European Food Safety Authority

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SCIENTIFIC OPINION

Flavouring Group Evaluation 80, Revision 1 (FGE.80Rev1):

Consideration of alicyclic, alicyclic-fused and aromatic-fused ring lactones evaluated by JECFA (61st meeting) structurally related to a aromatic lactone evaluated by EFSA in FGE.27 (2008)

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

(Question No EFSA-Q-2009-00559)

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PANEL MEMBERS

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SUMMARY

The Scientific Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (the Panel) was asked to provide scientific advice to the Commission on the implications for human health of chemically defined flavouring substances used in or on foodstuffs in the Member States. In particular, the Panel was requested to consider the Joint FAO/WHO Expert Committee on Food Additives (the JECFA) evaluations of flavouring substances assessed since 2000, and to decide whether no further evaluation is necessary, as laid down in Commission Regulation (EC) No 1565/2000. These flavouring substances are listed in the Register, which was adopted by Commission Decision 1999/217 EC and its consecutive amendments.

The present consideration concerns 13 alicyclic, alicyclic-fused and aromatic-fused ring lactones evaluated by the JECFA (61st meeting) and will be considered in relation to the European Food

Safety Authority (EFSA) evaluation of one aromatic lactone (phthalide [FL-no: 10.056]) evaluated in the Flavouring Group Evaluation 27 (FGE.27). Furthermore, the JECFA evaluation is supported by a group of lactones evaluated in FGE.10 as well as by alicyclic secondary and tertiary alcohols in FGE.09 and FGE.18, respectively.

The Panel agrees with the way the application of the Procedure has been performed by the JECFA for all 13 substances considered in this FGE. However, for six of 13 substances [FL-no: 10,050, 10.061, 10.069, 10.070, 10.072 and 13.161] the JECFA evaluation is only based on the Maximised Survey-derived Daily Intake (MSDI) value derived from a production figure from the USA. Accordingly, the safety in use in Europe could not be assessed using the Procedure, so EU production figures are needed in order to finalise the evaluation of these six substance.

For all 13 substances evaluated through the Procedure use levels are needed to calculate the mTAMDIs in order to identify those flavouring substances that need more refined exposure assessment and to finalise the evaluation.

In order to determine whether the conclusion for the 13 JECFA evaluated substances can be applied to the materials of commerce, it is necessary to consider the available specifications. Adequate specifications including complete purity criteria and identity are available for six of the 13 JECFA evaluated substances. For seven substances [FL-no: 10.050, 10.061, 10.069, 10.070, 10.072, 10.169 and 13.161] information on the stereoisomeric composition is lacking and in addition for two of the substances [FL-no: 10.069 and 10.169] further information on the composition is requested.

Thus, for seven substances [FL-no: 10.050, 10.061, 10.069, 10.070, 10.072, 10.169 and 13.161] the Panel has reservations (no European production volumes available, preventing them from being evaluated using the Procedure, and/or missing data on stereoisomerism and/or further information on the composition of the mixture). For the remaining six of the 13 JECFA evaluated alicyclic, alicyclic-fused and aromatic-fused ring lactones [FL-no: 10.005, 10.024, 10.025, 13.009, 13.012 and 16.055] the Panel agrees with the JECFA conclusion “No safety concern at estimated levels of intake as flavouring substances” based on the MSDI approach.

KEYWORDS

Alicyclic, alicyclic-fused, aromatic-fused ring, lactones, JECFA, 61th meeting, FGE.27.

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BACKGROUND

Regulation (EC) No 2232/96 of the European Parliament and the Council (EC, 1996) lays down a Procedure for the establishment of a list of flavouring substances, the use of which will be authorised to the exclusion of all other substances in the EU. In application of that Regulation, a Register of flavouring substances used in or on foodstuffs in the Member States was adopted by Commission Decision 1999/217/EC (EC, 1999a), as last amended by Commission Decision 2009/163/EC (EC, 2009a). Each flavouring substance is attributed a FLAVIS-number (FL-number) and all substances are divided into 34 chemical groups. Substances within a group should have some metabolic and biological behaviour in common.

Substances which are listed in the Register are to be evaluated according to the evaluation programme laid down in Commission Regulation (EC) No 1565/2000 (EC, 2000a), which is broadly based on the Opinion of the Scientific Committee on Food (SCF, 1999).

Commission Regulation (EC) No 1565/2000 lays down that substances that are contained in the Register and will be classified in the future by the Joint FAO/WHO Expert Committee on Food Additives (the JECFA) so as to present no safety concern at current levels of intake will be considered by the European Food Safety Authority (EFSA), who may then decide that no further evaluation is necessary.

In the period 2000 – 2008, during its 55th, 57th, 59th, 61st, 63rd, 65th, 68th and 69th meetings, the JECFA evaluated about 1000 substances which are in the EU Register.

TERMS OF REFERENCE

EFSA is requested to consider the JECFA evaluations of flavouring substances assessed since 2000, and to decide whether no further evaluation is necessary, as laid down in Commission Regulation (EC) No 1565/2000 (EC, 2000a). These flavouring substances are listed in the Register, which was adopted by Commission Decision 1999/217/EC (EC, 1999a) and its consecutive amendments.

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ASSESSMENT

The approach used by EFSA for safety evaluation of flavouring substances is referred to in Commission Regulation (EC) No 1565/2000 (EC, 2000a), hereafter named the "EFSA Procedure". This Procedure is based on the Opinion of the Scientific Committee on Food (SCF, 1999), which has been derived from the evaluation procedure developed by the Joint FAO/WHO Expert Committee on Food Additives (JECFA, 1995; JECFA, 1996a; JECFA, 1997a; JECFA, 1999b), hereafter named the "JECFA Procedure". The Scientific Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (the Panel) compares the JECFA evaluation of

structurally related substances with the result of a corresponding EFSA evaluation, focussing on specifications, intake estimations and toxicity data, especially genotoxicity data. The evaluations by EFSA will conclude whether the flavouring substances are of no safety concern at their estimated levels of intake, whether additional data are required or whether certain substances should not be evaluated through the EFSA Procedure.

The following issues are of special importance.

Intake

In its evaluation, the Panel as a default uses the Maximised Survey-derived Daily Intake (MSDI) approach to estimate the per capita intakes of the flavouring substances in Europe.

In its evaluation, the JECFA includes intake estimates based on the MSDI approach derived from both European and USA production figures. The highest of the two MSDI figures is used in the evaluation by the JECFA. It is noted that in several cases, the MSDI figures only from the USA were available, meaning that certain flavouring substances have been evaluated by the JECFA only on the basis of these figures. For Register substances for which this is the case the Panel will need EU production figures in order to finalise the evaluation.

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. It is noted that the JECFA, at its 65th meeting, considered ”how to improve the identification and assessment of flavouring agents, for which the MSDI estimates may be substantially lower than the dietary exposures that would be estimated from the anticipated average use levels in foods” (JECFA, 2006c).

In the absence of more accurate 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.

As information on use levels for the flavouring substances has not been requested by the JECFA or has not otherwise been provided to the Panel, it is not possible to estimate the daily intakes using the mTAMDI approach for the substances evaluated by the JECFA. The Panel will need information on use levels in order to finalise the evaluation.

Threshold of 1.5 Microgram/Person/Day (Step B5) Used by the JECFA

The JECFA uses the threshold of concern of 1.5 microgram/person/day as part of the evaluation procedure:

“The Committee noted that this value was based on a risk analysis of known carcinogens which involved several conservative assumptions. The use of this value was supported by additional information on developmental toxicity, neurotoxicity and immunotoxicity. In the judgement of the Committee, flavouring substances for which insufficient data are available for them to be evaluated using earlier steps in the Procedure, but for which the intake would not exceed 1.5 microgram per person per day would not be expected to present a safety concern. The Committee recommended that the Procedure for the Safety Evaluation of Flavouring Agents used at the forty-sixth meeting be

amended to include the last step on the right-hand side of the original procedure ("Do the condition of use result in an intake greater than 1.5 microgram per day?")" (JECFA, 1999b).

In line with the Opinion expressed by the Scientific Committee on Food (SCF, 1999), the Panel does not make use of this threshold of 1.5 microgram per person per day.

Genotoxicity

As reflected in the Opinion of SCF (SCF, 1999), the Panel has in its evaluation focussed on a possible genotoxic potential of the flavouring substances or of structurally related substances. Generally, substances for which the Panel has concluded that there is an indication of genotoxic potential *in vitro,* will not be evaluated using the EFSA Procedure until further genotoxicity data are provided. Substances for which a genotoxic potential *in vivo* has been concluded, will not be evaluated through the Procedure.

Specifications

Regarding specifications, the evaluation by the Panel could lead to a different opinion than that of the JECFA, since the Panel requests information on e.g. isomerism.

Structural Relationship

In the consideration of the JECFA evaluated substances, the Panel will examine the structural relationship and metabolism features of the substances within the flavouring group and compare this with the corresponding FGE.

HISTORY OF THE EVALUATION OF THE SUBSTANCES IN THE PRESENT FGE

At its 61st meeting the JECFA evaluated a group of 16 flavouring substances consisting of alicyclic, alicyclic-fused and aromatic-fused ring lactones. One of the JECFA evaluated substances is not in the Register (dihydro-5-((Z,Z)octa-2,5-dienyl)-2(3H)-furanone) (JECFA no: 1160) and four of the substances evaluated by the JECFA [FL-no: 10.034, 10.036, 10.169, 13.012] were concluded to be possible precursors for alpha,beta-unsaturated ketones. As the alpha,beta-unsaturated aldehyde and ketone structures are considered by the Panel to be structural alerts for genotoxicity (EFSA, 2008b), they have been given special considerations in the Flavouring Group Evaluation 19 (FGE.19). The remaining 11 flavouring substances have originally been considered by EFSA in the FGE.80 (EFSA, 2008ax).

FGE.19 contains 360 flavouring substances from the EU Register being alpha, beta-unsaturated aldehydes or ketones and precursors which could give rise to such carbonyl substances via hydrolysis and / or oxidation (EFSA, 2008b). The alpha, beta-unsaturated carbonyls were subdivided into 28 subgroups on the basis of structural similarity (EFSA, 2008b). In an attempt to decide which of the substances could go through the Procedure, a (quantitative) structure-activity relationship ((Q)SAR) prediction of the genotoxicity of these substances was undertaken. The Panel took note of the (Q)SAR predictions by using two ISS Local Models (Benigni & Netzeva, 2007a; Benigni & Netzeva, 2007b) and four DTU-NFI MultiCASE Models (Gry et al., 2007; Nikolov et al., 2007) and the fact that there are available data on genotoxicity, *in vitro* and *in vivo*, as well as data on carcinogenicity for several substances. The Panel decided that 11 subgroups (1.1.2, 1.1.3, 1.1.4, 2.4, 2.6, 2.7, 3.1, 3.3, 4.1, 4.2 and 4.4) (EFSA, 2008b) should be further examined to determine whether evaluation through the Procedure is feasible. Corresponding to these 11 subgroups 11 Flavouring Group Evaluations (FGEs) were established (FGE.201, 202, 203, 210, 212, 213, 214, 216, 217, 218 and 220).

History of FGE.80:

FGE	Opinion Adopted by EFSA	Link	No. of Candidate Substances
FGE.80	1 April 2008	http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1211902220401.htm	11
FGE.80rev1	17 June 2009	http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_ScientificDocuments.htm	13

The present Revision of FGE.80, FGE.80Rev1, includes the assessment of two additional substances, 6-Methylcoumarin [FL-no: 13.012] originally considered in FGE.217 (EFSA, 2009ad) (subgroup 4.1 in FGE.19) and for which the Panel concluded that the genotoxicity data available do not preclude its evaluation through the Procedure and 5,6,7,7-alpha-tetrahydro-4,4,7alpha-trimethyl-2-(4H)-benzofuranone [FL-no: 10.169], which was also considered a precursor for an alpha,beta-unsaturated ketone. However, it has been recognised that upon hydrolysis a tertiary alcohol would be formed and therefore the substance would not be of concern with respect to genotoxicity. Accordingly, the substance is considered in this Revision 1 of FGE.80.

1. Presentation of the Substances in the JECFA Flavouring Group

1.1. Description

1.1.1. JECFA Status

The JECFA has evaluated a group of 16 flavouring substances consisting of alicyclic, alicyclic-fused and aromatic-fused ring lactones.

1.1.2. EFSA Considerations

Eleven of 16 flavouring substances have originally been considered by EFSA in the FGE.80 (EFSA, 2008ax). One of the 16 JECFA evaluated substances is not in the Register (dihydro-5-((Z,Z)octa-2,5-dienyl)-2(3H)-furanone) (JECFA no: 1160) and four substances [FL-no: 10.034, 10.036, 10.169, 13.012] were concluded to be possible precursors for alpha,beta-unsaturated ketones. As the alpha,beta-unsaturated aldehyde and ketone structures are considered by the Panel to be structural alerts for genotoxicity (EFSA, 2008b), these four substances have been given special considerations.

The genotoxicity of three [FL-no: 10.034, 10.036 and 13.012] of the four alpha,beta-unsaturated carbonyl substances has been considered in FGE.217 (EFSA, 2009ad). For 6-methylcoumarin [FL-no: 13.012] the Panel concluded that the data available did rule out the concern for genotoxicity and thus concluded that 6-methylcoumarin can be evaluated through the Procedure. 6-Methylcoumarin will accordingly be considered in this Revision of FGE.80, FGE.80Rev1. For the two substances [FL-no: 10.034 and 10.036] the Panel concluded that the data available on genotoxicity were of limited validity and furthermore that the data available for 6-methylcoumarin could not support their evaluation as they are structurally different from 6-methylcoumarin. Accordingly, the genotoxic potential of [FL-no: 10.034 and 10.036] could not be evaluated and additional data are required in FGE.217 (EFSA, 2009ad). 5,6,7,7-Alpha-tetrahydro-4,4,7alpha-trimethyl-2-(4H)-benzofuranone [FL-no: 10.169] was also considered a precursor for an alpha,beta-unsaturated ketone. However, it has been recognised that upon hydrolysis a tertiary alcohol would be formed and therefore the substance would not be of concern with respect to genotoxicity. Accordingly, the substance should therefore be considered in this FGE.80Rev1.

The present FGE.80Rev1 therefore deals with 13 flavouring substances (see Table 1).

The Panel concluded that the 13 substances [FL-no: 10.005, 10.024, 10.025, 10.050, 10.061, 10.069, 10.070, 10.072, 10.169, 13.009, 13.012, 13.161 and 16.055] in the JECFA flavouring group of alicyclic, alicyclic-fused and aromatic-fused ring lactones are structurally related to the one aromatic lactone evaluated by EFSA in the FGE.27 (phthalide [FL-no: 10.056] (EFSA, 2008a)). Furthermore, the JECFA evaluation is supported by a group of lactones evaluated in FGE.10 as well as by alicyclic secondary and tertiary alcohols in FGE.09 and FGE.18, respectively.

1.2. Isomers

1.2.1. JECFA Status

The following nine substances [FL-no: 10.025, 10.050, 10.061, 10.069, 10.070, 10.072, 10.169, 13.161 and 16.055] in the group of JECFA evaluated alicyclic, alicyclic-fused and aromatic-fused ring lactones have one or more chiral centres and [FL-no: 10.061] can exist as geometrical isomers.

1.2.2. EFSA Considerations

Information has not been provided about the stereoisomerism for seven substances [FL-no: 10.050, 10.061, 10.069, 10.070, 10.072, 10.169 and 13.161].

1.3. Specifications

1.3.1. JECFA Status

The JECFA specifications are available for all 13 substances (JECFA, 2003b). See Table 1.

1.3.2. EFSA Considerations

The available specifications are considered adequate for six substances. For seven substances [FL-no: 10.050, 10.061, 10.069, 10.070, 10.072, 10.169 and 13.161] information on stereoisomerism has not been provided (see Section 1.2.) and in addition for two of the substances [FL-no: 10.069 and 10.169] further information on the composition is requested. See Table 1.

2. Intake Estimations

2.1. JECFA Status

For seven substances [FL-no: 16.055, 10.005, 10.024, 10.025, 10.169, 13.009 and 13.012] evaluated through the JECFA Procedure intake data are available for the EU (see Table 3.1). For the remaining six substances [FL-no: 10.050, 10.061, 10.069, 10.070, 10.072 and 13.161] production figures are only available for the USA.

2.2. EFSA Considerations

As production figures are only available for the USA for six substances [FL-no: 10.050, 10.061, 10.069, 10.070, 10.072 and 13.161], MSDI values for the EU cannot be calculated for these.

3. Genotoxicity Data

3.1. Genotoxicity Studies - Text Taken[1] from the JECFA (JECFA, 2004b)

In vitro

Testing for genotoxicity *in vitro* has been performed with five representative members [FL-no: 13.161, 10.005, 10.169, 13.009 and 13.012] of the group of alicyclic, alicyclic-fused and aromatic-fused ring lactones used as flavouring agents (see Table 2.1).

Negative results were reported in the Ames assay when *Salmonella typhimurium* strains (TA97, TA98, TA100, TA1535, TA1537 and TA1538) were incubated with 100 µg of (+/-) (2,6,6-trimethyl-2-hydroxycyclohexylidene) acetic acid γ-lactone [FL-no: 10.169] per plate (Kinae et al., 1981a), up to 5000 µg of octahydrocoumarin [FL-no: 13.161] per plate (Watanabe & Morimoto, 1989a), or up to 75 µl (88 950 µg) of dihydrocoumarin [FL-no: 13.009] per plate (Brusick, 1982b; Prival et al., 1982; NTP, 1993c), with and without metabolic activation.

In a similar assay for reverse mutation, concentrations of up to 400 µg of 3-propylidenephthalide [FL-no:10.005] per plate yielded a mutagenic response in the presence of metabolic activation in *S. typhimurium* strains TA97, TA98, TA100, TA1535 and TA1537 (Zeiger et al., 1988). As the purity of the 3-propylidenephthalide sample was unknown, the authors could not conclusively determine whether the mutagenic response was caused by the test material or by possible contaminants present in the sample. These results *in vitro* have not been confirmed by a standard *in vivo* assay.

At concentrations of up to 3.6 mg of 6-methylcoumarin [FL-no: 13.012] per plate, a slight but significant increase in the number of revertants of one strain of *S. typhimurium*, TA100, was reported, but only in the presence of metabolic activation (Wild et al., 1983). Negative results were reported in four strains (TA98, TA1535, TA1537 and TA1538) with or without metabolic activation (Wild et al., 1983). In further assays for reverse mutation, 6-methylcoumarin yielded negative results in *S. typhimurium* strains TA98, TA100, TA1535, TA1537 and TA1538 at concentrations of up to 5000 µg/plate, with or without metabolic activation (Brusick, 1982a; Haworth, 1987). On this basis, the marginally positive result in one strain is considered to be an isolated incident, and cannot be used to conclusively characterize the mutagenic potential of 6-methylcoumarin.

Negative results were reported in an assay for DNA repair in which (+/-)-(2,6,6-trimethyl-2-hydroxycyclohexylidene) acetic acid γ -lactone [FL-no: 10.169] was incubated with *Bacillus subtillis* strains (H17 and M45) at concentrations of up to 10 mg/disk (10000 µg/disk) (Kinae et al., 1981a).

Concentrations of dihydrocoumarin of up to 2500 nl/ml (2965 µg/ml) were reported to be mutagenic in mouse lymphoma L5178Y *Tk* $^{+/-}$ cells only in the presence of metabolic activation (Cifone, 1982b; Cifone, 1984). The authors did not consider the positive results to be a conclusive determination of mutagenicity because increases in mutant frequency were only detected at cytotoxic concentrations in the presence of metabolic activation (Cifone, 1982b; Cifone, 1984). It has since been proven that non-physiological culture conditions, such as low pH and high osmolality, may produce positive results in similar assays in the absence of genotoxic materials (Brusick, 1986). The effect of low pH has been observed mainly in the presence of metabolic activation and is believed to be an effect of the acidic environment created by the S9 constituents

[11] *The text is taken verbatim from the indicated reference source, but text related to substances not included in the present FGE has been removed.*

produced at low pH (Cifone, 1985; Brusick, 1987). In similar assays, negative results were reported for dihydrocoumarin in assays for forward mutation in mouse lymphoma L5178Y *Tk*$^{+/-}$ cells at concentrations of up to 2500 nl/ml (2965 µg/ml) in the absence of metabolic activation (Cifone, 1982b; Cifone, 1984). 6-Methylcoumarin was not mutagenic in mouse lymphoma L5178Y *Tk*+/- cells at concentrations of up to 250 µg/ml, with or without metabolic activation (Cifone, 1982a).

Dihydrocoumarin did not induce unscheduled DNA synthesis in rat hepatocytes at concentrations of up to 4.0 µl/ml (4744 µg/ml) (Curren, 1986).

Dihydrocoumarin did not induce chromosomal aberrations in Chinese hamster ovary (CHO) cells, at doses of up to 1600 µg/ml with metabolic activation and up to 500 µg/ml without metabolic activation (Galloway, 1983; NTP, 1993c). Dihydrocoumarin induced a dose-related increase in sister chromatid exchange in CHO cells at a concentration of up to 300 µg/ml, in the absence of metabolic activation (NTP, 1993c). In the presence of metabolic activation, a significant increase in sister chromatid exchange was observed in CHO cells only at the two highest dihydrocoumarin doses tested (1600 and 2000 µg/ml). However, cytotoxicity was clearly evident at a dose of 2000 µg/ml (NTP, 1993c). The isolated positive results from assays for cytogenetic indicator sister chromatid exchange in CHO cells are clearly out-weighed by the overwhelming negative evidence from the studies of chromosomal aberration in the same cell type.

In vivo

The genotoxic potential of 6-methylcoumarin was studied in a Basc test for induction of sex-linked recessive lethal mutations in adult *Drosophila melanogaster* (Wild et al., 1983). The observed frequency of mutation was not increased when a 10 mmol/l (1602 µg/ml) solution of 6-methylcoumarin was fed to the flies for three days.

No significant increase in the frequency of micronucleated erythrocytes was reported in peripheral blood samples obtained from male and female $B6C3F_1$ mice after 13 weeks of treatment with dihydrocoumarin at doses of up to 1600 mg/kg bw per day (NTP, 1993c). A test for micronucleus formation in peripheral blood from $B6C3F_1$ mice given 6-methylcoumarin at a dose of 200 or 400 mg/kg bw per day was reported to produce negative results in females and equivocal results in males, owing to the very small increase in the frequency of micronucleus normochromatic erythrocytes (NCE) observed (<0.5 increase per 1000 NCE) (Witt et al., 2000). In a similar study, groups of NMRI mice given 6- methylcoumarin intraperitoneally at a dose of 160, 240 or 320 mg/kg bw showed no increase in micronucleated erythrocytes in samples of bone marrow, 30 h after treatment (Wild et al., 1983).

Conclusion on genotoxicity

Alicyclic, alicyclic-fused and aromatic-fused ring lactones used as flavouring agents are not mutagenic *in vitro* in the Ames or DNA repair assays. In the assay in mouse lymphoma cells, positive results obtained only in the presence of metabolic activation from S9 could be explained as a well-known artefact of the presence of S9. The negative results obtained at the same concentrations in the absence of metabolic activation support this possibility. The predominance of negative results for dihydrocoumarin in CHO cells *in vitro* and in assays *in vivo* suggests a lack of genotoxicity. Taking into account the above results and the fact that these substances are rapidly metabolized *in vivo* to compounds of lower toxicological potential, it is concluded that the alicyclic, alicyclic-fused and aromatic-fused ring lactones used as flavouring agents exhibit low genotoxic potentials.

For a summary of *in vitro/in vivo* genotoxicity data considered by the JECFA, see Table 2.1.

3.2. Genotoxicity Studies – Text taken[2] from FGE.27 (EFSA, 2008a)

In vitro / in vivo

There are no data available on the candidate substance, phthalide [FL-no: 10.056].

Data from *in vitro* tests are available for the supporting substance 3-propylidenephthalide [FL-no: 10.005].

When 3-propylidenephthalide was tested for reverse mutations *in vitro* (Ames test) a weak mutagenic response was observed in the presence of metabolic activation in *S. typhimurium* strains TA100, but not in TA97, TA98 and TA1535. There are no further genotoxicity data available on this compound.

Conclusion on genotoxicity

The genotoxicity for the candidate substance could not be assessed adequately. However, this does not preclude evaluation of phthalide [FL-no: 10.056] through the Procedure in FGE.27.

3.3. Genotoxicity and Carcinogenicity – Text taken[3] from FGE.217 (EFSA, 2009ad)

"6-Methylcoumarin was found negative in two valid Ames tests (Haworth et al., 1983; Brusick, 1982a); equivocal results were obtained in a valid study with strain TA100 (Wild et al., 1983). It was found negative in a valid mouse lymphoma tk assay (Cifone, 1982a). Furthermore, it was found negative in the following three *in vivo* studies considered of limited validity: a *Drosophila melanogaster* Sex-linked recessive lethal test (Wild et al., 1983), a mouse bone marrow micronucleus assay (Wild et al., 1983) and a mouse peripheral blood micronucleus 90-day assay reported by Witt et al. (Witt et al., 2000).

Overall, the Panel concluded that the data available do not indicate a genotoxic potential for 6-methylcoumarin."

"Groups of 25 male and 25 female weanling Osborne-Mendel rats were fed diets containing 0, 500, 1000, 3500, 5000, 7500 or 15000 mg/kg body weight (bw)/day 6-methylcoumarin [FL-no: 13.012] for two years, corresponding to 0, 25, 50, 175, 250, 375 or 750 mg 6-methylcoumarin/kg bw/day. Growth depression was observed in males at 375 mg 6-methylcoumarin/kg bw/day (moderate effect) and at 750 mg/kg bw/day (severe effect) paralleled by decreased food intake. In the liver, slight fatty metamorphosis and very slight bile duct proliferation was observed at the highest dose level. In addition, moderate testicular atrophy was seen in the high-dose males, presumably due to the severe growth depression. No other toxicological effects, including carcinogenicity, were seen. The Panel noted that in parallel studies the same research group was able to clearly demonstrate the liver carcinogenicity of safrole after dietary administration to rats (Hagan et al., 1967).

The Panel also noted that this study was performed before OECD test guidelines 451/453 (1981) were established and that it does not meet the criteria of these OECD test guidelines with respect to the number of animals. However, the Panel agreed with the conclusion of the authors that 6-methylcoumarin was not carcinogenic in rats under the study conditions."

Conclusion on Genotoxicity and Carcinogenicity

"The data available do not indicate a genotoxic or carcinogenic potential for 6-methylcoumarin."

[22] *The text is taken verbatim from the indicated reference source, but text related to substances not included in the present FGE has been removed.*

[33] *The text is taken verbatim from the indicated reference source, but text related to substances not included in the present FGE has been removed.*

For a summary of *in vitro/in vivo* genotoxicity data evaluated in FGE.217 for 6-methylcoumarin, see Table 2.4 and Table 2.5.

3.4. EFSA Considerations

In its evaluation of phthalide [FL-no: 10.056] in Flavouring Group Evaluation 27 (FGE.27) the Panel used 3-propylidenephthalide [FL-no: 10.005], 3-butylidenephthalide [FL-no: 10.024] and 3-butylphthalide [FL-no: 10.025] as supporting substances. Genotoxicity data were only available for 3-propylidenephthalide However, the available data did not preclude the evaluation of phthalide through the Procedure, and consequently the three JECFA evaluated substances 3-propylidenephthalide [FL-no: 10.005], 3-butylidenephthalide [FL-no: 10.024] and 3-butylphthalide [FL-no: 10.025] can also be evaluated through the Procedure in this FGE.

For 3,4-dihydrocoumarin [FL-no: 13.009] and the structurally similar dimethyl-3,6-benzo-2(3H)-furanone [FL-no: 10.072] the Panel noted that 3,4-dihydrocoumarin [FL-no: 13.009] was negative in bacterial tests for mutagenicity and in four studies of chromosomal aberrations in CHO cells *in vitro*. In an assay in mouse lymphoma cells, positive results were obtained in the presence of metabolic activation from S9. However, 3,4-dihydrocoumarin did not induce micronuclei in mouse peripheral blood cells *in vivo*. The Panel concludes that the data do not indicate that 3,4-dihydrocoumarin [FL-no: 13.009] and dimethyl-3,6-benzo-2(3H)-furanone [FL-no: 10.072] are genotoxic and accordingly can be evaluated through the Procedure. The Panel also noted that 3,4-dihydrocoumarin, when tested for long-term toxicity and carcinogenicity in mice and rats, did not increase neoplasms relevant for the safety evaluation in humans (JECFA, 2004b; NTP, 1993c). Overall the Panel agrees with JECFA that there is no genotoxic concern with 3,4-dihydrocoumarin [FL-no: 13.009] (or with dimethyl-3,6-benzo-2(3H)-furanone [FL-no: 10.072]).

For 6-methylcoumarin [FL-no: 13.012] the Panel concluded that the data available do not indicate a genotoxic or carcinogenic potential.

Hexahydro-3,6-dimethyl-2-(3H)-benzofuranone [FL-no: 10.050], 5,6,7,7alpha-tetrahydro-4,4,7alpha-trimethyl-2-(4H)-benzofuranone [FL-no: 10.169] and octahydrocoumarin [FL-no: 13.161] are anticipated to be hydrolysed in the lactone ring to form monocyclic secondary or tertiary alcohols structurally related to monocyclic secondary alcohols evaluated in FGE.09 ("Secondary alicyclic saturated and unsaturated alcohols, ketones and esters containing secondary alicyclic alcohols") or tertiary alcohols evaluated in FGE.18 ("Aliphatic, alicyclic and aromatic saturated and unsaturated tertiary alcohols, aromatic tertiary alcohols and their esters"). The genotoxicity data available do not preclude an evaluation of these flavourings through the Procedure and accordingly not either for [FL-no: 10.050, 10.169 and 13.161].

Sclareolide [FL-no: 16.055] is anticipated to be hydrolysed to a bicyclic tertiary alcohol structurally related to bicyclic tertiary alcohols in FGE.18 ("Aliphatic, alicyclic and aromatic saturated and unsaturated tertiary alcohols, aromatic tertiary alcohols and their esters"). The genotoxicity data available do not give rise to safety concern for these flavourings and accordingly not either for [FL-no: 16.055].

cis-5-Hexenyldihydro-5-methylfuran-2(3H)-one [FL-no: 10.061], 3-methyl gamma-decalactone [FL-no: 10.069] and 4-methyl-5-hexen-1,4-olide [FL-no: 10.070] are structurally related to a group of lactones evaluted in FGE.10. These substances did not give rise to concern with respect to genotoxicity.

Overall, the Panel agreed with the JECFA and concluded that the available data on genotoxicity do not preclude evaluation of the 13 flavouring substances in the present group using the Procedure.

4. Application of the Procedure

4.1. Application of the Procedure to 13 Alicyclic, Alicyclic-fused and Aromatic-fused Ring Lactones by the JECFA (JECFA, 2004b):

According to the JECFA three of the substances belong to structural class I, and ten to structural class III using the decision tree approach presented by Cramer *et al.* (Cramer et al., 1978).

The JECFA concluded seven alicyclic, alicyclic-fused and aromatic-fused ring lactones [FL-no: 10.050, 10.061, 10.069, 10.070, 10.169, 13.161 and 16.055] at step A3 in the JECFA Procedure, i.e. the substances are expected to be metabolised to innocuous products (step 2) and the intakes for all substances are below the threshold for structural class III (step A3).

Six substances [FL-no: 10.005, 10.024, 10.025, 10.072, 13.009 and 13.012] were evaluated via the B-side of the Procedure as the substances could not be anticipated to be metabolised to innocuous products. For four substances the intakes were below the threshold for the structural class (step B3) and a no-observed adverse effect level (NOAEL) exists for propylidenephthalide [Fl-no: 10.005] to provide an adequate margin of safety of the estimated intake as flavouring substance (step B4). For two substances, 3,4-dihydrocoumarin [FL-no: 13.009] and 6-methylcoumarin [FL-no: 13.012], the intakes were above the threshold for the structural class and accordingly data must be available for a safety evaluation of the substances or a closely related substances. The JECFA therefore considered toxicological studies carried out with 3,4-dihydrocoumarin and 6-methylcoumarin.

In a 13-week study in rats given 3,4-dihydrocoumarin, a NOAEL of 150 mg/kg body weight (bw) per day was identified (NTP, 1993c). This NOAEL is 7500 times greater than the estimated per capita intake of dihydrocoumarin in Europe (20 µg/kg bw per day). In rats, the NOAEL for 3,4-dihydrocoumarin in a 2-year study by gavage was 300 mg/kg bw per day (NTP, 1993c). This NOAEL is 15000 times greater than the estimated per capita intake of 3,4-dihydrocoumarin in Europe (20 µg/kg bw per day).

In a 13-week study in rats, a NOEL of 150 mg/kg bw per day was found (National Toxicology Program, 2002). This NOEL on 6-methylcoumarin is about 35000 times greater than the estimated intake of 6-methylcoumarin in Europe (4 µg/kg bw per day).

Understanding of their metabolism and the available data on toxicity led the Committee to conclude that the safety of 3,4-dihydrocoumarin and 6-methylcoumarin would not be expected to present a safety concern at the current levels of intake.

In conclusion, the JECFA evaluated all 13 substances as to be of no safety concern at the estimated levels of intake as flavouring substances based on the MSDI approach.

4.2. Application of the Procedure to the One Aromatic Lactone in FGE.27 (EFSA, 2008a):

One candidate substance was evaluated in FGE.27. The substance is classified into structural class III using the decision tree approach presented by Cramer *et al.* (Cramer et al., 1978).

The substance was concluded at step A3, i.e. the substance is expected to be metabolised to innocuous products (step 2) and the estimated daily intake is below the threshold for the structural class III (step A3).

In conclusion, the Panel evaluated the substance as to be of no safety concern at the estimated level of intake as a flavouring substance based on the MSDI approach.

The stepwise evaluations of the substance is summarised in Table 3.2: Summary of Safety Evaluation Applying the Procedure (EFSA / FGE.27).

Further, the Panel noted that a group of structurally related lactones in FGE.10 were all concluded to be hydrolysed to innocuous products and to be of no safety concern at their estimated levels of intake.

4.3. EFSA Considerations

The Panel agrees with the way the application of the Procedure has been performed by the JECFA for all 13 substances in the group of alicyclic, alicyclic-fused and aromatic-fused ring lactones.

However, for six of 13 substances [FL-no: 10,050, 10.061, 10.069, 10.070, 10.072 and 13.161] no European production figure was available and consequently no European exposure estimate could be calculated. Accordingly, the safety in use in Europe could not be assessed using the Procedure for these substances.

5. Conclusion

The JECFA has evaluated a group of 16 flavouring substances consisting of alicyclic, alicyclic-fused and aromatic-fused ring lactones.

Eleven of the 16 flavouring substances have originally been considered by EFSA in the FGE.80 (EFSA, 2008ax). One of the 16 JECFA evaluated substances is not in the Register (dihydro-5-((Z,Z)octa-2,5-dienyl)-2(3H)-furanone) (JECFA no: 1160) and four [FL-no: 10.034, 10.036, 10.169 13.012] were evaluated as precursors for alpha,beta-unsaturated ketones. As the alpha,beta-unsaturated aldehyde and ketone structures are considered by the Panel to be structural alerts for genotoxicity (EFSA, 2008b), these four substances have been given special considerations.

The genotoxicity of three [FL-no: 10.034, 10.036 and 13.012] of the four alpha,beta-unsaturated carbonyl substances has been considered in FGE.217 (EFSA, 2009ad). For 6-methylcoumarin [FL-no: 13.012] the Panel concluded that the data available did rule out the concern for genotoxicity and thus concluded that 6-methylcoumarin can be evaluated through the Procedure. 6-Methylcoumarin will accordingly be considered in this Revision of FGE.80, FGE.80Rev1. For the two substances [FL-no: 10.034 and 10.036] the Panel concluded that the data available on genotoxicity were of limited validity and furthermore that the data available for 6-methylcoumarin could not support their evaluation as they are structurally different from 6-methylcoumarin. Accordingly, the genotoxic potential of [FL-no: 10.034 and 10.036] could not be evaluated and additional data are required in FGE.217 (EFSA, 2009ad). 5,6,7,7-Alpha-tetrahydro-4,4,7alpha-trimethyl-2-(4H)-benzofuranone [FL-no: 10.169] was also considered a precursor for an alpha,beta-unsaturated ketone. However, it has been recognised that upon hydrolysis a tertiary alcohol would be formed and therefore the substance would not be of concern with respect to genotoxicity. Accordingly, the substance should therefore be considered in this FGE.80Rev1.

The present FGE.80Rev1 therefore only deals with 13 flavouring substances (see Table 1).

The Panel concluded that the 13 substances [FL-no: 10.005, 10.024, 10.025, 10.050, 10.061, 10.069, 10.070, 10.072, 10.169, 13.009, 13.012, 13.161 and 16.055] in the JECFA flavouring group

of alicyclic, alicyclic-fused and aromatic-fused ring lactones are structurally related to the one aromatic lactone evaluated by EFSA in the FGE.27 (phthalide [FL-no: 10.056]). Furthermore, the JECFA evaluation is supported by a group of lactones evaluated in FGE.10 as well as by alicyclic secondary and tertiary alcohols in FGE.09 and FGE.18, respectively.

The Panel agrees with the way the Procedure was applied by the JECFA for the 13 substances considered in this FGE. However, for six of 13 substances [FL-no: 10,050, 10.061, 10.069, 10.070, 10.072 and 13.161] the JECFA evaluation is based on MSDI values derived from production figures only from the USA. EU production figures are needed in order to finalise the evaluation of these six substances.

For all 13 substances evaluated through the Procedure use levels are needed to calculate the mTAMDIs in order to identify those flavouring substances that need more refined exposure assessment and to finalise the evaluation.

In order to determine whether the conclusion for the 13 JECFA evaluated substances can be applied to the materials of commerce, it is necessary to consider the available specifications. Adequate specifications including complete purity criteria and identity are available for six of the 13 JECFA evaluated substances. For seven substances [FL-no: 10.050, 10.061, 10.069, 10.070, 10.072, 10.169 and 13.161] information on the stereoisomeric composition is lacking and in addition for two of the substances [FL-no: 10.069 and 10.169] further information on the composition is requested.

Thus, for seven substances [FL-no: 10.050, 10.061, 10.069, 10.070, 10.072, 10.169 and 13.161] the Panel has reservations (no European production volumes available, preventing them from being evaluated using the Procedure, and/or missing data on stereoisomerism and/or further information on the composition of the mixture). For the remaining six of the 13 JECFA evaluated alicyclic, alicyclic-fused and aromatic-fused ring lactones [FL-no: 10.005, 10.024, 10.025, 13.009, 13.012 and 16.055] the Panel agrees with the JECFA conclusion “No safety concern at estimated levels of intake as flavouring substances” based on the MSDI approach.

TABLE 1: SPECIFICATION SUMMARY FOR JECFA EVALUATED SUBSTANCES IN THE PRESENT GROUP

Table 1: Specification Summary of the Substances in the JECFA Flavouring Group of Alicyclic, Alicyclic-fused and Aromatic-fused Ring Lactones

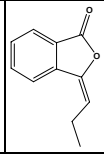
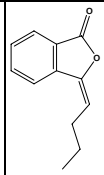
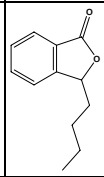
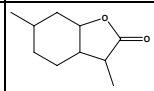
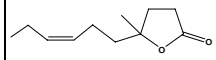
FL-no JECFA-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	EFSA comments
10.005 1168	3-Propylidenephthalide		2952 494 17369-59-4	Liquid $C_{11}H_{10}O_2$ 174.20	Insoluble Soluble	169-171 (17hPa) NMR 96 %	1.557-1.562 1.127-1.132	
10.024 1170	3-Butylidenephthalide		3333 10083 551-08-6	Liquid $C_{12}H_{12}O_2$ 188.23	Insoluble Soluble	114-116(0.07hPa NMR 99 %	1.554-1.559 1.098-1.103	
10.025 1169	3-Butylphthalide		3334 10084 6066-49-5	Liquid $C_{12}H_{14}O_2$ 190.24	Slightly soluble Soluble	113 (0.3 hPa) IR NMR 97 %	1.524-1.529 1.068-1.074	Racemate.
10.050 1161	Hexahydro-3,6-dimethyl-2(3H)-benzofuranone 6)		4032 92015-65-1	Liquid $C_{10}H_{16}O_2$ 168.24	Soluble Soluble	274-276 (17hPa) IR NMR 99.4 %	1.464-1.470 1.016-1.022 (20°)	CASrn in Register does not specify stereoisomers.
10.061 1159	cis-5-Hexenyldihydro-5-methylfuran-2(3H)-one 6)		3937 70851-61-5	Liquid $C_{11}H_{18}O_2$ 182.26	Insoluble Soluble	150 (8 hPa) IR NMR 97 %	1.463-1.468 0.960-0.967	CASrn in Register does not specify stereoisomers. Register name to be changed so position of double bond is indicated.

Table 1: Specification Summary of the Substances in the JECFA Flavouring Group of Alicyclic, Alicyclic-fused and Aromatic-fused Ring Lactones

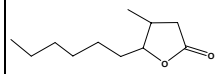
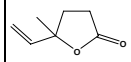
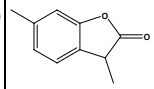
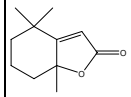
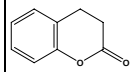
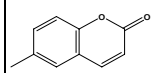
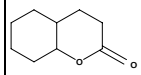
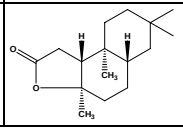
FL-no JECFA-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	EFSA comments
10.069 1158	3-Methyl gamma-decalactone 6)		3999 67663-01-8	Liquid $C_{11}H_{20}O_2$ 184.28	Insoluble Soluble	110-115 (5 hPa) NMR 94 %	1.446-1.452 0.938-0.944	CASrn in Register does not specify stereoisomers. JECFA name: ± 3-methyl gamma-decalactone. According to JECFA: Min. assay value is "94 % (sum of cis and trans isomers)" and secondary components "heptan-1-ol" Composition of mixture to be specified.
10.070 1157	4-Methyl-5-hexen-1,4-olide 6)		4051 1073-11-6	Liquid $C_7H_{10}O_2$ 126.15	Insoluble Soluble	219 IR NMR 97 %	1.457-1.462 1.015-1.025 (20°)	CASrn in Register does not specify stereoisomers.
10.072 1167	Dimethyl-3,6-benzo-2(3H)-furanone 6)		3863 65817-24-5	Liquid $C_{10}H_{10}O_2$ 162.19	Insoluble Soluble	64 (0.1 hPa) IR NMR 98 %	1.518-1.524 1.099-1.104	CASrn in Register does not specify stereoisomers.
10.169 1164	5,6,7,7alpha-Tetrahydro-4,4,7alpha-trimethyl-2-(4H)-benzofuranone 6)		1020 15356-74-8	Liquid $C_{11}H_{16}O_2$ 180.25	Insoluble Soluble	90 NMR 90 %	1.499-1.505 1.051-1.058	CASrn in Register does not specify stereoisomers. According to JECFA: Min. assay value is "90 %" and secondary components "2,9-dimethyl 3,8-decanedione, 4-hydroxy-5,6-oxo beta-ionone". Composition of mixture to be specified.
13.009 1171	3,4-Dihydrocoumarin		2381 535 119-84-6	Liquid $C_9H_8O_2$ 148,16	Slightly soluble Soluble	272 IR 99 %	1.555-1.559 1.186-1.192	
13.012 1172	6-Methylcoumarin		2699 579 92-48-8	Solid $C_{10}H_8O_2$ 160.17	Insoluble Soluble	 73-79 IR 99 %	n.a. n.a.	

Table 1: Specification Summary of the Substances in the JECFA Flavouring Group of Alicyclic, Alicyclic-fused and Aromatic-fused Ring Lactones

FL-no JECFA-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	EFSA comments
13.161 1166	Octahydrocoumarin 6)		3791 4430-31-3	Liquid $C_9H_{14}O_2$ 154.21	Insoluble Soluble	293-298 NMR 99 %	1.489-1.493 1.090-1.096	CASrn in Register does not specify stereoisomers.
16.055 1165	Sclareolide		3794 564-20-5	Solid $C_{16}H_{26}O_2$ 250.38	Insoluble Slightly soluble	124.4 IR NMR 98 %	n.a. n.a.	Register name to be changed to (R)-(+)-Sclareolide.

1) Solubility in water, if not otherwise stated.

2) Solubility in 95% ethanol, if not otherwise stated.

3) At 1013.25 hPa, if not otherwise stated.

4) At 20°C, if not otherwise stated.

5) At 25°C, if not otherwise stated.

6) Stereoisomeric composition not specified.

TABLE 2: GENOTOXICITY DATA

Table 2.1: Genotoxicity Data (*in vitro / in vivo*) for 13 Alicyclic, Alicyclic-fused and Aromatic-fused Ring Lactones (JECFA, 2004b)

Table 2.1: Summary of Genotoxicity Data of 13 Alicyclic, Alicyclic-fused and Aromatic-fused Ring Lactones evaluted by JECFA

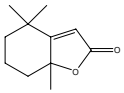
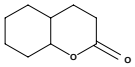
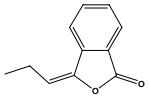
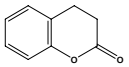
FL-no JECFA-no	EU Register name JECFA name	Structural formula	End-point	Test system	Concentration	Results	Reference
In vitro							
10.169 1164	5,6,7,7alpha-Tetrahydro-4,4,7alpha-trimethyl-2-(4H)-benzofuranone		Reverse mutation	*S. typhimurium* TA97, TA98, TA1535, TA1537 and TA1538	100 µg/plate	Negative	(Kinae et al., 1981a)
			DNA repair	*B. Subtilis* H-17 (rec$^+$) and M-45 (rec$^-$)	10000 µg/plate	Negative	(Kinae et al., 1981a)
13.161 1166	Octahydrocoumarin		Reverse mutation	*S. typhimurium* TA98 and TA100	≤5000 µg/plate	Negative[1,2]	(Watanabe & Morimoto, 1989a)
10.005 1168	3-Propylidenephthalide		Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA97 and TA1537	3.3–400 µg/plate	Positive[1,2,5]	(Zeiger et al., 1988)
13.009 1171	3,4-Dihydrocoumarin		Reverse mutation	*S. typhimurium* TA1535, TA1537, TA1538, TA98 and TA100	≤75 µl/plate (88 950 µg/plate)	Negative[1]	(Brusick, 1982b)
			Reverse mutation	*S. typhimurium* TA98, TA100, TA1535 and TA1537	10–6666 µg/plate	Negative[1]	(NTP, 1993c)
			Reverse mutation	*S. typhimurium* and TA98, TA100, TA1535 and TA1537	≤10 mg/plate (≤10 000 µg/plate)	Negative[1,3]	(Prival et al., 1982)
			Forward mutation	Mouse lymphoma L5178Y TK +/- cells	200–500 nl/ml (237–593 µg/ml)	Weakly positive[4]	(Cifone, 1982b)
			Forward mutation	Mouse lymphoma L5178Y TK +/- cells	400–800 nl/ml (474–949 µg/ml)	Negative[6]	(Cifone, 1982b)
			Forward mutation	Mouse lymphoma L5178Y TK +/- cells	≤2500 nl/ml (2965 µg/ml)	Positive[4]	(Cifone, 1984)
			Forward mutation	Mouse lymphoma L5178Y TK +/- cells	≤2500 nl/ml (2965 µg/ml)	Negative	(Cifone, 1984)

Table 2.1: Summary of Genotoxicity Data of 13 Alicyclic, Alicyclic-fused and Aromatic-fused Ring Lactones evaluted by JECFA

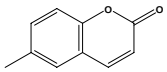
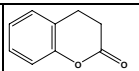
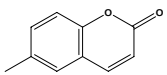
FL-no JECFA-no	EU Register name JECFA name	Structural formula	End-point	Test system	Concentration	Results	Reference
			Unscheduled DNA synthesis	Rat hepatocytes	0.03–4.0 µl/ml (35.6–4744 µg/ml)	Negative	(Curren, 1986)
			Chromosomal aberrations	Chinese hamster ovary cells	0.01–1.0 µl/ml (11.9–1186 µg/ml)	Negative[4]	(Galloway, 1983)
			Chromosomal aberrations	Chinese hamster ovary cells	33.3–333 nl/ml (39.5–395 µg/ml)	Negative[4]	(Galloway, 1983)
			Chromosomal aberrations	Chinese hamster ovary cells	500, 1000 and 1600 µg/ml	Negative[4]	(NTP, 1993c)
			Chromosomal aberrations	Chinese hamster ovary cells	100, 160 and 500 µg/ml	Negative	(NTP, 1993c)
			Sister chromatid exchanges	Chinese hamster ovary cells	50–300 µg/ml	Positive	(NTP, 1993c)
			Sister chromatid exchanges	Chinese hamster ovary cells	50–1000 µg/ml	Negative[4]	(NTP, 1993c)
			Sister chromatid exchanges	Chinese hamster ovary cells	1600 and 2000 µg/ml	Positive[4]	(NTP, 1993c)
13.012 1172	6-Methylcoumarin		Reverse mutation	*S. typhimurium* TA100	≤3.6 mg/plate (≤3600 µg/plate)	Marginally positive[4]	(Wild et al., 1983)
			Reverse mutation	*S. typhimurium* TA100	≤3.6 mg/plate (≤3600 µg/plate)	Negative	(Wild et al., 1983)
			Reverse mutation	S. *typhimurium* TA98, TA1535, TA1537 and TA1538	≤3.6 mg/plate (≤3600 µg/plate)	Negative[1]	(Wild et al., 1983)
			Reverse mutation	S. *typhimurium* TA98, TA100, TA1535 and TA1537	33-3333 µg/plate	Negative[1,2]	(Haworth et al., 1983)
			Reverse mutation	S. *typhimurium* TA98, TA100, TA1535, TA1537 and TA1538	1-5000 µg/plate	Negative[1]	(Brusick, 1982a)
			Forward mutation	Mouse lymphoma L5178Y *Tk* +/- cells	6.25-100 µg/plate	Negative[4]	(Cifone, 1982a)
			Forward mutation	Mouse lymphoma L5178Y *Tk* +/- cells	15.6-250 µg/plate	Negative	(Cifone, 1982a)
In vivo							
13.009 1171	3,4-Dihydrocoumarin		Micronucleus formation	Mouse peripheral blood cells	400, 800 and 1600 mg/kg bw	Negative	(NTP, 1993c)
13.012 1172	6-Methylcoumarin		Sex-linked recessive lethal mutation	*Drosophila melanogaster*	10 mmol/l (1602 µg/ml)	Negative	(Wild et al., 1983)
			Micronucleus formation	Mouse peripheral blood cells	200 and 400 mg/kg	Equivocal (M)[7] Negative (F)	(Witt et al., 2000)

Table 2.1: Summary of Genotoxicity Data of 13 Alicyclic, Alicyclic-fused and Aromatic-fused Ring Lactones evaluted by JECFA

FL-no JECFA-no	EU Register name JECFA name	Structural formula	End-point	Test system	Concentration	Results	Reference
			Micronucleus formation	Mouse bone-marrow cells	160, 240 and 320 mg/kg	Negative[8]	(Wild et al., 1983)

1 With and without metabolic activation.

2 Pre-incubation method.

3 Plate incorporation method.

4 With metabolic activation.

5 A two-fold increase in revertants was reported at one concentration only.

6 Without metabolic activation.

7 Although the statistical analysis yielded a positive trend test (p = 0.006), and the frequency of micronucleus formation was significantly elevated above the control value (p = 0.0072), the result was concluded to be equivocal in male mice due to the very small increase in the frequency of micronucleus normochromatic erythrocytes (NCE) observed (<0.5 per 1000 NCE).

8 Administered by intraperitoneal injection.

Table 2.2: Genotoxicity (*in vitro*) FGE.27 (EFSA, 2008a)

Substances listed in brackets are JECFA evaluated substances

Table 2.2: Summary of Genotoxicity Data (*in vitro*) EFSA

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
(3-Propylidenephthalide [10.005])	Ames test (preincubation method)	*Salmonella typhimurium* TA97, TA98, TA100 and TA1535	0, 3.3, 10, 33, 100, 200 µg/plate (in addition 300 and 400 µg/plate in TA100 +S9 from rat liver)	Negative[1] Positive[2]	(Zeiger et al., 1988)	Published summary report including limited results from the testing of 300 chemicals in various laboratories. Purity of substance not indicated. Due to limitations of the study with respect to the unknown purity of the test substance the authors could not conclusively determine if the mutagenic response was due to the test material or the possible contaminants present in the sample. Therefore, the results are considered of limited validity. Cytotoxicity was observed at 200 µg/plate in the absence of S9 in all strains and at 300 µg/plate and higher in the presence of S9 in TA100. A positive response was observed in TA100 in the presence of S9 from rat liver but not in the presence of hamster liver. (A two-fold increase in revertants was reported at one concentration only).

NR: Not reported.

[1] *Without metabolic activation.*

[2] *With metabolic activation.*

Table 2.3: Genotoxicity (*in vivo*) FGE.27 (EFSA, 2008a)

No data.

Table 2.4 Genotoxicity (*in vitro*) FGE.217 (EFSA, 2009ad)

Table 2.4: GENOTOXICITY (*in vitro*)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments [d]
6-methylcoumarin [13.012]	Reverse mutation	S. *typhimurium* TA100	5 concentrations up to cytotoxicity, or max (≤3600 µg/plate)	Marginally positive[c]	(Wild et al., 1983)	Valid, however the results are considered equivocal (+ S9: dose-response showed positive trend, but was never above twice control frequency; - S9: negative).
	Reverse mutation	*S. typhimurium* TA98, TA1535, TA1537, and TA1538	5 concentrations up to cytotoxicity, or max. 3600 µg/plate	Negative[a]	(Wild et al., 1983)	Valid.
	Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, and TA1537	33–3333 µg/plate	Negative[a,b]	(Haworth et al., 1983)	Valid.
	Reverse mutation	S. *typhimurium* TA98, TA100, TA1535, TA1537 and TA1538	1–5000 µg/plate	Negative[a]	(Brusick, 1982a)	Valid. Unpublished GLP study carried out according to current OECD guideline; Result is considered as valid.
	Forward mutation	Mouse lymphoma L5178Y *Tk* +/-cells	6.25–100 µg/ml	Negative[c]	(Cifone, 1982a)	Valid. Unpublished GLP study carried out according to current OECD guideline; Result is considered as valid.
	Forward mutation	Mouse lymphoma L5178Y *Tk* +/-cells	15.6–250 µg/ml	Negative	(Cifone, 1982a)	Valid. Unpublished GLP study carried out According to current OECD guideline; Result is considered as valid.

a: With and without metabolic activation.

b: Pre-incubation method.

c: With metabolic activation.

d: Validity of genotoxicity studies:

Valid.

Limited validity (e.g. if certain aspects are not in accordance with OECD guidelines or current standards and / or limited documentation).

Insufficient validity (e.g. if main aspects are not in accordance with any recognised guidelines (e.g. OECD) or current standards and/or inappropriate test system).

Validity cannot be evaluated (e.g. insufficient documentation, short abstract only, too little experimental details provided).

Table 2.5: Genotoxicity (*in vivo*) FGE.217 (EFSA, 2009ad)

Table 2.5: GENOTOXICITY (*in vivo*)

Chemical Name [FL-no]	Test System	Test Object	Route	Dose	Result	Reference	Comments [a]
6-methylcoumarin [13.012]	Sex-linked recessive lethal mutation	*Drosophila melanogaster*	Feed	10 mmol/l (1602 µg/ml)	Negative	(Wild et al., 1983)	Limited validity (limited reporting, study system considered of limited relevance).
	Micronucleus formation	Mouse peripheral blood cells	Oral (Gavage)	200 and 400 mg/kg for 90 days	Equivocal (M) Negative (F)	(Witt et al., 2000)	Limited validity (not a standard protocol; exposure for 90 days; no information on cytotoxicity; no positive controls).
	Micronucleus formation	Mouse bone-marrow cells	i.p.	160, 240, and 320 mg/kg	Negative	(Wild et al., 1983)	Limited validity (only analysis at one time point; no PCE/NCE ratio reported).

a: Validity of genotoxicity studies:

Valid.

Limited validity (e.g. if certain aspects are not in accordance with OECD guidelines or current standards and / or limited documentation).

Insufficient validity (e.g. if main aspects are not in accordance with any recognised guidelines (e.g. OECD) or current standards and/or inappropriate test system).

Validity cannot be evaluated (e.g. insufficient documentation, short abstract only, too little experimental details provided).

TABLE 3: SUMMARY OF SAFETY EVALUATION TABLES

Table 3.1: Summary of Safety Evaluation of Alicyclic, Alicyclic-fused and Aromatic-fused Ring Lactones (JECFA, 2004b)

Table 3.1: Summary of Safety evaluation of 12 JECFA-evaluated Substances

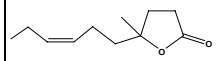
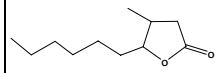
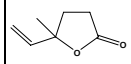
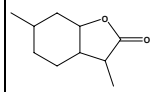
FL-no JECFA-no	EU Register name	Structural formula	EU MSDI 1) US MSDI (µg/*capita*/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [(4) or 5) or 6)]	EFSA conclusion on the named compound (Procedure steps, intake estimates, NOAEL, genotoxicity)	EFSA conclusion on the material of commerce
10.061 1159	cis-5-Hexenyldihydro-5-methylfuran-2(3H)-one		ND 13	Class I A3: Intake below threshold	4)	MSDI based on USA production figure.	CASrn in Register does not specify stereoisomers. Stereoisomeric composition to be specified. MSDI based on USA production figure.
10.069 1158	3-Methyl gamma-decalactone		ND 5	Class I A3: Intake below threshold	4)	MSDI based on USA production figure.	According to JECFA: Min. assay value is "94 % (sum of cis and trans isomers)" and secondary components "heptan-1-ol". CASrn in Register does not specify stereoisomers. Stereoisomeric composition to be specified. Composition of mixture to be specified. MSDI based on USA anticipated production figure.
10.070 1157	4-Methyl-5-hexen-1,4-olide		ND 3	Class I A3: Intake below threshold	4)	MSDI based on USA production figure.	CASrn in Register does not specify stereoisomers. Stereoisomeric composition to be specified. MSDI based on USA production figure.
10.050 1161	Hexahydro-3,6-dimethyl-2(3H)-benzofuranone		ND 12	Class III A3: Intake below threshold	4)	MSDI based on USA production figure.	CASrn in Register does not specify stereoisomers. Stereoisomeric composition to be specified. MSDI based on USA production figure.

Table 3.1: Summary of Safety evaluation of 12 JECFA-evaluated Substances

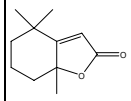
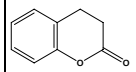
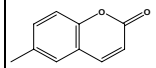
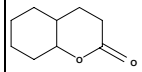
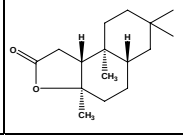
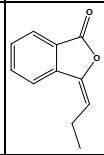
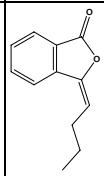
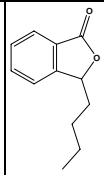
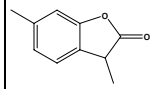
FL-no JECFA-no	EU Register name	Structural formula	EU MSDI 1) US MSDI (µg/*capita*/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [(4) or 5) or 6)]	EFSA conclusion on the named compound (Procedure steps, intake estimates, NOAEL, genotoxicity)	EFSA conclusion on the material of commerce
10.169 1164	5,6,7,7alpha-Tetrahydro-4,4,7alpha-trimethyl-2-(4H)-benzofuranone		0.12 0.9	Class III A3: Intake below threshold	4)	No safety concern at estimated level of intake as flavouring substance based on the MSDI approach.	According to JECFA: Min. assay value is "90 %" and secondary components "2,9-dimethyl 3,8-decanedione, 4-hydroxy-5,6-oxo beta-ionone". CASrn in Register does not specify stereoisomers. Stereoisomeric composition to be specified. Composition of mixture to be specified.
13.009 1171	3,4-Dihydrocoumarin		1200 1111	Class III B3: Intake above threshold. But adequate data are available for a safety evaluation.	6)	Adequate data are available to reach the conclusion "No safety concern at estimated level of intake as flavouring substance based on the MSDI approach".	No safety concern at estimated level of intake as flavouring substance based on the MSDI approach.
13.012 1172	6-Methylcoumarin		250 96	Class III B3: Intake above threshold. But adequate data are available for a safety evaluation.	6)	Adequate data are available to reach the conclusion "No safety concern at estimated level of intake as flavouring substance based on the MSDI approach".	No safety concern at estimated level of intake as flavouring substance based on the MSDI approach.
13.161 1166	Octahydrocoumarin		ND 0.07	Class III A3: Intake below threshold	4)	MSDI based on USA production figure.	CASrn in Register does not specify stereoisomers. Stereoisomeric composition to be specified. MSDI based on USA production figure.
16.055 1165	Sclareolide		1.1 6	Class III A3: Intake below threshold	4)	No safety concern at estimated level of intake as flavouring substance based on the MSDI approach.	Register name to be changed to (R)-(+)-Sclareolide. No safety concern at estimated level of intake as flavouring substance based on the MSDI approach.

Table 3.1: Summary of Safety evaluation of 12 JECFA-evaluated Substances

FL-no JECFA-no	EU Register name	Structural formula	EU MSDI 1) US MSDI (µg/*capita*/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [(4) or 5) or 6)]	EFSA conclusion on the named compound (Procedure steps, intake estimates, NOAEL, genotoxicity)	EFSA conclusion on the material of commerce
10.005 1168	3-Propylidenephthalide		17 52	Class III B3: Intake below threshold, B4: Adequate NOAEL exists	4)	No safety concern at estimated level of intake as flavouring substance based on the MSDI approach.	No safety concern at estimated level of intake as flavouring substance based on the MSDI approach.
10.024 1170	3-Butylidenephthalide		8.6 7	Class III B3: Intake below threshold, B4: Adequate NOAEL exists	4)	No safety concern at estimated level of intake as flavouring substance based on the MSDI approach.	No safety concern at estimated level of intake as flavouring substance based on the MSDI approach.
10.025 1169	3-Butylphthalide		0.49 0.4	Class III B3: Intake below threshold, B4: Adequate NOAEL exists	4)	No safety concern at estimated level of intake as flavouring substance based on the MSDI approach.	No safety concern at estimated level of intake as flavouring substance based on the MSDI approach.
10.072 1167	Dimethyl-3,6-benzo-2(3H)-furanone		ND 2	Class III B3: Intake below threshold, B4: Adequate NOAEL exists	4)	MSDI based on USA production figure.	CASrn in Register does not specify stereoisomers. Stereoisomeric composition to be specified. MSDI based on USA production figure.

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

2) Thresholds of concern: Class I = 1800, Class II = 540, Class III = 90 µg/person/day.

3) Procedure path A substances can be predicted to be metabolised to innocuous products. Procedure path B substances cannot.

4) No safety concern based on intake calculated by the MSDI approach of the named compound.

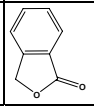
5) Data must be available on the substance or closely related substances to perform a safety evaluation.

6) No safety concern based on intake calculated by the MSDI approach of the named compound and on adequate data available for a safety evaluation.

ND: not determined.

Table 3.2: Summary of Safety Evaluation Applying the Procedure (EFSA / FGE.27)

Table 3.2: Summary of Safety Evaluation Applying the Procedure of substances in FGE.27 (based on intakes calculated by the MSDI approach)

FL-no	EU Register name	Structural formula	MSDI 1) (µg/*capita*/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
10.056	Phthalide		0.8	Class III A3: Intake below threshold	4)	8)	

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

2) Thresholds of concern: Class I = 1800, Class II = 540, Class III = 90 µg/person/day.

3) Procedure path A substances can be predicted to be metabolised to innocuous products. Procedure path B substances cannot.

4) No safety concern based on intake calculated by the MSDI approach of the named compound.

5) Data must be available on the substance or closely related substances to perform a safety evaluation.

6) No safety concern at estimated level of intake of the material of commerce meeting the specification of Table 1 (based on intake calculated by the MSDI approach).

7) 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.

8) No conclusion can be drawn due to lack of information on the purity of the material of commerce.

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ABBREVIATIONS

CAS	Chemical Abstract Service
CEF	Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
CHO	Chinese hamster ovary (cells)
CoE	Council of Europe
DNA	Deoxyribonucleic acid
DTU-NFI	Danish Technical University – National Food Institute
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
FLAVIS (FL)	Flavour Information System (database)
GLP	Good Laboratory Practise
ID	Identity
IR	Infrared spectroscopy
ISS	Istituto Superiore di Sanita
JECFA	The Joint FAO/WHO Expert Committee on Food Additives
LD_{50}	Lethal Dose, 50%; Median lethal dose
MSDI	Maximised Survey-derived Daily Intake
mTAMDI	Modified Theoretical Added Maximum Daily Intake
NMR	Nuclear magnetic resonance
No	Number
NOAEL	No observed adverse effect level
NTP	National Toxicology Program
OECD	Organisation for Economic Co-operation and Development
PCE/NCE	Polychromatic eryhtrocyte/normochromatic erythrocyte ratio
(Q)SAR	(Quantitative) structure-activity relationship
SCE	Sister chromatid exchange
SCF	Scientific Committee on Food
SLRL	Sex-linked recessive lethal mutations

TAMDI	Theoretical Added Maximum Daily Intake
UDS	Unscheduled DNA synthesis
US EPA	United States Environmental Protection Agency
WHO	World Health Organisation.