	2 10	3 15	2 10	– –	4 20	2 10	5 25	1 5	1 5	– –	– –	2 10	3 15	2 10	4 20	5 25	2 10

FL-no	Food categories Normal use levels (mg/kg) Maximum use levels (mg/kg)																
	01.0	**02.0**	**03.0**	**04.1**	**04.2**	**05.0**	**06.0**	**07.0**	**08.0**	**09.0**	**10.0**	**11.0**	**12.0**	**13.0**	**14.1**	**14.2**	**15.0**	**16.0**
07.199	3 15	2 10	3 15	2 10	– –	4 20	2 10	5 25	1 5	1 5	– –	– –	2 10	3 15	2 10	4 20	5 25	2 10
07.201	3 15	2 10	3 10	2 10	– –	4 20	2 10	5 25	1 5	1 5	– –	– –	2 10	3 15	2 10	4 20	5 25	2 10
07.204	3 15	2 10	3 15	2 10	– –	4 20	2 10	5 25	1 5	1 5	– –	– –	2 10	3 15	2 10	4 20	5 25	2 10
07.205	3 15	2 10	3 15	2 10	– –	– –	4 20	5 25	1 5	1 5	– –	– –	2 10	3 15	2 10	– –	5 25	2 10
07.210	3 15	2 10	3 15	2 10	– –	4 20	2 10	5 25	1 5	1 5	– –	– –	2 10	3 15	2 10	4 20	5 25	2 10
07.236	3 15	2 10	3 15	2 10	– –	4 20	2 10	5 25	1 5	1 5	– –	– –	2 10	3 15	2 10	4 20	5 25	2 10
07.239	3 15	2 10	3 15	2 10	– –	4 20	2 10	5 25	1 5	1 5	– –	– –	2 10	3 15	2 10	4 20	5 25	2 10
07.262	10 30	– –	5 15	10 30	10 30	30 150	– –	– –	– –	– –	– –	– –	– –	10 50	5 25	10 50	– –	30 150
09.304	7 35	5 25	10 50	7 35	– –	10 50	5 25	10 50	2 10	2 10	– –	– –	5 25	10 50	5 25	10 50	20 100	5 25
09.323	7 35	5 25	10 50	7 35	– –	10 50	5 25	10 50	2 10	2 10	– –	– –	5 25	10 50	5 25	10 50	20 100	5 25
09.325	7 35	5 25	10 50	7 35	– –	10 50	5 25	10 50	2 10	2 10	– –	– –	5 25	10 50	5 25	10 50	20 100	5 25
09.328	7 35	5 25	10 50	7 35	– –	10 50	5 25	10 50	2 10	2 10	– –	– –	5 25	10 50	5 25	10 50	20 100	2 25
09.332	7 35	5 25	10 50	7 35	– –	10 50	5 25	10 50	2 10	2 10	– –	– –	5 25	10 50	5 25	10 50	20 100	5 25
09.386	7 35	5 25	10 50	7 35	– –	10 50	5 25	10 50	2 10	2 10	– –	– –	5 25	10 50	5 25	10 50	20 100	5 25
09.388	7 35	5 25	10 50	7 35	– –	10 50	5 25	10 50	2 10	2 10	– –	– –	5 25	10 50	5 25	10 50	20 100	5 25

FL-no	Food categories Normal use levels (mg/kg) Maximum use levels (mg/kg)																
	01.0	**02.0**	**03.0**	**04.1**	**04.2**	**05.0**	**06.0**	**07.0**	**08.0**	**09.0**	**10.0**	**11.0**	**12.0**	**13.0**	**14.1**	**14.2**	**15.0**	**16.0**
09.391	7 35	5 25	10 50	7 35	– –	10 50	5 25	10 50	2 10	2 10	– –	– –	5 25	10 50	5 25	10 50	20 100	5 25
09.604	7 35	5 25	10 50	7 35	– –	10 50	5 25	10 50	2 10	2 10	– –	– –	5 25	10 50	5 25	10 50	20 100	5 25
09.605	7 35	5 25	10 50	7 35	– –	10 50	5 25	10 50	2 10	2 10	– –	– –	5 25	10 50	5 25	10 50	20 100	5 25
09.606	7 35	5 25	10 50	7 35	– –	10 50	5 25	10 50	2 10	2 10	– –	– –	5 25	10 50	5 25	10 50	20 100	5 25
09.608	7 35	5 25	10 50	7 35	– –	10 50	5 25	10 50	2 10	– –	– –	– –	5 25	10 50	5 25	10 50	20 100	5 25
09.609	7 35	5 25	10 50	7 35	– –	10 50	5 25	10 50	2 10	2 10	– –	– –	5 25	10 50	5 25	10 50	– –	5 25
09.676	7 35	5 25	10 50	7 35	– –	10 50	5 25	10 50	2 10	2 10	– –	– –	5 25	10 50	5 25	10 50	20 100	5 25
09.880	7 35	5 25	10 50	7 35	– –	10 50	5 25	10 50	2 10	2 10	– –	– –	5 25	10 50	5 25	10 50	20 100	5 25
09.926	7 35	5 25	10 50	7 35	– –	10 50	5 25	10 50	2 10	2 10	– –	– –	5 25	10 50	5 25	10 50	20 100	5 25

The mTAMDI values (see Table C.5) are presented for each of the 49 flavouring substances in the present flavouring group, for which industry has provided use and use levels (EFFA, 2002a,c, 2007a,b, c; Flavour Industry, 2006, 2009). The mTAMDI values are only given for highest reported normal use levels (see Table C.4).

Table C.5: Estimated intakes based on the mTAMDI approach

FL-no	EU register name	mTAMDI (μg/person per day)	Structural class	Threshold of concern (μg/person per day)
02.077	Pentan-3-ol	3,900	Class I	1,800
02.124	6-Methylhept-5-en-2-ol	3,900	Class I	1,800
02.142	3,3-Dimethylbutan-2-ol	3,900	Class I	1,800
02.148	Dodecan-2-ol	3,900	Class I	1,800
02.177	2-Methylhexan-3-ol	3,900	Class I	1,800
02.182	3-Methylpentan-2-ol	3,900	Class I	1,800
02.183	4-Methylpentan-2-ol	3,900	Class I	1,800
02.190	Nonan-3-ol	3,900	Class I	1,800
02.255	(*Z*)-4-Hepten-2-ol	2,500	Class I	1,800
07.084	Pentan-3-one	1,600	Class I	1,800
07.178	3-Methylbutan-2-one	1,600	Class I	1,800
07.239	[*R*-(*E*)]-5-Isopropyl-8-methylnona-6,8-dien-2-one	1,600	Class I	1,800
09.304	*sec*-Heptyl isovalerate	3,900	Class I	1,800
09.323	*sec*-Butyl acetate	3,900	Class I	1,800
09.325	*sec*-Butyl butyrate	3,900	Class I	1,800
09.328	*sec*-Butyl formate	3,900	Class I	1,800
09.332	*sec*-Butyl hexanoate	3,900	Class I	1,800
09.386	*sec*-Hept-4(*cis*)-enyl acetate	3,900	Class I	1,800
09.388	*sec*-Heptyl acetate	3,900	Class I	1,800
09.391	*sec*-Heptyl hexanoate	3,900	Class I	1,800
09.604	Isopropyl decanoate	3,900	Class I	1,800
09.605	Isopropyl dodecanoate	3,900	Class I	1,800
09.606	Isopropyl hexadecanoate	3,900	Class I	1,800
09.608	Isopropyl octanoate	3,900	Class I	1,800
09.609	Isopropyl valerate	3,500	Class I	1,800
09.676	*sec*-Octyl acetate	3,900	Class I	1,800
09.880	(*Z*)-Hept-4-enyl-2 butyrate	3,900	Class I	1,800
09.926	Octan-3-yl formate	3,900	Class I	1,800
02.145	2,6-Dimethylocta-1,5,7-trien-3-ol	3,900	Class II	540
02.194	Octa-1,5-dien-3-ol	3,900	Class II	540
02.211	Undeca-1,5-dien-3-ol	3,900	Class II	540
07.072	6-Methylheptan-3-one	1,600	Class II	540
07.150	Decan-2-one	1,600	Class II	540
07.156	2,6-Dimethyloct-6-en-3-one (mixture of *E* and *Z*)	1,600	Class II	540
07.157	6,10-Dimethylundecan-2-one	1,500	Class II	540
07.158	Dodecan-2-one	1,600	Class II	540
07.160	Heptadecan-2-one	1,600	Class II	540
07.162	Hex-5-en-2-one	1,600	Class II	540
07.181	6-Methylheptan-2-one	1,600	Class II	540
07.185	3-Methylpentan-2-one	1,600	Class II	540
07.189	Nonan-4-one	1,600	Class II	540
07.198	Pseudo-ionone	1,600	Class II	540

FL-no	EU register name	mTAMDI (μg/person per day)	Structural class	Threshold of concern (μg/person per day)
07.199	Tetradecan-2-one	1,600	Class II	540
07.201	Tridec-12-en-2-one	1,600	Class II	540
07.204	3,3,6-Trimethylhepta-1,5-dien-4-one	1,600	Class II	540
07.205	6,10,14-Trimethylpentadecan-2-one	1,500	Class II	540
07.236	(*Z*)-5-Octen-2-one	1,600	Class II	540
07.262	9-Decen-2-one	6,600	Class II	540
07.182	5-Methylheptan-3-one	1,600	Class II	540
02.131	But-3-en-2-ol	3,900	Class II	540
02.187	Non-1-en-3-ol	3,900	Class II	540
07.161	Hex-1-en-3-one	1,600	Class II	540
07.210	1-Nonene-3-one	1,600	Class II	540

mTAMDI: modified Theoretical Added Maximum Daily Intake.

Appendix D – Metabolism

General information

The present flavouring group evaluation consists of 53 candidate substances of which seven are saturated aliphatic acyclic secondary alcohols [FL-nos: 02.077, 02.142, 02.148, 02.177, 02.182, 02.183 and 02.190]; seven are unsaturated aliphatic secondary alcohols [FL-nos: 02.124, 02.131, 02.145, 02.187, 02.194, 02.211 and 02.255] of which five contain a terminal double bond [FL-nos: 02.131, 02.145, 02.187, 02.194 and 02.211]; 13 are saturated aliphatic ketones [FL-nos: 07.072, 07.084, 07.150, 07.157, 07.158, 07.160, 07.178, 07.181, 07.182, 07.185, 07.189, 07.199 and 07.205], 10 are unsaturated aliphatic ketones [FL-nos: 07.156, 07.161, 07.162, 07.198, 07.201, 07.204, 07.210, 07.236, 07.239 and 07.262] of which seven contain a terminal double bond [FL-nos: 07.161, 07.162, 07.201, 07.204, 07.210, 07.239 and 07.262] and 16 are esters of aliphatic acyclic secondary alcohols and linear aliphatic carboxylic acids [FL-nos: 09.304, 09.323, 09.325, 09.328, 09.332, 09.386, 09.388, 09.391, 09.604, 09.605, 09.606, 09.608, 09.609, 09.676, 09.880 and 09.926]. The general metabolic reactions that the candidate substances may be expected to undergo, and which are discussed below, are one or several of the following:

- conjugation of secondary alcohols with glucuronic acid
- oxidation of secondary alcohols
- reduction of ketones
- oxidation of ketones
- oxidation of double bonds
- oxidation of terminal double bonds
- hydrolysis of esters.

A general discussion on the biotransformation of Saturated Aliphatic Acyclic Secondary Alcohols, Ketones, and Related Saturated and Unsaturated Esters may be found in the reports from the 51st, 59th and 69th meetings of the JECFA (1999a, 2002a, 2009b). The discussions and conclusions related to these supporting substances essentially apply also to the candidate substances. There is one candidate substance 5-methylheptan-3-one [FL-no: 07.182] that may be oxidised to yield a neurotoxic gamma-diketone and therefore it may potentially give rise to concern.

Absorption

In general, aliphatic secondary alcohols and ketones are expected to be rapidly absorbed in the gastrointestinal tract (JECFA, 1999a).

Peak blood levels were obtained 1–2 h after dosing when isopropanol was given orally to rats as well as when the same substance was administered intravenously to dogs (Lehman et al., 1945; Nordmann et al., 1973). Peak blood levels were also obtained within 2 h when 1- and 2-propanol, or 1- and 2-isobutanol were given orally to human volunteers together with ethanol (Bonte et al., 1981).

In a pharmacokinetic experiment, 2-butanol (2.2 mL/kg bw or 1,776 mg/kg bw), 2-butanone (2.1 mL/kg bw or 1,690 mg/kg bw) and 2,3-butanediol (0.68 mL/kg bw or 676 mg/kg bw), respectively, were administered orally in aqueous solutions to male Sprague–Dawley rats. Peak blood concentrations after administration of 0.95 mg/L 2-butanone were detected after 4 h and declined to 0.07 mg/mL after 18 h. The concentrations of the metabolites 2,3-butanediol, 2-butanol and 3-hydroxy-2-butanone peaked at 0.26, 0.033 and 0.027 mg/L at 18, 6 and 8 h, respectively, after 2-butanone administration. Total area under the curve (AUC) values for 2-butanone, 2,3-butanediol, 2-butanol and 3-hydroxy-2-butanone were 10,899 ± 824, 3,863 ± 238, 414 ± 38 and 382 ± 38 mg h/L, respectively. Blood concentration after administration of 2-butanol peaked after 2 h at 0.59 mg/L and declined to 0.05 mg/L after 16 h. The blood concentrations of 2-butanone, 3-hydroxy-2-butanone and 2,3-butanediol rose to maximums after 8, 12 and 18 h and were 0.78, 0.04 and 0.21 mg/L, respectively. Total AUC values were 3,254 ± 258 mg h/L for 2-butanol, 9,868 ± 566 for 2-butanone, 443 ± 93 for 3-hydroxy-2-butanone and 3,167 ± 503 mg h/L for 2,3-butanediol (Dietz et al., 1981).

Rats were administered 1 g/kg bw 2-pentanol, 3-pentanol and 3-methyl-2-butanol, via intraperitoneal (IP) injection. The alcohols were eliminated within 13–16 h (Haggard et al., 1945).

Metabolism and elimination

Secondary alcohols

Oxidation and glucuronic acid conjugation: Secondary alcohols may undergo oxidation to the corresponding ketone. However, this reaction is generally unfavoured *in vivo*, since the alcohol is removed from the equilibrium by conjugation with glucuronic acid, which represents the major biotransformation pathway for secondary alcohols (Kasper and Henton, 1980; JECFA, 1999a). Glucuronidation is a phase-II-reaction, which involves the transfer of glucuronic acid in an activated form to functional groups of the substrate, in this case to the hydroxyl groups of the molecules. This renders highly polar products, for which excretion is facilitated. The reaction is catalysed by UDP-glucuronyl transferase, which exists in several isoforms with different substrate specificities. The enzymes are located in the endoplasmic reticulum, and are found in most tissues including the liver. The glucuronic acid conjugates are primarily excreted in the urine or bile, depending on the relative molecular mass and the animal species. For the candidate secondary alcohols, the urine is expected to be the main route of elimination.

Ketones

In addition to reduction and oxidation pathways, low molecular weight ketones (carbon chain length < 5) may be excreted unchanged in expired air (Brown et al., 1987). In mammals, oral doses of volatile ketones or their corresponding alcohols are mainly eliminated as the ketone in expired air. Lower amounts are excreted in the urine (Haggard et al., 1945; Scopinaro et al., 1947; Schwartz, 1989).

In the rat, 2-pentanone in expired air was the major metabolite following administration of 2-pentanol by IP injection. Lower amounts of 2-pentanol were also exhaled and both metabolites were detected in the urine (Haggard et al., 1945). Similarly, unchanged 2-pentanone administered orally to dogs has been identified in the expired air (Schwartz, 1989).

Reduction of ketones: In general, the major metabolic pathway for the detoxification and excretion of aliphatic ketones involves reduction of the ketone to the corresponding secondary alcohol with subsequent excretion as conjugate of glucuronic acid. This reaction is reversible under physiologic conditions, but *in vivo* the secondary alcohols are removed from the equilibrium by conjugation to glucuronic acid, as is stated above, and the reaction proceeds to form further secondary alcohols (Felsted and Bachur, 1980; JECFA, 1999a). Reduction of aliphatic ketones is mediated by alcohol dehydrogenase and NADH/NADPH-dependent cytosolic carbonyl reductases (Bosron and Li, 1980). According to Felsted and Bachur (1980), the reaction catalysed by carbonyl reductase is stereoselective and favours formation of the (*S*)-enantiomer of the alcohol (Felsted and Bachur, 1980).

In studies limited to the identification of urinary glucuronide, relatively high single dose levels of a homologous series of aliphatic secondary alcohols and ketones were administered individually by gavage to rabbits. The urinary excretion of glucuronic acid conjugates was determined after 24 h (Kamil et al., 1953). The substances, dose levels and average urinary output of glucuronide (UGAC) are shown below in Table D.1.

Oxidation of ketones: Ketones may also be metabolised via omega- or omega-1-oxidation. Participation in these pathways depends on chain length, position of the carbonyl function and dose (Dietz et al., 1981; Topping et al., 1994).

Table D.1: The urinary excretion of glucuronic acid conjugates (UGAC, determined after 24 h) of aliphatic secondary alcohols and ketones after administration by gavage to rabbits (Kamil et al., 1953)

Substance	Dose (mg/kg bw)	UGAC (%)
2-Pentanol	735	44.8
2-Heptanone	950	41.0
2-Heptanol	965	54.6
3-Heptanol	965	61.9
2-Octanol	1,081	15.5

bw: body weight; UGAC: average urinary output of glucuronide.

Short chain ketones (C < 5) that contain a carbonyl function at the C-2 may undergo oxidation of the terminal methyl group and subsequent oxidation to yield an alpha-keto carboxylic acid. As intermediary metabolites, alpha-keto acids undergo oxidative decarboxylation to yield carbon dioxide and a simple aliphatic carboxylic acid, which may be completely metabolised in the fatty acid pathway and citric acid cycle. Alternatively, omega-oxidation may occur to yield a hydroxy-ketone, which may

be further reduced to a diol, e.g. 2,3-butanediol from butanone, and excreted in the urine as a glucuronic acid conjugate.

Longer chain aliphatic ketones (carbon chain length ≥ 5) are primarily metabolised via reduction, but omega- and omega-1-oxidation are competing pathways at high concentrations (Dietz et al., 1981; Topping et al., 1994).

Studies with specific substances: 4-Methylpentan-2-ol [FL-no: 02.183] and 4-hydroxy-4-methylpentan-2-one were detected in serum after IP injection of 4-methylpentan-2-one in guinea pigs. The half-life and clearance times of 4-methylpentan-2-one were 66 min and 6 h, respectively. 4-Hydroxy-4-methylpentan-2-one was the principal metabolite and was cleared in 16 h. The concentration of 4-methylpentan-2-ol [FL-no: 02.183] was too low for quantification. 4-Methylpentan-2-one is metabolised by reduction of the carbonyl group to form the secondary alcohol, 4-methylpentan-2-ol [FL-no: 02.183], and by oxidation at the omega-1 carbon atom to form the hydroxylated ketone, 4-hydroxy-4-methylpentan-2-one (DiVincenzo et al., 1976).

Gamma-Diketone formation: Omega-1-oxidation of aliphatic ketones with special structural features may yield neurotoxic gamma-diketones. The metabolic pathway includes oxidation of the omega-1-carbon, first to a hydroxy-ketone and then to a diketone. The gamma-spacing of the carbonyl functions has been shown to be a prerequisite for neurotoxic effects, only ketones with this structural feature may yield the neurotoxic metabolites. One of the candidate substances 5-methyl-3-heptanone [FL-no: 07.182], may potentially be oxidised to a gamma-diketone 3-methyl-2,5-heptanedione.

Studies have shown that neurotoxicity of selected ketones is related to a common metabolic pathway leading to the formation of a gamma-diketone, which is the metabolite that produces neuropathy. The neurotoxic effects show a specific anatomic and morphological type of nerve degeneration characterised by large multifocal axonal swellings, referred to as 'giant-axonal' neuropathy. Clinical symptomatology in humans includes bilaterally symmetrical paraesthesia, 'pins and needles' feeling, and muscle weakness, primarily in arms and legs. Except for 3,6-octanedione, all metabolic interconversions are oxidation of the omega-1-carbon, first to a hydroxy-ketone and then to a gamma-diketone. When the omega-carbon is oxidised in preference to the omega-1-carbon, no gamma-diketone is formed (Topping et al., 1994).

Induction of clear and typical signs of neurotoxicity in male rats dosed with 5-methyl-3-heptanone [FL-no: 07.182] in a subchronic study (IBM Corp., 1989) supported the hypothesis that a gamma-diketone may be formed as toxic metabolite.

Data suggest that the neurotoxicity of the diketone decreases as chain length increases, possibly owing to steric hindrance. However, chain length may not be important to some materials, as in the case of 5-nonanone. Another factor modifying the neurotoxic potential of these substances is the number and size of substituent groups located between the gamma-spaced carbonyls. Single methyl groups on the carbons located between the carbonyl groups increase the potential neurotoxicity, whereas two methyl groups positioned on one of the carbon atoms between the carbonyls eliminate neurotoxicity (Topping et al., 1994).

Among the supporting substances, 3-heptanone [FL-no: 07.003], 2-methylheptan-3-one [FL-no: 07.240], 3-heptanol [FL-no: 02.044] and 3-heptyl acetate [FL-no: 09.924] are the only substances that may be metabolised to yield neurotoxic gamma-diketones (Topping et al., 1994). The neurotoxicity for these substances is observed only at high doses.

In a study reported as a meeting abstract, aliphatic ketones (hexane-2-one, pentane-3-one, heptane-3-one, 4-methyl-2-pentanone and 3,3-dimethyl-2-butanone) were administered in drinking water to female Wistar rats. It was concluded that administration of approximately 1 g/kg bw per day of hexane-2-one for 120 days produced muscle weakness, atrophy and peripheral neuropathy. None of the other ketones produced significant neurological alterations (Homan and Maronpot, 1978).

In an oral gavage study Crl rats, two per group, were given 3-heptanone [FL-no: 07.003] (0.25, 0.5, 1 or 2 g/kg bw per day, for 5 days/week for 14 weeks. The highest dose group (approaching the LD50 value in rats = 2,760 mg/kg bw) was the only one developing treatment-related neuropathologic lesions of typical 'giant-axonal' type. No neuropathology was observed in the lower dose groups (O'Donoghue et al., 1984). This study determined that 3-heptanone has a low neurotoxic potential; however, when its intake was combined with-methyl ethyl ketone, neurotoxic effects were potentiated, by stimulating 3-heptanone metabolism to 2,5-heptandione, a neurotoxic gamma-diketone (O'Donoghue et al., 1984).

Oxidation of terminal double bonds in secondary alcohols and in ketones

Twelve of the candidate substances, but-3-en-2-ol, 2,6-dimethylocta-1,5,7-triene-3-ol, non-1-en-3-ol, octa-1,5-dien-3-ol, undeca-1,5-dien-3-ol, hex-1-en-3-one, hex-5-en-2-one, tridec-12-en-2-one, 3,3,6-trimethylhepta-1,5-dien-4-one, 1-nonene-3-one, ([*R*-(*E*)]-5-isopropyl-8-methylnona-6,8-dien-2-one and 9-decen-2-one [FL-nos: 02.131, 02.145, 02.187, 02.194, 02.211, 07.161, 07.162, 07.201, 07.204, 07.210, 07.239 and 07.262] have terminal double bonds. These double bonds may be oxidised to the corresponding epoxides. Epoxides are highly reactive molecules, due to the large strain associated with this three-membered ring structure, and they react easily with nucleophilic sites of cellular macromolecules. However, epoxides will be conjugated with glutathione by glutathione *S*-transferases or hydrolysed to diols by epoxide hydrolases. These two reactions can be considered to be detoxications (Sanchez and Kauffman, 2010). 1-Alkenes are metabolised by P450 through both double bond oxidation to the corresponding epoxide and allylic oxidation (Chiappe et al., 1998). The rates of the two reactions measured with different P450 isoforms indicate that epoxide formation is generally favoured (Chiappe et al., 1998).

Based on the low levels of intake of alkenones and alkenols characterised by a carbonyl or an alcohol group in addition to the terminal double bond, it is expected that the detoxication reactions of the formed epoxides (conjugation with glutathione or epoxide hydrolase mediated hydrolysis) would not be saturated and would outweigh the rate of epoxide formation. The presence of the terminal double bond is therefore not considered of concern under the intended conditions of use.

In addition to reduction and oxidation pathways, low molecular weight alcohols and ketones may be excreted unchanged in expired air (Brown et al., 1987).

Ester hydrolysis

The aliphatic esters among the candidate substances are expected to be hydrolysed to their component secondary alcohols and carboxylic acids. The carboxylesterase or esterase classes of enzymes, the most important of which are the beta-esterases, catalyse ester hydrolysis (Heymann, 1980). In mammals, these enzymes occur within the body in most tissues including the gut lumen and intestinal wall, but predominate in the hepatocytes (Heymann, 1980). The wide range of tissue distribution and the multiplicity of esterases generally give rise to rapid hydrolysis of esters *in vivo*.

There are no hydrolysis studies on the candidate substances, but there are *in vitro* hydrolysis data for structurally related esters.

In vitro hydrolysis studies of esters have been performed with specific carboxylesterase isoenzymes isolated from pig and rat livers (Arndt and Krisch, 1973; Junge and Heymann, 1979). The isoenzyme I exhibits an increase in enzyme binding (lower K_m) and maximum velocity (V_{max}) as the carbon chain length of either the alcohol or carboxylic acid component of the substrate increases. It is also shown that different isoenzymes show great differences in the hydrolysis rates. Isoenzyme *V* had an optimum for the C-5 compound, while this isoenzyme exhibited a minimum activity with the butyl and pentyl acetates. Results of *in vitro* studies indicate that the rate of hydrolysis of straight chain esters is approximately 100 times faster than the rate of hydrolysis of branched-chain esters.

Incubation of isopropyl butanoate, isopropyl phenylacetate, isoamyl acetate and isoamyl phenylacetate with pancreatin produced 40%, 50%, 20% and 100% hydrolysis, respectively, after 2 h (Leegwater and van Straten, 1974a; Grundschober, 1977). Also, isoamyl acetate incubated with intestinal mucosa homogenates obtained from pigs demonstrated complete hydrolysis (Leegwater and van Straten, 1974b; Grundschober, 1977).

Esters formed from aliphatic secondary alcohols were hydrolysed to their corresponding alcohols and carboxylic acids when incubated with liver homogenates or small intestinal homogenates obtained from male Wistar albino rats, artificial gastric juice or artificial pancreatic juice with half-lives ranging from less than one-second to several hours depending on the incubation medium (Gangolli and Shilling, 1968; Longland et al., 1977). Rat liver homogenates and small intestinal preparations were found to be much more efficient than artificial pancreatic juice for hydrolysis of a variety of aliphatic esters. Also, hydrolysis in simulated intestinal fluid with pancreatin was much faster than in simulated gastric juice (Longland et al., 1977).

The data on substances structurally related to the candidate substances indicate that hydrolysis is the major pathway for the candidate substances that are esters of secondary alcohols, and that they will be hydrolysed to their component alcohols and carboxylic acids within a relatively short time.

Conclusion

In conclusion, it may be anticipated that 52 of the candidate substances (the seven saturated aliphatic acyclic secondary alcohols [FL-nos: 02.077, 02.142, 02.148, 02.177, 02.182, 02.183 and 02.190], the seven unsaturated aliphatic secondary alcohols [FL-nos: 02.124, 02.131, 02.145, 02.187, 02.194, 02.211 and 02.255], the 12 of the 13 saturated aliphatic ketones [FL-nos: 07.072, 07.084, 07.150, 07.157, 07.158, 07.160, 07.178, 07.181, 07.185, 07.189, 07.199 and 07.205], the 10 unsaturated aliphatic ketones [FL-nos: 07.156, 07.161, 07.162, 07.198, 07.201, 07.204, 07.210, 07.236, 07.239 and 07.262] and the 16 esters of aliphatic acyclic secondary alcohols and linear aliphatic carboxylic acids [FL-nos: 09.304, 09.323, 09.325, 09.328, 09.332, 09.386, 09.388, 09.391, 09.604, 09.605, 09.606, 09.608, 09.609, 09.676, 09.880 and 09.926]) will be metabolised to innocuous substances at the estimated levels of intake, based on the MSDI approach, as flavouring substances.

One candidate substance, 5-methyl-3-heptanone [FL-no: 07.182], may be oxidised to a potentially neurotoxic gamma-diketone, 3-methyl-2,5-heptanedione.

Appendix E – Toxicity summary tables

Oral acute toxicity data are available for 12 candidate substances of the present Flavouring Group Evaluation from chemical group 5, and for 25 supporting substances evaluated by JECFA at the 51st and 59th meetings (JECFA, 1999a, 2003). The supporting substances are listed in brackets.

Table E.1: Acute toxicity

Chemical name [FL-no]	Species	Sex	LD_{50} (mg/kg bw)	Reference
(Acetone [07.050])	Rat	M	8,452	Smyth et al. (1970)
	Rat	NR	8,930	Smyth et al. (1969)
	Rat	NR	9,750	FDA (1975)
	Rat	NR	6,800	Kimura et al. (1971)
	Rat	NR	3,465	Kohli et al. (1967)
	Mouse	M	5,250	Tanii et al. (1986)
	Rabbit	NR	5,300	Krasavage et al. (1982)
(Isopropyl alcohol [02.079])	Rat	NR	5,840	Smyth and Carpenter (1948)
	Rat	NR	5,280	Lehman and Chase (1944)
	Rat	NR	5,300	Kimura et al. (1971)
	Rat	NR	5,330	FDA (1975)
	Mouse	NR	5,070	FDA (1975)
	Rabbit	NR	5,040	Lehman and Chase (1944)
	Rabbit	NR	7,990	Munch (1972)
	Dog	NR	4,830	Lehman and Chase (1944)
(2-Butanone [07.053])	Rat	M	5,490	Smyth et al. (1962)
	Rat	NR	2,730	Kimura et al. (1971)
	Rat	NR	3,980	Union Carbide Corp. (1956)
	Rat	F	5,525	Pozzani et al. (1959)
	Mouse	M	3,137	Zakhari et al. (1977)
	Mouse	M	4,050	Tanii et al. (1986)
(2-Pentanone [07.054])	Rat	M	3,730	Smyth et al. (1962)
	Mouse	M	2,205	Tanii et al. (1986)
(2-Pentanol [02.088])	Rabbit	NR	2,820	Munch (1972)

Chemical name [FL-no]	Species	Sex	LD_{50} (mg/kg bw)	Reference
Pentan-3-one [07.084]	Rat	NR	2,900	BASF (1969)
	Rat	NR	2,140	Panson and Winek (1980)
	Rat	NR	2,140	Eder et al. (1982)
	Rat	NR	2,140	Kennedy and Graepel (1991)
	Rat	NR	3,100	Ibatullina and Larionova (1997)
Pentan-3-ol [02.077]	Rat	NR	1,870	Eder et al. (1982)
(3-Hexanone [07.096])	Rat	NR	2,727	Carpenter et al. (1974)
(2-Heptanone [07.002])	Rat	M	1,670	Smyth et al. (1962)
	Mouse	M	2,407	Tanii et al. (1986)
	Mouse	NR	1,088	Schafer and Bowles (1985)
	Mouse	NR	730	Srepel and Akacic (1962)
(2-Heptanol [02.045])	Rat	M, F	2,580	Eder et al. (1982)
(3-Heptanone [07.003])	Rat	NR	2,760	Smyth et al. (1949)
(3-Heptanol [02.044])	Rat	NR	1,870	Smyth et al. (1951)
(4-Heptanone [07.058])	Rat	NR	3,049	Carpenter et al. (1974)
(2-Octanone [07.019])	Rat	NR	> 5,000	Katz et al. (1980)
	Mouse	M	3,823	Tanii et al. (1986)
	Mouse	NR	3,870	Tanii et al. (1986)
(2-Octanol [02.022])	Rat	NR	3,200	Patty et al. (1935)
(3-Octanone [07.062])	Rat	NR	5,000	Shelanski and Moldovan (1973)
(2-Nonanone [07.020])	Mouse	M	7,992	Tanii et al. (1986)
Decan-2-one [07.150]	Mouse	M	7,936	Tanii et al. (1986)
(2-Undecanone [07.016])	Mouse	NR	950	Schafer and Bowles (1985)
	Mouse	M	5,460	Tanii et al. (1986)
Methyl-3-butan-2-one [07.178]	Mouse	M	2,572	Tanii et al. (1986)
	Rat	NR	148	Kennedy and Graepel (1991)
(4-Methyl-2-pentanone [07.017])	Rat	NR	2,080	Smyth et al. (1951)
	Mouse	M	2,670	Tanii et al. (1986)
	Mouse	NR	1,200	McOmie and Anderson (1949)
Methyl-4-pentan-2-ol [02.183]	Rat	NR	2,590	Smyth et al. (1951)
	Mouse	NR	1,500	McOmie and Anderson (1949)
Methyl-6-heptan-2-one [07.181]	Rat	NR	6,700	BASF (1975)

Chemical name [FL-no]	Species	Sex	LD_{50} (mg/kg bw)	Reference
Methyl-5-heptan-3-one [07.182]	Rat	NR	3,500	Kennedy and Graepel (1991)
(2,6-Dimethyl-4-heptanone [07.122])	Rat	NR	5,750	Smyth et al. (1949)
	Mouse	NR	2,800	McOmie and Anderson (1949)
	Mouse	NR	1,416	RTECS (1975)
Trimethyl-6,10,14-pentadecan-2-one [07.205]	Rat	NR	> 2,000	BASF (1988)
(6-Methyl-5-hepten-2-one [07.015])	Mouse	M, F	3,609	Colaianni (1967)
	Rat	M, F	4,100	Keating (1972)
(3,4,5,6-Tetra-hydropseudoionone [07.069])	Mouse	M, F	5,200	Moreno (1982)
	Rat	M, F	> 5,000	Moreno (1977)
(6,10-Dimethyl-5,9-undecadien-2-one [07.123])	Mouse	M, F	8,650	Moreno (1976)
	Rat	M, F	> 6,800	Hofmann (1978)
(2,6,10-Trimethyl-2,6,10-pentadecatrien-14-one [07.114])	Rat	M, F	> 5,000	deGroot et al. (1974)
(Isopropyl formate [09.165])	Rat	NR	4,300	FDA (1975)
	Rabbit	NR	2,500	FDA (1975)
	Guinea Pig	NR	2,700	FDA (1975)
	Chicken	NR	2,100	FDA (1975)
(Isopropyl acetate [09.003])	Rat	M, F	6,750	Eder et al. (1982)
	Rat	NR	3,000	FDA (1975)
	Rabbit	NR	6,945	Munch (1972)
Isopropyl hexadecanoate [09.606]	Rat	M, F	> 40,000	Food and Drug Research Laboratories, Inc. (1976)
	Rat	M, F	> 8,000	Kolmar Research Center (1972)
	Rat	M, F	> 64,000	Bio-Toxicology Laboratories (1982)
	Rat	NR	> 5,000	Moreno (1978)
sec-Butyl formate [09.328]	Rat	NR	11,300	Union Carbide Corp. (1980)
9-Decen-2-one [07.262]	Rat	F	2,500	Flavour Industry (2009)
(6-Methylhepta-3,5-dien-2-one [07.099])	Mouse	M, F	3,200	Colaianni (1967)
Pseudo-ionone [07.198]	Rat	NR	> 5,000	Moreno (1976)

FL-no: FLAVIS number; LD_{50}: lethal dose, 50%; bw: body weight.
NR: Not Reported; M = Male; F = Female.

Subacute and subchronic toxicity data are available for three candidate substances and for 10 supporting substances of the present flavouring group. They were evaluated at the 51st and 59th JECFA meetings (JECFA, 1999a, 2003). No carcinogenicity data are available. The supporting substances are listed in brackets.

Table E.2: Subacute and subchronic toxicity studies

Chemical name [FL-no]	Species/sex No/group	Route	Dose levels (mg/kg per day)	Duration	NOAEL (mg/kg per day)	Reference	Comments
(Acetone [07.050])	Rat/M,F 10	Drinking water	0, 250, 500, 1,000, 2,000, 5,000	13 weeks	1,000[(a)]	Diet (1991)	[(c)] NTP study
	Mouse/M,F 10	Drinking water	0, 312, 625, 1,250, 2,500, 5,000 (M) 0, 625, 1,250, 2,500, 5,000, 12,500 (F)	13 weeks	2,500[(a)]	Dietz (1991)	[(c)] NTP study
	Rat/M,F 30	Gavage	0, 100, 500, 2,500	90 days	100	Sonawane et al. (1986)	[(c)] Meeting abstract
	Rat/NR 3	Drinking water	1,000	4 weeks	1,000[(a),(b)]	Spencer et al. (1978)	[(c)] Examinations were limited to specific neurotoxic effects. No other parameter was monitored
(Isopropyl alcohol [02.079])	Human/M 8	Oral	0, 2.6, 6.4	6 weeks	6.4[(b)]	Wills et al. (1969)	[(c)] Paper published in a peer-reviewed journal
	Rat/M 22	Drinking water	0, 870, 1,280, 1,680, 2,520	12 weeks	870	Pilegaard and Ladefoged (1993)	[(c)] Good quality study
Pentan-3-one [07.084]	Rat/F 5	Drinking water	0, 1,860	120 days	Not detected (< 1,860)	Union Carbide Corp. (1977)	Good quality unpublished report. Focused on neurotoxic effect
(2-Heptanone [07.002])	Rat/M,F 15	Gavage (dissolved in corn oil)	0, 20, 100, 500	13 weeks	20	Gaunt et al. (1972)	[(c)] Good quality study – peer-reviewed journal
	Rat/NR 5	Drinking Water	0, 500	12 weeks	500[(a),(b)]	Spencer et al. (1978)	[(c)] Good quality study – peer-reviewed journal
(3-Heptanone [07.003])	Rat/M 2	Gavage	0, 250, 500, 1,000, 2,000, 4,000	14 weeks	1,000	O'Donoghue et al. (1984)	[(c)] Good quality study – peer-reviewed journal
	Rat/F NR	Drinking Water	1,000	120 days	1,000[(a)]	Homan and Maronpot (1978)	[(c)] Meeting abstract
	Rat/F 5	Drinking water	0, 27	120 days	27[(b)]	Union Carbide Corp. (1977)	Good quality unpublished report. Focused on neurotoxic effect

Chemical name [FL-no]	Species/sex No/group	Route	Dose levels (mg/kg per day)	Duration	NOAEL (mg/kg per day)	Reference	Comments
(4-Heptanone [07.058])	Rat/M 8	Gavage	0, 1,000	90 days	Not detected (< 1,000)	O'Donoghue and Krasavage (1980)	(c) Good quality unpublished report
	Rat/M 3	Gavage (undiluted)	0, 1,000, 2,000, 4,000	3 weeks	Not detected (< 1,000)	Krasavage and O'Donoghue (1979)	(c) Good quality unpublished report
(2-Nonanone [07.020])	Rat/M 3	Gavage (undiluted)	0, 1,000, 2,000, 4,000	3 weeks	Not detected (< 1,000)	Krasavage and O'Donoghue (1979)	(c) Good quality unpublished report
	Rat/M 8	Gavage	0, 2,000	90 days	Not detected (< 2,000)	O'Donoghue and Krasavage (1980)	(c) Good quality unpublished report
(4-Methyl-2-pentanone [07.017])	Rat/M,F 5	Drinking water	0, 1,040	120 days	Not detected (< 1,040)	Union Carbide Corp. (1977)	Good quality unpublished report. Focused on neurotoxic effect
	Rat/F NR	Drinking water	1,000	120 days	1,000(b)	Homan and Maronpot (1978)	(c) Meeting abstract
Methyl-5-heptan-3-one [07.182]	Rat/M 5	Gavage (in distilled water)	82, 410, 820	13 weeks (5 days/ week)	82	IBM Corp. (1989)	Good quality unpublished report submitted to EPA
(2,6-Dimethyl-4-heptanone [07.122])	Rat/M 8	Gavage	0, 2,000	90 days	Not detected (< 2,000)	O'Donoghue and Krasavage (1980)	(c) Good quality unpublished report
(5-Methyl-5-hexen-2-one [07.100])	Rat/M,F 5	Diet	0, 10	14 days	10(b)	Gill and Van Miller (1987)	(d) GLP study – unpublished report
(2,6,10-Trimethyl-2,6,10-pentadecatrien-14-one [07.114])	Rat/M,F 5	Oral (gavage in maize oil)	0, 0.35, 3.5	14 days	3.5	deGroot et al. (1974)	(d) TNO Unpublished Report
9-Decen-2-one [07.262]	Rat/M,F 5	Oral (gavage in corn oil)	0, 250, 500, 1,000	28 days	1,000(e)	Flavour Industry (2009)	Good study, OECD 407

FL-no: FLAVIS number; NOAEL: no observed adverse effect level.
NR = sex not reported; M = Male; F = Female.
(a): Concentrations converted to mg/kg bw per day using conversion table for test chemical treatment doses used in PAFA (FDA, 1993).
(b): This study was performed at a single dose level that produced no adverse effects.
(c): Summarised by JECFA, 51st meeting (JECFA, 1999a).
(d): Summarised by JECFA 59th meeting (JECFA, 2003).
(e): The highest dose tested.

Developmental and reproductive toxicity data are available for two candidate substance of the present Flavouring Group Evaluation from chemical group 5 and for one supporting substance evaluated by JECFA at the 51st meetings (JECFA, 1999a). The supporting substance is listed in brackets.

Table E.3: Developmental and reproductive toxicity studies

Chemical name [FL-no]	Study type/duration	Species/sex No/group	Route	NOAEL mg/kg per day including information on possible maternal toxicity	Reference	Comments
(Isopropyl alcohol [02.079])	Reproductive toxicity: two generations with 10 weeks of dosing prior to mating	Rat/M, F 4/60	Gavage	500	Bevan et al. (1995)	(a) EPA Guideline compliance
	Developmental toxicity: gestation days 6–15	Rat/F 4/25	Gavage	400 (maternal) 400 (fetal)	Tyl et al. (1994)	(a) EPA Guideline compliance
	Developmental toxicity: gestation days 6–18	Rabbit/F 4/15	Gavage	240 (maternal) 480 (fetal)	Tyl et al. (1994)	(a) EPA Guideline compliance
Pentan-3-one [07.084]	Fertility screen: 28 daily doses with mating starting on day 10	Mouse/F 2/8	IP	50	Carlson et al. (1975)	Few details given in the paper
Pseudo-ionone [07.198]	Developmental toxicity: gestation days 8	Hamster/F 3/20 (control) and 7 or 10	Oral	960	Willhite (1986)	

FL-no: FLAVIS number; NOAEL: no observed adverse effect level.
M = Male; F = Female.
(a): Summarised by JECFA, 51st meeting (JECFA, 1999a).

In vitro mutagenicity/genotoxicity data are available for nine candidate substances of the present flavouring group evaluation from chemical group 5 and for 10 supporting substances evaluated at the 51st and 59th JECFA meetings. The supporting substances are listed in brackets.

Table E.4: Genotoxicity (*in vitro*)

Chemical name [FL-no]	Test system	Test object	Concentration	Result	Reference	Comments
(Acetone [07.050])	Rec assay	*B. subtilis*	NR	Negative[(a)]	Kawachi et al. (1980)	(h)
	Rec assay	*B. subtilis*	NR	Negative	Ishizaki et al. (1979)	(h)
	Ames test	*S.* Typhimurium TA100	0.1–1,000 μg/plate	Negative	Rapson et al. (1980)	(h)
	Ames test	*S.* Typhimurium TA98, TA100, TA1535, TA1537	174 μg/plate	Negative[(a)]	Florin et al. (1980)	(h)
	Ames test	*S.* Typhimurium TA98, TA100	NR	Negative[(a)]	Kawachi et al. (1980)	(h)
	Ames test[(b)]	*S.* Typhimurium TA98, TA100	30 μL/plate	Negative[(d)]	Yamaguchi (1985)	(h)
	Ames test	*S.* Typhimurium TA97, TA98, TA100, TA1535, TA1537	Up to 10,000 μg/plate	Negative[(a)]	McCann et al. (1975)	(h)
	Ames test[(b)]	*S.* Typhimurium TA97, TA98, TA100, TA1535, TA1537	Up to 10,000 μg/plate	Negative[(a)]	Zeiger et al. (1992)	(h)
	Ames test	*S.* Typhimurium TA100	500 μg/plate	Negative[(a)]	Yamaguchi (1982)	(h)
	Ames test	*S.* Typhimurium TA97, TA98, TA100	20–40 μg	Negative[(a)]	Azizan and Blevins (1995)	(h)
	Sister chromatid exchange	Human embryo fibroblasts	NR	Negative[(d)]	Kawachi et al. (1980)	(h)
	Sister chromatid exchange	Hamster lung fibroblasts	NR	Negative[(d)]	Kawachi et al. (1980)	(h)
	Sister chromatid exchange	Chinese hamster ovary cells	Up to 10 μg/mL	Negative	Sasaki et al. (1980)	(h)
	Sister chromatid exchange	Chinese hamster ovary cells	Up to 5,020 μg/mL	Negative[(a)]	Loveday et al. (1990)	(h)
	Sister chromatid exchange	Diploid human fibroblasts	5 μg/mL	Negative	Sasaki et al. (1980)	(h)
	Sister chromatid exchange	Human lymphocytes	395 μg/mL	Negative	Norppa et al. (1983)	(h)
	Sister chromatid exchange	Human lymphocytes	0.1–1 mM	Negative	Zarani et al. (1999)	(h)
	Chromosomal aberrations	Chinese hamster ovary cells	Up to 5,020 μg/mL	Negative[(a)]	Loveday et al. (1990)	(h)
	Chromosomal aberrations	Hamster lung fibroblasts	NR	Positive[(d)]	Kawachi et al. (1980)	(h)
	Aneuploidy induction	*S. cerevisiae*	6.98–7.83%	Positive[(d)]	Zimmermann et al. (1985)	(k)

Chemical name [FL-no]	Test system	Test object	Concentration	Result	Reference	Comments
(Isopropyl alcohol [02.079])	Ames test	*S.* Typhimurium TA98, TA100, TA1535, TA1537	174 μg/plate	Negative[a]	Florin et al. (1980)	(h)
	Ames test[b]	*S.* Typhimurium TA98, TA100, TA1535, TA1537, *E. coli* WP2uvrA	5–5,000 μg/plate	Negative[a]	Shimizu et al. (1985)	(h)
	Ames test[b]	*S.* Typhimurium TA97, TA98, TA100, TA102, TA104, TA1535, TA1537	Up to 10 mg/plate[5]	Negative[a]	Zeiger et al. (1992)	(h)
	Forward mutation	Chinese hamster ovary cells[f]	0.5–5.0 mg/mL	Negative[a]	CMA (1990)	(h)
	Forward mutation	Chinese hamster ovary cells[f]	0.5–5.0 mg/mL	Negative[a]	Kapp et al. (1993)	(h)
(2-Butanone [07.053])	Ames test	*S.* Typhimurium TA98, TA100, TA1535, TA1537, TA1538	10,000 μg/plate	Negative[a]	Douglas et al. (1980)	(h)
	Ames test	*S.* Typhimurium TA102, TA104	1 mg/plate	Negative	Marnett et al. (1985)	(h)
(2-Butanone [07.053]) continued	Ames test[b]	*S.* Typhimurium TA98, TA100, TA1535, TA1537, TA1538	5–5,000 μg/plate	Negative[a]	Shimizu et al. (1985)	(h)
	Ames test	*S.* Typhimurium TA98, TA100, TA1535, TA1537, TA1538	0.04–26 μg/plate	Negative[a]	O'Donoghue et al. (1988)	(h)
	Ames test[b]	*S.* Typhimurium TA97, TA98, TA100, TA104, TA1535, TA1537	Up to 10,000 μg/plate	Negative[a]1	Zeiger et al. (1992)	(h)
	Ames test	*S.* Typhimurium TA102	5,000 μg/plate	Negative[d]	Müller et al. (1993)	(h)
	Ames test	*S.* Typhimurium TA98, TA100, TA1535, TA1537, TA1538, *E. coli* WP2uvrA	4,000 μg/plate	Negative	Brooks et al. (1988)	(h)
	Gene conversion	*S. cerevisiae*	5 mg/mL	Negative[a]	Brooks et al. (1988)	(h)
	Forward Mutation	L5178Y/TL+/− mouse lymphoma cells	0.67–12 μg/mL	Negative[a]	O'Donoghue et al. (1988)	(h)
	Unscheduled DNA synthesis	Human lymphocytes	0.72 mg/mL	Negative[a]	Perocco et al. (1983)	(h)
	Unscheduled DNA synthesis	Rat hepatocytes	7.2–360 mg/mL	Negative	O'Donoghue et al. (1988)	(h)
	Chromosomal aberrations	Rat hepatocytes	1,000 μg/mL	Negative	Brooks et al. (1988)	(h)
	Chromosomal aberrations	Chinese hamster ovary cells	1,000 μg/mL	Negative[a]	Brooks et al. (1988)	(h)
	Cell transformation assay[a]	BALB/3T3 cells (clone A31-1)	6–18 μL/mL	Negative	O'Donoghue et al. (1988)	
	Aneuploidy induction	*S. cerevisiae*	3.38%	Positive[d]	Zimmermann et al. (1985)	(k)
Pentan-3-one [07.084]	Aneuploidy induction	*S. cerevisiae*	1.48%	Positive[d]	Zimmermann et al. (1985)	(k)

Chemical name [FL-no]	Test system	Test object	Concentration	Result	Reference	Comments
Pentan-3-ol [02.077]	Chromosomal aberrations	Chinese hamster ovary cells	0.5–10%	Negative[a]	Abbondandolo et al. (1980)	
	Forward mutation	*S. pombe*	0.5–10%	Negative[a]	Abbondandolo et al. (1980)	
(2-Heptanone [07.002])	Unscheduled DNA synthesis	Rat hepatocytes	1,000 ppm	Negative	Barber et al. (1999)	
Methyl-3-butan-2-one [07.178]	Aneuploidy induction	*S. cerevisiae*	1.23–1.36%	Negative[d]	Zimmermann et al. (1985)	(k)
	Aneuploidy induction	*S. cerevisiae*	0.84–1.23%	Negative[d]	Zimmermann et al. (1985)	(k)
(4-Methyl-2-pentanone [07.017])	Ames test	*S.* Typhimurium TA98, TA100, TA1535, TA1537, TA1538	0.03–3 mg/plate	Negative[a]	O'Donoghue et al. (1988)	(h)
	Ames test[b]	*S.* Typhimurium TA97, TA98, TA100, TA1535	Up to 6,667 μg/plate	Negative[a]	Zeiger et al. (1992)	(h)
	Ames test	*E. coli* WP2uvrA	8,000 μg/plate	Negative[d]	Brooks et al. (1988)	(h)
	Gene conversion	*S. cerevisiae*	5 mg/mL	Negative[a]	Brooks et al. (1988)	(h)
	Forward mutation	L5178Y/TL+/– mouse lymphoma cells	0.26–4.2 μg/mL	Negative[a]	O'Donoghue et al. (1988)	(h)
	Unscheduled DNA synthesis	Rat hepatocytes	8–80 μg/mL	Negative	O'Donoghue et al. (1988)	(h)
	Chromosomal aberrations	Rat hepatocytes	1,000 μg/mL	Negative	Brooks et al. (1988)	(h)
	Cell transformation assay[a]	BALB/3T3 cells (clone A31-1)	1–7 μL/mL	Negative	O'Donoghue et al. (1988)	
	Chromosomal aberrations	Chinese hamster ovary cells	1,000 μg/mL	Negative[a]	Brooks et al. (1988)	(h)
Methyl-4-pentan-2-ol [02.183]	Ames test[b]	*S.* Typhimurium TA98, TA100, TA1535, TA1537, TA1538, *E. coli* WP2uvrA	5,000 μg	Negative[a]	Shimizu et al. (1985)	
Methyl-6-heptan-2-one [07.181]	Ames test	*S.* Typhimurium TA98, TA100, TA1535, TA1537	5,000 μg/plate	Negative[a]	BASF (1989a)	
(2,6-Dimethyl-4-heptanone [07.122])	Ames test[b]	*S.* Typhimurium TA98, TA100, TA1535, TA1537	1–333 μg/plate	Negative[a]	Mortelmans et al. (1986)	(h)
Trimethyl-6,10,14-pentadecan-2-one [07.205]	Ames test	*S.* Typhimurium TA98, TA100, TA1535, TA1537	5,000 μg/plate	Negative[a]	BASF (1989b)	
(6-Methyl-5-hepten-2-one [07.015])	Reverse mutation	*S.* Typhimurium TA98, TA100, TA1535, TA1537	380 μg/plate	Negative[a]	(Florin et al. 1980)	(i)

Chemical name [FL-no]	Test system	Test object	Concentration	Result	Reference	Comments
(Isopropyl acetate [09.003])	Ames test[b]	*S.* Typhimurium TA97, TA98, TA100, TA1537, TA1538	Up to 10 mg/plate	Negative[a]	Zeiger et al. (1992)	[h]
(Isopropyl myristate [09.105])	Ames test[g]	*S.* Typhimurium TA98, TA100, TA1535, TA1537, TA1538	50 μg/plate	Negative[a]	Blevins and Taylor (1982)	[h]
Isopropyl hexadecanoate [09.606]	Ames test[g]	*S.* Typhimurium TA98, TA100, TA1535, TA1537, TA1538	50 μg/plate	Negative[a]	Blevins and Taylor (1982)	
9-Decen-2-one [07.262]	Ames test[j]	*S.* Typhimurium TA98, TA100, TA1535, TA1537	Up to 5 μL/plate	Negative[a]	Flavour Industry (2009)	
	Ames test[j]	*E. coli* WP2 (pKM 101)	Up to 5 μL/plate	Negative[a]	Flavour Industry (2009)	
(6-Methylhepta-3,5-dien-2-one [07.099])	Reverse mutation	*S.* Typhimurium TA98, TA100, TA1535, TA1537	370 μg/plate	Negative[a]	Florin et al. (1980)	
	Reverse Mutation	*S.* Typhimurium TA98, TA100, TA1535, TA1537, TA 102	1.6, 8, 40, 200, 1,000 and 5,000 μg/plate	Negative[a]	Williams (2009)	Toxicity observed in all strains at 2,000 μg/plate or greater in the absence of S9 and at 800 μg/plate in the presence of S9. Study design complied with current recommendations. Acceptable top concentration was achieved
	Micronucleus induction	Human peripheral blood lymphocytes	225, 325 and 450 μg/mL[m] 225, 300 and 350 μg/mL[n]	Negative	Whitwell (2010)	Complies with draft OECD guideline 487. Acceptable levels of cytotoxicity achieved at the top concentrations used in all parts of the study

Chemical name [FL-no]	Test system	Test object	Concentration	Result	Reference	Comments
Pseudo-ionone [07.198]	Ames test	*S.* Typhimurium TA98, TA100, TA1535, TA1537	20.48, 51.2, 128, 320, 800, 2000 and 5,000 μg/plate [(l)]	Negative[(a)]	Florin et al. (1980)	
	Reverse Mutation	*S.* Typhimurium TA98, TA100, TA1535, TA1537, TA 102	0.128, 0.64, 3.2, 16, 80, 400 and 2,000 μg/plate	Negative[(a)]	(Beevers, 2009)	Toxicity was observed in all strains at 400 μg/plate and greater in the presence and absence of S9 in this experiment
			0, 12.5, 25, 50, 100, 200 and 400 μg/plate[(l)]	Negative[(a)]		Precipitation was observed in the 400 μg/plate concentration in the presence and absence of S9 in this experiment. Study design complies with current recommendations. Acceptable top concentrations were achieved
	Micronucleus induction	Human peripheral blood lymphocytes	30, 50 and 60 μg/mL[(m)]; 100, 110 and 120 μg/mL[(n)]	Negative	Lloyd (2010)	Complies with draft OECD guideline 487. Acceptable levels of cytotoxicity achieved at the top concentrations used in all parts of the study
	Micronucleus induction	Human peripheral blood lymphocytes	10, 15 and 20 μg/mL[(o)]	Negative	Lloyd (2010)	Complies with draft OECD guideline 487. Acceptable levels of cytotoxicity achieved at the top concentrations used in all parts of the study

FL-no: FLAVIS number.
(a): Assay performed with and without metabolic activation.
(b): Modified Ames (pre-incubation) protocol.
(c): Assay performed with S9 metabolic activation.
(d): Assay performed without S9 metabolic activation.
(e): Maximum non-toxic dose.
(f): HGPRT locus.
(g): Spot test.
(h): Summarised by JECFA, 51st meeting (JECFA, 1999a).
(i): Summarised by JECFA 59th meeting (JECFA, 2003).
(j): Direct incorporation method.

(k): Unusual experimental protocol for detection of aneuploidy, which can be considered a threshold effect not mediated by a direct interaction with DNA. Positive results were obtained at concentrations approaching cytotoxic levels and are very likely due to the presence of technical artefacts (low temperature treatment inducing tubulin dissociation). Indeed, absence of effect was recorded when the ice treatment was skipped. – The limited relevance of fungal systems together with the uncertain quality of these results make questionable their extrapolation to the *in vivo* situation in humans.
(l): Assay modified with pre-incubation in the presence of S9.
(m): Without metabolic activation, 3 h treatment + 21 h recovery.
(n): With metabolic activation, 3 h treatment + 21 h recovery.
(o): Without metabolic activation, 24 h + 0 h recovery.

In vivo mutagenicity/genotoxicity data available for four supporting substances evaluated at the 51st and 59th JECFA meetings. The supporting substances are listed in brackets.

Table E.5: Genotoxicity *in vivo*

Chemical name	Test system	Test object	Route	Dose	Result	Reference	Comments
(Isopropyl alcohol [02.079])	Micronucleus test	ICR Mouse (15 M & 15 F)	IP injection in 0.9% NaCl	350–2,500 mg/kg	Negative	Kapp et al. (1993)	(a)
(Acetone [07.050])	Micronucleus test	Chinese hamster (5 M & 5 F)	IP injection in corn oil	865 mg/kg	Negative	Basler (1986)	(a)
(2-Butanone [07.053])	Micronucleus test	CD-1 mice (5 M & 5 F)	IP injection in corn oil	LD_{20} (1.96 mL/kg)	Negative	O'Donoghue et al. (1988)	(a)
	Micronucleus test	Chinese hamster (5 M & 5 F)	IP injection in corn oil	411 mg/kg	Negative	Basler (1986)	(a)
(4-Methyl-2-pentanone [07.017])	Micronucleus test	CD-1 mice (5 M & 5 F)	IP injection in corn oil	LD_{20} (0.73 mL/kg)	Negative	Basler (1986)	(a)

M = Male; F = Female; IP: intraperitoneal; LD_{20}: lethal dose, 20%.
(a): Summarised by JECFA, 51st meeting (JECFA, 1999a).

Table E.6: Summary of *in vitro* mutagenicity study considered by the Panel in FGE.205Rev1

Chemical name FL-no	Test	Test object	Concentration tested and test conditions	Result	Reference	Comments
Oct-1-en-3-one [07.081]	Bacterial reverse mutation assay	*S.* Typhimurium TA100	7.8–500 μg/plate[(a),(b)]	Positive	Bowen (2013)	

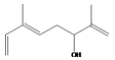
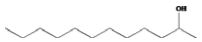
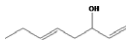
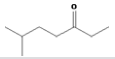
FL-no: FLAVIS number.
(a): With and without metabolic activation.
(b): The following free radical/electrophile scavengers were added: glutathione, *N*-acetyl cysteine, catalase, 2,5-dimethylfuran.

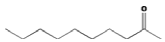
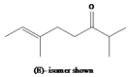
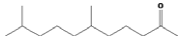
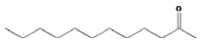
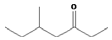
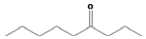
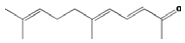
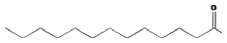
Table E.7: Summary of *in vivo* genotoxicity data considered by the Panel in FGE.205Rev1

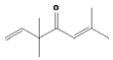
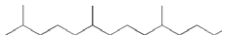
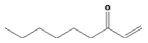
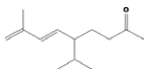
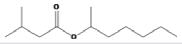
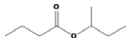
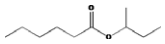
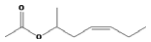
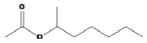
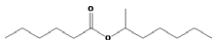
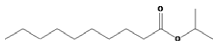
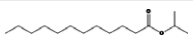
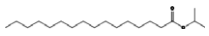
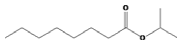
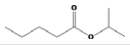
Chemical name FL-no	Test system *in vivo*	Test object	Route	Dose	Result	Reference	Comments
Pent-1-en-3-one [07.102]	Micronucleus Assay	Han Wistar Rat; M	Gavage	0, 10, 20 and 40 mg/kg bw per day	Negative	Keig-Shevlin (2015b,c)	
	Comet assay	Han Wistar Rat; M	Gavage		Negative[(a),(b)]		
Oct-1-en-3-one [07.081]	Comet assay	Han Wistar Rat; M	Gavage	0, 45, 90 and 180 mg/kg bw per day	Negative[(a)]	Keig-Shevlin (2015a)	

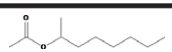
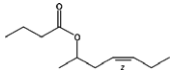
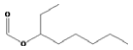
FL-no: FLAVIS number; bw: body weight.
(a): Scored in liver cells.
(b): Scored in duodenum cells.

Appendix F – Natural food occurrence

FL-no	EU register name	Structural formula	CAS no	VCF* online search 15-11-2016
02.077	Pentan-3-ol		584-02-1	Quantified in apricot, grape, grape brandy, guinea hen, loquat, milk and milk products, mushroom, olive, papaya, red currants, rum, shrimps from trace amount up to 1.3 mg/kg and up to 34 mg/kg in tea. Has been identified in a further 36 food items
02.124	6-Methylhept-5-en-2-ol		1569-60-4	Quantified in annatto, litchi, macadamia nut, tomato from 0.0125 mg/kg and up to 50 mg/kg in citrus fruits. Has been identified in a further 18 food items
02.131	But-3-en-2-ol		598-32-3	Identified in citrus fruits
02.142	3,3-Dimethylbutan-2-ol		464-07-3	Identified in melon
02.145	2,6-Dimethylocta-1,5,7-trien-3-ol		29414-56-0	Quantified in *Salvia* species up to 100 mg/kg
02.148	Dodecan-2-ol		10203-28-8	Quantified in mastic up to 1,300 mg/kg. Identified in apple, banana, beer and cheddar cheese
02.177	2-Methylhexan-3-ol		617-29-8	Quantified in tomato up to 2.5 mg/kg
02.182	3-Methylpentan-2-ol		565-60-6	Quantified in pineapple up to 0.009 mg/kg. Identified in *Capsicum* species, date, shrimps and tea
02.183	4-Methylpentan-2-ol		108-11-2	Quantified in annatto and citrus fruits from 0.027 up to 0.111 mg/kg. Identified in apple brandy, bantu beer, cocoa, peanut and peas
02.187	Non-1-en-3-ol		21964-44-3	Identified in banana, beef, chervil, date and rambutan
02.190	Nonan-3-ol		624-51-1	Identified in banana, beef, cherimoya, chervil, date, guava and feyoa, mentha oils and passion fruit
02.194	Octa-1,5-dien-3-ol		83861-74-9	Quantified in cheese (various types), fish and oysters from 0.025 up to 0.26 mg/kg. Identified in chicken, scallop and tea
02.211	Undeca-1,5-dien-3-ol		56722-23-7	Identified in fish and katsuobushi
02.255	(*Z*)-4-Hepten-2-ol		66642-85-1	Identified in maize
07.072	6-Methylheptan-3-one		624-42-0	Identified in melon and potato
07.084	Pentan-3-one		96-22-0	Quantified in guava and feyoa, *Mangifera* species, milk and milk products, mushroom, olive, passion fruit and shrimps from 0.0007 up to 14 mg/kg. Identified in a further 41 food items

FL-no	EU register name	Structural formula	CAS no	VCF* online search 15-11-2016
07.150	Decan-2-one		693-54-9	Quantified in blue cheeses, cheese various types, chicken, milk and milk products, mountain papaya and shrimps from trace amounts up to 2.5 mg/kg and up to 2,600 mg/kg hop oil. Identified in a further 42 food items
07.156	2,6-Dimethyloct-6-en-3-one (mixture of *E* and *Z*)		90975-15-8 Search on substance name	Quantified up to 0.05 mg/kg in citrus fruits
07.157	6,10-Dimethylundecan-2-one		1604-34-8 Search on substance name	Quantified in up to 0.002 mg/kg in *Vaccinium* species. Identified in buckwheat, coffee, mate, rooibos tea and tea
07.158	Dodecan-2-one		6175-49-1	Quantified in blue cheeses, chicken, cocoa category and milk and milk products from 0.0014 up to 1.8 mg/kg and up to 2,700 mg/kg in hop oil
07.160	Heptadecan-2-one		2922-51-2	Quantified in blue cheeses, cocoa category, *Mangifera* species and milk and milk products from trace amount up to 8.7 mg/kg and up to 100 mg/kg in hop oil
07.161	Hex-1-en-3-one		1629-60-3	Quantified in artichoke up to 0.00014 mg/kg. Identified in cocoa category, dill, honey, milk and milk products and passion fruit
07.162	Hex-5-en-2-one		109-49-9	No product occurrence data
07.178	3-Methylbutan-2-one		563-80-4	Quantified in cheese various types, guava and feyoa, guinea hen, honey, milk and milk products, passion fruit, peanut and strawberry from trace amount up to 1.56 mg/kg and up to 14 mg/kg in hog plum. Identified in a further 23 food items
07.181	6-Methylheptan-2-one		928-68-7	Quantified in chicken, guinea hen and wine from 0.001 up to 0.1 mg/kg. Identified in beef, buckwheat, mate, peas and tea
07.182	5-Methylheptan-3-one		541-85-5	Quantified in lemon grass oil (14,300 mg/kg), mentha oils (1 mg/kg) and papaya (0.02 mg/kg). Identified in tomato
07.185	3-Methylpentan-2-one		565-61-7	Quantified in beer, dill, Filbert hazelnut, plum and tea from trace amount up to 1.7 mg/kg and up to 100 mg/kg in hop oil. Identified in apple brandy, beef, blue cheeses, cheese various types, egg, grape and peanut
07.189	Nonan-4-one		4485-09-0	Quantified in passion fruit up to 0.01 mg/kg and identified in beef
07.198	Pseudo-ionone		141-10-6	Quantified in licorice, tea and tomato from trace amount up to 5 mg/kg. Identified in mate, passion fruit and tamarind
07.199	Tetradecan-2-one		2345-27-9	Quantified in milk and milk products, mountain papaya and passion fruit from 0.01 up to 2.5 mg/kg and up to 1,600 mg/kg in hop. Identified in beef, cherimoya, ginger, lamb and mutton and mate
07.201	Tridec-12-en-2-one		60437-21-0	No product occurrence data

FL-no	EU register name	Structural formula	CAS no	VCF* online search 15-11-2016
07.204	3,3,6-Trimethylhepta-1,5-dien-4-one		546-49-6	Quantified in camomile from 500 up to 5,100 mg/kg and identified in tarragon
07.205	6,10,14-Trimethylpentadecan-2-one		502-69-2	Quantified in camomile, grape, lemon balm, mastic, tea and *Vaccinium* species from 0.007 up to 2,000 mg/kg and up to 50,000 mg/kg in maize. Identified in a further 12 food items
07.210	1-Nonene-3-one		24415-26-7	No product occurrence data
07.236	(*Z*)-5-Octen-2-one		22610-86-2	Identified in beans
07.239	[*R*-(*E*)]-5-Isopropyl-8-methylnona-6,8-dien-2-one		2278-53-7	No product occurrence data
07.262	9-Decen-2-one		35194-30-0	No product occurrence data
09.304	*sec*-Heptyl isovalerate		238757-71-6	Identified in banana
09.323	*sec*-Butyl acetate		105-46-4	Quantified in vinegar from 43 up to 67 mg/kg. Identified in banana, beans, beer, cheddar cheese, cheese various types, cocoa category, coffee, potato and walnut
09.325	*sec*-Butyl butyrate		819-97-6	Quantified in strawberry from 0.0054 up to 0.0086 mg/kg and identified in cheddar cheese, cheese various types, custard apple, atemoya, plum and tomato
09.328	*sec*-Butyl formate		589-40-2	Identified in apple fresh and cheese various types
09.332	*sec*-Butyl hexanoate		820-00-8	No product occurrence data
09.386	*sec*-Hept-4(*cis*)-enyl acetate		94088-33-2	Quantified in banana up to 0.18 mg/kg
09.388	*sec*-Heptyl acetate		5921-82-4	Quantified in guava, feyoa and passion fruit from 0.01 up to 0.563 mg/kg and up to 400 mg/kg in cloves. Identified in banana, beans, soybean and strawberry
09.391	*sec*-Heptyl hexanoate		6624-58-4	Quantified in passion fruit from 0.036 up to 6,634 mg/kg and identified in banana and strawberry
09.604	Isopropyl decanoate		2311-59-3	Identified in blue cheeses, citrus fruits and strawberry
09.605	Isopropyl dodecanoate		10233-13-3	Identified in blue cheeses and melon
09.606	Isopropyl hexadecanoate		142-91-6	Quantified in macadamia nut up to 0.04 mg/kg and identified in buckwheat and citrus fruits
09.608	Isopropyl octanoate		5458-59-3	Identified in blue cheeses, nectarine and strawberry
09.609	Isopropyl valerate		18362-97-5	Identified in cashew apple, cheddar cheese and vanilla

FL-no	EU register name	Structural formula	CAS no	VCF* online search 15-11-2016
09.676	*sec*-Octyl acetate		2051-50-5	Identified in chicken
09.880	(*Z*)-Hept-4-enyl-2 butyrate		233666-01-8	No product occurrence data
09.926	Octan-3-yl formate		84434-65-1	No product occurrence data

*: Triskelion, VCF online, Volatile Compounds in Food. Version 16.2 released by 16 January 2016.