EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF); Scientific Opinion on Flavouring Group Evaluation 22, Revision 1 (FGE.22Rev1): Ring substituted phenolic substances from chemical groups 21 and 25

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SCIENTIFIC OPINION

Scientific Opinion on Flavouring Group Evaluation 22, Revision 1 (FGE.22Rev1):

Ring-substituted phenolic substances from chemical groups 21 and 25[1]

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

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

The Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids of the European Food Safety Authority was requested to evaluate 28 flavouring substances in the Flavouring Group Evaluation 22, Revision 1, using the Procedure in Commission Regulation (EC) No 1565/2000. The substance 3,4-methylenedioxyphenol [FL-no: 04.080] was reported to have a genotoxic potential *in vitro*, while *in vivo* studies were not available. Therefore, the Panel concluded that the Procedure could not be applied to this substance until adequate genotoxicity data become available. The remaining 27 substances were evaluated through a stepwise approach (the Procedure) that integrates information on structure-activity relationships, intake from current uses, toxicological threshold of concern, and available data on metabolism and toxicity. The Panel concluded that these 27 candidate substances do not give rise to safety concerns at their levels of dietary intake, estimated on the basis of the MSDI approach. Adequate specifications for the materials of commerce are available for all 27 flavouring substances evaluated through the Procedure.

1 On request from the Commission, Question No EFSA-Q-2011-00045-49, adopted on 4 February 2011.

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SUMMARY

The European Food Safety Authority (EFSA) asked the Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (the Panel) to provide scientific advice to the Commission on the implications for human health of chemically defined flavouring substances used in or on foodstuffs in the Member States. In particular, the Panel was requested to evaluate 28 flavouring substances in the Flavouring Group Evaluation 22, Revision 1 (FGE.22Rev1) using the Procedure as referred to in the Commission Regulation (EC) No 1565/2000. The flavouring substance belongs to chemical group 30, Annex I of the Commission Regulation (EC) No 1565/2000.

The 28 candidate substances are ring substituted phenolic substances belonging to chemical groups 21 and 25.

Two of the candidate substances have a chiral centre, the commercial product of both substances are the racemate.

Twenty-five of the flavouring substances are classified into structural class I and three are classified into structural class II [FL-no: 04.091, 04.092 and 07.234].

Twenty-two of the flavouring substances in the present group have been reported to occur naturally in a wide range of food items.

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 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 28 flavouring substances in this group have intakes in Europe from 0.001 to 610 microgram/capita/day, which are all below the thresholds of concern for their respective class of 1800 microgram/person/day for structural class I or 540 microgram/person/day for structural class II substances.

One of the flavourings substances, 3,4-methylenedioxyphenol [FL-no: 04.080], was reported to have a genotoxic potential *in vitro*, while *in vivo* studies were not available for this candidate substance. Therefore, the Panel decided that the Procedure could not be applied to this candidate substance until adequate genotoxicity data become available. The genotoxicity data available did not preclude an evaluation of the other 27 candidate flavouring substances in this Flavouring Group Evaluation through the Procedure.

The candidate substances in this group are conjugated with glucuronic acid or sulphate very efficiently and these pathways are not easily saturated. At high dose levels reactive metabolites (quinones, catechols, quinone methides) may be formed, but it is not expected that, at the levels of intake from the use as flavouring substances, the formation of these metabolites would overwhelm detoxication capacity through conjugation with sulphate, glucuronic acid or in particular glutathione. Thus, it is

concluded that all 27 candidate substances in this group, which have been evaluated using the Procedure, may be expected to be metabolised to innocuous products at the estimated levels of intake, based on the MSDI approach.

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 was considered that on the basis of the default MSDI approach the 27 candidate substances evaluated through the Procedure would not give rise to safety concerns at the estimated levels of intake arising from their use as flavouring substances. The total combined intake of the candidate and supporting substances belonging to structural class I is approximately 3900 microgram/capita/day, which exceeds the threshold of concern for a compound belonging to structural class I of 1800 microgram/person/day. The main contribution to the MSDI of 3900 microgram/capita/day originates from one supporting substance, 4-(p-hydroxyphenyl)butan-2-one [FL-no: 07.055], for which the estimated daily per capita intake is 2400 microgram. For this supporting substance a 13 week study provides a NOAEL of 100 mg/kg bw/day, which gives a margin of safety of 2500 to the MSDI value.

The estimated intakes for the 24 candidate substances evaluated through the Procedure and belonging to structural class I, range from 74 to 35000 microgram/person/day based on the mTAMDI. The mTAMDI is above the threshold of concern of 1800 microgram/person/day for two of the candidate substances [FL-no: 08.134 and 09.946].

The estimated intakes of the three substances evaluated through the Procedure and belonging to structural class II, are 420 microgram/person/day based on the mTAMDI, which is below the threshold of concern for structural class II substances of 540 microgram/person/day.

Thus, for two candidate substances [FL-no: 08.134 and 09.946], for which the mTAMDI is above the threshold, further information is required. This would include more reliable intake data and then, if required, additional toxicological data.

The 25 substances which have mTAMDI intake estimates below the threshold of concern for their structural class are also expected to be metabolised to innocuous products.

In order to determine whether the conclusion for the 27 candidate substances evaluated using the Procedure can be applied to the materials of commerce, it is necessary to consider the available specifications. Adequate specifications including complete purity criteria and identity tests for the materials of commerce have been provided for all 27 flavouring substances evaluated through the Procedure.

Thus, the Panel concluded that for one candidate substance [FL-no: 04.080] additional genotoxicity data are required and that the remaining 27 candidate substances would present “no safety concern at the estimated level of intakes, based on the MSDI approach.

KEYWORDS

Phenolic, ring-alkyl, ring-alkoxy, flavourings, food safety, FGE.22.

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BACKGROUND

Regulation (EC) No 2232/96 of the European Parliament and the Council (EC, 1996a) lays down a Procedure for the establishment of a list of flavouring substances the use of which will be authorised to the exclusion of all other substances in the EU. In application of that Regulation, a Register of flavouring substances used in or on foodstuffs in the Member States was adopted by Commission Decision 1999/217/EC (EC, 1999a), as last amended by Commission Decision 2008/163/EC (EC, 2009a). Each flavouring substance is attributed a FLAVIS-number (FL-number) and all substances are divided into 34 chemical groups. Substances within a group should have some metabolic and biological behaviour in common.

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

The FGE is revised to include substances for which data were submitted after the deadline as laid down in Commission Regulation (EC) No 622/2002 and to take into account additional information that has been made available since the previous Opinion on this FGE.

The Revision also includes newly notified substances belonging to the same chemical groups evaluated in this FGE.

After the completion of the evaluation programme the Union 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, 1996a).

HISTORY OF THE EVALUATION

FGE	Opinion adopted by EFSA	Link	No. of candidate substances
FGE.22	27 September 2006	http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1178620772628.htm	23
FGE.22Rev1	4 February 2011		28

The present revision of FGE.22, FGE.22Rev1, includes the assessment of five newly notified candidate substances [FL-no: 08.134, 09.943, 09.944, 09.945 and 09.946]. For one of these substances [FL-no: 08.134] supporting metabolism data have been submitted. No other data have been provided.

A search in open literature did not provide any new pertinent data on toxicity or metabolism.

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 Union List according to Commission Regulation (EC) No 1565/2000 (EC, 2000a).

In addition, in letter of 11 May 2009 the Commission requested EFSA to carry out a risk assessment on guaiacol propionate [FL-no: 09.943], guaiacol butyrate [FL-no: 09.944], guaiacol isobutyrate [FL-no: 09.945], dihydrogalangal acetate [FL-no: 09.946] and nibovan [FL-no: 08.134] in accordance with Commission Regulation (EC) No 1565/2000 (EC, 2000a):

"The European Commission requests the European Food Safety Authority to carry out a risk assessment on eighteen new flavouring substances in accordance with Commission Regulation (EC) No 1565/2000, if possible by the end of the evaluation programme, if not within nine months from the finalisation of that programme".

The deadline of the "Terms of Reference" was negotiated to 31 May 2011.

The remaining 13 substances of the "Terms of Reference" of 11 May 2009 were evaluated in other FGEs.

ASSESSMENT

1. Presentation of the Substances in Flavouring Group Evaluation 22, Revision 1

1.1. Description

The present Flavouring Group Evaluation 22, Revision 1, FGE.22Rev1, using the Procedure as referred to in the Commission Regulation EC No 1565/2000 (EC, 2000a) (the Procedure – shown in schematic form in Annex I), deals with 28 ring-substituted phenolic substances. The group comprises:

- nine mono-, di- or tri-alkyl substituted phenols,
- three esters of alkyl-substituted phenols,
- three alkoxy-substituted phenols,
- three esters of alkoxy-substituted phenols,
- three alkoxy-substitued phenols with an alkenyl substituent or an alkyl substituent containing a keto-function or an hydroxycarboxylic acid,
- 4-hydroxyacetophenone,
- two alkoxy-substituted 4-hydroxyacetophenones,
- an alkoxy-substituted 4-hydroxypropiophenone,
- two 4-hydroxybenzylethers,
- an ester of 4-hydroxy-1-phenyl-1-propanol.

These flavouring substances belong to chemical groups 21 and 25, Annex I of Commission Regulation (EC) No 1565/2000 (EC, 2000a).

The 28 flavouring substances (candidate substances) under consideration, as well as their chemical Register names, FLAVIS- (FL-), Chemical Abstract Service- (CAS-), Council of Europe- (CoE-), and Flavor and Extract Manufactures Association- (FEMA-) numbers, structure and specifications, are listed in Table 1.

The outcome of the safety evaluation is summarised in Table 2a.

The 28 candidate substances are structurally closely related to 40 flavouring substances (supporting substances in the group of "Phenol and phenol derivatives" evaluated at the 55th JECFA meeting (JECFA, 2001a). These substances, with the respective structural formulas, FEMA, CoE, and CAS

register numbers, evaluation status by Scientific committee on Food (SCF), JECFA, and by CoE and the European Maximised Survey-derived Daily Intake (MSDI) values, are listed in Table 3.

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

In the original JECFA evaluation, from which the supporting substances for this FGE have been taken, phenol [FL no: 04.041] was also included. However, the Panel concluded (EFSA, 2006a) that phenol should not be used as supporting substance to evaluate the toxicity of the ring-substituted phenols in this FGE, because – in contrast to phenol – the formation of toxic metabolites like hydroquinones and quinones is not expected to be of significance for the candidate ring-substituted phenols at their estimated levels of intake as flavouring substances.

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 variability in 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 (CAS number, FLAVIS number etc.).

Two of the candidate substances possess a chiral centre [FL-no: 08.134 and 09.946]. For both substances industry has informed that the commercial compound is the racemate (Flavour Industry, 2009k).

1.3. Natural Occurrence in Food

Twenty-two of the 28 candidate substances have been reported to occur in one or more of the following food items: coffee, fish, alcoholic beverages, spices, milk powder, fruits, asparagus, pork and wort. Quantitative data on the natural occurrence in food have been reported for 16 of the candidate substances. These reports include:

- 3,5-Dimethylphenol [FL-no: 04.020]: 0.1 mg/kg in tamarind.
- 3-Ethylphenol [FL-no: 04.021]: up to 1.4 mg/kg in coffee.
- 2,6-Dimethoxy-4-vinylphenol [FL-no: 04.061]: up to 0.03 mg/kg in beer, 1.8 mg/kg in coffee, and 0.05 mg/kg in wort.
- 2,3-Dimethylphenol [FL-no: 04.065]: 2.1 mg/kg in beer.
- 2,4-Dimethylphenol [FL-no: 04.066]: 2 mg/kg in coffee.
- 2-Ethylphenol [FL-no: 04.070]: 1.7 mg/kg in coffee, up to 0.4 mg/kg in lean fish, 0.05 mg/kg in sherry, 0.002 mg/kg in whisky, 0.001 mg/kg in red wine.
- 4-Isopropylphenol [FL-no: 04.073]: up to 1900 mg/kg in cumin seed.
- 4-Methoxyphenol [FL-no: 04.077]: less than 500 mg/kg in galangal.
- 5-Methyl-2-(*tert*-butyl)phenol [FL-no: 04.078]: 0.0051 mg/kg in milk powder.

- 4-Hydroxybenzyl methyl ether [FL-no: 04.092]: 200 mg/kg in bourbon vanilla.
- Acetovanillone [FL-no: 07.142]: 0.08 mg/kg in asparagus, 0.15 mg/kg in cloudberry, up to 4 mg/kg in coffee, up to 0.6 mg/kg in lean fish, 9.3 mg/kg in pork, up to 0.03 mg/kg in beer, 0.15 mg/kg in sherry, up to 1227 mg/kg in red wine, up to 0.09 mg/kg in wort.
- 4-Hydroxy-3,5-dimethoxyacetophenone [FL-no: 07.164]: 4.4 mg/kg in pork, 0.025 mg/kg in sherry.
- 4-Hydroxyacetophenone [FL-no: 07.243]: 2.1 mg/kg in cloudberry, up to 3.2 mg/kg in coffee, trace amounts in cranberry, up to 0.04 mg/kg in mango, 0.4 mg/kg in sherry, 0.01 mg/kg in wort.
- 2-Isopropyl-5-methylphenyl acetate [FL-no: 09.253]: less than 1000 mg/kg in thyme.
- Carvacryl acetate [FL-no: 09.337]: up to 1100 mg/kg in thyme.
- Dihydrogalangal acetate [FL-no: 09.946]: 5 mg/kg in galangal roots (Yang et al., 2009)

The remaining six substances (3,4-methylenedioxyphenol [FL-no: 04.080], 2-isopropyl-5-methylphenyl formate [FL-no: 09.893], nibovan [FL-no: 08.134], guaiacol propionate [FL-no: 09.943], guaiacol butyrate [FL-no: 09.944] and guaiacol isobutyrate [FL-no: 09.945] have not been reported to occur naturally in any food items according to TNO (TNO, 2000; TNO, 2010)

2. Specifications

Purity criteria for the 28 substances have been provided by the Flavour Industry (EFFA, 2004c) (Table 1).

Judged against the requirements in Annex II of Commission Regulation (EC) No 1565/2000 (EC, 2000a), this information is adequate for all 28 candidate substances (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, 1999a).

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, 1999a).

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 by 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, 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 intake estimation is based on the Maximised Survey-derived Daily Intake (MSDI) approach, which involves the acquisition of data on the amounts used in food as flavourings (SCF, 1999a). These 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[4] (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, 1999a).

In the present FGE.22Rev1 the total annual production volume of the 28 candidate substances for use as flavouring substances in Europe was reported to be 7120 kg (EFFA, 2004h; Flavour Industry, 2009k). For the 40 supporting substances the total annual volume of production in Europe is approximately 25000 kg, (4-(*p*-hydroxyphenyl)butan-2-one [FL-no: 07.055] accounts for approximately 19000 kg) (JECFA, 2001b).

On the basis of the annual volumes of production reported for the 28 candidate substances, the MSDI values for each of these flavourings have been estimated (Table 2a). Approximately 99 % of the total annual volume of production for the candidate substances is accounted for by the five new substances included in FGE.22Rev1 [FL-no: 08.134, 09.943, 09.944, 09.945 and 09.946]. The estimated daily *per capita* intakes of these five candidate substances from use as flavouring substances are 120, 43, 43, 43 and 610 microgram, respectively. The daily *per capita* intakes for each of the remaining substances is 2.2 microgram or less (Table 2a).

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.

[4] EU figure 375 millions. 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 28 candidate substances, information on food categories and normal and maximum use levels[5,6,7] has been submitted by the Flavour Industry (EFFA, 2004g; EFFA, 2007a; Flavour Industry, 2009k). The 28 candidate substances are used in flavoured food products divided into the food categories, outlined in Annex III of the Commission Regulation 1565/2000 (EC, 2000a), as shown in Table 3.1. For the calculation of the 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.

Table 3.1 Use of Candidate Substances

Food category	Description	Flavourings used
01.0	Dairy products, excluding products of category 2	All but [FL-no: 09.943, 09.944, 09.945, 09.946]
02.0	Fats and oils, and fat emulsions (type water-in-oil)	All but [FL-no: 08.134, 09.943, 09.944, 09.945, 09.946]
03.0	Edible ices, including sherbet and sorbet	All but [FL-no: 09.946]
04.1	Processed fruits	All but [FL-no: 08.134, 09.943, 09.944, 09.945, 09.946]
04.2	Processed vegetables (incl. mushrooms & fungi, roots & tubers, pulses and legumes), and nuts & seeds	None
05.0	Confectionery	All 28
06.0	Cereals and cereal products, incl. flours & starches from roots & tubers, pulses & legumes, excluding bakery	All but [FL-no: 09.946]
07.0	Bakery wares	All but [FL-no: 09.946]
08.0	Meat and meat products, including poultry and game	All but [FL-no: 08.134, 09.943, 09.944, 09.945]
09.0	Fish and fish products, including molluscs, crustaceans and echinoderms	All but [FL-no: 08.134, 09.943, 09.944, 09.945]
10.0	Eggs and egg products	None
11.0	Sweeteners, including honey	[FL-no: 09.943, 09.944, 09.945, 09.946]
12.0	Salts, spices, soups, sauces, salads, protein products etc.	All but [FL-no: 09.943, 09.944, 09.945]
13.0	Foodstuffs intended for particular nutritional uses	All but [FL-no: 08.134, 09.943, 09.944, 09.945, 09.946]
14.1	Non-alcoholic ("soft") beverages, excl. dairy products	All 28
14.2	Alcoholic beverages, incl. alcohol-free and low-alcoholic counterparts	All 28
15.0	Ready-to-eat savouries	All but [FL-no: 08.134]
16.0	Composite foods (e.g. casseroles, meat pies, mincemeat) - foods that could not be placed in categories 1 – 15	All but [FL-no: 09.946]

According to the Flavour Industry the normal use levels for the 28 candidate substances are in the range of 0.1 to 500 mg/kg food, and the maximum use levels are in the range of 1 to 1000 mg/kg (EFFA, 2004g; EFFA, 2007a; Flavour Industry, 2009k).

The mTAMDI values for the 25 candidate substances from structural class I (see Section 5) range from 74 to 35000 microgram/person/day. For each of the remaining three candidate substances from structural class II (see section 5) the mTAMDI values are 420 microgram/person/day.

[5] "Normal use" is defined as the average of reported usages and "maximum use" is defined as the 95th percentile of reported usages (EFFA, 2002i).
[6] 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).
[7] 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).

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 phenolic substances in this group are assumed to be well absorbed. The substances are readily conjugated with sulphate or glucuronic acid at the hydroxy group, followed by rapid excretion usually via urine. The seven phenol esters will be hydrolysed, probably in the intestinal lumen by pancreatic enzymes to the corresponding phenols and carboxylic acids (see Table 2b). The carboxylic acids will be rapidly metabolised and have been considered innocuous in previous evaluations ([FL no: 08.001, 08.002, 08.003 and 08.005] in FGE.02Rev1 (EFSA, 2008ag) and [FL no: 08.006] in FGE.01Rev2 (EFSA, 2010t)

Sulphation and glucuronidation of phenols have been found in many species including humans, but species differences in efficacy of the two routes have been reported especially with phenol itself. The available data indicate that the efficacy of the conjugation reactions is not substantially influenced by the presence of ring substituents, provided that these are not too bulky.

Ring hydroxylation, side chain oxidation and O-demethylation occur to a much smaller extent than conjugation, owing to the efficiency of the conjugation reactions. Products of the oxidative metabolic pathways may include quinones and catechols which may trigger a cytotoxic response if their formation rate overwhelms their detoxication via glutathione conjugation. Reduced quinones (i.e. hydroquinones) and catechols are also substrates for sulphate and glucuronic acid conjugation.

In vitro metabolism studies have provided evidence of a bio-activation pathway for para-substituted alkylphenols. This pathway involves formation of quinone methides, which can be effectively detoxified by enzymatic or spontaneous conjugation with glutathione. As long as the intra-cellular concentration of glutathione is maintained, these quinone methides may not reach concentrations high enough to trigger a toxic response. Also the presence of other ring substituents affects the reactivity of the quinone methide intermediates, e.g. for 2,6-dimethoxy-4-vinylphenol [FL-no: 04.061] the two methoxy groups may mitigate cytotoxic properties.

The candidate substances with a ketonic side chain possess an additional option for metabolism. Such phenol derivatives may also be excreted in the urine as glucuronic acid or sulphate conjugates but reduction of the ketone to the secondary alcohol has also been reported. Minor metabolites may be formed through ring hydroxylation and side chain omega-oxidation may result in chain length reduction.

The candidate substance nibovan [FL-no: 08.134] is an endogenous metabolite of adrenalin and nor-adrenalin. This substance is rapidly eliminated from the blood and excreted via the kidneys. Data on conjugations are not available, but due to the chemical structure, conjugation may be anticipated similar to what has been described above for phenol. In addition, direct conjugation of the carboxylic acid moiety with amino acids has been described for phenylacetic acid (EFSA, 2009f) and this may also occur with nibovan. It has been described that decarboxylation of the carboxylic acid moiety is also an option, leading to the formation of benzoic acid which can be converted to the corresponding glycine conjugate.

The candidate substances in this group are conjugated with glucuronic acid or sulphate very efficiently, and these pathways are not easily saturated at the expected levels of intake resulting from their use as flavouring substances. At high dose levels reactive metabolites (quinones, catechols, quinone methides) may be formed. However, the formation of these metabolites is not expected to overwhelm the detoxication capacity through conjugation with sulphate, glucuronic acid or in particular glutathione, when the substances are used as flavouring substances.

Thus, it is concluded that in general, the substances in this group may be expected to be metabolised to innocuous substances at the estimated levels of intake, based on the MSDI approach.

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.

One of the candidate substances, 3,4-methylenedioxyphenol [FL-no: 04.080] showed genotoxic potential *in vitro*. Therefore, the Panel decided that the Procedure could not be applied to this candidate substance until adequate genotoxicity data become available.

For the safety evaluation of the remaining 27 candidate substances from chemical groups 21 and 25 the Procedure as outlined in Annex I was applied, based on the MSDI approach. The stepwise evaluations of the 27 substances are summarised in Table 2a.

Step 1

Twenty-four of the 27 candidate substances to which the Procedure could be applied are classified into structural class I, and three candidate substances are classified into structural class II.

Step 2

Based on the available data the 24 candidate substances from structural class I and three candidate substances from structural class II may be expected to be metabolised to innocuous substances at the estimated levels of intake. Therefore, these 27 flavouring substances proceed along the A-side of the Procedure.

Step A3

According to the default MSDI approach, the 27 flavouring substances in this group have intakes in Europe from 0.001 to 610 microgram/*capita*/day, which are all below the thresholds of concern for their respective class of 1800 microgram/person/day for structural class I or 540 microgram/person/day for structural class II substances.

Accordingly, these 27 candidate substances are not expected to be of safety concern at their 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 24 candidate substances evaluated through the Procedure and belonging to structural class I, range from 74 to 35000 microgram/person/day based on the mTAMDI. The mTAMDI is above the threshold of concern of 1800 microgram/person/day for two of the candidate substances [FL-no: 08.134 and 09.946].

The estimated intakes of the three substances evaluated through the Procedure and belonging to structural class II, are 420 microgram/person/day based on the mTAMDI, which is below 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

Table 6.1 Estimated intakes based on the MSDI approach and the mTAMDI approach

FL-no	EU Register name	MSDI (µg/*capita*/day)	mTAMDI (µg/person/day)	Structural class	Threshold of concern (µg/person/day)
04.020	3,5-Dimethylphenol	0.037	420	Class I	1800
04.021	3-Ethylphenol	0.073	420	Class I	1800
04.061	2,6-Dimethoxy-4-vinylphenol	1.2	420	Class I	1800
04.065	2,3-Dimethylphenol	0.013	420	Class I	1800
04.066	2,4-Dimethylphenol	0.011	420	Class I	1800
04.070	2-Ethylphenol	0.037	420	Class I	1800
04.072	3-Isopropylphenol	0.0012	420	Class I	1800
04.073	4-Isopropylphenol	0.24	420	Class I	1800
04.076	3-Methoxyphenol	0.011	420	Class I	1800
04.077	4-Methoxyphenol	0.12	420	Class I	1800
04.078	5-Methyl-2-(tert-butyl)phenol	0.061	420	Class I	1800
04.095	2,4,6-Trimethylphenol	0.0097	420	Class I	1800
07.142	Acetovanillone	2.2	420	Class I	1800
07.154	1-(3,5-Dimethoxy-4-hydroxyphenyl)propan-1-one	0.026	420	Class I	1800
07.164	4-Hydroxy-3,5-dimethoxyacetophenone	0.24	1600	Class I	1800
07.243	4-Hydroxyacetophenone	0.016	1600	Class I	1800
08.134	Nibovan	120	5000	Class I	1800
09.253	2-Isopropyl-5-methylphenyl acetate	1.1	420	Class I	1800
09.337	Carvacryl acetate	0.61	420	Class I	1800
09.893	2-Isopropyl-5-methylphenyl formate	0.52	420	Class I	1800
09.943	Guaiacol propionate	43	74	Class I	1800
09.944	Guaiacol butyrate	43	74	Class I	1800
09.945	Guaiacol isobutyrate	43	74	Class I	1800
09.946	Dihydrogalangal acetate	610	35000	Class I	1800
04.080	3,4-Methylenedioxyphenol	1.7	420	Class I	1800
04.091	Ethyl 4-hydroxybenzyl ether	0.0012	420	Class II	540
04.092	4-Hydroxybenzyl methyl ether	0.61	420	Class II	540
07.234	5-Paradol	0.012	420	Class II	540

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.

One of the candidate substances, 3,4-methylenedioxyphenol [FL-no: 04.080] showed genotoxic potential *in vitro* and therefore the Procedure could not be applied to this substance. Accordingly, the substance will not be considered in the combined intake.

On the basis of the reported annual production volumes in Europe (EFFA, 2004h; Flavour Industry, 2009k), the combined estimated daily per capita intake as flavourings of the 24 candidate flavouring substances assigned to structural class I and of the three candidate substances belonging to structural class II are 867 and 0.6 microgram, respectively. These values do not exceed the thresholds of concern for substances belonging to structural class I of 1800 microgram/person/day and to structural class II of 540 microgram/person/day.

The 27 candidate substances are structurally related to 40 supporting substances evaluated by JEFCA at its 55th meeting (JECFA, 2001b). Based on reported production volumes, European per capita intakes (MSDI) could be estimated for all 40 supporting substances.

The 40 supporting substances belong to structural class I. The total combined intake of the candidate and supporting substances belonging to structural class I is approximately 3900 microgram/capita/day, which exceeds the threshold of concern for a compound belonging to structural class I of 1800 microgram/person/day. The main contribution to the MSDI of 3900 microgram/capita/day originates from one supporting substance, 4-(p-hydroxyphenyl)butan-2-one [FL-no: 07.055], for which the estimated daily per capita intake is 2400 microgram. For this supporting substance a 13 week study provides a NOAEL of 100 mg/kg body weight (bw)/day, which gives a margin of safety of 2500 to the MSDI value (Gaunt et al., 1970).

Therefore, it can be concluded that the total combined intakes of the 27 candidate substances and the 40 supporting substances do not pose a safety concern.

8. Toxicity

8.1. Acute Toxicity

Data are available for six of the 28 candidate substances [FL-no: 04.021, 04.065, 04.020, 04.078, 04.077 and 07.243] and for 21 supporting substances. Oral LD_{50} values are in the range of 120 to more than 5000 mg/kg bw in rat, mice and rabbit.

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

8.2. Subacute, Subchronic, Chronic and Carcinogenicity Studies

Data are available for three of the 23 candidate substances [FL-no: 04.066, 04.077 and 04.080] and for 10 supporting substances.

Groups of male (N=10) and female (N=10) rats were dosed with 0, 60, 180 or 540 mg/kg bw/day of 2,4-dimethylphenol [FL-no: 04.066] by gavage for 90 days. No significant differences related to the treatment with 2,4-dimethylphenol were observed on clinical chemistry and haematological parameters. Histopathological examination of the stomach was performed on all surviving animals. Further, other tissues from the control group and the groups dosed with 180 and 540 mg/kg bw/day, respectively, were also examined histologically. Significant findings were hyperkeratosis and epithelial hyperplasia of the forestomach in 100 % of the males (540 and 180 mg/kg bw/day), 100 % of the females (540 mg/kg bw/day) and 60 % of the females (180 mg/kg bw/day). The effect was ascribed to the irritant properties of 2,4-dimethylphenol. The No Observed Adverse Effect Level (NOAEL) derived was 60 mg/kg bw/day (Daniel et al., 1993).

Three short term oral dosing studies in rats and rabbits ranging from 4 to 9 weeks are available on 4-methoxyphenol [FL-no: 04.077]. In two of these studies the only effects observed were changed body and organ weights (Hodge et al., 1949). In another study 4-methoxyphenol was reported to strongly affect the forestomach mucosa with hyperplasia and mild keratosis in rats fed 2 % of the substance in the feed for 4 to 8 weeks (Altmann et al., 1985; Altmann et al., 1986).

Groups of 15 male hamsters were administered diets containing 0 or 1.5 % 4-methoxyphenol for 20 weeks. Induction of hyperplasia in the forestomach was observed in all dosed animals (Hirose et al., 1986).

Groups of 10-11 male rats were administered diets containing 0 or 2 % 4-methoxyphenol for 24-48 weeks. One group in addition received basal diet for 24 weeks after dosing. Hyperplasia in the

forestomach was induced in all treated animals. Simple or papillary hyperplasia but not basal cell hyperplasia was clearly regressed in the recovery group receiving basal diet after cessation of dosing (Kagawa et al., 1993).

Male and female rats (N=30/sex) were given diets with 0 or 2 % 4-methoxyphenol for 104 weeks. Histopathological examination of the forestomach showed atypical hyperplasia (male, 67 %, female, 37 %), papillomas (50 %, 23 %) and squamos cell carcinomas (77 %, 20 %) (Asakawa et al., 1994).

In a study on induction of forestomach tumours groups of male rats (N= 26-28) were given diets containing 0 or 0.4 % 4-methoxyphenol for 104 weeks. Treated animals had lower body weights than controls and an increased incidence of forestomach hyperplasia was observed. No carcinomas were detected (Hirose et al., 1997).

Groups of 10-11 male rats were administered diets containing 0, 0.25, 0.5, 1 and 2 % of 4-methoxyphenol for 51 weeks. Mild to severe hyperplasia was induced in the forestomach of rats given 0.5 – 2 % of the test substance. Histopathological examination was only performed on liver, stomach, oesophagus and kidney. A NOAEL of 0.25 % corresponding to 125 mg/kg bw/day was derived (Wada et al., 1990).

In an initiation-promotion study groups of male rats were given diets containing 0, 0.08 or 0.4 % of 4-methoxyphenol for 24 weeks after initiation for 4 weeks with a combination of diethylnitrosamine, N-methylnitrosourea, 1,2-dimethylhydrazine, N-butyl-N-(4-hydroxybutyl)-nitrosamine and 2,2'-dihydroxy-di-n-propylnitrosamine. An increase in forestomach papillomas was observed in the high-dose group but not in the low-dose group. Histopathological examination of other major organs showed no significant findings (Hirose et al., 1997). A NOAEL of 0.08 % in the diet corresponding to 40 mg/kg bw/day was derived.

Groups of 30 male and 30 female mice were fed a diet containing 0 or 2 % 3,4-methylenedioxyphenol [FL-no: 04.080] for 96 weeks. Squamous cell carcinomas were induced in the forestomach of 11 (38 %) male and 5 (17 %) female mice (Tamano et al., 1992).

Groups of 30 male and 30 female rats were fed a diet containing 0 or 2 % 3,4-methylenedioxyphenol for 104 weeks. Squamous cell carcinomas were induced in the forestomach of 9 (31 %) male and 3 (10 %) female rats. Papillomas were observed in 10 (34 %) male and in 14 (47 %) female rats, but not in the mice. Hyperplasia was detected in almost all of the animals (Tamano et al., 1992).

Groups of 10-11 male rats were dosed with 0 or 2 % 3,4-methylenedioxyphenol in the diet for 24-48 weeks. In addition, one group received basal diet for 24 weeks after dosing. Hyperplasia in the forestomach was induced in all animals. Simple or papillary hyperplasia but not basal cell hyperplasia clearly regressed in the recovery group receiving basal diet after dosing (Kagawa et al., 1993).

In a study on induction of forestomach tumours groups of male rats (N= 15-25) were dosed with 0 or 0.4 % 3,4-methylenedioxyphenol in the diet for 104 weeks. Dosed animals had a lower body weight and increased relative liver and kidney weight compared to controls. There were no significant histopathological findings in the forestomach. No carcinomas were detected. (Hirose et al., 1997).

In an initiation-promotion study groups of male rats were dosed with 0, 0.08 or 0.4 % of 3,4-methylenedioxyphenol for 24 weeks after initiation for 4 weeks with a combination of diethylnitrosamine, N-methylnitrosourea, 1,2-dimethylhydrazine, N-butyl-N-(4-hydroxybutyl)-nitrosamine and 2,2'-dihydroxy-di-n-propylnitrosamine. Histopathological examination of major organs showed no significant findings. The NOAEL derived was 0.4 % in the diet corresponding to 200 mg/kg bw/day (Hirose et al., 1997).

Forestomach hyperplasia were reported in studies on the candidate substances 2,4-dimethylphenol [FL-no: 04.066], 4-methoxyphenol [FL-no: 04.077] and 3,4-methylenedioxyphenol [FL-no: 04.080] and the supporting substances 4-methylphenol [FL-no: 04.028] and 4-(1,1-dimethylethyl)phenol [FL-

no: 04.064]. 4-Methoxyphenol and 3,4-methylenedioxyphenol were also studied for tumour induction in long-term studies. The induction of tumours of the forestomach is generally considered to result from irritant effects. Both duration of exposure and the dose are important determinants in the subsequent formation of forestomach tumours. These were observed in long term rodent studies (96-104 weeks) with 4-methoxyphenol and 3,4-methylenedioxyphenol at 2 % but not at 0.4 % in diet. They are considered unlikely to occur at the estimated intakes when used as flavouring substances. In the light of the genotoxicity data discussed later for 3,4-methylenedioxyphenol, it is not possible to disregard the forestomach tumours, although it is recognised that they may also be formed via a non-genotoxic mechanism.

A 13-week oral feeding study in male and female rats given doses of 0.1, 0.2, 0.4 and 1 % in the diet is available for the supporting substance 4-(p-hydroxyphenyl)butan-2-one [FL-no: 07.055]. A slight increase in relative liver, adrenal and kidney weights was detected in male rats given 0.4 and 1 %. The NOAEL derived was 0.2 % in the diet corresponding to 100 mg/kg bw/day (Gaunt et al., 1970).

The repeated dose toxicity data are summarised in Annex IV, Table IV.2

8.3. Developmental / Reproductive Toxicity Studies

Data on developmental and reproductive toxicity are available for three candidate substances [FL-no: 04.076, 04.077 and 07.243] and for five supporting substances. No data are available on teratogenicity.

Groups of 12-16 female rats were dosed orally with 0, 333, 667 or 1000 mg/kg bw of either 3-methoxyphenol [FL-no: 04.076] or 4-methoxyphenol [FL-no: 04.077] or 4-hydroxyacetophenone [FL-no: 07.243], once on day 11 of gestation. Maternal weight was measured at 24 and 72 hours, respectively, and litter size and pup weight were determined after delivery.

Maternal weight change significantly different from the control was observed for 3-methoxyphenol and 4-methoxyphenol at all doses. 4-Hydroxyacetophenone did not result in maternal weight change different from the control at any dose. The only fetal effect detected was reduced litter size and fetal weight for 4-hydroxyacetophenone at the highest dose (Kavlock, 1990).

The developmental / reproductive toxicity data are summarised in Annex IV, Table IV.3

8.4. Genotoxicity Studies

Data from *in vitro* tests are available for 12 candidate [FL-no: 04.020, 04.021, 04.065, 04.066, 04.070, 04.076, 04.077, 04.080, 04.095, 07.142, 07.164 and 07.243] and 18 supporting substances. Data from *in vivo* tests are available for one candidate [FL-no: 04.077] and four supporting substances. Most studies are of limited or insufficient quality or are inadequately reported, thus for some of the studies the validity of the results could not be evaluated.

Positive results were observed with three candidate substances [FL-no: 04.077, 04.080 and 07.142].

4-Methoxyphenol [FL-no: 04.077] did not induce gene mutations in bacteria (Haworth et al., 1983). In a gene mutation assay in mammalian cells (MLTK assay) a positive result was observed for 4-methoxyphenol without metabolic activation and a negative result with metabolic activation using an S9 homogenate (Rogers-Back, 1986). In the test without metabolic activation an increase in the percentage of small colonies was noted indicating a potential for chromosomal aberrations. 4-Methoxyphenol induced chromosomal aberrations in CHO cells in the presence and absence of metabolic activation (Putman, 1986). 4-Methoxyphenol did not induce sister chromatid exchanges in human lymphocytes (Jansson et al., 1988), however, the study was of limited quality. Since 4-methoxyphenol did not induce chromosomal aberration *in vivo* in rat bone marrow cells after oral

application (Esber, 1986) the results observed *in vitro* with 4-methoxyphenol were considered to be of no concern.

3,4-Methylenedioxyphenol [FL-no: 04.080] was reported to be negative in a bacterial mutagenicity assay in the presence and absence of metabolic activation while a positive result was reported in a gene mutation assay in mammalian cells (MLTK assay) both in the presence and absence of metabolic activation (Longfellow, 1985/1986). However, this information was only available as a very short abstract and the study reports were not available for evaluation. *In vivo* studies were not available for this candidate substance.

Acetovanillone [FL-no: 07.142] was positive in a yeast assay without metabolic activation (Nestmann & Lee, 1983). This result is not considered to preclude the substance to be evaluated through the Procedure. The substance was negative in bacterial mutagenicity assays in the presence and absence of metabolic activation (Nestmann et al., 1980; Xu et al., 1984). However, reporting of the bacterial assays and the quality of data were insufficient and the validity of the results could not be evaluated.

With the candidate substances 2-ethylphenol [FL-no: 04.070] and 2,4-dimethylphenol [FL-no: 04.066] negative results were observed in bacterial gene mutation assays (Zeiger et al., 1992; Mortelmans et al., 1986; Pool & Lin, 1982). All other results observed in several assays with these two and seven further candidate substances for which data were available were negative. However, these data were of limited or insufficient quality and the validity of the studies could not be evaluated.

With supporting substances positive and negative results were obtained in *in vitro* tests.

2-Methylphenol [FL-no: 04.027], 3-methylphenol [FL-no: 04.026], 4-methylphenol [FL-no: 04.028], 2-methoxyphenol [FL-no: 04.005] and 2,6-dimethoxyphenol [FL-no: 04.036] did not induce gene mutations in bacterial assays of acceptable quality (Haworth et al., 1983; Pool & Lin, 1982). The validity of a positive result observed with 2-methylphenol FL-no: 04.027] in bacteria (Claxton, 1985) cannot be evaluated.

2,6-Dimethylphenol [FL-no: 04.042] induced chromosomal aberrations in mammalian cells in the presence of S9 while the result was negative in the absence of metabolic activation (Völkner, 1994). The *in vitro* genotoxic potential of 2,6-dimethylphenol does not give rise to concern with respect to other alkylated phenols in this FGE, as they are alkyl substituted in either m- or p-positions. Phenols, substituted in m- or p-position are expected to be metabolised differently from 2,6-dimethylphenol.

2-Methoxyphenol [FL-no: 04.005], 2-methoxy-4-methylphenol [FL-no: 04.007], 2-methylphenol [FL-no: 04.027] and a mixture of 2-methylphenol [FL-no: 04.027], 3-methylphenol [FL-no: 04.026] and 4-methylphenol [FL-no: 04.028] induced sister chromatid exchanges in human lymphocytes or CHO cells. In most cases the effects were observed in the presence and absence of metabolic activation.

The mixture of 2-methylphenol [FL-no: 04.027], 3-methylphenol [FL-no: 04.026] and 4-methylphenol [FL-no: 04.028] resulted in an equivocal response in a UDS assay (Myhr & Brusick, 1980) while induction of UDS was observed with 4-methylphenol [FL-no: 04.028] in another in *vitro* study (Crowley & Margard, 1978).

All other results observed in several *in vitro* assays with these and the remaining supporting substances were negative, however, these data were of limited or insufficient quality and the validity of the studies could not be evaluated.

With the supporting substances 2-methylphenol [FL-no: 04.027], 3-methylphenol [FL-no: 04.026] and 4-methylphenol [FL-no: 04.028] negative results were obtained in *in vivo* SCE assays (Cheng & Kligerman, 1984). However, these data were of limited quality. 3-Methylphenol [FL-no: 04.026] did not induce chromosomal aberrations in mice (Ivett et al., 1989). However, the validity of the result could not be evaluated as the study was inadequately reported. 2-Methylphenol [FL-no: 04.027] and carvacrol [FL-no: 04.031] did not induce mutations in *Drosophila* (Sernau, 1989; Kono et al., 1995).

Overall, the available genotoxicity data on the supporting substances would not preclude evaluation of the candidate substances through the Procedure. One of the candidate substances, 3,4-methylenedioxyphenol [FL-no: 04.080], was reported to have genotoxic potential *in vitro*. *In vivo* studies were not available for this candidate substance. Therefore, the Panel decided that the Procedure could not be applied to this candidate substance until adequate genotoxicity data become available.

The genotoxicity data are summarised in Annex IV, Table IV.4 and IV.5

9. Conclusions

The 28 candidate substances are ring substituted phenolic substances belonging to chemical groups 21 and 25.

Two of the candidate substances have a chiral centre, the commercial product of both substances are the racemate.

Twenty-five of the flavouring substances are classified into structural class I and three are classified into structural class II [FL-no: 04.091, 04.092 and 07.234].

Twenty-two of the flavouring substances in the present group have been reported to occur naturally in a wide range of food items.

According to the default MSDI approach, the 28 flavouring substances in this group have intakes in Europe from 0.001 to 610 microgram/capita/day, which are all below the thresholds of concern for their respective class of 1800 microgram/person/day for structural class I or 540 microgram/person/day for structural class II substances.

One of the flavourings substances, 3,4-methylenedioxyphenol [FL-no: 04.080], was reported to have a genotoxic potential *in vitro*, while *in vivo* studies were not available for this candidate substance. Therefore, the Panel decided that the Procedure could not be applied to this candidate substance until adequate genotoxicity data become available. The genotoxicity data available did not preclude an evaluation of the other 27 candidate flavouring substances in this Flavouring Group Evaluation through the Procedure.

The candidate substances in this group are conjugated with glucuronic acid or sulphate very efficiently and these pathways are not easily saturated. At high dose levels reactive metabolites (quinones, catechols, quinone methides) may be formed, but it is not expected that, at the levels of intake from the use as flavouring substances, the formation of these metabolites would overwhelm detoxication capacity through conjugation with sulphate, glucuronic acid or in particular glutathione. Thus, it is concluded that all 27 candidate substances in this group, which have been evaluated using the Procedure, may be expected to be metabolised to innocuous products at the estimated levels of intake, based on the MSDI approach.

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 was considered that on the basis of the default MSDI approach the 27 candidate substances evaluated through the Procedure would not give rise to safety concerns at the estimated levels of intake arising from their use as flavouring substances. The total combined intake of the candidate and supporting substances belonging to structural class I is approximately 3900 microgram/capita/day, which exceeds the threshold of concern for a compound belonging to structural class I of 1800 microgram/person/day. The main contribution to the MSDI of 3900 microgram/capita/day originates from one supporting substance, 4-(p-hydroxyphenyl)butan-2-one [FL-no: 07.055], for which the estimated daily per capita intake is 2400 microgram. For this supporting substance a 13 week study provides a NOAEL of 100 mg/kg bw/day which gives a margin of safety of 2500 to the MSDI value.

The estimated intakes for the 24 candidate substances evaluated through the Procedure and belonging to structural class I, range from 74 to 35000 microgram/person/day based on the mTAMDI. The mTAMDI is above the threshold of concern of 1800 microgram/person/day for two of the candidate substances [FL-no: 08.134 and 09.946].

The estimated intakes of the three substances evaluated through the Procedure and belonging to structural class II, are 420 microgram/person/day based on the mTAMDI, which is below the threshold of concern for structural class II substances of 540 microgram/person/day.

Thus, for two candidate substances [FL-no: 08.134 and 09.946], for which the mTAMDI is above the threshold, further information is required. This would include more reliable intake data and then, if required, additional toxicological data.

The 25 substances which have mTAMDI intake estimates below the threshold of concern for their structural class are also expected to be metabolised to innocuous products.

In order to determine whether the conclusion for the 27 candidate substances evaluated using the Procedure can be applied to the materials of commerce, it is necessary to consider the available specifications. Adequate specifications including complete purity criteria and identity tests for the materials of commerce have been provided for all 27 flavouring substances evaluated through the Procedure.

Thus, the Panel concluded that for one candidate substance [FL-no: 04.080] additional genotoxicity data are required and that the remaining 27 candidate substances would present “no safety concern at the estimated level of intakes, based on the MSDI approach.

TABLE 1: SPECIFICATION SUMMARY OF THE SUBSTANCES IN THE FLAVOURING GROUP EVALUATION 22, REVISION 1

Table 1: Specification Summary of the Substances in the FGE.22Rev1

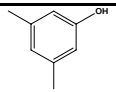
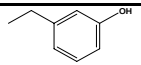
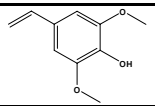
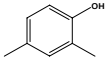
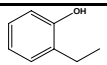
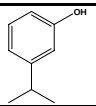
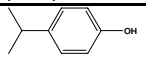
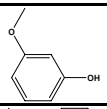
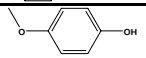
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	Specification comments
04.020	3,5-Dimethylphenol		538 108-68-9	Solid $C_8H_{10}O$ 122.17	Slightly soluble Freely soluble	220 63 MS 95 %	n.a. n.a.	
04.021	3-Ethylphenol		549 620-17-7	Liquid $C_8H_{10}O$ 122.17	Slightly soluble Freely soluble	217 MS 95 %	1.529-1.535 1.019-1.025	
04.061	2,6-Dimethoxy-4-vinylphenol		11229 28343-22-8	Solid $C_{10}H_{12}O_3$ 180.20	Practically insoluble or insoluble Freely soluble	314 135 NMR 95 %	n.a. n.a.	
04.065	2,3-Dimethylphenol		11258 526-75-0	Solid $C_8H_{10}O$ 122.17	Slightly soluble Freely soluble	218 76 MS 95 %	n.a. n.a.	
04.066	2,4-Dimethylphenol		11259 105-67-9	Solid $C_8H_{10}O$ 122.17	Very slightly soluble Freely soluble	210 26 MS 95 %	n.a. n.a.	
04.070	2-Ethylphenol		11232 90-00-6	Liquid $C_8H_{10}O$ 122.17	Slightly soluble Freely soluble	204 MS 95 %	1.534-1.540 1.020-1.026	
04.072	3-Isopropylphenol		618-45-1	Solid $C_9H_{12}O$ 136.19	Practically insoluble or insoluble Freely soluble	228 26 MS 95 %	n.a. n.a.	
04.073	4-Isopropylphenol		99-89-8	Solid $C_9H_{12}O$ 136.19	Practically insoluble or insoluble Freely soluble	223 64 MS 95 %	n.a. n.a.	
04.076	3-Methoxyphenol		150-19-6	Liquid $C_7H_8O_2$ 124.14	Slightly soluble Freely soluble	244 MS 95 %	1.547-1.553 1.063-1.069	
04.077	4-Methoxyphenol		11241 150-76-5	Solid $C_7H_8O_2$ 124.14	Slightly soluble Freely soluble	243 57 MS	n.a. n.a.	

Table 1: Specification Summary of the Substances in the FGE.22Rev1

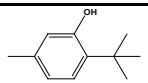
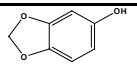
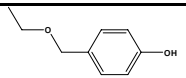
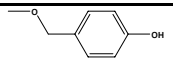
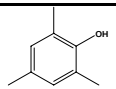
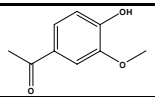
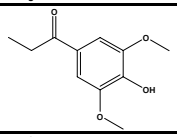
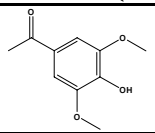
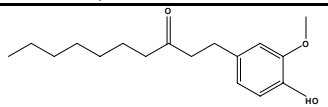
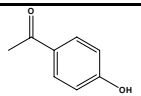
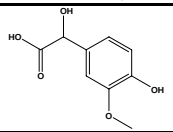
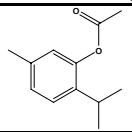
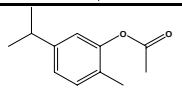
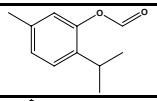
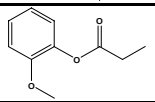
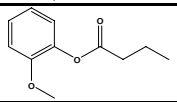
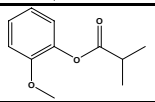
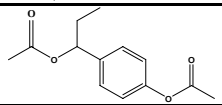
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	Specification comments
						95 %		
04.078	5-Methyl-2-(tert-butyl)phenol		88-60-8	Solid $C_{11}H_{16}O$ 164.25	Practically insoluble or insoluble Freely soluble	244 22 MS 95 %	n.a. n.a.	
04.080	3,4-Methylenedioxyphenol		533-31-3	Solid $C_7H_6O_3$ 138.12	Slightly soluble Freely soluble	115 (3 hPa) 63 MS 95 %	n.a. n.a.	
04.091	Ethyl 4-hydroxybenzyl ether		57726-26-8	Solid $C_9H_{12}O_2$ 152.19	Practically insoluble or insoluble Freely soluble	105 (1 hPa) 53 MS 95 %	n.a. n.a.	
04.092	4-Hydroxybenzyl methyl ether		5355-17-9	Solid $C_8H_{10}O_2$ 138.17	Practically insoluble or insoluble Freely soluble	175 82 MS 95 %	n.a. n.a.	
04.095	2,4,6-Trimethylphenol		4329 527-60-6	Solid $C_9H_{12}O$ 136.19	Practically insoluble or insoluble Freely soluble	221 70 MS 95 %	n.a. n.a.	
07.142	Acetovanillone		11035 498-02-2	Solid $C_9H_{10}O_3$ 166.18	Slightly soluble Freely soluble	297 115 MS 95 %	n.a. n.a.	
07.154	1-(3,5-Dimethoxy-4-hydroxyphenyl)propan-1-one		11106 5650-43-1	Solid $C_{11}H_{14}O_4$ 210.23	Practically insoluble or insoluble Freely soluble	394 109 NMR 95 %	n.a. n.a.	
07.164	4-Hydroxy-3,5-dimethoxyacetophenone		11105 2478-38-8	Solid $C_{10}H_{12}O_4$ 196.20	Practically insoluble or insoluble Freely soluble	371 122 MS 95 %	n.a. n.a.	
07.234	5-Paradol			Solid $C_{17}H_{26}O_3$ 278.39	Practically insoluble or insoluble Freely soluble	168 (0.1 hPa) 31 MS 95 %	n.a. n.a.	Register name to be changed to 1-(4-Hydroxy-3-methoxyphenyl)-3-decanone; CASrn: 27113-22-0 and EINECS: 248-208-1.

Table 1: Specification Summary of the Substances in the FGE.22Rev1

FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	Specification comments
07.243	4-Hydroxyacetophenone		4330 99-93-4	Solid $C_8H_8O_2$ 136.15	Practically insoluble or insoluble Freely soluble	286 110 MS 95 %	n.a. n.a.	
08.134 -	Nibovan		4660 - 55-10-7	Solid $C_9H_{10}O_5$ 198.17	Slightly soluble Freely soluble	n.a. 126.7 IR NMR MS 96 %	n.a. n.a.	Racemate. Register name to be changed to: 4-Hydroxy-3-methoxy-mandelic acid.
09.253	2-Isopropyl-5-methylphenyl acetate		2308 528-79-0	Solid $C_{12}H_{16}O_2$ 192.26	Practically insoluble or insoluble Freely soluble	243 30 MS 95 %	1.531-1.537 n.a.	
09.337	Carvacryl acetate		6380-28-5	Solid $C_{12}H_{16}O_2$ 192.26	Practically insoluble or insoluble Freely soluble	245 30 MS 95 %	1.489-1.495 n.a.	
09.893	2-Isopropyl-5-methylphenyl formate			Solid $C_{11}H_{14}O_2$ 178.23	Practically insoluble or insoluble Freely soluble	286 41 NMR 95 %	n.a. n.a.	CASrn 406700-80-9 to be introduced in the Register (EFFA, 2004g).
09.943 -	Guaiacol propionate		4609 - 7598-60-9	Liquid $C_{10}H_{12}O_3$ 180.2	Slightly soluble Soluble	118 (8.3 Torr) n.a. NMR MS 99 %	1.5276-1.5376 1.0926-1.1026	
09.944 -	Guaiacol butyrate		4607 - 4112-92-9	Liquid $C_{11}H_{14}O_3$ 194.23	Slightly soluble Soluble	96-97(1.5 Torr) n.a. NMR MS 99 %	1.522-1.532 1.0644-1.0744	
09.945 -	Guaiacol isobutyrate		4608 - 723759-62-4	Liquid $C_{11}H_{14}O_3$ 194.23	Slightly soluble Soluble	78-80(0.75Torr) n.a. NMR MS 99 %	1.5175-1.5275 1.0559-1.0659	
09.946 -	Dihydrogalangal acetate		4555 - 129319-15-9	Solid $C_{13}H_{16}O_4$ 236	Insoluble Slightly soluble	n.a. 41.5-43.5 NMR MS 99.5 %	n.a. n.a.	Racemate.

1) Solubility in water, if not otherwise stated.

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

3) At 1013.25 hPa, if not otherwise stated.

4) At 20°C, if not otherwise stated.

5) At 25°C, if not otherwise stated.

TABLE 2A: SUMMARY OF SAFETY EVALUATION APPLYING THE PROCEDURE (BASED ON INTAKES CALCULATED BY THE MSDI APPROACH)

Table 2a: Summary of Safety Evaluation Applying the Procedure (based on intakes calculated by the MSDI approach)

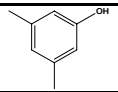
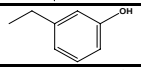
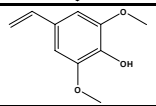
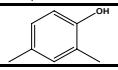
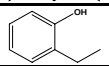
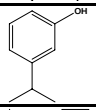
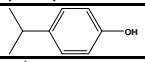
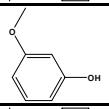
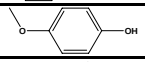
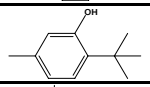
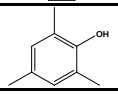
FL-no	EU Register name	Structural formula	MSDI 1) (µg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
04.020	3,5-Dimethylphenol		0.037	Class I A3: Intake below threshold	4)	6)	
04.021	3-Ethylphenol		0.073	Class I A3: Intake below threshold	4)	6)	
04.061	2,6-Dimethoxy-4-vinylphenol		1.2	Class I A3: Intake below threshold	4)	6)	
04.065	2,3-Dimethylphenol		0.013	Class I A3: Intake below threshold	4)	6)	
04.066	2,4-Dimethylphenol		0.011	Class I A3: Intake below threshold	4)	6)	
04.070	2-Ethylphenol		0.037	Class I A3: Intake below threshold	4)	6)	
04.072	3-Isopropylphenol		0.0012	Class I A3: Intake below threshold	4)	6)	
04.073	4-Isopropylphenol		0.24	Class I A3: Intake below threshold	4)	6)	
04.076	3-Methoxyphenol		0.011	Class I A3: Intake below threshold	4)	6)	
04.077	4-Methoxyphenol		0.12	Class I A3: Intake below threshold	4)	6)	
04.078	5-Methyl-2-(tert-butyl)phenol		0.061	Class I A3: Intake below threshold	4)	6)	
04.095	2,4,6-Trimethylphenol		0.0097	Class I A3: Intake below threshold	4)	6)	

Table 2a: Summary of Safety Evaluation Applying the Procedure (based on intakes calculated by the MSDI approach)

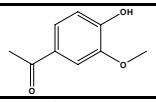
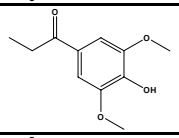
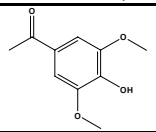
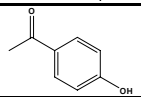
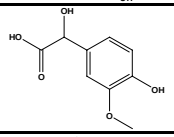
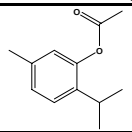
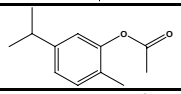
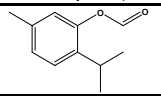
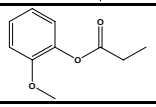
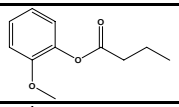
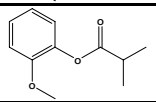
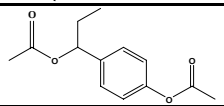
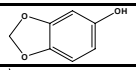
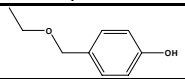
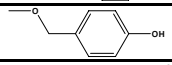
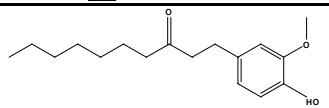
FL-no	EU Register name	Structural formula	MSDI 1) (µg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
07.142	Acetovanillone		2.2	Class I A3: Intake below threshold	4)	6)	
07.154	1-(3,5-Dimethoxy-4-hydroxyphenyl)propan-1-one		0.026	Class I A3: Intake below threshold	4)	6)	
07.164	4-Hydroxy-3,5-dimethoxyacetophenone		0.24	Class I A3: Intake below threshold	4)	6)	
07.243	4-Hydroxyacetophenone		0.016	Class I A3: Intake below threshold	4)	6)	
08.134 -	Nibovan		120	Class I A3: Intake below threshold	4)	6)	
09.253	2-Isopropyl-5-methylphenyl acetate		1.1	Class I A3: Intake below threshold	4)	6)	
09.337	Carvacryl acetate		0.61	Class I A3: Intake below threshold	4)	6)	
09.893	2-Isopropyl-5-methylphenyl formate		0.52	Class I A3: Intake below threshold	4)	6)	
09.943 -	Guaiacol propionate		43	Class I A3: Intake below threshold	4)	6)	

Table 2a: Summary of Safety Evaluation Applying the Procedure (based on intakes calculated by the MSDI approach)

FL-no	EU Register name	Structural formula	MSDI 1) (µg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
09.944 -	Guaiacol butyrate		43	Class I A3: Intake below threshold	4)	6)	
09.945 -	Guaiacol isobutyrate		43	Class I A3: Intake below threshold	4)	6)	
09.946 -	Dihydrogalangal acetate		610	Class I A3: Intake below threshold	4)	6)	
04.080	3,4-Methylenedioxyphenol		1.7	Class I No evaluation			a)
04.091	Ethyl 4-hydroxybenzyl ether		0.0012	Class II A3: Intake below threshold	4)	6)	
04.092	4-Hydroxybenzyl methyl ether		0.61	Class II A3: Intake below threshold	4)	6)	
07.234	5-Paradol		0.012	Class II A3: Intake below threshold	4)	6)	

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

2) Thresholds of concern: Class I = 1800, 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.)

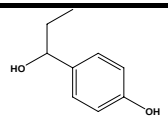
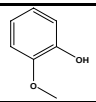
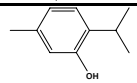
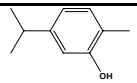
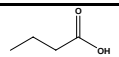
7) Tentatively regarded as presenting no safety concern (based on intake calculated by the MSDI approach) pending further information on the purity of the material of commerce and/or information on stereoisomerism.

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

a) Evaluation deferred pending further genotoxicity data.

TABLE 2B: EVALUATION STATUS OF HYDROLYSIS PRODUCTS OF CANDIDATE ESTERS

Table 2b: Evaluation Status of Hydrolysis Products of Candidate Esters

FL-no	EU Register name JECFA no	Structural formula	SCF status 1) JECFA status 2) CoE status 3) EFSA status	Structural class 4) Procedure path (JECFA) 5)	Comments
Non-register	Dihydrogalangal		Not evaluated as flavouring substance		Not in Register
04.005	2-Methoxyphenol 713		No safety concern a) Category B b)	Class I A3: Intake below threshold	
04.006	Thymol 709		No safety concern a) Category B b)	Class I A3: Intake below threshold	
04.031	Carvacrol 710		No safety concern a) Category B b)	Class I A3: Intake below threshold	
08.001	Formic acid 79		Category 1 c) No safety concern d) Deleted b)	Class I A3: Intake below threshold	
08.002	Acetic acid 81		Category 1 c) No safety concern d) Category A b)	Class I A3: Intake above threshold, A4: Endogenous	
08.003	Propionic acid 84		Category 1 c) No safety concern d) Category A b)	Class I A3: Intake above threshold, A4: Endogenous	
08.005	Butyric acid 87		Category 1 c) No safety concern d) Category A b)	Class I A3: Intake above threshold, A4: Endogenous	
08.006	2-Methylpropionic acid 253		Category 1 c) No safety concern d) Category A b)	Class I A3: Intake below threshold	

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 µg/person/day, Class II = 540 µg/person/day, 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, 2001a).

b) (CoE, 1992).

c) (SCF, 1995).

d) (JECFA, 1999b).

TABLE 3: SUPPORTING SUBSTANCES SUMMARY

Table 3: Supporting Substances Summary

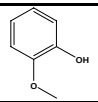
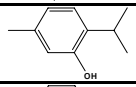
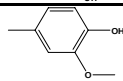
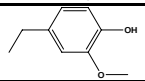
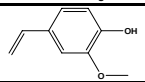
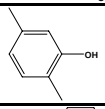
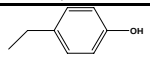
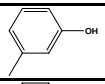
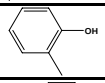
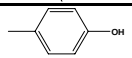
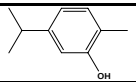
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (µg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
04.005	2-Methoxyphenol		2532 173 90-05-1	713 JECFA specification (JECFA, 2000d)	44	No safety concern a) Category B b)	
04.006	Thymol		3066 174 89-83-8	709 JECFA specification (JECFA, 2000d)	51	No safety concern a) Category B b)	
04.007	2-Methoxy-4-methylphenol		2671 175 93-51-6	715 JECFA specification (JECFA, 2000d)	31	No safety concern a) Category B b)	
04.008	4-Ethylguaiacol		2436 176 2785-89-9	716 JECFA specification (JECFA, 2000d)	6.9	No safety concern a) Category B b)	
04.009	2-Methoxy-4-vinylphenol		2675 177 7786-61-0	725 JECFA specification (JECFA, 2000d)	2.6	No safety concern a) Deleted b)	
04.019	2,5-Dimethylphenol		3595 537 95-87-4	706 JECFA specification (JECFA, 2000d)	0.49	No safety concern a) Deleted b)	
04.022	4-Ethylphenol		3156 550 123-07-9	694 JECFA specification (JECFA, 2000d)	3.5	No safety concern a) Category B b)	
04.026	3-Methylphenol		3530 617 108-39-4	692 JECFA specification (JECFA, 2000d)	0.12	No safety concern a) Category A b)	
04.027	2-Methylphenol		3480 618 95-48-7	691 JECFA specification (JECFA, 2000d)	250	No safety concern a) Category A b)	
04.028	4-Methylphenol		2337 619 106-44-5	693 JECFA specification (JECFA, 2000d)	0.97	No safety concern a) Category A b)	
04.031	Carvacrol		2245 2055 499-75-2	710 JECFA specification (JECFA, 2000d)	14	No safety concern a) Category B b)	

Table 3: Supporting Substances Summary

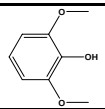
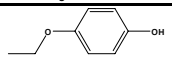
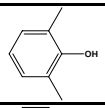
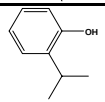
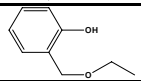
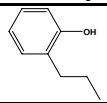
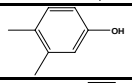
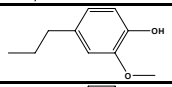
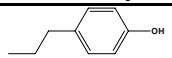
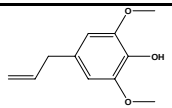
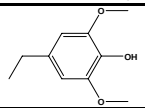
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (μg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
04.036	2,6-Dimethoxyphenol		3137 2233 91-10-1	721 JECFA specification (JECFA, 2000d)	5.4	No safety concern a) Category B b)	
04.037	4-Ethoxyphenol		3695 2258 622-62-8	720 JECFA specification (JECFA, 2001c)	0.37	No safety concern a) Category B b)	
04.042	2,6-Dimethylphenol		3249 11261 576-26-1	707 JECFA specification (JECFA, 2000d)	1.7	No safety concern a)	
04.044	2-Isopropylphenol		3461 11234 88-69-7	697 JECFA specification (JECFA, 2000d)	14	No safety concern a)	
04.045	2-(Ethoxymethyl)phenol		3485 11905 20920-83-6	714 JECFA specification (JECFA, 2000d)	1.5	No safety concern a)	
04.046	2-Propylphenol		3522 11908 644-35-9	695 JECFA specification (JECFA, 2000d)	0.12	No safety concern a)	
04.048	3,4-Dimethylphenol		3596 11262 95-65-8	708 JECFA specification (JECFA, 2000d)	5.7	No safety concern a)	
04.049	2-Methoxy-4-propylphenol		3598 2785-87-7	717 JECFA specification (JECFA, 2000d)	180	No safety concern a)	
04.050	4-Propylphenol		3649 645-56-7	696 JECFA specification (JECFA, 2000d)	0.049	No safety concern a)	
04.051	4-Allyl-2,6-dimethoxyphenol		3655 11214 6627-88-9	726 JECFA specification (JECFA, 2001c)	0.012	No safety concern a)	
04.052	4-Ethyl-2,6-dimethoxyphenol		3671 11231 14059-92-8	723 JECFA specification (JECFA, 2001c)	1.3	No safety concern a)	

Table 3: Supporting Substances Summary

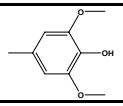
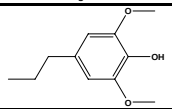
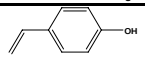
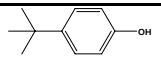
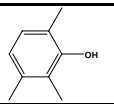
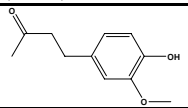
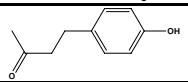
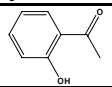
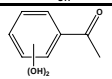
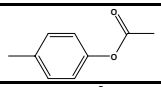
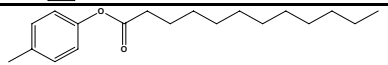
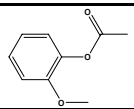
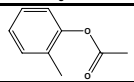
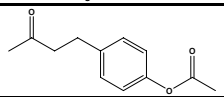
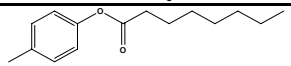
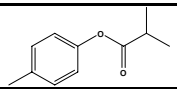
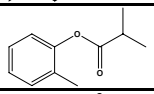
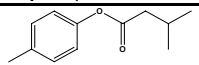
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (µg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
04.053	4-Methyl-2,6-dimethoxyphenol		3704 6638-05-7	722 JECFA specification (JECFA, 2000d)	0.054	No safety concern a)	
04.056	2,6-Dimethoxy-4-propylphenol		3729 6766-82-1	724 JECFA specification (JECFA, 2001c)	0.061	No safety concern a)	
04.057	4-Vinylphenol		3739 11257 2628-17-3	711 JECFA specification (JECFA, 2001c)	0.12	No safety concern a)	
04.064	4-(1,1-Dimethylethyl)phenol		3918 98-54-4	733 JECFA specification (JECFA, 2000d)	0.012	No safety concern a)	
04.085	2,3,6-Trimethylphenol		3963 2416-94-6	737 JECFA specification (JECFA, 2003b)	0.24	No safety concern a)	
07.005	Vanillyl acetone		3124 139 122-48-5	730 JECFA specification (JECFA, 2000d)	34	No safety concern a) Category B b)	
07.055	4-(p-Hydroxyphenyl)butan-2-one		2588 755 5471-51-2	728 JECFA specification (JECFA, 2000d)	2400	No safety concern a) Category A b)	
07.124	2-Hydroxyacetophenone		3548 11784 118-93-4	727 JECFA specification (JECFA, 2000d)	0.12	No safety concern a)	
07.135	2,4-Dihydroxyacetophenone		3662 11884 28631-86-9	729 JECFA specification (JECFA, 2002d)	0.012	No safety concern a)	JECFA evaluated a mixture of dihydroxyacetophenones
09.036	p-Tolyl acetate		3073 226 140-39-6	699 JECFA specification (JECFA, 2000d)	0.047	No safety concern a) Category B b)	
09.102	p-Tolyl dodecanoate		3076 378 10024-57-4	704 JECFA specification (JECFA, 2003b)	0.24	No safety concern a) Category B b)	

Table 3: Supporting Substances Summary

FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (µg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
09.174	2-Methoxyphenyl acetate		3687 552 613-70-7	718 JECFA specification (JECFA, 2000d)	0.012	No safety concern a) Category B b)	
09.228	o-Tolyl acetate		3072 2078 533-18-6	698 JECFA specification (JECFA, 2001c)	0.12	No safety concern a) Category B b)	
09.288	4-(4-Acetoxyphenyl)butan-2-one		3652 3572-06-3	731 JECFA specification (JECFA, 2000d)	0.12	No safety concern a)	
09.301	p-Tolyl octanoate		3733 59558-23-5	703 JECFA specification (JECFA, 2000d)	0.024	No safety concern a)	
09.429	p-Tolyl isobutyrate		3075 304 103-93-5	701 JECFA specification (JECFA, 2000d)	0.037	No safety concern a) Category B b)	
09.480	o-Tolyl isobutyrate		3753 681 36438-54-7	700 JECFA specification (JECFA, 2000d)	0.024	No safety concern a) Category B b)	
09.518	4-Methylphenyl isovalerate		3387 10545 55066-56-3	702 JECFA specification (JECFA, 2000d)	0.37	No safety concern a)	

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, 2001a).

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, 1999a), 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[8] (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[9] (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.

[8] *"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).*

[9] *"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).*

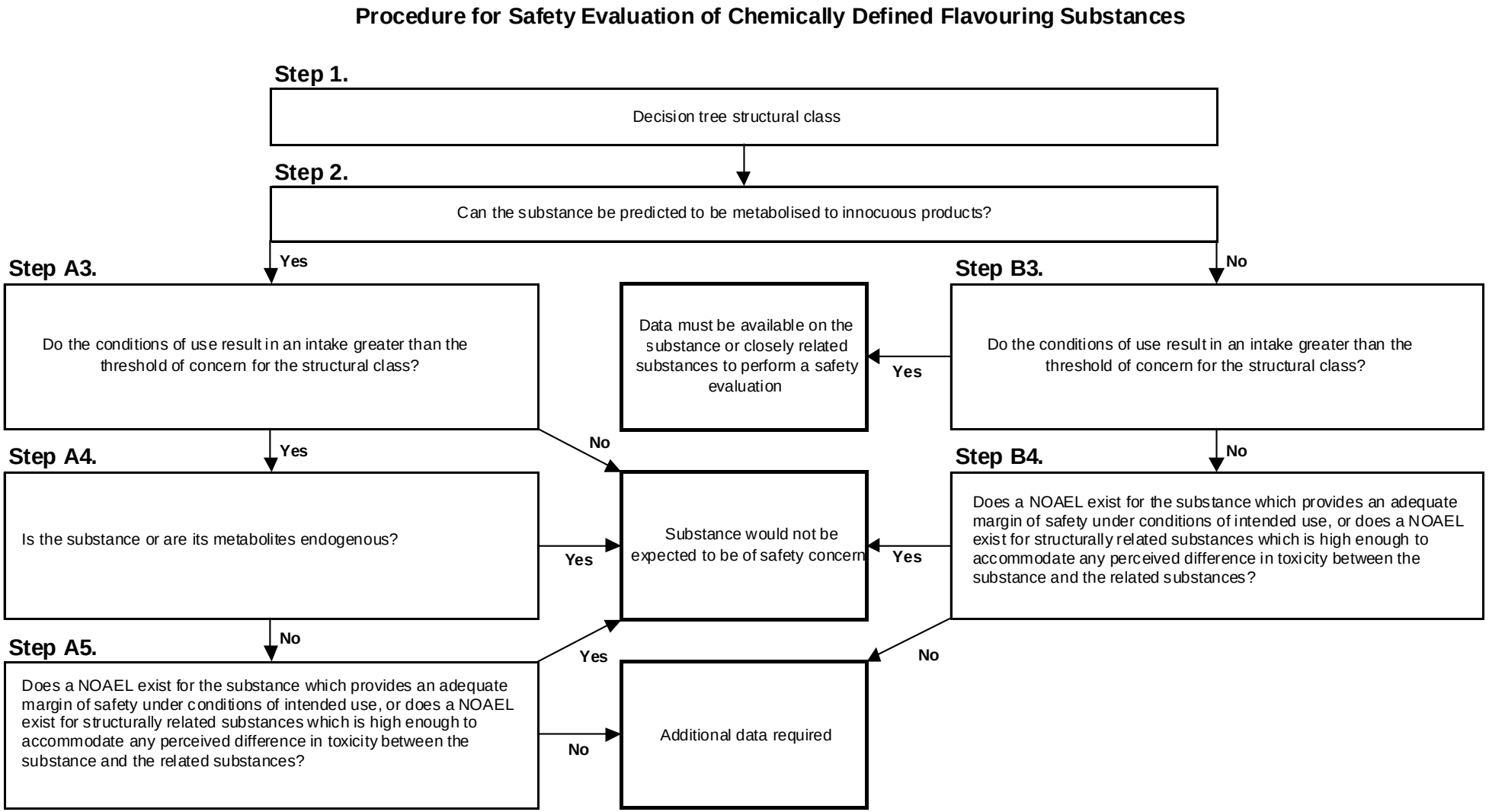


Figure I.1 *Procedure for Safety Evaluation of Chemically Defined Flavouring Substances*

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 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)

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

The "normal and maximum use levels" are provided by Industry for the 23 candidate substances in the present flavouring group (Table II.1.2).

Table II.1.2 Normal and Maximum use levels (mg/kg) for the candidate substances in FGE.22 (EFFA, 2004g; EFFA, 2007a; Flavour Industry, 2009k).

FL-no	Food Categories Normal use levels (mg/kg) Maximum use levels (mg/kg)																	
	01.0	02.0	03.0	04.1	04.2	05.0	06.0	07.0	08.0	09.0	10.0	11.0	12.0	13.0	14.1	14.2	15.0	16.0
04.020	0,5 2,5	0,2 1	0,5 2,5	0,4 2	- -	1 5	0,2 1	2 10	0,2 1	0,2 1	- -	- -	0,3 1,5	0,5 2,5	0,2 1	1 5	2 10	0,4 2
04.021	0,5 2	0,2 1	0,5 2,5	0,4 2	- -	1 5	0,2 1	2 10	0,2 1	0,2 1	- -	- -	0,3 1,5	0,5 2,5	0,2 1	1 5	2 10	0,4 2
04.061	0,5 2,5	0,2 1	0,2 2,5	0,4 2	- -	1 5	0,2 1	2 10	0,2 1	0,2 1	- -	- -	0,3 1,5	0,5 2,5	0,2 1	1 5	2 10	0,4 2
04.065	0,5 2,5	0,2 1	0,5 2,5	0,4 2	- -	1 5	0,2 1	2 10	0,2 1	0,2 1	- -	- -	0,3 1,5	0,5 2,5	0,2 1	1 5	2 10	0,4 2
04.066	0,5 2,5	0,2 1	0,5 2,5	0,4 2	- -	1 5	0,2 1	2 10	0,2 1	0,2 1	- -	- -	0,3 1,5	0,5 2,5	0,2 1	1 5	2 10	0,4 2
04.070	0,5 2,5	0,2 1	0,5 2,5	0,4 2	- -	1 5	0,2 1	2 10	0,2 1	0,2 1	- -	- -	0,3 1,5	0,5 2,5	0,2 1	1 5	2 10	0,4 2
04.072	0,5 2,5	0,2 1	0,5 2,5	0,4 2	- -	1 5	0,2 1	2 10	0,2 1	0,2 1	- -	- -	0,3 1,5	0,5 2,5	0,2 1	1 5	2 10	0,4 2
04.073	0,5 2,5	0,2 1	0,5 2,5	0,4 2	- -	1 5	0,2 1	2 10	0,2 1	0,2 1	- -	- -	0,3 1,5	0,5 2,5	0,2 1	1 5	2 10	0,4 2
04.076	0,5 2,5	0,2 1	0,2 2,5	0,4 2	- -	1 5	0,2 1	2 10	0,2 1	0,2 1	- -	- -	0,3 1,5	0,5 2,5	0,2 1	1 5	2 10	0,4 2

Table II.1.2 Normal and Maximum use levels (mg/kg) for the candidate substances in FGE.22 (EFFA, 2004g; EFFA, 2007a; Flavour Industry, 2009k).

FL-no	Food Categories																	
	Normal use levels (mg/kg) Maximum use levels (mg/kg)																	
	01.0	**02.0**	**03.0**	**04.1**	**04.2**	**05.0**	**06.0**	**07.0**	**08.0**	**09.0**	**10.0**	**11.0**	**12.0**	**13.0**	**14.1**	**14.2**	**15.0**	**16.0**
04.077	0,5	0,2	0,5	0,4	-	1	0,2	2	0,2	0,2	-	-	0,3	0,5	0,2	1	2	0,4
	2,5	1	2,5	2	-	5	1	10	1	1	-	-	1,5	2,5	1	5	10	2
04.078	0,5	0,2	0,5	0,4	-	1	0,2	2	0,2	0,2	-	-	0,3	0,5	0,2	1	2	0,4
	2,5	1	2,5	2	-	5	1	10	1	1	-	-	1,5	2,5	1	5	10	2
04.080	0,5	0,2	0,5	0,4	-	1	0,2	2	0,2	0,2	-	-	0,3	0,5	0,2	1	2	0,4
	2,5	1	2,5	2	-	5	1	10	1	1	-	-	1,5	2,5	1	5	10	2
04.091	0,5	0,2	0,5	0,4	-	1	0,2	2	0,2	0,3	-	-	0,3	0,5	0,2	1	2	0,4
	2,5	1	2,5	2	-	5	1	10	1	1	-	-	1,5	2,5	1	5	10	2
04.092	0,5	0,2	0,5	0,4	-	1	0,2	2	0,2	0,2	-	-	0,3	0,5	0,2	1	2	0,4
	2,5	1	2,5	2	-	5	1	10	1	1	-	-	1,5	2,5	1	5	10	2
04.095	0,5	0,2	0,5	0,4	-	1	0,2	2	0,2	0,2	-	-	0,3	0,5	0,2	1	2	0,4
	2,5	1	2,5	2	-	5	1	10	1	1	-	-	1,5	2,5	1	5	10	2
07.142	0,5	0,2	0,5	0,4	-	1	0,2	2	0,2	0,2	-	-	0,3	0,5	0,2	1	2	0,4
	2,5	1	2,5	2	-	5	1	10	1	1	-	-	1,5	2,5	1	5	10	2
07.154	0,5	0,2	0,5	0,4	-	1	0,2	2	0,2	0,2	-	-	0,3	0,5	0,2	1	2	0,4
	2,5	1	2,5	2	-	5	1	10	1	1	-	-	1,5	2,5	1	5	10	2
07.164	3	2	3	2	-	4	2	5	1	1	-	-	2	3	2	4	5	2
	15	10	15	10	-	20	10	25	5	5	-	-	10	15	10	20	25	10
07.234	0,5	0,2	0,5	0,4	-	1	0,2	2	0,2	0,2	-	-	0,3	0,5	0,2	1	2	0,4
	2	1	2,5	2	-	5	1	10	1	1	-	-	1,5	2,5	1	5	10	2
07.243	3	2	3	2	-	4	2	5	1	1	-	-	2	3	2	4	5	2
	15	10	15	10	-	20	10	25	5	5	-	-	10	15	10	20	25	10
08.134	10	-	10	-	-	10	10	10	-	-	-	-	10	-	5	10	-	20
	50	-	100	-	-	20	20	20	-	-	-	-	20	-	10	20	-	50
09.253	0,5	0,2	0,5	0,4	-	1	0,2	2	0,2	0,2	-	-	0,3	0,5	0,2	1	2	0,4
	2,5	1	2,5	2	-	5	1	10	1	1	-	-	1,5	2,5	1	5	10	2
09.337	0,5	0,2	0,5	0,4	-	1	0,2	2	0,2	0,2	-	-	0,3	0,5	0,2	1	2	0,4
	2,5	1	2,5	2	-	5	1	10	1	1	-	-	1,5	2,5	1	5	10	2
09.893	0,5	0,2	0,5	0,4	-	1	0,2	2	0,2	0,2	-	-	0,3	0,5	0,2	1	2	0,4
	2,5	1	2,5	2	-	5	1	10	1	1	-	-	1,5	2,5	1	5	10	2
09.943	-	-	0,1	-	-	0,2	0,2	0,2	-	-	-	0,1	-	-	0,1	0,2	0,3	0,2
	-	-	5	-	-	5	5	5	-	-	-	5	-	-	5	5	10	5
09.944	-	-	0,1	-	-	0,2	0,2	0,2	-	-	-	0,1	-	-	0,1	0,2	0,3	0,2
	-	-	5	-	-	5	5	5	-	-	-	5	-	-	5	5	10	5
09.945	-	-	0,1	-	-	0,2	0,2	0,2	-	-	-	0,1	-	-	0,1	0,2	0,3	0,2
	-	-	5	-	-	5	5	5	-	-	-	5	-	-	5	5	10	5
09.946	-	-	-	-	-	25	-	-	100	100	-	0,1	500	-	0,5	25	500	-
	-	-	-	-	-	500	-	-	500	500	-	5	1000	-	5	100	1000	-

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 may consume 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)

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

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 II.2.2 Distribution of the 18 food categories listed in Commission Regulation (EC) No 1565/2000 (EC, 2000a) into the seven SCF food categories used for TAMDI calculation (SCF, 1995)

	Food categories according to Commission Regulation 1565/2000	**Distribution of the seven SCF food categories**		
Key	Food category	Food	Beverages	Exceptions
01.0	Dairy products, excluding products of category 02.0	Food		
02.0	Fats and oils, and fat emulsions (type water-in-oil)	Food		
03.0	Edible ices, including sherbet and sorbet	Food		
04.1	Processed fruit	Food		
04.2	Processed vegetables (incl. mushrooms & fungi, roots & tubers, pulses and legumes), and nuts & seeds	Food		
05.0	Confectionery			Exception a
06.0	Cereals and cereal products, incl. flours & starches from roots & tubers, pulses & legumes, excluding bakery	Food		
07.0	Bakery wares	Food		
08.0	Meat and meat products, including poultry and game	Food		
09.0	Fish and fish products, including molluscs, crustaceans and echinoderms	Food		
10.0	Eggs and egg products	Food		
11.0	Sweeteners, including honey			Exception a
12.0	Salts, spices, soups, sauces, salads, protein products, etc.			Exception d
13.0	Foodstuffs intended for particular nutritional uses	Food		
14.1	Non-alcoholic ("soft") beverages, excl. dairy products		Beverages	
14.2	Alcoholic beverages, incl. alcohol-free and low-alcoholic counterparts			Exception c
15.0	Ready-to-eat savouries			Exception b
16.0	Composite foods (e.g. casseroles, meat pies, mincemeat) - foods that could not be placed in categories 01.0 - 15.0	Food		

The mTAMDI values are presented for each of the 28 flavouring substances in the present flavouring group, for which Industry has provided use and use levels (EFFA, 2004g; Flavour Industry, 2009k). The mTAMDI values are only given for normal (average) use levels and are presented as a range corresponding to the lowest and highest normal use level within a food category (see Table II.2.3).

TableII.2.3 Estimated intakes based on the mTAMDI approach

FL-no	**EU Register name**	**mTAMDI (µg/person/day)**	**Structural class**	**Threshold of concern (µg/person/day)**
04.020	3,5-Dimethylphenol	420	Class I	1800

TableII.2.3 Estimated intakes based on the mTAMDI approach

FL-no	EU Register name	mTAMDI (µg/person/day)	Structural class	Threshold of concern (µg/person/day)
04.021	3-Ethylphenol	420	Class I	1800
04.061	2,6-Dimethoxy-4-vinylphenol	420	Class I	1800
04.065	2,3-Dimethylphenol	420	Class I	1800
04.066	2,4-Dimethylphenol	420	Class I	1800
04.070	2-Ethylphenol	420	Class I	1800
04.072	3-Isopropylphenol	420	Class I	1800
04.073	4-Isopropylphenol	420	Class I	1800
04.076	3-Methoxyphenol	420	Class I	1800
04.077	4-Methoxyphenol	420	Class I	1800
04.078	5-Methyl-2-(tert-butyl)phenol	420	Class I	1800
04.095	2,4,6-Trimethylphenol	420	Class I	1800
07.142	Acetovanillone	420	Class I	1800
07.154	1-(3,5-Dimethoxy-4-hydroxyphenyl)propan-1-one	420	Class I	1800
07.164	4-Hydroxy-3,5-dimethoxyacetophenone	1600	Class I	1800
07.243	4-Hydroxyacetophenone	1600	Class I	1800
08.134	Nibovan	5000	Class I	1800
09.253	2-Isopropyl-5-methylphenyl acetate	420	Class I	1800
09.337	Carvacryl acetate	420	Class I	1800
09.893	2-Isopropyl-5-methylphenyl formate	420	Class I	1800
09.943	Guaiacol propionate	74	Class I	1800
09.944	Guaiacol butyrate	74	Class I	1800
09.945	Guaiacol isobutyrate	74	Class I	1800
09.946	Dihydrogalangal acetate	35000	Class I	1800
04.080	3,4-Methylenedioxyphenol	420	Class I	1800
04.091	Ethyl 4-hydroxybenzyl ether	420	Class II	540
04.092	4-Hydroxybenzyl methyl ether	420	Class II	540
07.234	5-Paradol	420	Class II	540

ANNEX III: METABOLISM

III.1. Introduction

The candidate flavouring substances in this group are ring-substituted phenols and esters thereof. Twenty of the substances [FL-no: 04.020, 04.021, 04.061, 04.065, 04.066, 04.070, 04.072, 04.073, 04.076, 04.077, 04.078, 04.080, 04.091, 04.092, 04.095, 07.142, 07.154, 07.164, 07.243 and 07.234] are phenols, which bear one or more substituents. These substituents include methyl, ethyl, vinyl, isopropyl, tert-butyl, methoxy, methyl- and ethyl-benzyl ether, 1-acetyl, 1-propionyl or decan-3-one groups. One of these phenols [FL-no: 04.080] bears a methylenedioxy-ring.

Six other substances in this group are esters: the formate and acetate of thymol (= 2-isopropyl-5-methylphenol) [FL-no: 09.253 and 09.893], the acetate of carvacrol (= 2-methyl-5-isopropylphenol) [FL-no: 09.337] and the propionate-, butyrate- and isobutyrate esters of guaiacol (= 2-methoxyphenol) [FL no: 09.943, 09.444 and 09.945]. One substance (dihydrogalangal acetate) [FL no: 09.946] is a diacetate ester of dihydrogalangal (= 1-(4-(hydroxymethyl)phenyl)-propan-1-ol), which is not in the Register.

The remaining substance (nibovan) [FL no: 08.134] bears as a ring substituent 2-hydroxyacetic acid.

A number of structurally related phenolic supporting substances have been evaluated previously by the JECFA (JECFA, 2001b).

III.2. Absorption, Distribution and Elimination

General data

No detailed absorption and distribution studies for the candidate flavouring substances in this group have been submitted. However, uptake, metabolism and elimination of phenols have been studied quite extensively, and from the high fractions of the dose that can be found in urine usually within 24 hours, it must be concluded that in general these substances are readily and almost completely absorbed and excreted via urine. However, data cited by Williams may indicate that the intestinal absorption of carvacrol may be considerably less than for other phenols (Williams, 1959a).

It is believed that p-cresol, found in serum from healthy human volunteers (0.23 ± 0.17 mg/100 ml), is also formed via bacterial degradation of proteins in the intestinal lumen (Wengle & Hellström, 1972). In addition, in an extensive review on biotransformations the natural occurrence of p-ethylphenol, p-cresol, catechol and quinol in urine from various mammals, including humans has been reported (Williams, 1959a).

Candidate substances

4-Methoxyphenol [FL-no: 04.077]

Female rabbits (2.5 to 3.5 kg) were administered a suspension of 0.7 g p-hydroxy-anisole (= 4-methoxyphenol) once via oral gavage. The mean quantity of the administered dose excreted via the urine within 24 hours following administration was 82 % (Bray et al., 1955), which indicates rapid absorption and elimination.

2,4-Dimethylphenol [FL-no: 04.066]

2,4-Dimethylphenol (2,4-DMP) was administered to male Sprague-Dawley rats by constant intravenous infusion at 17 mg/hour for six hours, intravenous bolus injection at 30 mg/kg bw, or intraperitoneal bolus injection at 30 mg/kg bw. Blood samples were collected at the end of the 6 hours infusion period, at 5, 10, 20, 30 and 60 min after the intravenous bolus injection or at 30 min after the intraperitoneal bolus injection for at least 3 animals per treatment / time point. At the same time points brain, liver and fat tissue samples were collected. 2,4-DMP distributed rapidly in the brain and liver following an intravenous bolus injection reaching peak concentrations in these tissues and plasma by the first measurement point at 5 minutes and declining rapidly over the next 5 to 30 minute intervals. Plasma, liver and fat concentrations of the parent compound were undetectable at 60 minutes; however, 2,4-DMP was detectable in brain tissue at 60 minutes at about 10 % of its value measured at 5 minutes. Also after intravenous infusion for six hours 2,4-DMP showed higher tissue than plasma-concentrations (ratios about 2.8, 1.4 and 2.1 for brain, liver and fat, respectively). The authors concluded that, "The probability of accumulation of 2,4-DMP in brain, liver and fat appears to be minimal, due to rapid metabolism" (Kaka et al., 1982)

III.3. Metabolism

General data

Various ring-substituted phenols were administered at dose levels of 150 - 450 mg/kg bw via gavage to fasted rabbits and urine samples were collected until renal excretion had been completed (usually between 6-16 hours after administration of the substance) (Bray et al., 1952d). In some of the studies (see below) the phenols were given together with sodium sulphite or L-cysteine, which served as "sulphate donors" (in various amounts at various time points). Glucuronic acid conjugation of phenol appeared to be proportional to the administered dose. The rate constants for glucuronidation were not affected by the co-administration of a "sulphate donor". In addition, ring substitution did not affect the rate constant for glucuronidation, unless two methyl groups were present in *ortho*-position (i.e. 2,6-dimethylphenol) or a ring substituent heavier than ~ 35 D (chlorine) was present at *para*-position. In these cases a lower rate constant for glucuronidation was observed.

The same authors also studied rates of sulphation and the dependence of this conjugation reaction upon availability of sulphate (as sulphite or L-cycteine). In fasted rabbits with various phenols given at 250 mg/kg bw by gavage, the sulphate conjugation ranged from 7 to 28 % of the dose excreted via the urine in control animals. When phenols were given in combination with either sulphite or L-cysteine, urinary excretion of sulphate conjugates ranged from 22 – 75 %. The rate of sulphate conjugation was not influenced in a significant way by the nature of the ring substituents. The authors concluded that sulphate conjugation was dependent upon sulphate availability (Bray et al., 1952c).

Ester hydrolysis

Limited data have been submitted to support ester hydrolysis of the candidate esters. 60 % of *o*-cresyl acetate was hydrolysed *in vitro* following incubation with pancreatin for two hours at 37°C and pH 7.5 (Grundschober, 1977). Methyl salicylate has been shown to be hydrolysed *in vivo* in rats, dogs and humans (Davison et al., 1961). A male human volunteer was orally administered one capsule of 90 mg phenyl salicylate per hour for 8 hours. Urine was collected for 72 hours beginning with the first dose, and fractionated into 8-hours collection periods. Analysis of total urinary phenol showed a 472 mg/l peak during the second 8 hours collection period. The level of free urinary phenol peaked at 25 mg/l during the same period. Approximately sixty hours after the first dose, both total and free urinary phenol levels returned to baseline levels (7 ppm and 1 ppm, respectively). Based on the presented data, for the phenol moiety a total urinary recovery of 130 % could be calculated after subtraction of the baseline phenol excretion (Fishbeck et al., 1975). From the urinary excretion of phenol, it can be concluded that phenyl salicylate is probably completely hydrolysed, either in the GI-tract or after absorption.

Although these data are fairly limited, it is expected that the esters of the three alkyl-substituted phenolic candidate flavouring substances undergo ester hydrolysis *in vivo*. The hydrolysis products are formic and acetic acid from [FL-no: 09.893] and [FL-no: 09.253 and 09.337], respectively. These acids have previously been evaluated (SCF, 2002d; SCF, 2002e). The alcohols released after ester hydrolysis are 2-methyl-5-isopropylphenol (= carvacrol) and 5-methyl-2-isopropylphenol (= thymol) for [FL-no: 09.337] and [FL-no: 09.893 and 09.253], respectively. These two alcohols have no special structural features as compared to the other candidate phenols in this FGE. Ester hydrolysis may also be expected for [FL-no: 09.943, 09.944, 09.945 and 09.946] which would yield the free phenolic substances guaiacol [FL-no: 04.005] and hydroxygalangal (not in the register), together with 4 different short-chain carboxylic acids acetic acid, propionic acid, isobutanoic acid and butanoic acid and propionic acid. The alcoholic fragments of these esters do not bear special structural features as compared to the other candidate phenols in this FGE. The carboxylic acid fragments have been evaluated previously to be of no safety concern (EFSA, 2008ag).

Alkyl-substituted phenols

[FL-no: 04.020, 04.021, 04.065, 04.066, 04.070, 04.072, 04.073, 04.078 and 04.095; alcohol moieties of FL-no: 09.253, 09.893 and 09.337].

In rabbits, given a single oral 200 mg/kg bw dose of 2- and 3-methylphenols, these phenols were hydroxylated to 2,5-dihydroxytoluene to a minor extent (< 3 % of the dose), while up to 10 % of *p*-cresol was oxidised and excreted as *p*-hydroxybenzoic acid. With both 3- and 4-methylphenol, also trace amounts of 3,4-dihydroxytoluene were found. The non-acidic phenolic metabolites (i.e. the parent cresols and the dihydroxytoluenes) were excreted mainly as the glucuronic acid (60-72 % of the dose) and sulphate conjugates (10-15 % of the dose) within 24 hours. *p*-Hydroxybenzoic acid was excreted as an unidentified conjugate and as free acid (Bray et al., 1950a).

One gram doses (~ 300 – 500 mg/kg bw) of 2,3-, 2,4- 2,5-, 2,6-, 3,4- or 3,5- dimethylphenol given to rabbits were excreted as xylenyl sulphates (8 – 16 %) and ether glucuronides (50 – 72 %). Small amounts (1-2 %) of ester glucuronides were also observed. Only 1 – 3 % of the administered dose was excreted unconjugated. Small amounts of ring and side-chain hydroxylation products were also formed, and for the 2,6- and 3,5-xylenols these could be identified as 2,5-dihydroxy-1,3-dimethylbenzene (= 1,3-dimethyldihydroquinone). Total urinary recoveries ranged from 84 – 96 % of the dose (Bray et al., 1950b). Based on another study by the same research group (Bray et al., 1950a), it is likely that these amounts were excreted within 24 hours after dosing.

According to data presented in an extensive review on biotransformations, monoalkyl-substituted phenols are mainly conjugated with sulphate or glucuronic acid (Williams, 1959a). To a small extent, they undergo side-chain oxidation and ring hydroxylation to form a second hydroxyl group, in ortho or para position to the existing hydroxyl group. Thymol (5-methyl-2-isopropylphenol) and carvacrol (2-methyl-5-isopropylphenol) are also excreted via conjugation with sulphates and glucuronic acid. Thymoquinol (2-isopropyl- 5-methyl-1,4-benzenediol) has been detected as a minor oxidation product of thymol. At least for thymol a higher urinary excretion of free parent compound than with phenol may occur, as has been observed in the dog (Williams, 1959a).

In rats, less than 0.2 % of a 100 mg/kg bw oral dose of *p*-cresol was ring hydroxylated and *o*-methylated to yield 4- and 5-methylguaiacol [2-methoxy-4- and 5-methylphenol; ratio 2 to 1] (Bakke, 1970).

In rat liver slice incubations with 2-, 3- or 4-methylphenol, the latter isomer appeared to be ~ 10 times more cytotoxic than the *o*- and *m*- forms. Especially for the *p*-isomer (i.e. 4-methylphenol), cytotoxicity was mediated by bioactivation and could be influenced by modification of the intracellular GSH levels. 4-Methylphenol caused profound depletion of GSH, which preceded onset of cytotoxicity. Reactive intermediates in 4-methylphenol metabolism were also capable of covalent binding to protein. No cytotoxicity was observed with *p*-hydroxybenzyl alcohol, a primary metabolite of 4-methylphenol

(Thompson et al., 1994). The authors speculated that a reactive intermediate leading to *p*-hydroxybenzyl alcohol could be the cause of the cytotoxicity of 4-methylphenol. In subsequent studies it was demonstrated with 4-methylphenol, 4-ethylphenol and *p*-isopropylphenol that the reactive intermediate is a quinone methide, which can be detoxified by conjugation with GSH. The most cytotoxic congener of this series appeared to be the *p*-isopropylphenol. 4-Methylphenol appeared to be the least cytotoxic of these three *para*-substitued phenols (Thompson et al., 1995a).

Despite the fact that 2- and 3-methylphenols are considerably less cytotoxic than the *p*-isomer, also with 2- and 3-methylphenol some decrease of hepato-cellular GSH has been observed, which could indicate the involvement of an epoxide in the metabolism towards 2,5-dihydroxytoluene (Bray et al., 1950a; Thompson et al., 1994). Further, from the data by Thompson et al., (1994), the involvement of a quinone-hydroquinone redox cycle in hepatocellular GSH depletion by 2- and 3-methylphenol cannot be excluded.

The kinetic behaviour of 4-methylphenol was investigated in male OFA rats (mean body weight = 310 g) that received an intravenous bolus of 2 ml isotonic saline or 3 mg 4-methylphenol in 2 ml isotonic saline. Blood samples were collected at 0, 5, 30, 60, 120, 180 and 240 minutes after administration of 4-methylphenol and urine was collected at one hour intervals. The serum concentration of 4-methylphenol at the 5-minute time point was 6.7 ± 1.4 mg/l and decreased gradually to 0.6 ± 0.3 mg/l at 240 minutes post-administration. No 4-methylphenol was found in the serum of control rats. Urinary excretion of 4-methylphenol was 23 ± 10 % of the administered dose over the 4 hours observation period, and the half-life was 1.5 ± 0.8 hours. Total clearance (CLt) was 23.2 ± 4.5 ml/hr/kg, but renal clearance (CLr) was substantially lower at 4.8 ± 2.0 ml/hr/kg. The volume of distribution (Vd) was determined to be 2.9 ± 1.4 l/kg. No attention was paid to the possible renal excretion of conjugation products. A putative urinary metabolite of 4-methylphenol was not identified (Lesaffer et al., 2001).

The systemic availability and the pharmacokinetics of thymol were determined in 12 fasted healthy human male volunteers that each received a single 1.08 mg oral dose of thymol. Venous blood samples and urine were collected at various time intervals up to 72 hours post administration of the test substance. Thymol was absorbed rapidly as evidenced by the detection of thymol sulphate in plasma 20 minutes after dose administration. Mean peak plasma concentration of thymol sulphate, achieved at 1.97 ± 0.77 hours, was 93.1 ± 24.5 ng/ml. The mean terminal elimination half-life for thymol was calculated to be 10.2 ± 1.4 hours. The same metabolite profile was seen for all the subjects. Free thymol or thymol glucuronic acid were undetectable in human plasma. In contrast, in urine the metabolites thymol sulphate and thymol glucuronic acid were found. The combined amount of thymol sulphate and thymol glucuronide excreted in 24-hour urine was 16.2 ± 4.5 % of the administered dose of thymol, with a sulphate / glucuronide ratio of ~ 1.15 /1, as determined in one of the volunteers. No further renal elimination could be observed at later time points. The authors argued that the urinary glucuronic acid could have been formed in the kidney subsequent to renal hydrolysis of the sulphate conjugate present in the blood (Kohlert et al., 2002).

Alkoxy-substituted phenols

[FL-no: 04.076, 04.077, 04.091 and 04.092]

Female rabbits (2.5 to 3.5 kg) were administered a suspension of 0.7 g 1-methoxy-4-methylbenzene or 0.7 g 4-methoxyanisole once via oral gavage. The mean quantity of the administered dose excreted in the urine within 24 hours following administration was 85 and 94 % of the dose, respectively. With 4-methylanisole 27 % of the dose was converted into phenols, mainly 4-methylphenol (i.e. a O-demethylation product) or some 4-hydroxybenzoic acid, which were excreted as their sulphate and glucuronic acid conjugates. The major part of the dose (on average 58 %) was excreted as glucuronide and glycine conjugates of 4-methoxybenzoic acid (i.e. oxidised at the methyl group). With 4-methoxyanisole only glucuronic acid and sulphate conjugates of phenolic metabolites (mainly 4-methoxyphenol and small amounts of quinol) were seen together with some unconjugated phenols. In a similar study, also 0.7 g of anisic acid (4-methoxy benzoic acid was given to rabbits. No O-demethylation was observed, but within 24 hours on average 57 %

and 38 % of the dose were recovered as the glucuronide ester and glycine conjugate of the parent compound, respectively (Bray et al., 1955).

In cats, a single 20 or 40 mg/kg bw intravenous dose of ^{14}C-2,6-dimethoxyphenol was excreted mainly as conjugated (90-93 % as 2,6-dimethoxy-4-hydroxyphenol disulphate) and unconjugated (3 % as 2,6-dimethoxy-4-hydroxyphenol) within 24 hours of dosing. Free (5 %) and conjugated (2 % with glucuronic acid) forms of the parent phenol (all expressed as percentage of urinary radioactivity) were also detected. Total urinary excretion over the first 24 hours post dosing amounted to 75 - 91 % of the dose in males and 85-80 % of the dose in females (for 20 and 40 mg/kg bw, respectively). Biliary excretion was only observed during the first 6 hours post dosing and amounted to ~ 3 - 7 % of the dose (metabolites not further identified) (Miller et al., 1976).

In humans, 73 % of a 50 mg dose of guaiacol (i.e. 2-methoxyphenol) was excreted probably as conjugates (not further identified) within 14 hours (Sedivec & Flek, 1970) (paper in Czech language with abstract in English).

Phenols with alkoxy-, alkenyl- or ketonic ring substituents

[FL-no: 04.061 (vinyl) and FL-no: 07.142; 07.154; 07.164; 07.243 and 07.234 (ketonic)]

Like the *p*-alkylphenols, such as 4-methylphenol, 4-ethylphenol and, 4-isopropyl-phenol (see above), 2-methoxy-4-alkylphenols can form electrophilic quinone methide intermediates at high cellular concentrations. An *in vitro* study revealed that 2-methoxy-4-methylphenol, 2-methoxy-4-ethylphenol, 2-methoxy-4-propylphenol, 2-methoxy-4-isopropylphenol, 2-methoxy-4-allylphenol (= eugenol) and 4-allyl-2,6-dimethoxyphenol are oxidised to quinone methide intermediates in rat liver slices. The half-lives of quinone methide intermediates formed from these substances were 1, 6, 6, 87, 408 and 4332 seconds, respectively. The data suggest that quinone methide intermediates with half-lives in the 10 seconds to 10 minutes range have optimal reactivity and stability for causing cytotoxicity. Outside this range, the quinone methide either reacts with the solvent water rather than the target biomacromolecules, or has only low reactivity towards cellular nucleophiles. In both cases this is reflected in lower cytotoxicity of the respective parent phenol (Thompson et al., 1995b). If concentrations of the quinone methide intermediates are insufficient to deplete intracellular GSH, they may be detoxicated via conjugation with this scavenger (Thompson et al., 1994; Thompson et al., 1995a; Thompson et al., 1995b).

Vinyl- and alkoxy- substituted phenol

[FL-no: 04.061]

No data have been submitted to describe or support the metabolism of 2,6-dimethoxy-4-vinylphenol [FL-no: 04.061] with respect to the vinyl ring substituent. However, 4-vinylphenol is a known metabolite of styrene (vinyl benzene) and data from the metabolism of the latter substance may be useful to support the metabolism of [FL-no: 04.061].

When animals or humans are exposed to styrene, 4-vinylphenol can be identified in the urine as glucuronic acid or sulphate conjugates (Bakke & Scheline, 1970; Pfäffli et al., 1981; Manini et al., 2003). In addition, after intraperitoneal injection of styrene in rats, *p*-hydroxymandelic acid, *p*-hydroxy-benzoic acid and *p*-hydroxyhippuric acid, which may be secondary metabolites of 4-vinylphenol after oxidation of the vinyl double bond, have been identified (Pantarotto et al., 1978). 4-Vinylphenol was shown to be an intermediate in the subsequent and rapid formation of 1-(4-hydroxyphenyl)ethane-1,2-diol as a metabolite of styrene (Watabe et al., 1984). The putative intermediate 4-hydroxystyrene-7,8-epoxide was extremely unstable in aqueous environment but could be trapped in the presence of mercaptoethanol. 4-Vinylphenol is a known liver and lung toxicant. To elicit a toxic response in these tissues, bioactivation of 4-vinylphenol is required via cytochromes P450 (CYP 2E1 and 2F2). Microsomal preparations of mouse liver and lung appeared to be

far more active than microsomes from the same tissues in the rat. In addition, metabolism of 4-vinylphenol in mouse lung microsomes was also observed to occur with glutathione, also in absence of an active cytochrome P450 system (Carlson et al., 2001; Carlson et al., 2002). However, 4-vinylphenol is not a potent GSH-depleting agent in liver and lung tissue *in vivo* (Turner et al., 2005). It is noted that no specific metabolism studies with 4-vinylphenol in which metabolites have been identified, have been done up to now, apart from one study in a bacterial system. In this study it has been shown that Nocardia is capable of producing 1-(4-hydroxyphenyl)ethanol and 4-hydroxyacetophenone when incubated with 4-vinylphenol. The authors hypothesised that in this conversion a quinone methide intermediate might be involved (Lee & Rosazza, 2002).

Alkoxy and ketone-substituted phenols

[FL-no: 07.142, 07.154, 07.164, 07.243 and 07.234]

No data have been submitted for any of these five candidate flavouring substances. However, for some structural analogues, data were submitted or could be retrieved from the public domain literature.

Acetophenone [FL-no: 07.004] is an intermediate in the metabolism of ethylbenzene. It was made conceivable by studying various intermediates that acetophenone may undergo side-chain omega oxidation and keto-reduction, leading via phenyl glycol aldehyde to mandelic acid. When methyl-^{14}C-acetophenone was given to rats, 10 % of the radioactivity was exhaled as carbon dioxide within 4 hours and 30 % within 13 hours post dosing, which demonstrated length reduction of the side chain. Benzoic acid is a known metabolite of ethylbenzene (Sullivan et al., 1976). From the pathway indicated above it may be concluded that the carbon dioxide is released by decarboxylation of mandelic acid, which results in the formation of benzoic acid.

In rabbit liver S9 fraction, incubated with phenylacetone (1-phenyl-2-propanone) the following metabolites could be detected: 1-phenyl-2-propanol from ketoreduction (~ 90 % of all material retrieved), 1-phenyl-1,2-propanediol, 1-phenyl-1-hydroxypropanone and benzoic acid. Within 60 minutes almost all parent compound had been converted. Reduction of the keto-group was also observed with 1-phenyl-butan-2-one and with 1-phenyl-3-methyl-butan-2-one (Kammerer et al., 1978).

Vanillyl acetone [FL-no: 07.005] (i.e. 4-(4-hydroxy-3-methoxyphenyl)butan-2-one)) administered to rats in single oral or intraperitoneal doses (100 mg/kg bw) was excreted in the urine mainly as glucuronic acid and/or sulphate conjugates, quantitatively in an essentially similar way, after both routes. Vanillyl acetone (conjugates) comprised 54 % of the dose and total urinary excretion amounted to 96 %[10] of the dose within 24 hours post dosing, and was complete within 48 hours. In some separately treated animals (with 400 mg/kg bw) biliary excretion of vanillyl acetone (25-46 % of the dose) was observed together with zingerol (3-7 %) and trace amounts of other metabolites, indicating a significant enterohepatic cycle. The only metabolite which was excreted in appreciable amounts in unconjugated form was homovanillic acid (= (4-hydroxy-3-methoxyphenyl)acetic acid, which was excreted in total amounts of ~ 7 % of the dose. Reduction of the ketone to zingerol (12 %) was also observed along with lesser amounts of ring O-demethylation to 4-(3,4-dihydroxyphenyl)butan-2-one (6 %). Other metabolites included homovanillyl alcohol, 4-hydroxy- and 1-hydroxy-(4-hydroxy-3-methoxyphenyl)butan-2-one, 4-(4-hydroxy-3-methoxyphenyl)butane-1,2- and -2,3-diol, dihydro-ferulic acid (= 3-(4-hydroxy-3-methoxyphenyl)-2-propanoic acid), homo-proto-catechuyl alcohol (2-(3,4-dihydroxyphenyl)ethanol), and 4-(3,4-di-hydroxyphenyl)butan-2-one and -butan-2-ol. In bile duct ligated animals, no O-demethylation products could be detected in the urine and in separate *in vitro* studies it was demonstrated that gut microflora can O-demethylate vanillyl acetone. These findings indicate that the urinary O-demethylation products probably result from conversions carried out by the intestinal flora (Monge et al., 1976).

[10] Percentages of excretion are the averages for both routes.

When rats were dosed intravenously with 4-phenyl-2-butanone, in the blood small amounts of the reduction product 4-phenyl-2-butanol could be detected. The analytical methods were not designed to include conjugated products (Kitamura et al., 1999).

With several zingerone analogues 5-hydroxylation (producing a catechol) of the vanillyl ring (= 4 hydroxy - 3-methoxyphenyl) has been observed in phenobarbital-pretreated rat liver microsomes. The efficiency of 5-hydroxylation depended on the length of the alkenyl-ketonic side chain and the position of the double bond in this chain (Lee & Kumar, 1982). The paper was not sufficiently detailed to evaluate the rate of this conversion. In addition, the fact that only microsomes from phenobarbital-pretreated rats were used may reduce the applicability of this finding for human risk assessments.

Polyphenols (catechol, resorcinol or quinol)

Polyphenols are not represented in this FGE as candidate flavouring substances. However, in general ring hydroxylation of phenols may result in the formation of polyphenolic metabolites. For that reason some attention is given below to the metabolism of polyphenols.

Rabbits were fed single 100 mg/kg bw doses of benzene-1,3-diol [FL-no: 04.047] or 100 – 200 mg/kg bw doses of quinol via stomach tube. Urinary analysis revealed that within 24 h after administration, 52 % of the dose was excreted as glucuronic acid conjugates, 13.5 % as sulphate conjugates, and 11.4 % as free benzene-1,3-diol. Quinol was excreted for 43 % as glucuronide and for 30 % as sulphate. Only trace amounts of parent quinol could be found. In this paper results from the same research group have been quoted, which show that catechol is excreted in rabbit urine for 2 % as parent substance, 70 % as glucuronide and for 18 % as sulphate conjugate. With catechol, but not with quinol or benzene-1,3-diol a trace amount of the sulphate conjugate of the oxidation product hydroxyquinol (= 1,2,4-trihydroxybenzene) was found (Garton & Williams, 1949).

Single 112 or 225 mg/kg bw doses of ^{14}C-benzene-1,3-diol were administered orally to male and female F344 rats. Within 24 hours ca. 94 % of the low dose was excreted, mainly via the urine. The high dose was eliminated slightly slower (~ 80 % in 24 hours urine). Virtually no radioactivity was exhaled. Approximately 70 % of the dose was excreted as glucuronic acid conjugates in the urine of both sexes. A greater portion of sulphate conjugates was detected in the urine of females. Males excreted a larger percentage of a diconjugate containing both sulphate and glucuronic acid conjugates. About 2 % of the dose was excreted in the faeces of both sexes. In addition, daily oral doses of 225 mg benzene-1,3-diol/kg bw were administered for five consecutive days. This regimen did not significantly alter the ratio of metabolic products observed in the single dose experiment (Kim & Matthews, 1987).

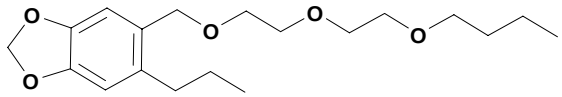
Candidate Substances

3,4-Methylenedioxyphenol [FL-no: 04.080]

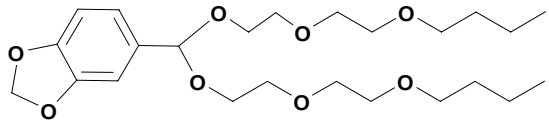
No studies have been submitted to support the evaluation of this substance. As the methylenydioxy-ring may be looked at as a (cyclic) acetal, it might be expected that this ring may be opened and demethylated, which would result in the formation of 1,2,4-trihydroxybenzene (and formaldehyde). The same loss of the methylenedioxy-ring has been reported for safrole, myristicin (Figure III.1) and some related compounds, for which the loss of the methylenedioxy-group may be as much as 61-92 % of the dose after oral administration, based on urinary metabolites or exhalation of methylenedioxy-derived carbondioxide (Klungsoeyr & Scheline,1982; Kamienski & Casida, 1970).

Also with piperonyl butoxide (=[3,4-(methylenedioxy)-6-propylbenzyl]butyl diethyleneglycol ether (Figure III.1), considerable loss of the dimethylendioxy-ring was found (~ 70 % of the dose), but no loss of the entire propyl or butyl diethyleneglycol side chains. In contrast to e.g. safrole or piperonyl butoxide, when rats were given piperonyl alcohol, piperonal or piperonylic acid (3,4-methylenedioxylbenzyl alcohol, -

benzaldehyde or -benzoic acid, respectively (Figure III.1)) no loss of the dimethylenedioxy-ring was observed. With tropital (= piperonal bis(2-(2-butoxyethoxy)ethyl) acetal; Figure III.1) extensive loss of both alcohol side chains was observed (resulting in the formation of piperonyl aldehyde and further metabolic products, including glycine and glucuronic acid conjugates of piperonylic acid), but hardly any loss of the methylenedioxy-ring. From these observations it may be concluded that loss of the methylenedioxy-ring is a major route of metabolism when more rapid elimination is not possible, e.g. because no rapid options for conjugation are present. On the basis of this conclusion, it may be expected that the candidate flavouring substance 3,4-methylenedioxyphenol [FL-no: 04.080] is not metabolised via loss of the methylenedioxy-ring but that it may rather be conjugated with sulphate or glucuronic acid, similar to the other phenols. In addition, some ring hydroxylation at the *ortho*-position cannot be excluded but this may also be expected to represent a minor route of metabolism.



Piperonyl butoxide (=[3,4-(methylenedioxy)-6-propylbenzyl] butyl diethyleneglycol ether)



Tropital (=piperonal bis(2-(2-butoxyethoxy)ethyl)acetal)

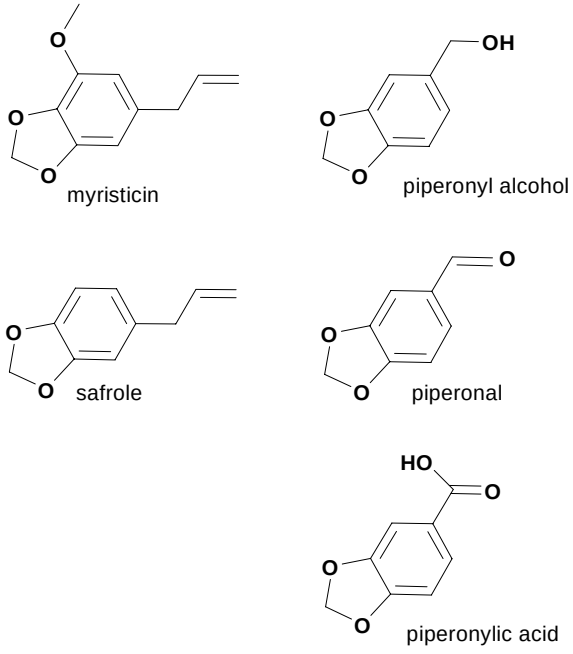


Figure III.1: *Chemical structures of methylendioxybenzene derivatives*

4-Isopropylphenol [FL-no: 04.073]

Hepatocellular toxicity was suggested as time-dependent intracellular potassium leakage from precision-cut rat liver slices incubated with 2 mM 4-isopropylphenol for six hours. A significant depletion of intracellular glutathione levels in rat liver slices after one hour of incubation suggested the formation of intermediates that react with glutathione. Supplementation of the incubates with N-acetyl cysteine or metyrapone reduced

cytotoxicity. An *in vitro* study using rat liver microsomes in the presence of NADPH and 3H-glutathione provided evidence that 4-isopropylphenol may be oxidised to reactive quinone methide intermediates and then conjugated with glutathione (Thompson et al., 1995a).

2,4-Dimethylphenol [FL-no: 04.066]

2,4-Dimethylphenol (2,4-DMP) was administered to male Sprague-Dawley rats by intravenous bolus injection at 30 mg/kg bw or intraperitoneal bolus injection at 30 mg/kg bw and concentrations of 2,4-DMP and its conjugated metabolites in plasma were measured. Only minute amounts of unchanged parent compound were detected one hour after intravenous bolus injection of 2,4-dimethylphenol, indicating rapid and extensive metabolism. Thirty minutes after the intravenous bolus injection of 2,4-dimethylphenol, the concentration of unchanged compound in plasma was 0.12 ± 0.02 µg/ml, and the concentration of total conjugates was 8.55 ± 0.61 µg/ml. Thirty minutes after the intraperitoneal bolus injection, the concentration of unchanged compound in plasma was 1.16 ± 0.30 µg/ml, and the concentration of total conjugates was 19.45 ± 3.80 µg/ml. The relative amounts of 2,4-dimethylphenol and metabolites in plasma amounted to ~ 5.6, 53 and 41 % for parent compound, glucuronic acid conjugate and other conjugates (probably sulphate), respectively (Kaka et al., 1982).

2,4-Dimethylphenol [FL-no: 04.066] and 2,4,6-Trimethylphenol [FL-no: 04.095]

In vitro metabolism studies provide evidence of a bioactivation pathway for some *o*-substituted *p*-alkylphenols, but not for others. For 2,4-dimethylphenol and 2,4,6-trimethylphenol potential for cytotoxicity arises from the oxidative formation of reactive quinone methide (QM) intermediates. QM formation was estimated to be 0.28 ± 0.07 and 0.17 ± 0.02 nmol per nmol of P450 per min using rat liver microsomes and 16 ± 3 and 17 ± 2 pmol per 10^6 cells per min using rat hepatocytes, respectively. These QM formation levels were considerably lower than the rates for other investigated phenols containing tert-butyl groups, which were in the range of 0.40 – 0.70 nmol per nmol of P450 per min using rat liver microsomes and 38 – 62 pmol per 10^6 cells per min using rat hepatocytes. At an incubation concentration of 0.30 mM of the parent phenol, QM reactive intermediates are effectively detoxicated by the available glutathione for 2,4-dimethylphenol, 2,4,6-trimethylphenol and 2,6-di-tert-butyl-4-methylphenol since they were not cytotoxic when incubated with rat hepatocytes for two hours. However, under the same experimental conditions, 2-tert-butyl-4-methylphenol, 2-tert-butyl-4,6-dimethylphenol, and 6-tert-butyl-2-(hydroxy-tert-butyl)-4-methylphenol destroyed 100 % of rat hepatocytes after complete glutathione depletion (Bolton et al., 1992). The results of this study demonstrate that the nature of the *o*-substituents to the ring affects the stability of charge separation within the various quinone methide metabolites and thereby their reactivity and toxicity.

3-Methoxyphenol [FL-no: 04.076]

Cytotoxicity studies in isolated rat hepatocytes were used to elucidate the cytochrome P450- catalysed bioactivation pathway of 3-methoxyphenol to form reactive metabolites. No *in vivo* data on metabolism were presented, but it was shown that intraperitoneal administration of 300 or 400 mg 3-methoxyphenol/kg bw to mice resulted in profound hepatotoxicity, and even death. Using "intervention agents" such as cytochrome P450 inhibitors, radical scavengers and DT-diaphorase inhibitors, it was demonstrated that 3-methoxyphenol could be metabolised to a reactive intermediate that could generate reactive oxygen species and might be able to result in alkylation of protein SH groups. As in microsomal incubations with 3-methoxyphenol no formaldehyde production was observed[11], it was concluded that no O-demethylation took place. In addition, it was demonstrated that upon metabolic activation, 3-methoxyphenol is a more potent depletor of intracellular GSH than 4-methoxyphenol and 2-methoxyphenol. In microsomal preparations with 3-methoxyphenol, fortified with GSH, no indications of ring epoxidation were obtained, because no 3-methoxyphenol-SG or resorcinol-SG conjugates were found. Instead, formation of a GSH-conjugate of 4-

[11] Moridani, 2005; Written correspondence between FLAVIS working group and study authors, 2005.

methoxycathecol was observed. In separate studies it could be demonstrated that 4-methoxycatechol may trigger toxicity to hepatocytes via redox reactions involving 4-methoxy-*ortho*-quinone (Moridani et al., 2003).

4-Methoxyphenol [FL-no: 04.077]

Studies in isolated rat hepatocytes and liver microsomal incubates were used to elucidate the cytochrome P450- catalysed bioactivation pathway of 4-methoxyphenol (= 4-hydroxyanisole) to form reactive metabolites and trigger cytotoxicity. Cytotoxicity of 4-methoxyphenol could be influenced by co-incubation of hepatocytes and 4-methoxyphenol with inhibitors of CYP2E1 or DT-diaphorase or with GSH or depletors thereof. *o*-Quinone traps or scavengers of reactive oxygen species were not very effective. When the substance was incubated in microsomal preparations, no formaldehyde was released, indicating absence of O-demethylation. However, hydroquinone and *p*-quinone could be detected, and in addition also the GSH conjugate of hydroquinone was found if GSH was included in the incubates. The authors propose that ring epoxidation and/or P450-mediated one electron oxidation as bioactivation routes to convert 4-methoxyphenol to the reactive intermediate species, 4-hydroxyanisole epoxide and *p*-quinone, occurs rather than O-demethylation / ring hydroxylation / ipso attack mechanism. The cytotoxicity of 4-methoxyphenol results from covalent binding of hydroquinone / *p*-quinone to proteins rather than from oxidative stress (Moridani et al., 2002).

The human metabolism of 4-hydroxyanisole (= 4-methoxyphenol) was investigated using urine samples from three melanoma patients being treated therapeutically with this substance and five untreated healthy volunteers (Pavel et al., 1989). The substance was administered to patients by intra-arterial infusion at a concentration of 20 mg/ml isotonic saline at a dose rate of 40 g/24 hours for four days. In the urine samples of the patients, 4-hydroxyanisole, 3,4-dihydroxyanisole 3-hydroxy-4-methoxyanisole, 4-hydroxy-3-methoxyanisole and hydroquinone were detected. However, none of these compounds were detected in the urine of healthy volunteers except for minute quantities of hydroquinone. All metabolites in the urine of the patients were excreted both as sulphates and glucuronides with a small percentage found in the unconjugated form. Of the urinary metabolites, 3,4-dihydroxyanisole made up 85 – 90 % of the total, and 3-hydroxy-4-methoxyanisole and 4-hydroxy-3-methoxyanisole made up 10 – 15 % of the total. The presence of "considerable amounts" of hydroquinone in the urine samples of patients (in contrast to very low quantities of hydroquinone detected in the urine of healthy volunteers) was attributed to O-demethylation of 4-hydroxyanisole as a possible metabolic route based on previous research, although it was mentioned above that O-demethylation is not a major metabolic pathway for 4-methoxyphenol. The authors concluded that: "*…the metabolism of 4-methoxyphenol proceeds preferentially via ring hydroxylation and is in accordance with the generally recognised phase I step of drug metabolism. The o-hydroxyphenolic compounds formed are, however, potentially toxic, and the ring hydroxylation is usually coupled to the phase II reaction, such as glucuronidation or sulphation…thus forming metabolites that are more readily eliminated than the parent compound*" (Pavel et al., 1989). Because of the high amounts of catechol derivatives in human urine, as observed by Pavel *et al.*, it has been speculated that the human metabolism may differ from that of rats because no catecholic metabolites of 4-methoxyphenol could be detected in rat liver microsomal incubates (see above) (Moridani et al., 2002).

Female rabbits (2.5 to 3.5 kg) were administered a suspension of 0.7 g 4-methoxyphenol once via oral gavage. The mean quantity of the administered dose excreted via the urine within 24 hours following administration was 13 % (10 – 15 %) for the ethereal sulphate, 69 % (65 – 73 %) for the ether glucuronide, and 1 % (0 – 2 %) for the free 4-methoxyphenol. 4-Methoxyphenol was also determined to be partially demethylated resulting in ~ 3 % of the dose being excreted as quinol (Bray et al., 1955).

Acetovanillone [FL-no: 07.142]

Male Wistar rats (200 – 350 g) were administered a 1 mmol (166 mg) acetovanillone/kg bw dose via stomach tube as a solution in propylene glycol. Control animals received only propylene glycol. Urine

samples were collected at 24-hours intervals for five days. Acetovanillone was excreted rapidly in the urine (80 % of the dose within the first 24 hours), mainly as the parent compound but also as the demethylated compound (3,4-dihydroxyacetophenone; 6 %) and three ring-hydroxylated metabolites (together 11.3 %) (see Figure III.2). Additionally, minor metabolic pathways produced the *p*-methoxy derivative (acetoisovanillone), a dimethoxyhydroxy derivative, and two 1-phenylethanol derivatives (1-(4-hydroxy-3-methoxyphenyl)ethanol and 1-(3,4-dihydroxyphenyl)ethanol) formed by ketone reduction of acetovanillone and 3,4-dihydroxyacetophenone, respectively. The positions of the substituents of the three ring-hydroxylated metabolites and the dimethoxy-hydroxy metabolite, were not fully ascertained in the study, but based on mass spectrometry and comparison with fragmentation patterns of know metabolites, they were tentatively deduced to be 2,4-dihydroxy-3-methoxyacetophenone, 4,5-dihydroxy-3-methoxyacetophenone, 4,6-dihydroxy-3-methoxyacetophenone and 3,4-dimethoxy-5-hydroxy-cetophenone, respectively. Hardly any metabolites were found in the faeces. The total urinary excretion of all metabolites over the entire observation period was 96 ± 4 % of the dose. The metabolites excreted in the urine were mainly glucuronic acid and/or sulphate conjugates. From unpublished data it was concluded that the amounts of free metabolites were 15-40 % of total amounts of metabolites excreted (Gjertsen et al., 1988).

5-Paradol [FL no: 07.234]

When studying the metabolism of shogaol (= 1-(4-hydroxy-3-methoxyphenyl)-deca-4-ene-3-one) (Surh & Lee, 1992), it was found that the keto-reduction of 5-paradol (= 1-(4-hydroxy-3-methoxyphenyl)-3-decanone [FL no: 07.234]), which is an intermediate in shogaol metabolism, is mediated by liver cytosolic reductases. When 5-paradol was given to bile duct-cannulated rats via intraperitoneal injection, neither parent substance nor metabolites were excreted via the urine. In bile, free and conjugated 5-paradol and the keto-reduced metabolite (1-(4-hydroxy-3-methoxyphenyl)-decan-3-ol) could be detected. No further details about dose, recovery or nature of the conjugates (whether phenolic or alcoholic; sulphate or glucuronide) were available. Based on observations with vanillyl acetone (see above) ring hydroxylation may also have occured with 5-paradol, but such metabolites were not reported and it is not clear whether such metabolites have been looked for (Watabe et al., 1981; only abstract available).

Nibovan [FL-no: 08.134]

Nibovan [FL-no: 08.134] (4-hydroxy-3-methoxymandelic acid or vanillylmandelic acid) has been identified as an endogenous break-down of adrenalin and nor-adrenalin in humans. The substance can be determined in plasma and is eliminated via the kidneys. A plasma half-life of approximately 0.5 hours has been mentioned. In contrast to other phenolic metabolites of adrenalin and nor-adrenalin, vanillylmandelic acid is not conjugated to sulphate, but no systematic study on metabolism of [FL-no: 08.134] has been submitted (Eisenhofer et al., 1996; Eisenhofer et al., 2004; Fukuda et al., 1996; Yang et al., 1996). For the related substance L(+) mandelic acid (2-phenyl-2-hydroxyacetic acid), which in humans is virtually completely eliminated via the urine, metabolic options are conversion into oxo(phenyl) acetic acid (*ca.* 20 % of the administered dose) and benzoic acid (*ca.* 2 % of the administered dose). The D(-) stereoisomer is metabolised to a less extent (Nagwekar & Kostenbauder, 1970). Benzoic acid may be further metabolised with glycine to yield hippuric acid. Whether other conjugates of mandelic acid can be formed is not clear from the submitted data. Direct conjugation of the carboxylic acid moiety with amino acids has been described for phenylacetic acid (EFSA, 2009f).

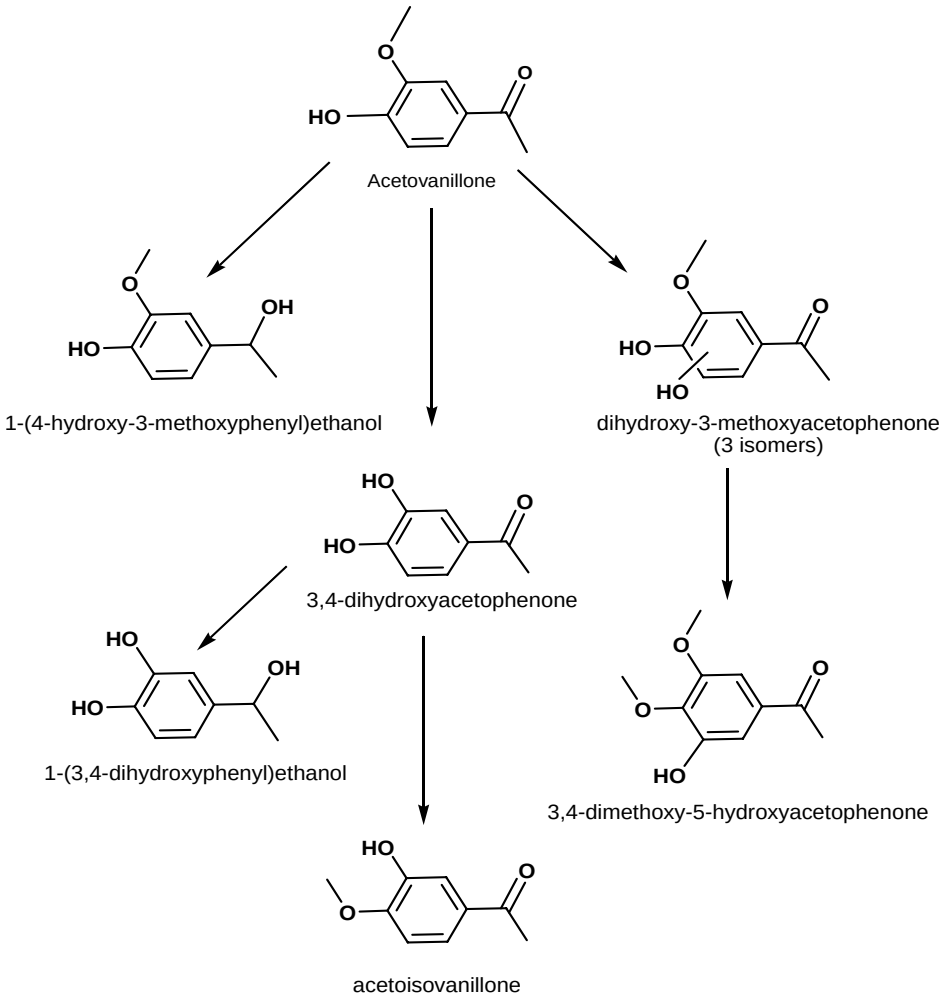


Figure III.2. *Metabolic pathways for acetovanillone in the rat (Gjertsen et al., 1988).*

III.4. Summary and Conclusions

It can be assumed that the phenolic substances within this group are well absorbed. The phenol esters in the group will be hydrolysed, probably already in the intestinal lumen by pancreatic enzymes.

After absorption, the substances in this group are easily metabolised and excreted. The most important metabolic conversions are straightforward conjugation of the phenolic group with sulphate or glucuronic acid at the hydroxy group, followed by rapid excretion usually via urine. For 5-paradol, considerable excretion via the bile has been observed.

Conjugation with sulphate or glucuronic acid has been observed in many species, but in cats and pigs, in contrast to other species including humans, enzymes catalysing sulphation and glucuronidation of phenols, respectively, are not very active, if not absent. As a result of the limited capacity of these two species to conjugate phenols, toxicity data obtained with cats or pigs may be considered of limited relevance for humans. With rabbits and rats, it has been observed that sulphation may have limited capacity at high dose levels. At those high dose levels, glucuronidation increases in importance. This may be due to depletion of body sulphate stores, but also differences in substrate affinity and/ or maximum conjugation velocities of the various enzymes have been shown to play a role in the observed shift in conjugation ratio when dose levels

are increased. The available data indicate that the efficiency of the conjugation reactions is not very much influenced by the presence of ring substituents, provided that these are not too bulky.

Ring hydroxylation, side chain oxidation and O-demethylation occur to a much smaller extent than conjugation. This may be related to the high velocity with which these phenolic substances are conjugated in the first place. Products of the oxidative metabolic pathways may include quinones and catechols, which may trigger a cytotoxic response if their concentration or formation rate overwhelms their detoxication via glutathione conjugation. Reduced quinones (i.e. hydroquinones) and catechols are also substrates for sulphate and glucuronic acid conjugation.

In vitro metabolism studies with rat liver slices have provided evidence of a bioactivation pathway for *para*-substituted alkylphenols at high concentrations. With some *p*-isomers of alkyl-substituted phenols, as well as some *o*-methoxy-4-alkylphenols, cytotoxicity arises from the oxidative formation of reactive quinone methide intermediates. At sufficiently low concentrations this reactive intermediate is inactivated by conjugation with glutathione. At higher concentrations, the quantity of quinone methide intermediates exceeds glutathione availability which resulted in cytotoxicity. Based on an observation in a bacterial incubation system with 4-vinylphenol, formation of a quinone methide intermediate can also not be excluded from 2,6-dimethoxy-4-vinylphenol [FL-no: 04.061], but because of the presence of the two methoxy groups, this intermediate substance is probably not reactive enough to cause cytotoxicity.

Some of the candidate substances possess a ketone function in the side chain, which provides an additional metabolic option for metabolism. In principle, such phenol derivatives may also be excreted in the urine as conjugates of glucuronic acid and sulphate. In addition to this, minor metabolites may be formed through ring hydroxylation, or side chain omega-oxidation, which may result in chain length reduction. Reduction of the ketone to the corresponding phenyl alcohol has also been reported. With 5-paradol conjugation and keto-reduction, but no chain length reduction or oxidations on the aromatic ring, have been reported. The candidate substance nibovan [FL-no: 08.134] is an endogenous metabolite of adrenalin and nor-adrenalin. This substance is rapidly eliminated from the blood and excreted via the kidneys. Data on conjugations are not available.

Overall, for the candidate substances in this group the phenolic groups are conjugated with glucuronic acid or sulphate very efficiently and these conjugation pathways are not easily saturated. Although especially at high dose levels reactive metabolites such as quinones, catechols or quinone methides may be formed, it is not expected that the formation of these metabolites would overwhelm the capacity of conjugation with glutathione at the estimated levels of intake as flavouring substance. Even for an exceptionally susceptible species such as the cat, acute dose levels of > 20 mg phenol/kg bw seem to be necessary to result in a production of quinones which is large enough to trigger toxicity. Therefore, in general it is concluded that the substances in this group of chemically defined flavouring substances may be expected to be metabolised to innocuous products at the estimated levels of intake.

ANNEX IV: TOXICITY

Oral acute toxicity data are available for six candidate substances of the present flavouring group evaluation from chemical groups 21 and 25, and for 21 supporting substances evaluated by JECFA at the 55th meeting (JECFA, 2001b). The supporting substances are listed in brackets.

TABLE IV.1: ACUTE TOXICITY

Chemical Name [FL-no]	Species	Sex	Route	LD_{50} (mg/kg bw)	Reference	Comments
(2-Methylphenol [04.027])	Rat	F	Oral	1000 – 2000*	(Pinkerton & Daniel, 1978)	*Results are for the *o*-, *m*- and *p*-isomers.
	Rat	NR	Oral	121	(E.I. du Pont de Nemours & Company, 1969b)	
	Rat	M, F	Gavage	300	(Bailey, 1983)	
	Rat	M, F	Gavage	530	(Deichmann & Witherup, 1944)	Substance administered as a 2 % or 5 % aqueous solution.
	Rat	M, F	Gavage	540	(Deichmann & Witherup, 1944)	Substance administered as a 10 % aqueous solution.
	Rat	M, F	Gavage	340	(Deichmann & Witherup, 1944)	Substance administered as a 20 % aqueous solution.
	Rat	F	Gavage	400	(Berman et al., 1995)	
(3-Methylphenol [04.026])	Rat	F	Oral	1000 – 2000*	(Pinkerton & Daniel, 1978)	Results are for the *o*-, *m*- and *p*-isomers.
	Rat	M	Oral	242	(E.I. du Pont de Nemours & Company, 1969a)	
	Rat	M	Gavage	520	(Carpenter, 1949b)	
(4-Methylphenol [04.028])	Rat	F	Oral	1000 – 2000*	(Pinkerton & Daniel, 1978)	Results are for the *o*-, *m*- and *p*-isomers.
3-Ethylphenol [04.021]	Rat	M, F	Oral	M: 252 F: 400	(Eastman Kodak Co., 1980b)	
(4-Ethylphenol [04.022])	Rat	NR	Oral	>5000	(Blaszcak & Auletta, 1986)	
(2-Propylphenol [04.046])	Rat	NR	Oral	500	(Unigovski & Veldre, 1975)	
(4-Propylphenol [04.050])	Mouse	NR	Oral	700	(Pellmont, 1973a)	
(4-(1,1-Dimethylethyl)phenol [04.064])	Rat	M, F	Gavage	M: 5360 F: 3620	(Klonne et al., 1988)	
	Rat	M	Gavage	3.25 ml/kg (3250)	(Smyth et al., 1969a)	
	Rat	NR	Oral	≈ 3500	(Zelle, 1982)	
	Rat	NR	Oral	1400	(Palanker & Denine, 1973)	
	Guinea pig	NR	Oral	1400* 400**	(Dow Chemical Company, 1943)	*Value = LD_{100} **Value = LD_0
(*p*-Tolyl acetate [09.036])	Rat	NR	Oral	1900	(Palanker & Denine, 1973)	
2,3-Dimethylphenol [04.065]	Rat	NR	Oral	<5000	(De Cresente, 1982)	
(2,5-Dimethylphenol [04.019])	Rat	NR	Oral	444	(Maazik, 1968) (Veldre, 1972)	
	Rat	NR	Gavage	<5000	(De Cresente, 1982)	
	Mouse	NR	Oral	383	(Maazik, 1968)	

TABLE IV.1: ACUTE TOXICITY

Chemical Name [FL-no]	Species	Sex	Route	LD_{50} (mg/kg bw)	Reference	Comments
	Rabbit	NR	Oral	938	(Maazik, 1968)	
(2,6-Dimethylphenol [04.042])	Rat	NR	Oral	296	(Maazik, 1968)	
	Rat	NR	Oral	406	(Larionov, 1976)	
	Mouse	NR	Oral	479	(Maazik, 1968)	
	Mouse	NR	Oral	450	(Larionov, 1976)	
	Rabbit	NR	Oral	700	(Maazik, 1968)	
(3,4-Dimethylphenol [04.048])	Rat	NR	Oral	727	(Maazik, 1968)	
	Rat	NR	Oral	1600	(Eastman Kodak Co., 1991c)	
	Mouse	NR	Oral	400	(Maazik, 1968); (Eastman Kodak Co., 1991c)	
	Rabbit	NR	Oral	800	(Maazik, 1968)	
3,5-Dimethylphenol [04.020]	Rat	NR	Oral	2250	(Uzhdavini et al., 1979)	
	Rat	NR	Oral	608	(Maazik, 1968)	
	Mouse	NR	Oral	477	(Maazik, 1968)	
	Mouse	M	Gavage	620	(McOmie et al., 1949)	
	Rabbit	NR	Oral	1313	(Maazik, 1968)	
5-Methyl-2-(tert-butyl)phenol [04.078]	Mouse	M	Gavage	1080	(McOmie et al., 1949)	
(4-Vinylphenol [04.057])	Rat	M, F	Gavage	>5000	(Piccirillo & Hartman, 1982)	
(2-Methoxyphenol [04.005])	Rat	M, F	Gavage	725	(Taylor et al., 1964)	
	Mouse	NR	Oral	621	(Cioli et al., 1980)	
4-Methoxyphenol [04.077]	Rat	M, F	Gavage	>5000	(Blaszcak & Auletta, 1987)	
(2,6-Dimethoxyphenol [04.036])	Rat	M, F	Oral	790	(Piccirillo & Hartman, 1982)	
(4-Methyl-2,6-dimethoxyphenol [04.053])	Rat	M, F	Oral	1700	(Piccirillo & Hartman, 1982)	
(4-Ethyl-2,6-dimethoxyphenol [04.052])	Rat	M, F	Oral	2300	(Piccirillo & Hartman, 1982)	
(2,6-Dimethoxy-4-propylphenol [04.056])	Rat	M, F	Oral	>5000	(Piccirillo & Hartman, 1982)	
(4-Allyl-2,6-dimethoxyphenol [04.051])	Rat	M, F	Oral	2000	(Piccirillo & Hartman, 1982)	
4-Hydroxyacetophenone [07.243]	Mouse	M	Oral	1780	(Procter & Gamble Company, 1977)	
(4-(*p*-Hydroxyphenyl)butan-2-one [07.055])	Rat	NR	Oral	>5000	(Mc Gee Laboratories, Inc., 1974)	
	Rat	M, F	Gavage	M: 1320 F: 1400	(Gaunt et al., 1970)	
(Vanillyl acetone [07.005])	Rat	NR	Oral	2580	(Moreno, 1977ae)	
(4-(4-Acetoxyphenyl)butan-2-one [09.288])	Rat	M, F	Gavage	<5000	(Sauer & Salem, 1980)	

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

Subacute / subchronic / chronic / carcinogenic toxicity data are available for three candidate substance of the present flavouring group evaluation from chemical groups 21 and 25 and for ten supporting substances evaluated by JECFA at the 55th meeting (JECFA, 2001b). The supporting substances are listed in brackets.

TABLE IV.2: SUBACUTE / SUBCHRONIC / CHRONIC / CARCINOGENICITY STUDIES

Chemical Name [FL-no]	Species; Sex No./Group	Route	Dose levels	Duration	NOAEL (mg/kg bw/day)	Reference	Comments
(2-Methylphenol [04.027])	Mouse; M, F 20	Diet	0, 1250, 2500, 5000, 10.000, 20.000 ppm (equal to M: 0, 199, 400, 794, 1490, 2722 mg/kg bw; F: 0, 237, 469, 935, 1663, 3205 mg/kg bw)	13 weeks	M: 199 F: 496	(NTP, 1992c)	5.
	Rat; M, F 60	Gavage	0, 50, 175, 600 mg/kg bw	13 weeks	175	(EPA, 1988b)	5.
	Rat; M, F 40	Diet	0, 1880, 3750, 7500, 15.000, 30.000 ppm (equal to M: 0, 126, 247, 510, 1017, 2028 mg/kg bw; F: 0, 129, 256, 513, 1021, 2004 mg/kg bw)	13 weeks	250	(NTP, 1992c)	5.
(3-Methylphenol [04.026])	Mouse; M, F 20	Diet	0, 625, 1250, 2500, 5.000, 10.000 ppm (equal to M: 0, 96, 194, 402, 776, 1513 mg/kg bw; F: 0, 116, 239, 472, 923, 1693 mg/kg bw)	13 weeks	M: 402[1] F: 923[1]	(NTP, 1992c)	5.
	Rat; M, F 40	Diet	0, 1880, 3750, 7500, 15.000, 30.000 ppm (equal to M: 0, 123, 241, 486, 991, 2014 mg/kg bw; F: 0, 131, 254, 509, 1024, 2050 mg/kg bw)	13 weeks	250[1]	(NTP, 1992c)	5.
	Rat; M, F 24	Gavage	0, 10, 30, 100, 300 mg/kg bw	Neonatal toxicity study: Postnatal days 4 – 21	Newborn: 30	(Koizumi et al., 2003)	5.
	Rat; M, F 14	Gavage	0, 100, 300, 1000 mg/kg bw	28 days	300	(Koizumi et al., 2003)	5.
(4-Methylphenol [04.028])	Mouse; M, F 20	Diet	0, 625, 1250, 2500, 5.000, 10.000 ppm (equal to M: 0, 96, 194, 402, 776, 1513 mg/kg bw; F: 0, 116, 239, 472, 923, 1693 mg/kg bw)	13 weeks	M: 402[1] F: 923[1]	(NTP, 1992c)	5.
	Rat; M, F 60	Gavage	0, 50, 175, 600 mg/kg bw	13 weeks	M: 50 F: 175	(EPA, 1988a)	5
	Rat; M, F 40	Diet	0, 1880, 3750, 7500, 15.000, 30.000 ppm	13 weeks	250[1]	(NTP, 1992c)	5

TABLE IV.2: SUBACUTE / SUBCHRONIC / CHRONIC / CARCINOGENICITY STUDIES

Chemical Name [FL-no]	Species; Sex No./Group	Route	Dose levels	Duration	NOAEL (mg/kg bw/day)	Reference	Comments
			(equal to M: 0, 123, 241, 486, 991, 2014 mg/kg bw; F: 0, 131, 254, 509, 1024, 2050 mg/kg bw)				
	Hamster; M 15	Diet	1,5 % (equivalent to 1600 mg/kg bw)	20 weeks	No NOAEL derived	(Hirose et al., 1986)	5. Induction of hyperplasia in forestomach. Only effects on forestomach studied.
(4-Ethylphenol [04.022])	Rat; M, F 20 – 32	Diet	M: 0.199 mg/kg bw F: 0.219 mg/kg bw	90 days	M: 0.199 F: 0.219	(Posternak et al., 1969)	5.
(4-(1,1-Dimethylethyl)phenol [04.064])	Hamster; M 15	Diet	1.5 % (equivalent to 1600 mg/kg bw)	20 weeks	No NOAEL derived	(Hirose et al., 1986)	5. Induction of hyperplasia in forestomach. Only effects on forestomach studied.
2,4-Dimethylphenol [04.066]	Rat; M, F 20 – 32	Gavage	0, 60, 180, 540 mg/kg bw	90 days	60	(Daniel et al., 1993)	Well conducted non GLP study. Significant findings were hyperkeratosis and hyperplasia of the forestomach in the two highest dose groups.
(2,6-Dimethylphenol [04.042])	Rat; NR 13	Gavage	0.06 and 6.0 mg/kg bw	8 months	0.06	(Maazik, 1970)	5
(3,4-Dimethylphenol [04.048])	Rat; NR 13	Gavage	0.14 and 14 mg/kg bw	8 months	0.14	(Maazik, 1970)	5
(Thymol [04.006])	Rat; M, F 10	Diet	1000 and 10.000 ppm (equivalent to 50, 500 mg/kg bw)	19 weeks	500	(Hagan et al., 1967)	5
4-Methoxyphenol [04.077]	Rat; M, F 20	Diet	0, 0.02, 0.1, 0.5, 2 and 5 % (equivalent to 0, 20, 100, 500, 2000, 5000 mg/kg bw)	5 – 7 weeks	100	(Hodge et al., 1949)	Study not in accordance with modern guidelines, no statistical evaluation. Only effect observed on body- and organ weight.
	Rabbit 6	Diet	0, 1 and 5 % (equivalent to 0, 300 and 1500 mg/kg bw)	5 – 9 weeks	300	(Hodge et al., 1949)	Study not in accordance with modern guidelines, no statistical evaluation. Only effect observed on body- and organ weight.
	Rat; NR 5 – 10	Diet	2 % (equivalent to 2000 mg/kg bw)	4 – 8 weeks	No NOAEL derived	(Altmann et al., 1985; Altmann et al., 1986)	Hyperplasia induced in all dosed animals. Only forestomach effects investigated.
	Hamster; M 15	Diet	1,5% (equivalent to 1600 mg/kg bw)	20 weeks	No NOAEL derived	(Hirose et al., 1986)	Induction of hyperplasia in forestomach. Only effects on forestomach studied.
	Rat; M 10 – 11	Diet	0, 0.25, 0.5, 1 and 2 % (equivalent to 0, 125, 250, 500 and 1000 mg/kg bw)	51 weeks	125	(Wada et al., 1990)	Histological examination of liver, forestomach, stomach, esophagus and kidney.
	Rat; M 10 - 11	Diet	2 % (equivalent to 1000 mg/kg bw)	24 – 48 weeks	No NOAEL derived	(Kagawa et al., 1993)	Hyperplasia induced in all dosed animals, no carcinomas. Only forestomach effects investigated.

TABLE IV.2: SUBACUTE / SUBCHRONIC / CHRONIC / CARCINOGENICITY STUDIES

Chemical Name [FL-no]	Species; Sex No./Group	Route	Dose levels	Duration	NOAEL (mg/kg bw/day)	Reference	Comments
	Rat; M, F 60	Diet	2 % (equivalent to 1000 mg/kg bw)	104 weeks	No NOAEL derived	(Asakawa et al., 1994)	The only dose of 2 % tested induced hyperplasia, papillomas and squamos cell carcinomas in the forestomach. Only effects on forestomach investigated.
	Rat; M 30 – 31	Diet	0.4 % (equivalent to 200 mg/kg bw)	104 weeks	No NOAEL derived	(Hirose et al., 1997)	Study on induction of forestmach tumors. Reduced body weight in dosed animals. Increase in forestomach hyperplasia, no carcinomas.
	Rat; M 10 – 15	Diet	0.08 and 0.4 % (equivalent to 40 and 200 mg/kg bw)	24 weeks after 4 weeks initiation	40	(Hirose et al., 1997)	Initiation with a combination of diethylnitrosamine, N-methylnitrosourea, 1,2-dimethylhydrazine, N-butyl-N-(4-hydroxybutyl)-nitrosamine and 2,2'-dihydroxy-di-n-propylnitrosamine. All major organs were examined histopathologically. Increase in forestomach papillomas in highest dosed group. No carcinomas.
(2,6-Dimethoxyphenol [04.036])	Rat; M, F 20 – 32	Diet	M: 5.99 mg/kg bw F: 6.85 mg/kg bw	90 days	M: 5.99[2] F: 6.85	(Posternak et al., 1969)	5.
3,4-Methylenedioxyphenol [04.080]	Mouse; M, F 60	Diet	2 % (equivalent to 1000 mg/kg bw)	96 weeks	No NOAEL derived	(Tamano et al., 1992)	Hematological and clinical biochemical as well as histopathological examination of all major organs. Significant different findings were induction of forestomach hyperplasia and squamous cell carcinomas in both males and females and hyperplasia in the glandular stomach in females only.
	Rat; M, F 60	Diet	2 % (equivalent to 1000 mg/kg bw)	104 weeks	No NOAEL derived	(Tamano et al., 1992)	Hematological and clinical biochemical as well as histopathological examination of all major organs. Significant different findings were induction of forestomach hyperplasia and squamous cell carcinomas in both males and females.
	Rat; M 10 - 11	Diet	2 % (equivalent to 1000 mg/kg bw)	24 – 48 weeks	No NOAEL derived	(Kagawa et al., 1993)	Hyperplasia induced in all dosed animals, no carcinomas. Only forestomach effects investigated.
	Rat; M 30 - 31	Diet	0.4 % (equivalent to 200 mg/kg bw)	104 weeks	No NOAEL derived	(Hirose et al., 1997)	Study on induction of forestomach tumors. Reduced body weight in dosed animals. Increase in forestomach hyperplasia, no carcinomas.
	Rat; M 10 - 15	Diet	0.08 and 0.4 % (equivalent to 40 and 200 mg/kg bw)	24 weeks after 4 weeks initiation	200	(Hirose et al., 1997)	Initiatioan with a combination of diethylnitrosamine, N-methylnitrosourea, 1,2-dimethylhydrazine, N-butyl-N-(4-hydroxybutyl)-nitrosamine and 2,2'-dihydroxy-di-n-propylnitrosamine. All major organs were examined histopathologically. No significant differences were observed.
(4-(*p*-Hydroxyphenyl)butan-2-one	Rat; M, F	Diet	0, 0.1, 0.2, 0.4 and 1 %	13 weeks	100	(Gaunt et al., 1970)	5.

TABLE IV.2: SUBACUTE / SUBCHRONIC / CHRONIC / CARCINOGENICITY STUDIES

Chemical Name [FL-no]	Species; Sex No./Group	Route	Dose levels	Duration	NOAEL (mg/kg bw/day)	Reference	Comments
[07.055])	30		(equivalent to 0, 50, 100, 200 and 500 mg/kg bw)				Valid study. Reduced body weight of males dosed 1 %. Slight increase in relative weight of liver, adrenal and kidney in male rats dosed 0.4 and 1 %. No difference between animals dosed 1 % and control animals in haematological, clinical chemical, urine and histological examination. Histopathological examination therefore not conducted on the middle dosed groups.

M = Male; F = Female.
NR = Not reported.
1 Administered as a mixture: m-cresol, 60 %; p-cresol, 40 %.
2 This study was performed at either a single dose level or multiple dose levels that produced no adverse effects.
3 NOAEL not determined. Study evaluated induction of fore stomach lesions and carcinogenicity.
4 Did not act as a tumour promoter.
5 Summarised by JECFA, 55th meeting (JECFA, 2001b).

Developmental and reproductive toxicity data are available for three candidate substances of the present flavouring group evaluation from chemical groups 21 and 25 and for five supporting substance evaluated by the JECFA at the 55th meeting (JECFA, 2001b). Supporting substance listed in brackets.

TABLE IV.3: DEVELOPMENTAL AND REPRODUCTIVE TOXICITY STUDIES

Chemical Name [FL-no]	Study type Duration	Species / Sex No / group	Route	Dose levels	NOAEL (mg/kg bw/day), including inf. of possible maternal toxicity	Reference	Comments
(2-Methylphenol [04.027])	Two generation Reproductive Toxicity: 5 days/week	Rat; M, F 50	Gavage	30, 175 and 450 mg/kg bw	Parental: 30 Offspring: 175	(Tyl & Neeper-Bradley, 1989a)	2.
	Developmental Toxicity: Gestation Days 6 - 15	Rat; F 25	Gavage	30, 175 and 450 mg/kg bw	Maternal: 175 Foetal: 175	(Tyl, 1988a)	2.
	Developmental Toxicity: Gestation Days 6 - 18	Rabbit; F 14	Gavage	5, 50, 100 mg/kg bw	Maternal: 5 Foetal: 50	(Tyl, 1988b)	2.
(3-Methylphenol [04.026])	Two generation Reproductive Toxicity: 5 days/week	Rat; M, F 50	Gavage	30, 175 and 450 mg/kg bw	Parental: < 30 Offspring: 175	(Tyl & Neeper-Bradley, 1989b)	2.
	RACB: (Task 1) 2 weeks	Mouse; M, F	Diet	370, 1500 and 2100 mg/kg bw	1500[1]	(Heindel et al., 1992)	2. Number of animals not given, but may be known from the study design; (RACB protocol).
	RACB: (Task 2) 18 weeks	Mouse; M, F	Diet	370, 1500 and 2100 mg/kg bw	Reproductive: 370[1]	(Heindel et al., 1992)	2. Number of animals not given, but may be known from the study design; (RACB protocol).
	RACB: (Task 3) 14 weeks	Mouse; M, F	Diet	450, 1700 and 2100 mg/kg bw	< 1700[1]	(Heindel et al., 1992)	2. Number of animals not given, but may be known from the study design; (RACB protocol).
	RACB: (Task 4) 32 weeks	Mouse; M, F	Lactation/diet	450, 1700 and 2100 mg/kg bw	< 450[1]	(Heindel et al., 1992)	2. Number of animals not given, but may be known from the study design; (RACB protocol).
	Developmental Toxicity: Gestation Days 6 - 15	Rat; F 25	Gavage	30, 175 and 450 mg/kg bw	Maternal: 175 Foetal: 450	(Tyl, 1988a)	2.
	Developmental Toxicity: Gestation Days 6 - 18	Rabbit; F 14	Gavage	5, 50 and 100 mg/kg bw	Maternal: 5 Foetal: 100	(Tyl, 1988b)	2.

TABLE IV.3: DEVELOPMENTAL AND REPRODUCTIVE TOXICITY STUDIES

Chemical Name [FL-no]	Study type Duration	Species / Sex No / group	Route	Dose levels	NOAEL (mg/kg bw/day), including inf. of possible maternal toxicity	Reference	Comments
(4-Methylphenol [04.028])	Two generation Reproductive Toxicity: 5 days/week	Rat; M, F 50	Gavage	30, 175 and 450 mg/kg bw	Parental: 30 Offspring: 175	(Tyl & Neeper-Bradley, 1989c)	2.
	RACB: (Task 1) 2 weeks	Mouse; M, F	Diet	370, 1500 and 2100 mg/kg bw	2100[1]	(Heindel et al., 1992)	2. Number of animals not given, but may be known from the study design; RACB.
	RACB: (Task 2) 18 weeks	Mouse; M, F	Diet	370, 1500 and 2100 mg/kg bw	Reproductive: 370[1]	(Heindel et al., 1992)	2. Number of animals not given, but may be known from the study design; RACB.
	RACB: (Task 3) 14 weeks	Mouse; M, F	Diet	450, 1700 and 2100 mg/kg bw	< 2100[1]	(Heindel et al., 1992)	2. Number of animals not given, but may be known from the study design; RACB.
	RACB: (Task 4) 32 weeks	Mouse; M, F	Lactation/diet	450, 1700 and 2100 mg/kg bw	< 450[1]	(Heindel et al., 1992)	2. Number of animals not given, but may be known from the study design; RACB.
	Developmental Toxicity: Gestation Days 6 - 15	Rat; F 25	Gavage	30, 175 and 450 mg/kg bw	Maternal: 175 Foetal: 175	(Tyl, 1988a)	2.
	Developmental Toxicity: Gestation Day 11	Rat; F 13	Gavage	100, 333, 667 and 1000 mg/kg bw	Maternal: 100 Foetal: 1000	(Kavlock, 1990)	2.
	Developmental Toxicity: Gestation Days 6 - 18	Rabbit; F 3/14	Gavage	5, 50 and 100 mg/kg bw	Maternal: 5 Foetal: 100	(Tyl, 1988b)	2.
(Thymol [04.006])	Developmental Toxicity: Gestation Days 19 - 26	Rabbit; F 3	Gel capsule		< 294	(Savignoni & de Maria, 1933)	2.
3-Methoxyphenol [04.076]	Developmental Toxicity: Gestation Day 11	Rat; F 16	Gavage	0, 333, 667, 1000 mg/kg bw	Maternal: < 333 Foetal: 1000	(Kavlock, 1990)	The study is considered valid.
4-Methoxyphenol [04.077]	Developmental Toxicity: Gestation Day 11	Rat; F 16	Gavage	0, 333, 667, 1000 mg/kg bw	Maternal: <333 Foetal: 1000	(Kavlock, 1990)	The study is considered valid.
(4-Ethoxyphenol [04.037])	Developmental Toxicity: Gestation Day 11	Rat; F 15	Gavage	0, 333, 667, 1000 mg/kg bw	Maternal: 333 Foetal: 1000	(Kavlock, 1990)	2. The study is considered valid.
4-Hydroxyacetophenone [07.243]	Developmental Toxicity: Gestation Day 11	Rat; F 16	Gavage	0, 333, 667, 1000 mg/kg bw	Maternal: 1000 Foetal: 667	(Kavlock, 1990)	The study is considered valid.

M = Male; F = Female.
NR = Not reported.
RACB = Reproductive and fertility assessment by continuous breeding.
Task 1 = Dose range-finding phase.
Task 2 = Continuous breeding phase. Mice were exposed to the test article for a 7-day premating phase, 98-day cohabitation period and a 21-day segregation period.

Task 3 = Crossover mating trial. Mice were exposed to the test article for a 7-day premating period, followed by a 14-week cohabitation/breeding period.
Task 4 = Offspring reproductive performance phase.
1) Study evaluated a mixture of m- and p-cresol.
2) Summarised by JECFA, 55th meeting (JECFA, 2001b).

In vitro mutagenicity/genotoxicity data are available for 12 candidate substances of the present flavouring group evaluation from chemical groups 21 and 25 and for 18 supporting substances evaluated by JECFA at the 55th meeting (JECFA, 2001b). Supporting substances are listed in brackets.

TABLE IV.4: GENOTOXICITY (*IN VITRO*)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
(2-Methylphenol [04.027])	Ames assay	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	2.5 µl/plate (26,200µg/plate)	Negative[1, 2]	(Douglas et al., 1980)	17.
	Ames assay (preincubation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	324 µg/plate	Negative[1, 2]	(Florin et al., 1980)	17. Insufficient quality. Not in accordance with OECD guideline 471. Inadequate study design (Spot test).
	Ames assay	*S. typhimurium* TA98; TA100; TA1535; TA1537	5 concentrations from 1 to 100 µg/plate	Negative[1, 2]	(Haworth et al., 1983)	17. Acceptable quality. Published summary report including detailed results from studies on 250 chemicals tested in various laboratories within the NTP. In accordance with OECD guideline 471 (1983).
	Ames assay	*S. typhimurium* TA98; TA100	5 µg/plate	Negative[1, 2]	(Massey et al., 1994)	17.
	Ames assay	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	Up to 2600 µg/plate (range not reported)	Negative[1, 2, 3]	(Nestmann et al., 1980)	17. Insufficient quality as main details of method and results were not reported. Additionally, the test was not repeated.
	Ames assay	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	4 concentrations from 5 to 5000 µg/plate	Negative[1, 2]	(Pool & Lin, 1982)	17. Acceptable quality.
	Ames assay (preincubation assay)	*S. typhimurium* TA98; TA100; TA1535; TA1537	1000 µg/plate	Negative[1, 2]	(Canter, 1981)	17.
	Ames assay	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	500 µg/plate	Negative[1, 2]	(Nuodex Inc., 1980a)	17.
	Ames assay	*S. typhimurium* TA98; TA100	Not reported	Negative[4] Positive[5]	(Claxton, 1985)	17. Result cannot be evaluated since it was reported only as a very short summary in table format. The paper was on methodological aspects of the assay and not specifically on this compound.
	Sister chromatid exchange	Human lymphocytes	5 concentrations up to 0.5 mM (54 µg/ml)	Negative	(Jansson et al., 1986; Jansson et al., 1988)	17. Limited quality (selection of maximum concentration not justified and experiment not repeated).
	Sister chromatid exchange	Human fibroblasts	86.5 - 865 µg/ml without S9	Equivocal	(Cheng & Kligerman, 1984)	17. Limited quality. Only the highest concentration resulted in a result statistically significantly different from control (1.2-fold increase only). A second experiment was not

TABLE IV.4: GENOTOXICITY (*IN VITRO*)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
						performed.
	Sister chromatid exchange	Chinese Hamster ovary cells	4 concentrations from 12.5 to 75 nl/ml (78.6 µg/ml) without S9, 11 concentrations from 1.56 to 700 nl/ml (733 µg/ml) with S9	Positive[1] Positive[2]	(Galloway & Brusick, 1981)	17. Acceptable quality. Statistically significant dose-related increase (up to two-fold).
	Sister chromatid exchange	Chinese hamster ovary cells	Up to 500 nl/ml (524 µg/ml)[4]	Positive[1, 2]	(Galloway & Brusick, 1980)	17. Acceptable quality but limited relevance because the test material was a mixture of *p*-cresol, *m*-cresol and *o*-cresol (33 1/3 % each).
	Unscheduled DNA synthesis	Rat primary hepatocytes	7 concentrations from 0.5 to 50 nl/ml (52.4 µg/ml)[4]	Equivocal	(Myhr & Brusick, 1980)	17 . Acceptable quality but limited relevance because the test material was a mixture of *p*-cresol, *m*-cresol and *o*-cresol (33 1/3 % each). Response was not dose-related. A slight response was observed at concentrations up to 5.0 nl/ml, while UDS was not observed at concentrations from 10 to 50 nl/ml.
	DNA Repair assay	*E. coli* W3110	5000 µg/ml	Negative[1, 2]	(Pepper Hamilton & Scheetz, 1980)	17. Test substance included 60 % *o*-cresol.
	DNA repair assay	*E. coli*	5000 µg/ml	Negative[1, 2]	(Nuodex Inc., 1980b)	17.
(3-Methylphenol [04.026])	Ames assay	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	2000 µg/plate	Negative[1, 2]	(Douglas et al., 1980)	17.
	Ames assay (preincubation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	324 µg/plate	Negative[1, 2]	(Florin et al., 1980)	17. Insufficient quality. Not in accordance with OECD guideline 471. Inadequate study design (Spot test).
	Ames assay	*S. typhimurium* TA98; TA100; TA1535; TA1537	5 concentrations from 3.3 to 333 µg/plate	Negative[1, 2]	(Haworth et al., 1983)	17. Acceptable quality. Published summary report including detailed results from studies on 250 chemicals tested in various laboratories within the NTP. In accordance with OECD guideline 471 (1983).
	Ames assay	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	Up to 2000 µg/plate (range not reported)	Negative[1, 2, 3]	(Nestmann et al., 1980)	17. Insufficient quality as main details of method and results were not reported. Additionally, the test was not repeated.
	Ames assay	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	5 concentrations from 0.5 to 5000 µg/plate	Negative[1, 2]	(Pool & Lin, 1982)	17. Acceptable quality.
	Ames assay (preincubation assay)	*S. typhimurium* TA98; TA100; TA1535; TA1537	3333 µg/plate	Negative[1, 2]	(Canter, 1981)	17.
	Sister chromatid exchange	Human lymphocytes	5 concentrations up to	Negative	(Jansson et al., 1986);	17.

TABLE IV.4: GENOTOXICITY (*IN VITRO*)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
			1 mmol/L (108 µg/ml)		(Jansson et al., 1988)	Limited quality (selection of maximum concentration not justified and experiment not repeated).
	Sister chromatid exchange	Human fibroblasts	865 µg/ml	Negative	(Cheng & Kligerman, 1984)	17.
	Sister chromatid exchange	Chinese hamster ovary cells	Up to 500 nl/ml (524 µg/ml)[4]	Positive[1, 2]	(Galloway & Brusick, 1980)	17. Acceptable quality but limited relevance because the test material was a mixture of *p*-cresol, *m*-cresol and *o*-cresol (33 1/3 % each).
	Unscheduled DNA synthesis	Rat primary hepatocytes	10 µg/ml	Negative	(Cifone, 1988a)	17.
	Unscheduled DNA synthesis	Rat primary hepatocytes	7 concentrations from 0.5 to 50 nl/ml (51.7 µg/ml)[4]	Equivocal	(Myhr & Brusick, 1980)	17. Limited relevance because the test material was a mixture of *p*-cresol, *m*-cresol and *o*-cresol (33 1/3 % each). Response was not dose-related. A slight response was observed at concentrations up to 5.0 nl/ml, while UDS was not observed at concentrations from 10 to 50 nl/ml.

TABLE IV.4: GENOTOXICITY (*IN VITRO*)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
(4-Methylphenol [04.028])	Ames assay	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	1000 µg/plate	Negative[1, 2]	(Douglas et al., 1980)	17.
	Ames assay (preincubation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	324 µg/plate	Negative[1, 2]	(Florin et al., 1980)	17. Insufficient quality. Not in accordance with OECD guideline 471. Inadequate study design (Spot test).
	Ames assay	*S. typhimurium* TA98; TA100; TA1535; TA1537	5 concentrations from 3.3 to 333 µg/plate	Negative[1, 2]	(Haworth et al., 1983)	17. Acceptable quality. Published summary report including detailed results from studies on 250 chemicals tested in various laboratories within the NTP. In accordance with OECD guideline 471 (1983).
	Ames assay	*S. typhimurium* TA98; TA100	5 µg/plate	Negative[1, 2]	(Massey et al., 1994)	17.
	Ames assay	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	Up to 1000 µg/plate (range not reported)	Negative[1, 2, 3]	(Nestmann et al., 1980)	17. Insufficient quality as main details of method and results were not reported. Additionally, the test was not repeated.
	Ames assay	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	5 concentrations from 0.5 to 5000 µg/plate	Negative[1, 2]	(Pool & Lin, 1982)	17. Acceptable quality.
	Ames assay (preincubation assay)	*S. typhimurium* TA98; TA100; TA1535; TA1537	1000 µg/plate	Negative[1, 2]	(Canter, 1981)	17.
	Ames assay	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	1 µl/plate (1030 µg/plate)	Negative[1, 2]	(Crowley & Margard, 1978)	17.
	Sister chromatid exchange	Human lymphocytes	5 concentrations up to 0.5 mmol/L (54 µg/ml)	Negative	(Jansson et al., 1986)	17. Limited quality (selection of maximum concentration not justified and experiment not repeated).
	Sister chromatid exchange	Human fibroblasts	865 µg/ml	Negative	(Cheng & Kligerman, 1984)	17.
	Sister chromatid exchange	Chinese hamster ovary cells	Up to 500 nl/ml (524 µg/ml)[4]	Positive[1, 2]	(Galloway & Brusick, 1980)	17. Acceptable quality but limited relevance because the test material was a mixture of *p*-cresol, *m*-cresol and *o*-cresol (33 1/3 % each).
	Unscheduled DNA synthesis	Human lymphocytes	25 µM (2.7 µg/ml)	Negative	(Daugherty & Franks, 1986)	17. Not relevant since only an inhibition of UV-induced UDS was measured.. Additionally, a result is reported only for one concentration (resulting in inhibition by 30 %) and a negative control was not included.
	Unscheduled DNA synthesis	Rat primary hepatocytes	7 concentrations from 0.5 to 50 nl/ml (51.5 µg/ml)	Equivocal	(Myhr & Brusick, 1980)	17. Acceptable quality but limited relevance because the test material was a mixture of *p*-cresol, *m*-cresol and *o*-cresol (33 1/3 % each).

TABLE IV.4: GENOTOXICITY (*IN VITRO*)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
						Response was not dose-related. A slight response was observed at concentrations up to 5.0 nl/ml, while UDS was not observed at concentrations from 10 to 50 nl/ml.
	Unscheduled DNA synthesis	WI-38 human embryonic lung fibroblast cells	Not unambiguously reported	Positive	(Crowley & Margard, 1978)	17. Unpublished study report of limited quality because concentrations were not unambiguously reported and only 3 concentrations have been tested. However, the result was reproducible. Liquid scintillation counting.
2-Ethylphenol [04.070]	Ames assay (preincubation method)	*S. typhimurium* TA97; TA98; TA100; TA1535	5 doses from 0.01 to 10 mg/plate	Negative[1, 2]	(Zeiger et al., 1992)	Acceptable quality.
	Ames assay (preincubation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537	3 µmol/plate (367 µg/plate)	Negative[1, 2]	(Florin et al., 1980)	Insufficient quality. Not in accordance with OECD guideline 471. Inadequate study design (Spot test).
	Sister chromatid exchange	Human lymphocytes	5 concentrations up to 0.25 mmol/L (30.5 µg/ml)	Negative[6]	(Jansson et al., 1986)	Limited quality (selection of maximum concentration not justified and experiment not repeated).
3-Ethylphenol [04.021]	Ames assay (preincubation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537	3 µmol/plate (366 µg/plate)	Negative[1, 2]	(Florin et al., 1980)	Insufficient quality. Not in accordance with OECD guideline 471. Inadequate study design (Spot test).
	Sister chromatid exchange	Human lymphocytes	5 concentrations up to 0.25 mmol/L (30.5 µg/ml)	Negative[6]	(Jansson et al., 1986)	Limited quality (selection of maximum concentration not justified and experiment not repeated).
(4-Ethylphenol [04.022])	Ames assay (preincubation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	367 µg/plate	Negative[1, 2]	(Florin et al., 1980)	17. Insufficient quality. Not in accordance with OECD guideline 471. Inadequate study design (Spot test).
	Ames assay	*S. typhimurium* TA98; TA100	Not reported	Negative[1, 2]	(Epler et al., 1979)	17. Insufficient quality. Not in accordance with OECD guideline 471. Only two strains tested. Results not reported in detail.
	Sister chromatid exchange	Human lymphocytes	5 concentrations up to 0.25 mmol/L (27 µg/ml)	Negative	(Jansson et al., 1986)	17. Limited quality (selection of maximum concentration not justified and experiment not repeated).
(4-(1,1-Dimethyl)ethyl phenol [04.064])	Ames assay	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	2000 µg/plate	Negative[1, 2]	(Dean et al., 1985)	17. Insufficiently reported. Validity cannot be evaluated as the results were not reported in detail.
	Mutation assay	*E. coli* WP2 and WP2 *uvrA*	2000 µg/plate	Negative[1, 2]	(Dean et al., 1985)	17. Insufficiently reported. Validity cannot be evaluated as main details of the method (e.g. concentration range tested) were not reported. Additionally, the result was not reported in detail.

TABLE IV.4: GENOTOXICITY (*IN VITRO*)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
	Mutation assay	*S. cerevisiae* JD1	2000 µg/plate	Negative[1, 2]	(Dean et al., 1985)	17. Insufficiently reported. Validity cannot be evaluated as main details of the method (e.g. concentration range tested) were not reported. Additionally, the result was not reported in detail.
	Chromosomal aberration assay	Rat liver cell RL_1, RL_2	Not specifically indicated[7]	Negative	(Dean et al., 1985)	17. Insufficiently reported. Validity cannot be evaluated as main details of the method (e.g. concentrations tested) were not reported. Additionally, the result was not reported in detail.
	Chromosome aberration assay	Chinese hamster lung cells	Not reported	Negative[8]	(Kusakabe et al., 2002)	17.
	Mouse lymphoma assay	L5178Y *tk* +/- mouse lymphoma cells	80 µg/ml	Negative	(Honma et al., 1999b)	17.
2,3-Dimethylphenol [04.065]	Ames assay	*S. typhimurium* TA98; TA100	Not reported	Negative[1, 2]	(Epler et al., 1979)	Insufficient quality. Not in accordance with OECD guideline 471. Only two strains tested. Results not reported in detail.
	Sister chromatid exchange	Human lymphocytes	5 concentrations up to 0.5 mmol/L (61 µg/ml)	Negative[6]	(Jansson et al., 1986)	Limited quality (selection of maximum concentration not justified and experiment not repeated).
2,4-Dimethylphenol [04.066]	Ames assay (preincubation method)	*S. typhimurium* TA97; TA98; TA100; TA1535; TA1537	0, 0.33, 1, 3.3, 10, 33 µg/plate	Negative[1, 2]	(Mortelmans et al., 1986)	Acceptable quality.
	Ames assay (preincubation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537	3 µmol/plate[9] (366 µg/plate)	Negative[1, 2]	(Florin et al., 1980)	Insufficient quality. Not in accordance with OECD guideline 471. Inadequate study design (Spot test).
	Ames assay	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	5 concentrations from 0.5 to 5000 µg/plate[10]	Negative[1, 2]	(Pool & Lin, 1982)	Acceptable quality.
	Sister chromatid exchange	Human lymphocytes	5 concentrations up to 0.1 mmol/L (12 µg/ml)	Negative[6]	(Jansson et al., 1986)	Limited quality (selection of maximum concentration not justified and experiment not repeated).
(2,5-Dimethylphenol [04.019])	Ames assay (preincubation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	367 µg/plate	Negative[1, 2]	(Florin et al., 1980)	17. Insufficient quality. Not in accordance with OECD guideline 471. Inadequate study design (Spot test).
	Ames assay	*S. typhimurium* TA98; TA100	Not reported	Negative[1, 2]	(Epler et al., 1979)	17. Insufficient quality. Not in accordance with OECD guideline 471. Only two strains tested. Results not reported in detail.
(2,6-Dimethylphenol [04.042])	Ames assay (preincubation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	367 µg/plate	Negative[1, 2]	(Florin et al., 1980)	17 Insufficient quality. Not in accordance with OECD guideline 471. Inadequate study design (Spot test).
	Ames assay (preincubation method)	*S. typhimurium* TA98; TA100; TA1535;	5 mg/plate (5000 µg/plate)	Negative[1, 2]	(Schechtman et al., 1980)	17.

TABLE IV.4: GENOTOXICITY (*IN VITRO*)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
		TA1537; TA1538				
	Chromosome aberration assay	Chinese hamster V79 cells	3 concentrations from 10 to 100 µg/ml (without S9) and 5 concentrations from 30 to 600 µg/ml (with S9)	Negative[1] Positive[2]	(Völkner, 1994)	17. Acceptable quality. This GLP-study was in accordance with OECD guideline 473 (1983). A final report was not available and the draft was not signed. However, the results and conclusions available as draft report are considered valid.
	Sister chromatid exchange	Human lymphocytes	5 concentrations up to 0.25 mmol/L (31 µg/ml)	Negative	(Jansson et al., 1986)	17. Limited quality (selection of maximum concentration not justified and experiment not repeated).
(3,4-Dimethylphenol [04.048])	Ames assay (preincubation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	367 µg/plate	Negative[1, 2]	(Florin et al., 1980)	17. Insufficient quality. Not in accordance with OECD guideline 471. Inadequate study design (Spot test).
	Ames assay	*S. typhimurium* TA98; TA100	Not reported	Negative[1, 2]	(Epler et al., 1979)	17. Insufficient quality. Not in accordance with OECD guideline 471. Only two strains tested. Results not reported in detail.
3,5-Dimethylphenol [04.020]	Ames assay (plate incorporation, preincubation, spot test, and treat-and-plate methods)	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538; *E. coli* WP2; WP2*uvrA*	6 concentrations from 125 to 4000 µg/plate	Negative[1, 2]	(Dean et al., 1985)	Insufficiently reported. Validity cannot be evaluated as the results were not reported in detail.
	Mitotic gene conversion assay	*S. cerevisiae* JD1	Not reported	Negative[1, 2]	(Dean et al., 1985)	Insufficiently reported. Validity cannot be evaluated as main details of the method (e.g. concentration range tested) were not reported. Additionally, the result was not reported in detail.
	Chromosome aberration assay	Rat liver cells RL_4	3 concentrations from 0.125 to 0.5 of GI_{50} (50% growth inhibition). Values in µg/ml or µmol/ml not reported.	Negative[1, 2]	(Dean et al., 1985)	Insufficiently reported. Validity cannot be evaluated as main details of the method (e.g. concentrations tested) were not reported. Additionally, the result was not reported in detail.
2,4,6-Trimethylphenol [04.095]	Ames assay (preincubation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537	3 µmol/plate[10] (409 µg/plate)	Negative[1, 2]	(Florin et al., 1980)	Insufficient quality. Not in accordance with OECD guideline 471. Inadequate study design (Spot test).
	Ames assay	*S. typhimurium* TA98; TA100	Not reported	Negative[1, 2]	(Epler et al., 1979)	Insufficient quality. Not in accordance with OECD guideline 471. Only two strains tested. Results not reported in detail.
(Thymol [04.006])	Ames assay	*S. typhimurium* TA97; TA98; TA100	1000 µg/ml	Negative[1, 2]	(Azizan & Blevins, 1995)	17.
	Ames assay (preincubation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	451 µg/plate	Negative[1, 2]	(Florin et al., 1980)	17. Insufficient quality. Not in accordance with OECD guideline 471. Inadequate study design (Spot test).

TABLE IV.4: GENOTOXICITY (*IN VITRO*)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
	Ames assay (preincubation method)	*S. typhimurium* TA97; TA98; TA100	Not reported	Negative[1, 2]	(Azizan & Blevins, 1995)	17.
	Sister chromatid exchange	SHE cells	5 concentrations from 0.3 to 30 µg/ml	Equivocal	(Fukuda, 1987)	17 . Validity cannot be evaluated since the study was published in Japanese (e.g. presence or absence of S9 is not clear). However, the results reported in a table were not dose-related.
	Unscheduled DNA synthesis	SHE cells	4 concentrations from 0.3 to 10 µg/ml	Equivocal	(Fukuda, 1987)	17. Validity cannot be fully evaluated since the study was published in Japanese (e.g. presence or absence of S9 is not clear). However, the results reported in a table were not dose-related.
(Carvacrol [04.031])	Ames assay	*S. typhimurium* TA98; TA100	2 concentrations (8 and 16 ppm)	Negative[1, 2]	(Kono et al., 1995)	17. Not in accordance with OECD guideline 471 (only two strains used and only two concentrations tested). In Japanese with a short summary in English.
	Ames assay (plate incorporation assay)	*S. typhimurium* TA98; TA100	3 concentrations from 0.6 to 2.5 µmol/plate	Negative[1, 2]	(Stammati et al., 1999)	17. This study was not in accordance with OECD guideline 471 (only two strains used and only 3 concentrations tested).
	Bacterial DNA repair test	*E. coli* WP2 *trpE65*; CM8781 *trpE65; uvrA155, recA56, lexA*	4 concentrations from 2.5 to 6 µmol/paper disk	Positive	(Stammati et al., 1999)	17. Effects were measured as inhibition zones. This assay is considered to be of minor relevance. Positive results from such assays may be interpreted as an indication of a genotoxic potential which needs to be clarified by other assays.
	SOS Chromotest	*E. coli* PQ37	4 concentrations (not unambiguously reported)	Negative	(Stammati et al., 1999)	17. Concentrations not unambiguously reported, only without S9 tested. This assay is considered to be of minor relevance. Positive results from such assays may be interpreted as an indication of a genotoxic potential which needs to be clarified by other assays.
(4-Vinylphenol [04.057])	Sister chromatid exchange	Human lymphocytes	5 concentrations up to 0.1 mmol/L (12 µg/ml)	Negative	(Jansson et al., 1988)	17. Limited quality (selection of maximum concentration not justified and experiment not repeated).

TABLE IV.4: GENOTOXICITY (*IN VITRO*)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
(2-Methoxyphenol [04.005])	Ames assay	*S. typhimurium* TA98; TA100; TA102	111,726 µg/plate	Negative[1, 2]	(Aeschbacher et al., 1989)	17.
	Ames assay	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	16,000 µg/plate	Negative[1, 2]	(Douglas et al., 1980)	17.
	Ames assay	*S. typhimurium* TA98; TA100; TA1535; TA1537	5 concentrations from 333 to 11,740 µg/plate in one experiment and 5 concentrations from 33 to 3333 µg/plate in two further experiments performed in another laboratory	Negative[1, 2]	(Haworth et al., 1983)	17. Acceptable quality. Published summary report including detailed results from studies on 250 chemicals tested in various laboratories within the NTP. Three experiments performed in two laboratories. In accordance with OECD guideline 471 (1983).
	Ames assay	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	Up to 16,000 µg/plate (range not reported)	Negative[1, 2]	(Nestmann et al., 1980)	17. Insufficient quality as main details of method and results were not reported. Additionally, the test was not repeated.
	Ames assay	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	5 conentrations from 0.5 to 5000 µg/plate	Negative[1, 2]	(Pool & Lin, 1982)	17. Acceptable quality.
	Sister chromatid exchange	Human lymphocytes	5 concentration up to 0.5 mmol/L (62 µg/ml)	Positive	(Jansson et al., 1988)	17. Acceptable quality. Only the highest concentration resulted in a statistically significant increase. The effect was very waek but reproducible.
3-Methoxyphenol [04.076]	Ames assay (preincubation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537	30 µmol/plate (3724 µg/plate)	Negative[1, 2]	(Florin et al., 1980)	Insufficient quality. Not in accordance with OECD guideline 471. Inadequate study design (Spot test).
	Sister chromatid exchange	Human lymphocytes	5 concentrations up to 1 mmol/L (124 µg/ml)	Negative[6]	(Jansson et al., 1986)	Limited quality (selection of maximum concentration not justified and experiment not repeated).
4-Methoxyphenol [04.077]	Ames assay (preincubation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537	5 concentrations from 3.3 to 167 µg/plate in the first experiment and 5 concentrations from 100 to 5000 µg/plate in the second experiment performed in another laboratory	Negative[2,]	(Haworth et al., 1983)	Acceptable quality. Published summary report including detailed results from studies on 250 chemicals tested in various laboratories within the NTP. Experiments performed in two laboratories. In accordance with OECD guideline 471 (1983).
	Ames assay (preincubation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537	30 µmol/plate (3724 µg/plate)	Negative[1, 2]	(Florin et al., 1980)	Not in accordance with OECD guideline 471. Inadequate study design (Spot test).
	Ames assay (plate incorporation method)	*S. typhimurium* TA100; TA1530	Up to 4 µmol/plate	Negative[1, 2, 12]	(Bartsch et al., 1980)	As only two strains were used the quality of the study must be considered insufficient for the purpose of this Flavouring Group Evaluation Validity cannot be evaluated as details of the result were not reported.
	Mouse lymphoma assay	Mouse L5178Y TK +/- lymphocytes	27 to 2000 µg/ml	Positive[1]	(Rogers-Back, 1986)	The validity of this unpublished report cannot

TABLE IV.4: GENOTOXICITY (*IN VITRO*)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
			(without S9) 1.3 to 100 µg/ml (with S9)	Negative[2]		fully be evaluated since all pages in table format are lacking.
	Sister chromatid exchange	Human lymphocytes	5 concentrations up to 0.05 mmol/L (6.2 µg/ml)	Negative[6]	(Jansson et al., 1986)	Limited quality (selection of maximum concentration not justified and experiment not repeated).
	Chromosome aberration assay	Chinese hamster ovary cells	954, 1269, and 1692 µg/ml (each in the presence and absence of S9)	Positive[1, 2]	(Putman, 1986)	The validity of this unpublished report cannot fully be evaluated since all pages in table format are lacking.
	Unscheduled DNA synthesis	Human lymphocytes	25 µM (3.1 µg/ml)	Equivocal	(Daugherty & Franks, 1986)	Not relevant since only an inhibition of UV-induced UDS was measured. Additionally, a result is reported only for one concentration (resulting in inhibition by 30 %) and a negative control was not included.
(2-Methoxy-4-methylphenol [04.007])	Sister chromatid exchange	Human lymphocytes	5 concentrations up to 1 mmol/L (138 µg/ml)	Positive	(Jansson et al., 1988)	17. Acceptable quality. The effect was weak (twofold increase) but dose-related and statistically significant.
(4-Ethylguaiacol [04.008])	Sister chromatid exchange	Human lymphocytes	5 concentration up to 1 mmol/L (152 µg/ml)	Negative	(Jansson et al., 1988)	17. Limited quality (selection of maximum concentration not justified and experiment not repeated).
(2,6-Dimethoxyphenol [04.036])	Ames assay	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	16,000 µg/plate	Negative	(Douglas et al., 1980)	17.
	Ames assay (preincubation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	463 µg/plate	Negative[1, 2]	(Florin et al., 1980)	17. Insufficient quality. Not in accordance with OECD guideline 471. Inadequate study design (Spot test).
	Ames assay	*S. typhimurium* TA98; TA100; TA1535; TA1537	1000 µg/ml	Negative[1, 2]	(McMahon et al., 1979)	17.
	Ames assay	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	5 concentrations from 0.5 to 5000 µg/plate	Negative[1, 2]	(Pool & Lin, 1982)	17. Acceptable quality.
	Mutation assay	*E. coli*	1000 µg/ml	Negative[1, 2]	(McMahon et al., 1979)	17.
	Sister chromatid exchange	Human lymphocytes	4 concentrations up to 0.5 mmol/L (77 µg/ml)	Negative	(Jansson et al., 1986)	17. Limited quality (selection of maximum concentration not justified and experiment not repeated).
4-Hydroxy-3,5-dimethoxyacetophenone [07.164]	Ames assay (plate incorporation assay)	*S. typhimurium* TA97; TA98; TA100; TA102	6 concentrations from 10 to 4000 µg/plate	Negative[1, 2]	(Pfuhler et al., 1995)	Limited quality. Strain TA 1535 was not used although recommended by OECD 471 (1983 and 1997) which may be acceptable but the test was not repeated.
	Ames assay	*S. typhimurium* TA98; TA100; TA1535;	Up to 1 mg/plate (range not reported)	Negative[1, 2]	(Nestmann et al., 1980)	Insufficient quality as main details of method and results were not reported. Additionally, the

TABLE IV.4: GENOTOXICITY (*IN VITRO*)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
		TA1537; TA1538				test was not repeated.
	Mutagenicity assay	*S. cerevisiae* D7; XV185-14C	Not reported	Negative[1]	(Nestmann & Lee, 1983)	Insufficient quality. Details of concentrations and results not reported.
	Sister chromatid exchange	Human peripheral lymphocytes	4 concentrations from 3.3 to 100 µg/ml	Negative[1, 2]	(Pfuhler et al., 1995)	Limited quality as the test was not repeated in an independent experiment. Otherwise in accordance with OECD 479 (1986).
(2-Methoxy-4-vinylphenol [04.009])	Sister chromatid exchange	Human lymphocytes	5 concentrations up to 0.5 mmol/L (75 µg/ml)	Equivocal	(Jansson et al., 1988)	17. Limited quality (selection of maximum concentration not justified and experiment not repeated). Weak effect (only the highest concentration resulted in a twofold increase of SCE frequency which was statistically significant but was not repeated in a second experiment).
3,4- Methylenedioxyphenol [04.080]	Ames assay (preincubation method)	*S. typhimurium* TA98; TA100; TA102	4 concentrations from 1 to 10 µM/plate (1381 µg/plate)	(Not applicable)[13]	(Kaur & Saini, 2000)	Limited relevance. Antimutagenic activity was investigated only. The substance was tested only in combination with mutagens.
	Ames Assay (plate incorporation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	33 - 3333 µg/plate	Negative[1, 2, 14]	(Longfellow, 1985/1986)	Validity cannot be evaluated. The information was generated from the Chemical Carcinogenesis Research Information System database. Details of methods and results were not available.
	Mouse lymphoma assay	Mouse L5178Y TK +/- lymphocytes	25 - 215 µg/ml	Positive[1, 2]	(Longfellow, 1985/1986)	Validity cannot be evaluated. The information was generated from the CCRIS database. Details of methods and results were not available.
(2-Hydroxyacetophenone [07.124])	Ames assay (preincubation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	408 µg/plate	Negative[1, 2]	(Florin et al., 1980)	17. Insufficient quality. Not in accordance with OECD guideline 471. Inadequate study design (Spot test).
4- Hydroxy acetophenone [07.243]	Ames assay (preincubation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537	30 µmol/plate (4085 µg/plate)	Negative[1, 2]	(Florin et al., 1980)	Insufficient quality. Not in accordance with OECD guideline 471. Inadequate study design (Spot test).
Acetovanillone [07.142]	Ames assay (preincubation and plate incorporation methods)	*S. typhimurium* TA98; TA100	Not reported	Negative[1, 2]	(Xu et al., 1984)	Insufficient quality. Not in accordance with OECD guideline 471. Only two strains used. Concentration range not reported. Details of results not reported.
	Ames assay	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	Up to 1 mg/plate (range not reported)	Negative[1, 2]	(Nestmann et al., 1980)	Insufficient quality as main details of method and results were not reported. Additionally, the test was not repeated.
	Mutagenicity assay	*S. cerevisiae* D7; *S. cerevisiae* XV185-14C	6 concentrations from 100 to 1000 µg/ml	Negative[15] Positive[15]	(Nestmann & Lee, 1983)	Tested only without S9, however the positive results reported seem to be reliable.
(Vanillyl acetone [07.005])	Ames assay (plate incorporation method)	*S. typhimurium* TA98; TA100	1000 µg/plate	Negative[2, 16]	(Mikulasova & Bohovicova, 2000)	17.
	DNA Repair test	*E. coli* WP2, WP2*uvrA*, CM611;	2000 µg/ml	Negative	(Mikulasova & Bohovicova, 2000)	17.

TABLE IV.4: GENOTOXICITY (*IN VITRO*)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
		CM561				

GI = Growth inhibition.
IP = Intraperitoneal.
1) Without metabolic activation.
2) With metabolic activation.
3) Presumably non-mutagenic but solubility did not allow testing in amounts that result in lethality.
4) Negative results in TA100, with and without S9 metabolic activation.
5) Positive results in TA98, with and without metabolic activation.
6) The use of metabolic activation was not reported.
7) The concentrations selected for this assay corresponded to 0.5, 0.25, and 0.125 of the concentration causing 50 % growth inhibition (this concentration was not specified) as determined from a cytotoxicity assay.
8) Test substance was negative in a short-term assay without S9 metabolic activation and in a long-term assay (48 hours.) with and without S9 metabolic activation. The test substance gave positive results in the short-term assay with S9 metabolic activation.
9) Tested quantitatively with TA100. Substance was cytotoxic at 30 µmol/plate.
10) 5000 µg/plate resulted in cytotoxicity which was defined as a thinning of the background lawn.
11) Tested quantitatively with TA98. Substance was cytotoxic at 30 µmol/plate.
12) The presentation of the result in the publication obviously led the petitioner to the interpretion that the substance was positive in TA1530 but this is not correct. From the footnotes of the publication it becomes clear that the substance was tested in TA100 and TA1530 and that the result was negative. However, as only two strains were used the quality of the study must be considered insufficient for the purpose of this Flavouring Group Evaluation.
13) Antimutagenicity study. Sesamol greatly reduced the mutagenic effects of *t*-BOOH.
14) Test with both rat and mouse S-9 metabolic activation.
15) Negative response for gene conversion (strain D7) and a positive response for reversion (strain XV185-14C).
16) Dose level was the highest non-toxic dose level examined. At 2500 µg/ml cytotoxicity was observed.
17) Summarised by JECFA, 55th meeting (JECFA, 2001b).

In vivo mutagenicity/genotoxicity data are available for one candidate substance of the present flavouring group evaluation from chemical groups 21 and 25 and for four supporting substances evaluated by the JECFA at the 55th meeting (JECFA, 2001b). Supporting substances are listed in brackets.

TABLE IV.5: GENOTOXICITY (*IN VIVO*)

Chemical Name [FL-no]	Test System	Test Object	Route	Dose	Result	Reference	Comments
(2-Methylphenol [04.027])	*In vivo* Sister chromatid exchange	Mouse bone marrow cells, alveolar macrophages, and regenerating liver cells	IP injection	0, 200 mg/kg	Negative	(Cheng & Kligerman, 1984)	1. Limited quality since only two animals were used and only 20 metaphases were analysed for each cell type from each animal. Only one dose tested.
	In vivo Sex- linked recessive lethal test	*D. melanogaster*	Oral	0, 100, 500, 1000 µg/ml	Negative	(Sernau, 1989)	1. Acceptable quality. GLP study generally in accordance with OECD 477 (1984).
(3-Methylphenol [04.026])	*In vivo* Sister chromatid exchange	Mouse bone marrow cells, alveolar macrophages, and regenerating liver cells	IP injection	0, 200 mg/kg	Negative	(Cheng & Kligerman, 1984)	1. Limited quality since only three animals were used and only 20 metaphases were analysed for each cell type from each animal. Only one dose tested.
	In vivo Chromosome aberration assay	Mouse bone marrow	Oral (gavage)	0, 96, 320, 960 mg/kg	Negative	(Ivett et al., 1989)	1. GLP study in accordance with OECD guideline 475 (1984). However, the validity of the result cannot be evaluated as all pages with results in table format are lacking.
(4-Methylphenol [04.028])	*In vivo* Sister chromatid exchange	Mouse bone marrow cells, alveolar macrophages, and regenerating liver cells	IP injection	0, 75 mg/kg	Negative	(Cheng & Kligerman, 1984)	1. Limited quality since only three animals were used and only 20 metaphases were analysed for each cell type from each animal. Only one dose tested.
(Carvacrol [04.031])	*In vivo* Spot test	*D. melanogaster* BINSC; Oregon-R		1.40 ppm; 0.35 ppm	Negative	(Kono et al., 1995)	1. Validity cannot be evaluated. Publication is in Japanese with a short summary in English. Results reported only for two doses in table format. Not clear if control groups were treated concomitantly.
4-Methoxyphenol [04.077]	*In vivo* Chromosome aberration assay	Rat	Oral (gavage)	0, 100, 333, 1000 mg/kg bw	Negative	(Esber, 1986)	The study design was in accordance with OECD guideline 475 (1984). The study was incompletely reported, however, the study report contained sufficient details to conclude that the outcome of the study is negative.

1) Summarised by JECFA, 55th meeting (JECFA, 2001b).

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ABBREVIATIONS

ADI	Acceptable Daily Intake
BW	Body weight
CAS	Chemical Abstract Service
CEF	Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids Chemical Abstract Service
CHO	Chinese hamster ovary (cells)
CL_r	Renal Clearance
CL_t	Total Clearance
CoE	Council of Europe
DMP	Dimethylphenol
DNA	Deoxyribonucleic acid
EC	European Commission
EFSA	The European Food Safety Authority
EU	European Union
FAO	Food and Agriculture Organization of the United Nations
FEMA	Flavor and Extract Manufacturers Association
FGE	Flavouring Group Evaluation
FLAVIS (FL)	Flavour Information System (database)
GI	Gastro intestinal
GLP	Good Laboratory Practice
GSH	Glutathione
ID	Identity
IOFI	International Organization of the Flavour Industry
IR	Infrared spectroscopy
JECFA	The Joint FAO/WHO Expert Committee on Food Additives
LD_{50}	Lethal Dose, 50%; Median lethal dose
MS	Mass spectrometry
MSDI	Maximised Survey-derived Daily Intake
mTAMDI	Modified Theoretical Added Maximum Daily Intake
NAD	Nicotinamide Adenine Dinucleotide
NADP	Nicotinamide Adenine Dinucleotide Phosphate
NADPH	Nicotinamide Adenine Dinucleotide Phosphate, reduced form
No	Number
NOAEL	No Observed Adverse Effect Level
NOEL	No Observed Effect Level
NTP	National Toxicology Program

OECD	Organisation for Economic Co-operation and Development
QM	Quinone methide
SCE	Sister Chromatid Exchange
SCF	Scientific Committee on Food
SMART	Somatic Mutation and Recombination Test
TAMDI	Theoretical Added Maximum Daily Intake
UDS	Unscheduled DNA Synthesis
Vd	Volume of distribution
WHO	World Health Organisation