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)

ADOPTED ON 17 JUNE 2009

PANEL MEMBERS

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, 61\(^{th}\) 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, focusing 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:

<table>
<thead>
<tr>
<th>FGE</th>
<th>Opinion Adopted by EFSA</th>
<th>Link</th>
<th>No. of Candidate Substances</th>
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<td>FGE.80</td>
<td>1 April 2008</td>
<td><a href="http://www.efsa.europa.eu/EFSA/efsaloa-1178620753812_1211902220401.htm">http://www.efsa.europa.eu/EFSA/efsaloa-1178620753812_1211902220401.htm</a></td>
<td>11</td>
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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 subtilis* 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

\(^1\) 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 B6C3F1 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 B6C3F1 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\textsuperscript{2} from FGE.27 (EFSA, 2008a)

\textit{In vitro / in vivo}

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

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

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

\textit{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\textsuperscript{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 \textit{in vivo} studies considered of limited validity: a \textit{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.”

\textit{Conclusion on Genotoxicity and Carcinogenicity}

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

\textsuperscript{2} The text is taken verbatim from the indicated reference source, but text related to substances not included in the present FGE has been removed.

\textsuperscript{3} 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].

Selareolide [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 evaluated 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 mTAMDIIs 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>
<thead>
<tr>
<th>FL-no</th>
<th>JECFA-no</th>
<th>EU Register name</th>
<th>Structural formula</th>
<th>FEMA no</th>
<th>CoE no</th>
<th>CAS no</th>
<th>Phys.form</th>
<th>Solubility 1)</th>
<th>Solubility in ethanol 2)</th>
<th>Boiling point, °C</th>
<th>Melting point, °C</th>
<th>Refrac. Index 4)</th>
<th>Spec.gravity 5)</th>
<th>EFSA comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>10.005</td>
<td>1168</td>
<td>3-Propylidenephthalide</td>
<td><img src="image" alt="Structure" /></td>
<td>2952</td>
<td>494</td>
<td>17369-59-4</td>
<td>Liquid</td>
<td>C_3H_3O_4</td>
<td>Insoluble</td>
<td>Soluble</td>
<td>169-171 (17hPa)</td>
<td>NMR 96 %</td>
<td>1.557-1.562</td>
<td>1.127-1.132</td>
</tr>
<tr>
<td>10.024</td>
<td>1170</td>
<td>3-Butylidenephthalide</td>
<td><img src="image" alt="Structure" /></td>
<td>3333</td>
<td>10083</td>
<td>551-08-6</td>
<td>Liquid</td>
<td>C_3H_3O_4</td>
<td>Insoluble</td>
<td>Soluble</td>
<td>114-116 (0.07hPa)</td>
<td>NMR 99 %</td>
<td>1.554-1.559</td>
<td>1.098-1.103</td>
</tr>
<tr>
<td>10.025</td>
<td>1169</td>
<td>3-Butylphthalide</td>
<td><img src="image" alt="Structure" /></td>
<td>3344</td>
<td>10084</td>
<td>6066-49-5</td>
<td>Liquid</td>
<td>C_3H_3O_4</td>
<td>Slightly soluble</td>
<td>Soluble</td>
<td>113 (0.3 hPa)</td>
<td>IR NMR 97 %</td>
<td>1.524-1.529</td>
<td>1.068-1.074</td>
</tr>
<tr>
<td>10.050</td>
<td>1161</td>
<td>Hexahydro-3,6-dimethyl-2(3H)-benzofuranone</td>
<td><img src="image" alt="Structure" /></td>
<td>4032</td>
<td>92015-65-1</td>
<td>Liquid</td>
<td>C_3H_3O_4</td>
<td>Soluble</td>
<td>Soluble</td>
<td>274-276 (17hPa)</td>
<td>IR NMR 99.4 %</td>
<td>1.464-1.470</td>
<td>1.016-1.022</td>
<td></td>
</tr>
<tr>
<td>10.061</td>
<td>1159</td>
<td>cis-5-Hexenylidihydro-5-methylfuran-2(3H)-one</td>
<td><img src="image" alt="Structure" /></td>
<td>3937</td>
<td>70851-61-5</td>
<td>Liquid</td>
<td>C_3H_3O_4</td>
<td>Insoluble</td>
<td>Soluble</td>
<td>150 (8 hPa)</td>
<td>IR NMR 97 %</td>
<td>1.463-1.468</td>
<td>0.960-0.967</td>
<td></td>
</tr>
</tbody>
</table>

Racemate.

CASrn in Register does not specify stereoisomers.

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

<table>
<thead>
<tr>
<th>FL-no</th>
<th>EU Register name</th>
<th>Structural formula</th>
<th>FEMA no</th>
<th>CAS no</th>
<th>Phys.form</th>
<th>Mol.formula</th>
<th>Mol.weight</th>
<th>Solubility 1)</th>
<th>Boiling point, °C 3)</th>
<th>Refrac. Index 4)</th>
<th>EFSA comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>10.069</td>
<td>3-Methyl gamma-decalactone</td>
<td><img src="image1" alt="Structural formula" /></td>
<td>5999</td>
<td>67663-01-8</td>
<td>Liquid</td>
<td>C₇H₁₀O₂</td>
<td>184.28</td>
<td>Insoluble</td>
<td>110-115 (5 hPa)</td>
<td>1.446-1.452</td>
<td>CASrn in Register does not specify stereoisomers. JECFA name: ± 3-methyl gamma-decalactone. According to JECFA: Min. assay value is &quot;94 % (sum of cis and trans isomers)&quot; and secondary components &quot;heptan-1-ol&quot; Composition of mixture to be specified.</td>
</tr>
<tr>
<td>10.070</td>
<td>4-Methyl-5-hexen-1,4-olide</td>
<td><img src="image2" alt="Structural formula" /></td>
<td>4051</td>
<td>1073-11-6</td>
<td>Liquid</td>
<td>C₇H₁₀O₂</td>
<td>126.15</td>
<td>Insoluble</td>
<td>97 %</td>
<td>1.457-1.462</td>
<td>CASrn in Register does not specify stereoisomers.</td>
</tr>
<tr>
<td>10.072</td>
<td>Dimethyl-3,6-benzo-2(3H)-furanone</td>
<td><img src="image3" alt="Structural formula" /></td>
<td>3863</td>
<td>65817-24-5</td>
<td>Liquid</td>
<td>C₈H₁₀O₂</td>
<td>162.19</td>
<td>Insoluble</td>
<td>64 (0.1 hPa)</td>
<td>1.518-1.524</td>
<td>CASrn in Register does not specify stereoisomers.</td>
</tr>
<tr>
<td>10.169</td>
<td>5,6,7,7alpha-Tetrahydro-4,4,7alpha-trimethyl-2-(4H)-benzofuranone</td>
<td><img src="image4" alt="Structural formula" /></td>
<td>1020</td>
<td>15356-74-8</td>
<td>Liquid</td>
<td>C₉H₁₄O₂</td>
<td>180.25</td>
<td>Insoluble</td>
<td>IR NMR 98 %</td>
<td>1.499-1.505</td>
<td>CASrn in Register does not specify stereoisomers. According to JECFA: Min. assay value is &quot;90 %&quot; and secondary components &quot;2,9-dimethyl 3,8-decanedione, 4-hydroxy-5,6-oxo beta-ionone&quot;. Composition of mixture to be specified.</td>
</tr>
<tr>
<td>13.009</td>
<td>3,4-Dihydrocoumarin</td>
<td><img src="image5" alt="Structural formula" /></td>
<td>2381</td>
<td>535-119-84-6</td>
<td>Liquid</td>
<td>C₅H₇O₂</td>
<td>148.16</td>
<td>Slightly soluble</td>
<td>IR NMR 99 %</td>
<td>1.555-1.559</td>
<td></td>
</tr>
<tr>
<td>13.012</td>
<td>6-Methylcoumarin</td>
<td><img src="image6" alt="Structural formula" /></td>
<td>2699</td>
<td>579-92-48-8</td>
<td>Solid</td>
<td>C₆H₈O₂</td>
<td>160.17</td>
<td>Insoluble</td>
<td>73-79</td>
<td>n.a.</td>
<td></td>
</tr>
</tbody>
</table>

**Notes:**
- **Solubility 1):** Solubility in ethanol
- **Boiling point, °C 3):** Melting point, °C
- **Refrac. Index 4):** Refractive Index
- **EFSA comments:** Additional information from EFSA Journal (2009) 1169, 17-32
Table 1: Specification Summary of the Substances in the JECFA Flavouring Group of Alicyclic, Alicyclic-fused and Aromatic-fused Ring Lactones

<table>
<thead>
<tr>
<th>FL-no</th>
<th>EU Register name</th>
<th>Structural formula</th>
<th>FEMA no</th>
<th>CAS no</th>
<th>Phys.form</th>
<th>Mol.formula</th>
<th>Mol.weight</th>
<th>Solubility 1)</th>
<th>Solubility in ethanol 2)</th>
<th>Boiling point, °C 3)</th>
<th>Melting point, °C</th>
<th>ID test</th>
<th>Assay minimum</th>
<th>Refrac. Index 4)</th>
<th>Spec.gravity 5)</th>
<th>EFSA comments</th>
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<tbody>
<tr>
<td>13.161 1166</td>
<td>Octahydrocoumarin 6)</td>
<td><img src="image1.png" alt="Structural formula" /></td>
<td>3791</td>
<td>4430-31-3</td>
<td>Liquid</td>
<td>C₉H₁₄O₂</td>
<td>154.21</td>
<td>Insoluble</td>
<td>Soluble</td>
<td>293-298</td>
<td>NMR</td>
<td>99 %</td>
<td>1.489-1.493</td>
<td>1.090-1.096</td>
<td>CASRN in Register does not specify stereoisomers.</td>
<td></td>
</tr>
<tr>
<td>16.055 1165</td>
<td>Sclareolide</td>
<td><img src="image2.png" alt="Structural formula" /></td>
<td>3794</td>
<td>564-20-5</td>
<td>Solid</td>
<td>C₁₆H₂₆O₂</td>
<td>250.38</td>
<td>Insoluble</td>
<td>Slightly soluble</td>
<td>124.4</td>
<td>IR NMR</td>
<td>98 %</td>
<td>n.a.</td>
<td>n.a.</td>
<td>Register name to be changed to (R)-(+)-Sclareolide.</td>
<td></td>
</tr>
</tbody>
</table>

1) Solubility in water, if not otherwise stated.
2) Solubility in 95% ethanol, if not otherwise stated.
3) At 1013.25 kPa, 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>
<thead>
<tr>
<th>FL-no</th>
<th>EU Register name</th>
<th>Structural formula</th>
<th>End-point</th>
<th>Test system</th>
<th>Concentration</th>
<th>Results</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>10.169 1164</td>
<td>5,6,7alpha-Tetrahydro-4,4,7alpha-trimethyl-2-(4H)-benzofuranone</td>
<td></td>
<td>Reverse mutation</td>
<td>S. typhimurium TA97, TA98, TA1535, TA1537 and TA1538</td>
<td>100 µg/plate</td>
<td>Negative</td>
<td>(Kinae et al., 1981a)</td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>DNA repair</td>
<td>B. Subtilis H-17 (rec+) and M-45 (rec-)</td>
<td>10000 µg/plate</td>
<td>Negative</td>
<td>(Kinae et al., 1981a)</td>
</tr>
<tr>
<td>13.161 1166</td>
<td>Octahydrocoumarin</td>
<td></td>
<td>Reverse mutation</td>
<td>S. typhimurium TA98 and TA100</td>
<td>≤5000 µg/plate</td>
<td>Negative</td>
<td>(Watanabe &amp; Morimoto, 1989a)</td>
</tr>
<tr>
<td>10.005 1168</td>
<td>3-Propylidenephthalide</td>
<td></td>
<td>Reverse mutation</td>
<td>S. typhimurium TA98, TA100, TA1535, TA1537 and TA1538</td>
<td>3.3–400 µg/plate</td>
<td>Positive</td>
<td>(Zeiger et al., 1988)</td>
</tr>
<tr>
<td>13.009 1171</td>
<td>3,4-Dihydrocoumarin</td>
<td></td>
<td>Reverse mutation</td>
<td>S. typhimurium TA1535, TA1537, TA1538, TA98 and TA100</td>
<td>≤75 µl/plate (88 950 µg/plate)</td>
<td>Negative</td>
<td>(Brusick, 1982b)</td>
</tr>
<tr>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Reverse mutation</td>
<td>S. typhimurium TA98, TA100, TA1535 and TA1537</td>
<td>10–6666 µg/plate</td>
<td>Negative</td>
<td>(NTP, 1993c)</td>
</tr>
<tr>
<td></td>
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<td></td>
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<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Reverse mutation</td>
<td>S. typhimurium TA98 and TA100, TA1535 and TA1537</td>
<td>≤10 mg/plate (≤10 000 µg/plate)</td>
<td>Negative</td>
<td>(Prival et al., 1982)</td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Forward mutation</td>
<td>Mouse lymphoma L5178Y TK +/- cells</td>
<td>200–500 nM/ml (237–593 µg/ml)</td>
<td>Weakly positive</td>
<td>(Cifone, 1982b)</td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Forward mutation</td>
<td>Mouse lymphoma L5178Y TK +/- cells</td>
<td>400–800 nM/ml (474–949 µg/ml)</td>
<td>Negative</td>
<td>(Cifone, 1982b)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Forward mutation</td>
<td>Mouse lymphoma L5178Y TK +/- cells</td>
<td>≥2500 nM/ml (2965 µg/ml)</td>
<td>Positive</td>
<td>(Cifone, 1984)</td>
</tr>
<tr>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Forward mutation</td>
<td>Mouse lymphoma L5178Y TK +/- cells</td>
<td>≥2500 nM/ml (2965 µg/ml)</td>
<td>Negative</td>
<td>(Cifone, 1984)</td>
</tr>
</tbody>
</table>
Table 2.1: Summary of Genotoxicity Data of 13 Alicyclic, Alicyclic-fused and Aromatic-fused Ring Lactones evaluated by JECFA

<table>
<thead>
<tr>
<th>FL-no</th>
<th>EU Register name</th>
<th>JECFA name</th>
<th>Structural formula</th>
<th>End-point</th>
<th>Test system</th>
<th>Concentration</th>
<th>Results</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Unscheduled DNA synthesis</td>
<td>Rat hepatocytes</td>
<td>0.03–4.0 µl/ml (35.6–4744 µg/ml)</td>
<td>Negative</td>
<td>(Curren, 1986)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Chromosomal aberrations</td>
<td>Chinese hamster ovary cells</td>
<td>0.01–1.0 µl/ml (11.9–1186 µg/ml)</td>
<td>Negative&lt;sup&gt;4&lt;/sup&gt;</td>
<td>(Galloway, 1983)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Chromosomal aberrations</td>
<td>Chinese hamster ovary cells</td>
<td>33.3–333 nl/ml (39.5–395 µg/ml)</td>
<td>Negative&lt;sup&gt;4&lt;/sup&gt;</td>
<td>(Galloway, 1983)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Chromosomal aberrations</td>
<td>Chinese hamster ovary cells</td>
<td>500, 1000 and 1600 µg/ml</td>
<td>Negative&lt;sup&gt;4&lt;/sup&gt;</td>
<td>(NTP, 1993c)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Sister chromatid exchanges</td>
<td>Chinese hamster ovary cells</td>
<td>50–300 µg/ml</td>
<td>Positive&lt;sup&gt;1&lt;/sup&gt;</td>
<td>(NTP, 1993c)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Sister chromatid exchanges</td>
<td>Chinese hamster ovary cells</td>
<td>50–1000 µg/ml</td>
<td>Negative&lt;sup&gt;3&lt;/sup&gt;</td>
<td>(NTP, 1993c)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Sister chromatid exchanges</td>
<td>Chinese hamster ovary cells</td>
<td>1600 and 2000 µg/ml</td>
<td>Positive&lt;sup&gt;3&lt;/sup&gt;</td>
<td>(NTP, 1993c)</td>
</tr>
<tr>
<td>13.012</td>
<td>6-Methylcoumarin</td>
<td></td>
<td></td>
<td>Reverse mutation</td>
<td>S. typhimurium TA100</td>
<td>&lt;3.6 mg/plate (&lt;3600 µg/plate)</td>
<td>Marginally positive&lt;sup&gt;1&lt;/sup&gt;</td>
<td>(Wild et al., 1983)</td>
</tr>
<tr>
<td>1172</td>
<td></td>
<td></td>
<td></td>
<td>Reverse mutation</td>
<td>S. typhimurium TA100</td>
<td>&lt;3.6 mg/plate (&lt;3600 µg/plate)</td>
<td>Negative</td>
<td>(Wild et al., 1983)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Reverse mutation</td>
<td>S. typhimurium TA98, TA1535, and TA1537</td>
<td>&lt;3.6 mg/plate (&lt;3600 µg/plate)</td>
<td>Negative&lt;sup&gt;1&lt;/sup&gt;</td>
<td>(Wild et al., 1983)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Reverse mutation</td>
<td>S. typhimurium TA98, TA100, TA1535 and TA1537</td>
<td>&lt;33-3333 µg/plate</td>
<td>Negative&lt;sup&gt;3&lt;/sup&gt;</td>
<td>(Haworth et al., 1983)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Reverse mutation</td>
<td>S. typhimurium TA98, TA100, TA1535, TA1537 and TA1538</td>
<td>&lt;1-5000 µg/plate</td>
<td>Negative&lt;sup&gt;1&lt;/sup&gt;</td>
<td>(Brusick, 1982a)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Forward mutation</td>
<td>Mouse lymphoma L5178Y Tk +/- cells</td>
<td>6.25-100 µg/plate</td>
<td>Negative&lt;sup&gt;3&lt;/sup&gt;</td>
<td>(Cifone, 1982a)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Forward mutation</td>
<td>Mouse lymphoma L5178Y Tk +/- cells</td>
<td>15.6-250 µg/plate</td>
<td>Negative</td>
<td>(Cifone, 1982a)</td>
</tr>
</tbody>
</table>

**In vivo**

<table>
<thead>
<tr>
<th>FL-no</th>
<th>EU Register name</th>
<th>JECFA name</th>
<th>Structural formula</th>
<th>End-point</th>
<th>Test system</th>
<th>Concentration</th>
<th>Results</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>13.009</td>
<td>3,4-Dihydrocoumarin</td>
<td></td>
<td></td>
<td>Micronucleus formation</td>
<td>Mouse peripheral blood cells</td>
<td>400, 800 and 1600 mg/kg bw</td>
<td>Negative</td>
<td>(NTP, 1993c)</td>
</tr>
<tr>
<td>1171</td>
<td></td>
<td></td>
<td></td>
<td>Sex-linked recessive lethal mutation</td>
<td>Drosophila melanogaster</td>
<td>10 mmol/l (1602 µg/ml)</td>
<td>Negative</td>
<td>(Wild et al., 1983)</td>
</tr>
<tr>
<td>13.012</td>
<td>6-Methylcoumarin</td>
<td></td>
<td></td>
<td>Micronucleus formation</td>
<td>Mouse peripheral blood cells</td>
<td>200 and 400 mg/kg</td>
<td>Equivocal (M)&lt;sup&gt;7&lt;/sup&gt;</td>
<td>(Witt et al., 2000)</td>
</tr>
</tbody>
</table>
Table 2.1: Summary of Genotoxicity Data of 13 Alicyclic, Alicyclic-fused and Aromatic-fused Ring Lactones evaluated by JECFA

<table>
<thead>
<tr>
<th>FL-no</th>
<th>JECFA-no</th>
<th>EU Register name</th>
<th>JECFA name</th>
<th>Structural formula</th>
<th>End-point</th>
<th>Test system</th>
<th>Concentration</th>
<th>Results</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Micronucleus formation</td>
<td>Mouse bone-marrow cells</td>
<td>160, 240 and 320 mg/kg</td>
<td>Negative(^3)</td>
<td>(Wild et al., 1983)</td>
</tr>
</tbody>
</table>

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 \textit{(in vitro)} FGE.27 (EFSA, 2008a)
Substances listed in brackets are JECFA evaluated substances

<table>
<thead>
<tr>
<th>Chemical Name [FL-no]</th>
<th>Test System (preincubation method)</th>
<th>Test Object</th>
<th>Concentration</th>
<th>Result</th>
<th>Reference</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>(3-Propylidenephthalide [10.005])</td>
<td>Ames test</td>
<td>Salmonella typhimurium TA97, TA98, TA100 and TA1535</td>
<td>0, 3.3, 10, 33, 100, 200 µg/plate (in addition 300 and 400 µg/plate in TA100 +S9 from rat liver)</td>
<td>Negative$^1$</td>
<td>(Zeiger et al., 1988)</td>
<td>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).</td>
</tr>
</tbody>
</table>

NR: Not reported.
$^1$ Without metabolic activation.
$^2$ With metabolic activation.

Table 2.3: Genotoxicity \textit{(in vivo)} FGE.27 (EFSA, 2008a)
No data.
## Table 2.4 Genotoxicity (in vitro) FGE.217 (EFSA, 2009ad)

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

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

*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>
<thead>
<tr>
<th>FL-no</th>
<th>EU Register name</th>
<th>Structural formula</th>
<th>EU MSDI 1) US MSDI (μg/capita/day)</th>
<th>Class 2) Evaluation procedure path 3)</th>
<th>Outcome on the named compound 4) or 5) or 6)</th>
<th>EFSA conclusion on the named compound (Procedure steps, intake estimates, NOAEL, genotoxicity)</th>
<th>EFSA conclusion on the material of commerce</th>
</tr>
</thead>
<tbody>
<tr>
<td>10.061 1159</td>
<td>cis-5-Hexenyldihydro-5-methylfuran-2(3H)-one</td>
<td>ND 13</td>
<td>Class I A3: Intake below threshold</td>
<td>4) MSDI based on USA production figure.</td>
<td>CASrn in Register does not specify stereoisomers. Stereoisomeric composition to be specified. MSDI based on USA production figure.</td>
<td>CASrn in Register does not specify stereoisomers. Stereoisomeric composition to be specified. MSDI based on USA production figure.</td>
<td>According to JECFA: Min. assay value is &quot;94 % (sum of cis and trans isomers)&quot; and secondary components &quot;heptan-1-ol&quot;. 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.</td>
</tr>
<tr>
<td>10.069 1158</td>
<td>3-Methyl gamma-decalactone</td>
<td>ND 5</td>
<td>Class I A3: Intake below threshold</td>
<td>4) MSDI based on USA production figure.</td>
<td>CASrn in Register does not specify stereoisomers. Stereoisomeric composition to be specified. MSDI based on USA production figure.</td>
<td>CASrn in Register does not specify stereoisomers. Stereoisomeric composition to be specified. MSDI based on USA production figure.</td>
<td></td>
</tr>
<tr>
<td>10.070 1157</td>
<td>4-Methyl-5-hexen-1,4-olide</td>
<td>ND 3</td>
<td>Class I A3: Intake below threshold</td>
<td>4) MSDI based on USA production figure.</td>
<td>CASrn in Register does not specify stereoisomers. Stereoisomeric composition to be specified. MSDI based on USA production figure.</td>
<td>CASrn in Register does not specify stereoisomers. Stereoisomeric composition to be specified. MSDI based on USA production figure.</td>
<td></td>
</tr>
<tr>
<td>10.050 1161</td>
<td>Hexahydro-3,6-dimethyl-2(3H)-benzofuranone</td>
<td>ND 12</td>
<td>Class III A3: Intake below threshold</td>
<td>4) MSDI based on USA production figure.</td>
<td>CASrn in Register does not specify stereoisomers. Stereoisomeric composition to be specified. MSDI based on USA production figure.</td>
<td>CASrn in Register does not specify stereoisomers. Stereoisomeric composition to be specified. MSDI based on USA production figure.</td>
<td></td>
</tr>
</tbody>
</table>
Table 3.1: Summary of Safety evaluation of 12 JECFA-evaluated Substances

<table>
<thead>
<tr>
<th>FL-no</th>
<th>JECFA-no</th>
<th>EU Register name</th>
<th>Structural formula</th>
<th>EU MSDI 1) (μg/capita/day)</th>
<th>US MSDI (μg/capita/day)</th>
<th>Class 2) Evaluation procedure path 3)</th>
<th>Outcome on the named compound [4) or 5) or 6)]</th>
<th>EFSA conclusion on the named compound (Procedure steps, intake estimates, NOAEL, genotoxicity)</th>
<th>EFSA conclusion on the material of commerce</th>
</tr>
</thead>
<tbody>
<tr>
<td>10.169</td>
<td>1164</td>
<td>5,6,7,7alpha-Tetrahydro-4,4,7alpha-trimethyl-2-(4H)-benzofuranone</td>
<td><img src="image1" alt="Structural formula" /></td>
<td>0.12</td>
<td>0.9</td>
<td>Class III A3: Intake below threshold</td>
<td>4) No safety concern at estimated level of intake as flavouring substance based on the MSDI approach.</td>
<td>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.</td>
<td></td>
</tr>
<tr>
<td>13.009</td>
<td>1171</td>
<td>3,4-Dihydrocoumarin</td>
<td><img src="image2" alt="Structural formula" /></td>
<td>1200</td>
<td>1111</td>
<td>Class III B3: Intake above threshold. But adequate data are available for a safety evaluation.</td>
<td>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”.</td>
<td>No safety concern at estimated level of intake as flavouring substance based on the MSDI approach.</td>
<td></td>
</tr>
<tr>
<td>13.012</td>
<td>1172</td>
<td>6-Methylcoumarin</td>
<td><img src="image3" alt="Structural formula" /></td>
<td>250</td>
<td>96</td>
<td>Class III B3: Intake above threshold. But adequate data are available for a safety evaluation.</td>
<td>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”.</td>
<td>No safety concern at estimated level of intake as flavouring substance based on the MSDI approach.</td>
<td></td>
</tr>
<tr>
<td>13.161</td>
<td>1166</td>
<td>Octahydrocoumarin</td>
<td><img src="image4" alt="Structural formula" /></td>
<td>ND</td>
<td>0.07</td>
<td>Class III A3: Intake below threshold</td>
<td>4) MSDI based on USA production figure.</td>
<td>CASrn in Register does not specify stereoisomers. Stereoisomeric composition to be specified. MSDI based on USA production figure.</td>
<td></td>
</tr>
<tr>
<td>16.055</td>
<td>1165</td>
<td>Sclareolide</td>
<td><img src="image5" alt="Structural formula" /></td>
<td>1.1</td>
<td>6</td>
<td>Class III A3: Intake below threshold</td>
<td>4) No safety concern at estimated level of intake as flavouring substance based on the MSDI approach.</td>
<td>Register name to be changed to (R)(+)-Sclareolide. No safety concern at estimated level of intake as flavouring substance based on the MSDI approach.</td>
<td></td>
</tr>
</tbody>
</table>
Table 3.1: Summary of Safety evaluation of 12 JECFA-evaluated Substances

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

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>
<thead>
<tr>
<th>FL-no</th>
<th>EU Register name</th>
<th>Structural formula</th>
<th>MSDI 1) (μg/capita/day)</th>
<th>Class 2) Evaluation procedure path 3)</th>
<th>Outcome on the named compound [4) or 5)]</th>
<th>Outcome on the material of commerce [6), 7), or 8])</th>
<th>Evaluation remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>10.056</td>
<td>Phthalide</td>
<td><img src="image" alt="Phthalide Structure" /></td>
<td>0.8</td>
<td>Class III A3: Intake below threshold</td>
<td>4)</td>
<td>8)</td>
<td></td>
</tr>
</tbody>
</table>

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.
REFERENCES:


Cifone, M.A., 1985. Relationship between increases in the mutant frequency in L5178Y TK+/- mouse lymphoma cells at low pH and metabolic activation. Environ. Mutat. 7(suppl. 3), 27.


NTP, 1993c. Toxicology and carcinogenesis studies of 3,4-dihydrocoumarin (CAS. no. 119-84-6) in F344/N rats and B6C3F1 mice (gavage studies). September 1993. NTP-TR 423. NIH Publication no. 93-3154.

NTP, 2002. Toxicology and carcinogenesis studies of methyl coumarin (CAS. no. 92-48-8) in F344/N rats and B6C3F1 mice (gavage studies).


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
LD50   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 erythrocyte/normochromatic erythrocyte ratio
(Q)SAR (Quantitative) structure-activity relationship
SCE    Sister chromatid exchange
SCF    Scientific Committee on Food
SLRL   Sex-linked recessive lethal mutations
<table>
<thead>
<tr>
<th>Acronym</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>TAMDI</td>
<td>Theoretical Added Maximum Daily Intake</td>
</tr>
<tr>
<td>UDS</td>
<td>Unscheduled DNA synthesis</td>
</tr>
<tr>
<td>US EPA</td>
<td>United States Environmental Protection Agency</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organisation.</td>
</tr>
</tbody>
</table>