SCIENTIFIC OPINION

Flavouring Group Evaluation 47, (FGE.47)¹

Bicyclic secondary alcohols, ketones and related esters from chemical group 8

Scientific Opinion of the Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food

(Question No EFSA-Q-2008-051)

Adopted on 22 May 2008

PANEL MEMBERS


SUMMARY

The Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food (the Panel) is asked to advise 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 is asked to evaluate four flavouring substances in the Flavouring Group Evaluation 47 (FGE.47), using the Procedure as referred to in the Commission Regulation (EC) No 1565/2000. These four flavouring substances belong to chemical group 8, Annex I of the Commission Regulation (EC) No 1565/2000.

The present Flavouring Group Evaluation deals with four bicyclic secondary alcohols, ketones and related esters from chemical group 8.

The four flavouring substances possess one or more chiral centres. For one of the flavouring substances [FL-no: 09.888] the stereoisomeric composition has not been specified.

Three of the four flavouring substances belong to structural class I and one belongs to structural class II according to decision tree approach presented by presented by Cramer et al., (1978).

Only one of the flavouring substances in the present group [FL-no: 07.171] has been reported to occur naturally.

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

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

According to the default MSDI approach, the four flavouring substances in this group have intakes in Europe from 0.011 to 0.085 microgram/capita/day, which are below the threshold of concern value for structural class I (1800 microgram/person/day) and structural class II (540 microgram/person/day) substances.

Genotoxicity data are available only for a limited number of substances, and the genotoxicity could not be assessed adequately. However, the data available do not preclude the evaluation of the four flavouring substances using the Procedure.

The four flavouring substances are expected to be metabolised to innocuous products.

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

It is considered that on the basis of the default MSDI approach the four flavouring substances would not give rise to safety concerns at the estimated levels of intake arising from their use as flavouring substances.

When the estimated intakes were based on the mTAMDI approach they ranged from 1900 to 2300 microgram/person/day for the three flavouring substances from structural class I. These intakes are above the threshold of concern for structural class I of 1800 microgram/person/day. The estimated intake of the one flavouring substance [FL-no: 07.171] assigned to structural class II, based on the mTAMDI, approach is 1000 microgram/person/day, which is above the threshold of concern for structural class II of 540 microgram/person/day.
Thus, for the all four flavouring substances the estimated intakes, based on the mTAMDI approach, exceed the relevant threshold for the structural class. Therefore, for all four substances more reliable exposure data are required. On the basis of such additional data, these flavouring substances should be reconsidered using the Procedure. Subsequently, additional data might become necessary.

In order to determine whether this evaluation could be applied to the materials of commerce, it is necessary to consider the available specifications.

Specifications including purity criteria for the materials of commerce have been provided for all four flavouring substances. Information on chirality is missing for one substance [FL-no: 09.888] and for all four substances identity tests are missing.

Thus, the final evaluation of the materials of commerce cannot be performed for the four substances [FL-no: 07.171, 09.584, 09.848 and 09.888], pending further information on specifications.

Keywords

Bicyclic secondary alcohols, ketones and related esters, flavourings, safety.
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Background

Regulation (EC) No 2232/96 of the European Parliament and the Council (EC, 1996) lays down a Procedure for the establishment of a list of flavouring substances, the use of which will be authorised to the exclusion of all other substances in the EU. In application of that Regulation, a Register of flavouring substances used in or on foodstuffs in the Member States was adopted by Commission Decision 1999/217/EC (EC, 1999a), as last amended by Commission Decision 2008/478/EC (EC, 2008). Each flavouring substance is attributed a FLAVIS-number (FL-number) and all substances are divided into 34 chemical groups. Substances within a group should have some metabolic and biological behaviour in common.

Substances which are listed in the Register are to be evaluated according to the evaluation programme laid down in Commission Regulation (EC) No 1565/2000 (EC, 2000a), which is broadly based on the Opinion of the Scientific Committee on Food (SCF, 1999). For the submission of data by the manufacturer, deadlines have been established by Commission Regulation (EC) No 622/2002 (EC, 2002b).

After the completion of the evaluation programme the positive list of flavouring substances for use in or on foods in the EU shall be adopted (Article 5 (1) of Regulation (EC) No 2232/96) (EC, 1996).

TERMS OF REFERENCE

The European Food Safety Authority (EFSA) is requested to carry out a risk assessment on flavouring substances in the Register prior to their authorisation and inclusion in a positive list according to Commission Regulation (EC) No 1565/2000 (EC, 2000a). In addition, the Commission requested EFSA to evaluate newly notified flavouring substances, where possible, before finalising the evaluation programme.

ACKNOWLEDGEMENTS

The Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food wishes to thank Vibe Beltoft, Jørn Gry, Pia Lund and Karin Nørby for their contribution to the draft opinion.
ASSESSMENT

1. Presentation of the Substances in the Flavouring Group Evaluation 47

1.1. Description


The four flavouring substances under consideration, as well as their chemical Register names, FLAVIS- (FL-), Chemical Abstract Service- (CAS-), Council of Europe- (CoE-) and Flavor and Extract Manufacturers Association- (FEMA-) numbers, structure and specifications, are listed in Table 1.

The hydrolysis products of candidate substances are listed in Table 2b.

The four candidate substances are structurally related to 15 flavouring substances (supporting substances) evaluated at the 63rd meeting of the Joint FAO/WHO Expert Committee on Food Additives (the JECFA) in the group “Monocyclic and Bicyclic Secondary Alcohols, Ketones and Related Esters” (JECFA, 2006a).

Furthermore, d-camphor [FL-no: 07.215], a structurally related substance to the substances in FGE.47 and which the Panel has evaluated in a separate Opinion (EFSA, 2008l), was also evaluated by JECFA at the 63rd meeting (JECFA, 2006a).

The names and structures of the 15 supporting substances are listed in Table 3, together with their evaluation status (CoE, 1992; SCF, 1995; JECFA, 2006a).

1.2. Stereoisomers

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

The four candidate substances, isopinocamphone [FL-no: 07.171], isobornyl isobutyrate [FL-no: 09.584], (1S-endo)-1,7,7-trimethylbicyclo[2.2.1]heptan-2-ol acetate [FL-no: 09.848] and isobornyl 2-methylbutyrate [FL-no: 09.888], possess one or more chiral centres. One of the substances [FL-no: 09.888] has been presented without specification of the stereoisomeric composition. See Table 1.
1.3. Natural Occurrence in Food

One of the four candidate substances has been reported to occur in apricot, cloudberry and essential oils (TNO, 2000). Quantitative data on the natural occurrence have been reported for this substance:

Isopinocamphone [FL-no: 07.171]: up to 2.2 mg/kg in apricot.

The remaining three substances (isobornyl isobutyrate [FL-no: 09.584], (1S-endo)-1,7,7-trimethylbicyclo[2.2.1]heptan-2-ol acetate [FL-no: 09.848] and isobornyl 2-methylbutyrate [FL-no: 09.888]) have not been reported to occur naturally in any food items according to TNO (TNO, 2000).

2. Specifications

Judged against the requirements in Annex II of Commission Regulation (EC) No 1565/2000 (EC, 2000), the purity criteria for all four candidate substances are deficient in one of the parameters as identification tests are missing for all four. Otherwise the specifications are adequate for all candidate substances, except that information on chirality is needed for one candidate substance (see Section 1.2 and Table 1).

3. Intake Data

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

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

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

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

One of the alternatives is the “Theoretical Added Maximum Daily Intake” (TAMDI) approach, which is calculated on the basis of standard portions and upper use levels (SCF, 1995) for flavourable beverages and foods in general, with exceptional levels for particular foods. This method is regarded as a conservative estimate of the actual intake in most consumers because it is based on the assumption that the consumer regularly eats and drinks several food products containing the same flavouring substance at the upper use level.
One option to modify the TAMDI approach is to base the calculation on normal rather than upper use levels of the flavouring substances. This modified approach is less conservative (i.e. it may underestimate the intake of consumers being loyal to products flavoured at the maximum use levels reported (EC, 2000a). However, it is considered as a suitable tool to screen and prioritise the flavouring substances according to the need for refined intake data (EFSA, 2004a).

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

The Maximised Survey-Derived Daily Intake (MSDI) (SCF, 1999) data are derived from surveys on annual production volumes in Europe. These surveys were conducted in 1995 by the International Organization of the Flavour Industry, in which flavour manufacturers reported the total amount of each flavouring substance incorporated into food sold in the EU during the previous year (IOFI, 1995). The intake approach does not consider the possible natural occurrence in food.

Average *per capita* intake (MSDI) is estimated on the assumption that the amount added to food is consumed by 10 % of the population\(^2\) (Eurostat, 1998). This is derived for candidate substances from estimates of annual volume of production provided by Industry and incorporates a correction factor of 0.6 to allow for incomplete reporting (60 %) in the Industry surveys (SCF, 1999).

In the present Flavouring Group Evaluation 47 (FGE.47) the total annual production volume of the four candidate substances for use as flavouring substances in Europe has been reported to be approximately 1.5 kg (EFFA, 2005f). For 14 of the 16 supporting substances, the total annual volume of production has been reported by the JECFA to be approximately 1100 kg (isobornyl acetate [FL-no: 09.218] accounts for 890 kg, borneol [FL-no: 02.016] for 130 kg and fenchyl alcohol [FL-no: 02.038] for 55 kg) (JECFA, 2005c). For the remaining two supporting substances [FL-no: 09.153 and 09.319] information is not available for Europe (JECFA, 2006a).

On the basis of the annual volume of production reported for the four candidate substances, MSDI values for each of these flavourings have been estimated (Table 2a). The estimated MSDI of isobornyl isobutyrate [FL-no: 09.584] from use as a flavouring substance is 0.085 microgram/capita/day, that of (1S-endo)-1,7,7-trimethylbicyclo[2.2.1]heptan-2-ol acetate [FL-no: 09.848] is 0.011 microgram/capita/day, that of isobornyl 2-methylbutyrate [FL-no: 09.888] is 0.061 microgram/capita/day and that of isopinocamphone [FL-no: 07.171] is 0.024 microgram/capita/day.

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

The method for calculation of modified Theoretical Added Maximum Daily Intake (mTAMDI) values is based on the approach used by SCF up to 1995 (SCF, 1995).

The assumption is that a person may consume a certain amount of flavourable foods and beverages per day.

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\(^2\) EU figure 375 millions (Eurostat, 1998). This figure relates to EU population at the time for which production data are available, and is consistent (comparable) with evaluations conducted prior to the enlargement of the EU. No production data are available for the enlarged EU.
For the present evaluation of the four candidate substances, information on food categories and normal and maximum use levels\(^1\) \(^4\) \(^5\) were submitted by the Flavour Industry (EFFA, 2005f; EFFA, 2007a). The four candidate substances are used in flavoured food products divided into the food categories, outlined in Annex III of the Commission Regulation (EC) No 1565/2000 (EC, 2000a), as shown in Table 3.1. For the present calculation of mTAMDI, the reported normal use levels were used. In the case where different use levels were reported for different food categories the highest reported normal use level was used.

According to the Flavour Industry the normal use levels for the four candidate substances are in the range of 1 - 20 mg/kg food, and the maximum use levels are in the range of 5 - 100 mg/kg (EFFA, 2002i; EFFA, 2005f; EFFA, 2007a).

The mTAMDI values for the three candidate substances from structural class I range from 1900 to 2300 microgram/person/day. For the remaining one candidate substances from structural class II the mTAMDI is 1000 microgram/person/day.

For detailed information on use levels and intake estimations based on the mTAMDI approach, see Section 6 and Annex II.

<table>
<thead>
<tr>
<th>Table 3.1 Use of Candidate Substances</th>
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<tr>
<td><strong>Food category</strong></td>
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<tr>
<td>Category 1</td>
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<td>Category 4.1</td>
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<td>Category 4.2</td>
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<td>Category 13</td>
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<td>Category 14.1</td>
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<td>Category 14.2</td>
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<tr>
<td>Category 15</td>
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<tr>
<td>Category 16</td>
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</tbody>
</table>

\(^1\) “Normal use” is defined as the average of reported usages and “maximum use” is defined as the 95\(^{th}\) percentile of reported usages (EFFA, 2002i).

\(^4\) The normal and maximum use levels in different food categories (EC, 2000) have been extrapolated from figures derived from 12 model flavouring substances (EFFA, 2004e).

\(^5\) The use levels from food category 5 “Confectionery” have been inserted as default values for food category 14.2 “Alcoholic beverages” for substances for which no data have been given for food category 14.2 (EFFA, 2007a).
4. Absorption, Distribution, Metabolism and Elimination

The available data indicate that the three esters in this group [FL-no: 09.584, 09.848 and 09.888] are readily hydrolysed to the corresponding bicyclic secondary alcohols, which are subsequently conjugated with glucuronic acid and excreted in the urine. The major metabolic pathway of the ketone [FL-no: 07.171] involves reduction to the corresponding secondary alcohol, which is subsequently excreted primarily as the glucuronic acid conjugate. In addition to reductive pathways, alicyclic ketones and, to a lesser extent, secondary alcohols containing an alkyl side chain undergo oxidation of the side chain to form polar oxygenated metabolites that are excreted either unchanged or as glucuronide, or as sulphate conjugates, mainly in the urine. It is therefore concluded that the candidate substances can be anticipated to be metabolised to innocuous products.

For more detailed information, see Annex III.

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

The application of the Procedure is based on intakes estimated on the basis of the MSDI approach. Where the mTAMDI approach indicates that the intake of a flavouring substance might exceed its corresponding threshold of concern, a formal safety assessment is not carried out using the Procedure. In these cases the Panel requires more precise data on use and use levels. For comparison of the intake estimations based on the MSDI approach and the mTAMDI approach, see Section 6.

For the safety evaluation of the four candidate substances from chemical group 8 the Procedure was applied (see Annex I). The stepwise evaluations of the four substances are summarised in Table 2a.

Step 1:
Three of the four candidate substances are classified in structural class I [FL-no: 09.584, 09.848 and 09.888] and one [FL-no: 07.171] in structural class II according to the decision tree approach presented by Cramer et al., (1978).

Step 2:
All four candidate substances in this group are expected to be metabolised to innocuous products. The evaluation of these substances therefore proceeded via the A-side of the Procedure scheme.

Step A3:
The estimated *per capita* daily intakes for all four candidate substances classified in structural classes I and II are below the human intake threshold of concern (i.e. 1800 microgram/person/day for class I and 540 microgram/person/day for class II).

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

The estimated intakes for the three candidate substances in structural class I, based on the mTAMDI, range from 1900 to 2300 microgram/person/day. For all these substances the mTAMDI is above the threshold of concern of 1800 microgram/person/day. For comparison of the intake estimates based on the MSDI approach and the mTAMDI approach, see Table 6.1.

The estimated intake of the substance assigned to structural class II, based on the mTAMDI, is 1000 microgram/person/day, which is above the threshold of concern for structural class II substances of 540 microgram/person/day. For comparison of the MSDI and mTAMDI values, see Table 6.1.

For all four candidate substances further information is required. This would include more reliable intake data and then, if required, additional toxicological data.

<table>
<thead>
<tr>
<th>FL-no</th>
<th>EU Register name</th>
<th>MSDI (µg/capita/day)</th>
<th>mTAMDI (µg/person/day)</th>
<th>Structural class</th>
<th>Threshold of concern (µg/person/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>09.584</td>
<td>Isobornyl isobutyrate</td>
<td>0.085</td>
<td>2300</td>
<td>Class I</td>
<td>1800</td>
</tr>
<tr>
<td>09.848</td>
<td>(1S-endo)-1,7,7-Trimethylbicyclo[2.2.1]heptan-2-ol acetate</td>
<td>0.011</td>
<td>2300</td>
<td>Class I</td>
<td>1800</td>
</tr>
<tr>
<td>09.888</td>
<td>Isobornyl 2-methylbutyrate</td>
<td>0.061</td>
<td>1900</td>
<td>Class I</td>
<td>1800</td>
</tr>
<tr>
<td>07.171</td>
<td>Isopinocamphone</td>
<td>0.024</td>
<td>1000</td>
<td>Class II</td>
<td>540</td>
</tr>
</tbody>
</table>

7. Considerations of Combined Intakes from Use as Flavouring Substances

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

The total estimated combined daily per capita intake of structurally related flavourings is estimated by summing the MSDI for individual substances.

On the basis of the reported annual production volumes in Europe (EFFA, 2005f) the combined estimated per capita intake as flavouring of the three candidate substances assigned to structural class I is 0.2 microgram/day, which does not exceed the threshold of concern for the structural class of 1800 microgram/person/day.

The four candidate substances are structurally related to 15 flavouring substances (13 are structural class I substances, two are structural class II substances) evaluated by JECFA at its 63rd session (JECFA, 2006a). The estimated total combined intake of candidate and
supporting substances (in Europe) would be 1100 microgram/capita/day for structural class I substances (European data were not available for two of the supporting substances), which is below the threshold of concern for structural class I of 1800 microgram/person/day. The estimated total combined intake of candidate and supporting substances (in Europe) would be 7 microgram/capita/day for structural class II substances, which is below the threshold of concern for structural class II of 540 microgram/person/day.

8. Toxicity

8.1. Acute Toxicity

Data are only available on the supporting substances. In rats, LD50 values ranged from 5000 mg/kg body weight (bw) to more than 10000 mg/kg bw.

The acute toxicity data are summarised in Annex IV, Table IV.1.

The Panel is aware that there are acute toxicity data on adults and children for one structurally related substance, (1R)-1,7,7-trimethylbicyclo[2.2.1]heptan-2-one (camphor [FL-no: 07.215]), mostly arising from the accidental ingestion of camphor-containing medications. The probable lethal oral bolus dose has been reported to be in the range of 50 to 500 mg/kg bw. No acute toxicity was reported after doses lower than 2 mg/kg bw and clinically insignificant signs of toxicity may be seen in sensitive individuals at doses of 5 mg/kg bw and higher, whereas clinically manifest toxicity in sensitive persons would require doses higher than 30 mg/kg bw. The Panel therefore suggested that maximum limits should be set to ensure that exposure to camphor does not exceed 2 mg/kg bw on a single day in any age group (EFSA, 2008).

As discussed in Annex III, camphor is rapidly metabolised to hydroxylated products which are then excreted. Under the anticipated conditions of dietary exposure from a food matrix, these metabolic pathways are the major routes for detoxification and would not be expected to be saturated. The reduction of camphor to borneol and isoborneol is only a minor metabolic pathway. In rat liver preparations the 2-keto group of d-camphor underwent no detectable reduction; l-camphor was reduced to a small extent. Rabbit liver cytosol mediated a vigorous stereospecific endo-reduction of d-camphor to borneol; a small amount (1%) of isoborneol was also formed. After oral administration of camphor to rabbits a reduction to borneol was observed to some extent, whereas in dogs only excretion of hydroxylated camphor was reported. In humans, admitted to hospital in a state of acute intoxication after ingestion of 6-10 g camphor, no reduction products, but only 5- and 8-(or 9)-hydroxycamphor and their conjugates, were detected as metabolites. Thus, the acute toxicity of camphor is not likely to be attributable to metabolism to borneol or isoborneol, which are hydrolysis products of candidate substances in this FGE.

The Panel therefore considers that the acute human toxicity findings on camphor, a substance structurally related to the candidate substances in this FGE, are not relevant for the safety assessment of the candidate flavouring substances or their hydrolysis products.

8.2. Subacute, Subchronic, Chronic and Carcinogenicity Studies

There are no data available on the four candidate substances, but there are data on three of the supporting substances.
The repeated dose studies are summarised in Annex IV, Table IV.2.

8.3. Developmental/Reproductive Toxicity Studies

There are no data available on the four candidate substances or for the supporting substances.

8.4. Genotoxicity Studies

There are no genotoxicity data available for the four candidate substances evaluated here, but there are data for two supporting substances.

*In vitro*

Borneol [FL-no: 02.016] and isobornyl propionate [FL-no: 09.131] were consistently tested negative in the Ames assay when a variety of *Salmonella typhimurium* strains including TA97, TA98, TA100, TA1535, TA1537 and TA1538 were incubated with up to 5,000 μg/plate with or without metabolic activation (Simmon et al., 1977; Wild et al., 1983; Azizan & Blevins, 1995).

Borneol showed no mutagenic activity when tested in *Escherichia coli* WP2 uvrA at concentrations up to 3,200 μg/plate (Yoo, 1986).

In the Rec assay, borneol was reported to induce growth inhibition in *Bacillus subtilis* strain M45- when tested at concentrations of up to 10 mg/disk (Yoo, 1986). This test has very limited relevance for the genotoxicity evaluation.

*In vivo*

The genotoxic potential of isobornyl propionate to induce somatic mutations in adult *Drosophila melanogaster* was studied in a Basc test. No increased frequency of mutation was observed when a 10 mM solution of isobornyl propionate was fed to the flies for 3 days (Wild et al., 1983).

In the micronucleus test, groups of NMRI mice administered intraperitoneal doses of 841, 1,893 or 2,944 mg/kg bw isobornyl propionate showed no increase in micronucleated erythrocytes in bone marrow samples, 30 hours post administration (Wild et al., 1983).

Conclusion on genotoxicity

Genotoxicity data are available only for a limited number of supporting substances, and the genotoxicity could not be assessed adequately. However, the data available do not preclude the evaluation of the candidate substances using the Procedure.

The *in vitro/in vivo* studies available are summarised in Annex IV, Table IV.4 and IV.5.
CONCLUSIONS

The four candidate substances are one bicyclic ketone [FL-no: 07.171] and three esters of bicyclic secondary alcohols [FL-no: 09.584, 09.848 and 09.888].

The four candidate substances possess one or more chiral centres. For one substance [FL-no: 09.888] the stereoisomeric composition has not been specified.

Three of the four candidate substances belong to structural class I while the fourth is assigned to structural class II [FL-no: 07.171], according to the decision tree approach as presented by Cramer et al., (1978).

Only one of the four candidate substances [FL-no: 07.171] in the present group has been reported to occur naturally.

According to the default MSDI approach, the four candidate substances in this group have intakes in Europe from 0.011 to 0.085 microgram/capita/day, which are below the thresholds of concern values for structural class I (1800 microgram/person/day) and structural class II (540 microgram/person/day) substances.

On the basis of the reported annual production volumes in Europe (MSDI approach), the combined intake of the three candidate substances belonging to structural class I would result in a combined intake of approximately 0.2 microgram/capita/day. This value is lower than the threshold of concern for structural class I substances (1800 microgram/person/day). The total combined intakes of candidate and supporting substances in Europe are approximately 1100 and 7 microgram/capita/day, for structural class I substances and for structural class II substances, respectively, which do not exceed the thresholds of concern for structural class I and II (1800 and 540 microgram/person/day, respectively).

Genotoxicity data are available only for a limited number of substances, and the genotoxicity could not be assessed adequately. However, the data available do not preclude the evaluation of the candidate substances using the Procedure.

The four candidate substances are expected to be metabolised to innocuous products.

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

It is considered that on the basis of the default MSDI approach the four candidate substances would not give rise to safety concerns at the estimated levels of intake arising from their use as flavouring substances.

When the estimated intakes were based on the mTAMDI approach, they ranged from 1900 to 2300 microgram/person/day for the three candidate substances from structural class I. These intakes are above the threshold of concern for structural class I of 1800 microgram/person/day. The estimated intake of the one candidate substance [FL-no: 07.171] assigned to structural class II, based on the mTAMDI approach, is 1000 microgram/person/day, which is above the threshold of concern for structural class II of 540 microgram/person/day.
Thus, for the three candidate substances from structural class I and for the one candidate substance allocated to structural class II, the intakes, estimated on the basis of the mTAMDI approach, exceed the relevant threshold for the structural class. Therefore, for all four substances more reliable exposure data are required. On the basis of such additional data, these flavouring substances should be reconsidered using the Procedure. Subsequently, additional data might become necessary.

In order to determine whether the conclusion for the candidate substances can be applied to the materials of commerce, it is necessary to consider the available specifications:

Specifications including purity criteria for the materials of commerce have been provided for all four flavouring substances. Information on chirality is missing for one substance [FL-no: 09.888] and for all four substances [FL-no: 07.171, 09.584, 09.848 and 09.888] identity tests are missing.

Thus, the final evaluation of the materials of commerce cannot be performed for the four substances [FL-no: 07.171, 09.584, 09.848 and 09.888], pending further information on specifications.
### Table 1  Specification Summary of the Substances in the Flavouring Group Evaluation 47

<table>
<thead>
<tr>
<th>FL-no</th>
<th>EU Register name</th>
<th>Structural formula</th>
<th>FEMA no CoE no CAS no</th>
<th>Phys. form Mol. formula Mol. weight</th>
<th>Solubility 1) Solubility in ethanol 2)</th>
<th>Boiling point, °C 3) Melting point, °C ID test Assay minimum</th>
<th>Refrac. Index 4) Spec. gravity 5)</th>
<th>Specification comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>07.171</td>
<td>Isopinocamphone</td>
<td></td>
<td>11125 18358-53-7</td>
<td>Liquid $\text{C}<em>{10}\text{H}</em>{16}\text{O}$ 152.24</td>
<td>Insoluble 1 ml in 1 ml 70 (7 hPa) 95 %</td>
<td>1.472-1.478 0.963-0.969</td>
<td>ID 7). CASrn in the Register refers to the racemate.</td>
<td></td>
</tr>
<tr>
<td>09.584</td>
<td>Isobornyl isobutyrate</td>
<td></td>
<td>85586-67-0</td>
<td>Liquid $\text{C}<em>{14}\text{H}</em>{24}\text{O}_{2}$ 224.34</td>
<td>Insoluble 1 ml in 1 ml 132 (25 hPa) 95 %</td>
<td>1.460-1.466 0.958-0.964</td>
<td>ID 7). CASrn in the Register refers to the racemate.</td>
<td></td>
</tr>
<tr>
<td>09.848</td>
<td>(1S-endo)-1,7,7-Trimethylbicyclo[2.2.1]heptan-2-ol acetate</td>
<td></td>
<td>5655-61-8</td>
<td>Solid $\text{C}<em>{12}\text{H}</em>{20}\text{O}_{2}$ 196.29</td>
<td>Insoluble 1 ml in 1 ml 225 29 95 %</td>
<td>1.456-1.462 0.981-0.987</td>
<td>ID 7). Name to be changed to (-)-bornyl acetate.</td>
<td></td>
</tr>
<tr>
<td>09.888</td>
<td>Isobornyl 2-methylbutyrate 6)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CASrn to be provided.</td>
<td></td>
</tr>
</tbody>
</table>

1) Solubility in water, if not otherwise stated.
2) Solubility in 95 % ethanol, if not otherwise stated.
3) At 1013.25 hPa, if not otherwise stated.
4) At 20°C, if not otherwise stated.
5) At 25°C, if not otherwise stated.
6) Stereoisomeric composition not specified.
7) ID: Missing identification test.
### Table 2a  Summary of Safety Evaluation Applying the Procedure (Based on Intakes Calculated by the MSDI Approach)

<table>
<thead>
<tr>
<th>FL-no</th>
<th>EU Register name</th>
<th>Structural formula</th>
<th>MSDI 1) (µg/capita/day)</th>
<th>Class 2) Evaluation procedure path 3)</th>
<th>Outcome on the named compound [4) or 5)]</th>
<th>Outcome on the material of commerce [6), 7), or 8)]</th>
<th>Evaluation remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>09.584 1863</td>
<td>Isobornyl isobutyrate</td>
<td><img src="image" alt="isobornyl isobutyrate" /></td>
<td>0.085</td>
<td>Class I A3: Intake below threshold</td>
<td>4)</td>
<td>7)</td>
<td></td>
</tr>
<tr>
<td>09.848 1864</td>
<td>(1S-endo)-1,7,7-Trimethylbicyclo[2.2.1]heptan-2-ol acetate</td>
<td><img src="image" alt="1S-endo)-1,7,7-Trimethylbicyclo[2.2.1]heptan-2-ol acetate" /></td>
<td>0.011</td>
<td>Class I A3: Intake below threshold</td>
<td>4)</td>
<td>7)</td>
<td></td>
</tr>
<tr>
<td>09.888 1869</td>
<td>Isobornyl 2-methylbutyrate</td>
<td><img src="image" alt="Isobornyl 2-methylbutyrate" /></td>
<td>0.061</td>
<td>Class I A3: Intake below threshold</td>
<td>4)</td>
<td>7)</td>
<td></td>
</tr>
<tr>
<td>07.171 1868</td>
<td>Isopinocamphone</td>
<td><img src="image" alt="Isopinocamphone" /></td>
<td>0.024</td>
<td>Class II A3: Intake below threshold</td>
<td>4)</td>
<td>7)</td>
<td></td>
</tr>
</tbody>
</table>

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

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

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

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

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

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

7) Tentatively regarded as presenting no safety concern (based on intake calculated by the MSDI approach) pending further information on the purity of the material of commerce and/or information on stereoisomerism.

8) No conclusion can be drawn due to lack of information on the purity of the material of commerce.
### Table 2b: Evaluation Status of Hydrolysis Products of Candidate Esters

<table>
<thead>
<tr>
<th>FL-no</th>
<th>EU Register name JECFA no</th>
<th>Structural formula</th>
<th>SCF status 1) JECFA status 2) CoE status 3)</th>
<th>Structural class 4) Procedure path (JECFA) 5)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>02.016 Borneol 1385</td>
<td><img src="image" alt="Structure" /></td>
<td>No safety concern a) Category B b)</td>
<td>Class I A3: Intake below threshold</td>
<td></td>
<td></td>
</tr>
<tr>
<td>02.059 Isoborneol 1386</td>
<td><img src="image" alt="Structure" /></td>
<td>No safety concern a) Category B b)</td>
<td>Class I A3: Intake below threshold</td>
<td></td>
<td></td>
</tr>
<tr>
<td>08.002 Acetic acid 81</td>
<td><img src="image" alt="Structure" /></td>
<td>Category 1 c) No safety concern d) Category A b)</td>
<td>Class I A3: Intake above threshold, A4: Endogenous</td>
<td></td>
<td></td>
</tr>
<tr>
<td>08.006 2-Methylpropionic acid 253</td>
<td><img src="image" alt="Structure" /></td>
<td>Category 1 c) No safety concern d) Category A b)</td>
<td>Class I A3: Intake below threshold</td>
<td></td>
<td></td>
</tr>
<tr>
<td>08.046 2-Methylbutyric acid 255</td>
<td><img src="image" alt="Structure" /></td>
<td>Category 1 c) No safety concern d) Category A b)</td>
<td>Class I A3: Intake below threshold</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1) Category 1: Considered safe in use, Category 2: Temporarily considered safe in use, Category 3: Insufficient data to provide assurance of safety in use, Category 4: Not acceptable due to evidence of toxicity.

2) No safety concern at estimated levels of intake.

3) Category A: Flavouring substance, which may be used in foodstuffs, Category B: Flavouring substance which can be used provisionally in foodstuffs.

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

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

a) (JECFA, 2005c).
b) (CoE, 1992).
c) (SCF, 1995).
d) (JECFA, 1999b).
<table>
<thead>
<tr>
<th>FL-no</th>
<th>EU Register name</th>
<th>Structural formula</th>
<th>FEMA no</th>
<th>CoE no</th>
<th>CAS no</th>
<th>JECFA no</th>
<th>Specification available</th>
<th>MSDI (EU) 1) (μg/capita/day)</th>
<th>SCF status 2)</th>
<th>JECFA status 3)</th>
<th>CoE status 4)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>02.016</td>
<td>Borneol</td>
<td></td>
<td>2157</td>
<td>64</td>
<td>507-70-0</td>
<td>1385</td>
<td>JECFA specification (JECFA, 2005b)</td>
<td>130</td>
<td>No safety concern a) Category B b)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>02.038</td>
<td>Fenchyl alcohol</td>
<td></td>
<td>2480</td>
<td>87</td>
<td>1632-73-1</td>
<td>1397</td>
<td>JECFA specification (JECFA, 2005b)</td>
<td>55</td>
<td>No safety concern a) Category B b)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>02.059</td>
<td>Isoborneol</td>
<td></td>
<td>2158</td>
<td>2020</td>
<td>124-76-5</td>
<td>1386</td>
<td>JECFA specification (JECFA, 2005b)</td>
<td>21</td>
<td>No safety concern a) Category B b)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>07.153</td>
<td>1,10-Dihydroneotkatone</td>
<td></td>
<td>3776</td>
<td>20489-53-6</td>
<td>1407</td>
<td>JECFA specification (JECFA, 2005b)</td>
<td>0.6</td>
<td>No safety concern a)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>07.159</td>
<td>d-Fenchone</td>
<td></td>
<td>2479</td>
<td>551</td>
<td>4695-62-9</td>
<td>1396</td>
<td>JECFA specification (JECFA, 2005b)</td>
<td>6</td>
<td>No safety concern a)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>09.017</td>
<td>Bornyl acetate</td>
<td></td>
<td>2159</td>
<td>207</td>
<td>76-49-3</td>
<td>1387</td>
<td>JECFA specification (JECFA, 2005b)</td>
<td>18</td>
<td>No safety concern a) Category B b)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>09.082</td>
<td>Bornyl formate</td>
<td></td>
<td>2161</td>
<td>349</td>
<td>7492-41-3</td>
<td>1389</td>
<td>JECFA specification (JECFA, 2005b)</td>
<td>1.2</td>
<td>No safety concern a) Category B b)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>09.131</td>
<td>Isobornyl propionate</td>
<td></td>
<td>2163</td>
<td>412</td>
<td>2756-56-1</td>
<td>1391</td>
<td>JECFA specification (JECFA, 2005b)</td>
<td>2.6</td>
<td>No safety concern a) Category B b)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>09.153</td>
<td>Bornyl valerate</td>
<td></td>
<td>2164</td>
<td>471</td>
<td>7549-41-9</td>
<td>1392</td>
<td>JECFA specification (JECFA, 2005b)</td>
<td>ND</td>
<td>No safety concern a) Category B b)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*CASrn in the Register refers to the racemate.*
Table 3 Supporting Substances Summary

<table>
<thead>
<tr>
<th>FL-no</th>
<th>EU Register name</th>
<th>Structural formula</th>
<th>FEMA no</th>
<th>CoE no</th>
<th>CAS no</th>
<th>JECFA no</th>
<th>Specification available</th>
<th>MSDI (EU) 1)</th>
<th>SCF status 2)</th>
<th>JECFA status 3)</th>
<th>CoE status 4)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>09.176</td>
<td>Isobornyl formate</td>
<td><img src="image1" alt="Structural formula" /></td>
<td>2162</td>
<td>565</td>
<td>1200-67-5</td>
<td>1390</td>
<td>JECFA specification (JECFA, 2005b)</td>
<td>0.61</td>
<td>No safety concern a) Category B b)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>09.218</td>
<td>Isobornyl acetate</td>
<td><img src="image2" alt="Structural formula" /></td>
<td>2160</td>
<td>2066</td>
<td>125-12-2</td>
<td>1388</td>
<td>JECFA specification (JECFA, 2005b)</td>
<td>890</td>
<td>No safety concern a) Category B b)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>09.269</td>
<td>Fenchyl acetate</td>
<td><img src="image3" alt="Structural formula" /></td>
<td>3390</td>
<td>11769</td>
<td>13851-11-1</td>
<td>1399</td>
<td>JECFA specification (JECFA, 2005b)</td>
<td>2.9</td>
<td>No safety concern a)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>09.319</td>
<td>Bornyl butyrate</td>
<td><img src="image4" alt="Structural formula" /></td>
<td>3907</td>
<td>13109-70-1</td>
<td>1412</td>
<td>JECFA specification (JECFA, 2005b)</td>
<td>ND</td>
<td>No safety concern a)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>09.456</td>
<td>Bornyl isovalerate</td>
<td><img src="image5" alt="Structural formula" /></td>
<td>2165</td>
<td>451</td>
<td>76-50-6</td>
<td>1393</td>
<td>JECFA specification (JECFA, 2005b)</td>
<td>0.12</td>
<td>No safety concern a) Category B b)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>09.457</td>
<td>Isobornyl isovalerate</td>
<td><img src="image6" alt="Structural formula" /></td>
<td>2166</td>
<td>452</td>
<td>7779-73-9</td>
<td>1394</td>
<td>JECFA specification (JECFA, 2005b)</td>
<td>0.012</td>
<td>No safety concern a) Category B b)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

ND) No intake data reported.
1) EU MSDI: Amount added to food as flavouring substance in (kg / year) x 10E9 / (0.1 x population in Europe (= 375 x 10E6) x 0.6 x 365) = µg/capita/day.
2) Category 1: Considered safe in use, Category 2: Temporarily considered safe in use, Category 3: Insufficient data to provide assurance of safety in use, Category 4: Not acceptable due to evidence of toxicity.
3) No safety concern at estimated levels of intake.
4) Category A: Flavouring substance, which may be used in foodstuffs, Category B: Flavouring substance which can be used provisionally in foodstuffs.
a) (JECFA, 2005c).
b) (CoE, 1992).
ANNEX I  PROCEDURE FOR THE SAFETY EVALUATION

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

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

Class I contains flavourings that have simple chemical structures and efficient modes of metabolism, which would suggest a low order of oral toxicity. Class II contains flavourings that have structural features that are less innocuous, but are not suggestive of toxicity. Class III comprises flavourings that have structural features that permit no strong initial presumption of safety, or may even suggest significant toxicity (Cramer et al., 1978). The thresholds of concern for these structural classes of 1800, 540 or 90 microgram/person/day, respectively, are derived from a large database containing data on subchronic and chronic animal studies (JECFA, 1996a).

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

- can the flavourings be predicted to be metabolised to innocuous products\(^6\) (Step 2)?
- do their exposures exceed the threshold of concern for the structural class (Step A3 and B3)?
- are the flavourings or their metabolites endogenous\(^7\) (Step A4)?
- does a NOAEL exist on the flavourings or on structurally related substances (Step A5 and B4)?

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

---
\(^6\) "Innocuous metabolic products": Products that are known or readily predicted to be harmless to humans at the estimated intakes of the flavouring agent" (JECFA, 1997a).
\(^7\) "Endogenous substances": Intermediary metabolites normally present in human tissues and fluids, whether free or conjugated; hormones and other substances with biochemical or physiological regulatory functions are not included (JECFA, 1997a).
The Procedure is not to be applied to flavourings with existing unresolved problems of toxicity.

Therefore, the right is reserved to use alternative approaches if data on specific flavourings warranted such actions.
Procedure for Safety Evaluation of Chemically Defined Flavouring Substances

Step 1. Decision tree structural class

Step 2. Can the substance be predicted to be metabolised to innocuous products?

Step A3. Do the conditions of use result in an intake greater than the threshold of concern for the structural class?

Yes

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

Yes

Do the conditions of use result in an intake greater than the threshold of concern for the structural class?

No

Substance would not be expected to be of safety concern

Yes

No

Step B3.

No

Step B4.

Does a NOAEL exist for the substance which provides an adequate margin of safety under conditions of intended use, or does a NOAEL exist for structurally related substances which is high enough to accommodate any perceived difference in toxicity between the substance and the related substances?

No

Additional data required

No

Yes

Is the substance or are its metabolites endogenous?

Yes

No

Step A4.

No

Step A5.

Does a NOAEL exist for the substance which provides an adequate margin of safety under conditions of intended use, or does a NOAEL exist for structurally related substances which is high enough to accommodate any perceived difference in toxicity between the substance and the related substances?

No

Yes

Yes

No

Figure I.1 Procedure for Safety Evaluation of Chemically Defined Flavouring Substances
Annex II Use Levels / mTAMDI

II.1 Normal and Maximum Use Levels

For each of the 18 Food categories (Table II.1.1) in which the candidate substances are used, Flavour Industry reports a “normal use level” and a “maximum use level” (EC, 2000a). According to the Industry the “normal use” is defined as the average of reported usages and “maximum use” is defined as the 95th percentile of reported usages (EFFA, 2002i). The normal and maximum use levels in different food categories (EC, 2000a) have been extrapolated from figures derived from 12 model flavouring substances (EFFA, 2004e).

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

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

The “normal and maximum use levels” are provided by Industry for the four candidate substances in the present flavouring group (Table II.1.2).

Table II.1.2 Normal and Maximum use levels (mg/kg) for candidate substances in FGE.47 (EFFA, 2005f; EFFA, 2007a)

<table>
<thead>
<tr>
<th>FL.-no</th>
<th>Normal use levels (mg/kg)</th>
<th>Maximum use levels (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>01.0</td>
<td>02.0</td>
</tr>
<tr>
<td>07.171</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>07.171</td>
<td>15</td>
<td>10</td>
</tr>
<tr>
<td>09.584</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td>09.584</td>
<td>35</td>
<td>25</td>
</tr>
<tr>
<td>09.848</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td>09.848</td>
<td>35</td>
<td>25</td>
</tr>
<tr>
<td>09.888</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td>09.888</td>
<td>35</td>
<td>25</td>
</tr>
</tbody>
</table>

II.2 mTAMDI Calculations
The method for calculation of modified Theoretical Added Maximum Daily Intake (mTAMDI) values is based on the approach used by SCF up to 1995 (SCF, 1995). The assumption is that a person consumes the amount of flavourable foods and beverages listed in Table II.2.1. These consumption estimates are then multiplied by the reported use levels in the different food categories and summed up.

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

The mTAMDI calculations are based on the normal use levels reported by Industry. The seven food categories used in the SCF TAMDI approach (SCF, 1995) correspond to the 18 food categories as outlined in Commission Regulation (EC) No 1565/2000 (EC, 2000a) and reported by the Flavour Industry in the following way (see Table II.2.2):

Beverages (SCF, 1995) correspond to food category 14.1 (EC, 2000a)

Foods (SCF, 1995) correspond to the food categories 1, 2, 3, 4.1, 4.2, 6, 7, 8, 9, 10, 13, and/or 16 (EC, 2000a)

Exception a (SCF, 1995) corresponds to food category 5 and 11 (EC, 2000a)

Exception b (SCF, 1995) corresponds to food category 15 (EC, 2000a)

Exception c (SCF, 1995) corresponds to food category 14.2 (EC, 2000a)

Exception d (SCF, 1995) corresponds to food category 12 (EC, 2000a)

Exception e (SCF, 1995) corresponds to others, e.g. chewing gum.

<table>
<thead>
<tr>
<th>Food categories according to Commission Regulation (EC) No 1565/2000</th>
<th>Distribution of the seven SCF food categories</th>
</tr>
</thead>
<tbody>
<tr>
<td>Key</td>
<td>Food category</td>
</tr>
<tr>
<td>-----</td>
<td>----------------</td>
</tr>
<tr>
<td>01</td>
<td>Dairy products, excluding products of category 02.0</td>
</tr>
<tr>
<td>02</td>
<td>Fats and oils, and fat emulsions (type water-in-oil)</td>
</tr>
<tr>
<td>03</td>
<td>Edible ices, including sherbet and sorbet</td>
</tr>
<tr>
<td>04.1</td>
<td>Processed fruit</td>
</tr>
<tr>
<td>04.2</td>
<td>Processed vegetables (incl. mushrooms &amp; fungi, roots &amp; tubers, pulses and legumes), and nuts &amp; seeds</td>
</tr>
<tr>
<td>05</td>
<td>Confectionery</td>
</tr>
<tr>
<td>06</td>
<td>Cereals and cereal products, incl. flours &amp; starches from roots &amp; tubers, pulses &amp; legumes, excluding bakery</td>
</tr>
<tr>
<td>07</td>
<td>Bakery wares</td>
</tr>
<tr>
<td>08</td>
<td>Meat and meat products, including poultry and game</td>
</tr>
<tr>
<td>09</td>
<td>Fish and fish products, including molluscs, crustaceans and echinoderms</td>
</tr>
<tr>
<td>10</td>
<td>Eggs and egg products</td>
</tr>
</tbody>
</table>
The mTAMDI values (see Table II.2.3) are presented for each of the four flavouring substances in the present Flavouring Group Evaluation, for which Industry has provided use and use levels (EFFA, 2005f; EFFA, 2007a). The mTAMDI values are only given for highest reported normal use.

<table>
<thead>
<tr>
<th>FL-no</th>
<th>EU Register name</th>
<th>mTAMDI (µg/person/day)</th>
<th>Structural class</th>
<th>Threshold of concern (µg/person/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>09.584</td>
<td>Isobornyl isobutyrate</td>
<td>2300</td>
<td>Class I</td>
<td>1800</td>
</tr>
<tr>
<td>09.848</td>
<td>(1S-endo)-1,7,7-Trimethylbicyclo[2.2.1]heptan-2-ol acetate</td>
<td>2300</td>
<td>Class I</td>
<td>1800</td>
</tr>
<tr>
<td>09.888</td>
<td>Isobornyl 2-methylbutyrate</td>
<td>1900</td>
<td>Class I</td>
<td>1800</td>
</tr>
<tr>
<td>07.171</td>
<td>Isopinocamphone</td>
<td>1000</td>
<td>Class II</td>
<td>540</td>
</tr>
</tbody>
</table>
### Annex III Metabolism

#### III.1 Introduction

The substances in this flavouring group evaluation consist of four substances of which three are esters of bicyclic secondary alcohols [FL-no: 09.584, 09.848 and 09.888] and one is a ketone [FL-no: 07.171].

#### III.2 Absorption, Distribution, Metabolism and Excretion

##### III.2.1 Hydrolysis of Esters

The esters within this group are expected to be hydrolysed in humans to their component alcohols and aliphatic carboxylic acids. Subsequently, the carboxylic acids are completely metabolised through recognised biochemical pathways (Nelson & Cox, 2000a). Ester hydrolysis is catalysed by classes of enzymes recognised as carboxylesterases (Heymann, 1980; White et al., 1990), the most important of which are the B-esterases. In mammals, these enzymes occur in most tissues (Heymann, 1980; Anders, 1989), but predominate in hepatocytes (Heymann, 1980).

##### III.2.2 Absorption, Distribution, Metabolism and Excretion of the alcohols and ketones

#### III.2.2.1 Absorption, Distribution and Excretion

In rabbits, greater than 90 % of an oral dose of d-, l-, or d,l-bornyl acetate was excreted in the urine as the glucuronic acid conjugate of borneol [FL-no: 02.016] (Williams, 1959).

Studies in humans, dogs and rabbits have shown that the secondary alcohols and ketones of this group are rapidly absorbed, distributed, metabolised and excreted mainly in the urine as glucuronide conjugates. Small amounts may be expired in exhaled air.

Case reports, in which ingestion of the structurally related substance camphor [FL-no: 07.215] resulted in toxicity in both adults and children within minutes of exposure (Jacobziner & Raybin, 1962; Phelan, 1976; Kopelman et al., 1979; Gibson et al., 1989), demonstrate rapid absorption of this substance. Rabbits gavaged with 1.9-3.5 mmol/kg bw [289-533 mg/kg bw] d-camphor excreted 59.1 % of the dose, conjugated with glucuronic acid in the urine within 24 hours (Robertson & Hussain, 1969). A group of 50 Sprague-Dawley rats was administered a single dose of 1,000 mg of 40 % camphor in cottonseed oil/kg bw (approximately 400 mg camphor) by gavage and killed at 0.25, 0.5, 0.75, 1.0, 1.5, 2.0, 4.0, 6.0, 8.0 or 10.0 hours following treatment. Blood samples were taken prior to death. Peak blood concentration of camphor occurred at 96 min, with an absorption half-life of 38 min and a plasma elimination half-life of 142 min. The authors considered these data to compare favourably to those in man (Dean et al., 1992).

The toxicokinetics of d,l-camphor were studied in B6C3F1 mice and F344 rats. In mice, camphor was rapidly eliminated from the plasma following a single intravenous injection of 50 mg/kg bw with an elimination rate constant of 0.0337 and 0.0335/min for males and females, respectively, and a half-live of 21 minutes. In rats, camphor underwent biphasic elimination from plasma following a single intravenous injection of 6 mg/kg bw with an
elimination rate constant of 0.0038 and 0.0059/min for males and females, respectively, and half-lifes of 185 (males) and 118 (females) minutes (Grizzle et al., 1996).

In a case report, a pregnant woman (week 40) accidentally ingested 12 g camphorated oil (% camphor not specified) and 36 hours later gave birth to a cyanotic baby exhibiting no respiration. The baby died within 30 min. The presence of camphor was noted at 15 min in maternal circulation, at 20 hours in amniotic fluid, and at 36 hours in cord blood, infant brain, liver and kidneys (Riggs et al., 1965).

Approximately 80 % of a 2,000 mg oral dose of d-borneol [FL-no: 02.016] given to humans (sex and number not specified) was excreted within 10 hours (Williams, 1959a).

### III.2.2.2 Metabolism

The major metabolic pathway of the ketones involves reduction to the corresponding secondary alcohols, which are subsequently excreted primarily as the glucuronic acid conjugates (Williams, 1959; Lington & Bevan, 1994; Topping et al., 1994). Metabolites excreted into the bile containing a double bond may be reduced to the corresponding dihydro derivatives by the gut microflora (Krasavage et al., 1982). In addition to reductive pathways, alicyclic ketones and, to a lesser extent, secondary alcohols containing an alkyl side chain undergo oxidation of the side chain to form polar poly-oxygenated metabolites that are excreted, either unchanged or as the glucuronide, or as sulphate conjugates, mainly in the urine.

The bicyclic secondary alcohols are rapidly conjugated in humans, dogs and rabbits with glucuronic acid and excreted via the urine. In humans (Figure III.1), 81 and 94 % of an oral dose of 1,000 and 2,000 mg of borneol [FL-no: 02.016], respectively, was excreted as the glucuronic acid conjugate within 24 hours (Wagreich et al., 1941). At 10 hours following ingestion of 2,000 mg of borneol, 81 % of the dose was detected as the glucuronic acid conjugate in human urine (Quick, 1928b). At a higher dose level (i.e. 3,500 mg borneol), 69 % of the dose was detected in human urine after 6 hours (Quick, 1928b). Similar conjugation has been reported in dogs (Quick, 1927; Pryde & Williams, 1934) and an increased level of β-glucuronidase has been reported in several tissues of dogs orally administered borneol (Fishman, 1940). At oral doses of 100 mg/kg per day and higher, rats fed borneol over a period of 10 days showed an increase in the urinary levels of total glucuronic acid, o-glucuronide, and ascorbic acid (Tamura et al., 1962). Fenchyl alcohol administered by gavage to rabbits also was excreted via urine as a glucuronide conjugate (Hämäläinen, 1912).

![Figure III.1 Metabolism of borneol in humans](image)

In rats, pre-treated for 3 days with borneol (intraperitoneal or dietary exposure), increases of approximately 25 % were reported in the activities of biphenyl 4-hydroxylase, glucuronyl...
transferase, 4-nitrobenzoate reductase and CYP-450 (Parke & Rahman, 1969). Rats (4/group) given 250 mg/kg bw of l-borneol by intraperitoneal injection daily for 3 days showed no significant increase in liver UDP-glucuronosyltransferase (UDPGT) activity. After daily treatment for up to four weeks slight increases in the activity were observed. The authors concluded that over the short periods of exposure, detoxication of borneol does not require the induction of UDPGT; however, longer exposure periods, at high dose levels, necessitate UDPGT induction (Boutin et al., 1983). Conversely, in rats intubated with 3 mmol/kg bw of borneol [463 mg/kg bw in olive oil] the activity of hepatic S-3-hydroxy-3-methylglutaryl coenzyme A reductase was decreased by approximately 50 % 17 hours after dosing (Clegg et al., 1980).

Cytochrome P4502B1 was induced in rat liver microsomes isolated from rats injected intraperitoneally with 300 mg/kg bw borneol (Hiroi et al., 1995), indicating that oxidation may occur to a limited extent. Rats injected intraperitoneally with 1,000 mg/kg bw isobornyl acetate [FL-no: 09.848] for 3 days showed a minimum 2.0-fold increase in the activities of N-demethylase and NADPH cytochrome c reductase, and in CYP-450 content indicating that isobornyl acetate induces the microsomal mixed-function oxidase system (Cinti et al., 1976), which also suggests that oxidation of ring positions and ring substituents may occur.

Ingestion of 6 to 10 gram of camphor by humans resulted in urinary excretion of 3-, 5-, 8-, and 9-hydroxycamphor, 5-ketocamphor and the carboxylic acid of either 8- or 9-hydroxycamphor, unconjugated or conjugated with glucuronic acid (Köppel et al., 1982). A minor amount was exhaled in expired air. Hydroxylation products, predominantly 5-endo- and 5-exo-hydroxycamphor and a compound resembling 3-endo-hydroxycamphor, also have been reported when camphor was orally administered to dogs (1,000 mg per animal, 4 times per day via gelatine capsule for 7 days) or rabbits (300 mg per animal, single dose by gavage) (Leibman & Ortiz, 1973). The same camphor hydroxylation products, with a small amount of 2,5-bornanedione, were similarly identified in vitro following incubation with rat and rabbit liver fractions (Leibman & Ortiz, 1973). Similar hydroxylation products (4- and 5-hydroxyfenchone and p-apofenchone-3-carboxylic acid) were detected in the urine of dogs fed d-fenchone (Reinartz & Zanke, 1936). The metabolism of d-fenchone also demonstrates that hydroxylation of ring methyl substituents leads to the corresponding carboxylic acid derivatives.

In rabbit liver cytosol, d-camphor was reduced via an NADPH-dependent pathway to borneol and a small amount of isoborneol (Robertson & Hussain, 1969; Leibman & Ortiz, 1973). In rat liver, camphor induced members of the P450IIB sub-family, most likely P450b and/or P450e (Austin et al., 1988). Female Swiss albino mice gavaged with 50, 150 or 300 mg camphor/kg bw per day in olive oil for 20 days showed a statistically significant increase in CYP-450 and cytochrome b5, aryl hydrocarbon hydrolase, and glutathione S-transferase activities only at the highest dose level (Banerjee et al., 1995).

Other minor routes of metabolism of the bicyclic secondary alcohols include hydroxylation of an allylic position and oxidative cleavage of the strained ring in the bicyclic substance.

III.3 Conclusion
The available data demonstrate that the esters in this group are readily hydrolysed to the corresponding bicyclic secondary alcohols and are subsequently conjugated with glucuronic acid and excreted in the urine. Minor metabolic routes include oxidation of ring positions and substituents to yield oxygenated metabolites that are also readily excreted. The bicyclic ketone in the group undergoes reduction to the corresponding secondary alcohol followed by conjugation with glucuronic acid and excretion in the urine.
Annex IV  Toxicity

Oral acute toxicity data are available for none of the candidate substances of the present flavouring group evaluation from chemical group 8, but for nine supporting substances evaluated by JECFA at the 63rd meeting. The supporting substances are listed in brackets.

Table IV.1  Acute Toxicity

<table>
<thead>
<tr>
<th>Chemical Name [FL-no]</th>
<th>Species</th>
<th>Sex</th>
<th>LD$_{50}$ (mg/kg bw)</th>
<th>Reference</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Bornyl isovalerate [09.456])</td>
<td>Rat</td>
<td>NR</td>
<td>&gt;5000</td>
<td>(Denine, 1973b)</td>
<td></td>
</tr>
<tr>
<td>(Isoborneol [02.059])</td>
<td>Rat</td>
<td>NR</td>
<td>5200</td>
<td>(Moreno, 1977abp)</td>
<td></td>
</tr>
<tr>
<td>(Isobornyl formate [09.176])</td>
<td>Rat</td>
<td>NR</td>
<td>&gt;5000</td>
<td>(Levenstein, 1975p)</td>
<td></td>
</tr>
<tr>
<td>(Isobornyl acetate [09.218])</td>
<td>Rat</td>
<td>M</td>
<td>&gt;10,000</td>
<td>(Fogleman &amp; Margolin, 1970)</td>
<td></td>
</tr>
<tr>
<td>(Isobornyl propionate [09.131])</td>
<td>Rat</td>
<td>NR</td>
<td>&gt;5000</td>
<td>(Moreno, 1973al)</td>
<td></td>
</tr>
<tr>
<td>(Fenchyl alcohol [02.038])</td>
<td>Rat</td>
<td>NR</td>
<td>ND</td>
<td>(Moreno, 1976ad)</td>
<td></td>
</tr>
<tr>
<td>(Fenchyl acetate [09.269])</td>
<td>Rat</td>
<td>NR</td>
<td>&gt;5000</td>
<td>(Moreno, 1975s)</td>
<td></td>
</tr>
<tr>
<td>(1,10 Dihydroneootkatone [07.153])</td>
<td>Rat</td>
<td>NR</td>
<td>&gt;5 ml/kg</td>
<td>(Sedlacek, 1985)</td>
<td></td>
</tr>
</tbody>
</table>

M=Male; F=Female; NR=Not Reported; ND=No Data.
Subacute/subchronic/chronic/carcinogenic toxicity data are available for none of the candidate substance of the present flavouring group evaluation from chemical group 8 but for three supporting substances evaluated by JECFA at the 63rd meeting. The supporting substances are listed in brackets.

**Table IV.2 Subacute/Subchronic/Chronic/Carcinogenicity Studies**

<table>
<thead>
<tr>
<th>Chemical Name [FL-no]</th>
<th>Species; Sex</th>
<th>Route</th>
<th>Dose levels</th>
<th>Duration</th>
<th>NOAEL (mg/kg bw/day)</th>
<th>Reference</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Borneol [02.016])</td>
<td>Dog; NR 1/3</td>
<td>Gavage</td>
<td>526 mg/kg bw/day</td>
<td>31 days</td>
<td>526</td>
<td>(Miller et al., 1933)</td>
<td></td>
</tr>
<tr>
<td>(Isobornyl acetate [09.218])</td>
<td>Rat; M, F 3/30</td>
<td>Gavage</td>
<td>0, 15, 90, 270 mg/kg bw/day</td>
<td>91 days</td>
<td>15 (M) 90 (F)6</td>
<td>(Gaunt et al., 1971b)</td>
<td></td>
</tr>
<tr>
<td>(d-Fenchone [07.159])</td>
<td>Dog; NR 1/1</td>
<td>Oral</td>
<td>1064 mg/kg bw/day</td>
<td>16 days</td>
<td>&lt;1064</td>
<td>(Rimini, 1901)</td>
<td></td>
</tr>
</tbody>
</table>

M=Male; F=Female; NR=Not reported

2 Total number of test groups does not include control animals.

3 Total number per test group includes both male and female animals.

4 Study performed with either a single dose or multiple doses that produced no adverse effect. The value is therefore not a true NOEL, but is the highest dose level tested that produced no adverse effects. The actual NOEL may be higher.

5 Animals were gradually introduced to the final dose level of 1,300 mg/kg bw per day over a 2-month period.

6 Author specified a single NOEL of 15 mg/kg bw per day, without distinguishing between male and female rats.
No developmental and reproductive toxicity data are available for the candidate substances of the present flavouring group evaluation or for the supporting substances evaluated by JECFA at the 63rd meeting.

*In vitro* mutagenicity/genotoxicity data are available for none of the candidate substances of the present flavouring group evaluation but for two supporting substances evaluated by JECFA at the 63rd meeting.

### Table IV.4 Genotoxicity (*in vitro*)

<table>
<thead>
<tr>
<th>Chemical Name [FL-no]</th>
<th>Test System</th>
<th>Test Object</th>
<th>Concentration</th>
<th>Result</th>
<th>Reference</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Borneol [02.016])</td>
<td>Reverse mutation</td>
<td>Salmonella typhimurium TA98, TA100, TA97</td>
<td>1 mg/ml (1000 μg/ml)</td>
<td>Negative¹</td>
<td>(Azizan &amp; Blevins, 1995)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Reverse mutation</td>
<td>S. typhimurium TA98, TA100, TA1535, TA1537, TA1538</td>
<td>Up to 5 mg/plate (5000 μg/plate)</td>
<td>Negative</td>
<td>(Simmon et al., 1977)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>DNA repair</td>
<td>Bacillus subtilis M45- and H17</td>
<td>Up to 10 mg/disk</td>
<td>Positive</td>
<td>(Yoo, 1986)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mutation test</td>
<td>Escherichia coli WP2 uvrA (trp-)</td>
<td>0.4-3.2 mg/plate</td>
<td>Negative</td>
<td>(Yoo, 1986)</td>
<td></td>
</tr>
<tr>
<td>(Isobornyl propionate [09.131])</td>
<td>Reverse mutation</td>
<td>S. typhimurium TA98, TA100, TA1535, TA1537, TA1538</td>
<td>Up to 3.6 mg/plate (3600 μg/plate)</td>
<td>Negative</td>
<td>(Wild et al., 1983)</td>
<td></td>
</tr>
</tbody>
</table>

¹ Tested with and without metabolic activation

*In vivo* mutagenicity/genotoxicity data are available for none of the candidate substance of the present flavouring group evaluation and only for one supporting substance evaluated by JECFA at the 63rd meeting.

### Table IV.5 Genotoxicity (*in vivo*)

<table>
<thead>
<tr>
<th>Chemical Name [FL-no]</th>
<th>Test System</th>
<th>Test Object</th>
<th>Route</th>
<th>Dose</th>
<th>Result</th>
<th>Reference</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Isobornyl propionate [09.131])</td>
<td>Somatic mutation and recombination</td>
<td><em>Drosophila melanogaster</em></td>
<td>Oral</td>
<td>10 mM (2103 μg/ml)</td>
<td>Negative</td>
<td>(Wild et al., 1983)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Micronucleus formation</td>
<td>Mouse bone marrow cells</td>
<td>IP</td>
<td>841, 1893, and 2944 mg/kg bw</td>
<td>Negative¹</td>
<td>(Wild et al., 1983)</td>
<td></td>
</tr>
</tbody>
</table>

¹ Administered via intraperitoneal injection
REFERENCES


Hämäläinen J., 1912. [Concerning the behavior of alicyclic compounds with glucuronic acid in organisms]. Skand. Arch. Physiol. 27, 141-226. (In German)


Pryde, J., Williams, R.T., 1934. The biochemistry and physiology of glucuronic acid. IV. The occurrence of conjugated glucuronic acids in the animal body; observations on the conjugation of d- and l-borneol. V. The site and mechanism of the formation of conjugated glucuronic acid. Biochem. J. 28, 131-142.


