EFSA CEF Panel (EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids), 2014. Scientific Opinion on Flavouring Group Evaluation 11, Revision 3 (FGE.11Rev3): Aliphatic dialcohols, diketones, and hydroxyketones from chemical groups 8 and 10

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SCIENTIFIC OPINION

Scientific Opinion on Flavouring Group Evaluation 11, Revision 3 (FGE.11Rev3): Aliphatic dialcohols, diketones, and hydroxyketones from chemical groups 8 and 10[1]

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

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

The Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids of the European Food Safety Authority was requested to evaluate 11 flavouring substances in the Flavouring Group Evaluation 11, Revision 3, using the Procedure in Commission Regulation (EC) No 1565/2000. The substances were evaluated through a stepwise approach (the Procedure) that integrates information on structure-activity relationships, intake from current uses, toxicological threshold of concern and available data on metabolism and toxicity. The present Revision of FGE.11, FGE.11Rev3, is prepared due to the evaluation of genotoxicity data, which have been requested in the previous version of FGE.11, for 3-methylnona-2,4-dione [FL-no: 07.184]. Additionally, new information on the stereoisomerism of [FL-no: 07.184 and 07.260] has become available. Based on the new data received the Panel concluded that all 11 flavouring substances [FL-no: 02.133, 07.071, 07.097, 07.152, 07.165, 07.167, 07.168, 07.184, 07.238, 07.248 and 07.260] do not give rise to safety concerns at their levels of dietary intake, estimated on the basis of the MSDI approach. Besides the safety assessment of these flavouring substances, the specifications for the materials of commerce have also been considered. Specifications including complete purity criteria and identity for the materials of commerce have been provided for all candidate substances.

KEY WORDS

flavourings, safety, alpha-diketones, ketals, hydroxyketones, diols, FGE.11

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[1] On request from the European Commission, Question No EFSA-Q-2014-00145, adopted on 23 October 2014.

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[3] Acknowledgement: The Panel wishes to thank the members of the Working Groups on Flavourings: Ulla Beckman Sundh, Leon Brimer, Angelo Carere, Karl-Heinz Engel, Henrik Frandsen, Rainer Gürtler, Trine Husøy, Wim Mennes, Gerard Mulder and Harriet Wallin and the hearing experts: Vibe Beltoft, Pia Lund and Karin Nørby for the preparatory work on this scientific opinion and EFSA staff: Maria Carfí, Annamaria Rossi and Kim Rygaard Nielsen for the support provided to this scientific opinion.

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SUMMARY

The European Food Safety Authority (EFSA) asked the Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (the Panel) to deliver a scientific advice to the Commission on the implications for human health of chemically defined flavouring substances used in or on foodstuffs in the Member States. In particular, the Panel was requested to evaluate 11 flavouring substances in the Flavouring Group Evaluation 11, Revision 3 (FGE.11Rev3), using the Procedure as referred to in the Commission Regulation (EC) No 1565/2000. These 11 flavouring substances belong to chemical group 8 and 10, Annex I of the Commission Regulation (EC) No 1565/2000.

The present flavouring group includes 11 candidate substances; eight alpha-diketones or their corresponding alcohols or ketals [FL-no: 02.133, 07.071, 07.152, 07.167, 07.168, 07.238, 07.248 and 07.260] and three β-diketones or their corresponding β-hydroxyketones (of which one is a tertiary alcohol) [FL-no: 07.165, 07.097 and 07.184] all belonging to chemical groups 8 and 10.

The present Revision of FGE.11, FGE.11Rev3, is prepared due to the evaluation of genotoxicity data, which have been requested in the previous version of FGE.11 for 3-methylnona-2,4-dione [FL-no: 07.184]. Additionally, new information on natural occurrence in food [FL no: 07.165, 07, 184 and 07.238] and on the stereoisomerism for [FL-no: 07.184 and 07.260] and composition for one substance [FL-no: 07.097] has become available.

Two of the flavouring substances possesses two chiral centres [FL-no: 02.133 and 07.168] and five substances possesses one chiral centre [FL-no: 07.097, 07.167, 07.184, 07.238 and 07.260]. For all substances, the stereoisomeric composition has been specified sufficiently.

Five of the flavouring substances are classified in structural class I, five in structural class II and one in structural class III.

Nine of the flavouring substances in the present group have been reported to occur naturally in a wide range of food items.

In its evaluation, the Panel as a default used the "Maximised Survey-derived Daily Intake" (MSDI) approach to estimate the per capita intakes of the flavouring substances in Europe. However, when the Panel examined the information provided by the European Flavour Industry on the use levels in various foods, it appeared obvious that the MSDI approach in a number of cases would grossly underestimate the intake by regular consumers of products flavoured at the use level reported by the Industry, especially in those cases where the annual production values were reported to be small. In consequence, the Panel had reservations about the data on use and use levels provided and the intake estimates obtained by the MSDI approach.

In the absence of more precise information that would enable the Panel to make a more realistic estimate of the intakes of the flavouring substances, the Panel has decided also to perform an estimate of the daily intakes per person using a "modified Theoretical Added Maximum Daily Intake" (mTAMDI) approach based on the normal use levels reported by Industry. In those cases where the mTAMDI approach indicated that the intake of a flavouring substance might exceed its corresponding threshold of concern, the Panel decided not to carry out a formal safety assessment using the Procedure. In these cases the Panel requires more precise data on use and use levels.

According to the default MSDI approach, the candidate substances have European intakes ranging from 0.0012 to 15 µg/*capita*/day, which are below the thresholds of concern for structural class I, II and III (1800, 540 and 90 µg/person/day, respectively). The total combined estimated levels of intake of candidate and supporting substances (all from structural class II) is approximately 4600 µg/*capita*/day, which exceeds the threshold of concern for structural class II (540 µg/person/day). However, based on information on efficient metabolism and on presence in the body as endogenous

compounds, there are no safety concerns from the combined intakes of the candidate and supporting substances.

Data available for the flavouring substance 3-methyl-2,4-nonadione [FL-no: 07.184] shows that this substance has no genotoxic potential *in vitro*. For the remaining flavouring substances in the present FGE, genotoxicity data are only available for a limited number, and the genotoxicity could not be assessed adequately. However, the genotoxicity data available do not preclude evaluation using the Procedure.

The candidate flavouring substances in this FGE are expected to be metabolised to innocuous products.

It was noted that where toxicity data were available they were consistent with the conclusions in the present flavouring group evaluation using the Procedure.

It is considered that on the basis of the default MSDI approach the 11 candidate substances evaluated through the Procedure [FL-no: 02.133, 07.071, 07.097, 07.152, 07.165, 07.167, 07.168, 07.184, 07.238, 07.248 and 07.260] would not give rise to safety concerns at the estimated levels of intake arising from their use as flavouring substances.

When the estimated intakes were based on the mTAMDI approach they ranged from 1600 to 3900 µg/person/day for the five candidate substances from structural class I, from 1500 to 5400 µg/ person/day for the five candidate substances from structural II and for the one substance from structural class II the mTAMDI is 1600 µg/person/day. So for seven candidate substances evaluated through the Procedure [FL-no: 02.133, 07.071, 07.152, 07.168, 07.184, 07.248 and 07.260] the intakes, estimated on the basis of the mTAMDI exceed the threshold for the structural class, to which the flavouring substances have been assigned. Therefore, more reliable exposure data are required. On the basis of such additional data, the substances should be reconsidered along the steps of the Procedure. Following this procedure additional toxicological data might become necessary. In order to determine whether the conclusion for the candidate substances can be applied to the materials of commerce, it is necessary to consider the available specifications. Adequate specification and data on stereoisomerism are available for all the candidate substances.

Thus, all 11 substances [FL-no: 02.133, 07.071, 07.097, 07.152, 07.165, 07.167, 07.168, 07.184, 07.238, 07.248 and 07.260] evaluated in the present FGE would not present any safety concern at the estimated levels of intake based on the MSDI approach.

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BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

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

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

EFSA has evaluated 12 flavouring substances in the Flavouring Group Evaluation 11 Revision 2 (FGE.11Rev2). The Opinion was adopted on17 June 2009. EFSA concluded that the final evaluation could not be performed for the substance [FL-no: 07.184], further data on genotoxicity are required before it can be evaluated through the Procedure.

The requested information on 3-methyl-2,4-nonadione [FL-no: 07.184] has now been submitted by the applicant.

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

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

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

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

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

[6] Commission Regulation 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. OJ L 180, 19.7.2000, p. 8-16.

ASSESSMENT

1. History of the evaluation of the substances in the present FGE

The first version of the Flavouring Group Evaluation 11, FGE.11 dealt with six flavouring substances, α- and β-diketones, one related ketal, hydroxyketones, and diols.

The first Revision of FGE.11, FGE.11Rev1 included the assessment of two additional candidate substances [FL-no: 07.238 and 07.260]. One flavouring substance, pentan-2,4-dione (former candidate substance [FL-no: 07.191]) was deleted from the Register of flavouring substances as it is considered genotoxic *in vitro* and *in vivo*.

The second Revision of FGE.11, FGE.11Rev2, included the assessment of five additional candidate substances [FL-no: 06.134, 07.097, 07.168, 07.184 and 07.248]. The candidate substance 3-methyl-2,4-nonadione [FL-no: 07.184] contains a structural 2,4-dione element which is considered genotoxic *in vitro* and *in vivo.* Due to the structural alert for genotoxicity the Procedure was not applied for 3-methyl-2,4-nonadione [FL-no: 07.184] and accordingly additional data on genotoxicity were required. For the candidate substance diacetyl trimer [FL-no: 06.134] additional metabolism and toxicity data were required.

Since the publication of FGE.11Rev1 and the Minutes from the 7th Plenary meeting in which the conclusion on the FGE.11Rev2 was summarised, information on stereoisomeric composition and a boiling point has been provided by EFFA on the following six substances: [FL-no: 02.133, 07.097, 07.167, 07.168, 07.238 and 07.260] (EFFA, 2010).

FGE	Opinion adopted by EFSA	Link	No. of candidate substances
FGE.11	9 December 2004	http://www.efsa.europa.eu/en/efsajournal/doc/166.pdf	6
FGE.11Rev1	17 April 2007	http://www.efsa.europa.eu/en/efsajournal/doc/493.pdf	7
FGE.11Rev2	17 June 2009	http://www.efsa.europa.eu/en/efsajournal/pub/1170.htm	12
FGE.11Rev3			11

The present Revision of FGE.11, FGE.11Rev3, includes the re-evaluation of 3-methylnona-2,4-dione [FL.no: 07.184] as additional genotoxicity data (*in vitro* micronucleus) have been submitted (Watters, 2013). A search in the open literature did not provide any further relevant data on toxicity or metabolism for the substance, but additional data on natural occurenec in food for [Fl-no: 07.165, 07.184 and 07.238] was found and has been included.

Information on stereoisomerism or on composition has been provided for three substances [FL-no: 07.097, 07.184 and 07.260] (EFFA, 2014). These data are also included in the present revision.

In addition, the publication of FGE.11Rev2, one of the 12 candidate substances is no longer supported by the Industry for use as flavouring substance in Europe (DG SANCO, 2012). The substance is diacetyl-trimer [FL-no: 06.134] and the substance will therefore not be considered any further.

2. Presentation of the Substances in Flavouring Group Evaluation 11, Revision 3

2.1. Description

The present Flavouring Group Evaluation 11, Revision 3 (FGE.11Rev3), using the Procedure as referred to in the Commission Regulation (EC) No 1565/2000 (the Procedure – shown in schematic form in Appendix A), deals with eight α-diketones or their corresponding alcohols or ketal and one β-diketone and two β-hydroxy ketones. These 11 flavouring substances (candidate substances) belong to chemical groups 8 and 10 of Annex I of Regulation (EC) No 1565/2000 (EC, 2000).

The 11 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, structures and specifications. This group of candidate substances includes eight α-diketones or their corresponding alcohols or ketals [FL-no: 02.133, 07.071, 07.152, 07.167, 07.168, 07.238, 07.248 and 07.260], and three β-diketone or their corresponding alcohols (of which one is a tertiary alcohol) [FL-no: 07.097, 07.165 and 07.184].

The outcome of the safety evaluation is summarised in Table 6. The hydrolysis products anticipated for the candidate ketals are listed in Table 7.

The 11 candidate substances are closely related structurally to 13 aliphatic acyclic α-diketones and related α-hydroxyketones (supporting substances) evaluated at the 51st meeting of the Joint FAO/WHO Expert Committee on Food Additives (the JECFA) in the group "Aliphatic acyclic and alicyclic α-diketones and related α-hydroxyketones" (JECFA, 1999a). The names and structures of the 13 supporting substances are listed in Table 8, together with their evaluation status.

2.2. 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 material of commerce. Flavouring substances with different configurations should have individual chemical names and codes (CAS number, FLAVIS number, etc.).

Two of the 11 candidate substances possess two chiral centres [FL-no: 02.133 and 07.168] and five substances possess one chiral centre [FL-no: 07.097, 07.167, 07.184, 07.238 and 07.260]. Adequate information on the stereoisomeric composition of these substances has been provided.

SUMMARY OF SPECIFICATION DATA

Table 1: Specification Summary of the Substances in the Flavouring Group Evaluation 11, Revision 3

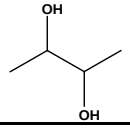
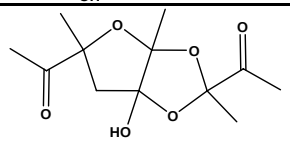
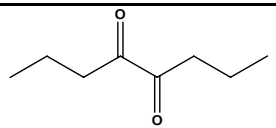
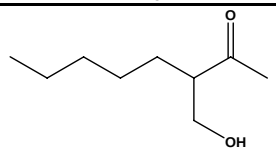
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility [a] Solubility in ethanol [b]	Boiling point, °C [c] Melting point, °C ID test Assay minimum	Refrac. Index [d] Spec.gravity [e]	Specification comments
02.133	Butane-2,3-diol		10181 513-85-9	Liquid $C_4H_{10}O_2$ 90.12	Soluble Freely soluble	181 MS 95 %	1.432-1.438 1.001-1.007	Racemate (EFFA, 2010).
06.134	Diacetyl-trimer		4303 18114-49-3	Solid $C_{12}H_{18}O_6$ 258.27	Soluble Soluble	 90 MS 95 %	n.a. n.a.	No longer supported by Industry, (DG SANCO, 2012). Stereoisomeric composition not specified by CASrn in Register. Register name to be changed to: 1,1'-(tetrahydro-6a-hydroxy-2,3a,5-trimethylfuro[2,3-d]-1,3-dioxole-2,5-diyl)bis-ethanone.
07.071	Octane-4,5-dione		4533 2141 5455-24-3	Liquid $C_8H_{14}O_2$ 142.20	Slightly soluble Freely soluble	168 MS 95 %	1.415-1.421 0.907-0.913	
07.097	3-(Hydroxymethyl)octan-2-one		3292 11113 59191-78-5	Liquid $C_9H_{18}O_2$ 158.24	Slightly soluble Freely soluble	80 (0.3 hPa) NMR 92 %	1.416-1.422 0.874-0.878	Racemate (EFFA, 2010). Min. Assay value 92 %, secondary component: 5 - 7 % 3-methylene-2-octanone (EFFA, 2014).

Table 1: Specification Summary of the Substances in the Flavouring Group Evaluation 11, Revision 3

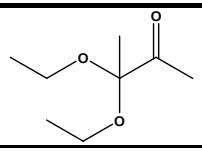
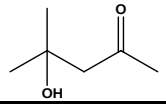
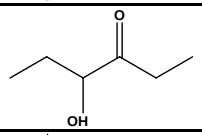
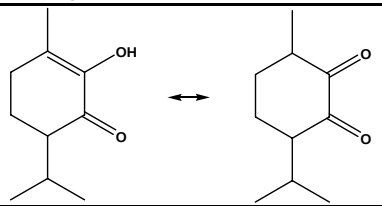
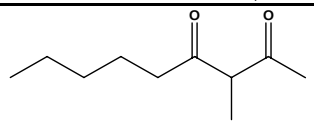
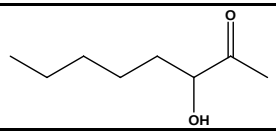
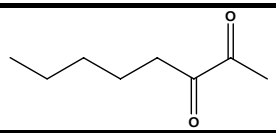
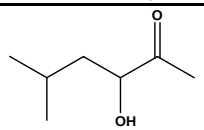
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility [a] Solubility in ethanol [b]	Boiling point, °C [c] Melting point, °C ID test Assay minimum	Refrac. Index [d] Spec.gravity [e]	Specification comments
07.152	3,3-Diethoxybutan-2-one		51933-13-2	Liquid $C_8H_{16}O_3$ 160.21	Slightly soluble Freely soluble	164 MS 95 %	1.400-1.406 0.919-0.925	
07.165	4-Hydroxy-4-methylpentan-2-one		123-42-2	Liquid $C_6H_{12}O_2$ 116.16	Slightly soluble Freely soluble	165 MS 95 %	1.418-1.424 0.929-0.935	
07.167	4-Hydroxyhexan-3-one		11108 4984-85-4	Liquid $C_6H_{12}O_2$ 116.16	Sparingly soluble Freely soluble	167 MS 95 %	1.422-1.428 0.949-0.955	Racemate (EFFA, 2010).
07.168	2-Hydroxypiperitone		4143 490-03-9	Solid $C_{10}H_{16}O_2$ 168.24	Slightly soluble Freely soluble	233 82 NMR MS 98 %	n.a. n.a.	Racemate (EFFA, 2010).
07.184	3-Methylnona-2,4-dione		4057 113486-29-6	Liquid $C_{10}H_{18}O_2$ 170.25	Practically insoluble or insoluble Freely soluble	52 (0.13 hPa) IR NMR MS 97 %	1.448-1.454 0.923-0.927	Racemate (EFFA, 2014).
07.238	3-Hydroxy-2-octanone		4139 37160-77-3	Liquid $C_8H_{16}O_2$ 144.21	Practically insoluble or insoluble Freely soluble	91 (13 hPa) MS 95 %	1.431-1.437 0.927-0.933	Racemate (EFFA, 2010).

Table 1: Specification Summary of the Substances in the Flavouring Group Evaluation 11, Revision 3

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
07.248	Octan-2,3-dione		4060 585-25-1	Liquid $C_8H_{14}O_2$ 142.2	Soluble Freely soluble	58 (1.3 hPa) IR NMR MS 95 %	1.419-1.424 0.905-0.913	
07.260	1- or 3-Hydroxy-5-methyl-2- or 3-hexanone	 3-Hydroxy-5-methyl-2-hexanone shown	3989 163038-04-8	Liquid $C_7H_{14}O_2$ 130.18	Soluble Soluble	171-173 MS 95 %	0.921-0.933 1.419-1.431	Register name to be changed to 1-hydroxy-5-methyl-3-hexanone and 3-hydroxy-5-methyl-2-hexanone. 75-77 % 3-hydroxy-5-methyl-2-hexanone and 20-22 % 2-hydroxy-5-methyl-3-hexanone. Mixture of diastereoisomers (25 % of each) (EFFA, 2014).

(a): Solubility in water, if not otherwise stated.
(b): Solubility in 95 % ethanol, if not otherwise stated.
(c): At 1013.25 hPa, if not otherwise stated.
(d): At 20°C, if not otherwise stated.
(e): At 25°C, if not otherwise stated.

2.3. Natural Occurrence in Food

Nine of the 11 candidate substances have been reported to occur in fruits, fruit juice, vegetables, bread, cheese, fish, meat, peanuts, cocoa, wine, drinks, beer, tea, and coffee. Quantitative data on the natural occurrence in food have been reported for seven of these substances:

Table 2: Candidate Substances Reported to Occur in Food (EFFA, 2004b; Flavour Industry, 2005b; TNO, 2000; TNO, 2014)

FL-no:	Name:	Quantitative data reported
02.133	Butane-2,3-diol	0.006 mg/kg in fish (lean), up to 90 mg/kg in cheddar cheese, up to 2.3 mg/kg in raspberry, up to 850 mg/kg in vinegar, up to 95 mg/kg in sherry and up to 2900 mg/kg in various types of wine
07.152	3,3-Diethoxybutan-2-one	Up to 0.1 mg/kg in cognac and weinbrand
07.165	4-Hydroxy-4-methylpentan-2-one	Up to 28.5 mg/kg in annatto, up to 0.07 mg/kg in roasted chicken, up to 2.7 mg/kg in honey, 0.41 mg/kg in passion fruit
07.168	2-Hydroxypiperitone	36 mg/kg in black currant (buds)
07.184	3-methyl-2,4-nonadione	0.083 mg/kg in tea
07.238	3-Hydroxy-2-octanone	Up to 15 mg/kg in lamb and mutton
07.248	Octan-2,3-dione	0.1 mg/kg in fish (lean), up to 0.2 mg/kg in turkey (roasted), up to 0.07 mg/kg in chicken (roasted), up to 0.112 mg/kg in Guinea hen, up to 0.03 mg/kg in beef (grilled, roasted), up to 108 mg/kg in lamb and mutton fat (heated), 0.01 mg/kg in peanuts

According to TNO two of the substances have not been reported to occur naturally in any food items:

Table 3: Candidate Substances Not Reported to Occur in Food (TNO, 2000; TNO, 2014)

FL-no:	Name:
07.097	3-(Hydroxymethyl)octan-2-one
07.260	1- or 3-hydroxy-5-methyl-2- or 3-hexanone

3. Specifications

Purity criteria for the 11 candidate substances have been provided by the Flavour Industry (EFFA, 2003a; EFFA, 2004b; EFFA, 2007b; EFFA, 2010; Flavour Industry, 2005a; Flavour Industry, 2005b) (see Table 1).

Judged against the requirements in Annex II of Commission Regulation (EC) No 1565/2000 (EC, 2000), the information is adequate for all candidate substances (see Section 2.2 and Table 1).

4. Intake Data

Annual production volumes of the flavouring substances as surveyed by the 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 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 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).

4.1. Estimated Daily *per Capita* Intake (MSDI Approach)

The intake estimation is based on the Maximised Survey-derived Daily Intake (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[7] (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 Flavouring Group Evaluation 11, Revision 3 (FGE.11Rev3) the total annual volume of production of the 11 candidate substances for use as flavouring substances in Europe has been reported to be approximately 170 kg (EFFA, 2003b; EFFA, 2004b; EFFA, 2007b; Flavour Industry, 2005a; Flavour Industry, 2005b). 120 kg is accounted for by 3-(hydroxymethyl)octan-2-one [FL-no: 07.097], 25 kg is accounted for by butane-2,3-diol [FL-no: 02.133] and 19 kg is accounted for by 1- or 3-hydroxy-5-methyl-2- or 3-hexanone [FL-no: 07.260]. For the 13 supporting substances the total annual volume of production has been reported by the JECFA to be approximately 38000 kg. Diacetyl [FL-no: 07.052] accounts for 18000 kg and 3-hydroxybutan-2-one [FL-no: 07.051] accounts for 19000 kg (JECFA, 2000a).

[7] EU figure 375 millions. 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.

On the basis of the annual volumes of production reported for the 11 candidate substances, the daily *per capita* intakes for each of these flavourings have been estimated (Table 5). The estimated daily *per capita* intake of 3-(hydroxymethyl)octan-2-one [FL-no: 07.097], butane-2,3-diol [FL-no: 02.133] and 1- or 3-hydroxy-5-methyl-2 or 3-hexanone [FL-no: 07.260] from use as a flavouring substance is 15, 3.0 µg and 2.3 µg, respectively. The daily *per capita* intakes for each of the remaining substances are equal to or less than 0.37 µg (Table 5).

4.2. Intake Estimated on the Basis of the Modified TAMDI (mTAMDI)

The method for calculation of the 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 11 candidate substances, information on food categories and normal and maximum use levels[8,9,10] were submitted by the Flavour Industry (EFFA, 2003a; EFFA, 2003b; EFFA, 2004b; EFFA, 2007a; EFFA, 2007b; Flavour Industry, 2005a; Flavour Industry, 2005b).

The 11 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 (EC, 2000), as shown in Table 4. 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 4: Use of candidate substances

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: 07.260]
03.0	Edible ices, including sherbet and sorbet	All
04.1	Processed fruits	All
04.2	Processed vegetables (incl. mushrooms & fungi, roots & tubers, pulses and legumes), and nuts & seeds	Only [FL-no: 07.260]
05.0	Confectionery	All
06.0	Cereals and cereal products, incl. flours & starches from roots & tubers, pulses & legumes, excluding bakery	All except [FL-no: 07.260]
07.0	Bakery wares	All
08.0	Meat and meat products, including poultry and game	All except [FL-no: 07.260]
09.0	Fish and fish products, including molluscs, crustaceans and echinoderms	All except [FL-no: 07.260]
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-no: 07.260]
13.0	Foodstuffs intended for particular nutritional uses	All except [FL-no: 07.260]
14.1	Non-alcoholic ("soft") beverages, excl. dairy products	All

[8] "Normal use" is defined as the average of reported usages and "maximum use" is defined as the 95th percentile of reported usages (EFFA, 2002).

[9] The normal and maximum use levels in different food categories (EC, 2000) have been extrapolated from figures derived from 12 model flavouring substances (EFFA, 2004a).

[10] 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).

Food category	Description	Flavourings used
14.2	Alcoholic beverages, incl. alcohol-free and low-alcoholic counterparts	All
15.0	Ready-to-eat savouries	All except [FL-no: 07.260]
16.0	Composite foods (e.g. casseroles, meat pies, mincemeat) - foods that could not be placed in categories 1 – 15	All except [FL-no: 07.260]

According to the Flavour Industry, the normal use levels for the candidate substances are in the range of 1 – 20 mg/kg food, and the maximum use levels are in the range of 3 – 100 mg/kg (EFFA, 2003a; EFFA, 2003b; EFFA, 2004b; EFFA, 2007b; Flavour Industry, 2005a; Flavour Industry, 2005b) (See Table B.1.2, Appendix B).

The mTAMDI values for the five candidate substances from structural class I (see Section 7) range from 1600 to 3900 µg/person/day. For the six candidate substances from structural class II the mTAMDI values range from 1500 to 1600 µg/person/day. For the one candidate substance [FL-no: 07.168] from structural class III the mTAMDI value is 1600 µg/person/day.

For detailed information on use levels and intake estimations based on the mTAMDI approach, see Section 7 and Appendix B.

5. Absorption, Distribution, Metabolism and Elimination

Ketals are expected to be readily hydrolysed after ingestion under the acidic conditions in the stomach to the corresponding alcohols and ketones.

The candidate substances, which are α- and β-diketones, ketal, hydroxyketones or diols, are expected to be absorbed from the gastrointestinal tract. The metabolic fate of acyclic aliphatic diketones depends primarily on the position of the carbonyl function and the chain length. Aliphatic acyclic diketones and α-hydroxyketones which contain a carbonyl function at the 2-position (i.e. a methyl ketone) may undergo α–hydroxylation and subsequent oxidation of the terminal methyl group to eventually yield corresponding ketocarboxylic acids. The ketoacids are intermediary metabolites (e.g. α-ketoacids), which may undergo oxidative decarboxylation to yield carbon dioxide and an aliphatic carboxylic acid. The acid may be completely metabolised in the fatty acid pathway and citric acid cycle. β-Keto-acids and derivatives readily undergo decarboxylation. Along with α-keto- and α-hydroxyacids, they yield breakdown products, which are incorporated into normal biochemical pathways.

Alternatively, the methyl-substituted diketones may be successively reduced to the corresponding hydroxyketones and diols, which are excreted in the urine as glucuronic acid conjugates. This pathway is favoured at elevated *in vivo* concentrations, especially for longer chain length ketones. α-Hydroxyketones or their diol metabolites may be excreted as glucuronic acid conjugates. If the carbonyl function is located elsewhere on the chain or in a ring, reduction is the predominant detoxification pathway.

A more detailed discussion on the metabolism of these α-, β-diketones, a related ketal, hydroxyketones and diols follows in Appendix C.

6. Application of the Procedure for the Safety Evaluation of Flavouring Substances

In FGE.11, one of the six candidate substances, pentan-2,4-dione, was, based on the genotoxicity data available, considered genotoxic *in vitro* and *in vivo* and accordingly, the Procedure was not applied for this substance. The candidate substance 3-methyl-2,4-nonadione [FL-no: 07.184] contains a structural 2,4-dione element similar to pentan-2,4-dione. The genotoxicity data available for this substance are two valid un-published GLP studies in *Salmonella typhimurium* and *Escherichia coli* which were

negative, and a recent valid unpublished *in vitro* micronucleus assay, which was also negative. Based on the lack of genotoxicity at gene and chromosome levels, 3-methylnona-2,4-dione can be evaluated through the Procedure. 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, using the mTAMDI approach, 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 7.

For the safety evaluation of the 11 candidate substances from chemical groups 8 and 10 the Procedure was applied. The stepwise evaluations are summarised in Table 6.

Step 1

Five of the candidate substances [FL-no: 02.133, 07.097, 07.165, 07.167 and 07.238], are classified into structural class I according to the decision tree approach presented by Cramer et al. (Cramer et al., 1978). Five of the candidate substances are classified in structural class II [FL-no: 07.071, 07.152, 07.184, 07.248 and 07.260] and the remaining substance [FL-no: 07.168] is classified into structural class III.

Step 2

Ketals are expected to be readily hydrolysed after ingestion under the acid conditions in the stomach to the corresponding alcohols and ketones.

At the estimated levels of intake, the candidate substances would not be expected to saturate metabolic detoxification pathways. They are considered to be metabolised to innocuous products. The evaluation of the 11 candidate substances, therefore, proceeds via the A-side of the Procedure scheme.

Step A3

The estimated levels of intake for the five candidate substances classified into structural class I are in the range of 0.0012 - 15 µg/*capita*/day, which are below the human intake threshold of concern for structural class I (1800 µg/person/day). The intakes of five class II candidate substances are 0.0012 – 2.3 µg/*capita*/day, which also are below the human intake threshold for that class (540 µg/person/day). The intake of the one class III candidate substance [FL-no: 07.168] is 0.0012 µg/*capita*/day, which is below the human intake threshold for that class (90 µg/person/day) (Table 6).

Based on the results of the safety evaluation sequence of the Procedure, these 11 candidate substances proceeding via the A-side of the Procedure scheme do not pose a safety concern when used as flavouring substances at the estimated levels of intake, based on MSDI approach.

7. Comparison of the Intake Estimations Based on the MSDI Approach and the mTAMDI Approach

The estimated intakes for the five candidate substances in structural class I based on the mTAMDI approach range from 1600 to 3900 µg/person/day. For one of the substances the mTAMDI value is above the threshold of concern for structural class I of 1800 µg/person/day.

The estimated intakes for the five candidate substances assigned to structural class II based on the mTAMDI range from 1500 to 1600 µg/person/day, which are above the threshold of concern for structural class II substances of 540 µg/person/day.

The estimated intake for the one candidate substance 2-hydroxypiperitone [FL-no: 07.168] assigned to structural class III based on the mTAMDI is 1600 µg/person/day, which is above the threshold of concern for structural class III substances of 90 µg/person/day.

Thus, for seven candidate substances [FL-no: 02.133, 07.071, 07.152, 07.248, 07.260, 07.184 and 07.168] further information is required. This would include more reliable intake data and then, if required, additional toxicological data.

For comparison of the intake estimate based on the MSDI approach and the mTAMDI approach see Table 5.

Table 5: Estimated intakes based on the MSDI approach and the mTAMDI approach

FL-no	EU Register name	MSDI (μg/*capita*/day)	mTAMDI (μg/person/day)	Structural class	Threshold of concern (μg/person/day)
02.133	Butane-2,3-diol	3	3900	Class I	1800
07.097	3-(Hydroxymethyl)octan-2-one	15	1600	Class I	1800
07.165	4-Hydroxy-4-methylpentan-2-one	0.085	1600	Class I	1800
07.167	4-Hydroxyhexan-3-one	0.0012	1600	Class I	1800
07.238	3-Hydroxy-2-octanone	0.0049	1600	Class I	1800
07.071	Octane-4,5-dione	0.0012	1600	Class II	540
07.152	3,3-Diethoxybutan-2-one	0.088	1600	Class II	540
07.184	3-Methylnona-2,4-dione	0.35	1600	Class II	540
07.248	Octan-2,3-dione	0.37	1600	Class II	540
07.260	1- or 3-Hydroxy-5-methyl-2- or 3-hexanone	2.3	1500	Class II	540
07.168	2-Hydroxypiperitone	0.0012	1600	Class III	90

8. 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. Further, 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, 2003a; EFFA, 2003b; EFFA, 2004b; EFFA, 2007b; Flavour Industry, 2005a; Flavour Industry, 2005b) the estimated combined daily *per capita* intake as flavourings of the 11 candidate substances assigned to structural class I, II or III is 21 μg, which do not exceed the thresholds of concern for substances belonging to structural class I of 1800 or II of 540 or III of 90 μg/person/day, respectively.

The 11 candidate substances [FL-no: 02.133, 07.071, 07.097, 07.152, 07.165, 07.167, 07.168, 07.184, 07.238, 07.248 and 07.260] are structurally related to 13 supporting substances, which all are α-diketones or precursors evaluated by the JEFCA at its 51st meeting. For 12 of these supporting substances European annual production volumes have been provided by Flavour Industry. The 12 supporting substances are all assigned to structural class II. The total estimated combined daily intake of the candidate and supporting substances (in Europe) is approximately 4600 μg/*capita*, which would exceed the threshold of concern for structural class II (540 μg/person/day).

However, based on the high capacity of enzymes in the metabolic pathways, it is anticipated that the combined intake of candidate substances (21 µg/*capita*/day) and supporting substances (4600 µg/*capita*/day) would be metabolised efficiently and would not saturate these metabolic pathways. Further, based on the data available, two supporting substances (diacetyl [FL-no: 07.052] 2200 µg/*capita*/day and 3-hydroxybutan-2-one [FL-no: 07.051] 2300 µg/*capita*/day) out of the total of 23 candidate and supporting substances provide 95 % of the contribution. These are present in the body as endogenous compounds (Kawano, 1959; Gabriel et al., 1972) and they would not be expected to give rise to perturbations outside the physiological range (JECFA, 1999a). Therefore, at the level of exposure, based on the MSDI approach, the total combined intake as flavouring substances of the candidate and supporting substances would not be expected to be of safety concern.

9. Toxicity

9.1. Acute Toxicity

Data are available for three candidate substances ([FL-no: 02.133, 07.165 and 07.184]) and for pentan-2,4-dione and for six of the supporting substances [FL-no: 07.018, 07.051, 07.052, 07.060, 07.064 and 09.264] evaluated by JECFA (JECFA, 1999a).

Oral LD_{50} values in rats and mice are in the range from 600 to 9000 mg/kg body weight (bw).

The acute toxicity data are summarised in Table 9.

9.2. Subacute, Subchronic, Chronic and Carcinogenicity Studies

Data on oral subacute toxicity are available for one candidate substance ([FL-no: 07.165]), for the structurally related pentan-2,4-dione and for three supporting substances [FL-no: 07.051, 07.052, and 07.077] evaluated by the JECFA (JECFA, 1999a). There are no studies available on chronic toxicity and carcinogenicity for the candidate substances. No Observed Adverse Effect Levels (NOAELs) in the range of 10 - 100 mg/kg bw/day in rats (and rabbits) have been derived from subacute studies for one candidate substance [FL-no: 07.165] and for pentan-2,4-dione and in the range of 90 - 330 mg/kg bw/day from subchronic studies in rats for two supporting substances [FL-no: 07.051 and 07.052].

Repeated dose toxicity data are summarised in Table 10.

9.3. Developmental / Reproductive Toxicity Studies

There are no data available for candidate substances. For supporting substances there is one developmental toxicity study available for diacetyl [FL-no: 07.052] in which no adverse effects were observed at the applied dose levels up to 1600 mg/kg bw/day in hamsters, mice and rats.

The developmental/reproductive toxicity study is summarised in Table 11.

9.4. Genotoxicity Studies

In vitro data are available for three candidate substances [FL-no: 02.133, 07.165 and 07.184], diacetyl-trimer former candidate substance [FL-no: 06.134], for the structurally related pentan-2,4-dione and for five supporting substances [FL-no: 07.051, 07.052, 07.060, 07.018 and 07.077].

For one of the candidate substances 4-hydroxy-4-methylpentan-2-one [FL-no: 07.165], *in vitro* studies have been reported with negative results obtained in bacterial gene mutation assays with and without metabolic activation as well as in a chromosomal aberration assay in rat liver cells *in vitro*. For a second candidate substance butane-2,3-diol [FL-no: 02.133], there is only one Ames test reported to be negative, but the validity of the study cannot be evaluated. No evidence of mutagenicity was reported in standard or modified Ames assays considered valid when 3-methyl-2,4-nonanedione [FL-no: 07.184] and diacetyl-trimer, a former candidate substance ([FL-no: 06.134]) were incubated with

various strains of *S. typhimurium* or *E. coli* at concentrations up to 5000 µg/plate, with and without metabolic activation (Stien, 2005; Sasaki, 2006).

For pentan-2,4-dione, both *in vitro* and *in vivo* studies are available. In the various *in vitro* studies reported (reverse mutation assays (Ames Tests), microbial DNA repair tests and tests on primary DNA damage, gene mutation and chromosomal aberrations) negative results were observed in one Ames Test with five tester strains of *S. typhimurium* with and without metabolic activation. Positive results were found in two Ames Tests with *S. typhimurium* strain TA104 with or without metabolic activation. Positive results were also observed in the chromosomal aberrations test in the absence of metabolic activation. For the three tests on microbial DNA repair both positive and negative results have been reported. However, they followed unusual study protocols and experimental details are insufficiently reported. Thus, the results are of limited validity. In two *in vivo* micronucleus studies using intraperitoneal dosing, which were performed in compliance with GLP and in accordance with OECD Guideline 474 in mice significant increases in micronucleated polychromatic erythrocytes were observed in peripheral blood as well as in bone marrow. Test concentrations used were high and close to the LD_{50} determined in the same test system, however, there was no decrease in the ratio of PCE/NCE, on the contrary an increase was reported. The results were clearly positive in both studies which are considered as valid. The same test protocol was used in an *in vivo* micronucleus study in rats. However, test concentrations had to be reduced due to excessive mortality. Under these conditions, negative results were observed in the micronucleus test in rats. There were no significant changes in the proportion of PCE. Therefore, the validity of the results of this study is limited.

3-Methylnona-2,4-dione [FL-no: 07.184] was tested in an *in vitro* micronucleus assay (Watters, 2013) using duplicate human lymphocyte cultures prepared from the pooled blood of two female donors in a single experiment. It is a valid GLP study carried out in compliance with OECD Guideline 487. Treatments covering ranges of concentrations from 80 – 815 µg/mL, depending on the tested conditions, separated by narrow intervals, were performed both in the absence and presence of metabolic activation (S-9) from Aroclor 1254-induced rats. The test article was formulated in anhydrous analytical grade dimethyl sulphoxide (DMSO) and the highest concentrations tested in the micronucleus experiment (limited by toxicity) were determined following a preliminary cytotoxicity range-finder experiment.

Treatments were conducted 48 hours following mitogen stimulation by phytohaemagglutinin (PHA). The test article concentrations for micronucleus analysis were selected by evaluating the effect of 3-methylnona-2,4-dione on the replication index (RI).

Appropriate positive and negative (vehicle) control cultures were included in the test system under each treatment condition. The proportion of micronucleated binucleate (MNBN) cells in the vehicle cultures fell within the 95th percentile of the current observed historical vehicle control (normal) ranges.

Treatment of cells with 3-methylnona-2,4-dione in the absence and presence of S-9 resulted in frequencies of MNBN cells that were generally similar to those observed in concurrent vehicle controls for all concentrations analysed under all three treatment conditions.

The MNBN cell frequency of all but a single treated culture fell within the normal ranges. This isolated increase in MNBN cell frequency occurred at an intermediate concentration (400 µg/mL) following 3+21 hour treatment in the absence of S-9. As this small increase was not reproduced in the replicate culture or any other culture analysed and the mean MNBN cell frequencies of all concentrations analysed fell within the normal range, it was considered of no biological relevance.

It is concluded that 3-methylnona-2,4-dione did not induce micronuclei in cultured human peripheral blood lymphocytes following treatment in the absence and presence of a rat liver metabolic activation system (S-9), when tested up to cytotoxic concentrations.

Genotoxicity studies were also performed with five supporting substances:

For 3-hydroxybutan-2-one (acetoin) [FL-no: 07.051] there is only one valid negative Ames test while data from other *in vitro* studies (results of which were reported to be negative) cannot be considered as valid. Diacetyl [FL-no: 07.052] was found able to induce gene mutations in *S. typhimurium* TA100 and TA104. Diacetyl was reported to produce mutations in the TK +/- locus of L5178Y mouse lymphoma cells. However, the concentration required for a two-fold increase in mutations results in a 62 % growth reduction, rendering this effect questionable (Whittaker et al., 2008). In an unpublished GLP study on *in vivo* micronucleus formation in B6C3F1 mice diacetyl was reported negative, however, since the PCE/NCE ratio was not reported it is not clear whether the test substance reached the target organ (NTP, 1994). Hexan-3,4-dione [FL-no: 07.077] slightly induced gene mutations in bacteria. No genotoxic activity was observed in valid *in vitro* studies with pentan-2,3-dione [FL-no: 07.060] and hexan-2,3-dione [FL-no: 07.018] (see Table 12.).

Conclusion on genotoxicity:

There are mutagenicity data on four candidate substances ([FL-no: 02.133, 06.134, 07.165 and 07.184]) and for the structurally related pentan-2,4-dione in this flavouring group evaluation.

4-Hydroxy-4-methylpentan-2-one [FL-no: 07.165] was not mutagenic in various *in vitro* studies in bacteria and yeast and did not induce chromosomal aberrations in rat liver cells. For butane-2,3-diol [FL-no: 02.133] negative results were reported in an *in vitro* gene mutation study, of which, however, the validity cannot be evaluated. No evidence of mutagenicity was reported in Ames assays considered valid when 3-methyl-2,4-nonanedione [FL-no: 07.184] and diacetyl-trimer, a former candidate substance ([FL-no: 06.134]) were incubated with various strains of *S. typhimurium* or *E. coli*. Furthermore, the results of a valid *in vitro* micronucleus assay indicate that 3-methyl-2,4-nonanedione [FL-no: 07.184] does not induce chromosomal damage with and without metabolic activation.

Mutagenicity data are available for five of the 13 supporting substances, giving mainly negative results. There is indication that diacetyl [FL-no: 07.052] has a weak genotoxic activity *in vitro*. However, diacetyl is reported to be endogenous in humans and is reported to be rapidly reduced to acetoin and further to butan-2,3-diol, for which there are no indication of mutagenicity.

Overall, the genotoxicity data available on candidate and supporting substances do not preclude evaluation of the candidate substances in the present group using the Procedure. Data on genotoxicity are summarised in Table 12 and 13.

CONCLUSIONS

The present flavouring group includes 11 candidate substances; eight α-diketones or their corresponding alcohols or ketals [FL-no: 02.133, 07.071, 07.152, 07.167, 07.168, 07.238, 07.248 and 07.260], and three β-diketones or their corresponding β-hydroxyketones (of which one is a tertiary alcohol) [FL-no: 07.165, 07.097 and 07.184] all belonging to chemical groups 8 and 10.

The present Revision of FGE.11, FGE.11Rev3 is prepared due to the evaluation of genotoxicity data, which have been requested in the previous version of FGE.11, for 3-methylnona-2,4-dione [FL-no: 07.184]. Additionally, new information on natural occurrence in food [FL no: 07.165, 07, 184 and 07.238] and on the stereoisomerism for [FL-no: 07.184 and 07.260] and composition for one substance [FL-no: 07.097] has become available.

Two of the candidate substances possesses two chiral centres [FL-no: 02.133 and 07.168] and five substances possesses one chiral centre [FL-no: 07.097, 07.167, 07.184, 07.238 and 07.260]. For all substances, the stereoisomeric composition has been specified sufficiently.

Five of the candidate substances are classified in structural class I, and five are classified in structural class II and one is classified in structural class III.

Nine of the candidate substances in the present group have been reported to occur naturally in a wide range of food items.

According to the default MSDI approach, the candidate substances, assigned to structural class I, II or III, have European daily *per capita* intakes ranging from 0.0012 to 15 µg, which are below the thresholds of concern for their respective structural class of 1800, 540 and 90 µg/person/day.

The total combined MSDI of candidate and supporting substances (all from structural class II) is approximately 4600 µg/*capita*, which exceeds the threshold of concern for structural class II (540 µg/person/day). However, based on information on efficient metabolism and on presence in the body as endogenous compounds, there are no safety concerns from the combined intakes of the candidate and supporting substances.

Data available for the candidate substance, 3-methyl-2,4-nonadione [FL-no: 07.184], shows that this substance has no genotoxic potential *in vitro*. For the remaining candidate substances in the present group genotoxicity data are only available for a limited number and the genotoxicity could not be assessed adequately. However, the genotoxicity data available do not preclude evaluation using the Procedure.

It was noted that where toxicity data were available they were consistent with the conclusions in the present flavouring group evaluation using the Procedure.

It is considered that on the basis of the default MSDI approach the 11 candidate substances evaluated through the Procedure [FL-no: 02.133, 07.071, 07.097, 07.152, 07.165, 07.167, 07.168, 07.184, 07.238, 07.248 and 07.260] would not give rise to safety concerns at the estimated levels of intake arising from their use as flavouring substances.

When the estimated intakes were based on the mTAMDI they ranged from 1600 to 3900 µg/person/day for the five candidate substances from structural class I. For one of these candidate substances [FL-no: 02.133] the estimated intake is above the threshold of concern of 1800 µg/person/day for structural class I. For the five candidate substances which are allocated to structural class II, the estimated intake based on the mTAMDI range from 1500 to 1600 µg/ person/day, which is above the threshold of concern for structural class II of 540 µg/person/day. For the one candidate substance [FL-no: 07.168] from structural class III the mTAMDI value is 1600 µg/person/day, which exceeds the threshold of concern for structural class III of 90 µg/person/day.

Thus, for seven of the 11 candidate substances evaluated through the Procedure [FL-no: 02.133, 07.071, 07.152, 07.168, 07.184, 07.248 and 07.260] the intakes, estimated on the basis of the mTAMDI exceed the threshold for the structural class, to which the flavouring substances have been assigned. Therefore, more reliable exposure data are required. On the basis of such additional data, the substances should be reconsidered along the steps of the Procedure. Following this procedure additional toxicological data might become necessary. The four candidate substances [FL-no: 07.097, 07.165, 07.167 and 07.238], which have mTAMDI intake estimates below the threshold of concern for structural class I are also expected to be metabolised to innocuous products.

In order to determine whether the conclusion for the candidate substances can be applied to the materials of commerce, it is necessary to consider the available specifications. Adequate specification and data on stereoisomerism are available for all the candidate substances.

Thus, all 11 substances [FL-no: 02.133, 07.071, 07.097, 07.152, 07.165, 07.167, 07.168, 07.184, 07.238, 07.248 and 07.260] evaluated in the present FGE would not present any safety concern at the estimated levels of intake based on the MSDI approach.

SUMMARY OF SAFETY EVALUATION

Table 6: Summary of Safety Evaluation Applying the Procedure

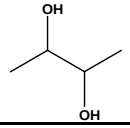
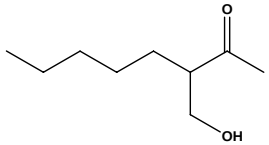
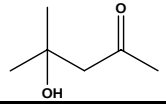
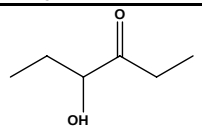
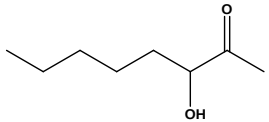
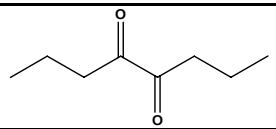
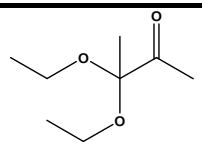
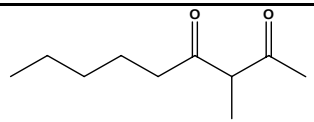
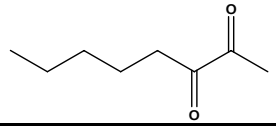
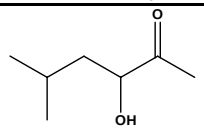
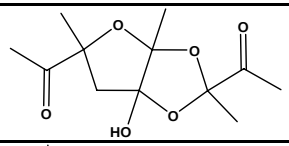
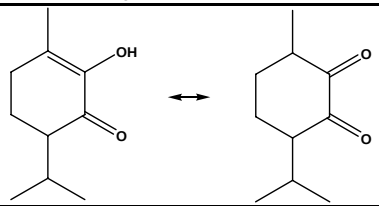
FL-no	EU Register name	Structural formula	MSDI [(a)] (μg/*capita*/day)	Class [(b)] Evaluation procedure path [(c)]	Outcome on the named compound [[(d)] or [(e)]]	Outcome on the material of commerce [[(f)], [(g)] or [(h)]]	Evaluation remarks
02.133	Butane-2,3-diol		3	Class I A3: Intake below threshold	d	f	
07.097	3-(Hydroxymethyl)octan-2-one		15	Class I A3: Intake below threshold	d	f	
07.165	4-Hydroxy-4-methylpentan-2-one		0.085	Class I A3: Intake below threshold	d	f	
07.167	4-Hydroxyhexan-3-one		0.0012	Class I A3: Intake below threshold	d	f	
07.238	3-Hydroxy-2-octanone		0.0049	Class I A3: Intake below threshold	d	f	
07.071	Octane-4,5-dione		0.0012	Class II A3: Intake below threshold	d	f	

Table 6: Summary of Safety Evaluation Applying the Procedure

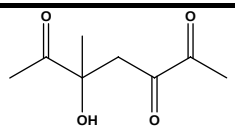
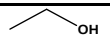
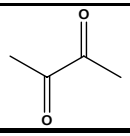
FL-no	EU Register name	Structural formula	MSDI [(a)] (µg/*capita*/day)	Class [(b)] Evaluation procedure path [(c)]	Outcome on the named compound [(d) or (e)]	Outcome on the material of commerce [(f), (g) or (h)]	Evaluation remarks
07.152	3,3-Diethoxybutan-2-one		0.088	Class II A3: Intake below threshold	d	f	
07.184	3-Methylnona-2,4-dione		0.35	Class II A3: Intake below threshold	d	f	
07.248	Octan-2,3-dione		0.37	Class II A3: Intake below threshold	d	f	
07.260	1- or 3-Hydroxy-5-methyl-2- or 3-hexanone	 3-Hydroxy-5-methyl-2-hexanone shown	2.3	Class II A3: Intake below threshold	d	f	
06.134	Diacetyl-trimer		1.2	Class II B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		No longer supported by Industry, (DG SANCO, 2012).
07.168	2-Hydroxypiperitone		0.0012	Class III A3: Intake below threshold	d	f	

(a): EU MSDI: Amount added to food as flavour in (kg / year) x 10E9 / (0.1 x population in Europe (= 375 x 10E6) x 0.6 x 365) = µg/capita/day.
(b): Thresholds of concern: Class I = 1800 µg/person/day, Class II = 540 µg/person/day, Class III = 90 µg/person/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.

EVALUATION STATUS OF HYDROLYSIS PRODUCTS OF CANDIDATE KETAL

Table 7: Evaluation Status of Hydrolysis Products of Candidate Ketal

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
	3-Hydroxy-3-methyl-hept-2,5,6-trione		Not in register	Not in register	Anticipated hydrolysis products of diacetyl-trimer, a former candidate substance [FL-no: 06.134].
02.078	Ethanol 41		Category 1 (SCF, 1995) No safety concern (JECFA, 1997)	No evaluation	At the forty-sixth JECFA meeting (JECFA, 1997), the Committee concluded that ethanol posed no safety concern at its current level of intake when ethyl esters are used as flavouring agents.
07.052	Diacetyl 408		No safety concern (JECFA, 2000a) Category A (CoE, 1992)	Class II A3: Intake above threshold, A4: Endogenous	

(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 = 1800 µg/person/day, Class II = 540 µg/person/day, Class III = 90 µg/person/day.
(e): Procedure path A substances can be predicted to be metabolised to innocuous products. Procedure path B substances cannot.

SUPPORTING SUBSTANCES SUMMARY

Table 8: Supporting Substances Summary

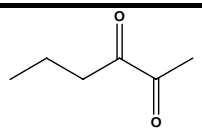
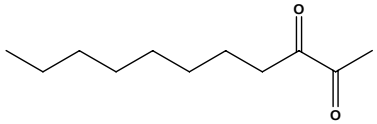
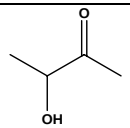
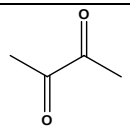
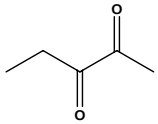
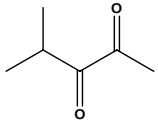
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) [a] (μg/*capita*/day)	SCF status [b] JECFA status [c] CoE status [d]	Comments
07.018	Hexan-2,3-dione		2558 152 3848-24-6	412 JECFA specification (JECFA, 2000b)	8.5	No safety concern (JECFA, 2000a) Category A (CoE, 1992)	
07.021	Undeca-2,3-dione		3090 155 7493-59-6	417 JECFA specification (JECFA, 2003)	0.0037	No safety concern (JECFA, 2000a) Category A (CoE, 1992)	
07.051	3-Hydroxybutan-2-one		2008 749 513-86-0	405 JECFA specification (JECFA, 1998)	2300	No safety concern (JECFA, 2000a) Category A (CoE, 1992)	JECFA evaluated acetoin (CASrn as in Register). (R)- or (S)-enantiomer not specified by CASrn in Register.
07.052	Diacetyl		2370 752 431-03-8	408 JECFA specification (JECFA, 1998)	2200	No safety concern (JECFA, 2000a) Category A (CoE, 1992)	
07.060	Pentan-2,3-dione		2841 2039 600-14-6	410 JECFA specification (JECFA, 2003)	130	No safety concern (JECFA, 2000a) Category A (CoE, 1992)	
07.063	4-Methylpentan-2,3-dione		2730 2043 7493-58-5	411 JECFA specification (JECFA, 2000b)	0.3	No safety concern (JECFA, 2000a) Category A (CoE, 1992)	

Table 8: Supporting Substances Summary

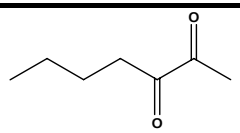
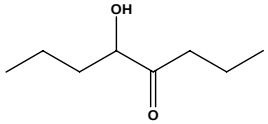
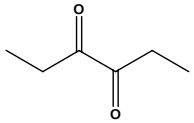
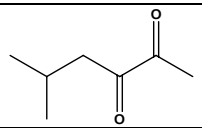
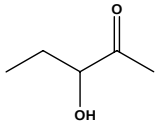
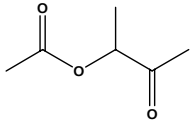
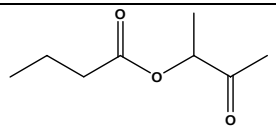
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) [a] (μg/*capita*/day)	SCF status [b] JECFA status [c] CoE status [d]	Comments
07.064	Heptan-2,3-dione		2543 2044 96-04-8	415 JECFA specification (JECFA, 1998)	0.97	No safety concern (JECFA, 2000a) Category A (CoE, 1992)	
07.065	5-Hydroxyoctan-4-one		2587 2045 496-77-5	416 JECFA specification (JECFA, 2001)	0.012	No safety concern (JECFA, 2000a) Deleted (CoE, 1992)	JECFA evaluated 5-hydroxy-4-octanone (CASrn as in Register). CASrn in Register refers to the racemate.
07.077	Hexan-3,4-dione		3168 2255 4437-51-8	413 JECFA specification (JECFA, 1998)	21	No safety concern (JECFA, 2000a) Category A (CoE, 1992)	
07.093	5-Methylhexan-2,3-dione		3190 11148 13706-86-0	414 JECFA specification (JECFA, 2000b)	1.1	No safety concern (JECFA, 2000a)	
07.125	3-Hydroxypentan-2-one		3550 11115 3142-66-3	409 JECFA specification (JECFA, 2003)	ND	No safety concern (JECFA, 2000a)	JECFA evaluated 3-hydroxy-2-pentanone (CASrn as in Register). (R)- or (S)-enantiomer not specified by CASrn in Register.
09.186	sec-Butan-3-onyl acetate		3526 608 4906-24-5	406 JECFA specification (JECFA, 2000b)	0.024	No safety concern (JECFA, 2000a) Category A (CoE, 1992)	JECFA evaluated 2-acetoxy-3-butanone (CASrn as in Register). (R)- or (S)-enantiomer not

Table 8: 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*/day)	SCF status [b] JECFA status [c] CoE status [d]	Comments
							specified by CASrn in Register.
09.264	sec-Butan-3-onyl butyrate		3332 10525 84642-61-5	407 JECFA specification (JECFA, 2000b)	0.012	No safety concern (JECFA, 2000a)	JECFA evaluated butan-3-one-2-yl butanoate (CASrn as in Register). (R)- or (S)-enantiomer not specified by CASrn in Register.

(a): EU MSDI: Amount added to food as flavouring substance in (kg / year) x 10E9 / (0.1 x population in Europe (= 375 x 10E6) x 0.6 x 365) = µg/capita/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.

TOXICITY DATA

Table 9: Acute Toxicity

Chemical Name [FL-no][(a)]	Species	Sex	Route	LD_{50} (mg/kg bw)	Reference	Comments
(Acetoin [07.051])	Rat	NR	Oral	>5000	(Moreno, 1977)	
Butane-2,3-diol [02.133]	Mouse	NR	Oral	9000	(Kopf et al., 1950)	
(Butan-3-one-2-yl butanoate [09.264])	Mouse	NR	Oral	>8000	(Pellmont, 1969)	
	Rat	NR	Oral	>8000	(Pellmont, 1969)	
(Diacetyl [07.052])	Rat	M, F	Gavage	M: 3400; F: 3000	(Colley et al., 1969)	
	Rat	NR	Gavage	1580	(Jenner et al., 1964)	
	Guinea pig	NR	Gavage	990	(Jenner et al., 1964)	
4-Hydroxy-4-methylpentan-2-one [07.165]	Rat	M	Gavage	4920	(Myers et al., 1977)	
	Rat	M	Oral	4000	(Smyth, 1946a)	
(2,3-Pentanedione [07.060])	Rat	NR	Oral	3000	(Moreno, 1977)	
[Pentan-2,4-dione]	Rat	M	Oral	1000	(Smyth, 1941)	
	Rat	M, F	Gavage	M: 780; F: 590	(Ballantyne et al., 1986) (Myers et al., 1985)	
	Rat	M	Oral	800	(Eastman Kodak Co., 1992)	
	Mouse	M	Oral	951	(Eastman Kodak Co., 1992)	
(2,3-Hexanedione [07.018])	Rat	NR	Oral	>5000	(Moreno, 1977)	
(2,3-Heptanedione [07.064])	Rat	NR	Oral	>5000	(Moreno, 1979)	
1- or 3-Hydroxy-5-methyl-2- or 3-hexanone [07.260]	Rat	M, F	Gavage	>2000	(Strobel, 1998)	

NR: Not reported
M= Male; F= Female
(a): Supporting substances are listed in brackets.

Table 10: Subacute / Subchronic / Chronic / Carcinogenicity Studies

Chemical Name [FL-no]*	Species; Sex No./Group	Route	Dose levels	Duration	NOAEL (mg/kg/day)	Reference	Comments
(Acetoin [07.051])	Rat; M, F 30	Drinking water	0, 85, 330, 1345 mg/kg bw/d	90 Days	330	(Gaunt et al., 1972)	Non-GLP study of good quality carried out as part of the BIBRA safety evaluation programme; published in a peer reviewed journal.
(Diacetyl [07.052])	Rat; M, F 30	Gavage	0, 10, 30, 90, 540 mg/kg bw/d	90 Days	90	(Colley et al., 1969)	Non-GLP study of good quality carried out as part of the BIBRA safety evaluation programme; published in a peer reviewed journal.
4-Hydroxy-4-methylpentan-2-one [07.165]	Rat; NR 10	Drinking water	0, 10, 40, 130 mg/kg bw/d	30 Days	10	(Smyth, 1946b)	Unpublished non-GLP study of poor quality with respect to study protocol. No histopathological examination of high dose and control group. No details available for method and results.
[Pentan-2,4-dione]	Rat; M 5	Gavage	0, 100, 500, 1000 mg/kg bw/d	1-15 Days[(a)]	100	(Eastman Kodak Co., 1992)	Non-GLP study of 1979 in unpublished summary report. No details available for method and results. Quality of study limited with respect to study design. Results of the study have been published in Neurotoxicity of Industrial and Commercial Chemicals, Vol. 2, I.L. O'Donoghue, Editor, CRC Press, Boca Raton, Florida. p.77 (1985).
	Rat; M 5	Gavage	0, 100 mg/kg bw/d	14 Days[(b)]	100[(c)]	(Eastman Kodak Co., 1992)	
	Rat; M 5	Gavage	0, 200 to 500 mg/kg bw/d	126 Days[(d)]	<200[(e)]	(Eastman Kodak Co., 1992)	
	Rabbit; M 2	Gavage	0, 250, 500, 1000 mg/kg bw/d	14 Days[(b)]	250[(f)]	(Eastman Kodak Co., 1992)	
(3,4-Hexanedione [07.077])	Rat; M, F 10-16	Diet	0, 17 mg/kg bw/d	90 Days	17[(c)]	(Posternak et al., 1969)	Summary of an unpublished non-GLP study (on 42 flavouring substances carried out in 1962-1967) prepared by BIBRA and published in a peer-reviewed journal.

NR= not reported.
M = Male; F = Female
*: Supporting substances are listed in brackets.
(a): Animals dosed daily, between 1 and 11 times.
(b): Animals dosed ten times in fourteen days.
(c): The study was performed at a single dose level or multiple dose levels that produced no adverse effects.
(d): Animals dosed twice per day over a 126 day period at doses ranging from 100 to 250 mg/kg/day; animals that died or were killed during the study due to poor condition were replaced.

(e): NOAEL for the central nervous system was determined to be <200 mg/kg/day. The NOAEL for thymus toxicity was determined to be 200 mg/kg/day.
(f): One rabbit (50 % of the group population) died due to possible aspiration of the test substance.

Table 11: Developmental and reproductive toxicity studies

Chemical Name [FL-no]*	Study type Duration	Species/Sex No/Group	Route	Dose levels mg/kg bw/day	NOAEL (mg/kg/day), Including information of possible maternel toxicity	Reference	Comments
(Diacetyl [07.052])	Developmental toxicity: Gestation days 6-10	Hamster; F 21-25	Gavage	0, 16, 74.3, 345, 1600 mg/kg bw/d	1600 (maternal)[(a,b)] 1600 (foetal)[(b,c)]	(FDA, 1973)	Unpublished non-GLP study of limited quality with respect to possible developmental effects.
	Developmental toxicity: Gestation days 6-15	Mouse; F 21-24	Gavage	0, 16, 74.3, 345, 1600 mg/kg bw/d	1600 (maternal)[(a,c)] 1600 (foetal)[(b,c)]	(FDA, 1973)	Unpublished non-GLP study of limited quality with respect to possible developmental effects.
	Developmental toxicity: Gestation days 6-15	Rat; F 21-23	Gavage	0, 16, 74.3, 345, 1600 mg/kg bw/d	1600 (maternal)[(a,c)] 1600 (foetal)[(b,c)]	(FDA, 1973)	Unpublished non-GLP study of limited quality with respect to possible developmental effects.

M = Male; F = Female.

* Supporting substances are listed in brackets.

(a): Based on observations of maternal survival, body weight and reproductive parameters.

(b): Based on observations of foetal survival and microscopic examination of foetal external, skeletal and soft tissues.

(c): The study was performed at a single dose level or multiple dose levels that produced no adverse effects and, therefore, a NOAEL was not determined. The NOAEL is probably higher than the reported dose level that produced no adverse effects.

GENOTOXICITY DATA

Table 12: Genotoxicity (*in vitro*)

Chemical Name [FL-no]*	Test System	Test Object	Concentration	Result	Reference	Comments
(Acetoin [07.051])	Ames Test	*S. typhimurium* TA100	up to 4500 µg/plate	Negative[1]	(Garst et al., 1983)	Non-GLP study. Outcome of the study is only summarised with limited experimental details and no test results reported. Validity of the study cannot be evaluated.
	Ames Test	*S. typhimurium* TA100	390 µg/plate	Negative[2]	(Kim et al.,, 9987)	Non-GLP study with limited information given on study protocol and results. Validity of the study cannot be evaluated.
	Ames Test	*S. typhimurium* TA98, TA100, TA102	0.44 – 44000 µg/plate	Negative[3]	(Aeschbacher et al., 1989)	Good quality, non-GLP study.
	Ames Test	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538; *E. coli* WP2 uvrA	1 – 5000 µg/plate	Negative[2]	(Iwata et al., 1984)	Published study in Japanese. Results (i.e. average number of revertant colonies per plate from three plates for each test concentration, including positive and negative controls) are given in table. Only tested without metabolic activation. Validity of the study cannot be evaluated.
Butane-2,3-diol [02.133]	Ames Test	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538; *E. coli* WP2 uvrA	1 – 5000 µg/plate	Negative[2]	(Iwata et al., 1984)	Published study in Japanese. Results (i.e. average number of revertant colonies per plate from three plates for each test concentration, including positive and negative controls) are given in table. Only tested without metabolic activation. Validity of the study cannot be evaluated.
(Diacetyl [07.052])	Ames Test	*S. typhimurium* TA100	90 µg/plate	Negative[3]	(Kim et al.,, 9987)	Non-GLP study with limited information given on study protocol and results. Validity of the study cannot be evaluated.
	Modified Ames Test	*S. typhimurium* TA98, TA100, TA104;	NR	Positive[1]	(Kato et al., 1989)	Only poorly reported abstract. Validity of the study cannot be

Table 12: Genotoxicity (*in vitro*)

Chemical Name [FL-no]*	Test System	Test Object	Concentration	Result	Reference	Comments
		E. coli WP2 uvrA/pKM101				evaluated.
	Modified Ames Test	*S. typhimurium* TA104	530 µg/plate	Positive	(Marnett et al., 1985)	Published non-GLP study assessing the sensitivity of the new base substitution strains TA102 and TA104 to the mutagenic effects of carbonyls. Metabolic activation not reported. Due to the limited details reported on experimental design and results the validity of the study cannot be evaluated
	Ames Test	*S. typhimurium* TA104	5 – 500 µg/plate[4]	Positive[1] Negative[2]	(Shane et al., 1988)	Poorly reported non-GLP study of limited validity. Results are difficult to interpretate.
	Ames Test	*S. typhimurium* TA100, TA102	5 – 500 µg/plate[4]	Negative[3]	(Shane et al., 1988)	Poorly reported non-GLP study of limited validity. Results are difficult to interpretate.
	Ames Test	*S. typhimurium* TA100	152 – 950 µg/plate	Positive[2]	(Dorado et al., 1992)	Published non-GLP study of good quality. The number of revertants at the highest dose duplicated that of spontanous revertants. The effect was dose-related.
	Ames Test	*S. typhimurium* TA100	Approx. 400-600 µg/plate	Positive[3]	(Bjeldanes and Chew, 1979)	Published non-GLP study. Due to the limited details reported on experimental design and results the validity of the study cannot be evaluated.
	Ames Test	*S. typhimurium* TA98	10 – 10000 µg/plate	Negative	(Bjeldanes and Chew, 1979)	Published non-GLP study. Due to the limited details reported on experimental design and results the validity of the study cannot be evaluated.
	Ames Test	*S. typhimurium* TA102	0.17 – 17200 µg/plate	Positive[3,]	(Aeschbacher et al., 1989)	Good quality, non-GLP study. The number of revertants at the highest dose duplicated that of spontanous revertants.
	Ames Test	*S. typhimurium* TA98, TA100	0.17 – 17200 µg/plate	Negative[3]	(Aeschbacher et	Good quality, non-GLP study.

Table 12: Genotoxicity (*in vitro*)

Chemical Name [FL-no]*	Test System	Test Object	Concentration	Result	Reference	Comments
					al., 1989)	
	Modified Ames Test	*S. typhimurium* TA100	1.8 and 4 mM[4] (107 and 238 µg/pl)	Positive[2]	(Suwa et al., 1982)	Published non-GLP study with limited details reported on experimental design and results. The validity of the study is considered limited.
	Ames Test	*S. typhimurium* TA98, TA100, TA1535, TA1537,TA 1538; *E. coli* WP2 uvrA	1 – 5000 µg/plate	Negative[2]	(Iwata et al., 1984)	Published study in Japanese. Results (i.e. average number of revertant colonies per plate from three plates for each test concentration, including positive and negative controls) are given in table. Only tested without metabolic activation. Validity of the study cannot be evaluated.
	Ames Suspension Test	*S. typhimurium* TA1535, TA1537, TA1538	1 %	Negative[3]	(FDA, 1974)	(not evaluated).
	Mutation	*S. cerevisiae*	NR	Negative[3]	(FDA, 1974)	(not evaluated).
	Chromosomal Malsegregation Assay[5]	*S. cerevisiae* D61.M	148 – 393 µg/ml	Negative	(Zimmermann and Mohr, 1992)	Published non-GLP study. Study is considered valid.
	Forward Mutation	Mouse lymphoma L5178Y TK+/- cells	100 – 250 µg/ml	Positive	(Whittaker et al., 2008)	Published non-GLP study. The result was positive. However, the concentration required for a two-fold increase in mutations result in a 62 % growth reduction, rendering this effect questionable.
4-Hydroxy-4-methylpentan-2-one [07.165]	Ames Test	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538;	100 – 10000 µg/plate	Negative[3]	(San and Klug, 1993)	Plate incorporation assay. Non-published GLP-study.
	Ames Test	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538; *E. coli* WP2, WP2 uvrA	Up to 4000 µg/plate	Negative[3]	(Brooks et al., 1988)	Plate incorporation assay. Published study summarising an extended industry report. Study is considered valid.
	Mitotic Gene Conversion Assay	*S. cerevisiae*	10-5000 µg/ml	Negative[3]	(Brooks et al., 1988)	Published study summarising an extended industry report. Study is considered valid.
	Chromosome Aberrations	Rat liver epithelial type- cell line RL4	750, 1500, 2000, 3000, 4000 µg/ml	Negative	(Brooks et al., 1988)	Published study summarising an extended industry report. Study is

Table 12: Genotoxicity (*in vitro*)

Chemical Name [FL-no]*	Test System	Test Object	Concentration	Result	Reference	Comments
						considered valid.
(2,3-Pentanedione [07.060])	Ames Test	*S. typhimurium* TA100	105 µg/plate	Negative[2]	(Kim et al.,, 9987)	Non-GLP study with limited information given on study protocol and results. Validity of the study cannot be evaluated.
	Ames Test	*S. typhimurium* TA98, TA100, TA102	0.9 – 90000 µg/plate	Negative[3]	(Aeschbacher et al., 1989)	Good quality, non-GLP study
[Pentan-2,4-dione]	Ames Test	*S. typhimurium* TA98, TA100, TA1535, 1537, 1538;	300 – 30000 µg/plate	Negative[3, 6]	(Guzzie and Morabit, 1985)	Valid unpublished GLP-study carried out according to US EPA test guidelines.
	Ames Test	*S. typhimurium* TA92, TA98, TA100, TA104	1.9 – 48 µmol/plate (190 –4805 µg/plate)[7]	Negative Positive [8]	(Gava et al., 1989)	No data on cytotoxicity reported. Metabolic activation not reported. Due to the limited experimental details reported the validity of the study cannot be evaluated.
	Modified Ames Test	*S. typhimurium* TA98, TA100, TA104; *E. coli* WP2 uvrA/pKM 101	NR	Negative[3] Positive[9] Positive[10]	(Kato et al., 1989)	Only abstract reported. Validity of the study cannot be evaluated.
	Umu Test (DNA repair test)	*S. typhimurium* TA1535/ pSK1002	196, 410, 1235 µg/ml	Positive[3, 11,12]	(Ono et al., 1991)	Published non-GLP study. Unusual study design. Due to the limited experimental details and results reported the validity of the study cannot be evaluated. Relevance of results are questioned.
	Rec-Assay (DNA repair test)	*B. subtilis* H17 (Rec^+), M45 (Rec^-)	CR_{50} Rec^+=209 µg/ml, $CR_{50}Rec^-$ = 195 µg/ml[13,2] CR_{50} Rec^+=235 µg/ml, $CR_{50}Rec^-$ = 173 µg/ml[13,1]	Negative[2] Very weakly positive[1]	(Matsui et al., 1989)	Insufficient report of study design and experimental details. Detailed results not reported. Validity of the study cannot be evaluated.
	Mitotic aneuploidy (DNA repair test)	*S. cerevisiae* D61.M	0.74 – 1.96 % (7400 – 19600µg/ml)	Negative[2]	(Zimmermann et al., 1985)	Published non-GLP study. Study is considered valid.
	Sister Chromatid	Chinese Hamster Ovary Cells	~0.01 – 1.0 µmol/ml	Positive	(Gava et al., 1989)	Published non-GLP study. Due to

Table 12: Genotoxicity (*in vitro*)

Chemical Name [FL-no]*	Test System	Test Object	Concentration	Result	Reference	Comments
	Exchange		(~1 – 100µg/ml)[14]			the limited experimental details and the incomplete cytotoxicity data reported the validity of the study cannot be evaluated.
	Sister Chromatid Exchange	Chinese Hamster Ovary Cells	20, 30, 100 µg/ml 30, 100, 300 µg/ml	Positive[2, 19] Positive[1, 19]	(Slesinski, 1986)	Unpublished GLP study. Study is considered valid**.**
	HGPRT Mutation Assay	Chinese Hamster Ovary Cells	10, 50, 100, 500, 1000 µg/ml	Negative[2, 15] Negative[1, 15]	(Slesinski, 1986)	Cytotoxic effects at 1 mg/ml. Unpublished GLP study. Study is considered valid**.**
	Chromosomal Aberrations	Chinese Hamster Ovary Cells	40 – 120 µg/ml (80, 100, 120 µg/ml) 60 – 140 µg/ml (100, 120, 140 µg/ml)	Positive[2,16, 20] Negative[1,16]	(Guzzie and Morabit, 1986)	Three highest conentrations analysed. Mitotic cell division not excessively reduced at conentrations used (i.e. not cytotoxic). Effects observed were chromatid breaks. Good quality unpublished GLP study.
(2,3-Hexanedione [07.018])	Chromosomal Malsegregation Assay[17]	*S. cerevisiae* D61.M	372 – 833 µg/ml	Negative[18]	(Zimmermann and Mohr, 1992)	Published non-GLP study. Study is considered valid.
(3,4-Hexanedione [07.077])	Ames Test	*S. typhimurium* TA100	228 – 4900 µg/plate	Very weakly positive[2]	(Dorado et al., 1992)	Published non-GLP study of good quality.
3-Methylnona-2,4-dione [07.184]	Revere mutation	*S. typhimurium* TA100, TA1535, TA98, TA1537	39, 78, 156, 313, 625 and 1250 µg/plate	Negative	(Sasaki, 2006)	Unpublished GLP study. Study considered valid
	Revere mutation	*E. Coli* WP2*uvrA*	39, 78, 156, 313, 625 and 1250 µg/plate	Negative	(Sasaki, 2006)	Unpublished GLP study. Study considered valid
	Micronucleus	Human peripheral lymphocytes	200, 400, 800 and 815 µg/mL[21]	Negative	(Watters, 2013)	Valid GLP study, in compliance with OECD Guideline 487
			400, 600, and 800 µg/mL[22]	Negative	(Watters, 2013)	
			80, 145, 190 and 210 µg/mL[23]	Negative	(Watters, 2013)	
Diacetyl-trimer , former candidate substance [06.134]	Revere mutation	*S. typhimurium* TA98, TA100, TA102, TA1535, TA1537	100, 316, 1000, 3160 or 5000 µg/plate	Negative[3,24]	(Stien, 2005)	Unpublished GLP study. Study considered valid

Table 12: Genotoxicity (*in vitro*)

Chemical Name [FL-no]*	Test System	Test Object	Concentration	Result	Reference	Comments
			10, 31.6, 100, 316 or 1000 µg/plate	Negative[25]		

* Supporting substances are listed in brackets.
NR = Not reported.
CR50 = 50 % survival concentrations for the B. subtilis strains.
[1] With metabolic activation.
[2] Without metabolic activation.
[3] With and without metabolic activation.
[4] Estimated from graphical data.
[5] Pure chemical at 28°C, starting titer 14.6 x 10^6 cells/ml; and pure chemical, cold shock, starting titer 9.1 x 10^6 cells/ml.
[6] Test substance was cytotoxic at 30000 µg/plate.
[7] Calculated based on molecular weight = 100.12.
[8] Test substance was inactive towards TA92, TA98 and TA100 strains; however, it was mutagenic towards the TA104 strain.
[9] TA104 was positive in the absence of metabolic activation (specific activity = 2.25 revertants/µg); TA92, TA98 and TA100 were negative.
[10] WP2 uvrA/pKM 101 was positive in the presence of metabolic activation (specific activity = 7.73 revertants/µg) and negative in the absence of metabolic activation.
[11] The highest concentration was only used in a 2-hour test (short term reaction) for which weakly positive result were reported.
[12] At 410 µg/ml a strongly positive results was reported after 24 hrs (long term reaction) with S9 metabolic activation, negative results were reported at 410 µg/ml after 2, 4 and 6 hours with S9 metabolic activation and at 196 µg/ml after 2, 4 and 6 hours and 20 hours in the absence of S9 metabolic activation.
[13] The concentrations indicated are those of the test substance in the interaction period.
[14] Calculated based on molecular weight = 100.12.
[15] Cytotoxicity was observed, with and without S9 metabolic activation, at the 1.0 mg/ml dose level.
[16] Test substance was highly clastogenic in the absence of metabolic activation; however, in the presence of rat-liver S9 metabolic activation it was not clastogenic.
[17] Pure chemical at 28°C, starting titer 10.7 x 10^6 cells/ml; and pure chemical, cold shock, starting titer 11.3 x 10^6 cells/ml.
[18] In the pure form the test substance did not induce mitotic chromosome loss; however, almost all of the white colonies scored at the highest concentrations turned out to be respiratory deficient. The authors concluded that the test substance induces mitochondrial mutation under these experimental conditions.
[19] Pentan-2,4-dione-(purity 99.2 %) was tested for sister chromatid exchange in Chinese hamster ovary (CHO) cells at culture concentrations of 6 to 100 µg/ml without metabolic activation and 10 to 300 µg/ml with metabolic activation. Experiments were carried out in duplicate. In preliminary experiments the appropriate range of test concentrations was determined for which the highest concentration would not kill more than 90% of the treated cells. Cytotoxic effects have been reported at concentrations of ≥1 mg/ml, concentrations above 2 mg/ml were lethal. Result: At the highest three doses evaluated for SCE (10, 30 and 100 µgs/ml in the absence and 30, 100 and 300 in the presence of metabolic activation), pentan-2,4-dione produced significant ($p<0.001$) increases in the incidence of SCE in CHO cells both with and without metabolic activation. The SCE increase was greater without metabolic activation than with metabolic activation. A steep dose-response relationship was observed without S9, but not with S9. However, reproducible and statistically significant ($p<0.001$) increases were apparent in both tests. A remarkably high increase in the incidence of SCEs, which was higher than the positive control, was observed at 100 µg/ml without metabolic activation..Mitotic inhibition was evident only with the 300 µg /ml dose without S9.
[20] Pentan-2,4-dione (purity 99.2 %) was tested in a chromosomal aberration assay in Chinese Hamster Ovary Cells at concentrations of 40–120 µg/ml without metabolic activation and 60-140 µg/ml with metabolic activation. The three highest concentrations (80, 100 and 120 µg/ml without metabolic activation and 100, 120 and 140 µg/ml with metabolic activation) were analysed for chromosomal damage. Preliminary tests performed to assess effects on cell cycle division, indicated that pentan-2,4-dione produced a significant delay in cell division cycle, which was

more pronounced in the absence than in the presence of S9. Test concentrations were selected on the basis of cytotoxicity data from preliminary experiments. Result: Statistically significant ($p>0.001$) increases in numbers of chromosome aberrations were observed at the three highest concentrations without S9 activation. However, in the presence of metabolic activation the cells tested did not demonstrate increased numbers of chromosome aberrations at any concentrations compared to control values.

[21] Incubation for 3-hour followed by 21-hour recovery period, in the absence of S-9.
[22] Incubation for 3-hour followed by 21-hour recovery period, in the presence of S-9.
[23] Incubation for 24-hour without recovery period, in the absence of S-9.
[24] Standard plate-incorporation method, with and without S9.
[25] Modified pre-incubation method, with and without S9.

Table 13: Genotoxicity (*in vivo*)

Chemical Name [FL-no]*	Test system	Test Object	Route	Dose	Result	Refence	Comments
(Diacetyl [07.052])	*In vivo* Mouse Micronucleus Assay(bone marrow)	Mouse	Oral administration	300, 600, 1200, 2400 mg/kg; 300 mg/kg × 4 doses	Negative	(Iwata et al., 1984)	Published study in Japanese. Results (i.e. frequencies of PCEs and micronucleated PCEs, including positive and negative controls) are given in tables. No information can be found on sampling times. Validity of the study cannot be evaluated.
	In vivo Mouse Micronucleus Assay (bone marrow)	Mouse	Intraperitoneal injection	8, 16, 31, 62, 125, 250, 500 mg/kg	Negative	(NTP, 1994)	Sampling at 24 hours. Only summarised results of the study available. The PCE/NCE ratio was not reported so it is unclear whether the test substance has reached the bone marrow. Relevance of the results is limited.
[Pentan-2,4-dione]	*In vivo* Mouse Micronucleus Assay (peripheral blood)	Mouse	Intraperitoneal injection	200, 400, 650 mg/kg	Positive [(b)]	(Guzzie and Morabit, 1986)	Sampling at 30, 48, 72 hours. Toxic effects during the study not reported (LD_{50} of 808 mg/kg). Unpublished valid GLP-study.
	In vivo Mouse Micronucleus Assay (bone marrow)	Mouse	Intraperitoneal injection	400, 650 mg/kg	Positive [(c)]	(Vergnes and Kubena, 1994a)	Sampling at 6, 24, 48 hours. Toxic effects during the study not reported (LD_{50} of 808 mg/kg). Good quality GLP study carried out according to OECD and US EPA guidelines.
	In vivo Rat Micronucleus Assay (bone marrow)	Rat	Intraperitoneal injection	50, 100, 200 (400, 650) mg/kg[(a)]	Negative	(Vergnes and Kubena, 1994b)	Sampling at 6, 24, 48 hours. Only summarised results of the study available. Unpublished valid study carried out according to EPA standards. Due to the lack of an effect on the PCE/NCE ratio it is unclear whether the test substance has reached the bone marrow. Relevance of the results is limited.

* Supporting substances are listed in brackets.

(a): Excessive mortality was observed at 400 and 650 mg/kg dose levels; therefore, these dose levels were replaced with 50 and 100 mg/kg.

(b): Pentan-2,4-dione (purity 99.2 %) was tested in an *in vivo* Mouse Micronucleus Assay. Swiss Webster Mice (5 animals per sex/dose group) were given i.p. injections of 200, 400 and 650 mg/kg. Peripheral blood was sampled at 30, 48 and 72 hours post injection. Results: In a dose-finding study using 579-1200 mg/kg toxicity was observed from 694-1200 mg/kg (20 % to 100 % mortality) and an LD_{50} of 808 mg/kg i.p. was found (95 % confidential interval 731.6-889.9 mg/ml). In this study, at 48 hours post-injection, PCE/NCE ratio was reduced by 30 % and 23 % below the control levels for male and female animals that received a dose of 694 mg/kg, respectively. In the micronucleus study, the PCE/NCE ratio was determined in the 650 mg/kg group and in controls. No significant or dose-related decrease in the PCE/NCE ratio for either sex at any of the sample times (slight decrease with dose-related trend seen in females at 30 hours). In contrast, PCE/NCE ratio at 30 hours was increased over control values in males at 400 and 650 mg/kg. A similar effect was not observed at any concentration at 48 hours. A significant decrease in the PCE/NCE ratio (56.5 % of the control) was observed with the positive control. The mean percentages of micronucleated PCEs were 0.38 and 0.22 for the vehicle

control males and 0.12 and 0.14 for the vehicle control females sampled at 30 and 48 hours, respectively. Mean percentages of micronucleated PCEs in CP-treated positive controls were 2.36 in males and 2.52 in females at 30 hours post-treatment. At 30 hours, a statistically significant increase in the incidence of micronucleated PCE was observed in the peripheral blood at 400 and 650 mg/kg. The effect was not dose-related. The mean percentages of micronucleated PCEs were 1.42 and 0.80 at 400 mg/kg and 1.16 and 0.80 at 650 mg/kg in males and females, respectively. At 48 hours a lower increase in micronucleated PCE than at 30 hours was found at all concentrations tested. As there was no sex-related difference in the micronucleus response between males and females sampled at 48 and 72 hours post-treatment, male and female values were combined for statistical analysis. A dose-related and statistically significant ($p<0.001$) increase in the incidence of micronuclei was observed at the 48 hours sample period. A maximum incidence of 0.69 % (3.8 times the vehicle controls) micronucleated PCEs was observed for the highest dose level tested. The maximum ratio in the incidences of micronucleated PCEs compared to control was 6.7 (0.80 % at 400 mg/kg at 30 h in females compared to 0.12 % in control females). At 72 hours the micronucleus response had returned to baseline levels.

(c): Pentan-2,4-dione (purity >98 %) was tested in an *in vivo* Mouse Micronucleus Assay. Swiss Webster Mice (5 animals per sex/dose group) were given i.p injections of 400 and 650 mg/kg. Bone marrow was sampled 6, 24 and 48 hours post-injection. Results: Serious signs of toxicity were observed in both males and females at 650 mg/kg. Hypoactivity was seen in several males and females at 400 mg/kg. No serious signs of toxicity were observed in animals of either sex after day 1. In the micronucleus study, at 6 and 24 hours a significant ($p<0.05$) increase in the PCE/NCE ratio over control values was observed at 400 mg/kg in males. No changes in the PCE/NCE ratio was seen in males at 48 hours in either treatment group and in females of either treatment group at any sampling time. The mean percentages of micronucleated PCEs were 0.19, 0.29 and 0.18 for the vehicle control males and 0.20, 0.22 and 0.31 for the vehicle control females sampled at 6, 24 and 48 hours, respectively. At 24 hours the incidence of micronucleated PCEs was significantly increased in males and females at 400 and 650 mg/kg. The effect was not dose-related. The mean percentages of micronucleated PCEs were 0.81 and 0.97 at 400 mg/kg and 1.32 and 0.80 at 650 mg/kg in males and females, respectively. Mean percentages of micronucleated PCEs in CP-treated positive controls at 24 hours post-treatment were 1.47 and 1.63 % in males and females, respectively. The frequency of micronucleated PCEs was significantly increased at 24 hours at 400 mg/kg and at 650 mg/kg, both in males and females. A maximum incidence of 1.32 % (4.6 times the vehicle controls) micronucleated PCEs was observed for the highest dose level tested. This was also the maximum ratio in the incidences of micronucleated PCEs compared to control (1.32 % at 650 mg/kg at 24 hour in males compared to 0.29 % in control males). No significant increase of micronucleated PCEs was observed at 6 and 48 hours at 400 mg/kg and at 650 mg/kg in either sex.

DOCUMENTATION PROVIDED TO EFSA

1. DG SANCO (Directorate General for Health and Consumer Affairs), 2012. Information from DG SANCO 07/02 2012, concerning two lists of 85 and 15 non-supported substances and one list of 30 substances for which no data have been submitted or which are duplicates. FLAVIS.2.23rev1.
2. Eastman Kodak Company, 1992. Initial submission: The basic toxicity of 2,4-pentanedione with cover letter dated 093092. EPA Doc 88-920008917, microfiche no. 0TS0570692. December 19, 1979. Unpublished report submitted by EFFA to SCF.
3. EFFA (European Flavour and Fragrance Association), 2002. Letter from EFFA to Dr. Joern Gry, Danish Veterinary and Food Administration. Dated 31 October 2002. Re.: Second group of questions. FLAVIS/8.26.
4. EFFA (European Flavour and Fragrance Association), 2003a. Submission 2002-4. Flavouring group evaluation of six flavouring substances (candidate chemicals) of the chemical group 10 (Annex I of 1565/2000/EC), structurally related to aliphatic acyclic and alicyclic α-diketones and related α-hydroxyketones [FAO/WHO JECFA 42/51] used as flavouring substances. 30 October 2002. SCOOP/FLAV/8.17.
5. EFFA (European Flavour and Fragrance Association), 2003b. Submission 2002-4. Flavouring group evaluation of six flavouring substances (candidate chemicals) of the chemical group 10 (Annex I of 1565/2000/EC), structurally related to aliphatic acyclic and alicyclic α-diketones and related α-hydroxyketones [FAO/WHO JECFA 42/51] used as flavouring substances. 30 October 2002. SCOOP/FLAV/8.17. European inquiry on volume of use. IOFI, International Organization of the Flavor Industry, 1995. Private communication to FEMA. Unpublished report submitted by EFFA to SCF.
6. EFFA (European Flavour and Fragrance Association), 2004a. Intake - Collection and collation of usage data for flavouring substances. Letter from Dan Dils, EFFA to Torben Hallas-Møller, EFSA. May 31, 2004.
7. EFFA (European Flavour and Fragrance Association), 2004b. Submission of 2002-4 Addendum. Supplement of one flavouring substance (candidate chemicals) to the flavouring group evaluation of the chemical group 10 (Annex I of 1565/2000/EC) structurally related to aliphatic acyclic and alicyclic α-diketones and related α-hydroxyketones [FAO/WHO JECFA 42/51] used as flavouring substances. 31 March 2004. FLAVIS/8.67. Unpublished report submitted by EFFA to FLAVIS Secretariat.
8. EFFA (European Flavour and Fragrance Association), 2007a. E-mail from Jan Demyttenaere, EFFA to FLAVIS Secretariat, National Food Institute, Technical University of Denmark. Dated 8 February 2007. RE: FLAVIS submissions - use levels for Category 14.2 - Alcoholic beverages. FLAVIS/8.70.
9. EFFA (European Flavour and Fragrance Association), 2007b. Submission 2007-10. Safety evaluation of aliphatic acyclic and alicyclic α-diketones and related α-hydroketones used as flavouring agents (S08-J18). Unpublished report submitted by EFFA to FLAVIS Secretariat. FLAVIS/8.103
10. EFFA (European Flavour Association), 2010. EFFA Letters to EFSA for clarification of specifications and isomerism for which data were requested in published FGEs.
11. EFFA (European Flavour Association), 2014. E-mail from EFFA to FLAVIS Secretariat, Danish Food Institute, Technical University of Denmark. Dated 2 June 2014. Information on substances [FL-no: 07.097, 07.184 and 07.260] in FGE.11Rev3, [FL-no: 09.305] in FGE.73Rev3 and [FL-no: 07.170] in FGE.82Rev1. FLAVIS/8.240.
12. FDA (Food and Drug Administration), 1973. Teratologic evaluation of FDA 71-73 (Starter Distillate, Hansen). Food and Drug Research Labs., Inc. Morgareidge K. Lab. no. 1573p. FDABF-GRAS-152. August 20, 1973. Unpublished report submitted by EFFA to SCF.

13. FDA (Food and Drug Administration), 1974. Mutagenic evaluation of compound FDA 71-73 (starter distillate). Liotton Bionetics, Inc. Brusick, D. FDABF-GRAS-275. Unpublished report submitted by EFFA to SCF.

14. Flavour Industry, 2005a. Unpublished information submitted by Flavour Industry to DG SANCO and forwarded to EFSA. A-11.

15. Flavour Industry, 2005b. Unpublished information submitted by Flavour Industry to DG SANCO and forwarded to EFSA. A-11.

16. Guzzie PJ and Morabit ER, 1985. 2,4-pentanedione: Salmonella/microsome (Ames) bacterial mutagenicity assay. Project report 48-140. EPA Doc FYI-OTS-0286-0434, microfiche no. 0TS0000434-0. December 2, 1985. Unpublished report submitted by EFFA to SCF.

17. Guzzie PJ and Morabit ER, 1986. 2,4-pentanedione: In vivo mouse micronucleus study. Project report 49-124. EPA Doc 89-870000070, microfiche no. OTS0510542-1. November 21, 1986. Unpublished report submitted by EFFA to SCF.

18. Moreno OM, 1977. Acute oral toxicity in rats. Dermal toxicity in rabbits. Acetyl butyryl, project no. MB 77-1744, August 18, 1977. Acetyl methyl carbinol, project no. MB 77-1691, June 20, 1977. Acetyl propionyl, MB 76-1445, January 25, 1997. MB Research Laboratories, Inc. Unpublished data submitted by EFFA to SCF.

19. Moreno OM, 1979. Acute oral toxicity in rats. Acute dermal toxicity in rabbits. Acetyl valeryl. MB Research Laboratories, Inc. Project no. MB 78-3418. Date 3/22/79. Upublished data submitted by EFFA to SCF.

20. Myers RC, Carpenter CP and Cox EF, 1977. Initial submission: Silane coupling agent: Range finding toxicity studies with cover letter dated 090892. Carnegie-Mellon Institute. Kuryla WC. September 8, 1992. EPA Doc 88-920009321, microfiche no. 0TS0571073. Unpublished report submitted by EFFA to SCF.

21. Myers RC, Slesinski RS and Frank FR, 1985. Initial submission: 2,4-pentanedione: Acute toxicity and primary irritancy studies (final report) with cover letter dated 031892. Bushy Run Research CTR. Kuryla WC. October 4 1992. EPA Doc 88-920001502, microfiche no. OTS0536178. Unpublished data submitted by EFFA to SCF.

22. Pellmont B, 1969. Studies with rats and mice on substance no. R01-3801. Toxikologisches Labor 256, Bau 69. Unpublished data submitted by EFFA to FLAVIS Secretariat.

23. San RHC and Klug ML, 1993. Salmonella/mammalian-microsome plate incorporation mutagenicity assay (Ames test). 4-Hydroxy-4-methyl-2-pentanone. Microbiological Associates, Inc. Study no. C341.501017. January 28, 1993. Unpublished report submitted by EFFA to FLAVIS Secretariat.

24. Sasaki, 2006. 3-Methyl-2,4-nonedione: Reverse mutation test "Ames Test" with S. typhimurium and E.coli. Private communication to Research Institute Fragrance Manufacturers (RIFM). Unpublished report submitted by EFFA to FLAVIS Secretariat.

25. Slesinski RS, 1986. 2,4-pentanedione: In vitro genotoxicity studies; CHO/HGPRT gene mutation test; sister chromatid exchange assay (final report) with cover letter dated 013192. Union Carbide Chem. & Plas. Co. EPA Doc 86-92000782, microfiche no. OTS0535115. January 14, 1986. Unpublished report submitted by EFFA to FLAVIS Secretariat.

26. Smyth Jr HF, 1941. Toxicologic test preformed with 2,4-pentanedione with cover letter dated 053086. Union Carbide Corp. Heywood, D.L. EPA Doc 89-8600013, microfiche no. OTSO510542. June 5, 1986. Unpublished report submitted by EFFA to FLAVIS Secretariat.

27. Smyth Jr HF, 1946a. Letter from Union Carbide Corp. to USEPA regarding toxicology studies of diacetone alcohol, with attachments dated 08/25/95. Diaceton alcohol. Union Carbide Corp. EPA Doc 86950000301, microfiche no. 0TS0557741. September 14, 1995. Unpublished report submitted by EFFA to FLAVIS Secretariat.

28. Smyth Jr HF, 1946b. Letter from Union Carbide Corp. to USEPA regarding toxicology studies of diaceton alcohol, with attachments dated 08/25/95. Diaceton alcohol. Union Carbide Corp. EPA Doc 86950000301, microfiche no. 0TS0557741. September 14, 1995. Unpublished report submitted by EFFA to FLAVIS Secretariat.

29. Stien J, 2005. Mutagenicity study of diacetyl-trimer in the Salmonella typhimurium reverse mutation assay (in vitro). LPT Report no. 18432/8/04. Laboratory of Pharmacology and Toxicology KG, Hamburg Germany. Unpublished report submitted by EFFA to FLAVIS Secretariat.

30. Strobel R, 1998. Acute toxicity study in the rat. Oral administration (gavage). Fixed dose method. Study no. 016 G 98. Private communication to FEMA.

31. Vergnes JS and Kubena MF, 1994a. 2,4-pentanedione: Bone marrow micronucleus test in mice, with cover letter dated 11/14/94. Union Carbide Corp. EPA Doc 86950000030, microfiche no. OTSO557543. October 19, 1994. Unpublished report submitted by EFFA to FLAVIS Secretariat.

32. Vergnes JS and Kubena MF, 1994b. 2,4-pentanedione: Bone marrow micronucleus test in rats, with letter dated 12/20/94. Union Carbide Corp. EPA Doc 86950000061, microfiche no. OTS0557574. December 14, 1994. Unpublished report submitted by EFFA to FLAVIS Secretariat.

33. Watters B, 2013. 3-Methylnona-2,4-dione: Induction of micronuclei in cultured human peripheral blood lymphocytes. Covance Laboratories Ltd. Study no. 8283088. 03 December 2013. Unpublished report submitted by EFFA to FLAVIS Secretariat.

REFERENCES

Aeschbacher HU, Wolleb U, Loliger J, Spadone JC and Liardon R, 1989. Contribution of coffee aroma constituents to the mutagenicity of coffee. Food and Chemical Toxicology 27(4), 227-232.

Anders MW, 1989. Biotransformation and bioactivation of xenobiotics by the kidney. In: Hutson DH, Caldwell J and Paulson GD (Eds.). Intermediary xenobiotic metabolism in animals. Taylor and Francis, New York, pp. 81-97.

Ballantyne B, Dodd DE, Myers RC and Nachreiner DJ, 1986. The acute toxicity and primary irritancy of 2,4-pentanedione. Drug and Chemical Toxicology 9(2), 133-146.

Bjeldanes LF and Chew H, 1979. Mutagenicity of 1,2-dicarbonyl compounds: maltol, kojic acid, diacetyl and related substances. Mutation Research 67, 367-371.

Bosron WF and Li TK, 1980. Alcohol dehydrogenase. In: Jakoby WB (Ed.). Enzymatic Basis of Detoxification vol. 1. Academic Press, New York, pp. 231-248.

Brooks TM, Meyer AL and Hutson DH, 1988. The genetic toxicology of some hydrocarbon and oxygenated solvents. Mutagenesis 3(3), 227-232.

CoE, 1992. Flavouring substances and natural sources of flavourings. 4th Ed. vol. I. Chemically defined flavouring substances. Council of Europe, partial agreement in the social and public health field. Strasbourg.

Colley J, Gaunt IF, Lansdown ABG, Grasso P and Gangolli SD, 1969. Acute and short-term toxicity of diacetyl in rats. Food and Cosmetics Toxicology 7, 571-580.

Cramer GM, Ford RA and Hall RL, 1978. Estimation of toxic hazard - a decision tree approach. Food and Cosmetics Toxicology 16(3), 255-276.

Dawson J and Hullin RP, 1954. Metabolism of acetoin. 1. The formation and utilization of acetoin and butane-2:3-diol in the decerebrated cat. 2. Metabolic conversions of acetoin, pyruvate and acetate by rabbit-kidney tissue despersions. Biochemical Journal 57, 177-185.

DiVincenzo GD, Kaplan CJ and Dedinas J, 1976. Characterization of the metabolites of methyl n-butyl ketone, methyl iso-butyl ketone, and methyl ethyl ketone in guinea pig serum and their clearance. Toxicology and Applied Pharmacology 36, 511-522.

Dorado L, Montoya MR and Rodriguez Mellado JM, 1992. A contribution to the study of the structure-mutagenicity relationship for alpha-dicarbonyl compounds using the Ames test. Mutation Research 269(2), 301-306.

EC (European Commission), 2000. Commission Regulation 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, 8-16.

EFSA (European Food Safety Authority), 2004. Minutes of the 7th Plenary Meeting of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food, Held in Brussels on 12-13 July 2004. Brussels, 28 September 2004. [Online]. Available: http://www.efsa.europa.eu/en/events/event/afc040712-m.pdf/

EFSA, 2005. Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in contact with food on a request from the Commission related to Flavouring Group Evaluation 10: Aliphatic primary and secondary saturated and unsaturated alcohols, aldehydes, acetals, carboxylic acids and esters containing an additional oxygenated functional group and lactones from chemical groups 9, 13 and 30 (Commission Regulation (EC) No 1565/2000 of 18 July 2000). Adopted on 28 October 2005. EFSA-Q-2003-153a.

Eurostat, 1998. Total population. Cited in Eurostat, 2004. The EU population, Total population. [Online]. Available: http://epp.eurostat.ec.europa.eu/portal/page?_pageid=1090,30070682,1090_33076576&_dad=portal&_schema=PORTAL, Population and social conditions, Population, Demography, Main demographic indicators, Total population. December 2008.

Frantz SW, Ballantyne B and Leung H-W, 1998. Acute intravenous and inhalation pharmacokinetics of 2,4-pentanedione in the Fischer 344 rat. Toxicology and Industrial Health 14(3), 413-428.

Gabriel MA, Jabara H and Al-Khalidi UAS, 1971. Metabolism of acetoin in mammalian liver slices and extracts. Biochemical Journal 124, 793-800.

Gabriel MA, Ilbawi M and Al-Khalidi UAS, 1972. The oxidation of acetoin to CO2 in intact animals and in liver mince preparation. Comparative Biochemistry and Physiology 41B, 493-502.

Garst J, Stapleton P and Johnston J, 1983. Mutagenicity of alpha-hydroxy ketones may involve superoxide anion radical. Oxy Radicals and Their Scavenger Systems 2, 125-130.

Gaunt IF, Brantom PG, Kiss IS, Grasso P and Gangolli SD, 1972. Short-term toxicity of acetoin (acetylmethylcarbinol) in rats. Food and Cosmetics Toxicology 10, 131-141.

Gava C, Perazzolo M, Zentilin L, Levis AG, Corain B, Bombi GG, Palumbo M and Zatta P, 1989. Genotoxic potentiality and DNA-binding properties of acetylacetone, maltol, and their aluminium (III) and chromium(III) neutral complexes. Toxicological and Environmental Chemistry 22(1-4), 149-157.

Gordon AJ and Ford RA, 1972. The Chemist's Companion. A Handbook of Practical Data, Techniques, and References. John Wiley & Sons, New York, pp. 49-51.

Heymann E, 1980. Carboxylesterases and amidases. In: Jakoby WB (Ed.). Enzymatic basis of detoxication. 2nd Ed. Academic Press, New York, pp. 291-323.

IOFI (International Organization of the Flavor Industry), 1995. European inquiry on volume of use.

Iwata T, Kokuba S, Ariga F, Hiramatsu Y, Nose T and Aoyama T, 1984. [Mutagenicity of lenampicillin hydrochloride (KBT-1585)B and its metabolites]. Chemotherapy 32(8), 153-159. (In Japanese)

JECFA (Joint FAO/WHO Expert Committee on Food Additives), 1995. Evaluation of certain food additives and contaminants. Forty-fourth Meeting of the Joint FAO/WHO Expert Committee on Food Additives. 14-23 February 1995. WHO Technical Report Series, no. 859. Geneva.

JECFA (Joint FAO/WHO Expert Committee on Food Additives), 1996. Toxicological evaluation of certain food additives. Forty-fourth Meeting of the Joint FAO/WHO Expert Committee on Food Additives and contaminants. WHO Food Additives Series: 35. IPCS, WHO, Geneva.

JECFA (Joint FAO/WHO Expert Committee on Food Additives), 1997. Evaluation of certain food additives and contaminants. Forty-sixth report of the Joint FAO/WHO Expert Committee on Food Additives. Geneva, 6-15 February 1996. WHO Technical Report Series, no. 868. Geneva.

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

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

JECFA (Joint FAO/WHO Expert Committee on Food Additives), 1999b. Evaluation of certain food additives and contaminants. Forty-ninth report of the Joint FAO/WHO Expert Committee on Food Additives. Rome, 17-26 June 1997. WHO Technical Report Series, no. 884. Geneva.

JECFA (Joint FAO/WHO Expert Committee on Food Additives), 2000a. Evaluation of certain food additives. Fifty-first Meeting of the Joint FAO/WHO Expert Committee on Food Additives. Geneva, 9-18 June 1998. WHO Technical Report Series, no. 891. Geneva.

JECFA (Joint FAO/WHO Expert Committee on Food Additives), 2000b. Compendium of food additive specifications. Addendum 8. Joint FAO/WHO Expert Committee of Food Additives. Fifty-fifth Meeting. Geneva, 6-15 June 2000. FAO Food and Nutrition paper 52 Add. 8.

JECFA (Joint FAO/WHO Expert Committee on Food Additives), 2001. Compendium of food additive specifications. Addendum 9. Joint FAO/WHO Expert Committee of Food Additives 57th session. Rome, 5-14 June 2001. FAO Food and Nutrition paper 52 Add. 9.

JECFA (Joint FAO/WHO Expert Committee on Food Additives), 2003. Compendium of food additive specifications. Addendum 11. Joint FAO/WHO Expert Committee of Food Additives 61st session. Rome, 10-19 June 2003. FAO Food and Nutrition paper 52 Add. 11.

Jenner PM, Hagan EC, Taylor JM, Cook EL and Fitzhugh OG, 1964. Food flavorings and compounds of related structure. I. Acute oral toxicity. Food and Cosmetics Toxicology 2, 327-343.

Juni E and Heym GA, 1956. A cyclic pathway for the bacterial dissimilation of 2,3-butanediol, acetylmethylcarbinol, and diacetyl. Journal of Bacteriology 71, 425-432.

Järnefelt J, 1955. Studies on the enzymatic synthesis and breakdown of acetoin in the animal organism. Annales Academiae Scientiarum Fennicae: Medica-anthropologica 57, 7-78.

Kato F, Araki A, Nozaki K and Matsushima T, 1989. Mutagenicity of aldehydes and diketones. Mutation Research 216, 366-367.

Kawano T, 1959. On the relation of acetoin with pantothenic acid. Fukuoka Igaku Zasshi 50, 2939-2953.

Kim SB, Hayase F and Kato H, 1987b. Desmutagenic effect of α-dicarbonyl and α-hydroxycarbonyl compounds against mutagenic heterocyclic amines. Mutation Research 177, 9-15.

Kopf R, Loeser A and Meyer G, 1950. Untersuchungen über die Pharmakologie und Toxikologie mehrwertiger Alkohole (1,3-butylenglykol). Archiv für Experimentelle Pathologie und Pharmakologie 210, 346-360. (In German)

Marnett LJ, Hurd HK, Hollstein MC, Levin DE, Esterbauer H and Ames BN, 1985. Naturally-occurring carbonyl compounds are mutagens in Salmonella tester strain TA104. Mutation Research 148, 25-34.

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

Montgomery JA, David F, Garneau M and Brunengraber H, 1993. Metabolism of 2,3-butanediol stereoisomers in the perfused rat liver. Journal of Biological Chemistry 268(27), 20185-20190.

NTP (National Toxicology Program), 1994. Bone Marrow Micronucleus study (2,3-butanedione). Study no. A44706; http://ntp.niehs.nih.gov/

Ono Y, Somiya I and Kawamura M, 1991. The evaluation of genotoxicity using DNA repairing test for chemicals produced in chlorination and ozonation processes. Water Science & Technology 23, 329-338.

Otsuka M, Mine T, Ohuchi K and Ohmori S, 1996. A detoxication route for acetaldehyde: Metabolism of diacetyl, acetoin, and 2,3-butanediol in liver homogenate and perfused liver of rats. Journal of Biochemistry 119, 246-251.

Otsuka M, Harada N, Itabashi T and Ohmori S, 1999. Blood and urinary levels of ethanol, acetaldehyde, and C4 compounds such as diacetyl, acetoin, and 2,3-butanediol in normal male students after ethanol ingestion. Alcohol 17(2), 119-124.

Posternak NM, Linder A and Vodoz CA, 1969. Summaries of toxicological data. Toxicological tests on flavouring matters. Food and Cosmetics Toxicology 7, 405-407.

SCF (Scientific Committee for Food), 1995. Scientific Committee for Food. First annual report on chemically defined flavouring substances. May 1995, 2nd draft prepared by the SCF Working Group on Flavouring Substances (Submitted by the SCF Secretariat, 17 May 1995). CS/FLAV/FL/140-Rev2. Annex 6 to Document III/5611/95, European Commission, Directorate-General III, Industry.

SCF (Scientific Committee for Food), 1999. Opinion on a programme for the evaluation of flavouring substances (expressed on 2 December 1999). Scientific Committee on Food. SCF/CS/FLAV/TASK/11 Final 6/12/1999. Annex I to the minutes of the 119th Plenary meeting. European Commission, Health & Consumer Protection Directorate-General.

Shane BS, Troxclair AM, McMillin DJ and Henry CB, 1988. Comparative mutagenicity of nine brands of coffee to Salmonella typhimurium TA100, TA102, and TA104. Environmental and Molecular Mutagenesis 11, 195-206.

Suwa Y, Nagao M, Kosugi A and Sugimura T, 1982. Sulfite suppresses the mutagenic property of coffee. Mutation Research 102, 383-391.

TNO (Nederlandse Organisatie voor Toegepast Natuurwetenschappelijk Onderzoek), 2000. VCF Volatile Compounds in Food. Nijssen LM, van Ingen-Visscher CA and Donders JJH (Eds.). Database. Zeist, The Netherlands. TNO Triskelion, 1963-2000.

TNO (Nederlandse Organisatie voor Toegepast Natuurwetenschappelijk Onderzoek), 2014. VCF Volatile Compounds in Food. Nijssen LM, van Ingen-Visscher CA and Donders JJH (Eds.). Database version 15.1. Zeist, The Netherlands. TNO Triskelion, 1963-2014.

Veech RL, Gitomer WL and Casazza JP, 1987. Metabolic pathways leading to diol formation. Genetics and Alcoholism 241, 185-199.

Westerfeld WW and Berg RL, 1943. Observations on the metabolism of acetoin. Journal of Biological Chemistry 148(3), 523-528.

Whittaker P, Clarke JJ, San RHC, Begley TH and Dunkel VC, 2008. Evaluation of the butter flavouring chemical diacetyl and a fluorochemical paper additive for mutgenicity and toxicity using the mammalian cell gene mutation assay in L5178Y mouse lymphoma cells. Food and Chemical Toxicology 46, 2928-2933.

Zimmermann FK and Mohr A, 1992. Formaldehyde, glyoxal, urethane, methyl carbamate, 2,3-butanedione, 2,3-hexanedione, ethyl actylate, dibromoacetonitrile, 2-hydroxypropionitrile induce chromosome loss in Saccharomyces cerevisiae. Mutation Research 270, 151-166.

Zimmermann FK, Mayer VW, Scheel I and Resnick MA, 1985. Acetone, methyl ethyl ketone, ethyl acetate, acetonitrile and other polar aprotic solvents are strong inducers of aneuploidy in Saccharomyces cerevisiae. Mutation Research 149, 339-351.

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 (EC, 2000), 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; JECFA, 1996; JECFA, 1997; JECFA, 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, 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 1800, 540 or 90 µg/person/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[11] (Step 2)?
- do their exposures exceed the threshold of concern for the structural class (Step A3 and B3)?
- are the flavourings or their metabolites endogenous[12] (Step A4)?
- does a NOAEL exist on the flavourings or on structurally related substances (Step 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.

[11] "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).

[12] "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).

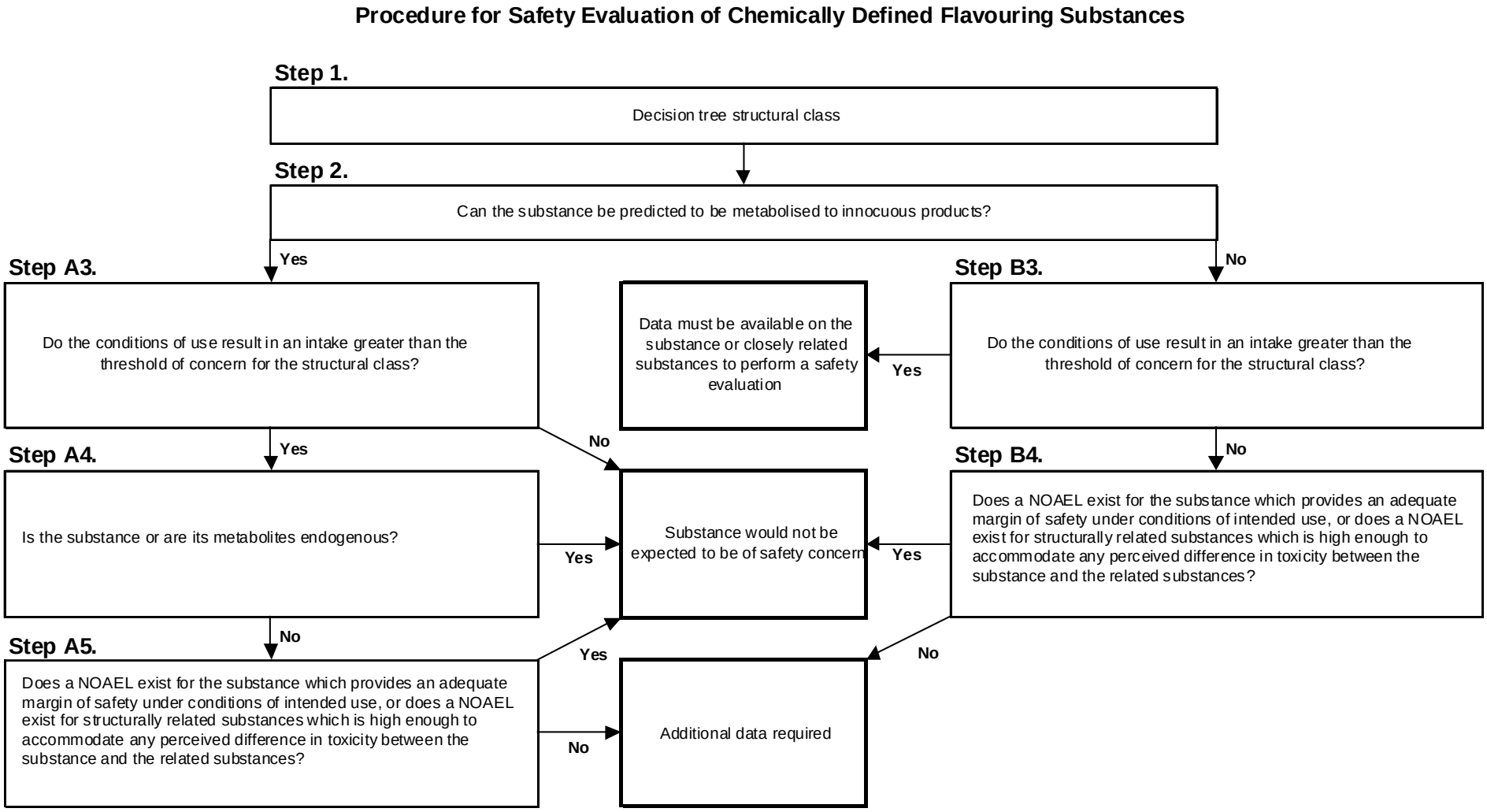


Figure A1: Procedure for Safety Evaluation of Chemically Defined Flavouring Substances

APPENDIX B: USE LEVELS / MTAMDI

B.1 Normal and Maximum Use Levels

For each of the 18 Food categories (Table B.1.1) in which the candidate substances are used, Flavour Industry reports a "normal use level" and a "maximum use level" (EC, 2000). According to the 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, 2002). The normal and maximum use levels in different food categories have been extrapolated from figures derived from 12 model flavouring substances (EFFA, 2004a).

Table B.1.1. Food categories according to Commission Regulation (EC) No 1565/2000

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 (incl. mushrooms & fungi, roots & tubers, pulses and legumes), and nuts & seeds
05.0	Confectionery
06.0	Cereals and cereal products, incl. 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, incl. 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 (EFFA, 2003a; EFFA, 2003b; EFFA, 2004b; EFFA, 2007a; EFFA, 2007b; Flavour Industry, 2005a; Flavour Industry, 2005b) for all the candidate substances in the present flavouring group (Table B.1.2).

Table B.1.2. Normal and Maximum use levels (mg/kg) for the candidate substances in FGE.11Rev3 (EFFA, 2003a; EFFA, 2003b; EFFA, 2004b; EFFA, 2007a; EFFA, 2007b; Flavour Industry, 2005a; Flavour Industry, 2005b)

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**
02.133	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
07.071	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
07.097	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
07.152	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
07.165	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
07.167	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
07.168	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
07.184	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
07.238	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
07.248	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
07.260	3 6	- -	3 7	3 6	3 6	3 6	- -	3 3	- -	- -	- -	- -	- -	- -	3 6	3 6	- -	- -

B.2 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 B.2.1. These consumption estimates are then multiplied by the reported use levels in the different food categories and summed up.

Table B2.1.: 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 and reported by the Flavour Industry in the following way (see Table B.2.2):

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

Table B2.2.: Distribution of the 18 food categories listed in Commission Regulation (EC) No 1565/2000 (EC, 2000) into the seven SCF food categories used for TAMDI calculation (SCF, 1995)

	Food categories according to Commission Regulation 1565/2000	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 (incl. mushrooms & fungi, roots & tubers, pulses and legumes), and nuts & seeds	Food		
05.0	Confectionery			Exception a
06.0	Cereals and cereal products, incl. 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		

Table B2.2.: Distribution of the 18 food categories listed in Commission Regulation (EC) No 1565/2000 (EC, 2000) into the seven SCF food categories used for TAMDI calculation (SCF, 1995)

	Food categories according to Commission Regulation 1565/2000	**Distribution of the seven SCF food categories**		
Key	**Food category**	**Food**	**Beverages**	**Exceptions**
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, incl. 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		

The mTAMDI values (see Table B.2.3) are presented for all candidate substances in the present flavouring group (EFFA, 2003a; EFFA, 2003b; EFFA, 2007b; EFFA, 2004b; Flavour Industry, 2005a; Flavour Industry, 2005b);. The mTAMDI values are only given for the highest reported normal use levels (see Table B.2.3).

Table B2.3.: Estimated intakes based on the mTAMDI approach

FL-no	**EU Register name**	**mTAMDI (µg/person/day)**	**Structural class**	**Threshold of concern (µg/person/day)**
02.133	Butane-2,3-diol	3900	Class I	1800
07.097	3-(Hydroxymethyl)octan-2-one	1600	Class I	1800
07.165	4-Hydroxy-4-methylpentan-2-one	1600	Class I	1800
07.167	4-Hydroxyhexan-3-one	1600	Class I	1800
07.238	3-Hydroxy-2-octanone	1600	Class I	1800
07.071	Octane-4,5-dione	1600	Class II	540
07.152	3,3-Diethoxybutan-2-one	1600	Class II	540
07.184	3-Methylnona-2,4-dione	1600	Class II	540
07.248	Octan-2,3-dione	1600	Class II	540
07.260	1- or 3-Hydroxy-5-methyl-2- or 3-hexanone	1500	Class II	540
07.168	2-Hydroxypiperitone	1600	Class III	90

APPENDIX C: METABOLISM

C.1 Absorption, Distribution and Elimination

The candidate substances and supporting substances which are aliphatic acyclic α-diketones participate in a keto-enol equilibrium with the corresponding ketoenol (see Figure C.1). The keto form predominates (Gordon and Ford, 1972).

In rats and mice, orally administered acetoin (3-hydroxybutan-2-one [FL-no: 07.051]) is rapidly absorbed from the gastrointestinal tract (Gabriel et al., 1972). Upon injection of acetoin-2,3-^{14}C to albino rats, $^{14}CO_2$ appears in the expired air. The average 12 - 20 hours $^{14}CO_2$ production from acetoin-2,3-^{14}C was found to be 15 % after intraperitoneal (i.p.) administration (12 hours) and 47.7 % after intracardial administration (20 hours) (Gabriel et al., 1972).

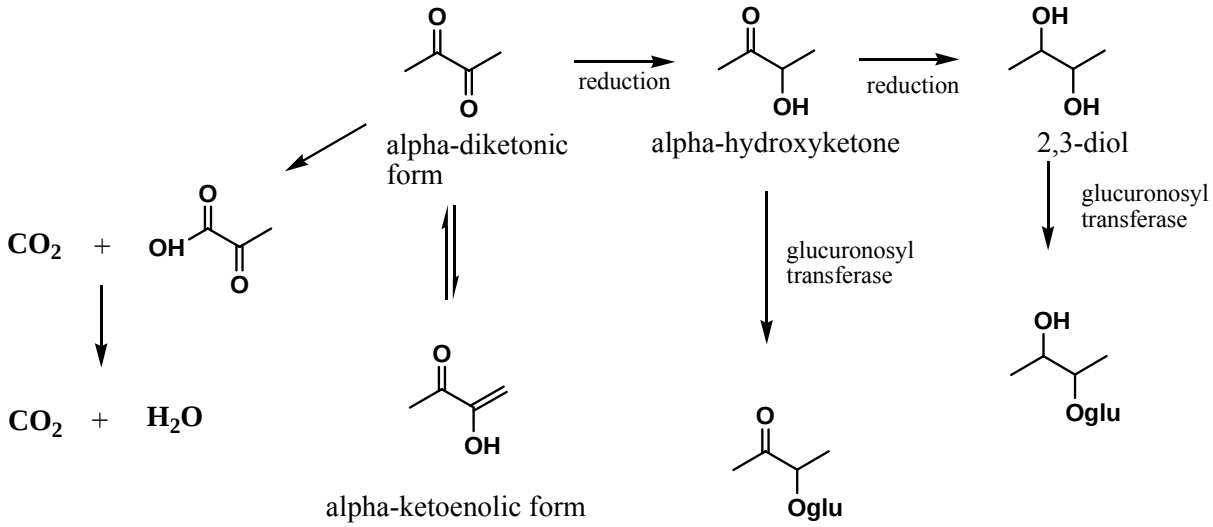


Figure C1: Keto-enol equilibrium and metabolism of aliphatic acyclic and alicyclic α-dicarbonyls

C.2 Biotransformation

C.2.1. Hydrolysis

Ketals are anticipated to be readily hydrolysed after ingestion under the acid conditions in the stomach to their corresponding alcohol and ketone prior to absorption.

In general, esters are hydrolysed to their corresponding alcohol and carboxylic acid. Classes of enzymes recognized as carboxylesterases or esterases, the most important of which are the B-esterases, catalyse hydrolysis. Acetyl esters are the preferred substrates of C-esterases (Heymann, 1980). In mammals these enzymes occur in most tissues throughout the body (Anders, 1989; Heymann, 1980) but predominate in hepatocytes (Heymann, 1980). As an example, it is expected that the supporting chemicals, 2-acetoxy-3-butanone and butanon-3-one-2-yl butanoate, are metabolised in humans to acetic acid and butanoic acid, respectively, and acetoin.

C.2.2. Metabolism of Aliphatic Acyclic Diketones

The metabolic fate of acyclic aliphatic diketones depends primarily on the position of the carbonyl function and the chain length. Aliphatic acyclic diketones and α–hydroxyketones, which contain a carbonyl function at the 2-position (i.e. a methyl ketone) may undergo α–hydroxylation and subsequent oxidation of the terminal methyl group to eventually yield corresponding ketocarboxylic acids. The ketoacids are intermediary metabolites (e.g. α-ketoacids), which may undergo oxidative decarboxylation to yield carbon dioxide and an aliphatic carboxylic acid. The acid may be completely metabolised in the fatty acid pathway and citric acid cycle (see Figure C.1). β-Keto-acids and derivatives readily undergo decarboxylation. Along with α-keto- and α-hydroxyacids, they yield breakdown products, which are incorporated into normal biochemical pathways (EFSA, 2005).

Alternately, the methyl-substituted diketones may be successively reduced to the corresponding hydroxyketones and diols, which are excreted in the urine as glucuronic acid conjugates. This pathway is favoured at elevated *in vivo* concentrations, especially for longer chain length ketones. If the carbonyl function is located elsewhere on the chain, reduction is the predominant detoxification pathway.

α-Hydroxyketones or their diol metabolites may be excreted as glucuronic acid conjugates (JECFA, 1999a).

Acetoin is metabolised primarily via oxidation at low concentrations *in vivo* and by reduction to 2,3-butanediol (butane-2,3-diol) at high concentrations. It is estimated that the rat liver is capable of oxidising 86 µg (1 µmol) acetoin/g liver per day (Gabriel et al., 1972).

Oxidation of the terminal methyl group may form an alpha-ketoacid, which undergoes cleavage to yield CO_2 and a carboxylic acid fragment.

A total dose of 78 g of acetoin was administered to a dog over a two-month period. The doses were given orally in a 3 to 4 percent solution and subcutaneously in a 20 percent solution.

Urine was collected under toluene from the beginning of the dosing period through 40 hours after the last treatment. Butane-2,3-diol was identified as the major urinary excretion product, ranging from 5 to 25 percent of the dose. The remainder of the dose was completely metabolised (Westerfeld and Berg, 1943).

In liver preparations obtained from rats and rabbits, greater than 95 % of the radioactivity of 2,3-^{14}C-acetoin was detected as a mixture of stereoisomers of butane-2,3-diol (Gabriel et al., 1971). Although reductions of diacetyl and acetoin have been observed in animals *in vivo* and in animal tissue preparation *in vitro* at high concentrations, it appears that oxidation of diacetyl is a major endogenous metabolic pathway.

Reduction of ketones is mediated by alcohol dehydrogenase and NADPH dependent cytosolic carbonyl reductases (Bosron and Li, 1980). Reduction of acetoin and diacetyl is catalysed by the substrate-specific enzymes diacetyl reductase and acetoin reductase, respectively. In rat liver mince, diacetyl, acetoin and butane-2,3-diol are interconvertible (Gabriel et al., 1972).

In male Wistar albino rats, a single oral dose of 5 mmol diacetyl/kg bw (430 mg diacetyl/kg bw) was metabolised by reduction to acetoin, which was present in high concentrations of major organs one hour after dosing. The subsequent reduction product, butane-2,3-diol, was detected in the liver, kidney and brain. Only 10 minutes incubation time was required to convert 10 nmol (9 x 10^{-4} mg) diacetyl to 3.7 nmol (3 x 10^{-4} mg) acetoin and 6.3 nmol (6 x 10^{-4} mg) butane-2,3-diol in rat liver homogenate (Otsuka et al., 1996). The organ-specific reductase activity was greatest in the liver and least in the brain (Otsuka et al., 1996).

Diacetyl and acetoin are reported to be endogenously formed in cats when pyruvate is converted to diacetyl and acetoin by pyruvate decarboxylase (Gabriel et al., 1972). Mean fasting blood acetoin levels of approximately 100 µg acetoin/100 ml blood have been reported (Dawson and Hullin, 1954). Pyruvate also forms diacetyl *in vitro* in rat liver preparations (Järnefelt, 1955) and in microorganisms (Juni and Heym, 1956).

Diacetyl, acetoin and butane-2,3-diol are also reported to be endogenous in humans at levels of 0.25 - 0.75 microM, 2.2 microM and 5 - 10 microM, respectively, in plasma. Plasma levels of diacetyl and acetoin, precursors of butane-2,3-diol were not affected by ethanol consumption, whereas plasma levels of butane-2,3-diol were elevated in individuals defective in aldehyde dehydrogenase (Otsuka et al., 1999) showing that acetoin is rapidly reduced to butan-2,3-diol in humans.

C.3 Studies on Candidate Substances

Butane-2,3-diol [FL-no: 02.133]:

Diacetyl, acetoin and butane 2,3-diol have been reported to be endogeous in humans. Higher levels of butane-2,3-diol, but not of diacetyl and acetoin were found in blood and urine of individuals defective in aldehyde dehydrogenase compared to normal individuals. This suggest that acetaldehyde formed from ethanol is converted to diacetyl, acetoin and eventually to butane-2,3-diol (Otsuka et al., 1999). The metabolic interrelationship of these chemicals is discussed above. Butane-2,3-diol may be an intermediate in the mammalian metabolism of acetaldehyde *in vitr*o, and butane-2,3-diol and its oxidation metabolite, acetoin, have been reported as intermediates in the mammalian metabolism of pyruvate *in vitro* (Veech et al., 1987; Montgomery et al., 1993).

Butane-2,3-diol, 2-butanol and 3-hydroxy-2-butanone were identified as metabolites in the serum of guinea pigs injected i.p. with methyl ethyl ketone. 3-Hydroxy-2-butanone forms by alpha-hydroxylation of methyl ethyl ketone that subsequently forms butane-2,3-diol by reduction of the ketone function. The half-life of methyl ethyl ketone in serum was 270 minutes, and the clearance time of butane-2,3-diol was 16 hours (DiVincenzo et al., 1976). A proposed pathway of butane-2,3-diol elimination is as 2,3-butanediol β-glucuronide after coupling with UDP-glucuronyltransferase (Otsuka et al., 1999).

4-Hydroxy-4-methylpentan-2-one [FL-no: 07.165]:

4-Hydroxy-4-methylpentan-2-one and 4-methyl-2-pentanol were detected in serum after i.p. injection of 4-methyl-2-pentanone to guinea pigs. 4-Hydroxy-4-methylpentan-2-one was the principal metabolite and was cleared in 16 hours. The concentration of 4-methyl-2-pentanol was too low for quantification. 4-Methyl-2-pentanone is metabolised by oxidation at the omega-1 carbon atom to form the hydroxylated ketone, 4-hydroxy-4-methylpentan-2-one, and to a lesser extent by reduction of the carbonyl group to form the secondary alcohol, 4-methyl-2-pentanol (DiVincenzo et al., 1976).

Pentan-2,4-dione:

Pentan-2,4-dione was investigated for its comparative pharmacokinetics in male F344 rats (4/1 group) by single intravenous (i.v.) injection of 4.3, 43, 148.5 and 430 mg/kg bw, or a 6-hour nose only inhalation exposure to ^{14}C-pentan-2,4-dione. Only the i.v. part of the study is reported here. The plasma concentration of ^{14}C-pentan-2,4-dione derived radioactivity declined in a biexponential fashion. The ^{14}C plasma concentration-time curves and derived pharmacokinetic parameters indicated that dose-linear kinetics occurred in the i.v. dose range of 4.3 to 148.5 mg/kg, but not at 430 mg/kg. Metabolism of pentan-2,4-dione was rapid in that the concentration of unmetabolised pentan-2,4-dione declined steadily to undetectable levels after eight hours. ^{14}C-pentan-2,4-dione derived radioactivity was eliminated mainly as exhaled $^{14}CO_2$ and in the urine. In the 48-hour samples for the 4.3, 43 and 148.5 mg/kg doses, $^{14}CO_2$ elimination was constant at 36.8, 38.8 and 42.3 %, and greater than the urinary ^{14}C excretion of 17.9, 14.3 and 29.6 %, respectively. However, at the 430 mg/kg i.v. dose there was a reversal of the excretion pattern, with the urine ^{14}C excretion (54.7 %) becoming greater than the

exhaled $^{14}CO_2$ (27.3 %). Free parent compound and seven metabolites were detected in the 12-hour urine samples. In the 24 - 48-hour urine samples only one metabolite was detectable in small amounts (Frantz et al., 1998). At lower dose levels oxidation predominates, whereas at the high dose urinary hydroxylated metabolites formed by hydroxylation and ketone reduction predominate. These mechanisms are similar to those observed for diacetyl.

C.4 Conclusions on Metabolism

It is anticipated that humans will metabolise aliphatic acyclic methyl ketones principally by oxidation of the terminal methyl group at low levels of exposure. At higher levels, reduction to the diol and subsequent conjugation with glucuronic acid is a competing detoxification pathway. Other aliphatic acyclic diketones and hydroxyketones are reduced, conjugated with glucuronic acid and excreted. The ketals in the present FGE are anticipated to be hydrolysed to the corresponding alcohols and ketones.

ABBREVIATIONS

ADI	Acceptable Daily Intake
BW	Body Weight
CAS	Chemical Abstract Service
CEF	Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids Chemical Abstract Service
CHO	Chinese hamster ovary (cells)
CoE	Council of Europe
DNA	Deoxyribonucleic acid
DMSO	Dimethyl sulphoxide
EC	European Commission
EFFA	European Flavour and Fragrance Association
EFSA	The European Food Safety Authority
EU	European Union
FAO	Food and Agriculture Organization of the United Nations
FEMA	Flavor and Extract Manufacturers Association
FGE	Flavouring Group Evaluation
GLP	Good Laboratory Practice
GSH	Glutathione
FLAVIS (FL)	Flavour Information System (database)
HPLC	High-performance liquid chromatography
ID	Identity
IOFI	International Organization of the Flavour Industry
IP	Intraparenteral
IR	Infrared spectroscopy
IV	Intravenous
JECFA	The Joint FAO/WHO Expert Committee on Food Additives
LD_{50}	Lethal Dose, 50 %; Median lethal dose
MNBN	Micronucleated binucleate cells
MS	Mass spectrometry
MSDI	Maximised Survey-derived Daily Intake
MTS	Minimum Toxicity Screen
mTAMDI	Modified Theoretical Added Maximum Daily Intake
NAD	Nicotinamide Adenine Dinucleotide
NADP	Nicotinamide Adenine Dinucleotide Phosphate
NADPH	Nicotinamide Adenine Dinucleotide Phosphate, reduced form
NMR	Nuclear magnetic resonance

No	Number
NOAEL	No Observed Adverse Effect Level
NOEL	No Observed Effect Level
NTP	National Toxicology Program
OECD	Organisation for Economic Co-operation and Development
PHA	Phytohaemagglutinin
RI	Replication Index
SC	Structural class
SCE	Sister Chromatid Exchange
SCF	Scientific Committee on Food
SMART	Somatic Mutation and Recombination Test
TAMDI	Theoretical Added Maximum Daily Intake
UDP	Uridine DiPhosphate
UDS	Unscheduled DNA Synthesis
WHO	World Health Organization