Flavouring Group Evaluation 27 (FGE.27): One aromatic lactone from chemical group 11

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

(Question No EFSA-Q-2003-170)

Adopted on 27 Sept 2007

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 Scientific Panel is asked to evaluate one flavouring substance in the Flavouring Group Evaluation 27 (FGE.27), using the procedure as referred to in the Commission Regulation (EC) No 1565/2000. This flavouring substance belongs to chemical group 11, Annex I of the Commission Regulation (EC) No 1565/2000.

The present Flavouring Group Evaluation 27 (FGE.27) deals with one aromatic lactone, phthalide [FL-no: 10.056]. No geometrical or optical isomers exist for this flavouring substance.

The flavouring substance is classified by the decision tree approach into structural class III.

The flavouring substance has been reported to occur naturally in food.

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 Flavouring 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

1 For citation purposes: Scientific Opinion of the Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food on a request from Commission on FGE.27: One aromatic lactone from chemical group 11. The EFSA Journal 2008) 806, 1-27
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 substance has a daily per capita intake as a flavouring of 0.8 microgram/capita/day, which is below the threshold of concern of 90 microgram/capita/day for a substance belonging to structural class III.

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

The candidate substance phthalide [FL-no: 10.056] is 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 candidate substance will not give rise to safety concerns at the estimated level of intake arising from its use as flavouring substance.

When using the mTAMDI approach the intake was estimated to be 3900 microgram/person/day for the candidate substance allocated to structural class III. The intake is above the threshold of concern of 90 microgram/person/day for structural class III. Therefore, for this substance more reliable exposure data are required. On the basis of such additional data, this flavouring substance should be reconsidered along the steps of the Procedure. Following this procedure additional toxicological data might become necessary.

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

Specifications including complete purity criteria for the material of commerce have been provided for the candidate substance. Information on identity test is missing for the candidate substance. Thus, the final evaluation of the material of commerce cannot be performed for the substance, pending further information.

**Key words:**

Flavourings, safety, aromatic lactone, phthalide.
Table of Contents
Panel Members .................................................................................................................. 1
Summary ............................................................................................................................. 1
Background ....................................................................................................................... 4
Terms of reference ........................................................................................................... 4
Acknowledgements .......................................................................................................... 4
Assessment ...................................................................................................................... 5
1. Presentation of the Substances in the Flavouring Group Evaluation 27 ...................... 5
   1.1. Description .................................................................................................................. 5
   1.2. Stereoisomers .............................................................................................................. 5
   1.3. Natural Occurrence in Food ....................................................................................... 5
2. Specifications ................................................................................................................. 5
3. Intake Data ..................................................................................................................... 5
   3.1. Estimated Daily per Capita Intake (MSDI Approach) .................................................. 6
   3.2. Intake Estimated on the Basis of the Modified TAMDI (mTAMDI) ............................. 7
4. Absorption, Distribution, Metabolism and Elimination ................................................. 8
5. Application of the Procedure for the Safety Evaluation of Flavouring Substances ....... 8
6. Comparison of the Intake Estimations Based on the MSDI Approach and mTAMDI Approach ............................................................. 9
7. Considerations of Combined Intakes from Use as Flavouring Substances .................. 9
8. Toxicity ........................................................................................................................ 9
   8.1. Acute Toxicity ........................................................................................................... 9
   8.2. Subacute, Subchronic, Chronic and Carcinogenicity Studies .................................... 9
   8.3. Developmental / Reproductive Toxicity Studies ....................................................... 10
   8.4. Genotoxicity Studies ............................................................................................. 10
Conclusions ..................................................................................................................... 10
Table 1: Specification Summary of the Substances in the Flavouring Group Evaluation 27 ........................................................................................................... 12
Table 2a: Summary of Safety Evaluation Applying the Procedure ..................................... 13
Table 2b: Evaluation Status of Hydrolysis Products of Candidate Esters ............................ 14
Table 3: Supporting Substances Summary ........................................................................ 15
Annex I: Procedure for the Safety Evaluation ................................................................. 16
Annex II: Use levels / mTAMDI ....................................................................................... 18
Annex III: Metabolism .................................................................................................... 20
Annex IV: Toxicity ......................................................................................................... 23
References ...................................................................................................................... 25
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 others 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, 2000), 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 prior to their authorisation and inclusion in a positive list according to Commission Regulation (EC) No 1565/2000 (EC, 2000).

ACKNOWLEDGEMENTS

The Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food wishes to thank Trine Husøy, Judith Amberg-Müller, Harriet Wallin, Jørn Gry, Vibe Belloft, Frederikke Bentzen, Karin Nørby and Trine Klein Reffstrup for their contribution to the draft opinion.
1. Presentation of the Substances in the Flavouring Group Evaluation 27

1.1. Description

The present Flavouring Group Evaluation 27 (FGE.27), using the procedure as referred to in the Commission Regulation (EC) No 1565/2000 (EC, 2000) (The Procedure – shown in schematic form in Annex I), deals with one aromatic lactone from chemical group 11, Annex I of Commission Regulation (EC) No 1565/2000 (EC, 2000). The flavouring substance under consideration, as well as its chemical Register name, FLAVIS- (FL-), Chemical Abstract Service- (CAS-), Council of Europe- (CoE-) and Flavor and Extract Manufacturers Association- (FEMA-) number, structure and specifications, are listed in Table 1.

The flavouring substance phthalide [FL-no: 10.056] (candidate substance) is closely related structurally to three flavouring substances (supporting substances) evaluated at the 61st JECFA meeting (JECFA, 2004a) in the group of “alicyclic, alicyclic-fused and aromatic-fused ring lactones”.

The candidate substance is listed in Tables 1 and 2a, the hydrolysis product in Table 2b and the supporting substances are listed in Table 3.

1.2. Stereoisomers

The candidate substance has no possibility for geometrical or optical isomers.

1.3. Natural Occurrence in Food

The candidate substance, phthalide [FL-no: 10.056] has been reported to occur in tomato and wine (TNO, 2000).

Quantitative data on the natural occurrence in food have been reported for the candidate substance: up to 0.07 mg/kg in wine.

2. Specifications

Purity criteria for the candidate substance have been provided by the Flavouring Industry (EFFA, 2004p) (Table 1).

Judged against the requirements in Annex II of Commission Regulation (EC) No 1565/2000 (EC, 2000), the purity criteria for the candidate substance is deficient in one of the parameters as no ID test is provided. Otherwise the specification is adequate (see 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 \textit{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 (e.g., it may underestimate the intake of consumers being loyal to products flavoured at the maximum use levels reported) (EC, 2000). 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. \textbf{Estimated Daily \textit{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 \textit{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 27 (FGE.27) the annual production volume of the candidate substance for use as flavouring substance in Europe was reported to be 6.6 kg (EFFA, 2004r). The estimated MSDI for the candidate substance from use as flavouring substance is 0.8 microgram/capita/day (Table 2a).

\(^2\) 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.
3.2. \textbf{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.

For the present evaluation of the candidate substance, information on food categories and normal and maximum use levels\textsuperscript{3,4,5} were submitted by the Flavour Industry (EFFA, 2004p; EFFA, 2007a). The candidate substance is used in flavoured food products divided into the food categories, outlined in Annex III of the Commission Regulation 1565/2000 (EC, 2000), 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.

\textbf{Table 3.1 Use of Candidate Substances}

<table>
<thead>
<tr>
<th>Food category</th>
<th>Description</th>
<th>Flavouring used</th>
</tr>
</thead>
<tbody>
<tr>
<td>Category 1</td>
<td>Dairy products, excluding products of category 2</td>
<td>Yes</td>
</tr>
<tr>
<td>Category 2</td>
<td>Fats and oils, and fat emulsions (type water-in-oil)</td>
<td>Yes</td>
</tr>
<tr>
<td>Category 3</td>
<td>Edible ices, including sherbet and sorbet</td>
<td>Yes</td>
</tr>
<tr>
<td>Category 4.1</td>
<td>Processed fruits</td>
<td>Yes</td>
</tr>
<tr>
<td>Category 4.2</td>
<td>Processed vegetables (incl. mushrooms &amp; fungi, roots &amp; tubers, pulses and legumes), and nuts &amp; seeds</td>
<td>No</td>
</tr>
<tr>
<td>Category 5</td>
<td>Confectionery</td>
<td>Yes</td>
</tr>
<tr>
<td>Category 6</td>
<td>Cereals and cereal products, incl. flours &amp; starches from roots &amp; tubers, pulses &amp; legumes, excluding bakery</td>
<td>Yes</td>
</tr>
<tr>
<td>Category 7</td>
<td>Bakery wares</td>
<td>Yes</td>
</tr>
<tr>
<td>Category 8</td>
<td>Meat and meat products, including poultry and game</td>
<td>Yes</td>
</tr>
<tr>
<td>Category 9</td>
<td>Fish and fish products, including molluscs, crustaceans and echinoderms</td>
<td>Yes</td>
</tr>
<tr>
<td>Category 10</td>
<td>Eggs and egg products</td>
<td>No</td>
</tr>
<tr>
<td>Category 11</td>
<td>Sweeteners, including honey</td>
<td>No</td>
</tr>
<tr>
<td>Category 12</td>
<td>Salts, spices, soups, sauces, salads, protein products etc.</td>
<td>Yes</td>
</tr>
<tr>
<td>Category 13</td>
<td>Foodstuffs intended for particular nutritional uses</td>
<td>Yes</td>
</tr>
<tr>
<td>Category 14.1</td>
<td>Non-alcoholic (&quot;soft&quot;) beverages, excl. dairy products</td>
<td>Yes</td>
</tr>
<tr>
<td>Category 14.2</td>
<td>Alcoholic beverages, incl. alcohol-free and low-alcoholic counterparts</td>
<td>Yes</td>
</tr>
<tr>
<td>Category 15</td>
<td>Ready-to-eat savouries</td>
<td>Yes</td>
</tr>
<tr>
<td>Category 16</td>
<td>Composite foods (e.g. casseroles, meat pies, mincemeat) - foods that could not be placed in categories 1 – 15</td>
<td>Yes</td>
</tr>
</tbody>
</table>

According to the Flavour Industry the normal use levels for the candidate substance are in the range of 2 - 20 mg/kg food, and the maximum use levels are in the range of 10 - 100 mg/kg (EFFA, 2002i; EFFA, 2004p; EFFA, 2007a).

\textsuperscript{3} "Normal use" is defined as the average of reported usages and "maximum use" is defined as the 95\textsuperscript{th} percentile of reported usages (EFFA, 2002i).

\textsuperscript{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).

\textsuperscript{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).
The mTAMDI value for the candidate substance belonging to structural class III is 3900 microgram/person/day.

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

4. Absorption, Distribution, Metabolism and Elimination

The candidate substance phthalide [FL-no: 10.056] is expected to be hydrolysed to the corresponding benzoic acid derivative, 2-hydroxymethyl benzoic acid, before absorption or upon entering systemic circulation. 2-Hydroxymethyl benzoic acid is anticipated to be further metabolised by conjugation to glycine and to be excreted in the urine, as the major pathway. As minor pathways it is likely that the hydroxymethyl group is conjugated with glucuronic acid, followed by excretion, or that the hydroxymethyl group is further metabolised to a carboxylic acid group yielding phthalic acid. As another minor pathway, phthalide might be hydroxylated at the benzene ring. Overall, it is concluded that phthalide is 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 one candidate substance from chemical group 11 the Procedure as outlined in Annex I was applied, based on the MSDI approach. The stepwise evaluations of the substance are summarised in Table 2a.

Step 1

The candidate substance phthalide [FL-no: 10.056] is classified according to the decision tree approach by Cramer et al. (Cramer et al., 1978) into structural class III.

Step 2

It can be anticipated that the candidate substance phthalide [FL-no: 10.056] is metabolised to innocuous products. Accordingly the evaluation of the candidate substance proceeds via the A-side of the Procedure scheme.

Step A3

The candidate substance [FL-no: 10.056], which has been assigned to structural class III, has an estimated European daily per capita intake (MSDI) of 0.8 microgram. This intake is below the threshold of concern of 90 microgram/person/day for structural class III. Accordingly, this candidate substance does not pose a safety concern when used as a flavouring substance at the estimated level of intake, based on the MSDI approach.
6. Comparison of the Intake Estimations Based on the MSDI Approach and mTAMDI Approach

The estimated intake for the candidate substance in structural class III based on the mTAMDI is 3900 microgram/person/day, which is above the threshold of concern of 90 microgram/person/day. For comparison of the intake estimates based on the MSDI approach and the mTAMDI approach see Table 6.1.

For the candidate substance 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>10.056</td>
<td>Phthalide</td>
<td>0.8</td>
<td>3900</td>
<td>Class III</td>
<td>90</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.

The candidate substance is structurally related to three supporting substances evaluated by the JECFA at its 61st meeting (JECFA, 2004a). Based on reported production volumes, European per capita intakes (MSDI) could be estimated for all three supporting substances. The total combined intake of the candidate and supporting substances is approximately 30 microgram/capita/day, which is below the threshold of concern of 90 microgram/person/day for structural class III substances.

8. Toxicity

8.1. Acute Toxicity

Data are available for the candidate substance [FL-no: 10.056] and for three structurally related supporting substances [FL-no: 10.005, 10.024 and 10.025] evaluated by JECFA in 2003 (JECFA, 2004a). The oral LD_{50} values are between 1700 and 5500 mg/kg body weight (bw) in rats and mice.

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

8.2. Subacute, Subchronic, Chronic and Carcinogenicity Studies

No data are available on subacute, subchronic, chronic or carcinogenic studies on the candidate substance. Only one poorly reported 90 day study is available on one of the supporting substances [FL-no: 10.005].
The study is summarised in Annex IV, Table IV.2.

8.3. Developmental / Reproductive Toxicity Studies

No data are available on developmental or reproductive toxicity for the candidate substance or for supporting substances.

8.4. Genotoxicity Studies

There are no data available on the candidate substance. Data from in vitro tests are available for the supporting substance 3-propylideneephthalide [FL-no: 10.005].

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

The only in vitro study available is summarised in Annex IV, Table IV.4.

Conclusion on genotoxicity:

The genotoxicity for the candidate substance could not be assessed adequately. However, this does not preclude evaluation of phthalide [FL-no: 10.056] through the Procedure (SCF, 1999).

CONCLUSIONS

The present Flavouring Group Evaluation 27 (FGE.27) deals with one aromatic lactone, phthalide [FL-no: 10.056].

No geometrical or optical isomers exist for this flavouring substance.

The flavouring substance is classified by the decision tree approach into structural class III.

It has been reported that the flavouring substance can occur naturally in wine and tomato.

According to the default MSDI approach, the substance has a daily per capita intake as a flavouring substance of 0.8 microgram/capita/day, which is below the threshold of concern of 90 microgram/person/day for a substance belonging to structural class III.

On the basis of the reported annual production in Europe (MSDI approach), the total combined estimated level of intake of the candidate substance and three supporting substances is approximately 30 microgram/capita/day, which is below the threshold of concern for structural class III substances of 90 microgram/person/day.

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

The candidate substance phthalide [FL-no: 10.056] is 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 on the basis of the default MSDI approach that the candidate substance will not give rise to safety concerns at the estimated level of intake arising from its use as flavouring substance.
When using the mTAMDI approach the intake was estimated to be 3900 microgram/person/day for the candidate substance allocated to structural class III. The intake is above the threshold of concern of 90 microgram/person/day for structural class III. Therefore, for this substance more reliable exposure data are required. On the basis of such additional data, this flavouring substance should be reconsidered along the steps of the Procedure. Following this procedure additional toxicological data might become necessary.

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

Specification including complete purity criteria for the material of commerce has been provided for the candidate substance. Information on identity test is missing for the candidate substance. Thus, the final evaluation of the material of commerce cannot be performed for the substance, pending further information.
**Table 1: Specification Summary of the Substances in the Flavouring Group Evaluation 27**

<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</th>
<th>Refrac. Index 4) Spec. gravity 5)</th>
<th>Specification comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>10.056</td>
<td>Phthalide</td>
<td><img src="image" alt="Phthalide structure" /></td>
<td>4195 87-41-2</td>
<td>Solid C₈H₆O₂ 134.13</td>
<td>Practically insoluble or insoluble 1 ml in 1 ml</td>
<td>290 73</td>
<td>n.a. n.a.</td>
<td>ID 6)</td>
</tr>
</tbody>
</table>

1) Solubility in water, if not otherwise stated.
2) Solubility in 95% ethanol, if not otherwise stated.
3) At 1013.25 kPa, if not otherwise stated.
4) At 20°C, if not otherwise stated.
5) At 25°C, if not otherwise stated.
6) ID: Missing identification test.

n.a.: not applicable.
### 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>
</table>
| 10.056 | Phthalide | ![Phthalide Structure](image) | 0.8 | Class III A3: Intake below threshold | 4) | 8) | 1) EU MSDI: Amount added to food as flavour in (kg / year) x 10E9 / (0.1 x population in Europe (= 375 x 10E6) x 0.6 x 365) = μg/capita/day. 
2) Thresholds of concern: Class I = 1800, Class II = 540, Class III = 90 μg/person/day. 
3) Procedure path A substances can be predicted to be metabolised to innocuous products. Procedure path B substances cannot. 
4) No safety concern based on intake calculated by the MSDI approach of the named compound. 
5) Data must be available on the substance or closely related substances to perform a safety evaluation. 
6) No safety concern at estimated level of intake of the material of commerce meeting the specification of Table 1 (based on intake calculated by the MSDI approach). 
7) Tentatively regarded as presenting no safety concern (based on intake calculated by the MSDI approach) pending further information on the purity of the material of commerce and/or information on stereoisomerism. 
8) No conclusion can be drawn due to lack of information on the purity of the material of commerce. |
### Table 2B: Evaluation Status of Hydrolysis Products of Candidate Esters

<table>
<thead>
<tr>
<th>FL-no</th>
<th>EU Register name</th>
<th>Structural formula</th>
<th>SCF status 1)</th>
<th>JECFA status 2)</th>
<th>CoE status 3)</th>
<th>EFSA status</th>
<th>Structural class 4)</th>
<th>Procedure path (JECFA) 5)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2-(Hydroxymethyl)benzoic acid</td>
<td><img src="image" alt="Structural formula" /></td>
<td>Not evaluated as flavour</td>
<td>Not evaluated as flavour</td>
<td>Not evaluated as flavour</td>
<td></td>
<td></td>
<td></td>
<td>Not in Register.</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.
### 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) (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>10.005</td>
<td>3-Propylidenephthalide</td>
<td><img src="image" alt="Formula" /></td>
<td>2852</td>
<td>494</td>
<td>17369-59-4</td>
<td>1168</td>
<td>JECFA specification (JECFA, 2003b)</td>
<td>17</td>
<td>No safety concern a) Category B b)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10.024</td>
<td>3-Butylidenephthalide</td>
<td><img src="image" alt="Formula" /></td>
<td>3333</td>
<td>10083</td>
<td>551-08-6</td>
<td>1170</td>
<td>JECFA specification (JECFA, 2003b)</td>
<td>8.6</td>
<td>No safety concern a)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10.025</td>
<td>3-Butylphthalide</td>
<td><img src="image" alt="Formula" /></td>
<td>3334</td>
<td>10084</td>
<td>6066-49-5</td>
<td>1169</td>
<td>JECFA specification (JECFA, 2003b)</td>
<td>0.49</td>
<td>No safety concern a)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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, 2004a).
   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, 2000), 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.

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.

---

\(^6\) "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).
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

Step A4.
Is the substance or are its metabolites endogenous?

Yes

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

Step B3.
Do the conditions of use result in an intake greater than the threshold of concern for the structural class?

Yes

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

Step B5.

No

Substance would not be expected to be of safety concern

Yes

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

Yes

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, 2000). According to the Industry the "normal use” is defined as the average of reported usages and "maximum use" is defined as the 95th percentile of reported usages (EFFA, 2002i). 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).

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

<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 (&quot;soft&quot;) 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 candidate substance 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.27 (EFFA, 2002i; EFFA, 2004p; EFFA, 2007a)

<table>
<thead>
<tr>
<th>FL-no</th>
<th>Food Category</th>
</tr>
</thead>
<tbody>
<tr>
<td>10.056</td>
<td>Normal use levels (mg/kg)</td>
</tr>
<tr>
<td>01.0</td>
<td>02.0</td>
</tr>
<tr>
<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 II.2.1 Estimated amount of flavourable foods, beverages, and exceptions assumed to be consumed per person per day (SCF, 1995)

<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, 2000) 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, 2000)
- 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, 2000)
- Exception a (SCF, 1995) corresponds to food categories 5 and 11 (EC, 2000)
- Exception b (SCF, 1995) corresponds to food category 15 (EC, 2000)
- Exception c (SCF, 1995) corresponds to food category 14.2 (EC, 2000)
- Exception d (SCF, 1995) corresponds to food category 12 (EC, 2000)
- Exception e (SCF, 1995) corresponds to others, e.g. chewing gum.

<table>
<thead>
<tr>
<th>Key</th>
<th>Food category</th>
<th>Distribution of the seven SCF food categories</th>
</tr>
</thead>
<tbody>
<tr>
<td>01</td>
<td>Dairy products, excluding products of category 02.0</td>
<td>Food</td>
</tr>
<tr>
<td>02</td>
<td>Fats and oils, and fat emulsions (type water-in-oil)</td>
<td>Food</td>
</tr>
<tr>
<td>03</td>
<td>Edible ices, including sherbet and sorbet</td>
<td>Food</td>
</tr>
<tr>
<td>04.1</td>
<td>Processed fruit</td>
<td>Food</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>
<td>Food</td>
</tr>
<tr>
<td>05</td>
<td>Confectionery</td>
<td>Exception a</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>
<td>Food</td>
</tr>
<tr>
<td>07</td>
<td>Bakery wares</td>
<td>Food</td>
</tr>
<tr>
<td>08</td>
<td>Meat and meat products, including poultry and game</td>
<td>Food</td>
</tr>
<tr>
<td>09</td>
<td>Fish and fish products, including molluscs, crustaceans and echinoderms</td>
<td>Food</td>
</tr>
<tr>
<td>10</td>
<td>Legumes and egg products</td>
<td>Food</td>
</tr>
<tr>
<td>11</td>
<td>Sweeteners, including honey</td>
<td>Exception a</td>
</tr>
<tr>
<td>12</td>
<td>Salts, spices, soups, sauces, salads, protein products, etc.</td>
<td>Exception d</td>
</tr>
<tr>
<td>13</td>
<td>Foods intended for particular nutritional uses</td>
<td>Food</td>
</tr>
<tr>
<td>14.1</td>
<td>Non-alcoholic (“soft”) beverages, excl. dairy products</td>
<td>Beverages</td>
</tr>
<tr>
<td>14.2</td>
<td>Alcoholic beverages, incl. alcohol-free and low-alcoholic counterparts</td>
<td>Exception c</td>
</tr>
<tr>
<td>15</td>
<td>Ready-to-eat savouries</td>
<td>Exception b</td>
</tr>
<tr>
<td>16</td>
<td>Composite foods (e.g. casseroles, meat pies, mincemeat) - foods that could not be placed in categories 01.0 - 15.0</td>
<td>Food</td>
</tr>
</tbody>
</table>

The mTAMDI value (see Table II.2.3) is presented for the flavouring substance in the present Flavouring Group Evaluation, for which Industry has provided use and use levels (EFFA, 2002i; EFFA, 2004p; EFFA, 2007a). The mTAMDI value is only given for highest reported normal use.
ANNEX III: METABOLISM

This flavouring group consists of one candidate substance, phthalide [FL-no: 10.056], which is an aromatic lactone.

III.1 Hydrolysis

No studies on the hydrolysis of the phthalide are found. Hydrolysis studies on structurally related gamma-lactones, like gamma-butyrolactone, have been performed. Whole rat blood was found to convert gamma-butyrolactone to gamma-hydroxybutyric acid very rapidly with a half-time of conversion of less than one minute. Serum was substantially more active than plasma and sera from rabbits, guinea pigs, cats and humans were also found to hydrolyse gamma-butyrolactone. Other tissues from the rat (brain, liver, kidney, heart, lung, skeletal muscle and intestine) were examined for lactonase activity, but only the liver was found to have any substantial activity (Roth & Giarman, 1966). These findings are supported by Fishbein and Bessman (Fishbein & Bessman, 1966).

A one-hour incubation of 1 mmol 4,4-dibutyl-gamma-butyrolactone with 50 ml of simulated intestinal fluid (consisting of an aqueous mixture of monobasic potassium phosphate, sodium hydroxide and pancreatin adjusted to pH 7.5) resulted in 92 % hydrolysis to yield the ring-opened hydroxycarboxylic acids (Morgareidge, 1962b). Incubation of 1 mmol gamma-valerolactone and gamma-undecalactone with 50 ml and 100 ml of simulated intestinal fluid resulted in 32 and 50 % hydrolysis of gamma-valerolactone and 58 and 62 % hydrolysis of gamma-undecalactone within four hours, respectively (Morgareidge, 1962b).

One hour after incubation of 200 mg gamma-valerolactone, gamma-nonalactone and gamma-undecalactone at pH 7.5 with 50 ml of whole rat liver homogenate 93, 80 and 30 % hydrolysis, respectively, had occurred (Morgareidge, 1963a). Increasing the pH to 8.0 with the same experimental conditions resulted in 87 % and 45 % hydrolysis of gamma-nonalactone and gamma-undecalactone, respectively (Morgareidge, 1963a).

In humans, paraoxonase (PON1), a serum enzyme belonging to the class of A-esterases, is known to rapidly hydrolyse a broad range of aliphatic lactone substrates including beta-, gamma- (such as gamma-butyrolactone, gamma-valerolactone and gamma-decanolactone), delta-, and omega-lactones, lactones fused to alicyclic rings such as 1,5-oxabicyclooctenone, and lactones fused to aromatic rings such as 2-coumaranone and 4-hydroxy-2-coumaranone (Billecke et al., 2000). The relative rates of hydrolysis are more rapid for lactones fused to benzene rings than for the non-fused aliphatic lactones. Incubation of 1 mM of R-type PON1 with 2-coumaranone resulted in a hydrolysis rate of 13.5 micromol/min/ml enzyme preparation suggesting that PON1 has a high affinity for aromatic lactones. Gamma-homogentisic acid lactone (2,5-dihydroxyphenylethanoic acid lactone) and 2-hydroxyphenylethanoic acid gamma-lactone are rapidly hydrolysed (50 and 13.5 micromol/min/ml, respectively) to the corresponding hydroxycarboxylic acids in the presence of 1 mM of R-type PON1 (Billecke et al., 2000).

These data on the hydrolysis on a wide range of lactones structurally related to phthalide suggest that phthalide will be hydrolysed either in the gastrointestinal tract prior to absorption or upon entering systemic circulation.

III.2 Absorption, Distribution and Excretion

No studies on absorption, distribution and excretion have been found for the candidate substance phthalide.
III.3 Metabolism

No information on biotransformation is available for the candidate substance, phthalide.

During absorption phthalide is anticipated to be hydrolysed to the benzoic acid derivative, 2-hydroxymethyl benzoic acid, which will be further metabolised. The metabolism of the structurally related compound benzoic acid is well known (JECFA, 1996a). The major urinary metabolite in rats exposed orally to benzoic acid is benzoic acid conjugated with glycine, which is excreted as hippuric acid.

Supported by the metabolism of benzoic acid, it is anticipated that 2-hydroxymethyl benzoic acid will be conjugated with glycine and excreted as the major pathway. In addition it is possible that the hydroxymethyl group will be oxidised to the corresponding aldehyde or alternatively that it will be conjugated to glucuronic acid or glycine and excreted.

It is likely that the hydroxymethyl group can be further metabolised to a carboxylic acid group yielding phthalic acid, although this is probably a minor metabolic pathway compared to glycine conjugation of the already present carboxylic acid group. As another minor pathway phthalide might be hydroxylated at the benzene ring.

The anticipated metabolism pathway of phthalide is shown in Figure III.1.

Figure III.1 Anticipated metabolism of phthalide. The arrows in bold show major metabolic pathways.

III.4 Conclusion on Metabolism
The candidate substance phthalide [FL-no: 10.056] is expected to be hydrolysed to the corresponding benzoic acid derivative, 2-hydroxymethyl benzoic acid, before absorption or upon entering systemic circulation. 2-Hydroxymethyl benzoic acid is anticipated to be further metabolised by conjugation to glycine and excreted in the urine as the major pathway. As a minor pathways it is likely that the hydroxymethyl group can be conjugated with glucuronic acid, followed by excretion, or that the hydroxymethyl group will be further metabolised to a carboxylic acid group yielding phthalic acid. As a further minor pathway phthalide might be hydroxylated at the benzene ring. Overall, it is concluded that phthalide is metabolised to innocuous products.
ANNEX IV: TOXICITY

Oral acute toxicity data are available for the candidate substance of the present Flavouring Group Evaluation from chemical group 11, and for the three supporting substances evaluated by JECFA at the 61st meeting. The supporting substances are listed in brackets.

Table IV.1: Acute Toxicity

<table>
<thead>
<tr>
<th>Chemical Name [FL-no]</th>
<th>Species; Sex</th>
<th>Route</th>
<th>LD₅₀ (mg/kg bw)</th>
<th>Reference</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phthalide [10.056]</td>
<td>Rat NR</td>
<td>Oral</td>
<td>5,500</td>
<td>(Moreno, 1979e)</td>
<td></td>
</tr>
<tr>
<td>(3-Propylidenephthalide [10.005])</td>
<td>Rat NR</td>
<td>Oral</td>
<td>1,650</td>
<td>(Moreno, 1975r)</td>
<td></td>
</tr>
<tr>
<td>(3-Butylphthalide [10.025])</td>
<td>Mouse NR</td>
<td>Oral</td>
<td>1,850</td>
<td>(Pellmont, 1970)</td>
<td></td>
</tr>
<tr>
<td>(3-Butylidenephthalide [10.024])</td>
<td>Rat</td>
<td>Oral</td>
<td>2,200</td>
<td>(Posternak, 1980n)</td>
<td></td>
</tr>
</tbody>
</table>

NR = Not reported.

Subacute / subchronic / chronic / carcinogenic toxicity data are not available for the candidate substance of the present Flavouring Group Evaluation from chemical group 11 but for one supporting substance evaluated by JECFA at the 61st meeting. The supporting substance is listed in brackets.

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

<table>
<thead>
<tr>
<th>Chemical Name [FL-no]</th>
<th>Species; Sex No./Group</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>(3-Propylidenephthalide [10.005])</td>
<td>Rat; M, F 28</td>
<td>Diet</td>
<td>5.42 (M) and 6.55 (F) mg/kg bw/day</td>
<td>90 days</td>
<td>&gt; 5.42 (M)¹ &gt; 6.55 (F)¹</td>
<td>(Posternak et al., 1969)</td>
<td>Haematologic and histological examination and weight of liver and kidney reported. No significant effects. Poorly reported study.</td>
</tr>
</tbody>
</table>

M=Male; F=Female.

¹ Study performed with either a single dose or multiple doses that produced no adverse effect.
Table IV.3: Developmental and Reproductive Toxicity Studies

No developmental and reproductive toxicity data are available for the candidate substance of the present Flavouring Group Evaluation from chemical group 11 or for the supporting substances evaluated by JECFA at the 61st meeting.

In vitro mutagenicity/genotoxicity data are not available for the candidate substance of the present Flavouring Group Evaluation from chemical group 11 but for one supporting substance evaluated by JECFA at the 61st meeting. The supporting substance is listed in brackets.

Table IV.4: Genotoxicity (in vitro)

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

\(^1\) Without metabolic activation
\(^2\) With metabolic activation

Table IV.5: Genotoxicity (in vivo)

In vivo mutagenicity/genotoxicity data are not available for the candidate substance of the present Flavouring Group Evaluation from chemical group 11 or for the supporting substances evaluated by JECFA at the 61st meeting.
REFERENCES


