EFSA Panel on Food Contact Material, Enzymes, Flavourings and Processing Aids (CEF); Scientific Opinion on Flavouring Group Evaluation 51, Revision 1: Consideration of alicyclic ketones and secondary alcohols and related esters evaluated by the JECFA (59th meeting) structurally related to alicyclic ketones secondary alcohols and related esters in FGE.09Rev3 (2011)

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SCIENTIFIC OPINION

Scientific Opinion on Flavouring Group Evaluation 51, Revision 1 (FGE.51Rev1):

Consideration of alicyclic ketones and secondary alcohols and related esters evaluated by the JECFA (59th meeting) structurally related to alicyclic ketones secondary alcohols and related esters in FGE.09Rev3 (2011)[1]

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

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

The Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids of the European Food Safety Authority was requested to consider evaluations of flavouring substances assessed since 2000 by the Joint FAO/WHO Expert Committee on Food Additives (the JECFA), and to decide whether further evaluation is necessary, as laid down in Commission Regulation (EC) No 1565/2000. The present consideration concerns a group of 20 alicyclic ketones and secondary alcohols and related esters evaluated by JECFA (59th meeting) in 2002. This revision is made due to inclusion of seven additional substances cleared for genotoxicity concern compared to the previous version. The substances were evaluated through a stepwise approach that integrates information on structure-activity relationships, intake from current uses, toxicological threshold of concern, and available data on metabolism and toxicity. The Panel agrees with the application of the Procedure as performed by the JECFA for all 20 substances considered in this FGE and agrees with the JECFA conclusion, "No safety concern at estimated levels of intake as flavouring substances" based on the MSDI approach. Besides the safety assessment of these flavouring substances, the specifications for the materials of commerce have also been considered and for all 20 substances, the information is adequate.

1 On request from the European Commission, Question No (EFSA-Q-2011-01040; EFSA-Q-2011-01041; EFSA-Q-2011-01042; EFSA-Q-2011-01043; EFSA-Q-2011-01044; EFSA-Q-2011-01045; EFSA-Q-2011-01046), adopted on 22 March 2012.

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KEY WORDS

Alicyclic ketones, JECFA 59th meeting, alicyclic secondary alcohols, FGE.09.

SUMMARY

The Scientific Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (the Panel) was asked to give 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.

This revision of FGE.51 is made due to the consideration of the seven the alpha,beta-unsaturated substances [FL-no: 07.034, 07.035, 07.098, 07.126, 07.129, 07.172 and 09.930] compared to the previous version of FGE.51. Furthermore, EU production volume on one substance [FL-no: 09.230] and data on stereoisomerism for four substances [FL-no: 02.209, 07.045, 07.095 and 07.257] have been provided since the publication of FGE.51.

The JECFA has evaluated a group of 25 flavouring substances consisting of alicyclic ketones, secondary alcohols and related esters at its 59th meeting. Two of the JECFA-evaluated substances are not in the Register (4-methyl cyclohexanone (JECFA no: 1104) and (E)-2-(2-octenyl) cyclopentanone (JECFA no: 1116)) and ten of the substances are alpha,beta-unsaturated ketones or precursors for such, which is recognized as a structural alert for genotoxicity. Seven of these 10 alpha,beta-unsaturated substances [FL-no: 07.034, 07.035, 07.098, 07.126, 07.129, 07.172 and 09.930] have been evaluated with respect to their genotoxic potential in FGE.211 (EFSA, 2011e) or in FGE.212Rev1 (EFSA, 2011f), and the Panel concluded that the data available ruled out the concern for genotoxicity and accordingly these seven substances can be evaluated through the Procedure.

The present consideration therefore concerns 20 alicyclic ketones, secondary alcohols and related esters evaluated by the JECFA at its 59th meeting and will be considered in relation to the European Food Safety Authority (EFSA) evaluation of 17 secondary alicyclic saturated and unsaturated alcohols, ketones and esters containing secondary alicyclic alcohols evaluated in the Flavouring Group Evaluation 09, Revision 3 (FGE.09Rev3).

The Panel agrees with the application of the Procedure as performed by the JECFA for the 20 substances considered in this FGE.

For all 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 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 tests are available for all 20 substances evaluated in this FGE.51Rev1.

For all 20 evaluated alicyclic ketones, secondary alcohols and related esters [FL-no: 02.209 07.034, 07.035, 07.045, 07.095, 07.098, 07.126, 07.129, 07.148, 07.149, 07.172, 07.179, 07.180, 07.257, 09.027, 09.140, 09.160, 09.230, 09.464 and 09.930], the Panel agrees with the JECFA conclusion, "No safety concern at estimated levels of intake as flavouring substance" based on the MSDI approach.

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BACKGROUND

Regulation (EC) No 2232/96 of the European Parliament and the Council (EC, 1996a) 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, 1999a).

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

The European Food Safety Authority (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.

The evaluation programme was finalised at the end of 2009.

After the finalisation of the evaluation programme, in their letters of the 7th May 2010 and 3rd June 2010, the Commission requested EFSA to carry out re-evaluation of the flavouring substances, tetramethyl ethylcyclohexenone [FL-no: 07.035], 3-methylcyclohex-2-en-1-one [FL-no: 07.098], 3,5,5-trimethylcyclohex-2-en-1-one (isophorone) [FL-no: 07.126], 3-methyl-5-propylcyclohex-2-en-1-one [FL-no: 07.129], 4-isopropylcyclohex-2-en-1-one [FL-no: 07.172], 2-hexylidenecyclopentan-1-one [FL-no: 07.034] and 1(7),8-p-menthadien-2-yl acetate (mixture of (E) and (Z) isomers) [FL-no: 09.930] based on additionally submitted data on genotoxicity, and depending on the outcome, to proceed to the evaluation of these flavouring substances through the Procedure, also according to Commission Regulation (EC) No 1565/2000 (EC, 2000a).

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, 1999a), 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 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, only the MSDI figures 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, 1999a), 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 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 59th meeting the JECFA evaluated a group of 25 flavouring substances consisting of alicyclic ketones, secondary alcohols and related esters. Two substances were not in the Register, and 10 are alpha,beta-unsaturated ketones or precursors for such which have been considered together with other alpha,beta-unsaturated substances. The remaining 13 flavouring substances have originally been considered by EFSA in the FGE.51 (EFSA, 2008aj).

FGE	Opinion adopted by EFSA	Link	No. of candidate substances
FGE.51	16 May 2007	http://www.efsa.europa.eu/en/efsajournal/doc/855.pdf	13
FGE.51Rev1	22 March 2012		20

The present revision of FGE.51, FGE.51Rev1, includes the consideration of seven additional substances [FL-no: 07.034, 07.035, 07.098, 07.126, 07.129, 07.172 and 09.930].

Six of the seven additional substances [FL-no: 07.034, 07.035, 07.098, 07.126, 07.129 and 07.172] are alpha,beta-unsaturated ketones originally allocated to FGE.211 (EFSA, 2011e) and FGE.212 (EFSA, 2009ai). The seventh substance [FL-no: 09.930] is a precursor for such ketones originally allocated to FGE.211. The seven substances have been considered with respect to genotoxicity and the Panel concluded in FGE.211 (EFSA, 2011e) and FGE.212Rev1 (EFSA, 2011f)) that the data available ruled out the concern for genotoxicity and accordingly the substances can be evaluated through the Procedure in this FGE.51Rev1. The information concerning genotoxicity of these seven substances is described in Section 3.3 and 3.4.

Since the publication of FGE.51, the EU production volume has been provided for the substance, [FL-no: 09.230] for which the evaluation could not be finalised in the previous version of this FGE, due to lack of these data. Based on the newly submitted EU production volume, the substance has already been evaluated in FGE.96[4] (EFSA, 2010al), but for the sake of completion, the information has also been included here as well.

[4] Consideration of 88 flavouring substances considered by EFSA for which EU production volumes / anticipated production volumes have been submitted on request by DG SANCO.

Finally, new information on the stereoisomeric composition has been provided for four substances [FL-no: 02.209, 07.045, 07.095 and 07.257] since the previous version of FGE.51 (EFFA, 2010a).

A search in open literature for the seven new substances did not provide any further data on toxicity or metabolism.

1. Presentation of the Substances in the JECFA Flavouring Group

1.1. Description

1.1.1. JECFA Status

The JECFA has evaluated at its 59th meeting a group of 25 flavouring substances consisting of alicyclic ketones, secondary alcohols and related esters (JECFA, 2002d; JECFA, 2003a).

1.1.2. EFSA Considerations

Two of the JECFA-evaluated substances are not in the Register (4-methyl cyclohexanone (JECFA no: 1104) and (E)-2-(2-octenyl) cyclopentanone (JECFA no: 1116)).

Seven of 10 alpha,beta-unsaturated ketones or precursors for such [FL-no: 07.034, 07.035, 07.098, 07.126, 07.129, 07.172 and 09.930] have been considered with respect to genotoxicity in FGE.211 (EFSA, 2011e) and FGE.212Rev1 (EFSA, 2011f), and the Panel concluded that the data available ruled out the concern for genotoxicity and accordingly the seven substances can be evaluated through the Procedure in this FGE.

For the remaining three substances [FL-no: 07.033, 07.094 and 07.112] considered with respect to genotoxicity in FGE.212Rev1, a final conclusion of genotoxic properties could not be reached and additional data were requested. Accordingly, these three substance will not be considered in this FGE.

This consideration will therefore deal with 20 JECFA-evaluated substances.

The Panel concluded that the 20 substances in the JECFA flavouring group of alicyclic ketones, secondary alcohols and related esters are structurally related to the group of secondary alicyclic saturated and unsaturated alcohols, ketones and esters with secondary alicyclic alcohol moieties evaluated by EFSA in Flavouring Group Evaluation 09, Revision 3 (FGE.09Rev3) (EFSA, 2011x).

1.2. Isomers

1.2.1. Status

Six of the substances have one chiral centre [FL-no: 07.045, 07.129, 07.172, 07.179, 07.180 and 07.257] and four substances have two or more chiral centres [FL-no: 02.209, 07.035, 07.095 and 09.930]. Two substances have possibility for cis/trans isomerism [FL-no: 07.034 and 07.257].

1.2.2. EFSA Considerations

Adequate information on isomeric composition is available for all substances.

1.3. Specifications

1.3.1. JECFA Status

The JECFA specifications are available for all the 20 substances (JECFA, 2002d). See Table 1.

1.3.2. EFSA Considerations

The available specifications are considered adequate for all the substances (See Section 1.2).

2. Intake Estimations

2.1. JECFA Status

For all the substances evaluated through the JECFA Procedure, intake data are available for the EU, see Table 3.1.

2.2. EFSA Considerations

Tonnage data are available for the EU allowing calculation of the intake estimates (MSDI). The Panel noted that since no use levels were submitted no mTAMDI values can be calculated.

3. Genotoxicity Data

3.1. Genotoxicity Studies – Text Taken[5] from the JECFA (JECFA, 2003a)

In vitro

Eight of the 13[6] alicyclic ketones, secondary alcohols and related esters have been tested for genotoxicity. Overall, negative results were reported in the standard assay for reverse mutation when various strains of *Salmonella typhimurium* (TA98, TA100, TA1535, TA1537 and TA1538) were incubated with up to 10000 microgram/plate of cyclohexanone [FL-no: 07.148]) or isophorone [FL-no: 07.126], 2.5 - 2500 µg/plate of cyclopentanone [FL-no: 07.149], up to 4200 µg/plate of 2,2,6-trimethylcyclohexanone [FL-no: 07.045] or up to 3600 µg/plate of 2-hexylidenecyclopentan-1-one [FL-no: 07.034] or tetramethyl ethylcyclohexanone [FL-no: 07.035], with or without metabolic activation (Florin et al., 1980; Haworth et al., 1983; Wild et al., 1983; Mortelmans et al., 1986). In another test for reverse mutation with *S. typhimurium* TA98, TA100, TA1535 and TA1537 (only an abstract), cyclohexanone was reported to produce 'a large number of revertants' in TA98, with no further elaboration and no results for the other strains. The concentrations and test conditions used were not specified (Massoud et al., 1980).

Both cyclohexyl acetate [FL-no: 09.027] and cyclohexyl butyrate [FL-no: 09.230] gave negative results for mutation in *Bacillus subtilis* M45 (*rec*$^-$) and H17 (*rec*$^+$) (Oda et al., 1979; Yoo, 1986). Positive results were reported with cyclohexanone in an assay for forward mutation assay in *B. subtilis* (Massoud et al., 1980); however, as previously stated, no concentrations or test conditions were reported in the abstract.

The results for forward mutation in mouse lymphoma cells were generally negative with isophorone, with or without metabolic activation (NTP, 1986d; McKee et al., 1987; O'Donoghue et al., 1988). An increased mutation frequency was reported in L5178Y Tk$^{+/-}$ mouse lymphoma cells without metabolic activation at concentrations of 400 and 800 µg/ml. Isophorone was lethal at 1600 µg/ml (MacGregor et al., 1988a).

Cyclohexanone [FL-no: 07.148] at concentrations up to 980 microgram/ml induced chromosomal aberrations in human lymphocytes with or without metabolic activation (Collin, 1971; Lederer et al., 1971; Dyshlovoi et al., 1981). It did not induce chromosomal aberrations in Chinese hamster ovary cells at a concentration of 7.5 µl/ml, with or without metabolic activation (Aaron et al., 1985). Isophorone [FL-no: 07.126] gave equivocal results in Chinese hamster ovary cells. In one study, no chromosomal aberrations were induced with or without metabolic activation at concentrations up to 1600 µg/ml (Gulati et al., 1989), whereas in another study isophorone at a concentration of 1200 µg/ml without metabolic activation or at a concentration of 1500 µg/ml with metabolic activation induced chromosomal aberrations (Matsuoka et al., 1996); however, lower concentrations of 250 - 1000 µg/ml tested without metabolic activation did not. In an assay for sister chromatid exchange,

[5] The text is taken verbatim from the indicated reference source, but text related to substances not included in FGE.51 has been removed.

[6] The genotoxicity data available for the seven new substances are summarised in Sections 3.3. and 3.4.

cyclohexanone at a concentration of 7.5 µl/ml gave weakly positive results in Chinese hamster ovary cells in the absence of metabolic activation and negative results in the presence of metabolic activation (Aaron et al., 1985). Similarly, isophorone induced sister chromatid exchange in Chinese hamster ovary cells only when tested without metabolic activation at concentrations of 500 - 1000 µg/ml and then only after delayed harvesting due to the cytostatic effect of isophorone (Gulati et al., 1989). At lower concentrations tested without metabolic activation or at concentrations up to 1600 µg/mL tested with metabolic activation, isophorone did not induce sister chromatid exchange (NTP, 1986d; Gulati et al., 1989). In an assay for unscheduled DNA synthesis in rat hepatocytes, isophorone showed no sign of genotoxicity at concentrations up to 200 µl/ml (McKee et al., 1987; O'Donoghue et al., 1988).

In vivo

When cyclohexanone [FL-no: 07.148], 2-hexylidenecyclopentan-1-one [FL-no: 07.034], tetramethyl ethylcyclohexanone [FL-no: 07.035] or isophorone [FL-no: 07.126] was fed to adult *Drosophila melanogaster* for 3 days, no mutations were observed (Goncharova, 1970; Wild et al., 1983; Foureman et al., 1994). In addition, negative results were obtained when *D. melanogaster* were injected with a single dose of 12 500 µg of isophorone (Foureman et al., 1994).

There was no increase in the frequency of micronucleated polychromatic erythrocytes in the bone marrow of male or female CD-1 mice given isophorone [FL-no: 07.126] at a dose of 540 µg/kg bw by intraperitoneal injection (McKee et al., 1987; O'Donoghue et al., 1988) or in NMRI mice injected intraperitoneally with 2-hexylidenecyclopentan-1-one at a dose of 170, 330 or 500 mg/kg bw or tetramethyl ethylcyclopentenone at a dose of 180, 310 or 450 mg/kg bw (Wild et al., 1983).

Conclusion on genotoxicity

Cyclohexyl acetate [FL-no: 09.027], cyclohexyl butyrate [FL-no: 09.230], cyclopentanone [FL-no: 07.149], 2,2,6-trimethyl cyclohexanone [FL-no: 07.045] and tetramethyl ethylcyclohexanone (mixed isomers) [FL-no: 07.035], gave negative results in assays for genotoxicity *in vitro*. The results reported for the genotoxicity of cyclohexanone [FL-no: 07.148] and isophorone [FL-no: 07.126] are conflicting. Most of the assays were conducted before 1986, when the pH and ionic strength of test media were often not adequately maintained. Mammalian cells *in situ* rely on complex regulatory mechanisms to maintain homeostatic conditions, and those in culture are not equipped to respond to environmental changes; therefore, it is important that the culture media used in mammalian cell assays be maintained at a pH of approximately 6.8 - 7.5. A lower pH or changes in osmolality due to the test agents can give rise to false-positive results, especially when metabolic activation systems are added. Acidity facilitates the breakdown of the components of such systems into mutagenic agents (Brusick, 1986).

The equivocal results of the assays for genotoxicity with cyclohexanone *in vitro* can be interpreted in terms of physiochemical properties. Compounds that are structurally similar to cyclohexanone have excellent membrane permeability and hydrogen bonding potential (Slater, 1963; Slater, 1967; Moreland, 1994). When cyclohexanone and related substances are tested *in vitro*, they may induce membrane expansion, leading to multiple effects on membrane-related processes. Membrane expansion may increase cell volume and lipid storage vacuoles, block ionic conductance channels, limit the availability of ATP and alter ion fluxes and metabolite distribution between the cytoplasm and organelles. Given these physiochemical properties, it is highly unlikely that any consistent pattern of genotoxicity would result from a battery of assays in bacterial and mammalian cells.

Overall, the tests for genotoxicity yielded mainly negative results. Positive results were reported in mammalian cells at cytotoxic concentrations, usually in the absence of biotransformation enzymes. The results of assays *in vivo* were negative.

For a summary of *in vitro* / *in vivo* genotoxicity data considered by the JECFA, see Table 2.1. Some of the studies, however, have only been summarised in Tables 2.5 - 2.6.

3.2. Genotoxicity Studies - Text Taken[7] from EFSA FGE.09Rev3 (EFSA, 2011x)

In vitro / in vivo

Genoxicity data are available for only three candidate substances cyclohexanol [FL-no: 02.070], cyclopentanol [FL-no: 02.135], methyl 3-oxo-2-pentyl-1-cyclopentylacetate [FL-no: 09.520] and for nine supporting substances and one structurally related substances.

Cyclohexanol [FL-no: 02.070] was not genotoxic in two Ames tests and in an *in vivo* micronucleus assay, which are all considered as valid studies. However, the results of the *in vivo* study are of limited relevance, due to the lack of evidence that the substance did reach the bone marrow. Inconclusive results were reported in an *in vitro* chromosomal aberration assay with human leukocytes and negative results were reported in a dominant lethal mutations assay with *Drosophila melanogaster;* both studies were considered inadequate. Cyclopentanol [FL-no: 02.135] was studied in a valid Ames test. No mutagenicity was found.

A battery of *in vitro* and *in vivo* genotoxicity studies were conducted on methyl 3-oxo-2-pentyl-1-cyclopentylacetate [FL-no: 09.520] including valid negative reverse mutation tests in *Escherichia coli* (Wagner and Klug, 2000) and *Salmonella typhimurium* (Thompson, 2000).

In a mouse lymphoma test, pre-dating GLP, a more than 2-fold increase of the mutant frequency over the solvent treated control values was found at the highest tested cytotoxic concentration of 300 µg/ml in the presence of metabolic activation, and at the two highest tested cytotoxic concentrations of 200 and 300 µg/ml, in the absence of metabolic activation. Only limited documentation is provided in the study report; together with the fact that several cultures were infected and a lack of a confirmatory test, it is impossible to assess the reliability of these results (Ross and Harris, 1979b).

No induction of forward mutations at the TK locus in L5178Y mouse lymphoma cells were found in a study performed in compliance with the current OECD test guidelines, both in the absence and in the presence of metabolic activation, up to and including cytotoxic concentrations (Cifone, 2001).

Methyl 3-oxo-2-pentyl-1-cyclopentylacetate was tested in a bone marrow micronucleus test in mice following a single intraperitoneal administration of 0, 280, 560 or 1120 mg/kg bw in corn oil. The study was performed in compliance with the current OECD test guidelines. The two highest doses chosen induced clear signs of toxicity; slight reductions (up to 12 %) in the ratio of polychromatic erythrocytes to total erythrocytes were found, indicating that the test material had reached the target cells. No increase in micronucleated cells was found in the groups treated with the test material. The positive control induced the expected increases (Gudi and Krsmanovic, 1998).

In an Unscheduled DNA Synthesis (UDS) study, the ability of methyl 3-oxo-2-pentyl-1-cyclopentylacetate to induce DNA repair was studied in isolated rat hepatocytes after administration *in vivo*. The study was performed in compliance with the current OECD Guideline 486 (OECD, 1997). Methyl 3-oxo-2-pentyl-1-cyclopentylacetate was administered to male Sprague-Dawley CD rats by intra-peritoneal injection in doses of 333.3 and 1000 mg/kg bw (the latter dose was the maximum tolerated dose) followed by liver perfusion at 2 or 16 hours after dosing. No marked increase in the incidence of UDS was observed at either dose level or perfusion time. Statistically significant differences were revealed in the positive control groups when compared to the negative control group and the test article (Durward, 2001).

Genotoxicity data are available for nine supporting substances [FL-no: 02.015, 02.062, 07.148, 07.176, 09.027, 09.215, 09.230, 07.149 and 07.045].

[7] The text is taken verbatim from the indicated reference source.

Cyclohexanone [FL-no: 07.148], structurally related to the alicyclic ketones and secondary alcohols in this FGE, was not mutagenic in an Ames test, considered to be valid. Negative and positive results were reported in several other *in vitro* studies at gene and chromosomal level, as well as a negative result in a sex-linked recessive lethal mutations in *D. melanogaster*. However, these studies were considered inadequate.

Menthol [FL-no: 02.015] gave negative results in an *in vitro* alkaline elution assay for detecting DNA single strand breaks in rat hepatocytes. With the same substance equivocal results in an *in vivo* host mediated mutation assay were observed at high dose levels and negative results in several Ames tests, a TK+/- mouse lymphoma assay, sister chromated exchange (SCE) tests in Chinese hamster ovary (CHO) cells and human lymphocytes, and chromosomal aberration assays with human embryonic lung cells, human lymphocytes and CHO cells. Negative results were also reported in two *in vivo* micronucleus and chromosomal aberration assays. However, the results of these studies have a limited relevance, due to the lack of bone marrow toxicity. In addition, an *in vivo* dominant lethal assay was available, from which also negative results were obtained. *trans*-Menthone [FL-no: 07.176] was genotoxic in an Ames test and in a somatic mutation and recombination test (SMART) with *Drosophila*. The observed effects were not very pronounced. Further, *trans*-menthone is easily converted to menthol, which is estimated to be overall negative in genotoxicity tests.

Carveol and carvyl acetate [FL-no: 02.062 and 09.215] were tested in Ames test at various doses from 10 - 560 µg/plate in the *Salmonella typhimurium* strains TA97, TA98, TA100, TA1535 and TA1537 with and without S9 mix in dimethyl sulphoxide. Positive and negative controls were used. No mutagenicity was observed (Mortelmans et al., 1986).

Conclusion on genotoxicity

Only for three of the candidate substances some genotoxicity data are available, and for these three mainly negative results were obtained. For the supporting substances mainly negative, but also some positive results were obtained. The positive results were obtained in poorly reported tests, or in tests, which are difficult to interpret with respect to their relevance for genotoxicity.

Overall, the genotoxic potential of this group of flavouring substances cannot be fully assessed as it is now. However, the data available do not indicate a genotoxic potential and therefore do not preclude their evaluation via the Procedure.

For a summary of *in vitro / in vivo* genotoxicity data considered by EFSA, see Table 2.2 and 2.3.

3.3. Genotoxicity Studies - Text Taken[8] from EFSA FGE.211 (EFSA, 2011e)

The following text is relevant for two substances [FL-no: 07.034 and 09.930] in this revision of FGE.51. These substances were evaluated based on structural similarity 1(7),8-p-menthadien-2-yl acetate [FL-no: 09.930].

The Industry has submitted data concerning genotoxicity studies for the one representative substance for subgroup 2.5 of FGE.19 (FGE.211), 1(7),8-p-menthadien-2-yl acetate [FL-no: 09.930] (structurally related to 1(7),8-p-menthadien-2-one).

In vitro data

The newly available data comprise a bacterial reverse mutation assay and an *in vitro* micronucleus assay with human peripheral blood lymphocytes. The genotoxicity assays have been performed on a commercial mixture of the representative substance 1(7),8-p-menthadien-2-yl acetate and a positional isomer, carvyl acetate. Carvyl acetate can be hydrolysed followed by oxidation to carvone, which has been evaluated by EFSA in FGE.212 (EFSA, 2009ai) and NTP (NTP, 1990b) as non-genotoxic. The

[8] The text is taken verbatim from the indicated reference source

highest concentration of d-carvone that could be tested without cytotoxicity was 333 µg/plate (Mortelmans et al., 1986), i.e. the cytotoxicity was in the same range as observed for the mixture of 1(7),8-p-menthadien-2-yl acetate/carvyl acetate. The Panel concluded that testing the commercial mixture of 1(7),8-p-menthadien-2-yl acetate/carvyl acetate for genotoxicity allows the evaluation of the genotoxic potential of 1(7),8-p-menthadien-2-yl acetate. The concentrations reported in Table 2.4 (FGE.51Rev1) are for the mixture of substances.

Bacterial Reverse Mutation Assay

1(7),8-p-menthadien-2-yl acetate/carvyl acetate was tested for mutagenic activity according to OECD guideline 471 and in compliance with GLP (Beevers, 2010a). The test material exhibited a marked toxicity as indicated by thinning of the background lawn, reduced revertant counts and complete killing of test bacteria. However, the Panel considered the remaining number of concentrations without signs of toxicity sufficient to draw a conclusion on mutagenicity in this system (for details, see Table 2.4 of this FGE.51Rev1).

Overall, the Panel concluded that there was no evidence of mutagenic activity of 1(7),8-p-menthadien-2-yl acetate/carvyl acetate at concentrations up to those causing bactericidal effects.

In vitro Micronucleus Test

1(7),8-p-menthadien-2-yl acetate/carvyl acetate was tested for induction of micronulei in human peripheral blood lymphocytes according to OECD guideline 487 and in compliance with GLP (Whitwell, 2010b). The Panel considered that acceptable levels of cytotoxicity as judged upon the replication index were achieved at the top concentrations (for details see Table 2.4 of this FGE.51Rev1).

Overall, the Panel concluded that no evidence of chromosomal damage or aneuploidy was observed by increased levels of micronucleated binucleate cells (MNBN) in the presence or absence of S9 metabolic activation.

A summary of the *in vitro* genotoxicity data is given in Table 2.4

Discussion of Mutagenicity/Genotoxicity Data

The commercial mixture of the representative substance 1(7),8-p-menthadien-2-yl acetate and a positional isomer, carvyl acetate, was tested for all three genetic endpoints: gene mutations, structural and numerical chromosomal aberrations. The test material did not induce gene mutations in bacteria and was not clastogenic and/or aneugenic in mammalian cells *in vitro*. Although this commercial mixture was cytotoxic at high concentrations, the remaining concentrations without signs of toxicity provide a valid data set.

Conclusion

The *in vitro* genotoxicity data on the commercial mixture of the representative substance 1(7),8-p-menthadien-2-yl acetate [FL-no: 09.930] and a positional isomer, carvyl acetate, do not indicate genotoxic potential. Accordingly the four substances in FGE.211 (subgroup 2.5 of FGE.19) would be of no safety concern with respect to genotoxicity.

3.4. Genotoxicity Studies - Text Taken[9] from EFSA FGE.212 (EFSA, 2009ai) and FGE.212Rev1 (EFSA, 2011f)

The following text is relevant for five substances [FL-no: 07.035, 07.098, 07.126, 07.129 and 07.172] in this revision of FGE.51. These substances were evaluated based on structural similarity with isophorone [FL-no: 07.126].

For tetramethyl ethylcyclohexenone (mixture of isomers) [FL-no: 07.035] one *in vitro* and one *in vivo* study are available and have been evaluated. Seven *in vitro* and three *in vivo* studies are available for 3,5,5 trimethylcyclohex-2-en-1-one [FL-no: 07.126] (isophorone).

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

Negative results were also observed with tetramethyl ethylcyclohexenone [FL-no: 07.035] in bacteria, in a sex-linked recessive lethal mutation assay in *Drosophila* (Wild et al., 1983) and in a mouse micronucleus assay (Wild et al., 1983); however, there was a mixture of isomers tested and the studies were only of limited validity.

Conclusion on Genotoxicity from FGE.212

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

Due to structural similarities and lack of data, the remaining substances cannot presently be evaluated through the Procedure [FL-no: 07.035, 07.098, 07.129 and 07.172]. Additional data on genotoxicity are requested for representative substances of this subgroup according to the opinion of the Panel on the Genotoxicity Test Strategy for Substances Belonging to Subgroups of FGE.19 (EFSA, 2008bb)

Data submitted from Industry in reply to request for additional genotoxicity data in FGE.212

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

[9] The text is taken verbatim from the indicated reference source, but text related to substances not included in FGE.51 has been removed.

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

A DNA binding study was conducted in which F344-rats and B6C3F1-mice (the strains used in the NTP carcinogenicity study) were exposed to isophorone (Thier et al., 1990). Animals of both sexes were dosed once or five times by gavage with 500 mg/kg bw of unlabelled isophorone spiked with [1,3,5-^{14}C]-isophorone (specific activity: 52 mCi per mmol, 1.92 GBq per mmol). An additional group of acute dosed male rats received undiluted ^{14}C-isophorone for increased sensitivity. Rats and mice were maintained for 24 hours in closed metabolic cages. Twenty four hours after exposure, livers and kidneys (the tumour target tissues) were removed from the animals. DNA was isolated through hydroxyapatite chromatography and radioactivity was measured by liquid scintillation counting. No positive controls were included. Also no untreated controls were included, but, except for the liver sample of one mouse in the five times dose group, radioactivity values were within 2σ of background (6 dpm). Radioactivity values therefore did not indicate significant attachment of radioactivity to DNA. From these results it can be concluded that neither isophorone nor its metabolites bind covalently to DNA.

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

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

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

Extraction of preputial gland and kidney DNA from rats treated with single 500 mg/kg labeled doses yielded no evidence of isophorone binding to DNA, whereas the positive control showed significant binding to DNA of preputial gland and kidney. These negative results with isophorone were confirmed in the ^{32}P -postlabeling assays.

Discussion of the additional data

Conflicting results were reported in two valid studies with the mouse lymphoma assay (MLA): one negative (O'Donoghue et al., 1988) and one positive (NTP, 1986d) at comparable concentrations. Mixed results were also reported in two studies of limited validity: one negative (Honma et al., 1999a) and one positive (Honma et al., 1999b). Another negative result was reported in a study (McKee et al., 1987), the validity of which cannot be evaluated. In the light of the clearly negative results in two valid bacterial gene mutation tests (Ames test) and in a valid Sex Linked Recessive Lethal Mutations test (SLRL) in *Drosophila*, and taking into account the lack of specificity and high sensitivity of the MLA, overall the results presently available are considered of questionable relevance. The Panel

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

agrees that isophorone demonstrates some genotoxic activity *in vitro* but that the new data demonstrate lack of clastogenicity *in vivo*. In addition, the new DNA-binding data from two separate studies provide convincing evidence that isophorone does not induce tumours via a genotoxic mechanism. On the basis of these data it may be argued that there is no need to perform further *in vivo* genotoxicity studies such as the Comet assay or bone marrow micronucleus test. Thus, based on the data available the Panel concluded in FGE.212Rev1 that there is no concern with respect to genotoxicity of isophorone.

A summary of the *in vitro* and *in vivo* genotoxicity data from FGE.212Rev1 is given in Tables 2.5 and 2.6.

3.5. EFSA Considerations

Data not available for the JECFA at the time of evaluation (59th meeting) for cyclohexanone [FL-no: 07.148] have been considered by EFSA. Results from *in vitro* genotoxicity studies with cyclohexanone, carried out by NTP, have been published on the NTP website (NTP, 2007). From the technical information also provided there, it can be concluded that the tests by NTP are reliable. A set of Ames tests with *Salmonella* strains TA98, TA100, TA1535 and TA1537) and a study with mouse lymphoma cells (L5178Y; tk^{+}/-), including cloning efficiency and colony sizing provided convincingly negative results. The tests were carried out with and without metabolic activation at cyclohexanone levels up to 10000 microg/plate in the Ames tests and up to 5000 microg/ml in the mouse lymphoma assay. For a summary of these studies see Table 2.7.

The Panel noted that cyclohexanone has also been studied in long term carcinogenicity studies in mice (up to 6.2 g/kg bw/day) and rats (up to 0.65 g/kg bw/day) (Lijinsky and Kovatch, 1986). The substance was tested up to the maximum tolerated dose levels and the overall conclusion from these studies was that cyclohexanone is not carcinogenic. In an evaluation of these studies the IARC concluded that the substance was not classifiable as to its carcinogenicity to humans (IARC, 1989).

For seven candidate substances [FL-no: 07.034, 07.035, 07.098, 07.126, 07.129, 07.172 and 09.930] it has been concluded in FGE.211 and FGE.212Rev1, that a concern for genotoxicity, indicated by the presence of a structural alert, could be ruled out based on experimental data for representative substances.

Based on these results the Panel concluded that the data available do not preclude evaluation of the 20 JECFA evaluated alicyclic ketones, secondary alcohols and related esters through the Procedure.

4. Application of the Procedure

4.1. Application of the Procedure to 20 Alicyclic Ketones, Secondary Alcohols or Related Esters Evaluated by the JECFA (JECFA, 2003a):

According to the JECFA six of the substances belong to structural class I and 14 to structural class II using the decision tree approach presented by Cramer *et al.* (Cramer et al., 1978).

The JECFA concluded all 20 alicyclic ketones, secondary alcohols or related esters 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 thresholds for structural classes I and II (step A3).

In conclusion, the JECFA evaluated all 20 substances as to be of no safety concern at the estimated levels of intake as flavouring substances based on the MSDI approach.

The evaluations of the 20 substances are summarised in Table 3.1: Summary of Safety Evaluation of Alicyclic Ketones, Secondary Alcohols or Related Esters (JECFA, 2003a).

4.2. Application of the Procedure to 17 Secondary Alicyclic Saturated and Unsaturated Alcohols, Ketones and Esters Containing Secondary Alicyclic Alcohols by EFSA in FGE.09Rev3 (EFSA, 2011x):

Seventeen flavouring substances were evaluated in FGE.09Rev3. Thirteen substances are classified into structural class I, three into structural class II and one into structural class III using the decision tree approach presented by Cramer *et al*. (Cramer et al., 1978).

Sixteen substances were concluded at step A3 using the EFSA Procedure – i.e. the substances are expected to be metabolised to innocuous products (step 2) and the estimated daily intakes for 15 substances are below the thresholds of concern for their structural classes (step A3).

For one substance methyl 3-oxo-2-pentyl-1-cyclopentylacetate [FL-no: 09.520] the estimated daily intake exceeds the threshold of concern for structural class II and since the substance is not endogenous the substance proceeds to step A5.

A 90 day study in rats has been performed for [FL-no: 09.520] from which a No Observed Adverse Effect Level (NOAEL) of 100 mg/kg body weight (bw)/day could be derived. This NOAEL provides a margin of safety of nearly 10^4 compared to the daily intake of 0.013 mg/kg bw/day for methyl 3-oxo-2-pentyl-1-cyclopentylacetate. Therefore, [FL-no: 09.520] does not pose a safety concern when used at estimated levels of intake, based on the MSDI approach, as a flavouring substance

One flavouring substance [FL-no: 07.207] was not expected to be metabolised to innocuous products and was therefore evaluated via the B-side in the EFSA Procedure. The estimated intake is below the threshold, but no adequate No Observed Adverse Effect Level (NOAEL) could be provided for the substance or a structurally related substance – therefore additional data are required for this substance.

In conclusion, the Panel considered that 16 of the substances evaluated through the Procedure were of no safety concern at the estimated levels of intakes based on the MSDI approach. For one substance additional data were required.

The stepwise evaluations of the 17 substances are summarised in Table 3.2: Summary of Safety Evaluation Applying the Procedure (EFSA, 2011x).

4.3. EFSA Considerations

The Panel agrees with the application of the Procedure as performed by the JECFA for the 20 substances in the group of alicyclic ketones, secondary alcohols and related esters.

5. Conclusions

The JECFA has evaluated a group of 25 flavouring substances consisting of alicyclic ketones, secondary alcohols and related esters at its 59th meeting. Two of the JECFA-evaluated substances are not in the Register (4-methyl cyclohexanone (JECFA no: 1104) and (E)-2-(2-octenyl) cyclopentanone (JECFA no: 1116)). Ten of the remaining 23 JECFA-evaluated substances are alpha,beta-unsaturated ketones or precursors for such, which structural property has been recognised as a structural alert for genotoxicity. Seven of these 10 candidate substances [FL-no: 07.034, 07.035, 07.098, 07.126, 07.129, 07.172 and 09.930] have been considered with respect to genotoxicity in FGE.211 (EFSA, 2011e) or FGE.212Rev1 (EFSA, 2011f), and the Panel concluded that the data available ruled out the concern for genotoxicity and accordingly these seven substances can be evaluated through the Procedure. For the remaining three substances [FL-no: 07.033, 07.094 and 07.112] considered with respect to genotoxicity in FGE.212Rev1 a final conclusion of genotoxic properties could not be reached and additional data were requested. These three substances will therefore not be considered in this revision of FGE. 51. This consideration therefore deals with 20 JECFA-evaluated substances.

The Panel concluded that the 20 substances in the JECFA group of alicyclic ketones, secondary alcohols and related esters are structurally related to the group of 17 secondary alicyclic saturated and unsaturated alcohols, ketones and esters containing secondary alicyclic alcohols evaluated in the Flavouring Group Evaluation 09, Revision 3 (FGE.09Rev3).

The Panel agrees with the application of the Procedure as performed by the JECFA for the 20 substances considered in this FGE.

For all 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 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 tests are available for all 20 substances.

For all 20 JECFA-evaluated alicyclic ketones, secondary alcohols and related esters [FL-no: 02.209 07.034, 07.035, 07.045, 07.095, 07.098, 07.126, 07.129, 07.148, 07.149, 07.172, 07.179, 07.180, 07.257, 09.027, 09.140, 09.160, 09.230, 09.464 and 09.930] the Panel agrees with the JECFA conclusion, "No safety concern at estimated levels of intake as flavouring substance" based on the MSDI approach.

TABLE 1: SPECIFICATION SUMMARY

Table 1: Specification Summary of the Substances in the JECFA Flavouring Group of Alicyclic Ketones, Secondary Alcohols and Related Esters (JECFA, 2002d)

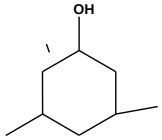
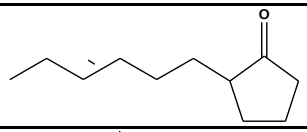
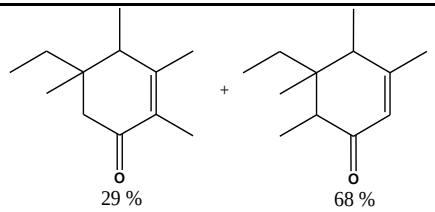
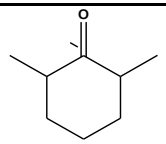
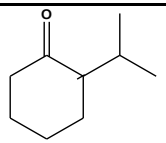
FL-no JECFA-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	EFSA comments
02.209 1099	3,3,5-Trimethylcyclohexan-1-ol		3962 116-02-9	Solid $C_9H_{18}O$ 142.24	Insoluble Soluble	193-196 30-34 IR MS 98 %	n.a. n.a.	Racemate (EFFA, 2010a).
07.034 1106	2-Hexylidenecyclopentan-1-one		2573 167 17373-89-6	Liquid $C_{11}H_{18}O$ 166.26	Insoluble Miscible	240 NMR 98 %	1.477-1.484 0.907-0.914	Mixture E/Z (50/50) (EFFA, 2012b).
07.035 1111	Tetramethyl ethylcyclohexenone (mixture of isomers)		3061 168 17369-60-7	Liquid $C_{12}H_{20}O$ 180.29	Slightly soluble Miscible	113-115 NMR 97 %	1.485-1.490 0.927-0.934	Mixture of of 5-ethyl-2,3,4,5-tetramethyl-2-cyclohexen-1-one and 5-ethyl-3,4,5,6-tetramethyl-2-cyclohexen-1-one. The predominant constituent is 5-ethyl-3,4,5,6-tetramethyl-2-cyclohexen-1-one. Mixture of diastereoisomers in approximately equal ratios (EFFA, 2012b).
07.045 1108	2,2,6-Trimethylcyclohexanone		3473 686 2408-37-9	Liquid $C_9H_{16}O$ 140.23	Insoluble Miscible	178-179 NMR 99 %	1.443-1.449 0.900-0.907	Racemate (EFFA, 2010a).
07.095 1109	2-(sec-Butyl)cyclohexanone		3261 11044 14765-30-1	Liquid $C_{10}H_{18}O$ 154.25	Insoluble Miscible	76-78 NMR 94 %	1.454-1.461 0.911-0.917	Mixture of diastereoisomers, approx. 25 % of each (EFFA, 2012b). Min assay 94 % secondary comp. 2-isobutyl cyclohexanone 2-2.5 % (EFFA, 2010a).

Table 1: Specification Summary of the Substances in the JECFA Flavouring Group of Alicyclic Ketones, Secondary Alcohols and Related Esters (JECFA, 2002d)

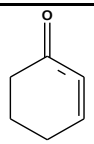
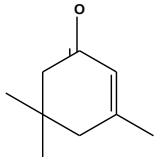
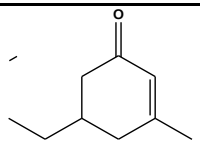
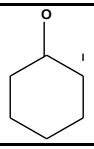
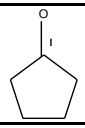
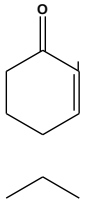
FL-no JECFA-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	EFSA comments
07.098 1107	3-Methylcyclohex-2-en-1-one		3360 11134 1193-18-6	Liquid $C_7H_{10}O$ 110.16	Miscible Miscible	199-200 NMR 98 %	1.490-1.498 0.967-0.972	
07.126 1112	3,5,5-Trimethylcyclohex-2-en-1-one		3553 11918 78-59-1	Liquid $C_9H_{14}O$ 138.21	Slightly soluble Miscible	213-215 NMR 97 %	1.474-1.481 0.919-0.927	
07.129 1113	3-Methyl-5-propylcyclohex-2-en-1-one		3577 3720-16-9	Liquid $C_{10}H_{16}O$ 152.23	Insoluble Miscible	242-244 NMR 95 %	1.481-1.486 0.924-0.928	Racemate (EFFA, 2012b).
07.148 1100	Cyclohexanone		3909 11047 108-94-1	Liquid $C_6H_{10}O$ 98.14	Miscible	154-156 IR NMR MS 99 %	1.447-1.453 0.947-0.950	
07.149 1101	Cyclopentanone		3910 11050 120-92-3	Liquid C_5H_8O 84.12	Miscible	130-131 IR NMR MS 99 %	1.432-1.438 0.950-0.960	
07.172 1110	4-Isopropylcyclohex-2-en-1-one		3939 11127 500-02-7	Liquid $C_9H_{14}O$ 138.21	Insoluble Miscible	198 NMR 97 %	1.481-1.490 0.930-0.950	Racemate (EFFA, 2012b).

Table 1: Specification Summary of the Substances in the JECFA Flavouring Group of Alicyclic Ketones, Secondary Alcohols and Related Esters (JECFA, 2002d)

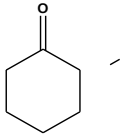
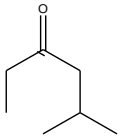
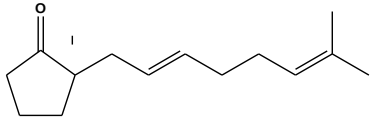
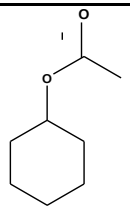
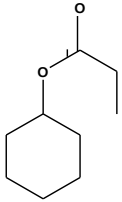
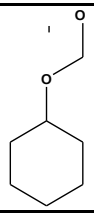
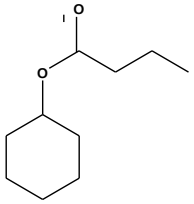
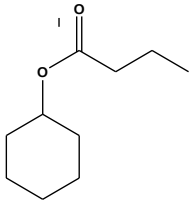
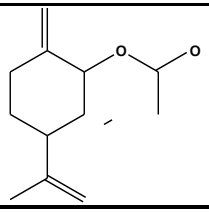
FL-no JECFA-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	EFSA comments
07.179 1102	2-Methylcyclohexanone		3946 583-60-8	Liquid $C_7H_{12}O$ 112.17	Insoluble Miscible	163-163 IR NMR MS 96 %	1.444-1.450 0.924-0.926	Racemate.
07.180 1103	3-Methylcyclohexanone		3947 591-24-2	Liquid $C_7H_{12}O$ 112.17	Insoluble Miscible	169-170 IR NMR MS 97 %	1.440-1.450 0.914-0.919	Racemate.
07.257 1117	2-(3,7-Dimethyl-2,6-octadienyl) cyclopentanone		3829 68133-79-9	Liquid $C_{15}H_{24}O$ 220.35	Insoluble Miscible	130 (4 hPa) NMR MS 95 %	1.482-1.489 0.911-0.916	Racemic mixture of (E)- and (Z)-isomers (EFFA, 2010a). The double bond occurs mainly as E-isomer (at least 80 % E and max. 20 % Z) (EFFA, 2012b).
09.027 1093	Cyclohexyl acetate		2349 217 622-45-7	Liquid $C_8H_{14}O_2$ 142.19	Insoluble Miscible	175-177 NMR 98 %	1.436-1.443 0.971-0.978	
09.140 1097	Cyclohexyl propionate		2354 421 6222-35-1	Liquid $C_9H_{16}O_2$ 156.23	Insoluble Miscible	193 NMR 97 %	1.439-1.446 0.969-0.974	

Table 1: Specification Summary of the Substances in the JECFA Flavouring Group of Alicyclic Ketones, Secondary Alcohols and Related Esters (JECFA, 2002d)

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
09.160 1095	Cyclohexyl formate		2353 498 4351-54-6	Liquid $C_7H_{12}O_2$ 128.17	Insoluble Miscible	162-163 NMR 97 %	1.439-1.445 1.052-1.060	
09.230 1094	Cyclohexyl butyrate		2351 2082 1551-44-6	Liquid $C_{10}H_{18}O_2$ 170.25	Practically insoluble Miscible	212 NMR 98 %	1.439-1.451 0.953-0.959	
09.464 1096	Cyclohexyl isovalerate		2355 459 7774-44-9	Liquid $C_{11}H_{20}O_2$ 184.28	Insoluble Miscible	58-62 NMR 95 %	1.439-1.445 0.945-0.952	
09.930 1098	1(7),8-p-Menthadien-2-yl acetate (mixture of (E) and (Z) isomers)		3848 71660-03-2	Liquid $C_{12}H_{18}O_2$ 194.27	Insoluble Miscible	77-79 (0.1 hPa) IR NMR MS 95 %	1.473-1.479 0.964-0970	Mixtures of diastereoisomers (25 % of each) (EFFA, 2012b). Registername to be changed to Cyclohexyl, 2-methylene-5-(1-methylethenyl) acetate.

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.

TABLE 2: GENOTOXICITY DATA

Table 2.1: Summary of Genotoxicity Data for Alicyclic Ketones, Secondary Alcohols and Related Esters (JECFA, 2003a)

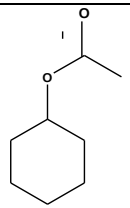
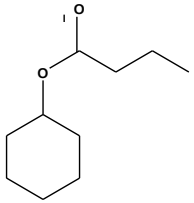
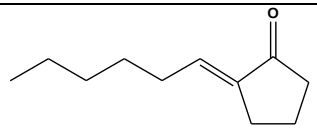
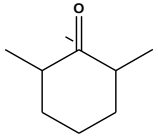
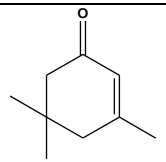
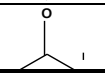
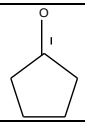
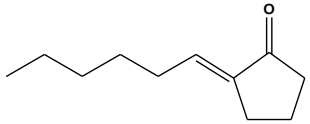
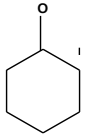
FL-no JECFA-no	EU Register name JECFA name	Structural formula	End-point	Test system	Maximum concentration	Results	Reference
In vitro							
09.027 1093	Cyclohexyl acetate		DNA damage	*B. subtilis* H17(*rec*$^{+}$), M45 (*rec*$^{-}$)	19 µg[d]/disc	Negative[a]	(Yoo, 1986)
09.230 1094	Cyclohexyl butyrate		DNA damage	*B. subtilis* H17(*rec*$^{+}$), M45 (*rec*$^{-}$)	19 µg[d]/plate	Negative[a]	(Oda et al., 1979)
07.034 1106	2-hexylidenecyclopentan-1-one		Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538	5 concentrations, up to cytotoxicity or max 36000 µg/plate.	Negative[a]	Wild et al., 1983.
07.045 1108	2,2,6-Trimethylcyclohexanone		Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537	4.2 - 3600 µg[d]/plate	Negative[a]	(Florin et al., 1980)
07.126	3,5,5-Trimethylcyclohex-2-en-1-one		Foreward mutation test	Mouse lymphoma L5178Y Tk$^{+/-}$ cells	0 – 1600 µg/ml	Positive[b]	MacGregor et al., 1988a
07.148 1100	Cyclohexanone		Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537	33 - 10 000 µg[d]/plate	Negative[a]	(Haworth et al., 1983)

Table 2.1: Summary of Genotoxicity Data for Alicyclic Ketones, Secondary Alcohols and Related Esters (JECFA, 2003a)

FL-no JECFA-no	EU Register name JECFA name	Structural formula	End-point	Test system	Maximum concentration	Results	Reference
			Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537	2.9 - 2900 µg[d]/plate	Negative[a]	(Florin et al., 1980)
			Chromosomal	Chinese hamster ovary cells aberration	7.5 µl/ml	Negative[a]	(Aaron et al., 1985)
			Chromosomal	Human lymphocytes aberration	9.8 - 980 µg[d]/ml	Positive[a]	(Lederer et al., 1971)
			Chromosomal	Human lymphocytes aberration	0.005 - 0.1 µg[d]/ml	Positive[a]	(Dyshlovoi et al., 1981)
			Sister chromatid exchange	Chinese hamster ovary cells	7.5 µl/ml	Negative[b] Positive[c]	(Aaron et al., 1985)
07.149 1101	Cyclopentanone		Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537	2.5 - 2500 µg[d]/plate	Negative[a]	(Florin et al., 1980)
In vivo							
07.034 1106	2-hexylidenecyclopentan-1-one		Sex-linked recessive lethal mutation	*D. melanogaster*	10 mM	Negative	Wild et al., 1983
			Micronucleus assay	NMRI mice (4/group)	0, 166, 333, 500 mg/kg bw; single dose, 30 hrs expression time	Negative	Wild et al., 1983
07.148 1100	Cyclohexanone		Sex-linked recessive lethal mutation	*D. melanogaster*	0.1 ml/100 ml	Negative	(Goncharova, 1970)

[a] With and without metabolic activation.

[b] Without metabolic activation.

[c] With metabolic activation.

[d] In the original JECFA report the figures for Maximum concentration were written as "mg". This is a mistake by the JECFA as the concentrationin the original references is reported in "µg". Therefore "mg" has been replaced by "µg".

Table 2.2: GENOTOXICITY (*in vitro*) EFSA / FGE.09Rev3 (EFSA, 2011x) (substances in brackets are JECFA-evaluated substances)

Chemical Name [FL-no]	Test system	Test Object	Concentration	Result	Reference	Comments
(Menthol [02.015])	Ames test	*S. typhimurium* TA92, TA94, TA98, TA100, TA1535, TA1537	0, and 6 concentrations up to 5000 µg/plate	Negative[1]	(Ishidate et al., 1984)	d,l-Menthol was used. The study is considered valid.
	Ames test (preincubation method)	*S. typhimurium* TA97, TA98, TA100, TA1535	3 - 666 µg/plate	Negative[1]	(Zeiger et al., 1988)	d,l-Menthol was used. The study is considered valid.
	Ames test	*S. typhimurium* TA98, TA100, TA2637	0, 5 - 500 µg/plate	Negative[1]	(Nohmi et al., 1985)	d,l-Menthol was tested. The highest concentrations were cytotoxic. The study is considered valid.
	Ames test	*S. typhimurium* TA98, TA100, TA2637	0, 20 - 500 µg/plate	Negative[1]	(Nohmi et al., 1985)	l-Menthol was tested. The highest concentrations were cytotoxic. The study is considered valid.
	Ames test	*S. typhimurium* TA98, TA100, TA1535, TA1537	0, 6.4, 32, 160, and 800 µg/plate	Negative[1]	(Andersen and Jensen, 1984b)	No indication of which enantiomer was used. In the absence of metabolic activation, the highest concentration was cytotoxic. The study is considered valid.
	Ames test	*E. coli WP2 uvrA* (Trp^-)	100 - 800 µg/plate	Negative	(Yoo, 1986)	l-Menthol was used. The article is not in English. The validity of the study cannot be evaluated. It is unclear whether metabolic activation or a control group was used.
	Ames test	*S. typhimurium* TA97A, TA98, TA100, TA102	0, 5 - 800 µg/plate	Negative[1]	(Gomes-Carneiro et al., 1998)	(-)-Menthol was used. The range of concentrations tested varied between the different strains. Cytotoxicity was observed with the highest concentrations tested with TA97A and, in the presence of metabolic activation, the highest concentration tested with TA102. The study is considered valid.
	Rec assay	*B. subtilis* H17, M45	Up to 10000 µg/disk	Positive	(Yoo, 1986)	l-Menthol was used. Inhibition zone for rec- and rec+ was 42 and 23 mm, respectively. The article is not in English. It is not clear from the study whether metabolic activation, or a control group was used. The validity of this study cannot be assessed. The method (*rec*-assay) has poor predictive value.
	Rec assay	*B. subtilis* H17, M45	20 µg/disk	Negative	(Oda et al., 1979)	l-Menthol was used. The article is not in English. Only one concentration level is mentioned at a table. No data on metabolic activation or control group. The validity of this study cannot be evaluated. The method (*rec*-assay) has poor predictive value.
	Alkaline elution assay	Rat hepatocytes	0, 0.1 - 1.3 mM (203.2 µg/ml[4])	Negative	(Storer et al., 1996)	The experiment employed *d*-Menthol. An increase in DNA breaks was only observed at concentrations associated with cytotoxicity. The authors concluded that this was a false-positive result. The study is considered valid.
	Sister chromatid exchange	Chinese hamster ovary cells	5 - 50 amd 0, 2 - 25 µg/ml[3] 0, 16 - 167 µg/ml [2]	Negative[1]	(Ivett et al., 1989)	d,l-Mentol was used. The compound was tested up to toxic or nearly toxic concentration levels. The study is considered valid.
	Sister chromatid exchange	Human lymphocytes	0, 0.1, 1, 10 mM (1563 µg/ml[4])	Negative[1]	(Murthy et al., 1991)	The study is considered valid.
	Cytogenetic assay	Human embryonic lung cells	0, 0.1, 1, 10 µg/ml	Negative	(Food and Drug Research Laboratories, Inc., 1975a)	The report does not mention exogenous metabolic activation. The study is considered valid.
	Chromosome aberration	Chinese hamster fibroblasts	0 and three concentrations up to 200 µg/ml	Negative[3]	(Ishidate et al., 1984)	The maximum concentration (cytotoxic) was selected by a preliminary test. The study is considered valid.
	Chromosome aberration	Chinese hamster ovary cells	0, 50 - 250 µg/ml	Negative[1]	(Ivett et al., 1989)	d,l-Mentol was used. The compound was tested up to

Table 2.2: GENOTOXICITY (*in vitro*) EFSA / FGE.09Rev3 (EFSA, 2011x) (substances in brackets are JECFA-evaluated substances)

Chemical Name [FL-no]	Test system	Test Object	Concentration	Result	Reference	Comments
						toxic or nearly toxic concentration levels. The study is considered valid.
	Chromosome aberration	Human lymphocytes	0, 0.1, 1, 10 mM (1563 µg/ml[4])	Negative[1]	(Murthy et al., 1991)	The study is considered valid.
	Gene mutation assay	Mouse lymphoma L5178Y TK+/-cells	0, 12.5 - 200 µg/ml	Negative[1]	(Myhr and Caspary, 1991)	d,l-Menthol was used. The maximum concentration was selected by a preliminary test. The study is considered valid.
(trans-Menthone [07.176])	Ames test	*S. typhimurium* TA97, TA98, TA100, TA1535, TA1537	0, 6.4 - 800 µg/plate	Positive[1]	(Andersen and Jensen, 1984b)	Concentrations were selected based on preliminary experiments. In absence of metabolic activation, menthone was mutagenic only to strain TA1537 at 6.4 and 32 µg/ml (slightly less than 2-fold increase in mutation frequency), but not at higher (toxic) concentrations. Also in absence of metabolic activation, there was a concentration dependent increase in number of TA97 strain revertants (up to 4-fold increase at 600 µg/l). It was stated that metabolic activation did not enhance the mutagenicity of menthone. The study is considered valid.
Cyclopentanol [02.135]	Modified Ames test	*S. typhimurium* G46, TA98, TA100, TA1535, C3076, TA1537, D3052, TA1538 *E. coli* WP2, WP2 *uvrA*$^-$	0, 0.1 - 1000 µg/ml	Negative[1]	(McMahon et al., 1979)	The study was performed with agar plates containing the following concentration gradients: 0.1 - 1, 1 - 10, 10 - 100, and 100 - 1000 µg/ml. The study is considered valid, although tabulated data on cyclopentanol were not presented.
(Cyclohexanone [07.148])	Ames test	*S. typhimurium* TA98, TA100, TA1535, TA1537	0, 33 - 10000 µg/plate	Negative[1]	(Haworth et al., 1983)	The highest level tested was the highest of either 10000 µg/plate, limit of solubility or maximal non-toxic concentration. The test was run twice. Both rat and hamster liver S9 were used. The test is considered valid.
	Ames test	*S. typhimurium* TA98, TA100, TA1535, TA1537	0, 3 µmol/plate	Negative[1]	(Florin et al., 1980)	A preliminary assay was performed with the four strains using only one concentration level (3 µmol/plate). This assay gave uncertain results. In addition, strains TA98 and TA100 were exposed to 0.03 - 30 µmol/plate. The validity of the study cannot be evaluated.
	Ames test	*S. typhimurium* TA98, TA100, TA1535, TA1537	NR	Positive	(Massoud et al., 1980)	Only an abstract is available. No reporting with respect to metabolic activation. The substance was also tested with *Bacillus subtilis*. With this specie, toxicity was found as well as a positive response. The validity of the study cannot be evaluated because of lack of experimental information.
	Cytogenetic assay	Human leukocytes	0.1 - 10 mM	Inconclusive[3]	(Collin, 1971)	The study report contains little experimental detail. Gaps, but no increase in breaks, were observed without any dose response relationship. There was no information with respect to cytotoxicity or presence of a control group. Only a statement on observations from 12 cells per concentration was given, but the total number of cells studied was not specified. The study is inadequate.

Table 2.2: GENOTOXICITY (*in vitro*) EFSA / FGE.09Rev3 (EFSA, 2011x) (substances in brackets are JECFA-evaluated substances)

Chemical Name [FL-no]	Test system	Test Object	Concentration	Result	Reference	Comments
	Chromosomal aberration	Human lymphocytes	0, 0.005 - 0.1 µg/ml	Positive	(Dyshlovoi et al., 1981)	Article is not in English. Only an abstract available in English. The validity of the study cannot be evaluated.
	Gene mutation (HPRT)	Chinese hamster ovary cells	0, 7.5 µg/ml	Negative[1]	(Aaron et al., 1985)	Only an abstract is available with limited experimental information. The validity of the study cannot be evaluated.
	Chromosomal aberration	Chinese hamster ovary cells	0, 7.5 µg/ml	Negative[1]	(Aaron et al., 1985)	Only an abstract is available with limited experimental information. The validity of the study cannot be evaluated.
	Sister chromatic exchange	Chinese hamster ovary cells	0, 7.5 µg/ml	Positive[3] Negative[2]	(Aaron et al., 1985)	Only an abstract is available with limited experimental information. The validity of the study cannot be evaluated.
Cyclohexanol [02.070]	Ames test	*S. typhimurium* TA98, TA1535, TA1537, TA1538	500 - 10000 µg/plate[3] 500 - 15000 µg/plate[2]	Negative[1]	(Barsky, 1976)	The highest concentrations showed cytotoxicity. The study is considered valid.
	Ames test	*S. typhimurium* TA98, TA100, TA1535, TA1537	0, 10 - 3333 µg/plate	Negative[1]	(Haworth et al., 1983)	The highest level tested was the highest of either 10000 µg/plate, limit of solubility or maximal non-toxic concentration. Both rat and hamster liver S9 were used. The test was run twice. The study is considered valid.
	Chromosomal aberration	Human leukocytes	0.1 - 10 mM	Inconclusive[3]	(Collin, 1971)	The study report contains little experimental detail. Gaps, but no increase in breaks, were observed without any dose response relationship. There was no information with respect to cytotoxicity or presence of a control group. Only a statement on observations from 12 cells per concentration was given, but the total number of cells studied was not specified. The study is inadequate.
(Cyclohexyl acetate [09.027])	DNA damage	*B. subtilis* H17(*rec*$^+$), M45 (*rec*$^-$)	19 mg/disc	Negative[1]	(Yoo, 1986)	
(Cyclohexyl butyrate [09.230])	DNA damage	*B. subtilis* H17(*rec*$^+$), M45 (*rec*$^-$)	19 mg/plate	Negative[1]	(Oda et al., 1979)	
(Cycopentanone [07.149])	Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537	2.5 - 2500 mg/plate	Negative[1]	(Florin et al., 1980)	
(2,2,6-Trimethyl cyclo-hexanone [07.045])	Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537	4.2 - 3600 mg/plate	Negative[1]	(Florin et al., 1980)	
Methyl 3-oxo-2-pentyl-1-cyclopentylacetate [09.520]	Reverse mutation	*S. typhimurium* TA98, TA100, TA102, TA1535,TA1537	5 mg/plate	Negative[1]	(Thompson, 2000)	Valid study in compliance with the OECD Guideline -471.
	Reverse mutation	*E. coli* WP2 *uvrA*	5 mg/plate	Negative[1]	(Wagner and Klug, 2000)	Valid study in compliance with the OECD Guideline -471.
	Forward mutation Test	Mouse lymphoma cells *L5178y*	200 & 300µg/L 300 µg/L	Positive[3] Positive[3]	(Ross and Harris, 1979b)	Pre-GLP study - not possible to assess the reliability of these studies.
	Forward mutation Test	Mouse lymphoma cells *L5178y*	100 - 325 µg/L	Negative[1]	(Cifone, 2001)	Valid study and in compliance with OECD Guideline 476.
(Carveol [02.062])	Ames test (pre-incubation)	*S. typhimurium* TA98, TA100, TA1535, TA1537	560 µg/plate	Negative	(Mortelmans et al., 1986)	
(Carvyl acetate [09.215])	Ames test (pre-incubation)	*S. typhimurium* TA98, TA100, TA1535, TA1537	333 µg/plate	Negative	(Mortelmans et al., 1986)	
(L-menthyl (R,S)-3-hydroxybutyrate)	Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537 and TA1538	78, 156, 312, 625, 1250, 2500 or 10 000 µg/plate	Negative[a,b]	(Morimoto, 2005)	The JECFA evaluated the racemate of L-menthyl (R,S)-3-hydroxybutyrate.

Table 2.2: GENOTOXICITY (*in vitro*) EFSA / FGE.09Rev3 (EFSA, 2011x) (substances in brackets are JECFA-evaluated substances)

Chemical Name [FL-no]	Test system	Test Object	Concentration	Result	Reference	Comments
	Reverse mutation	*E. coli* WP2uvrA	78, 156, 312, 625, 1250, 2500 or 10 000 µg/plate	Negative[a,b]	(Morimoto, 2005)	

NA: Not applicable.

NR: Not reported.

[1] With and without S9 metabolic activation.

[2] With S9 activation.

[3] Without S9 activation.

[4] Calculated based on molecular weight of menthol = 156.3 g/mol.

[5] Marked differential toxicity was seen at dose levels above 25 µmol/plate. No observations were noted at lower dose levels.

Table 2.3: GENOTOXICITY (*in vivo*) EFSA / FGE.09Rev3 (EFSA, 2011x) (substances in brackets are JECFA-evaluated substances)

Chemical Name	Test System	Test Object	Route	Dose	Result	Reference	Comments
(Menthol [02.015])	Host mediated mutation assay	*S. typhimurium* TA1530 and G46; *S. cerevisiae* D3 inoculated in mice (7-9 animals/group)	Gavage	0, 1.45 - 5000 mg/kg bw (single dose) 0, 1150 mg/kg bw/day (repeated doses)	Equivocal	(Food and Drug Research Laboratories, Inc., 1975a)	Negative results, with exception of the combination *S. typhimurium* TA1530 - 5000 mg/kg bw and *S. cerevisiae* D3 - 1150 mg/kg bw/day. This study is considered valid, but the equivocal result might have low relevance since the effect was only observed at very high (lethal) dose levels.
	In vivo cytogenetic assay	Male rat bone marrow cells	Gavage	0, 1.45 - 3000 mg/kg bw (single dose) 0, 1150 mg/kg bw/day (repeated doses)	Negative	(Food and Drug Research Laboratories, Inc., 1975a)	Oral DL_{50} was determined as 940 mg/kg bw. The study is considered valid but the negative result is of limited relevance, since no effect on mitotic index was observed. However, testing at higher dose levels may not have been possible, due to lethality.
	In vivo micronucleus assay	B6C3F1 male mouse bone marrow cells	Intra peritonal	0, 250 - 1000 mg/kg bw/day, during 3 days	Negative	(Shelby et al., 1993)	d,l-Menthol was used. The study is considered valid, but the negative result is of limited relevance, since no toxicity to the bone marrow was observed. However, testing at higher dose levels was not possible, because the highest dose caused 50 % lethality.
	In vivo dominant lethal assay	Male rat fertility, spermatozoa	Gavage	0, 1.45 - 3000 mg/kg bw (single dose) 0, 1150 mg/kg bw/day (repeated doses)	Negative	(Food and Drug Research Laboratories, Inc., 1975a)	This study is considered valid.
(trans-Menthone [07.176])	*In vivo* SMART assay	*D. melanogaster* – flr3 x mwh cross	Whole body	0, 1.3 µl/disk	Positive	(Franzios et al., 1997)	Somatic Mutation and Recombination Test. Only one dose level (1.29 µl/disk; slightlyhigher than the LD_{50}) was tested. A two-fold increase in mutation frequency as compared to control was observed. Menthone was not recombinogenic. The validity of this study is unclear.
(Cyclohexanone [07.148])	*In vivo* sex-linked recessive lethal mutation	*D. melanogaster*	NR 3 days exposure	0, 1 µl/ml	Negative	(Goncharova, 1970)	Article in Russian. Only an abstract available in English. The validity of this study cannot be assessed.
Cyclohexanol [02.070]	*In vivo* sex-linked recessive lethal mutation	*D. melanogaster*	NR 3 days exposure	0, 1 µl/ml	Negative	(Goncharova, 1970)	The validity of the study cannot be evaluated.
	In vivo micronucleus test	NMRI mouse bone marrow	Oral	500 - 1500 mg/kg bw	Negative	(Gelbke, 1991)	The study is considered valid. The negative result of this study is of limited relevance, since no bone marrow toxicity could be detected. Testing at higher dose levels might not have been possible due to observed general toxicity at the highest dose.

Table 2.3: GENOTOXICITY (*in vivo*) EFSA / FGE.09Rev3 (EFSA, 2011x) (substances in brackets are JECFA-evaluated substances)

Chemical Name	Test System	Test Object	Route	Dose	Result	Reference	Comments
Methyl 3-oxo-2-pentyl-1-cyclopentylacetate [09.520]	Micronucleus test	ICR mice	Intra peritonal	280, 560 & 1120 mg/kg bw	Negative	(Gudi and Krsmanovic, 1998)	Valid study in compliance with the OECD Guideline 474.
	Unscheduled DNA Synthesis	Rat hepatocytes	Intra peritonal	333.3 & 1000 mg/kg bw	Negative	(Durward, 2001)	Valid study in compliance with the OECD Guideline 486.

NR: Not reported

Table 2.4: GENOTOXICITY (*in vitro*) from FGE.211

FL-no JECFA-no	Chemical Name	Test System	Test Object	Concentrations of Substance and Test Conditions	Result	Reference	Comments
09.930 1098	1(7),8-*p*- Menthadien-2-yl acetate	Reverse Mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537 and TA102	1.6*, 8*, 40*, 200, 1000 and 5000 µg/plate [1,2]	Negative	(Beevers, 2010a)	* Concentration without cytotoxicity.
			S. typhimurium TA98, TA1535 and TA1537	15.6*, 31.3*, 62.5*, 125, 250 and 500 µg/plate [2,3]	Negative		
			S. typhimurium TA100 and TA102	78.1*, 156.3*, 312.5, 625, 1250 and 2500 µg/plate [2,3]	Negative		
			S. typhimurium TA98 and TA100	156.3*, 312.5, 625, 1250, 2500 and 5000 µg/plate [4,5]	Negative		
			S. typhimurium TA1535, TA1537 and TA102	78.1*, 156.3*, 312.5, 625, 1250 and 2500 µg/plate [4,5]	Negative		
			S. typhimurium TA100	25*, 50*, 100*, 200 and 400 µg/plate [2,3]	Negative		
			S. typhimurium TA98	50*, 100*, 200*, 400 and 800 µg/plate [4,5]	Negative		
			S. typhimurium TA100, TA1535, TA1537 and TA102	25*, 50*, 100*, 200 and 400 µg/plate [4,5]	Negative		
		Micronucleus induction	Human peripheral blood lymphocytes	80, 90 and 110 µg/ml [3,6]; 200, 300 and 400 µg/ml [5,6]	Negative	(Whitwell, 2010b)	50 to 65 % cytotoxicity at top concentrations.
				20, 50, 80 and 100 µg/ml [3,7]	Negative		

[1] With and without S9 metabolic activation.
[2] Plate incorporation method.
[3] Without S9 metabolic activation.
[4] Pre-incubation method.
[5] With S9 metabolic activation.
[6] 3-hour incubation with 21-hour recovery period.
[7] 24-hour incubation with no recovery period.

Table 2.5: GENOTOXICITY (*in vitro*) from FGE.212Rev1

Chemical Name [FL-no]	Test System	Test Object	Concentration	Reported Result	Reference	Comments [e]
Tetramethyl ethylcyclohexenone (mixture of isomers [07.035]	Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538	5 concentrations up to cytotoxicity, or max. 3600 µg/plate	Negative[a]	(Wild et al., 1983)	Limited validity (no TA 102 or *E. Coli*); possibly slightly low maximal concentration tested.
3,5,5-Trimethylcyclohex-2-en-1-one [07.126]	Reverse mutation	*S. typhimurium* TA97, TA98, TA100, TA1535, TA1537	33 - 10 000 µg/plate	Negative[a]	(Mortelmans et al., 1986)	Valid.
	Mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537	33 - 10 000 µg/plate	Negative[a]	(NTP, 1986d)	NTP study carried out according to standard US-EPA guideline; result is considered as valid.
	Mutation	L5178YTk+/– mouse lymphoma cells	67 - 810 µg/ml	Negative[b]	(McKee et al., 1987)	Validity cannot be evaluated (tested with S9; abstract only with very limitred information).
	Mutation	L5178YTk+/– mouse lymphoma cells	130 - 1300 µg/ml	Negative[c]	(McKee et al., 1987)	Validity cannot be evaluted (tested without S9; abstract only with very limitred information).
	Mutation	L5178YTk+/– mouse lymphoma cells	0.089 - 0.89 µg/ml	Negative[c]	(O'Donoghue et al., 1988)	Valid according to current guidelines.
	Mutation	L5178YTk+/– mouse lymphoma cells	0.13 - 1.3 µg/ml	Negative[b]	(O'Donoghue et al., 1988)	Valid according to current guidelines.
	Mutation	L5178YTk+/– mouse lymphoma cells	1200 µg/ml	Positive[b]	(NTP, 1986d)	NTP study carried out according to standard US-EPA guideline; Not tested with S9. Result is considered as valid.
	Mutation	L5178YTk+/– mouse lymphoma cells	Not reported (however, up to cytotoxic concentrations) for 3 hours exposure.	Negative[a]	(Honma et al., 1999a)	Limited validity since data were presented in a summarized table format only (as a result of an international collaborative study).
	Mutation	L5178YTk+/– mouse lymphoma cells	Up to 1500 µg /ml	Positive[b]	(Honma et al., 1999b)	Limited validity since mutation frequencies were not reported in table format. Tested only in the absence of S9. Isophorone was mutagenic after 24 hours treatments in the absence of S9. Although only graphs are plotted, it seems that increases in MF that exceeded the Global Evaluation Factor occurred at around 1250-1500 µg/ml where toxicity (by relative survival) reached 70-90 %.
	Chromosomal aberration	Chinese hamster ovary cells	5 - 1600 µg/ml	Negative[a]	(Gulati et al., 1989)	Limited validity (not clear if gaps were included in the scores).
	Chromosomal aberration	Chinese hamster ovary cells	250 - 1600 µg/ml	Negative[a]	(NTP, 1986d)	NTP study carried out according to standard US-EPA guideline; result is considered as valid.
	Chromosomal aberration	Chinese hamster lung fibroblasts	0 - 1250[b] µg/ml 0 - 1500[c] µg/ml	Positive[a]	(Matsuoka et al., 1996)	Valid. Exposed to isophorone for 6 hrs with a recovery period of 18 hours.
	Chromosomal aberration	Chinese hamster lung fibroblasts	250 - 1000 mg/ml	Negative[a]	(Matsuoka et al., 1996)	Valid. Exposed to isophorone without metabolic activation for 24 hours or 48 hours, cytotoxic at highest concentrations.
	Sister chromatid exchange	Chinese hamster ovary cells	5 - 1600 mg/ml	Positive[b,d]	(Gulati et al., 1989)	Valid (pos – S9; neg + S9).
	Sister chromatid exchange	Chinese hamster ovary cells	160 - 1000 mg/ml	Negative[a]	(NTP, 1986d)	NTP study carried out according to Standard US-EPA guideline; result is considered as valid.
	Unscheduled DNA synthesis	Rat hepatocytes	0.005 - 0.4 µl/ml	Negative	(O'Donoghue et al., 1988)	Valid according to current guidelines.
	Unscheduled DNA synthesis	Rat hepatocytes	5 - 200 µl/ml	Negative[a]	(McKee et al., 1987)	Validity cannot be evaluated (abstract only with very limited information).

Table 2.5: GENOTOXICITY (*in vitro*) from FGE.212Rev1

Chemical Name [FL-no]	Test System	Test Object	Concentration	Reported Result	Reference	Comments [e]
Carvone (isomer not specified)	Gene mutation	*S. typhimurium* TA1535, TA1537, TA98, TA100	3 µmol/plate	Negative	(Florin et al., 1980)	Insufficient validity (spot test, not according to OECD guideline, methods and results insufficiently reported). Isomer (D or L) not reported.
	Rec assay	*Bacillus subtilis* H17 (rec+) and M45 (rec-)	0.6 ml/disc	Negative	(Matsui et al., 1989)	The test system used is considered inappropriate.
d-Carvone [07.146]	Gene mutation	*S. typhimurium* TA1535, TA98, TA100, TA1537	333 µg/plate	Negative[a]	(NTP, 1990b)	Valid.
	Gene mutation (preincubation)	*S. typhimurium* TA1535, TA98, TA100, TA1537	560 µg/plate	Negative	(Mortelmans et al., 1986)	Valid.
	Sister chromatid exchange	Chinese hamster ovary cells	502 µg/ml	Positive[a]	(NTP, 1990b)	Valid.
	Chromosomal aberration	Chinese hamster ovary cells	400 µg/ml	Positive[a]	(NTP, 1990b)	Valid.

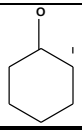
a: With and without metabolic activation.
b: Without metabolic activation.
c: With metabolic activation.
d: Cytotoxic at next highest dose tested (1600 mg/ml).
e: Validity of genotoxicity studies:
Valid.
Limited validity (e.g. if certain aspects are not in accordance with OECD guidelines or current standards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

Table 2.6: GENOTOXICITY (*in vivo*) from FGE.212Rev1

Chemical Name [FL-no]	Test System	Test Object	Route	Dose	Result	Reference	Comments [a]
Tetramethyl ethylcyclohexenone (mixture of isomers [07.035]	Sex-linked recessive lethal mutation	*D. melanogaster*	Feed	10 mM	Negative	(Wild et al., 1983)	Limited validity (low nr of chromosomes, limited reporting)
	Micronucleus formation	Mouse bone marrow	i.p.	180, 307, 450 mg/kg bw	Negative	(Wild et al., 1983)	Limited validity. Only analysis at one time point; no PCE/NCE ratio reported
3,5,5-Trimethylcyclohex-2-en-1-one [07.126]	Sex-linked recessive lethal mutation	*D. melanogaster*		2000[b] and 12 500[c] ppm	Negative	(Foureman et al., 1994)	Valid, however, only limited relevance.
	Micronucleus formation	CD-1 mice	i.p.	540 mg/kg bw (MTD)	Negative	(McKee et al., 1987)	Validity cannot be evaluated. Abstract only; very limited information nodata on PCE/NCE ratio.
	Micronucleus formation	CD-1 mice	i.p.	0.54 ml/kg bw	Negative	(O'Donoghue et al., 1988)	Limited validity. Only one dose level tested, this dose level corresponded to the LD20; sample schedule inadequate
	Chromosomal aberration	B6C3F1 mice	i.p.	125, 250, 500 mg/kg bw	Negative	NTP-Website	Valid. Submitted by Industry in 2009. The standard protocol for *in vivo* CA is not given on the NTP website. However, based on Shelby and Witt (1995), animals should have been sampled at 17 hours and, if negative, also at 36 hours. The data on the NTP website are only for bone marrow sampled at 36 hours. It is therefore possible that a 17 hours sample was also taken, and found to be negative, but the data not posted. Fifty cells per animal were scored for CA and no increases in CA were seen. No measures of toxicity were recorded, but i.p. dosing should have guaranteed systemic exposure.
	DNA binding	F344 rats	Gavage	500 mg unlabelled isophorone / kg bw spiked with C14-isophorone (0.4 mCi/rat)	Negative	Thier et al., 1990	Limited validity. Submitted by Industry in 2009. No positive controls and no untreated controls used. Liver and kidney were analysed.
	DNA binding	B6C3F1 mice	Gavage	500 mg unlabelled isophorone / kg bw spiked with C14-isophorone (0.08 mCi/mouse)	Negative	Thier et al., 1990	Limited validity. Submitted by Industry in 2009. No positive controls and no untreated controls used. Liver and kidney were analysed.
	DNA binding	F344 rats (10 males)	Gavage	500 mg/kg bw ^{14}C-isophorone (0.1 mCi/rat)	Negative	Morishita et al., 1997	Valid. Preputial glands and kidneys were analysed.
	DNA adducts (^{32}P-Postlabelling)	F344 rats (7 males and 7 females per dose group)	Gavage	0 and 500 mg/kg/day for 5 days.	Negative	Morishita et al., 1997	Valid. Preputial glands were analysed.

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

Table 2.7: Additional Genotoxicity Studies *(in vitro*)

FL-no JECFA-no	EU Register name JECFA name	Structural formula	End-point	Test system	Maximum concentration	Results	Reference
07.148 1100	Cyclohexanone		Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537	33–3333 µg/plate	Negative[a]	(NTP, 2007)
			Mutation	Mouse lymphoma L5178Y $Tk^{+/-}$ cells	312.5–5000 µg/ml	Negative	(NTP, 2007)

[a] With and without metabolic activation.

TABLE 3: SUMMARY OF SAFETY EVALUATIONS

Table 3.1: Summary of Safety Evaluation of Alicyclic Ketones, Secondary Alcohols and Related Esters (JECFA, 2003a)

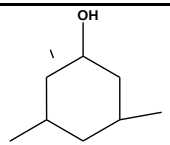
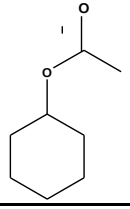
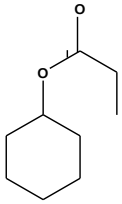
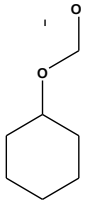
FL-no JECFA-no	EU Register name	Structural formula	EU MSDI 1) US MSDI (μg/*capita*/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	EFSA conclusion on the named compound (Procedure steps, intake estimates, NOAEL, genotoxicity)	EFSA conclusion on the material of commerce
02.209 1099	3,3,5-Trimethylcyclohexan-1-ol		0.12 0.1	Class I A3: Intake below threshold	4)	No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at the estimated level of intake based on the MSDI approach.
09.027 1093	Cyclohexyl acetate		12 10	Class I A3: Intake below threshold	4)	No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at the estimated level of intake based on the MSDI approach.
09.140 1097	Cyclohexyl propionate		0.012 0.05	Class I A3: Intake below threshold	4)	No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at the estimated level of intake based on the MSDI approach.
09.160 1095	Cyclohexyl formate		0.012 0.2	Class I A3: Intake below threshold	4)	No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at the estimated level of intake based on the MSDI approach.

Table 3.1: Summary of Safety Evaluation of Alicyclic Ketones, Secondary Alcohols and Related Esters (JECFA, 2003a)

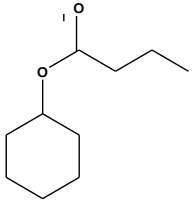
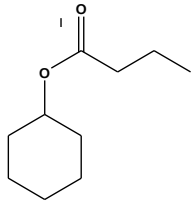
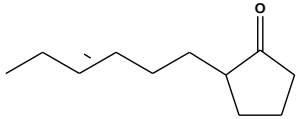
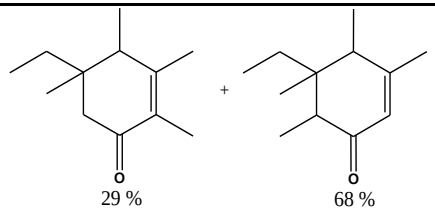
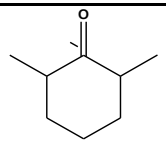
FL-no JECFA-no	EU Register name	Structural formula	EU MSDI 1) US MSDI (μg/*capita*/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	EFSA conclusion on the named compound (Procedure steps, intake estimates, NOAEL, genotoxicity)	EFSA conclusion on the material of commerce
09.230 1094	Cyclohexyl butyrate		0.89 0.1	Class I A3: Intake below threshold	4)	No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at the estimated level of intake based on the MSDI approach.
09.464 1096	Cyclohexyl isovalerate		0.28 0.05	Class I A3: Intake below threshold	4)	No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at the estimated level of intake based on the MSDI approach.
07.034 1106	2-Hexylidenecyclopentan-1-one		0.24 0.01	Class II A3: Intake below threshold	4)	Evaluated in FGE.211, genotoxicity concern could be ruled out. No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at the estimated level of intake based on the MSDI approach.
07.035 1111	Tetramethyl ethylcyclohexenone (mixture of isomers)		7.8 0.2	Class II A3: Intake below threshold	4)	Evaluated in FGE.212Rev1, genotoxic concern could be ruled out. No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at the estimated level of intake based on the MSDI approach.
07.045 1108	2,2,6-Trimethylcyclohexanone		2.1 0.04	Class II A3: Intake below threshold	4)	No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at the estimated level of intake based on the MSDI approach.

Table 3.1: Summary of Safety Evaluation of Alicyclic Ketones, Secondary Alcohols and Related Esters (JECFA, 2003a)

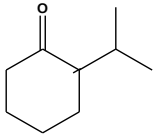
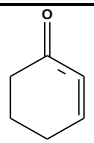
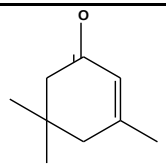
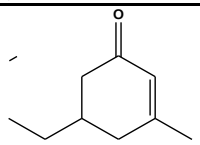
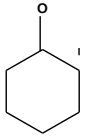
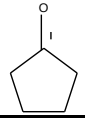
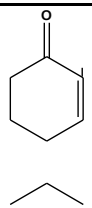
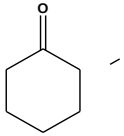
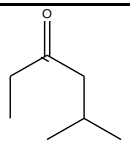
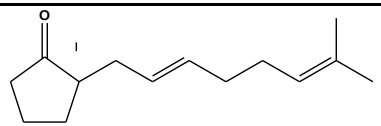
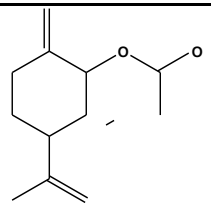
FL-no JECFA-no	EU Register name	Structural formula	EU MSDI 1) US MSDI (µg/*capita*/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	EFSA conclusion on the named compound (Procedure steps, intake estimates, NOAEL, genotoxicity)	EFSA conclusion on the material of commerce
07.095 1109	2-(sec-Butyl)cyclohexanone		5.1 ND	Class II A3: Intake below threshold	4)	No safety concern at the estimated level of intake based on the MSDI approach.	According to JECFA: Min. assay value is "94%" and secondary components "2-Isobutyl cyclohexanone" No safety concern at the estimated level of intake based on the MSDI approach.
07.098 1107	3-Methylcyclohex-2-en-1-one		0.012 0.1	Class II A3: Intake below threshold	4)	Evaluated in FGE.212Rev1, genotoxic concern could be ruled out. No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at the estimated level of intake based on the MSDI approach.
07.126 1112	3,5,5-Trimethylcyclohex-2-en-1-one		4.6 0.1	Class II A3: Intake below threshold	4)	Evaluated in FGE.212Rev1, genotoxic concern could be ruled out. No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at the estimated level of intake based on the MSDI approach.
07.129 1113	3-Methyl-5-propylcyclohex-2-en-1-one		0.097 4.1	Class II A3: Intake below threshold	4)	Evaluated in FGE.212Rev1, genotoxic concern could be ruled out. No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at the estimated level of intake based on the MSDI approach.
07.148 1100	Cyclohexanone		0.12 0.1	Class II A3: Intake below threshold	4)	No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at the estimated level of intake based on the MSDI approach.
07.149 1101	Cyclopentanone		0.018 0.02	Class II A3: Intake below threshold	4)	No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at the estimated level of intake based on the MSDI approach.

Table 3.1: Summary of Safety Evaluation of Alicyclic Ketones, Secondary Alcohols and Related Esters (JECFA, 2003a)

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)]	EFSA conclusion on the named compound (Procedure steps, intake estimates, NOAEL, genotoxicity)	EFSA conclusion on the material of commerce
07.172 1110	4-Isopropylcyclohex-2-en-1-one		0.0012 0.001	Class II A3: Intake below threshold	4)	Evaluated in FGE.212Rev1, genotoxic concern could be ruled out. No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at the estimated level of intake based on the MSDI approach.
07.179 1102	2-Methylcyclohexanone		0.12 0.1	Class II A3: Intake below threshold	4)	No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at the estimated level of intake based on the MSDI approach.
07.180 1103	3-Methylcyclohexanone		0.12 0.1	Class II A3: Intake below threshold	4)	No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at the estimated level of intake based on the MSDI approach.
07.257 1117	2-(3,7-Dimethyl-2,6-octadienyl) cyclopentanone		3.0 6.6	Class II A3: Intake below threshold	4)	No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at the estimated level of intake based on the MSDI approach.
09.930 1098	1(7),8-p-Menthadien-2-yl acetate (mixture of (E) and (Z) isomers)		0.61 0.6	Class II A3: Intake below threshold	4)	Evaluated in FGE.211, genotoxicity concern could be ruled out. No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at the estimated level of intake based on the MSDI approach.

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

ND: not determined

Table 3.2: Summary of Safety Evaluation Applying the Procedure (based on intakes calculated by the MSDI approach) (EFSA / FGE.09Rev3)

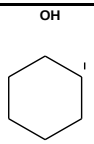
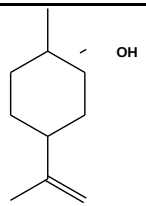
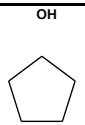
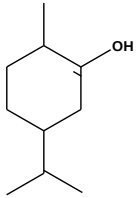
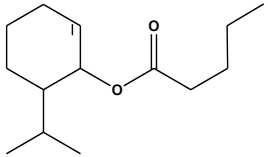
FL-no	EU Register name	Structural formula	MSDI 1) (µg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
02.070	Cyclohexanol		3.7	Class I A3: Intake below threshold	4)	6)	
02.075	neo-Dihydrocarveol		2.4	Class I A3: Intake below threshold	4)	6)	
02.135	Cyclopentanol		0.012	Class I A3: Intake below threshold	4)	6)	
02.167	Isodihydrocarveol		2.4	Class I A3: Intake below threshold	4)	6)	
09.154 1852	Menthyl valerate		1.0	Class I A3: Intake below threshold	4)	6)	

Table 3.2: Summary of Safety Evaluation Applying the Procedure (based on intakes calculated by the MSDI approach) (EFSA / FGE.09Rev3)

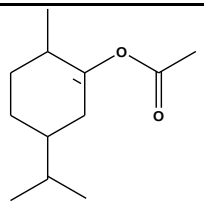
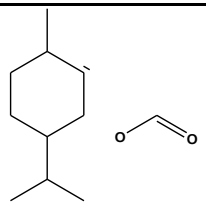
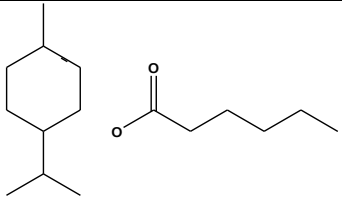
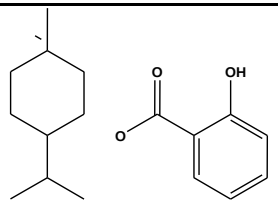
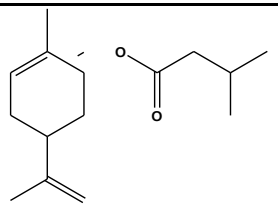
FL-no	EU Register name	Structural formula	MSDI 1) (µg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
09.355	neo-Dihydrocarvyl acetate		0.012	Class I A3: Intake below threshold	4)	6)	
09.618	Menthyl formate		0.73	Class I A3: Intake below threshold	4)	6)	
09.619	Menthyl hexanoate		0.37	Class I A3: Intake below threshold	4)	6)	
09.621	Menthyl salicylate		0.012	Class I A3: Intake below threshold	4)	6)	
09.870	Carvyl-3-methylbutyrate		0.0012	Class I A3: Intake below threshold	4)	6)	a)

Table 3.2: Summary of Safety Evaluation Applying the Procedure (based on intakes calculated by the MSDI approach) (EFSA / FGE.09Rev3)

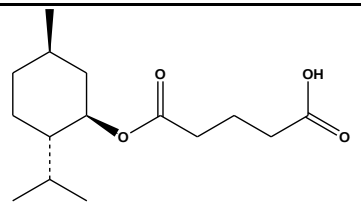
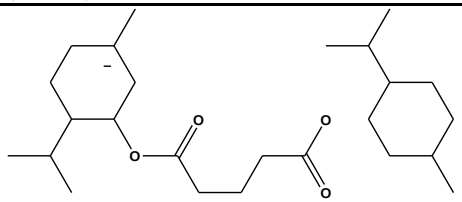
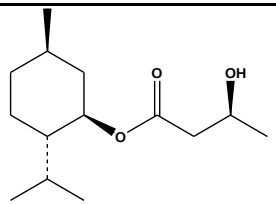
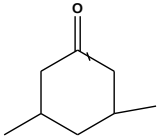
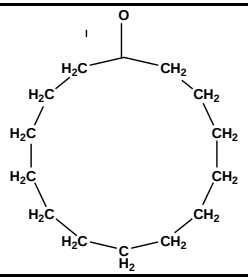
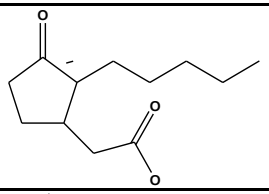
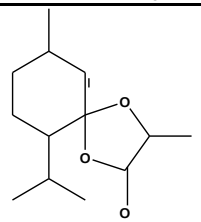
FL-no	EU Register name	Structural formula	MSDI 1) (µg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
09.929	L-Monomenthyl glutarate		110	Class I A3: Intake below threshold	4)	6)	
09.935	Dimenthyl glutarate		30	Class I A3: Intake below threshold	4)	6)	
09.949	L-Menthyl (S)-3-hydroxybutyrate		37	Class I A3: Intake below threshold	4)	6)	
07.203	3,3,5-Trimethylcyclohexan-1-one		0.0085	Class II A3: Intake below threshold	4)	6)	
07.207	Cyclotetradecanone		0.061	Class II B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		

Table 3.2: Summary of Safety Evaluation Applying the Procedure (based on intakes calculated by the MSDI approach) (EFSA / FGE.09Rev3)

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
09.520	Methyl 3-oxo-2-pentyl-1-cyclopentylacetate		770	Class II A3: Intake above threshold, A4: Not endogenous, A5: Adequate NOAEL exists	4)	6)	
06.136 1859	6-Isopropyl-3,9-dimethyl-1,4-dioxyspiro[4.5]decan-2-one		12	Class III A3: Intake below threshold	4)	6)	

1) EU MSDI: Amount added to food as flavour in (kg / year) x 10E9 / (0.1 x population in Europe (= 375 x 10E6) x 0.6 x 365) = µg/capita/day.
2) Thresholds of concern: Class I = 1800 µg/person/day, Class II = 540 µg/person/day, Class III = 90 µg/person/day.
3) Procedure path A substances can be predicted to be metabolised to innocuous products. Procedure path B substances cannot.
4) No safety concern based on intake calculated by the MSDI approach of the named compound.
5) Data must be available on the substance or closely related substances to perform a safety evaluation.
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.
a) Evaluated in FGE.212, genotoxic concern could be ruled out.

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ABBREVIATIONS

ATP	Adenosine Tri-Phosphate
BW	Body weight
CA	Chromosomal Aberration
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
EFSA	The European Food Safety Authority
EPA	United States Environmental Protection Agency
EU	European Union
FAO	Food and Agriculture Organization of the United Nations
FEMA	Flavor and Extract Manufacturers Association
FGE	Flavouring Group Evaluation
FLAVIS (FL)	Flavour Information System (database)
GEF	Global Evaluation Factor
GLP	Good laboratory practise
ID	Identity
Ip	Intraperitoneal
IR	Infrared spectroscopy
JECFA	The Joint FAO/WHO Expert Committee on Food Additives
MF	Mutation Frequency
MLA	Mouse Lymphoma Assay
MNBN	Micronucleated binucleate cells
MSDI	Maximised Survey-derived Daily Intake
mTAMDI	Modified Theoretical Added Maximum Daily Intake
NCE	Normochromatic erythrocyte
No	Number

NOAEL	No observed adverse effect level
NTP	National Toxicology Program
OECD	Organisation for Economic Co-operation and Development
PCE	Polychromatic erythrocyte
SCE	Sister chromatic exchange
SCF	Scientific Committee on Food
SLRL	Sex Linked Recessive Lethal Mutations test
SMART	Somatic Mutation And Recombination Test
UDS	Unscheduled DNA Synthesis
WHO	World Health Organisation