EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF); Scientific Opinion on Flavouring Group Evaluation 20, Revision 3 (FGE.20Rev3): Benzyl alcohols, benzaldehydes, a related acetal, benzoic acids, and related esters from chemical groups 23 and 30

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SCIENTIFIC OPINION

Scientific Opinion on Flavouring Group Evaluation 20, Revision 3 (FGE.20Rev3):

Benzyl alcohols, benzaldehydes, a related acetal, benzoic acids, and related esters from chemical groups 23 and 30[1]

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

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

The Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids of the European Food Safety Authority was requested to consider in this revision 3 of Flavouring Group Evaluation 20, the SCF Opinion on benzoic acid. Furthermore information on stereoisomeric composition for two substances [FL-no: 06.104 and 09.570] and new information to support the re-allocation of the structural class for the candidate substance piperonyl alcohol [FL-no: 02.205] has been submitted. The 41 flavouring substances in Flavouring Group Evaluation 20 were evaluated using the Procedure in Commission Regulation (EC) No 1565/2000. None of the substances were considered to have genotoxic potential. The 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 all the substances do not give rise to safety concerns at their levels of dietary intake, estimated on the basis of the MSDI approach.

1 On request from the Commission, Question No EFSA-Q-2011-00299, adopted on 19 May 2011.

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SUMMARY

The European Food Safety Authority (EFSA) asked the Scientific 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 41 flavouring substances in the Flavouring Group Evaluation 20, Revision 3 (FGE.20Rev3), using the Procedure as referred to in the Commission Regulation (EC) No 1565/2000. These 41 flavouring substances belong to chemical group 23 and 30, Annex I of the Commission Regulation (EC) No 1565/2000.

The present revision of FGE.20, FGE.20Rev3 includes the consideration of the SCF Opinion on benzoic acid (SCF, 2002c). Furthermore information on the stereoisomeric composition has become available for two substances [FL-no: 06.104 and 09.570] and new information to support the re-allocation of the structural class for the candidate substance piperonyl alcohol [FL-no: 02.205] has been submitted.

Four of the 41 flavouring substances can exist as optical isomers [FL-no: 06.104, 09.313, 09.317 and 09.852] and three of the 41 substances can exist as geometrical isomers [FL-no: 09.314, 09.560 and 09.570].

Thirty-seven candidate substances are classified into structural class I and four [FL-no: 02.205, 05.066, 05.221 and 06.104] are classified into structural class II according to the decision tree approach presented by Cramer et al., 1978.

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 Flavour Industry on the use levels in various foods, it appeared obvious that the MSDI approach in a number of cases would grossly underestimate the intake by regular consumers of products flavoured at the use level reported by the industry, especially in those cases where the annual production values were reported to be small. In consequence, the Panel had reservations about the data on use and use levels provided and the intake estimates obtained by the MSDI approach.

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

According to the default MSDI approach, the 37 flavouring substances allocated to structural class I have intakes in Europe from 0.001 to 610 microgram/*capita*/day, which are below the threshold of concern value for structural class I (1800 microgram/person/day). The four substances in structural class II [FL-no: 02.205, 05.066, 05.221 and 06.104] have estimated intakes of 0.011, 1.2, 0.61 and 100 microgram/*capita*/day, respectively. These intakes are below the threshold values of 540 microgram/person/day for structural class II.

On the basis of the reported annual production in Europe (MSDI approach), the combined intake of the 37 of the candidate substances belonging to structural class I is approximately 1300 microgram/*capita*/day. This value is lower than the threshold of concern for structural class I substances. Based on reported production volumes, European *per capita* intakes (MSDI) could be estimated for 76 of the 77 supporting substances. The total combined intakes of the candidate and

supporting substances are approximately 75000 and 7100 microgram/*capita*/day for structural class I and II, respectively, which exceed the thresholds of concern. However, the substances are expected to be efficiently metabolised and are not expected to saturate the metabolic pathways.

For the substances in this group the available genotoxicity data do not preclude the evaluation of the candidate substances using the Procedure.

It is anticipated that the candidate substances in FGE.20Rev3 would be metabolised to innocuous products.

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

It is considered that on the basis of the default MSDI approach the 41 candidate substances would not give rise to safety concerns at the estimated levels of intake arising from their use as flavouring substances.

When the estimated intakes were based on the mTAMDI approach they ranged from 1400 to 120000 microgram/person/day for the 37 flavouring substances from structural class I. The intakes were all above the threshold of concern for structural class I of 1800 microgram/person/day, except for five flavouring substances [FL-no: 05.129, 05.142, 05.153, 05.158 and 08.080]. The estimated intakes, based on the mTAMDI, of the four flavouring substances [FL-no: 02.205, 05.066, 05.221 and 06.104] assigned to structural class II were 3900, 1600, 7000 and 3900 microgram/person/day, respectively, which are above the threshold of concern for the structural class (540 microgram/person/day for structural class II). The five substances which have mTAMDI intake estimates below the threshold of concern for structural class I are also expected to be metabolised to innocuous products. Thus, on the basis of the mTAMDI, the estimated intakes for 36 of the 41 flavouring substances considered in this Opinion, exceed the relevant threshold for their structural class to which the flavouring substance has been assigned. Therefore, for these 36 substances more reliable exposure data are required. On the basis of such additional data, these flavouring substances should be re-evaluated using the Procedure. Subsequently, additional toxicological data might become necessary.

In order to determine whether the conclusion for the 41 candidate substances can be applied to the materials of commerce, it is necessary to consider the available specifications. Adequate specifications including complete purity criteria and identity for the materials of commerce have been provided for the 41 flavouring substances. For these 41 flavouring substances the Panel concluded that they would present no safety concern at their estimated levels of intake based of the MSDI approach.

KEYWORDS

Flavourings, safety, benzyl alcohols, benzaldehydes, benzoic acids, esters, acetals, benzyl, benzoate, aliphatic, acyclic, alicyclic, FGE.20.

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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.

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

The present flavouring group originally included two substances [FL-no: 09.693 and 09.696], which by hydrolysis and oxidation may be metabolised to alpha,beta-unsaturated aldehydes. As the alpha,beta-unsaturated aldehyde and ketone structures are considered a structural alerts for genotoxicity (EFSA, 2008b), they have been given special considerations in the Flavouring Group Evaluation 19 (FGE.19). The two substances [FL-no: 09.693 and 09.696] were considered with respect to genotoxicity in subgroup 1.1.3 of FGE.19 (FGE.202).

FGE.19 contains 360 flavouring substances from the EU Register being alpha, beta-unsaturated aldehydes or ketones and precursors which could give rise to such carbonyl substances via hydrolysis and / or oxidation (EFSA, 2008b). The alpha, beta-unsaturated carbonyls were subdivided into 28 subgroups on the basis of structural similarity (EFSA, 2008b). In an attempt to decide which of the substances could go through the Procedure, a (quantitative) structure-activity relationship (Q)SAR prediction of the genotoxicity of these substances was undertaken. The Panel took note of the (Q)SAR predictions by using two ISS Local Models (Benigni and Netzeva, 2007a; Benigni and Netzeva, 2007b) and four DTU-NFI MultiCASE Models (Gry et al., 2007; Nikolov et al., 2007) and the fact that there are available data on genotoxicity, *in vitro* and *in vivo*, as well as data on carcinogenicity for several substances. The Panel decided that 11 subgroups (1.1.2, 1.1.3, 1.1.4, 2.4, 2.6, 2.7, 3.1, 3.3, 4.1, 4.2 and 4.4) (EFSA, 2008b) should be further examined to determine whether evaluation through the Procedure is feasible. Corresponding to these 11 subgroups 11 Flavouring Group Evaluations (FGEs) were established: FGE.201, 202, 203, 210, 212, 213, 214, 216, 217, 218 and 220.

The revision 1 of FGE.20, FGE.20Rev1, included the assessment of one additional candidate substance, vanillin propylene glycol acetal [FL-no: 06.104]. For this substance there are hydrolysis data and for a related substance [FL-no: 02.248] *in vitro* genotoxicity data. Additional information for three substances [FL-no: 09.313, 09.317 and 09.852] has been made available since FGE.20 was published. Toxicity and metabolism data on a substance, vanillin 3-l-menthoxypropane-1,2-diol acetal [FL-no: 02.248], related to the candidate substance vanillin propylene glycol acetal [FL-no:06.104], are included.

The revision 2 of FGE.20, FGE.20Rev2, included the assessment of five additional candidate substances [FL-no: 05.221, 08.132, 08.133, 09.693 and 09.696]. Toxicity data are available for four of the five substances. *In vitro* genotoxicity data are available for [FL-no: 05.221 and 08.133], long-term toxicity data are available for [FL-no: 08.133] and acute toxicity data are available for [FL-no: 08.133, 09.693 and 09.696]. Two of the substances [FL-no: 09.693 and 09.696] were considered with respect to genotoxicity in FGE.202 in which the Panel concluded that the genotoxicity data available do not preclude their evaluation through the Procedure. So, [FL-no: 09.693 and 09.696] were evaluated through the Procedure in FGE.20Rev2.

History of FGE.20:

FGE	Opinion adopted by EFSA	Link	No. of candidate substances
FGE.20	7 December 2005	http://www.efsa.eu.int/science/afc/afc_opinions/1292_en.html	35
FGE.20Rev1	29 November 2007	http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1211902332837.htm	36
FGE.20Rev2	26 November 2009	http://www.efsa.europa.eu/en/efsajournal/pub/976.htm	41
FGE.20Rev3	17 May 2011		41

The present revision of FGE.20, FGE.20Rev3 includes the consideration of the SCF Opinion on benzoic acid (SCF, 2002c). Furthermore, the Industry has for two substances [FL-no: 06.104 and 09.570] submitted information on the stereoisomeric composition, which was missing in the previous version of the FGE. Finally, the Industry has submitted new information to support the re-allocation of structural class to the candidate substance piperonyl alcohol [FL-no: 02.205].

For piperonyl alcohol [FL-no: 02.205], the Flavouring Industry has submitted new information since the publication of FGE.20Rev2 that suggests that the natural occurrence in several food sources of closely structurally related substances, which are most likely metabolised to piperonyl alcohol. Therefore Flavouring Industry considered it correct to answer the question whether the substance occur naturally with a yes, and therefore the substance should be allocated to structural class II. The Panel agreed in this consideration and allocated [FL-no: 02.205] to structural class II.

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).

ASSESSMENT

1. Presentation of the Substances in Flavouring Group Evaluation 20, Revision 3

1.1. Description

The present Flavouring Group Evaluation 20, Revision 3 (FGE.20Rev3), 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 of this FGE), deals with 41 benzyl alcohols, benzaldehydes, a related acetal, benzoic acids and related esters. These flavouring substances belong to chemical groups 23 and 30, Annex I of Commission Regulation (EC) No 1565/2000 (EC, 2000a).

The 41 candidate substances under consideration with 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.

This group of candidate substances includes 15 benzyl derivatives (subgroup 1), 25 hydroxy- and alkoxy-substituted benzyl derivatives (subgroup 2) and 1 hydroxy- and alkoxy-substituted biphenyl derivative (subgroup 3).

- Subgroup 1 includes 14 alkyl esters of which nine are benzyl esters [FL-no: 09.152, 09.313, 09.314, 09.315, 09.316, 09.317, 09.318, 09.611 and 09.835] and five are benzoic acid esters [FL-no: 09.631, 09.656, 09.693, 09.779 and 09.825]. Three of these candidate substances contain a double-bond in the alkyl side chain [FL-no: 09.314, 09.656 and 09.693] and two contain an alkyl substituent at the aromatic ring [FL-no: 09.631 and 09.611]. The remaining substance [FL-no: 06.017] is an acetal.

- Subgroup 2 includes two benzyl alcohols [FL-no: 02.164, and the derivative piperonyl alcohol FL-no: 02.205], six benzaldehyde derivatives [FL-no: 05.066, 05.129, 05.142, 05.153, 05.158 and 06.104], four benzoic acids [FL-no: 08.080, 08.087, 08.132 and 08.133] and 13 related esters [FL-no: 09.362, 09.363, 09.367, 09.560, 09.570, 09.581, 09.623, 09.696, 09.762, 09.798, 09.799, 09.852 and 09.895]. One of the esters is a benzyl ester [FL-no: 09.895], all the others are benzoic acid esters. Three of the esters contain a double-bond in the alkyl side chain [FL-no: 09.560, 09.570 and 09.696].

- Subgroup 3 contains one biphenyl [FL-no: 05.221].

The 41 flavouring substances (candidate substances) are closely related structurally to 77 flavouring substances (supporting substances) evaluated at the 46th and 57th JECFA meeting (JECFA, 1997a; JECFA, 2002b). The names and structures of the 77 supporting substances are listed in Table 3, together with their evaluation status (CoE, 1992; JECFA, 1997a; JECFA, 2002b; SCF, 1995).

The 77 supporting substances include 34 benzyl derivatives (subgroup 1) and 43 hydroxy- and alkoxy-substituted benzyl derivatives (subgroup 2).

Two of the candidate substances [FL-no: 09.693 and 09.696] are alpha-beta unsaturated compounds and their possible genotoxicity has been evaluated in FGE.202 (EFSA, 2009ac). The Panel concluded that although the substances in FGE.202 have a structural alert for genotoxicity, the data available on one of the substances, citral [FL-no: 05.020], made it possible to conclude that there would be no safety concern with respect to genotoxicity for these substances and that they may be further evaluated through the Procedure.

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

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.).

Three of the 41 flavouring substances possess one chiral centre [FL-no: 09.313, 09.317 and 09.852] and one flavouring substance possesses two chiral centres [FL-no: 06.104]. Due to the presence and the position of double bonds, three of the 41 candidate substances can exist as geometrical isomers [FL-no: 09.314, 09.560 and 09.570].

Industry has for the two substances [FL-no: 06.104 and 09.570] submitted information on the stereoisomeric composition, which was missing in the previous version of the FGE. The candidate substance [FL-no: 06.104] consists of predominantly (up to 80 %) vanillin propylene glycol acetal, the parent compound, and up to 18 - 20 % vanillin. The candidate substance [FL-no: 09.570] has one double bond that exists in cis-form (Z). The Register name can thus be changed into (*Z*)-Hex-3-enyl salicylate (EFFA, 2010a) (See Table 1).

1.3. Natural Occurrence in Food

Twenty-two out of 41 candidate substances have been reported to occur in fruit (cherry, mango, papaya, bilberry, black currants, mulberry, sapodilla, cloudberry, pineapple, grape, cocoa), potato, coffee, beer, rum, sherry, whisky, wine, honey, spices, soybean, peanut, wort and pork. Quantitative data on the natural occurrence of these substances have been reported for the occurrence of 14 of these substances in food (TNO, 2000; EFFA, 2010a). These reports are:

- 2-Methoxybenzaldehyde [FL-no: 05.129]: 7000 mg/kg in cassia leaf, up to 1500 mg/kg in cinnamon bark.
- 3,4-Dihydroxybenzaldehyde [FL-no: 05.142]: up to 20 mg/kg in coffee, 313 mg/kg in bourbon vanilla.
- 4-Hydroxy-3,5-dimethoxybenzaldehyde [FL-no: 05.153]: up to 0.7 mg/kg in beer, up to 9.2 mg/kg in grape, up to 0.014 mg/kg in mango, 0.08 mg/kg in pineapple, 8.3 mg/kg in pork, up to 19.9 mg/kg in rum, 0.035 mg/kg in sherry, 1.9 mg/kg in bourbon vanilla, up to 8.7 mg/kg in whisky, up to 0.86 mg/kg in red wine, up to 0.04 mg/kg in wort.
- 3-Methoxybenzaldehyde [FL-no: 05.158]: 3900 mg/kg in clove bud.
- Gallic acid [FL-no: 08.080]: up to 0.6 mg/kg in beer, up to 7 mg/kg in cherry, up to 11 mg/kg in grape, up to 6.1 mg/kg in whisky, up to 35 mg/kg in wine.
- 4-Hydroxy-3,5-dimethoxybenzoic acid [FL-no: 08.087]: up to 1.1 mg/kg in beer, 1.3 mg/kg in grape, up to 0.096 mg/kg in mango, up to 18 mg/kg in rum, up to 34 mg/kg in soybean, up to 1.4 mg/kg in whisky, up to 10 mg/kg in wine.
- 3-Hydroxybenzoic acid [FL-no: 08.132]: up to 2.7 mg/kg in honey.
- 3,4-Dihydroxybenzoic acid [FL-no: 08.133]: up to 1.4 mg/kg in brandy, up to 0.4 mg/kg in beer, up to 52 mg/kg in black currants, up to 6.8 mg/kg in honey, 4.3 mg/kg in mulberry, 0.15 mg/kg in rum, 10 mg/kg in soybean, up to 0.3 mg/kg in whisky, up to 10 mg/kg in wine.
- Benzyl valerate [FL-no: 09.152]: 0.11 mg/kg in sea buckthorn.
- Benzyl crotonate [FL-no: 09.314]: 0.0001 mg/kg in papaya.
- Butyl benzoate [FL-no: 09.779]: 200 mg/kg in galanga, 2 mg/kg in hog plum, up to 0.05 mg/kg in papaya.
- Ethyl vanillate [FL-no: 09.798]: 0.3 mg/kg in rum, up to 113 mg/kg in red wine.

- Methyl vanillate [FL-no: 09.799]: 0.05 mg/kg in cloudberry, up to 214 mg/kg in red wine.
- Pentyl benzoate [FL-no: 09.825]: 0.001 mg/kg in bilberry, trace amounts in sapodilla fruit.

According to TNO the remaining 19 substances have not been reported to occur naturally in any food items (TNO, 2000). See Table 3.1

Table 1.3: Substances for which no occurrence in food has been reported

FL-no	EU Register name
02.164	4-Hydroxy-3,5-dimethoxybenzyl alcohol
09.315	Benzyl dodecanoate
09.317	Benzyl lactate
09.318	Benzyl octanoate
09.362	Ethyl 2-hydroxy-4-methylbenzoate
09.560	Hex-3(cis)-enyl anisate
09.581	Hexyl salicylate
09.611	4-Isopropylbenzyl acetate
09.623	Methyl 2,4-dihydroxy-3,6-dimethylbenzoate
09.656	3-Methylbut-3-enyl benzoate
09.693	Prenyl benzoate
09.696	Prenyl salicylate
09.762	Pentyl salicylate
09.835	Benzyl decanoate
09.852	2-Methylbutyl 2-hydroxybenzoate
09.895	4-Methoxybenzyl-2-methylpropionate
05.066	4-Ethoxy-3-methoxybenzaldehyde
05.221	6,6'-Dihydroxy-5,5'-dimethoxy-biphenyl-3,3'-dicarbaldehyde
06.104	Vanillin propylene glycol acetal

2. Specifications

Purity criteria for the 41 candidate substances have been provided by the Flavour Industry (EFFA, 2003u; EFFA, 2004c; EFFA, 2007d; Flavour Industry, 2008c).

Judged against the requirements in Annex II of Commission Regulation (EC) No 1565/2000 (EC, 2000a), this information is adequate for all the 41 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).

The total annual volume of production of the 41 candidate substances in the present Flavouring Group Evaluation (FGE.20Rev3) from use as flavouring substances in Europe has been reported to be approximately 11200 kg (EFFA, 2003u; EFFA, 2004d; EFFA, 2007d; Flavour Industry, 2008c). For 76 of the 77 supporting substances the total annual volume of production is approximately 660000 kg in Europe (vanillin [FL-no: 05.018] accounts for 390000 kg) (JECFA, 2002a). The annual volumes of production in Europe for 1 of the supporting substances [FL-no: 09.754] were not reported.

On the basis of the annual volumes of production reported for the 41 candidate substances, the daily *per capita* intakes for each of these flavourings have been estimated. Approximately 97 % of the annual volume of production for the candidate substances is accounted for by three substances [FL-no: 06.104, 08.132 and 08.133]. The estimated daily *per capita* intake of these three candidate substances from use as flavouring substances is 1320 microgram. For each of the remaining substances the estimated daily *per capita* intake is less than 10 microgram for each (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).

[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.

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

For the 41 candidate substances information on food categories and normal and maximum use levels[5,6,7] were submitted by the Flavour Industry(EFFA, 2003u; EFFA, 2004c; EFFA, 2007a; EFFA, 2007d; Flavour Industry, 2008c). The 41 candidate substances are used in flavoured food products divided into the food categories, outlined in Annex III of the Commission Regulation (EC) No 1565/2000 (EC, 2000a), as shown in Table 3.1. For the present calculation of mTAMDI, the reported normal use levels were used. In the case where different use levels were reported for different food categories the highest reported normal use level was used.

Table 3.1 Use of Candidate Substances

Food category	Description	Flavourings used
01.0	Dairy products, excluding products of category 2	All 41 except [FL-no: 08.132, 08.133]
02.0	Fats and oils, and fat emulsions (type water-in-oil)	All 41 except [FL-no: 08.132, 08.133]
03.0	Edible ices, including sherbet and sorbet	All 41 except [FL-no: 08.132, 08.133]
04.1	Processed fruits	All 41 except [FL-no: 05.221, 08.132, 08.133]
04.2	Processed vegetables (incl. mushrooms & fungi, roots & tubers, pulses and legumes), and nuts & seeds	None
05.0	Confectionery	All 41
06.0	Cereals and cereal products, incl. flours & starches from roots & tubers, pulses & legumes, excluding bakery	All 41 except [FL-no: 05.221, 08.132, 08.133]
07..0	Bakery wares	All 41 except [FL-no 05.129, 08.132, 08.133]
08.0	Meat and meat products, including poultry and game	All 41 except [FL-no: 05.221, 08.132, 08.133]
09.0	Fish and fish products, including molluscs, crustaceans and echinoderms	All 41 except [FL-no: 05.221, 08.132, 08.133, 09.825]
10.0	Eggs and egg products	None
11.0	Sweeteners, including honey	None
12.0	Salts, spices, soups, sauces, salads, protein products etc.	All 41 except [FL-no: 08.132, 08.133]
13.0	Foodstuffs intended for particular nutritional uses	All 41 except [FL-no: 05.221, 08.132, 08.133]
14.1	Non-alcoholic ("soft") beverages, excl. dairy products	All 41
14.2	Alcoholic beverages, incl. alcohol-free and low-alcoholic counterparts	All 41
15.0	Ready-to-eat savouries	All 41 except [FL-no: 08.132, 08.133, 09.693]
16.0	Composite foods (e.g. casseroles, meat pies, mincemeat) - foods that could not be placed in categories 1 – 15	All 41 except [FL-no: 08.132, 08.133]

[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).

According to the Flavour Industry the normal use levels for the candidate substances are in the range of 1 - 500 mg/kg food, and the maximum use levels are in the range of 5 - 2000 mg/kg (EFFA, 2003u; EFFA, 2004c; EFFA, 2007a; EFFA, 2007d; Flavour Industry, 2008c).

The mTAMDI value is 1400 - 120000 microgram/person/day for 37 of the 41 candidate substances from structural class I (see Section 5). For the candidate substances [FL-no: 02.205, 05.066, 05.221, and 06.104] from structural class II (see Section 5) the mTAMDIs are 3900, 1600, 7000 and 3900 microgram/person/day, respectively.

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 41 candidate substances are subdivided into three subgroups. Subgroup 1 includes 15 benzyl derivatives of which 14 are benzyl esters or benzoic acid esters and one is an acetal, [FL-no: 06.017] (diethoxymethyl)benzene. Subgroup 2 includes 25 hydroxy- and alkoxy-substituted benzyl derivatives of which 12 are benzyl alcohols, benzaldehydes or benzoic acids and 13 are related esters. Subgroup 3 contains one derivative of biphenyl [FL-no: 05.221] (6,6'-dihydroxy-5,5'dimethoxy-biphenyl-3,3'-dicarbaldehyde).

It is expected that esters in subgroup 1 and 2 will be hydrolysed *in vivo* to their component alcohols and acids. Eight of the 14 esters from subgroup 1 will yield benzyl alcohol which has previously been evaluated by the JECFA (JECFA, 1996b) and SCF (SCF, 2002b). One candidate ester, 4-isopropylbenzyl acetate [FL-no: 09.611] will yield 4-isopropyl benzyl alcohol. This substance has been previously evaluated by the JECFA (JECFA, 2002a). The benzyl alcohols are expected to be oxidised to corresponding benzoic acids, which will be conjugated with glycine and excreted as hippuric acids.

Of the remaining five candidate esters in subgroup 1, four are expected to yield benzoic acid and simple aliphatic alcohols upon hydrolysis, 3-methylbut-3-enyl benzoate [FL-no: 09.656], butyl benzoate [FL-no: 09.779], pentyl benzoate [FL-no: 09.825], prenyl benzoate [FL-no: 09.693], and one ester, methyl 4-methylbenzoate [FL-no: 09.631] will yield 4-methylbenzoic acid upon hydrolysis. Benzoic acid will mainly be conjugated with glycine and excreted as hippuric acid. Conjugation with glycine may be a saturable process and glucuronide conjugates increase with increasing dose.

One of the substances in subgroup 1 is an acetal, (diethoxymethyl)benzene [FL-no: 06.017]. This substance would be expected to yield benzaldehyde and ethanol upon hydrolysis. Benzaldehyde has been evaluated by the JECFA (JECFA, 1996b). Benzaldehyde is expected to be oxidized to benzoic acid.

Subgroup 2 includes 13 esters of which one, 4-methoxybenzyl-2-methylpropionate [FL-no: 09.895] will yield 4-methoxybenzyl alcohol (*p*-anisyl alcohol) (supporting substance [FL-no: 02.128]) upon hydrolysis. This substance has been evaluated by the JECFA (JECFA, 2002a). 4-Methoxybenzyl alcohol is expected to be excreted in the urine either unchanged or as glucuronic acid, glycine or sulphate conjugate. The same metabolic pathway is proposed for the candidate benzyl alcohol derivative, 4-hydroxy-3,5-dimethoxybenzyl alcohol [FL-no: 02.164].

The remaining 12 esters in subgroup 2, ethyl 2-hydroxy-4-methylbenzoate [FL-no: 09.362], ethyl 2-methoxybenzoate [FL-no: 09.363], ethyl 4-hydroxybenzoate [FL-no: 09.367], hex-3(cis)-enyl anisate [FL-no: 09.560] (hex-3(cis)-enyl 4-methoxybenzoate), hex-3-enyl salicylate [FL-no: 09.570] (hex-3-enyl 2-hydroxybenzoate), hexyl salicylate [FL-no: 09.581] (hexyl 2-hydroxybenzoate), methyl 2,4-dihydroxy-3,6-dimethylbenzoate [FL-no: 09.623], prenyl salicylate [FL-no: 09.696] (3-methylbut-2-enyl 2-hydroxybenzoate), pentyl salicylate [FL-no: 09.762] (pentyl 2-hydroxybenzoate), ethyl vanillate [FL-no: 09.798] (ethyl 3-methoxy-4-hydroxybenzoate), methyl vanillate [FL-no: 09.799]

(methyl 3-methoxy-4-hydroxybenzoate), 2-methylbutyl 2-hydroxybenzoate [FL-no: 09.852] (2-methylbutyl salicylate) will yield alkoxy- and/or hydroxy-substituted benzoic acids upon hydrolysis. The substituted benzoic acids that are hydrolysis products of candidate esters are expected to be excreted in the urine unchanged or as the glucuronic acid, glycine or sulphate conjugates. The same metabolic route is proposed for the candidate acids, 4-hydroxy-3,5-dimethoxybenzoic acid [FL-no: 08.087], 3-hydroxybenzoic acid [FL-no: 08.132] and 3,4-dihydroxybenzoic acid [FL-no: 08.133].

The main metabolic pathway for the acetal, vanillin propylene glycol acetal [FL-no: 06.104], after hydrolysis to the aldehyde, and for the five candidate aldehydes in subgroup 2, 4-ethoxy-3-methoxybenzaldehyde [FL-no: 05.066], 2-methoxybenzaldehyde [FL-no: 05.129], 3,4-dihydroxybenzaldehyde [FL-no: 05.142], 4-hydroxy-3,5-dimethoxybenzaldehyde [FL-no: 05.153], 3-methoxybenzaldehyde [FL-no: 05.158], is presumed to be oxidation of the aldehyde to the corresponding acids, followed by conjugation and excretion. The reduction to alcohols is a minor metabolic route and the oxidative pathway dominates clearly. To a minor extent O-demethylation followed by conjugation may occur.

The candidate substance piperonyl alcohol [FL-no: 02.205] (3,4-methylenedioxybenzylalcohol) is expected to mainly undergo oxidation and conjugation of the side chain, and be excreted as glycine conjugate. Demethylenation of the methylenedioxy moiety is a very minor metabolic path for this compound.

The main metabolite of gallic acid [FL-no: 08.080] (3,4,5-trihydroxybenzoic acid) is expected to be 4-O-methyl gallic acid (3,5-dihydroxy-4-methoxybenzoic acid), the product of O-methylation. Decarboxylation to pyrogallol (1,2,3-trihydroxybenzene) may occur as a very minor pathway, but no further dehydroxylation to catechol has been observed.

The biphenyl substance in subgroup 3 [FL-no: 05.221] is expected to be metabolised in a similar way to the benzaldehyde derivatives in subgroup 2. It is expected that the aldehyde group(s) will undergo oxidation to form the corresponding carboxylic acid which is likely to be conjugated and excreted. The reduction of the alcohol groups may again be a minor pathway, but some steric hindrance may occur making this less likely than for the benzaldehyde derivatives in subgroup 2.

Based on experimental evidence and general knowledge of toxicokinetics of structurally related compounds, it is expected that, at the reported levels of intake as flavouring substances, the candidate substances are metabolised to innocuous products.

For more detailed information, see Annex III.

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

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

For the safety evaluation of the 41 candidate substances from chemical groups 23 and 30 the Procedure as outlined in Annex I was applied, based on the MSDI approach. The stepwise evaluations of the substances are summarised in Table 2a.

Step 1

Thirty-seven of the flavouring substances are classified according to the decision tree approach by Cramer et al. (Cramer et al., 1978) into structural class I, four are classified into structural class II [FL-no: 02.205, 05.066, 05.221 and 06.104].

Step 2

Step 2 requires consideration of the metabolism of the candidate substances. It can be anticipated that at the estimated levels of intake all 41 candidate substances are expected to be metabolised to innocuous products. Accordingly, the evaluation of these 41 candidate substances proceeds via the A-side of the Procedure scheme.

Step A3

The estimated levels of the European daily *per capita* intake (MSDI) for the 37 candidate substances classified into structural class I are in the range of 0.0012 to 610 micrograms. For the four candidate substances [FL-no: 02.205, 05.066, 05.221 and 06.104] classified into structural class II, the intakes are 0.011, 1.2, 0.61 and 100 micrograms, respectively (Table 2a). These intakes are below the thresholds of concern of 1800 and 540 microgram/person/day for structural class I and II, respectively.

Based on results of the safety evaluation sequence of the Procedure, these 41 candidate substances, proceeding via the A-side of the Procedure scheme, do not pose a safety concern when used as flavouring substances at the estimated levels of intake, based on the MSDI approach.

6. Comparison of the Intake Estimations Based on the MSDI Approach and the mTAMDI Approach

The estimated intakes for the 37 candidate substances in structural class I based on the mTAMDI range from 1400 to 120000 microgram/person/day. For five of the substances [FL-no: 05.129, 05.142, 05.153, 05.158 and 08.080] the mTAMDI values are below the threshold of concern of 1800 microgram/person/day for structural class I. For the remaining 32 of the 37 substances in class 1, the mTAMDI is above the threshold of concern.

The estimated intake of the four candidate substances [FL-no: 02.205, 05.066, 05.221 and 06.104] assigned to structural class II, based on the mTAMDI, are 3900, 1600, 7000 and 3900 microgram/person/day, respectively. These intakes are above the threshold of concern of 540 microgram/person/day for structural classes II.

Thus, for 36 candidate substances, for which the mTAMDI is above the threshold of concern, further information is required. This would include more reliable intake data and subsequently, if required, additional toxicological data.

For comparison of the intake estimates based on the MSDI approach and the mTAMDI approach, 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)
02.164	4-Hydroxy-3,5-dimethoxybenzyl alcohol	0.037	3900	Class I	1800
05.129	2-Methoxybenzaldehyde	0.16	1400	Class I	1800
05.142	3,4-Dihydroxybenzaldehyde	8.5	1600	Class I	1800
05.153	4-Hydroxy-3,5-dimethoxybenzaldehyde	0.74	1600	Class I	1800
05.158	3-Methoxybenzaldehyde	0.011	1600	Class I	1800
06.017	(Diethoxymethyl)benzene	1.7	3900	Class I	1800
08.080	Gallic acid	0.011	1600	Class I	1800
08.087	4-Hydroxy-3,5-dimethoxybenzoic acid	1.2	3200	Class I	1800
08.132	3-Hydroxybenzoic acid	610	120000	Class I	1800
08.133	3,4-Dihydroxybenzoic acid	610	120000	Class I	1800
09.152	Benzyl valerate	1.7	3900	Class I	1800
09.313	Benzyl 2-methylbutyrate	7.3	3900	Class I	1800
09.314	Benzyl crotonate	0.37	3900	Class I	1800
09.315	Benzyl dodecanoate	0.13	3900	Class I	1800
09.316	Benzyl hexanoate	0.75	3900	Class I	1800

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)
09.317	Benzyl lactate	0.91	3900	Class I	1800
09.318	Benzyl octanoate	0.12	3900	Class I	1800
09.362	Ethyl 2-hydroxy-4-methylbenzoate	0.0012	3900	Class I	1800
09.363	Ethyl 2-methoxybenzoate	5.5	3900	Class I	1800
09.367	Ethyl 4-hydroxybenzoate	10	3900	Class I	1800
09.560	Hex-3(cis)-enyl anisate	0.12	3900	Class I	1800
09.570	Hex-3-enyl salicylate	0.13	3900	Class I	1800
09.581	Hexyl salicylate	0.018	3900	Class I	1800
09.611	4-Isopropylbenzyl acetate	0.012	3900	Class I	1800
09.623	Methyl 2,4-dihydroxy-3,6-dimethylbenzoate	0.012	3900	Class I	1800
09.631	Methyl 4-methylbenzoate	0.0012	3900	Class I	1800
09.656	3-Methylbut-3-enyl benzoate	0.12	3900	Class I	1800
09.693	Prenyl benzoate	0.012	4900	Class I	1800
09.696	Prenyl salicylate	0.011	3900	Class I	1800
09.762	Pentyl salicylate	0.24	3900	Class I	1800
09.779	Butyl benzoate	3.7	3900	Class I	1800
09.798	Ethyl vanillate	0.024	3900	Class I	1800
09.799	Methyl vanillate	0.011	3900	Class I	1800
09.825	Pentyl benzoate	1.1	3900	Class I	1800
09.835	Benzyl decanoate	0.35	3900	Class I	1800
09.852	2-Methylbutyl 2-hydroxybenzoate	0.011	3900	Class I	1800
09.895	4-Methoxybenzyl-2-methylpropionate	0.37	3900	Class I	1800
02.205	Piperonyl alcohol	0.011	3900	Class II	540
05.066	4-Ethoxy-3-methoxybenzaldehyde	1.2	1600	Class II	540
05.221	6,6'-Dihydroxy-5,5'-dimethoxy-biphenyl-3,3'-dicarbaldehyde	0.61	7000	Class II	540
06.104	Vanillin propylene glycol acetal	100	3900	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.

On the basis of the reported annual production volumes in Europe (EFFA, 2003u; EFFA, 2004d; EFFA, 2007d; Flavour Industry, 2008c) the combined estimated daily *per capita* intake as flavourings of the 37 candidate flavouring substances assigned to structural class I is 1300 microgram. This value does not exceed the threshold of concern for a substance belonging to structural class I of 1800 microgram/person/day.

The 41 candidate substances are structurally related to 77 supporting substances evaluated by JEFCA at its 46th and 57th meeting (JECFA, 1996b; JECFA, 2002a). Based on reported production volumes, European *per capita* intakes (MSDI) could be estimated for 76 of the 77 supporting substances. Production volumes in Europe were not reported for 1 of the supporting substances [FL-no: 09.754].

The total combined intakes of the candidate and supporting substances are approximately 75000 and 7100 microgram/*capita*/day for structural class I and II, respectively, which exceed the thresholds of concern of 1800 and 540 microgram/*capita*/day for structural classes I and II, respectively. However, the supporting substances were evaluated by the JECFA at the 46th and 57th meeting, where it was noted that although the combined intakes exceed the thresholds for the structural classes, the

substances are expected to be efficiently detoxicated and the available detoxication pathways would not be saturated.

The Panel agreed with this view and concluded that the contributions to the total combined intakes of the candidate substances of about 1300 and 100 microgram/*capita*/day for structural class I and II, respectively, would not alter the JECFA conclusion based on combined intakes of approximately 75000 and 7100 microgram/*capita*/day. The Panel noted that a considerable proportion of this combined intake is accounted for by the supporting substance vanillin [FL-no: 05.018] and for this compound the JECFA has allocated an Acceptable Daily Intake (ADI) of 0 - 10 mg/kg body weight (bw) (JECFA, 1967a; JECFA, 2002b).

8. Toxicity

8.1. Acute Toxicity

Data are available for 13 candidate substances and for 63 structurally related supporting substances evaluated by the JECFA (JECFA, 2002a). The LD_{50} values range from 500 to more than 5000 mg/kg body weight (bw) in four different animal species.

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

8.2. Subacute, Subchronic, Chronic and Carcinogenicity Studies

Benzyl Derivatives (Subgroup 1)

There are no data available on short-term and long-term toxicity of candidate substances from subgroup 1 (benzyl derivatives). Data on benzyl derivatives are available for 10 supporting substances, which have been tested for subacute oral toxicity [FL-no: 05.110], for subchronic oral toxicity [FL-no: 09.051, 09.725, 09.812, 09.803 and 05.027] and for chronic toxicity and carcinogenicity [FL-no: 02.010, 09.014, 05.013 and 08.021].

Results from carcinogenicity studies on benzyl alcohol, benzyl acetate and benzaldehyde by dietary administration were negative and they were not genotoxic. These substances have been evaluated by the JECFA (JECFA, 1996b). The JECFA concluded that "the data reviewed for compounds in this group were sufficient to demonstrate the lack of teratogenic, reproductive or carcinogenic potential". A group ADI of 0 – 5 mg/kg bw was allocated to these compounds. The SCF evaluated data on benzyl alcohol (SCF, 2002b) and concluded that it did not show compound-related effects with respect to carcinogenicity.

Four candidate esters in subgroup 1, 3-methylbut-3-enyl benzoate [FL-no: 09.656], butyl benzoate [FL-no: 09.779], pentyl benzoate [FL-no: 09.825], prenyl benzoate [FL-no: 09.693], yield benzoic acid and simple aliphatic alcohols upon hydrolysis. One of the substances in subgroup 1 is an acetal, (diethoxymethyl)benzene [FL-no: 06.017], which would be expected to yield benzaldehyde and ethanol upon hydrolysis, and in turn benzaldehyde is expected to be oxidized to benzoic acid. The SCF (2002) has established a group ADI of 5 mg/kg bw for benzoic acid and its salts including benzyl alcohol and related benzyl derivatives used as flavourings, based on a developmental toxicity study in rats (SCF, 2002c)[8].

Hydroxy-/Alkoxy- Substituted Benzyl Derivatives (Subgroup 2)

[8] The CEF panel is aware that the benzoic acid is currently under reviewing in the ANS Panel.

Short- and long-term toxicity data on hydroxy- and alkoxy-substituted benzyl derivatives (subgroup 2) are available for five candidate substances and eight supporting substances. The candidate substances have been tested for subacute oral toxicity [FL-no: 05.142, 08.080, 08.087 and 08.133] and for subchronic oral toxicity [FL-no: 08.080, 08.133 and 09.367]. There are data available on chronic toxicity and carcinogenicity for one candidate substance [FL-no: 08.133] in a study designed to evaluate incidences of lesions (hyperplasia, papillomas, squamous cell carcinoma and sarcoma incidence) in the forestomach in rats. No lesions developed in the forestomach of the rats. Other organs (oesophagus, stomach, intestines, liver and kidney) were inspected grossly. Mean body weight was not different from the control, but relative liver and kidney weight was significantly increased, but not further evaluated.

For the supporting substances, data are available on subacute oral toxicity [FL-no: 09.796], on subchronic oral toxicity [FL-no: 05.015 and 09.751] and on chronic toxicity and carcinogenicity [FL-no: 09.754, 05.018, 09.749, 05.019 and 05.016].

Ethyl 4-hydroxybenzoate and other parabens were evaluated by SCF in 1994 (SCF, 1996). From subchronic and chronic toxicity tests conducted in rats, dogs and mice an overall NOAEL of 1000 mg/kg bw/day was derived. This NOAEL value has been confirmed for ethyl- and methyl paraben by EFSA (EFSA, 2004b).

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

8.3. Developmental / Reproductive Toxicity Studies

There are data available for one candidate substance [FL-no: 09.367] (subgroup 2) and for 12 supporting substances of which four belong to subgroup 1 [FL-no: 02.010, 05.013, 08.021 and 09.014] and eight to subgroup 2 [FL-no: 05.016, 05.017, 05.018, 05.019, 08.076, 08.112, 09.749 and 09.754].

For the candidate substance ethyl-4-hydroxybenzoate (ethyl paraben) [FL-no: 09.367] a NOAEL of 2600 mg/kg bw/day has been reported for developmental toxicity in rats, while a NOAEL of 460 mg/kg bw/day was found in the same study for maternal toxicity. From another study a NOAEL of 1043 mg/kg bw/day is available for reproductive toxicity in male rats. Ethyl paraben has been evaluated as a food additive by the AFC panel, and the Panel considered 1000 mg/kg bw/day as the overall NOAEL, based on the absence of effects on sex hormones and on the male reproductive organs in juvenile rats at doses up to 1000 mg/kg bw/day in the above study (EFSA, 2004b).

As there are valid and sufficient studies available on the candidate substance ethyl paraben the data on the supporting substance butyl paraben [FL-no: 09.754] were not considered for the evaluation of ethyl paraben.

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

8.4. Genotoxicity Studies

Data from *in vitro* tests are available for 9 candidate substances [subgroup 1: FL-no: 09.631; subgroup 2: FL-no: 09.367, 05.129, 05.158, 08.080, 05.153, 08.087 and 08.133; subgroup 3: FL-no: 05.221], and for 28 supporting substances (twelve from subgroup 1 and 16 from subgroup 2) and for one related substance (vanillin 3-(l-menthoxy)propane-1,2-diol acetal [FL-no: 02.248] related to subgroup 2). Data from *in vivo* tests are available for two candidate substances from subgroup 2 [FL-no: 09.367 and 08.080] and for 10 supporting substances (three from subgroup 1 and seven from subgroup 2).

All the candidate substances [FL-no: 05.129, 05.153, 05.158, 05.221, 08.080, 08.087, 09.367 and 09.631] tested for bacterial gene mutations gave negative results. For six candidate substances [FL-no: 09.367, 05.129, 05.158, 08.080, 08.087 and 08.133] both positive and/or negative results were reported in various other *in vitro* test systems (Rec assay, chromosomal aberration test, sister

chromatid exchange (SCE) and mammalian cell gene mutation assay (mouse lymphoma tests)) for most of which the validity cannot be evaluated or are known to be of very limited relevance.

The same situation was observed for the supporting substances. All the available bacterial gene mutation assays on supporting substances gave negative results. For 14 of these substances, both positive and negative results were reported in other *in vitro* test systems (Rec assay, chromosomal aberration test, sister chromatid exchange (SCE) and mammalian cell gene mutation assay) for most of which, however, the validity cannot be evaluated.

The available *in vivo* studies on candidate substances reported negative results for ethyl 4-hydroxybenzoate [FL-no: 09.367] in a chromosome aberration assay in rat bone marrow cells and for gallic acid [FL-no: 08.080] in a bioassay in the rat liver. However, due to very limited details on method and results the validity of these studies cannot be evaluated.

The Panel noted that the supporting substance benzyl acetate [FL-no: 09.014] was positive in an *in vivo* Comet assay, which may indicate a genotoxic activity at high dose levels. The study was considered of limited validity. However, all other *in vivo* studies with benzyl acetate were negative and several of these studies, among which an unscheduled DNA synthesis (UDS) test in the liver and a mouse bone marrow micronucleus test were considered to be of good quality (NTP, 1993d). Additionally, in the long term carcinogenicity studies with benzyl acetate, no carcinogenic effects were observed in mice and rats after administration via the diet (NTP, 1993d). In a previous study by NTP (1986) in which this substance was administered by gavage in corn oil, concern was raised in particular about pancreatic tumours in rats, but for these tumours a confounding influence of the vehicle was suspected. In two other genotoxicity studies, specifically aiming at the determination of benzyl acetate-induced DNA damage (UDS test and alkaline elution assay) in rat pancreas, no indications of a genotoxic effect were obtained although these studies were of limited or inassessible validity. Taking all this information into account, the Panel considered the positive result from the *in vivo* Comet assay as insufficient grounds to preclude the evaluation of benzyl acetate via the Procedure.

Furthermore, all the studies carried out with 10 different supporting substances among which were benzyl alcohol, benzyl acetate and benzaldehyde, give no indication of a genotoxic potential *in vivo* in several studies for different genetic endpoints and by different routes of administration.

Conclusion on genotoxicity:

While some of the *in vitro* studies indicated equivocal weak positive or positive results, considering the weight of evidence from candidate and supporting substances and the *in vivo* studies the Panel concluded there was no safety concern with respect to genotoxicity of the substances in the present flavouring group.

Genotoxicity data are summaries in Annex IV, Table IV.4 and Table IV.5.

9. Conclusions

The present revision of FGE.20, FGE.20Rev3 includes the consideration of the SCF opinion on benzoic acid (SCF, 2002c). Furthermore information on the stereoisomeric composition has become available for two substances [FL-no: 06.104 and 09.570] and new information to support the re-allocation of structural class for the candidate substance piperonyl alcohol [FL-no: 02.205] has been submitted. The present FGE.20Rev3 deals in total with 41 benzyl alcohols, benzaldehydes, related acetals, benzoic acids and related esters and a hydroxy- and alkoxy-substituted biphenyl derivative. They belong to chemical groups 23 and 30.

Four of the 41 flavouring substances can exist as optical isomers [FL-no: 06.104, 09.313, 09.317 and 09.852] and three of the 41 substances can exist as geometrical isomers [FL-no: 09.314, 09.560 and 09.570].

Thirty-seven candidate substances are classified into structural class I and four [FL-no: 02.205, 05.066, 05.221 and 06.104] are classified into structural class II according to the decision tree approach presented by Cramer et al., 1978.

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 37 flavouring substances allocated to structural class I have intakes in Europe from 0.001 to 610 microgram/*capita*/day, which are below the threshold of concern value for structural class I (1800 microgram/person/day). The four substances in structural class II [FL-no: 02.205, 05.066, 05.221 and 06.104] have estimated intakes of 0.011, 1.2, 0.61 and 100 microgram/*capita*/day, respectively. These intakes are below the threshold values of 540 microgram/person/day for structural class II.

On the basis of the reported annual production in Europe (MSDI approach), the combined intake of the 37 of the candidate substances belonging to structural class I is approximately 1300 microgram/*capita*/day. This value is lower than the threshold of concern for structural class I substances. Based on reported production volumes, European *per capita* intakes (MSDI) could be estimated for 76 of the 77 supporting substances. The total combined intakes of the candidate and supporting substances are approximately 75000 and 7100 microgram/*capita*/day for structural class I and II, respectively, which exceed the thresholds of concern. However, the substances are expected to be efficiently metabolised and are not expected to saturate the metabolic pathways.

For the substances in this group the available genotoxicity data do not preclude the evaluation of the candidate substances using the Procedure.

It is anticipated that the candidate substances in FGE.20Rev3 would be metabolised to innocuous products.

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

It is considered that on the basis of the default MSDI approach the 41 candidate substances would not give rise to safety concerns at the estimated levels of intake arising from their use as flavouring substances.

When the estimated intakes were based on the mTAMDI approach they ranged from 1400 to 120000 microgram/person/day for the 37 flavouring substances from structural class I. The intakes were all above the threshold of concern for structural class I of 1800 microgram/person/day, except for five flavouring substances [FL-no: 05.129, 05.142, 05.153, 05.158 and 08.080]. The estimated intakes, based on the mTAMDI, of the four flavouring substances [FL-no: 02.205, 05.066, 05.221 and 06.104] assigned to structural class II were 3900, 1600, 7000 and 3900 microgram/person/day, respectively, which are above the threshold of concern for the structural class (540 microgram/person/day). The five substances which have mTAMDI intake estimates below the threshold of concern for structural class I are also expected to be metabolised to innocuous products. Thus, on the basis of the mTAMDI, the estimated intakes for 36 of the 41 flavouring substances considered in this Opinion, exceed the relevant threshold for their structural class to which the flavouring substance has been assigned. Therefore, for these 36 substances more reliable exposure data are required. On the basis of such additional data, these flavouring substances should be re-evaluated using the Procedure. Subsequently, additional toxicological data might become necessary.

In order to determine whether the conclusion for the 41 candidate substances can be applied to the materials of commerce, it is necessary to consider the available specifications. Adequate specifications including complete purity criteria and identity for the materials of commerce have been provided for the 41 flavouring substances. For these 41 flavouring substances the Panel concluded that they would present no safety concern at their estimated levels of intake based of the MSDI approach.

TABLE 1: SPECIFICATION SUMMARY OF THE SUBSTANCES IN THE FLAVOURING GROUP EVALUATION 20, REVISION 3

Table 1: Specification Summary of the Substances in the Flavouring Group Evaluation 20, Revision 3

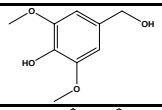
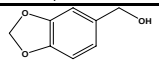
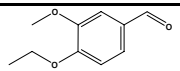
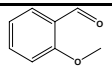
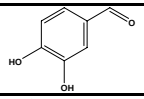
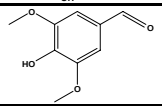
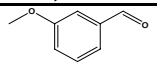
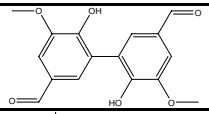
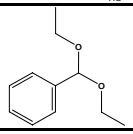
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	Specification comments
02.164	4-Hydroxy-3,5-dimethoxybenzyl alcohol		 530-56-3	Solid $C_9H_{12}O_4$ 184.19	Practically insoluble or insoluble Freely soluble	387 133 MS 95 %	n.a. n.a.	
02.205	Piperonyl alcohol		 10306 495-76-1	Solid $C_8H_8O_3$ 152.15	Very slightly soluble Freely soluble	161 (26 hPa) 55 MS 95 %	n.a. n.a.	
05.066	4-Ethoxy-3-methoxybenzaldehyde		 703 120-25-2	Solid $C_{10}H_{12}O_3$ 180.20	Practically insoluble or insoluble Freely soluble	168 (17 hPa) 63 MS 95 %	n.a. n.a.	
05.129	2-Methoxybenzaldehyde		4077 10350 135-02-4	Solid $C_8H_8O_2$ 136.15	Practically insoluble or insoluble Freely soluble	238 38 MS 97 %	1.556-1.562 1.128-1.136	
05.142	3,4-Dihydroxybenzaldehyde		 10328 139-85-5	Solid $C_7H_6O_3$ 138.12	Slightly soluble Freely soluble	323 154 MS 98 %	n.a. n.a.	
05.153 1878	4-Hydroxy-3,5-dimethoxybenzaldehyde		 10340 134-96-3	Solid $C_9H_{10}O_4$ 182.18	Practically insoluble or insoluble Freely soluble	192 (19 hPa) 113 MS 95 %	n.a. n.a.	
05.158	3-Methoxybenzaldehyde		 10351 591-31-1	Liquid $C_8H_8O_2$ 136.15	Practically insoluble or insoluble Freely soluble	230 MS 95 %	1.549-1.555 1.116-1.122	
05.221 1881	6,6'-Dihydroxy-5,5'-dimethoxy-biphenyl-3,3'-dicarbaldehyde		 2092-49-1	Solid $C_{16}H_{14}O_6$ 302.28	Practically insoluble or insoluble Soluble	 315 MS 91.4 %	n.a. n.a.	
06.017	(Diethoxymethyl)benzene		 517 774-48-1	Liquid $C_{11}H_{16}O_2$ 180.25	Practically insoluble or insoluble Freely soluble	222 MS 95 %	1.475-1.481 0.903-0.909	

Table 1: Specification Summary of the Substances in the Flavouring Group Evaluation 20, Revision 3

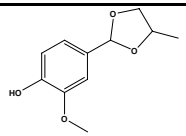
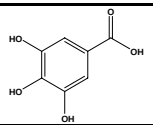
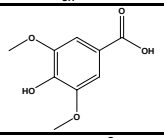
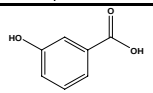
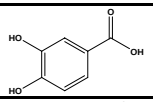
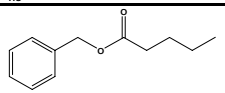
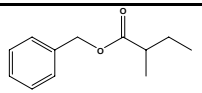
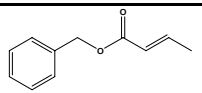
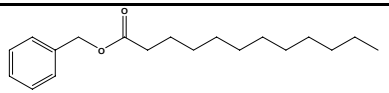
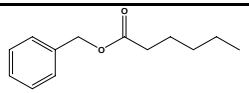
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	Specification comments
06.104 1882	Vanillin propylene glycol acetal		3905 68527-74-2	Liquid $C_{11}H_{14}O_4$ 210.23	Practically insoluble or insoluble Freely soluble	154 (0.1 hPa) NMR 97 %	1.537-1.543 1.190-1.206	Commercial compound: Vanillin propylene glycol acetal up to 80 % and with 18 - 20 % vanillin (EFFA, 2010a).
08.080	Gallic acid		 10170 149-91-7	Solid $C_7H_6O_5$ 170.12	Sparingly soluble Freely soluble	501 242 MS 95 %	n.a. n.a.	
08.087	4-Hydroxy-3,5-dimethoxybenzoic acid		 10111 530-57-4	Solid $C_9H_{10}O_5$ 198.18	Sparingly soluble Freely soluble	440 206 MS 95 %	n.a. n.a.	
08.132	3-Hydroxybenzoic acid		 99-06-9	Solid $C_7H_6O_3$ 138.12	Soluble Soluble	 202 IR NMR MS >99%	n.a. n.a.	
08.133	3,4-Dihydroxybenzoic acid		 99-50-3	Solid $C_7H_6O_4$ 154.12	Soluble Soluble	 221 IR NMR MS >99%	n.a. n.a.	
09.152	Benzyl valerate		 470 10361-39-4	Liquid $C_{12}H_{16}O_2$ 192.26	Practically insoluble or insoluble Freely soluble	236 MS 95 %	1.487-1.493 0.990-0.996	
09.313	Benzyl 2-methylbutyrate		 10523 56423-40-6	Liquid $C_{12}H_{16}O_2$ 192.26	Practically insoluble or insoluble Freely soluble	248 MS 99 %	1.486-1.495 0.982-0.994	Racemate.
09.314	Benzyl crotonate		 65416-24-2	Liquid $C_{11}H_{12}O_2$ 176.21	Practically insoluble or insoluble Freely soluble	138 (16 hPa) MS 95 %	1.515-1.521 1.029-1.035	
09.315	Benzyl dodecanoate		 140-25-0	Liquid $C_{19}H_{30}O_2$ 290.44	Practically insoluble or insoluble Freely soluble	210 (16 hPa) MS 95 %	1.479-1.485 0.937-0.943	
09.316	Benzyl hexanoate		 10521 6938-45-0	Liquid $C_{13}H_{18}O_2$ 206.28	Practically insoluble or insoluble Freely soluble	270 MS 99 %	1.486-1.492 0.978-0.985	

Table 1: Specification Summary of the Substances in the Flavouring Group Evaluation 20, Revision 3

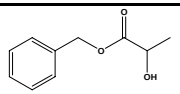
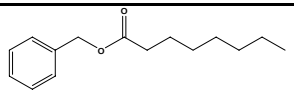
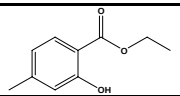
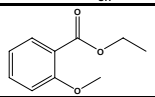
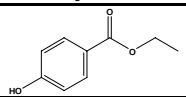
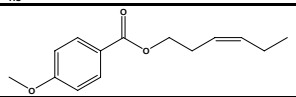
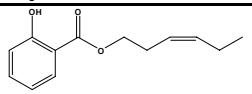
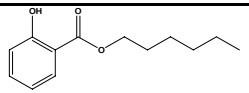
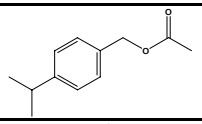
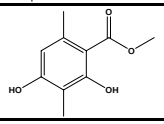
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	Specification comments
09.317	Benzyl lactate		2051-96-9	Liquid $C_{10}H_{12}O_3$ 180.20	Practically insoluble or insoluble Freely soluble	134 (13 hPa) MS 95 %	1.512-1.518 1.120-1.144	Racemate.
09.318	Benzyl octanoate		10276-85-4	Liquid $C_{15}H_{22}O_2$ 234.34	Practically insoluble or insoluble Freely soluble	153 (8 hPa) MS 95 %	1.484-1.490 0.960-0.966	
09.362	Ethyl 2-hydroxy-4-methylbenzoate		60770-00-5	Liquid $C_{10}H_{12}O_3$ 180.20	Practically insoluble or insoluble Freely soluble	254 MS 95 %	1.514-1.520 1.088-1.094	
09.363	Ethyl 2-methoxybenzoate		7335-26-4	Liquid $C_{10}H_{12}O_3$ 180.20	Practically insoluble or insoluble Freely soluble	235 MS 95 %	1.519-1.525 1.109-1.115	
09.367	Ethyl 4-hydroxybenzoate		120-47-8	Solid $C_9H_{10}O_3$ 166.18	Slightly soluble Freely soluble	298 118 MS 95 %	n.a. n.a.	
09.560	Hex-3(cis)-enyl anisate		121432-33-5	Solid $C_{14}H_{18}O_3$ 234.29	Practically insoluble or insoluble Freely soluble	363 73 NMR 95 %	n.a. n.a.	
09.570	Hex-3-enyl salicylate	 (Z)-form shown	10685 65405-77-8	Solid $C_{13}H_{16}O_3$ 220.26	Practically insoluble or insoluble Freely soluble	394 139 MS 98 %	1.518-1.522 1.057-1.065	Register name to be changed to (Z)-Hex-3-enyl salicylate (EFFA, 2010a).
09.581	Hexyl salicylate		10695 6259-76-3	Liquid $C_{13}H_{18}O_3$ 222.28	Practically insoluble or insoluble Freely soluble	290 MS 99 %	1.501-1.507 1.029-1.040	
09.611	4-Isopropylbenzyl acetate		59230-57-8	Liquid $C_{12}H_{16}O_2$ 192.26	Practically insoluble or insoluble Freely soluble	250 MS 95 %	1.494-1.500 0.998-1.004	
09.623	Methyl 2,4-dihydroxy-3,6-dimethylbenzoate		4707-47-5	Solid $C_{10}H_{12}O_4$ 196.20	Slightly soluble Freely soluble	246 143 MS 95 %	n.a. n.a.	

Table 1: Specification Summary of the Substances in the Flavouring Group Evaluation 20, Revision 3

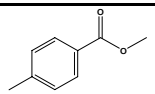
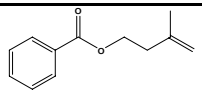
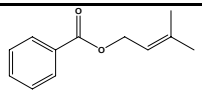
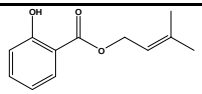
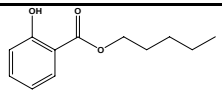
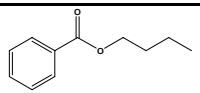
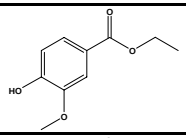
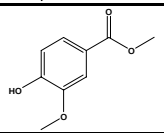
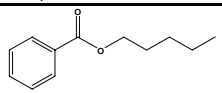
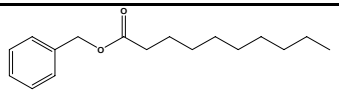
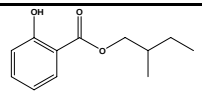
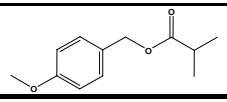
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	Specification comments
09.631	Methyl 4-methylbenzoate		 99-75-2	Solid $C_9H_{10}O_2$ 150.18	Practically insoluble or insoluble Freely soluble	421 33 MS 95 %	n.a. n.a.	
09.656	3-Methylbut-3-enyl benzoate		 5205-12-9	Liquid $C_{12}H_{14}O_2$ 190.24	Practically insoluble or insoluble Freely soluble	60 (0.1 hPa) MS 95 %	1.499-1.505 0.986-0.992	
09.693	Prenyl benzoate		4203 5205-11-8	Liquid $C_{12}H_{14}O_2$ 190.24	Practically insoluble or insoluble Freely soluble	60 (0.1 hPa) MS 95 %	1.505-1.511 0.982-0.988	
09.696	Prenyl salicylate		 68555-58-8	Solid $C_{12}H_{14}O_3$ 206.24	Practically insoluble or insoluble Freely soluble	370 113 MS 95 %	n.a. n.a.	
09.762	Pentyl salicylate		 613 2050-08-0	Liquid $C_{12}H_{16}O_3$ 208.26	Practically insoluble or insoluble Freely soluble	268 MS 95 %	1.533-1.539 1.062-1.068	
09.779	Butyl benzoate		 740 136-60-7	Liquid $C_{11}H_{14}O_2$ 178.23	Practically insoluble or insoluble Freely soluble	249 MS 95 %	1.493-1.499 1.003-1.009	
09.798	Ethyl vanillate		 2302 617-05-0	Solid $C_{10}H_{12}O_4$ 196.20	Practically insoluble or insoluble Freely soluble	292 44 MS 95 %	n.a. n.a.	
09.799	Methyl vanillate		 2305 3943-74-6	Solid $C_9H_{10}O_4$ 182.18	Sparingly soluble Freely soluble	286 63 MS 95 %	n.a. n.a.	
09.825	Pentyl benzoate		 2307 2049-96-9	Liquid $C_{12}H_{16}O_2$ 192.26	Practically insoluble or insoluble Freely soluble	260 MS 95 %	1.482-1.493 0.989-0.993	
09.835	Benzyl decanoate		 42175-41-7	Solid $C_{17}H_{26}O_2$ 262.39	Practically insoluble or insoluble Freely soluble	400 76 MS 95 %	n.a. n.a.	

Table 1: Specification Summary of the Substances in the Flavouring Group Evaluation 20, Revision 3

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
09.852	2-Methylbutyl 2-hydroxybenzoate		 51115-63-0	Solid $C_{12}H_{16}O_3$ 208.26	Practically insoluble or insoluble Freely soluble	366 117 MS 95 %	n.a. n.a.	Racemate.
09.895	4-Methoxybenzyl-2-methylpropionate			Solid $C_{12}H_{16}O_3$ 208.26	Practically insoluble or insoluble Freely soluble	287 40 MS 95 %	1.499-1.505 1.057-1.063	

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)

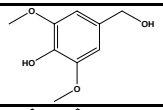
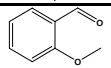
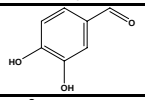
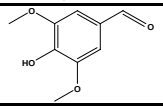
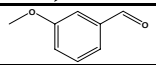
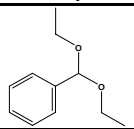
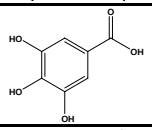
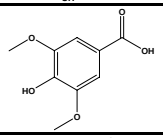
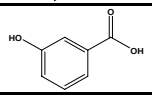
FL-no	EU Register name	Structural formula	MSDI 1) (µg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
02.164	4-Hydroxy-3,5-dimethoxybenzyl alcohol		0.037	Class I A3: Intake below threshold	4)	6)	
05.129	2-Methoxybenzaldehyde		0.16	Class I A3: Intake below threshold	4)	6)	
05.142	3,4-Dihydroxybenzaldehyde		8.5	Class I A3: Intake below threshold	4)	6)	
05.153 1878	4-Hydroxy-3,5-dimethoxybenzaldehyde		0.74	Class I A3: Intake below threshold	4)	6)	
05.158	3-Methoxybenzaldehyde		0.011	Class I A3: Intake below threshold	4)	6)	
06.017	(Diethoxymethyl)benzene		1.7	Class I A3: Intake below threshold	4)	6)	
08.080	Gallic acid		0.011	Class I A3: Intake below threshold	4)	6)	
08.087	4-Hydroxy-3,5-dimethoxybenzoic acid		1.2	Class I A3: Intake below threshold	4)	6)	
08.132	3-Hydroxybenzoic acid		610	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)

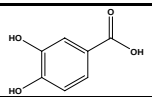
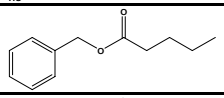
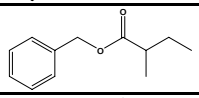
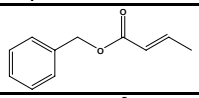
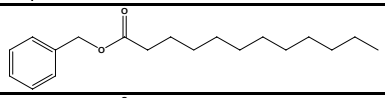
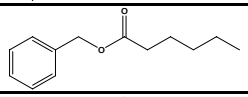
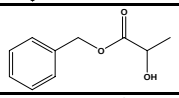
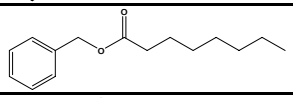
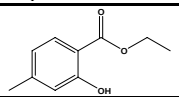
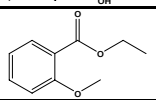
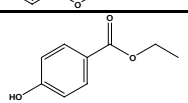
FL-no	EU Register name	Structural formula	MSDI 1) (µg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
08.133	3,4-Dihydroxybenzoic acid		610	Class I A3: Intake below threshold	4)	6)	
09.152	Benzyl valerate		1.7	Class I A3: Intake below threshold	4)	6)	
09.313	Benzyl 2-methylbutyrate		7.3	Class I A3: Intake below threshold	4)	6)	
09.314	Benzyl crotonate		0.37	Class I A3: Intake below threshold	4)	6)	
09.315	Benzyl dodecanoate		0.13	Class I A3: Intake below threshold	4)	6)	
09.316	Benzyl hexanoate		0.75	Class I A3: Intake below threshold	4)	6)	
09.317	Benzyl lactate		0.91	Class I A3: Intake below threshold	4)	6)	
09.318	Benzyl octanoate		0.12	Class I A3: Intake below threshold	4)	6)	
09.362	Ethyl 2-hydroxy-4-methylbenzoate		0.0012	Class I A3: Intake below threshold	4)	6)	
09.363	Ethyl 2-methoxybenzoate		5.5	Class I A3: Intake below threshold	4)	6)	
09.367	Ethyl 4-hydroxybenzoate		10	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)

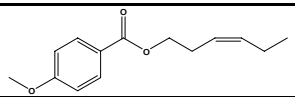
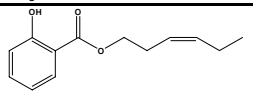
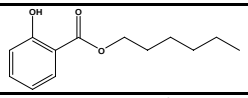
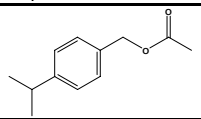
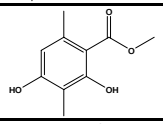
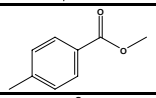
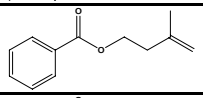
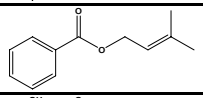
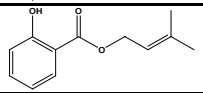
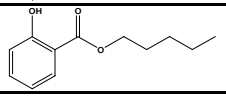
FL-no	EU Register name	Structural formula	MSDI 1) (µg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
09.560	Hex-3(cis)-enyl anisate		0.12	Class I A3: Intake below threshold	4)	6)	
09.570	Hex-3-enyl salicylate	 (Z)-form shown	0.13	Class I A3: Intake below threshold	4)	6)	
09.581	Hexyl salicylate		0.018	Class I A3: Intake below threshold	4)	6)	
09.611	4-Isopropylbenzyl acetate		0.012	Class I A3: Intake below threshold	4)	6)	
09.623	Methyl 2,4-dihydroxy-3,6-dimethylbenzoate		0.012	Class I A3: Intake below threshold	4)	6)	
09.631	Methyl 4-methylbenzoate		0.0012	Class I A3: Intake below threshold	4)	6)	
09.656	3-Methylbut-3-enyl benzoate		0.12	Class I A3: Intake below threshold	4)	6)	
09.693	Prenyl benzoate		0.012	Class I A3: Intake below threshold	4)	6)	
09.696	Prenyl salicylate		0.011	Class I A3: Intake below threshold	4)	6)	
09.762	Pentyl salicylate		0.24	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)

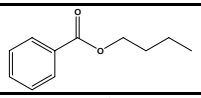
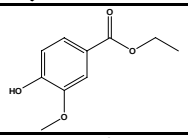
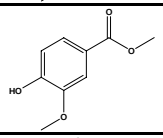
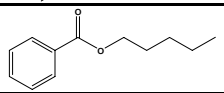
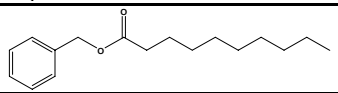
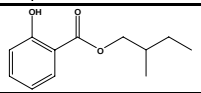
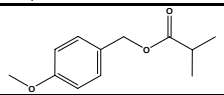
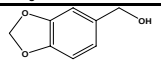
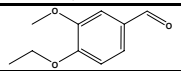
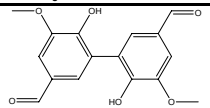
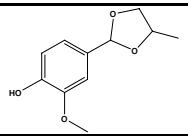
FL-no	EU Register name	Structural formula	MSDI 1) (µg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
09.779	Butyl benzoate		3.7	Class I A3: Intake below threshold	4)	6)	
09.798	Ethyl vanillate		0.024	Class I A3: Intake below threshold	4)	6)	
09.799	Methyl vanillate		0.011	Class I A3: Intake below threshold	4)	6)	
09.825	Pentyl benzoate		1.1	Class I A3: Intake below threshold	4)	6)	
09.835	Benzyl decanoate		0.35	Class I A3: Intake below threshold	4)	6)	
09.852	2-Methylbutyl 2-hydroxybenzoate		0.011	Class I A3: Intake below threshold	4)	6)	
09.895	4-Methoxybenzyl-2-methylpropionate		0.37	Class I A3: Intake below threshold	4)	6)	
02.205	Piperonyl alcohol		0.011	Class II A3: Intake below threshold	4)	6)	
05.066	4-Ethoxy-3-methoxybenzaldehyde		1.2	Class II A3: Intake below threshold	4)	6)	
05.221 1881	6,6'-Dihydroxy-5,5'-dimethoxy-biphenyl-3,3'-dicarbaldehyde		0.61	Class II 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
06.104 1882	Vanillin propylene glycol acetal		100	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 µg/person/day, Class II = 540 µg/person/day, Class III = 90 µg/person/day.

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

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

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

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

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

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

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

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

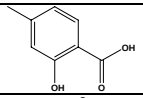
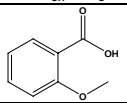
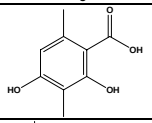
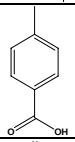
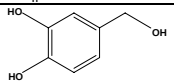
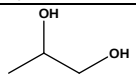
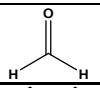
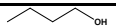
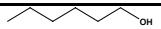
FL-no	EU Register name JECFA no	Structural formula	SCF status 1) JECFA status 2) CoE status 3) EFSA status	Structural class 4) Procedure path (JECFA) 5)	Comments
	4-Methylsalicylic acid		Not evaluated as flavouring substance		Not in EU-Register
	o-Methoxybenzoic Acid		Evaluated as flavouring substance by JECFA (881)		Not in EU-Register
	2,4-Dihydroxy-3,6-dimethylbenzoic acid		Not evaluated as flavouring substance		Not in EU-Register
	p-Toluic acid		Not evaluated as flavouring substance		Not in EU-Register
	Methanol		Not evaluated as flavouring substance		Not in EU-Register
	3,4-Dihydoxybenzyl alcohol		Not evaluated as flavouring substance		Not in EU-Register
	Propylene glycol 925		No evaluation Pending definition of "flavouring agent"		Not in EU-Register
	Formaldehylde		Not evaluated as flavouring substance		Not in EU-Register
02.004	Butan-1-ol 85		Category 1 a) No safety concern b) Category A c)	Class I A3: Intake above threshold, A4: Endogenous	
02.005	Hexan-1-ol 91		Category 1 a) No safety concern b) Category A c)	Class I A3: Intake above threshold, A4: Endogenous	

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

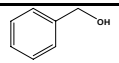
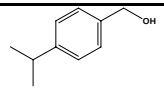
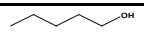
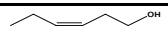
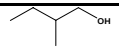
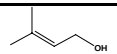
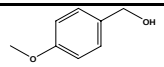
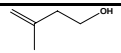
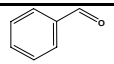
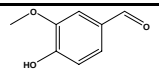
FL-no	EU Register name JECFA no	Structural formula	SCF status 1) JECFA status 2) CoE status 3) EFSA status	Structural class 4) Procedure path (JECFA) 5)	Comments
02.010	Benzyl alcohol 25		No safety concern d) Category A c)	Class I A3: Intake above threshold, A4: Endogenous	
02.039	4-Isopropylbenzyl alcohol 864		No safety concern d) Category B c)	Class I A3: Intake below threshold	
02.040	Pentan-1-ol 88		Category 1 a) No safety concern b) Category A c)	Class I A3: Intake below threshold	
02.056	Hex-3(cis)-en-1-ol 315		Category 1 a) No safety concern e) Category A c)	Class I A3: Intake above threshold, A4: Not endogenous, A5: Adequate NOAEL exists	
02.076	2-Methylbutan-1-ol 1199		Category 1 a) No safety concern f) Category B c)	Class I A3: Intake below threshold	
02.078	Ethanol 41		Category 1 a) No safety concern g)	No evaluation	At the forty-sixth JECFA meeting (JECFA, 1997a), the Committee concluded that ethanol posed no safety concern at its current level of intake when ethyl esters are used as flavouring agents.
02.109	3-Methylbut-2-en-1-ol 1200		No safety concern f)	Class I A3: Intake below threshold	This substance has been evaluated in FGE.202. it was concluded that there would be no safety concern with respect to genotoxicity or carcinogenicity.
02.128	p-Anisyl alcohol 871		No safety concern d) Category A c)	Class I A3: Intake below threshold	
02.176	3-Methylbut-3-en-1-ol		FGE.06	Class I A3: Intake below threshold	
05.013	Benzaldehyde 22		No safety concern d) Category A c)	Class I A3: Intake above threshold, A4: Endogenous	
05.018	Vanillin 889		No safety concern d) Category A c)	Class I A3: Intake above threshold, A4: Not endogenous, A5: Adequate NOAEL exists	

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

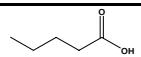
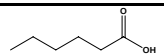
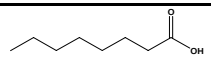
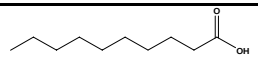
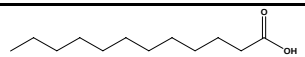
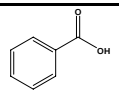
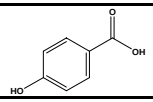
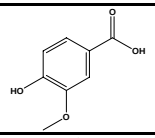
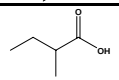
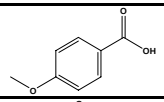
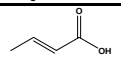
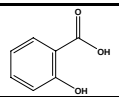
FL-no	EU Register name JECFA no	Structural formula	SCF status 1) JECFA status 2) CoE status 3) EFSA status	Structural class 4) Procedure path (JECFA) 5)	Comments
08.002	Acetic acid 81		Category 1 a) No safety concern b) Category A c)	Class I A3: Intake above threshold, A4: Endogenous	
08.004	Lactic acid 930		No safety concern d) Category A c)	Class I A3: Intake above threshold, A4: Endogenous	
08.006	2-Methylpropionic acid 253		Category 1 a) No safety concern b) Category A c)	Class I A3: Intake below threshold	
08.007	Valeric acid 90		Category 1 a) No safety concern b) Category A c)	Class I A3: Intake below threshold	
08.009	Hexanoic acid 93		Category 1 a) No safety concern b) Category A c)	Class I A3: Intake above threshold, A4: Endogenous	
08.010	Octanoic acid 99		Category 1 a) No safety concern b) Category A c)	Class I A3: Intake above threshold, A4: Endogenous	
08.011	Decanoic acid 105		Category 1 a) No safety concern b) Category A c)	Class I A3: Intake below threshold	
08.012	Dodecanoic acid 111		Category 1 a) No safety concern b) Category A c)	Class I A3: Intake below threshold	
08.021	Benzoic acid 850		No safety concern h) Deleted c)	Class I A3: Intake below threshold	Substances for which CoE Committee of Experts had no information as to real use in foodstuffs and/or for which insufficient technicological and/or toxicological information was available (CoE, 1992).
08.040	4-Hydroxybenzoic acid 957		No safety concern d) Category A c)	Class I A3: Intake below threshold	

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
08.043	Vanillic acid 959		No safety concern d) Category A c)	Class I A3: Intake below threshold	
08.046	2-Methylbutyric acid 255		Category 1 a) No safety concern b) Category A c)	Class I A3: Intake below threshold	
08.071	p-Anisic acid 883		No safety concern d)	Class I A3: Intake below threshold	
08.072	But-2-enoic acid (cis and trans)	 (E)-isomer shown	FGE.05	Class I A3: Intake below threshold	
08.112	Salicylic acid 958		No safety concern d)	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) (SCF, 1995).

b) (JECFA, 1999b).

c) (CoE, 1992).

d) (JECFA, 2002b).

e) (JECFA, 2000a).

f) (JECFA, 2004a).

g) (JECFA, 1997a).

h) (JECFA, 2002c).

ND: Not detected.

TABLE 3: SUPPORTING SUBSTANCES SUMMARY

Table 3: Supporting Substances Summary

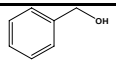
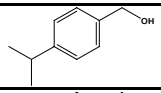
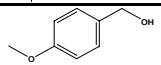
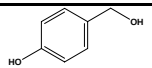
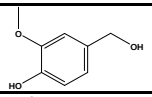
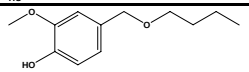
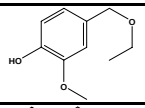
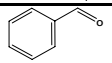
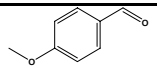
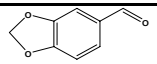
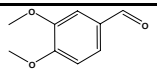
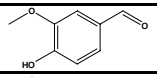
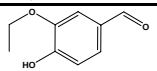
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (µg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
02.010	Benzyl alcohol		2137 58 100-51-6	25 JECFA specification (JECFA, 2001c)	13000	No safety concern a) Category A b)	GrADI: 0-5 (JECFA, 1997a).
02.039	4-Isopropylbenzyl alcohol		2933 88 536-60-7	864 JECFA specification (JECFA, 2001c)	0.24	No safety concern a) Category B b)	
02.128	p-Anisyl alcohol		2099 66 105-13-5	871 JECFA specification (JECFA, 2001c)	130	No safety concern a) Category A b)	
02.165	4-Hydroxybenzyl alcohol		3987 623-05-2	955 JECFA specification (JECFA, 2002d)	5.2	No safety concern a)	
02.213	Vanillyl alcohol		3737 690 498-00-0	886 JECFA specification (JECFA, 2001c)	5.4	No safety concern a) Category A b)	
04.093	Butyl vanillyl ether		3796 82654-98-6	888 JECFA specification (JECFA, 2001c)	1.4	No safety concern a)	
04.094	Ethyl 4-hydroxy-3-methoxybenzyl ether		3815 13184-86-6	887 JECFA specification (JECFA, 2001c)	20	No safety concern a)	
05.013	Benzaldehyde		2127 101 100-52-7	22 JECFA specification (JECFA, 2001c)	7900	No safety concern a) Category A b)	ADI: 0-5 (JECFA, 1997a).
05.015	4-Methoxybenzaldehyde		2670 103 123-11-5	878 JECFA specification (JECFA, 2001c)	370	No safety concern a) Category A b)	
05.016	Piperonal		2911 104 120-57-0	896 JECFA specification (JECFA, 2001c)	1500	No safety concern a) Category A b)	ADI: 0-2.5 (JECFA, 1968).
05.017	Veratraldehyde		3109 106 120-14-9	877 JECFA specification (JECFA, 2001c)	120	No safety concern a) Category A b)	
05.018	Vanillin		3107 107 121-33-5	889 JECFA specification (JECFA, 2001c)	47000	No safety concern a) Category A b)	ADI: 0-10 (JECFA, 1968).
05.019	Ethyl vanillin		2464 108 121-32-4	893 JECFA specification (JECFA, 2001c)	5400	No safety concern a) Category A b)	ADI: 0-3 (JECFA, 1995).

Table 3: Supporting Substances Summary

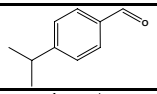
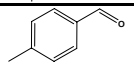
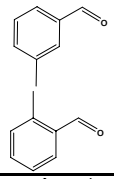
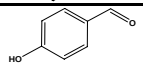
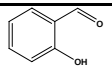
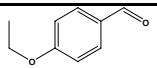
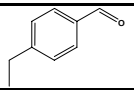
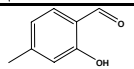
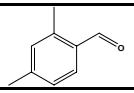
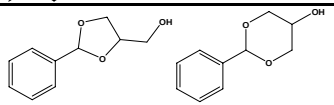
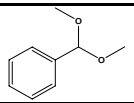
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (μg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
05.022	4-Isopropylbenzaldehyde		2341 111 122-03-2	868 JECFA specification (JECFA, 2001c)	110	No safety concern a) Category B b)	
05.027	Tolualdehyde	 	3068 115 1334-78-7	866 JECFA specification (JECFA, 2002d)	230	No safety concern a) Category A b)	CASrn does not specify position of methyl substituent, "Incompletely Defined Substance". Composition of mixture to be specified.
05.047	4-Hydroxybenzaldehyde		3984 558 123-08-0	956 JECFA specification (JECFA, 2002d)	55	No safety concern a) Category B b)	
05.055	Salicylaldehyde		3004 605 90-02-8	897 JECFA specification (JECFA, 2001c)	84	No safety concern a) Category B b)	
05.056	4-Ethoxybenzaldehyde		2413 626 10031-82-0	879 JECFA specification (JECFA, 2001c)	0.073	No safety concern a) Category B b)	
05.068	4-Ethylbenzaldehyde		3756 705 4748-78-1	865 JECFA specification (JECFA, 2001c)	0.37	No safety concern a) Category A b)	
05.091	2-Hydroxy-4-methylbenzaldehyde		3697 2130 698-27-1	898 JECFA specification (JECFA, 2001c)	0.61	No safety concern a) Category B b)	
05.110	2,4-Dimethylbenzaldehyde		3427 15764-16-6	869 JECFA specification (JECFA, 2001c)	0.37	No safety concern a)	
06.002	5-Hydroxy-2-phenyl-1,3-dioxane		2129 36 1319-88-6	838 JECFA specification (JECFA, 2001c)	13	No safety concern a) Category A b)	CASrn refers to benzaldehyde glyceryl acetate. Stereoisomeric composition and composition of mixture to be specified.
06.003	alpha,alpha-Dimethoxytoluene		2128 37 1125-88-8	837 JECFA specification (JECFA, 2001c)	0.12	No safety concern a) Category A b)	

Table 3: Supporting Substances Summary

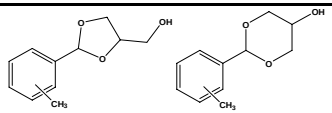
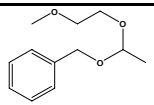
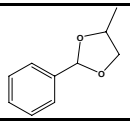
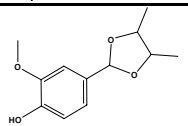
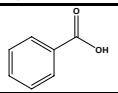
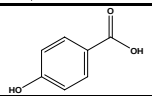
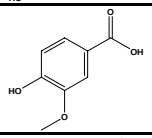
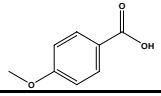
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (µg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
06.012	Tolualdehyde glyceryl acetal		3067 46 1333-09-1	867 JECFA specification (JECFA, 2001c)	0.012	No safety concern a) Category A b)	CASrn refers to named substance. Stereoisomeric composition and composition of mixture to be specified.
06.019	1-Benzyloxy-1-(2-methoxyethoxy)ethane		2148 523 7492-39-9	840 JECFA specification (JECFA, 2001c)	1.2	No safety concern a) Category B b)	CASrn refers to the racemate, according to JECFA: Min. assay value is "98 (sum of named compound and starting materials)", composition of mixture to be specified.
06.032	4-Methyl-2-phenyl-1,3-dioxolane		2130 2226 2568-25-4	839 JECFA specification (JECFA, 2001c)	0.037	No safety concern a) Category A b)	Stereoisomeric composition to be specified.
06.132	Vanillin butan-2,3-diol acetal (mixture of stereo isomers)		4023 63253-24-7	960 JECFA specification (JECFA, 2002d)	3.4	No safety concern a)	CASrn does not specify stereoisomers, stereoisomeric composition to be specified.
08.021	Benzoic acid		2131 21 65-85-0	850 JECFA specification (JECFA, 2001c)	34	No safety concern c) Deleted b)	GrADI: 0-5 (JECFA, 1997a).
08.040	4-Hydroxybenzoic acid		3986 693 99-96-7	957 JECFA specification (JECFA, 2002d)	16	No safety concern a) Category A b)	
08.043	Vanillic acid		3988 697 121-34-6	959 JECFA specification (JECFA, 2002d)	24	No safety concern a) Category A b)	
08.071	p-Anisic acid		3945 10077 100-09-4	883 JECFA specification (JECFA, 2001c)	1.7	No safety concern a)	

Table 3: Supporting Substances Summary

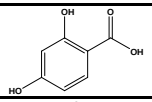
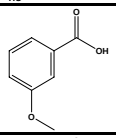
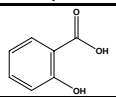
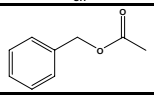
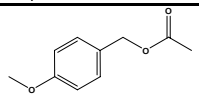
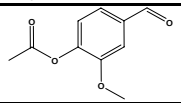
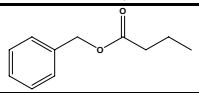
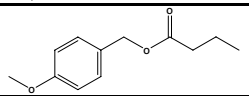
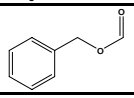
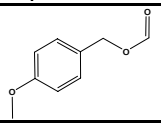
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (µg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
08.076	2,4-Dihydroxybenzoic acid		3798 89-86-1	908 JECFA specification (JECFA, 2001c)	5.5	No safety concern a)	
08.092	3-Methoxybenzoic acid		3944 586-38-9	882 JECFA specification (JECFA, 2001c)	0.012	No safety concern a)	
08.112	Salicylic acid		3985 10165 69-72-7	958 JECFA specification (JECFA, 2002d)	0.024	No safety concern a)	
09.014	Benzyl acetate		2135 204 140-11-4	23 JECFA specification (JECFA, 2001c)	1200	No safety concern a) Category B b)	GrADI: 0-5 (JECFA, 1997a).
09.019	p-Anisyl acetate		2098 209 104-21-2	873 JECFA specification (JECFA, 2001c)	50	No safety concern a) Category B b)	
09.035	Vanillyl acetate		3108 225 881-68-5	890 JECFA specification (JECFA, 2001c)	1.8	No safety concern a) Category B b)	
09.051	Benzyl butyrate		2140 277 103-37-7	843 JECFA specification (JECFA, 2001c)	100	No safety concern a) Category A b)	
09.058	p-Anisyl butyrate		2100 286 6963-56-0	875 JECFA specification (JECFA, 2001c)	29	No safety concern a) Category B b)	
09.077	Benzyl formate		2145 344 104-57-4	841 JECFA specification (JECFA, 2001c)	35	No safety concern a) Category A b)	
09.087	p-Anisyl formate		2101 354 122-91-8	872 JECFA specification (JECFA, 2002d)	39	No safety concern a) Category B b)	

Table 3: Supporting Substances Summary

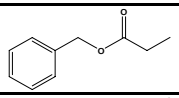
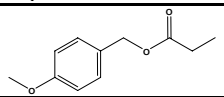
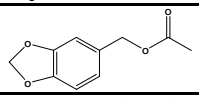
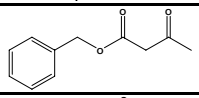
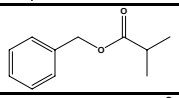
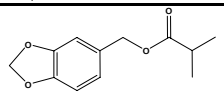
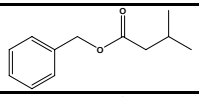
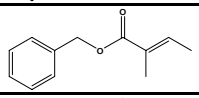
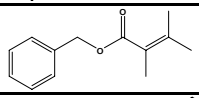
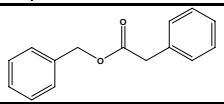
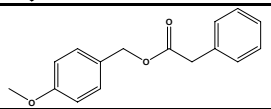
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (µg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
09.132	Benzyl propionate		2150 413 122-63-4	842 JECFA specification (JECFA, 2001c)	41	No safety concern a) Category A b)	
09.145	p-Anisyl propionate		2102 426 7549-33-9	874 JECFA specification (JECFA, 2001c)	0.42	No safety concern a) Category B b)	
09.220	Piperonyl acetate		2912 2068 326-61-4	894 JECFA specification (JECFA, 2001c)	34	No safety concern a) Category B b)	
09.406	Benzyl 3-oxobutyrate		2136 244 5396-89-4	848 JECFA specification (JECFA, 2001c)	0.24	No safety concern a) Category B b)	
09.426	Benzyl isobutyrate		2141 301 103-28-6	844 JECFA specification (JECFA, 2001c)	13	No safety concern a) Category B b)	
09.430	Piperonyl isobutyrate		2913 305 5461-08-5	895 JECFA specification (JECFA, 2001c)	0.085	No safety concern a) Category B b)	
09.458	Benzyl isovalerate		2152 453 103-38-8	845 JECFA specification (JECFA, 2001c)	12	No safety concern a) Category B b)	
09.494	Benzyl 2-methylcrotonate		3330 2184 37526-88-8	846 JECFA specification (JECFA, 2001c)	0.012	No safety concern a) Category B b)	
09.508	Benzyl 2,3-dimethylcrotonate		2143 11868 7492-69-5	847 JECFA specification (JECFA, 2002d)	0.012	No safety concern a)	
09.705	Benzyl phenylacetate		2149 232 102-16-9	849 JECFA specification (JECFA, 2001c)	4.3	No safety concern a) Category B b)	
09.706	Anisyl phenylacetate		3740 233 102-17-0	876 JECFA specification (JECFA, 2001c)	0.0024	No safety concern a) Category B b)	

Table 3: Supporting Substances Summary

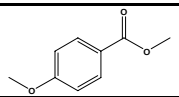
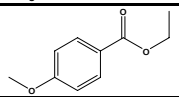
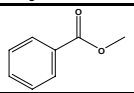
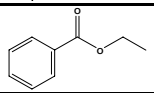
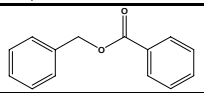
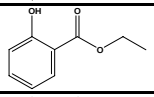
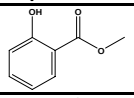
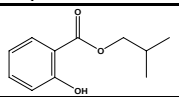
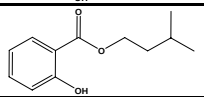
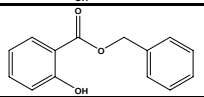
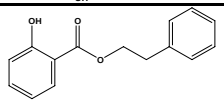
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (µg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
09.713	Methyl 4-methoxybenzoate		2679 248 121-98-2	884 JECFA specification (JECFA, 2001c)	0.97	No safety concern a) Category B b)	
09.714	Ethyl 4-methoxybenzoate		2420 249 94-30-4	885 JECFA specification (JECFA, 2001c)	9.1	No safety concern a) Category B b)	
09.725	Methyl benzoate		2683 260 93-58-3	851 JECFA specification (JECFA, 2001c)	40	No safety concern a) Category B b)	
09.726	Ethyl benzoate		2422 261 93-89-0	852 JECFA specification (JECFA, 2001c)	96	No safety concern a) Category B b)	
09.727	Benzyl benzoate		2138 262 120-51-4	24 JECFA specification (JECFA, 2001c)	1600	No safety concern a) Category A b)	GrADI: 0-5 (JECFA, 1980a).
09.748	Ethyl salicylate		2458 432 118-61-6	900 JECFA specification (JECFA, 2001c)	27	No safety concern a) Category B b)	
09.749	Methyl salicylate		2745 433 119-36-8	899 JECFA specification (JECFA, 2001c)	410	No safety concern a) Category A b)	ADI: 0-0.5 (JECFA, 1968).
09.750	Isobutyl salicylate		2213 434 87-19-4	902 JECFA specification (JECFA, 2001c)	0.97	No safety concern a) Category B b)	
09.751	Isopentyl salicylate		2084 435 87-20-7	903 JECFA specification (JECFA, 2001c)	41	No safety concern a) Category B b)	Composition of positional isomers to be identified.
09.752	Benzyl salicylate		2151 436 118-58-1	904 JECFA specification (JECFA, 2001c)	26	No safety concern a) Category B b)	
09.753	Phenethyl salicylate		2868 437 87-22-9	905 JECFA specification (JECFA, 2001c)	0.12	No safety concern a) Category B b)	

Table 3: Supporting Substances Summary

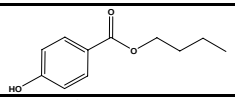
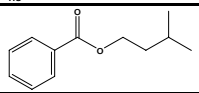
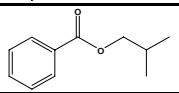
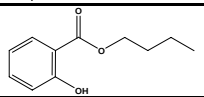
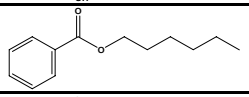
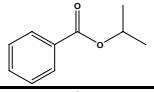
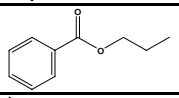
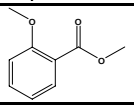
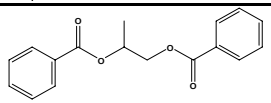
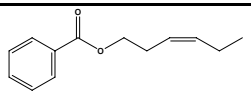
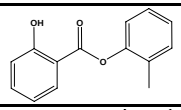
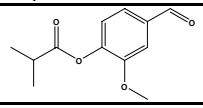
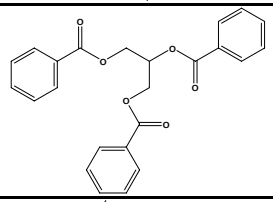
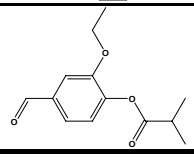
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (µg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
09.754	Butyl 4-hydroxybenzoate		2203 525 94-26-8	870 JECFA specification (JECFA, 2002d)	ND	No safety concern c) Deleted b)	
09.755	Isopentyl benzoate		2058 562 94-46-2	857 JECFA specification (JECFA, 2001c)	96	No safety concern a) Category B b)	Composition of mixture to be specified.
09.757	Isobutyl benzoate		2185 567 120-50-3	856 JECFA specification (JECFA, 2001c)	0.37	No safety concern a) Category B b)	
09.763	Butyl salicylate		3650 614 2052-14-4	901 JECFA specification (JECFA, 2001c)	0.012	No safety concern a) Category B b)	
09.768	Hexyl benzoate		3691 645 6789-88-4	854 JECFA specification (JECFA, 2001c)	320	No safety concern a) Category B b)	
09.770	Isopropyl benzoate		2932 652 939-48-0	855 JECFA specification (JECFA, 2001c)	0.0037	No safety concern a) Category B b)	
09.776	Propyl benzoate		2931 677 2315-68-6	853 JECFA specification (JECFA, 2001c)	0.012	No safety concern a) Category B b)	
09.796	Methyl 2-methoxybenzoate		2717 2192 606-45-1	880 JECFA specification (JECFA, 2001c)	49	No safety concern a) Deleted b)	
09.803	Propylene glycol dibenzoate		3419 10890 19224-26-1	862 JECFA specification (JECFA, 2002d)	13	No safety concern c)	CASrn refers to the racemate Stereoisomeric composition to be specified.
09.806	Hex-3-enyl benzoate	 (Z)-isomer shown	3688 11778 25152-85-6	858 JECFA specification (JECFA, 2001c)	6.7	No safety concern a)	CASrn refers to (Z)-isomer Stereoisomeric composition and composition of mixture to be specified.

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
09.807	o-Tolyl salicylate		3734 617-01-6	907 JECFA specification (JECFA, 2001c)	28	No safety concern a)	
09.811	Vanillin isobutyrate		3754 20665-85-4	891 JECFA specification (JECFA, 2001c)	55	No safety concern a)	
09.812	Glyceryl tribenzoate		3398 10656 614-33-5	861 JECFA specification (JECFA, 2002d)	45	No safety concern c)	
09.933	Ethyl vanillin isobutyrate		3837 188417-26-7	953 JECFA specification (JECFA, 2001c)	0.61	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, 2002b).

b) (CoE, 1992).

c) (JECFA, 2002c).

ND) No intake data reported.

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[9] (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[10] (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.

[9] *"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).*

[10] *"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).*

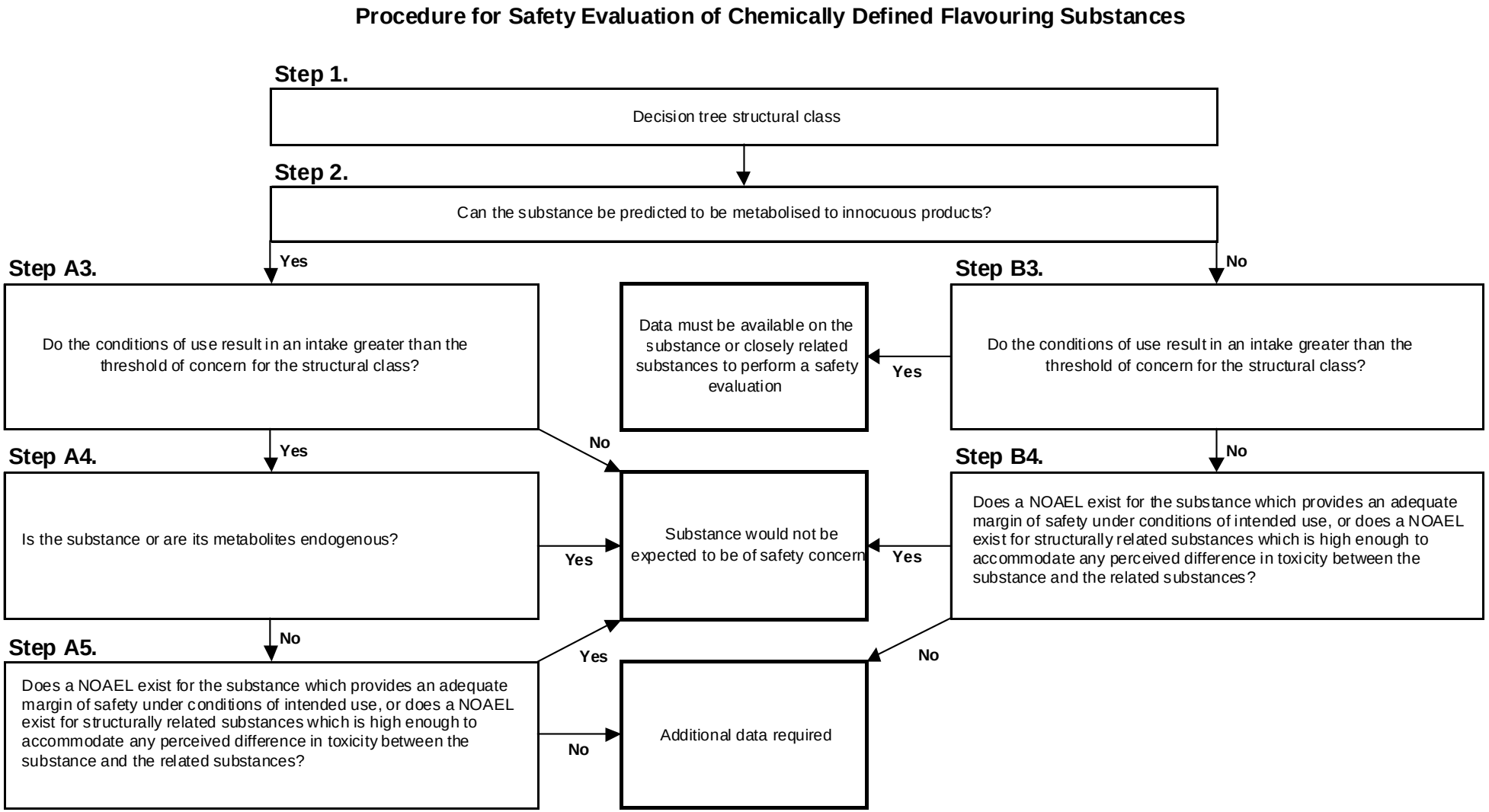


Figure I.1 *Procedure for Safety Evaluation of Chemically Defined Flavouring Substances*

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 41 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.20 (EFFA, 2003u; EFFA, 2004c; EFFA, 2007a; EFFA, 2007d; Flavour Industry, 2008c)

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
02.164	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
02.205	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
05.066	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
05.129	3	2	3	2	-	5	2	-	1	1	-	-	2	3	2	5	5	2
	15	10	15	10	-	25	10	-	5	5	-	-	10	15	10	25	25	10
05.142	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
05.153	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
05.158	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
05.221	10	15	10	-	-	10	-	30	-	-	-	-	10	-	5	15	30	10
	40	20	20	-	-	20	-	50	-	-	-	-	30	-	15	30	50	20
06.017	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25

Table II.1.2 Normal and Maximum use levels (mg/kg) for the candidate substances in FGE.20 (EFFA, 2003u; EFFA, 2004c; EFFA, 2007a; EFFA, 2007d; Flavour Industry, 2008c)

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
06.104	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
08.080	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
08.087	3	2	3	2	-	10	5	10	2	2	-	-	5	10	3	10	15	5
	15	10	15	10	-	50	25	50	10	10	-	-	25	50	15	50	75	25
08.132	-	-	-	-	-	500	-	-	-	-	-	-	-	-	300	300	-	-
	-	-	-	-	-	2000	-	-	-	-	-	-	-	-	500	500	-	-
08.133	-	-	-	-	-	500	-	-	-	-	-	-	-	-	300	300	-	-
	-	-	-	-	-	2000	-	-	-	-	-	-	-	-	500	500	-	-
09.152	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
09.313	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
09.314	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
09.315	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
09.316	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
09.317	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
09.318	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
09.362	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
09.363	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
09.367	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
09.560	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
09.570	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
09.581	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
09.611	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
09.623	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
09.631	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	34	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
09.656	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
09.693	7	5	10	7	-	10	5	10	2	2	-	-	5	20	5	10	-	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	100	25	50	-	25
09.696	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
09.762	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
09.779	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	11	10	-	-	25	50	25	50	100	25
09.798	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
09.799	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
09.825	7	5	10	7	-	10	5	10	2	-	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	-	-	-	25	50	25	50	100	25
09.835	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
09.852	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
09.895	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25

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		

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
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 (see Table II.2.3) are presented for each of the 41 flavouring substances in the present flavouring group, for which Industry has provided use and use levels (EFFA, 2003u; EFFA, 2004c; EFFA, 2007a; EFFA, 2007d; Flavour Industry, 2008c). The mTAMDI values are only given for the highest reported normal use levels.

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)
02.164	4-Hydroxy-3,5-dimethoxybenzyl alcohol	3900	Class I	1800
05.129	2-Methoxybenzaldehyde	1400	Class I	1800
05.142	3,4-Dihydroxybenzaldehyde	1600	Class I	1800
05.153	4-Hydroxy-3,5-dimethoxybenzaldehyde	1600	Class I	1800
05.158	3-Methoxybenzaldehyde	1600	Class I	1800
06.017	(Diethoxymethyl)benzene	3900	Class I	1800
08.080	Gallic acid	1600	Class I	1800
08.087	4-Hydroxy-3,5-dimethoxybenzoic acid	3200	Class I	1800
08.132	3-Hydroxybenzoic acid	120000	Class I	1800
08.133	3,4-Dihydroxybenzoic acid	120000	Class I	1800
09.152	Benzyl valerate	3900	Class I	1800
09.313	Benzyl 2-methylbutyrate	3900	Class I	1800
09.314	Benzyl crotonate	3900	Class I	1800
09.315	Benzyl dodecanoate	3900	Class I	1800
09.316	Benzyl hexanoate	3900	Class I	1800
09.317	Benzyl lactate	3900	Class I	1800
09.318	Benzyl octanoate	3900	Class I	1800
09.362	Ethyl 2-hydroxy-4-methylbenzoate	3900	Class I	1800
09.363	Ethyl 2-methoxybenzoate	3900	Class I	1800
09.367	Ethyl 4-hydroxybenzoate	3900	Class I	1800
09.560	Hex-3(cis)-enyl anisate	3900	Class I	1800
09.570	Hex-3-enyl salicylate	3900	Class I	1800
09.581	Hexyl salicylate	3900	Class I	1800
09.611	4-Isopropylbenzyl acetate	3900	Class I	1800
09.623	Methyl 2,4-dihydroxy-3,6-dimethylbenzoate	3900	Class I	1800
09.631	Methyl 4-methylbenzoate	3900	Class I	1800
09.656	3-Methylbut-3-enyl benzoate	3900	Class I	1800
09.693	Prenyl benzoate	4900	Class I	1800
09.696	Prenyl salicylate	3900	Class I	1800
09.762	Pentyl salicylate	3900	Class I	1800
09.779	Butyl benzoate	3900	Class I	1800
09.798	Ethyl vanillate	3900	Class I	1800
09.799	Methyl vanillate	3900	Class I	1800
09.825	Pentyl benzoate	3900	Class I	1800
09.835	Benzyl decanoate	3900	Class I	1800
09.852	2-Methylbutyl 2-hydroxybenzoate	3900	Class I	1800
09.895	4-Methoxybenzyl-2-methylpropionate	3900	Class I	1800
02.205	Piperonyl alcohol	3900	Class II	540
05.066	4-Ethoxy-3-methoxybenzaldehyde	1600	Class II	540

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)
05.221	6,6'-Dihydroxy-5,5'-dimethoxy-biphenyl-3,3'-dicarbaldehyde	7000	Class II	540
06.104	Vanillin propylene glycol acetal	3900	Class II	540

ANNEX III: METABOLISM

III.1. Introduction

The flavouring group consists of 41 substances out of which 15 are benzyl derivatives (subgroup 1) and 25 are hydroxy- and alkoxy-substituted benzyl derivatives (subgroup 2) and 1 hydroxy- and alkoxy- substituted biphenyl derivative (subgroup 3).

Subgroup 1 (benzyl derivatives) includes 14 alkyl esters of which eight contain benzyl alcohol in the alcohol moiety and straight or branched carboxylic acids as acid moiety [FL-no: 09.313 (benzyl 2-methylbutyrate; 09.314 (benzyl crotonate); 09.315 (benzyl dodecanoate); 09.316 (benzyl hexanoate); 09.317 (benzyl lactate); 09.318 (benzyl octanoate); 09.152 (benzyl valerate); 09.835 (benzyl decanoate)]. One of the esters contains isopropylbenzyl alcohol as alcohol moiety [FL-no: 09.611 (4-isopropylbenzyl acetate)]. Five of the esters contain benzoic acid in the acid moiety [FL-no: 09.631 (methyl 4-methylbenzoate); 09.656 (3-methylbut-3-enyl benzoate); 09.693 (prenyl benzoate); 09.779 (butyl benzoate); 09.825 (pentyl benzoate)].

One substance in subgroup 1 is an acetal [FL-no: 06.017 ((diethoxymethyl)benzene)].

Two of the substances in subgroup 1 contain an alkyl substituent on the aromatic ring [FL-no: 09.611 (4-isopropylbenzyl acetate); 09.631 (methyl 4-methylbenzoate)]. Three compounds contain a double bond in an alkyl chain [FL-no: 09.314 (benzyl crotonate); 09.656 (3-methylbut-3-enyl benzoate; 09.693 (prenyl benzoate)].

Subgroup 2 (hydroxy- and alkoxy-substituted benzyl derivatives) includes two derivatives of benzyl alcohol [FL-no: 02.164 (4-hydroxy-3,5-dimethoxybenzyl alcohol) and 02.205 (piperonyl alcohol; 3,4-methylenedioxybenzyl alcohol]. Piperonyl alcohol may also be considered as a cyclic acetal.

Six substances are derivatives of benzaldehyde [FL-no: 05.066 (4-ethoxy-3-methoxybenzaldehyde); 05.129 (2-methoxybenzaldehyde); 05.142 (3,4-dihydroxybenzaldehyde); 05.153 (4-hydroxy-3,5-dimethoxybenzaldehyde); 05.158 (3-methoxybenzaldehyde) and 06.104 (vanillin propylene glycol acetal].

Four are derivatives of benzoic acid [FL-no: 08.080 (gallic acid; 3,4,5-trihydroxybenzoic acid); 08.087 (4-hydroxy-3,5-dimethoxybenzoic acid); 08.132 (3-hydroxybenzoic acid) and 08.133 (3,4-dihydroxybenzoic acid)].

The remaining 13 substances are esters of which one is an ester with alkoxy substituted benzyl alcohol as the alcohol moiety [FL-no: 09.895 (4-methoxybenzyl-2-methylpropionate)] and 12 are esters with substituted benzoic acid as the acid moiety [FL-no: 09.362 (ethyl 2-hydroxy-4-methylbenzoate); 09.363 (ethyl 2-methoxybenzoate); 09.367 (ethyl 4-hydroxybenzoate); 09.560 (hex-3(cis)-enyl anisate; hex-3(cis)-enyl-4-methoxybenzoate); 09.570 (hex-3-enyl salicylate; hex-3-enyl 2-hydroxybenzoate); 09.581 (hexyl salicylate; hexyl 2-hydroxybenzoate); 09.623 (methyl 2,4-dihydroxy-3,6-dimethylbenzoate); 09.696 (prenyl salicylate; 3-methyl-but-2-enyl 2-hydroxybenzoate); 09.762 (pentyl salicylate; pentyl 2-hydroxybenzoate); 09.798 (ethyl vanillate; ethyl 3-methoxy-4-hydroxybenzoate); 09.799 (methyl vanillate; methyl 3-methoxy-4-hydroxybenzoate); 09.852 (2-methylbutyl 2-hydroxybenzoate; 2-methylbutyl salicylate)].

Three of the esters in subgroup 2 contain a double bond in an alkyl chain [FL-no: 09.560 (hex-3(cis)-enyl anisate); 09.570 (hex-3-enyl salicylate; 09.696 (prenyl salicylate)].

Subgroup 3 (biphenyl) contains one derivative of biphenyl [FL-no: 05.221 (6,6'-dihydroxy-5,5'dimethoxy-biphenyl-3,3'-dicarbaldehyde)].

III.2. Absorption, Distribution and Elimination

Subgroup 1 (benzyl derivatives)

Candidate substances from subgroup 1

There are no studies submitted on the candidate substances from subgroup 1.

Supporting substances from subgroup 1

Several studies have been submitted demonstrating efficient absorption, metabolism and excretion of the supporting substances benzyl alcohol [FL-no: 02.010], benzaldehyde [FL-no: 05.013], benzoic acid [FL-no: 08.021], and benzyl acetate [FL-no: 09.014].

Benzyl alcohol [FL-no: 02.010]

Similar intravenous doses (range 0.036 to 0.222 micromole/kg bw) of benzyl alcohol were given via medications to 14 term and nine pre-term infants, in order to estimate plasma levels of benzoic and hippuric acids. The mean peak concentrations of benzoic acid in the plasma of pre-term babies were almost 10 times higher than in full-term newborns. In the urine of pre-term infants a larger proportion of benzyl alcohol was found as benzoic acid and a smaller proportion as hippuric acid than in full-term infants (% of dose excreted in urine in 24 hours: pre-term: benzoic acid 75 %, hippuric acid 64 %; full-term: benzoic acid 38 %, hippuric acid 82 %). The results suggest that humans metabolise benzyl alcohol to both benzoic acid and hippuric acid but hippuric acid formation is deficient in pre-term newborns (LeBel et al., 1988).

Five minutes after single intraperitoneal doses of 500 - 1100 mg/kg bw of benzyl alcohol administered to CD1 mice, benzyl alcohol was detected in plasma (McCloskey et al., 1986).

Benzaldehyde [FL-no: 05.013]

In the rabbit, approximately 83 % of single oral doses of 350 or 750 mg/kg bw given orally of benzaldehyde was absorbed, since it was found in the urine of both dose groups. The aldehyde was oxidised mainly to benzoic acid and excreted predominantly as hippuric acid (approximately 68 %). Other urinary metabolites detected were benzoylglucuronic acid (10 %), benzoyl glucuronide (3 %), free benzoic acid (1.5 %) and trace amounts of benzyl mercapturic acid (Laham et al., 1988).

Benzoic acid [FL-no: 08.021]

Following administration of 375 mg [carboxyl-14C]-benzoic acid/kg bw to rats (orally) and mice intraperitonelly (i.p.), 88 - 89 % of the radioactivity was recovered in the urine of rats within 24 hours with 91 - 94 % recovery after 72 hours, and only 1 - 6 % was present in the faeces. In mice, 92 - 98 % of the radioactivity was recovered in the urine of rats within 24 hours and only 1 - 10 % was present in the faeces. It was possible to conclude that after both route of administration more than 95 % of benzoic acid is absorbed, metabolised and rapidly excreted. The following metabolites were identified: hippuric acid (70.2 - 84.2 %), benzoyl glucuronide (0.7 - 1.8 %), benzoic acid (0.4 - 12.8 %) and 3-hydroxy-3-phenyl propionic acid (0.1 - 0.2 %) (Nutley, 1990).

Benzyl acetate [FL-no: 09.014]

A study on benzyl acetate metabolism in male Fischer 344 rats and male $B6C3F_1$ mice was performed. Ring-labelled ^{14}C-benzyl acetate was used for single dose studies and unlabelled benzyl acetate was used for repeated dose studies. For intravenous administration of single doses three rats were injected with 5 mg 14C-benzyl acetate in the tail vein and three mice were similarly injected with 10 mg. For the single oral dose study, groups of three rats were given 5, 50 or 500 mg/kg bw and groups of three mice were given 10, 100 or 1000 mg/kg bw in corn oil by gavage. For the repeated dose studies, three rats and three mice were given unlabelled benzyl acetate in corn oil by gavage at 500 or 1000 mg/kg bw, respectively, once a day, 5 days a week for 2 weeks. Metabolites in urine were determined by High Performance Liquid Chromatography (HPLC). After administration of the radioactive compound, rats and mice were housed in metabolism cages and urine and faeces were collected during 24 hours. After intravenous administration CO_2 and volatiles were collected.

Benzyl acetate was rapidly and almost completely absorbed, based on the high recovery of radioactivity (nearly 90 % of the dose) in the urine in 24 hours, following both intravenous or oral dosing in rats and mice. Little radioactivity (0.3 - 1.3 % of the dose) was recovered in the faeces. Elimination as CO_2 or volatiles was minimal following intravenous administration and was not determined after oral dosing. This clearance pattern was not affected by repeated oral dosing, indicating no potential for bioacumulation, as supported also by the absence of radioactivity in tissues analysed at 24 hours after dosing. The major metabolite of benzyl acetate in the urine of rats and mice was hippuric acid, accounting for more than 90 % of the total metabolites excreted in urine of all dose groups. Mercapturic acid was detected as a minor metabolite in the urine of rats and mice (less than 1 %), but was not found in all dose groups and not in all animals of the dose groups where it was detected. Small amounts of unidentified metabolites were also present. The absorption, routes of metabolism and excretion of benzyl acetate were apparently unaffected by the size or number of doses administered in the metabolism study. There was no evidence to indicate a reduction or saturation of the metabolic capacity in tested animals in the tested dose range (Abdo et al., 1985).

Subgroup 2 (hydroxy- and alkoxy-substituted benzyl derivatives)

Candidate substances from subgroup 2

Piperonyl alcohol (1-hydroxymethyl-3,4-methylenedioxybenzene) [FL-no: 02.205]

In a study of several methylenedioxyphenyl (MDP) compounds, male Swiss-Webster mice were administered a dimethyl sulphoxide (DMSO) solution of radiolabelled piperonyl alcohol (1-hydroxymethyl-3,4-methylene-^{14}C-dioxybenzene) by oral gavage at a dose of 0.76 mg/kg bw. Total radiocarbon determinations were made on expired $^{14}CO_2$ at 0.5, 1, 2, 4 and 6 hours after dosing and at each six-hours interval thereafter; on urine and faeces samples taken at 12, 24 and 48 hours after treatment; on selected organs removed from the animals and the remaining carcass at 48 hours after treatment. Only the 12-hours samples were used for separation and characterisation of metabolites. Forty-eight hours after treatment, the distribution of radioactivity was as follows (averages of four experiments): CO_2, 3.0 %; urine, 93.3 %; faeces, 8.5 %; intestine, 0.2 %; liver, 0.1 %; and carcass, 0.3 %. These data indicate that piperonyl alcohol is almost completely absorbed in the gastrointestinal (GI) tract, then metabolised and rapidly and almost completely excreted, mostly via the urine. Less than 10 % was excreted in the faeces. In all cases, the major metabolite was the glycine conjugate of piperonylic acid. Free piperonylic acid was not detected. Minor amounts of two unidentified metabolites were also present (Klungsoeyr and Scheline, 1984).

Gallic acid (3,4,5-trihydroxybenzoic acid [FL-no: 08.080]

Gallic acid [FL-no: 08.080] was given orally to six week old male Wistar rats in order to determine the metabolic fate of the substance. After oral administration of 100 mg/kg bw, gallic acid was absorbed fairly quickly and reached the maximum concentration at 15 minutes in portal blood. The concentration was halved by 30 minutes and gallic acid had almost disappeared after six hours. The metabolite 4-O-methyl gallic acid also reached peak values within 15 minutes, and then decreased slowly. In the inferior vena cava, gallic acid

and its metabolite were detected in approximately equal proportions and both reached peak values at 30 minutes after oral administration and decreased gradually until six hours after administration. The main metabolite in urine was 4-O-methyl gallic acid, but unchanged gallic acid was also found in urine. The ratio of 4-O-methyl gallic acid to total gallic acid metabolites in urine ranged from 0.55 to 0.76, indicating that a significant amount of gallic acid was excreted without being metabolised (Zong et al., 1999).

Ethyl 4-hydroxybenzoate (ethyl paraben) [FL-no: 09.367]

Ethyl paraben [FL-no: 09.367] was given orally in capsules at a dose of 1 g/kg bw to groups of three fasted dogs and blood and urine were analysed at frequent predetermined intervals until 48 hours after dosing. Metabolites were detectable in the blood up to 24 hours post-ingestion. Recovery as urine metabolites was 66 % of the administered dose at 48 hours. Dogs were also administered a 100 mg/kg bw dose of ethyl paraben intravenously and then killed to determine the distribution of the parent material and its metabolites. The ester was detected only in the brain and pancreas, whereas high concentrations of metabolites were detected in the liver and kidneys (Jones et al., 1956).

Absorption, distribution, metabolism and excretion of ethylparaben [FL-no: 09.367] were investigated in Wistar rats administered 100 mg by oral gavage. Animals were held in metabolism cages for the collection of urine (at approximately 15, 30, 60, 75, 90, 120, 150 and 210 minutes) and blood (at approximately 30, 60, 90, 120, 180, 240 and 360 minutes), and samples were analysed to establish the excretion kinetics. Metabolites were detected in the urine starting at 30 minutes after dosing, and their concentration increased steadily during the next three to six hours. Absorption of ethylparaben was followed by metabolism and excretion of mainly free 4-hydroxybenzoic acid and its glucuronic and glycine conjugates. A small portion of the dose was excreted as sulphate conjugate (Derache and Gourdon, 1963).

^{14}C-labelled ethyl 4-hydroxybenzoate was orally given to four male cats in the diet at a single dose of 156 mg/kg bw. Essentially all (mean = 96.0 %) of the radioactiviy was excreted in urine within 72 hours as p-hydroxyhippuric acid and 4-hydroxybenzoic acid (Phillips et al., 1978).

Supporting substances from subgroup 2

3,4-Methylenedioxybenzaldehyde (piperonal) [FL-no: 05.016]

In male rats a 150 mg/kg bw dose of piperonal in propylene glycol was administered by gavage. Urine samples were collected at 24 and 48 hours. Recovery of urine metabolites made up 90 % of the given dose, and metabolite excretion occurred mainly within 24 hours. No unchanged compound was detected in the urine (Klungsoeyr and Scheline, 1984).

Veratraldehyde (3,4-dimethoxybenzaldehyde) [FL-no: 05.017]

A 1 g/kg bw oral dose of veratraldehyde was administered to rabbits by gavage and urine was collected for 24 hours. At least approximately 70 % of the aldehyde was absorbed as it was present in the urine mainly as the corresponding acid and its conjugates (Scheline, 1972).

Vanillin (4-hydroxy-3-methoxybenzaldehyde) [FL-no: 05.018]

Oral dosage of 100 mg/kg bw of vanillin to male albino rats resulted in an urinary excretion of most metabolites within 24 hours, mainly as glucuronide and sulphate conjugates, although vanillic acid was also excreted as free acid and as glycine conjugate. After 48 hours 94 % of the dose was excreted as different metabolites (Strand and Sheline, 1975).

A 100 mg dose of vanillin dissolved in water was given to an adult human and the urine was collected for 24 hours. During this period an increase, from a background level, in the vanillic acid output in the urine level was measured, accounting for approximately 94% of the vanillin dose (Dirscherl and Wirtzfeld, 1964).

4-Hydroxybenzoic acid [FL-no: 08.040]

Groups of four to eight rabbits were administered 100, 250, 500, 1000 or 1500 mg 4-hydroxybenzoic acid/kg bw by gavage. Urine was collected continuously and analysed for metabolites. Based on the total urinary recovery of the test material (84 to 104 %) the compound was almost completely absorbed, metabolised and excreted (Bray et al., 1947).

Concluding remarks on absorption, distribution and excretion

The results of these studies indicate that the benzyl derivatives in subgroup 1 as well as the hydroxy- and alkoxy-substituted benzyl derivatives in subgroup 2 are expected to be rapidly absorbed, metabolised and excreted mainly in the urine.

III.3. Metabolism

III.3.1. Hydrolysis of Esters and Acetals

In general, esters containing an aromatic ring system are expected to be hydrolysed *in vivo* to the component acid and alcohol through the catalytic activity of carboxylesterases or esterases. In mammals, esterases occur in most tissues throughout the body but predominate in the hepatocytes (Heymann, 1980).

Subgroup 1 (benzyl derivatives)

Candidate substances from subgroup 1

There are no studies submitted on the candidate substances from subgroup 1.

Supporting substances from subgroup 1

Benzyl acetate [FL-no: 09.014]

Neat benzyl acetate was spiked into control rat plasma (1 microlitre/0.5 ml), vortexed and incubated at room temperature for 0.5 – 36 minutes. Incubation was terminated by addition of acetonitrile. The plasma was centrifuged to precipitate plasma proteins and the clear plasma was analysed by (High Performance Liquid Chromatography) HPLC to determine benzyl acetate and benzyl alcohol. Benzyl acetate was found to be rapidly hydrolysed to benzyl alcohol. The half-life of benzyl acetate was about 4 minutes and 24 minutes after spiking virtually all benzyl acetate was hydrolysed to benzyl alcohol. Hydrolysis was partially inhibited by the esterase inhibitor sodium fluoride, which suggests that plasma esterases contribute to the rapid hydrolysis. When benzyl acetate was administered to rats and mice in gavage and dosed feed studies, benzyl acetate was not detected in any plasma samples collected (Yuan et al., 1995).

In vivo metabolism studies in mice and rats clearly indicate that radiolabelled benzyl acetate is readily hydrolysed, since more than 90 % of the radioactivity is demonstrated in the urine as benzoic or hippuric acid (Abdo et al., 1985).

Benzyl acetate was hydrolysed in pig liver homogenate. At pH 7.4 and 25° C the velocity was calculated to 27 micromole/min/mg, K_m 0.55 mM. (Greenzaid & Jenks, 1971 referred in (Heymann, 1980)).

Alkyl- and aryl-benzoates

The plasma half-lives ($t_{½}$) for the *in vitro* hydrolysis by plasma enzymes of a series of four alkyl benzoates (including supporting chemicals methyl benzoate [FL-no: 09.725], ethyl benzoate [FL-no: 09.726] and propyl benzoate [FL-no: 09.776]) and two aryl benzoates (including supporting substance benzyl benzoate [FL-no: 09.727]) in 80 % human blood plasma ranged from 24 to 210 minutes for the alkyl benzoates. By increasing chain length an increasing enzymatic degradation was seen, except when going from methyl to ethyl. The butyl ester was the least resistant (t½ 24 minutes), while the ethyl ester was the most resistant to hydrolysis (t½ 210 minutes). The plasma half-lives were 19 and 15 minutes for phenyl benzoate and benzyl benzoate, respectively (Nielsen and Bundgaard, 1987).

An *in vitro* hydrolysis study demonstrated that benzyl phenylacetate was 100 % hydrolysed within two hours of incubation with a pancreatin solution, whereas the supporting substance benzylacetate [FL-no: 09.014] was only 50 % hydrolysed after 2 hours incubation. Benzyl cinnamate and methyl phenylacetate were 80 and 70 % hydrolysed, respectively (Leegwater and Straten, 1974a).

Other related substances:

4-Methyl-2-phenyl-1,3-dioxolane (benzaldehyde propylene glycol acetal)

Benzaldehyde-related acetals readily hydrolyse to their component alcohols and benzaldehyde under acidic conditions. Hydrolysis of acetals in simulated gastric juice (pH 1.2) and simulated intestinal fluid (pH 7.5) was monitored by the formation rate of aldehyde liberated during treatment. Data show that non-cyclic acetals are completely hydrolysed at pH 1.2 but that hardly any hydrolysis occurs at pH 7.5. Benzaldehyde–propylene–glycol acetal (4-methyl-2-phenyl-1,3-dioxolane, MPD), a cyclic acetal, was hydrolysed to an extent of around 50 % after one hour in simulated gastric juice and no further hydrolysis was observed after five hours. Reflux of MPD for five hours in 0.1 N HCl also resulted in hydrolysis to an extent of 50 % of the theoretical maximum. Due to the same poor hydrolysis of MPD (to around 50 %) even after five hours reflux in 0.1 N HCl the author questioned the chemical identity of the substance (Morgareidge, 1962a). The result of this study on hydrolysis of a cyclic benzaldehyde acetal is inconclusive.

Subgroup 2 (hydroxy- and alkoxy-substituted benzylderivatives)

Candidate substances from subgroup 2

Benzyl 2-methylbutyrate (benzyl 2-methylbutanoate) [FL-no: 09.313]

Benzyl 2-methylbutyrate at a concentration of 40 microlitre/l (0.21 mM)was incubated in 0.5 M phosphate buffer at pH 7.5 and 37°C with a preparation of pancreatin for two hours. The extent of hydrolysis was 100 % as determined by gas-liquid chromatography. The supporting substance benzyl acetate [FL-no: 09.014] at a concentration of 70 microlitre/l (0.49 mM) was 50 % hydrolysed after 2 hours (Grundschober, 1977).

Ethyl 4-hydroxybenzoate (ethyl paraben) [FL-no: 09.367]

An *in vitro* assay demonstrated that ethylparaben is efficiently hydrolysed by the liver and kidney esterases: 96 % hydrolysis was measured after three minutes in dog liver tissue suspension and 100% hydrolysis after 30 minutes in dog kidney suspension (Jones et al., 1956).

Ethylparaben [FL-no: 09.367] was 80 % hydrolysed to free 4-hydroxybenzoic acid within 60 minutes in perfused mouse liver, only 2.3 % intact ester was recovered. Ethyl paraben was not detected in the blood of six humans 1-4½ hours after oral intake of 10 to 20 mg/kg bw. When given orally to dogs at doses between 25 and 500 mg/kg bw, high serum concentrations of 4-hydroxybenzoic acid were reported and no ethyl paraben was detected in the blood except for the 500 mg/kg bw dose (Heim et al., 1957).

Studies were conducted with methyl, ethyl [FL-no: 09.367], propyl and butyl 4-hydroxybenzoate [FL-no: 09.754] (supporting substance) in dogs. The results showed significantly higher rates of test material

recovery in the urine of dogs dosed orally 1 g/kg bw orally or 50 mg/kg bw by the intravenously route for the methyl, ethyl and propyl esters (% of dose excreted within 48 hours, oral: 89.0 and 66.0 and 57.6%, respectively; i.v. 85, 70 and 94 %, respectively) as compared to the butyl ester (oral 48.2 %; i.v. 40.1 %). The methyl, ethyl and propyl esters showed 100 % hydrolysis within 3 minutes when incubated with liver homogenate, whereas the butyl ester was completely hydrolysed only after 30-60 minutes. This finding suggests that an increase in the alkyl chain length in the homologous series of alkyl esters make the esters more resistant to hydrolysis (Jones et al., 1956).

Vanillin propylene glycol acetal [FL-no: 06.104]

Under acidic conditions, pH 2.6, vanillin propylene glycol acetal [FL-no: 06.104] began to hydrolyse immediately with approximately 3 % of the acetal disappearing and 92 % hydrolysed within two hours. At pH 1.8, approximately 90 % of vanillin propylene glycol acetal hydrolysed immediately and 93 % hydrolysed within five minutes (Bennett, 1997).

Supporting substances from subgroup 2

Methyl-2-hydroxybenzoate (methyl salicylate) [FL-no: 09.749]

An oral dose of methyl salicylate equivalent to 500 mg/kg bw of salicylic acid was dissolved in 2 % methyl cellulose and administered to male rats. The plasma levels measured within 20 minutes of dosing showed complete hydrolysis of methyl salicylate. A similar experiment was conducted with male dogs. Capsules containing 320 mg methyl salicylate/kg bw were given orally to three fasted dogs in five repeated experiments. Blood drawn 1 and 4 hours after dosing showed 95 % hydrolysis of methyl salicylate to salicylic acid at both time intervals. Six humans were given a 0.42 ml dose of methyl salicylate administered in ginger ale. Blood was drawn by venipuncture 15 and 90 minutes later. In contrast to the other two species an appreciable portion of unhydrolysed methyl salicylate was found, 39 % after 15 minutes and 21 % after 90 minutes (Davison et al., 1961).

Other related substances

At low pH similar to that found in the stomach, a structurally related substance, vanillin 3-(l-menthoxy)propane-1,2-diol acetal [FL-no: 02.248], is readily hydrolysed. In a hydrolysis study, 12-39 mM vanillin 3-l-menthoxypropane-1,2-diol acetal underwent 91 % hydrolysis at pH 2 within 45 minutes. At pH 3, approximately 86 % of vanillin 3-l-menthoxypropane-1,2-diol acetal was hydrolysed within 90 minutes. At pH 4, approximately 92 % of the acetal was hydrolysed within eight hours. At pH 5, approximately 12 % of the flavouring substance was hydrolysed within eight hours (Reitz, 1995).

Concluding remarks on hydrolysis

There is some information about hydrolysis of esters in *in vivo* as well as *in vitro* systems for some supporting substances in subgroup 1 and for some supporting and candidate substances in subgroup 2.

It is expected that esters in subgroup 1 and 2 will be hydrolysed *in vivo*. Eight of the candidate substances in subgroup 1 [FL-no: 09.313 (benzyl 2-methylbutyrate); 09.314 (benzyl crotonate); 09.315 (benzyl dodecanoate); 09.316 (benzyl hexanoate); 09.317 (benzyl lactate); 09.318 (benzyl octanoate); 09.152 (benzyl valerate); 09.835 (benzyl decanoate)] will yield benzyl alcohol and simple aliphatic carboxylic acids upon hydrolysis. One ester, 4-isopropylbenzyl acetate [FL-no: 09.611] will yield 4-isopropylbenzyl alcohol and acetic acid.

The acetal in subgroup 1, (diethoxymethyl)benzene [FL-no: 06.017], is expected to be efficiently hydrolysed to yield benzaldehyde and ethanol.

Four of the remaining esters in subgroup 1 are expected to yield benzoic acid and simple aliphatic alcohols upon hydrolysis, [FL-no: 09.656 (3-methylbut-3-enyl benzoate), 09.693 (prenyl benzoate), 09.779 (butyl benzoate), 09.825 (pentyl benzoate)]. One ester, [FL-no: 09.631] (methyl 4-methylbenzoate), will yield 4-methylbenzoic acid upon hydrolysis. The alcohol part of the candidate substance, [FL-no: 09.656] (3-methylbut-3-enyl benzoate), includes a terminal double bond.

Of the 13 esters in subgroup 2, one ester, [FL-no: 09.895 (4-methoxybenzyl-2-methylpropionate), will yield 4-methoxybenzyl alcohol (p-anisyl alcohol) [FL-no: 02.128] upon hydrolysis. The remaining 12 esters in subgroup 2, [FL-no: 09.362 (ethyl 2-hydroxy-4-methylbenzoate), 09.363 (ethyl 2-methoxybenzoate), 09.367 (ethyl 4-hydroxybenzoate), 09.560 (hex-3(cis)-enyl anisate, ((hex-3(cis)-enyl 4-methoxybenzoate)), 09.570 (hex-3-enyl salicylate), 09.581 (hexyl salicylate), 09.623 (methyl 2,4-dihydroxy-3,6-dimethylbenzoate), 09.696 (prenyl salicylate), 09.762 (pentyl salicylate), 09.798 (ethyl vanillate), 09.799 (methyl vanillate) and 09.852 (2-methylbutyl 2-hydroxybenzoate)], will yield hydroxy and/or alkoxy-substituted benzoic acid upon hydrolysis.

III.3.2 Metabolism Studies

Subgroup 1 (benzyl derivatives)

Candidate substances from subgroup 1

There are no metabolism studies submitted for the candidate substances belonging to subgroup 1.

Supporting substances from subgroup 1

Benzyl alcohol [FL-no: 02.010]

Five minutes after single intraperitoneal doses of 500 – 1100 mg/kg bw of benzyl alcohol administered to CD1 mice, benzyl alcohol was detected in plasma. At doses of 700 – 1100 mg/kg bw, plasma also contained measurable concentrations of benzaldehyde. Animals pre-treated with an alcohol dehydrogenase inhibitor (pyrazole) showed a 200 % increase in plasma benzyl alcohol levels, whereas pre-treatment with an aldehyde dehydrogenase inhibitor (disulphiram) resulted in a 368 % increase in plasma benzaldehyde levels as compared to control values (McCloskey et al., 1986).

Benzaldehyde [FL-no: 05.013]

The metabolism of benzaldehyde was investigated in Sprague Dawley rats (5/group/sex) which were administered single oral doses of 400, 750 or 1000 mg/kg bw of pure benzaldehyde by gavage once daily for 13 consecutive days. Urine was collected for 24 hours after the 2nd, the 8th and the 13th dose and analysed for the presence of metabolites. The major metabolites were benzoic acid acid conjugates and benzylmercapturic acid. Although females in the mid- and high-dose groups exhibited a slight decrease in excretion of benzylmercapturic acid after the 8th dose, all groups showed increased urinary levels after the 13th doses. An increase in dose from 400 to 1000 mg/kg bw/day resulted in a 7- to 8-fold increase in benzylmercapturic acid excretion. The amount of benzylmercapturic acid excreted in urine collected for 24 hours ranged between 0.13-2.05 mg/rat, the higher amounts collected from the rats in the highest dose groups. Benzaldehyde is reduced to benzyl alcohol only to a minor extent; the alcohol sulphate conjugate may further react with glutathione to form benzyl mercapturic acid (Laham and Potvin, 1987).

In the rabbits orally dosed with 350 or 750 mg/kg bw, the aldehyde was oxidised mainly to benzoic acid and excreted predominantly as hippuric acid (approximately 68 % of the administered dose). Other urinary metabolites detected were benzoylglucuronic acid (10 %), benzoyl glucuronide (3 %), free benzoic acid (1.5 %), and trace amounts of benzylmercapturic acid (Laham et al., 1988).

4-Isopropylbenzaldehyde (cuminaldehyde) [FL-no: 05.022]

High doses (2000 mg) of p-isopropylbenzaldehyde were given orally to male rabbits. Urine was collected for three days post-treatment. The yield of urinary oxidation metabolites was higher than that of reduction metabolites. This was in contrast to *o*-isopropylbenzaldehyde, the reduction of which was more extensive and the corresponding acids were not found. p-Isopropylbenzaldehyde mainly undergoes a combination of oxidation of the aldehyde function and the oxidation of the alkyl-side chain to yield 9-hydroxycuminic acid and 8-hydroxycuminic acid. Cumyl alcohol (cuminyl alcohol, 4-isopropylbenzyl alcohol) and 2-carboxyphenylpropionic acid were minor urinary metabolites. It was concluded that oxidation or reduction was controlled by the position of substituents, in that oxidation occurs with the *p*-isomer and reduction occurs with the o-isomer. In addition, stereoselective oxidation was found in the aromatic isopropyl group of the p-isomer (Ishida et al., 1989b).

Benzoic acid [FL-no: 08.021]

Ring labelled ^{14}C-benzoic acid was given orally at doses in the range of 1 – 400 mg/kg bw to various species including primates, pigs, rabbits, rodents, cats, dogs, hedgehogs, bats, birds and reptiles. Hippuric acid was the primary urinary metabolite in most species. The ornithine conjugate of benzoic acid, ornithic acid, was the major urinary metabolite excreted within 24 hours in chickens and reptiles. Benzoyl glucuronide was predominant in bats. In humans, more than 99 % of ^{14}C was excreted as hippuric acid within 24 hours (Bridges et al., 1970).

Following oral administration of 375 mg [^{14}C]-benzoic acid/kg bw to rats, 91 – 94 % of the radioactivity was recovered in the urine of rats after 72 hours, whereas only 1 – 6 % was present in the faeces. The following metabolites were identified: hippuric acid (70.2 – 84.2 %), benzoyl glucuronide (0.7 – 1.8 %), benzoic acid (0.4 – 12.8 %) and 3-hydroxy-3-phenyl propionic acid (0.1 – 0.2 %) (Nutley, 1990).

Urinary hippuric acid is used as a biologial marker of toluene exposure. In order to investigate the types and quantities of beverages that increase urinary hippuric acid excretion, 137 healthy students were recruited and divided into quintiles based on their consumption of non-alcoholic beverages containing benzoic acid. HPLC was used to determine benzoic acid intake from beverages and urinary hippuric acid before, and 1.5 and 3 hours after consumption of various beverages. The range of benzoic acid in 13 beverages was 0 – 1.02 mg/ml and the benzoic acid intakes from the beverages for groups 1 – 5, respectively, were: 0.4 mg ± 0.5; 23.4 mg ± 9.8; 55.2 mg ± 2.3; 76.3 mg ± 4.0; and 116.5 mg ± 16.5. Urinary hippuric acid geometric mean concentrations before consuming beverages in the five groups, respectively, were 0.276, 0.270, 0.207, 0.262 and 0.316 g/l; 1.5 hours after beverage consumption they were 0.210, 0.603, 1.026, 1.066 and 1.688 g/l and significantly increased ($p<0.001$) after adjustment for urinary hippuric acid before ingestion. Three hours after beverage consumption, urinary hippuric acid geometric mean concentrations in the five groups, respectively, were 0.160, 0.232, 0.306, 0.287 and 0.337 g/l ($p<0.001$). The authors concluded that beverages containing more than 100 mg benzoic acid may increase urinary hippuric acid significantly (Chang et al., 2000).

Benzyl acetate [FL-no: 09.014]

Following gavage administration of [methylene-^{14}C]-benzyl acetate to groups of three or more male Fischer 344 rats at a dose of 5, 250 or 500 mg/kg bw as the substance alone, in corn oil, or in propylene glycol, 70 – 89 % of the dose was excreted in the urine within 24 hours. Approximately 4 % of the radioactivity was detected in the faeces after 72 hours and about 1 % in the carcass after 72 hours. The elimination of benzyl acetate and metabolites, regardless of vehicle, was largely complete after three days. Urine was collected and urinary metabolites were assayed by Thin Layer Chromatography (TLC) and HPLC. In other animals ^{14}C plasma levels were measured, and variation of metabolites in plasma were assayed. No benzyl acetate was detected in the plasma or urine at any time point. Small amounts of benzyl alcohol were detected in the plasma at early time points after administration of the neat substance or dissolved in propylene glycol. After administration of 500 and 250 mg/kg bw, unconjugated benzoic acid was the major plasma metabolite. After the 5 mg/kg bw dose, hippuric acid was the major plasma metabolite. At the higher dose levels small

amounts of radioactivity (< 5 % of total plasma ^{14}C) was present as unknown metabolites of high and moderate polarity, but not in all samples. At the 5 mg/kg bw dose, 20 % of plasma ^{14}C was present as the unknown polar metabolite, although this became less important with time. When propylene glycol was used as vehicle, benzylmercapturic acid was detected in plasma, but only at the 5 mg/kg bw dose. Hippuric acid was always the major urinary metabolite but the proportion of dose present as benzoyl glucuronide increased with dose. Low levels (1.0 – 3.6 %) of benzoic acid and benzyl mercapturic acid (1.0 - 1.9 %) excreted in urine were not significantly affected by dose or vehicle (Chidgey and Caldwell, 1986).

Chidgey et al. (1986) suggest that formation of benzyl mercapturic acid occurs via formation of benzyl sulphate. In a study designed to define the route of metabolism of benzyl acetate leading to the formation of benzyl mercapturic acid, male Fischer 344 rats were dosed by gavage with [methylene-^{14}C]benzyl acetate (500 mg/kg bw) alone or together with pyrazole (200 mg/kg), pentachlorphenol (10 mg/kg bw) or both. Urine and faeces were collected and urinary metabolites were assayed by radio-TLC and HPLC. The excretion of ^{14}C was rapid in all cases, with most of the dose being excreted in urine within 24 hours. Co-administration of benzyl acetate with pyrazole, an inhibitor of alcohol dehydrogenase, caused an 11-fold increase in the excretion of benzyl mercapturic acid and halved the percentage of the dose excreted as benzoyl glucuronide. Pretreatment with pentachlorophenol, an inhibitor of sulphotransferase activity *in vivo*, abolished the excretion of benzyl mercapturic acid, while excretion of the mercapturate following treatment with both pyrazole and pentachlorophenol was higher than in control or pentacholorophenol treated rats, but much lower than in the animals given pyrazole alone. Taken together, these results suggest that the formation of benzyl mercapturic acid involves the sulphate ester of benzyl alcohol as an obligatory intermediate and that formation of reactive metabolites of toxicological significance is unlikely (Chidgey et al., 1986).

Fischer 344 rats and C57BL/6N mice were administered [ring-UL-^{14}C]benzyl acetate at single oral doses of 5 or 500 mg/kg bw in rats or 10 mg/kg bw in mice, and urine and faeces were collected for 96 hours to determine the effects of age on disposition of benzyl acetate. Age groups studied were 3 to 4, 9 and 25 month-old rats and 2, 13 and 25 month-old mice. In rats, approximately 80 % of radioactivity was recovered in the urine in the first 24 hours for all age groups. The major urinary metabolite was hippuric acid (> 90 % of total urinary radioactivity) and benzyl mercapturic acid (1-2 %) was the only other metabolite detected in the urine of rats. There were no age differences in the percentage of [^{14}C]benzyl acetate excreted as hippuric acid, but the amount of excreted benzyl mercapturic acid increased slightly in the 25 month-old rats as compared to younger rats. The percentage of radioactivity excreted in the faeces was slightly decreased in the 25 months old group. In mice, hippuric acid was the major urinary metabolite, constituting 93 – 96 % of the total dose after 96 hours. Less radioactivity was excreted in the urine of 25 month-old mice than in the younger groups. Faecal excretion was a minor route and the amount was similar for all age groups. The authors concluded that formation of hippuric acid is not affected by age, but aging does affect the minor routes of metabolism and excretion of benzyl acetate in rats and mice (McMahon et al., 1989).

Benzyl acetate was administered to rats and mice in gavage and dosed feed studies. For gavage study groups of six male F344/N rats and twelve male B6C3F$_1$ mice were administered benzyl acetate in corn oil at 500 mg/kg (rat) and 1000 mg/kg (mouse). Blood samples were collected 5 min – 24 hours after dosing. For dosed feed studies groups of ten rats and ten mice, of the same strains as in the gavage study, were dosed with benzyl acetate in feed (10,800 ppm for rats and 2700 ppm for mice) *ad libitum* during the study. The concentrations in feed were estimated to provide a daily benzyl acetate dose of 648 mg/kg for rats and 900 mg/kg for mice. At day 7 and 8 blood samples were collected at five time points during 15 hours, with two animals from each species sampled at each time point. Benzyl acetate was not detected in any plasma samples collected in the studies. Except for the 5 and 10 minutes rat plasma samples and the 5 minutes mice plasma samples in the gavage study, no benzyl alcohol was detected in plasma. Concentrations of benzoic acid and hippuric acid in plasma rapidly increased to peak concentrations within 3 hours after gavage with the peak benzoic acid concentrations being much higher (about 10- to 20-fold) than the peak hippuric acid concentrations. Plasma concentrations of benzoic acid in the dosed feed studies were much lower (more than 100-fold) than the concentrations in the gavage studies, consistently with the mode of administration (bolus

dose with gavage). Plasma concentrations of hippuric acid were comparable in both studies. The absence of benzyl acetate in plasma shows that benzyl acetate is rapidly hydrolysed to benzyl alcohol. The major metabolite of benzyl acetate, benzoic acid, is mainly dependent on the conjugation pathway involving Coenzyme A (CoA). This pathway would be saturated when plasma concentrations of benzoic acid are very high or when the CoA is depleted. Such conditions appear to have occurred after a bolus gavage dose of benzyl acetate to result in a brief peak in the plasma concentration of benzoic acid. When benzyl acetate was administered to rats and mice in dosed feed, it appears that the CoA conjugation pathway was never saturated and plasma concentration of benzoic acid remained low (Yuan et al., 1995).

Other related substances

Sodium benzoate

Male volunteers were given oral doses of 2000 to 5000 mg sodium benzoate. The 5000 mg dose group was given a 5000 mg dose of glycine one hour later and 2000 mg doses every two hours thereafter. Benzoate was excreted mainly as hippuric acid. No free benzoic acid was detected. Minor amounts of benzoyl glucuronide were detected at both doses. Co-administration of glycine with benzoate increased the rate of hippuric acid excretion, indicating that at high dose levels, glycine is rate limiting for formation of hippuric acid (Amsel and Levy, 1969).

After administration of oral doses of 40, 80 and 160 mg/kg bw of sodium benzoate to humans, the mean plasma Area Under Curves (AUCs) of benzoic acid increased disproportionately to the dose, 3.7 and 12.0 times greater, respectively, for the higher dosages than for the lowest dose, while the mean AUCs for hippuric acid was proportional to dose. Peak plasma concentrations of benzoic acid increased with increasing dose, while peak hippuric acid concentrations did not change. The data suggest that the conjugation with glycine to form hippuric acid is a saturable process in humans (Kubota et al., 1988; Kubota and Ishizaki, 1991).

Subgroup 2 (hydroxy- and alkoxy-substituted benzyl derivatives)

Candidate substances from subgroup 2

Piperonyl alcohol (3,4-methylenedioxybenzyl alcohol) [FL-no: 02.205]

The metabolism of piperonyl alcohol [FL-no: 02.205] and piperonal (3,4-methylenedioxybenzaldehyde) [FL-no: 05.016] (supporting substance) was studied in male Wistar rats. Piperonyl alcohol dissolved in propylene glycol was administered by oral gavage at a dose of 1 mmol/kg bw (corresponding to 152 mg/kg bw) and urine samples were taken at 24 and 48 hours. Recovery of urinary metabolites were 90 %, and metabolite excretion occurred mainly within 24 hours. Piperonyl glycine was identified as the major metabolite (70 ± 5 %; 24-hours analysis expressed as a percent of administered dose) and piperonylic acid (17 ± 3 %) was the other important metabolite. Demethylenation of the methylenedioxy moiety led to the excretion of three cathecol derivatives, which accounted for 0.7 % of the dose, protocatechuic acid (0.4 ± 0.1 %), protocatechuyl alcohol (0.3 ± 0.1 %). Other minor metabolites were piperonyl alcohol (1.4 ± 0.5 %) and vanillyl alcohol (0.05 ± 0.03 %) (Klungsoeyr and Scheline, 1984).

In a study of several methylenedioxyphenyl (MDP) compounds, radiolabelled piperonyl alcohol (1-hydroxymethyl-3,4-methylene-^{14}C-dioxybenzene) was administered to male Swiss-Webster mice in a DMSO solution by oral gavage at a dose of 0.76 mg/kg bw. Total radiocarbon determinations were made on expired $^{14}CO_2$ at 0.5, 1, 2, 4 and 6 hours after dosing and at each 6-hours interval thereafter. Urine and faeces samples were taken at 12, 24 and 48 hours after treatment. The 12-hours urine samples were used for separation and characterisation of metabolites. At the end of the experiment, $^{14}CO_2$ excretion amounted to 3 % of the dose, indicating that demethylenation of piperonyl alcohol only occurs as a minor metabolic pathway. The major part of radioactivity was retrieved in urine, 93.3 %, and less than 10 % in faeces. The major urinary metabolite after administration of piperonyl alcohol was piperonyl glycine. Other MDP

substances studied were safrole, dihydrosafrole, myristicin, Tropital, piperonyl butoxide, piperonal and piperonylic acid. The major metabolic pathway for piperonyl butoxide, safrole, dihydrosafrole and myristicin was demethylenation of the methylenedioxy moiety. As for piperonyl alcohol, oxidation and conjugation of the side chain is the major metabolic pathway for Tropital, piperonal and piperonylic acid. The authors discussed that the polar nature of these compounds or their ease of conversion to polar products may minimise their entrance to the lipid components of the microsomal enzymes so that no extensive demethylenation would occur (Kamienski and Casida, 1970).

Gallic acid (3,4,5-trihydroxybenzoic acid) [FL-no: 08.080]

Following administration of gallic acid [FL-no: 08.080] to rats either in the diet at a concentration of 0.5 % or in single doses of 100 mg/rat via oral gavage, the major urinary excretion products were the unchanged parent substance and one metabolite which was concluded to be 4-O-methyl gallic acid. A minor metabolite, 2-O-methylpyrogallol, was excreted mainly as an acid-labile conjugate. When the same compound was given at 100 mg/rat as intraperitoneal injection the results were similar to those obtained when the substance was given orally, however, a minor metabolite identified as pyrogallol was also present along with a trace of 2-O-methyl pyrogallol. Rabbits administered a diet containing 0.5 % gallic acid also excreted 4-O-methyl gallic acid, pyrogallol, and possibly also 2-O-methyl pyrogallol. The data indicated that mostly free benzoic acid derivatives were excreted, although rabbits excreted an acid-labile conjugate of 4-O-methyl gallic acid. The results indicate that O-methylation and decarboxylation are the reactions involved in the metabolic conversion of gallic acid. The authors stated that this selective O-methylation would prevent the formation of the catechol configurations (Booth et al., 1959).

Scheline (1966a) reported that rats that were administered 100 mg/kg bw of gallic acid [FL-no: 08.080] by oral gavage excreted the parent substance and the free and acid-labile conjugates of its 4-O-methyl ether. Pyrogallol and 2-O-methyl pyrogallol, the decarboxylated metabolites, were excreted in their conjugated forms. Dosing with 30 and 300 mg gallic acid/kg bw showed that excretion of decarboxylated metabolites increased with increasing dose. Intraperitoneal injection of gallic acid in four rats resulted in the urinary excretion of gallic acid and 3,5-dihydroxy-4-methoxybenzoic acid, but neither pyrogallol or 2-O-methyl pyrogallol were detected in these urines. A study of the ability of rat intestinal contents to metabolise gallic acid showed that it was decarboxylated to pyrogallol when test substance was added to medium containing extracts of caecal or colon contents. Test substance was recovered essentially unchanged when small intestine contents were used (Scheline, 1966a).

In order to examine decarboxylation and demethylation of some phenolic benzoic acid derivatives by rat caecal contents, test substances were incubated for 22 hours in medium containing ceacal contents. Solutions together with appropriate standards were then examined by TLC. Gallic acid [FL-no: 08.080] gave rise to pyrogallol which was present in five out of eight samples. When pyrogallol was absent after incubation large amounts of resorcinol were observed on the chromatograms. Dehydroxylation to resorcinol was also seen when pyrogallol itself was incubated with caecal extract. Unchanged gallic acid was found in 4 out of 8 samples. Pyrogallol was not dehydroxylated to catechol in these experiments. The main findings of the study that covered 27 phenolic benzoic acid derivatives was that decarboxylation only occurred when a free hydroxyl group was present in the *para* position (Scheline, 1966b).

The metabolic fate of gallic acid [FL-no: 08.080] in peripheral blood, liver and urine after oral administration was studied in six-week-old male Wistar rats in order to determine the most appropriate route of administration for the treatment of liver cancer, i.e. the route that gives the highest concentration of gallic acid in liver. Gallic acid was given orally to the rats at 50, 100 or 500 mg/kg bw (The number of animals is not reported, but results from the 100 mg/kg bw group are shown as the mean of 4-6 animals). Blood samples were taken from the portal vein and the inferior vena cava at 5, 15, 30, 60, 180 and 360 minutes after administration and urine was collected at the same time points. Intestinal contents were collected and, in order to avoid contamination due to enterohepatic circulation, bile duct was ligated before oral administration of 100 mg/kg bw. Animals were sacrificed, and the entire intestinal contents were collected.

The liver was removed from terminated animals after perfusion with saline to eliminate blood contamination. For analysis, samples from serum, urine and liver were processed and then analysed by HPLC. Gallic acid reached its peak concentration in the portal vein 15 minutes after oral administration of 100 mg/kg bw. After 30 minutes it had decreased to half the concentration and had almost disappeared after 6 hours. The only metabolite detected in the blood and urine was identified as 4-O-methyl gallic acid. 4-O-Methyl gallic acid also reached peak concentration in the portal vein after 15 minutes, and then decreased slowly. In the inferior vena cava both gallic acid and 4-O-methyl gallic acid reached peak concentration at 30 minutes after oral administration of 100 mg gallic acid/kg bw. In the portal vein, gallic acid was detected at about twice the concentration of 4-O-methyl gallic acid. In the inferior vena cava, 4-O-methyl gallic acid and gallic acid were detected in approximately equal proportions and in about the same amount as 4-O-methyl gallic acid in the portal vein. 4-O-Methyl gallic acid, but not gallic acid was found in the liver homogenate prepared after thorough perfusion with saline. The main metabolite of gallic acid in urine was 4-O-methyl gallic acid and its concentration was about 100 times higher than in the inferior vena cava. Gallic acid was also found in urine at a higher concentration than in the inferior vena cava, but at lower concentration than 4-O-methyl gallic acid in urine. In contrast to previously published studies (Booth et al., 1959; Scheline, 1966a), this study did not detect pyrogallol as a metabolite in blood or urine. The authors attribute this discrepancy to the earlier studies using TLC for determination of metabolites, but without proper determination of structures, and also comment that the time for collecting urine under unstable conditions may have led to the decomposition of gallic acid to pyrogallol (Zong et al., 1999).

Ethyl 4-hydroxybenzoate (ethyl paraben) [FL-no: 09.367]

Absorption, distribution, metabolism and excretion of ethylparaben [FL-no: 09.367] were investigated in Wistar rats administered 100 mg by oral gavage. Animals were held in metabolism cages for the collection of urine (at approximately 15, 30, 60, 75, 90, 120, 150 and 210 minutes) and blood (at approximately 30, 60, 90, 120, 180, 240 and 360 minutes), and samples were analysed to establish the excretion kinetics. Metabolites were detected in the urine starting at 30 minutes after dosing, but no unchanged ethyl paraben was identified by this time. p-Hydroxyhippuric acid appeared in the urine 30 minutes after dosing and its concentration increased steadily during the next three hours. The glucuronide and ethereal sulphate metabolites only appeared between 30 and 75 minutes post-ingestion. A continuous increase of free 4-hydroxybenzoic acid in the blood occurred during the first hour post-dosing, but its concentration then decreased over the next hour and plateaued for the remaining four hours of sample collection. Maximum urinary excretion of free 4-hydroxybenzoic acid occurred at 90 minutes post-dosing, whereas excretion of the glucuronic and glycine conjugates increased until the end of the collection period at 210 minutes post-dosing. In summary, absorption of ethylparaben was followed by metabolism and excretion of mainly free 4-hydroxybenzoic acid and its glucuronic and glycine conjugates. A small portion of the dose was excreted as sulphate conjugate (Derache and Gourdon, 1963).

The ^{14}C-labelled ethyl 4-hydroxybenzoate was orally given to four male cats in the diet at a concentration that provided a single dose of 156 mg/kg bw, equivalent to 130 mg/kg bw of the parent acid. Urine was collected at 24-, 48- and 72-hours intervals, total faeces were collected at 72-hours, and all samples were assayed for total radioactivity. The radioactive metabolites present in the 24-hours samples were isolated and identified. Essentially, all (mean = 96.0 %) of the radioactiviy was excreted within 72 hours with the breakdown expressed as mean values for the four animals as follows: 24-hours urine, 85.8 %; 48-hours urine, 3.6 %; 72-hours urine, 0.8 %; 72-hours faeces, 5.8 %. The 24-hours urine samples revealed two metabolites. Metabolite I contained between 54 and 69 % of the administered radioactivity and had a similar retention volume as p-hydroxyhippuric acid. Metabolite II contained between 31 and 46 % of the administered radioactivity and had a similar retention volume as 4-hydroxybenzoic acid. Additional evaluations confirmed the identity of the suggested metabolites (Phillips et al., 1978).

Ethyl 4-hydroxybenzoate (ethylparaben) [FL-no: 09.367] and supporting substances 4-hydroxy benzoic acid [FL-no 08.040] and butyl 4-hydroxybenzoate (butylparaben) [FL-no: 09.754]

Groups of three fasted dogs were administered single doses of 1 g/kg bw of 4-hydroxybenzoic acid (supporting substance [FL-no 08.040]) or its methyl, ethyl (candidate substance [FL-no: 09.367]), propyl and butyl (supporting substance [FL-no: 09.754]) esters orally or 50 mg/kg bw by intravenous injection. Blood and urine samples were collected at fixed intervals until 48 hours. Recovery of total test material as metabolites in urine after the oral and intravenous doses was 60 - 95 % for the acid and the methyl, ethyl and propyl esters. For the candidate substance ethyl 4-hydroxybenzoate [FL-no: 09.367] recovery of total material was 66 % of the oral and 70 % of the intravenous dose. Metabolites were detectable in the blood up to 24 hours post-ingestion. Of the dose 12.3 % was excreted as free acid and 32.5 % as the glucuronic acid conjugate. Recovery of the butyl ester was 48 % after oral and 40 % after intravenous dosing. After oral dosing about 5 % of the butyl ester was excreted as free 4-hydroxybenzoic acid and 27.5 % as the glucuronic acid conjugate. Other conjugates were not determined. After intravenous dosing 11.3 % of the given dose was recovered as free acid in urine and 20.1 % as the glucuronic acid conjugate. The test material was mainly excreted within 24 hours after dosing. The low rate of recovery seen with both dosing methods was attributed to incomplete hydrolysis of the butyl ester in the body. *In vitro* incubation of the butyl ester with freshly prepared liver homogenate showed complete hydrolysis within 30 – 60 minutes. Studies conducted with related benzoate esters, methyl and ethyl p-hydroxybenzoate, showed significantly higher rates of test material recovery when given to dogs by the oral and intravenous route, and showed 100 % hydrolysis within 3 minutes when incubated with liver homogenate. This finding suggests that an increase in the length of the alkyl rest in the homologous series of alkyl esters make the esters more resistant to hydrolysis and may result in the activation of other metabolic and excretion pathways (Jones et al., 1956).

Supporting substances from subgroup 2

4-Methoxybenzylalcohol (*p*-anisyl alcohol) [FL-no: 02.128]

In an *in vitro* study, 4-methoxybenzylalcohol (*p*-anisyl alcohol) [FL-no: 02.128] was incubated with rat caecal extract. Analysis after approximately 46 hours showed the presence of unchanged compound and anisic acid. No observation of O-demethylation was observed (Scheline, 1972).

Vanillyl alcohol (4-hydroxy-3-methoxy benzylalcohol) [FL-no: 02.213] and vanillin (4-hydroxy-3-methoxybenzaldehyde) [FL-no: 05.018]

In an *in vivo* study conducted in male albino rats, vanillyl alcohol [FL-no: 02.213] and vanillin [FL-no: 05.018] were administered by gavage in doses of 100 or 300 mg/kg bw. Urinary metabolites were collected over the first 24 - 48 hours period and analysed qualitatively. Vanillyl alcohol was mainly excreted as vanillyl alcohol or vanillic acid and related conjugates. The aldehyde intermediate was also detected. Conjugated fractions of vanillin, guaiacol, catechol, 4-methylguaiacol and 4-methylcatechol were also identified in smaller quantities. Oral dosage of 100 mg/kg bw of vanillin resulted in an urinary excretion of most metabolites within 24 hours, mainly as glucuronide and sulphate conjugates although vanillic acid was also excreted as free acid and as glycine conjugate. After 48 hours 94 % of the dose was accounted for as follows: vanillin 7 %, vanillyl alcohol 19 %, vanillic acid 47 %, vanilloylglycine 10 %, catechol 8 %, 4-methyl catechol 2 %, guaiacol 0.5 % and 4-methyl guaiacol 0.6 %. Vanillin and its primary reduction and oxidation metabolites were also excreted in appreciable amounts in the bile. Bile collected five hours after two rats were given 100 and 300 mg/kg bw oral doses of vanillin contained glucuronide conjugates of vanillin (6 %), vanillyl alcohol (8 %) and vanillic acid (9 %). The results show that both oxidative and reductive pathways exist for the metabolism of vanillin, although the oxidative metabolism dominates. At a dose level of 100 mg/kg bw, 57 % of the dose of vanillin was excreted as free vanillic acid or its conjugates and in total oxidation products amounted to approximately 65-70 % of the dose. The reduction pathway accounted for a little more than 20 % of the dose (Strand and Sheline, 1975).

In Sprague-Dawley albino rats 100 mg vanillin [FL-no: 05.018]/kg bw was given by intraperitoneal injection and 24-hours urine was collected and analysed. The main urinary metabolite was conjugated vanillic acid which accounted for 41 % of the administered dose, while free vanillic acid accounted for 6 %. In addition, there was a trace of catechol. Vanillyl alcohol, a reductive product, represented 10 % of the administered

dose. The presence of the urinary glycine conjugate of vanillic acid was not reported in this study. The oxidative path of metabolism was found to predominate, however, the importance of the minor reductive pathway may be magnified by inhibition of the oxidative process, as was achieved by administration of disulphiram in the study (Wong and Sourkes, 1966).

An experiment was conducted with the aim to determine whether man is capable of oxidising vanillin to vanillic acid. A 100 mg dose of vanillin dissolved in water was given to an adult human and the urine collected for 24 hours. Examination revealed an increase in the vanillic acid output in the urine from a background level of 0.3 mg/24 hours to 96 mg/24 hours. The observed increase accounted for approximately 94 % of the vanillin dose (Dirscherl and Wirtzfeld, 1964).

Veratraldehyde (3,4-dimethoxybenzaldehyde) [FL-no: 05.017]

A 1 g/kg bw oral dose of veratraldehyde (3,4-dimethoxybenzaldehyde) was administered to rabbits by gavage and urine was collected for 24 hours. Approximately 70 % of the aldehyde was accounted for in urine mainly as the corresponding acid, veratric acid (28 %) and its glucuronic acid (38 %) or sulphate (3-7 %) conjugate. To a small extent, veratric acid was decarboxylated and O-demethylated to yield catechol (Sammons and Williams, 1941). Presumably, veratric acid may enter the enterohepatic circulation where gut microflora decarboxylate the acid to yield catechol (o-hydroxyphenol). The observation that catechol was formed as a minor metabolite when veratraldehyde was incubated with rat caecal extract illustrates this decarboxylation pathway in gut bacteria (Scheline, 1972).

4-Hydroxybenzaldehyde [FL-no:05.047] and salicylaldehyde (2-hydroxybenzaldehyde) [FL-no: 05.055]

In rabbits, 96 % of a single oral dose of 400 mg/kg bw 4-hydroxybenzaldehyde was excreted in the urine within 24 hours as 4-hydroxybenzoic acid and its glycine, glucuronic acid and sulphate conjugates (Bray et al., 1952b).

A single dose of 400 mg)/kg bw 2-hydroxybenzaldehyde (salicylaldehyde was administered to a fasted rabbit in three or six experiments. Approximately 75 % of the dose was excreted as ether soluble acids in the urine collected over 24 hours, and 27 % and 3 % accounted for as glucuronic acid and sulphate conjugates, respectively (Bray et al., 1952b).

In a corresponding study, approximately 94 % of a single oral dose of 250 or 500 mg/kg bw salicylaldehyde administered to two groups of four rabbits was excreted unchanged or as the glucuronic acid and sulphate conjugates, while the major part was excreted as the unchanged acid (Bray et al., 1948).

Veratraldehyde (3,4-dimethoxybenzaldehyde) [FL-no: 05.017], vanillin (4-hydroxy-3-methoxybenzaldehyde) [FL-no: 05.018] and vanillic acid (4-hydroxy-3-methoxy benzoic acid) [FL-no: 08.043]

Veratraldehyde [FL-no: 05.017], vanillin [FL-no: 05.018] or vanillic acid [FL-no: 08.043] were given orally by gavage to six rabbits at a dose of approximately 1 g/kg bw. Urine was collected for 5 hours. After administration of veratraldehyde approximately 70 % of the material was recovered in the urine as free corresponding acid (28 %) and its glucuronic acid (38 %) or sulphate (3 - 7 %) conjugate. Approximately 69 % of vanillin was oxidised to vanillic acid, of which 44 % was recovered as free acid and 25 % conjugated acid. About 14 % of the dose was excreted as the glucuronic acid conjugate of vanillin. In the case of vanillic acid, 56 % was excreted as free vanilllic acid and 27 % as conjugated, as glucuronide conjugate or ethereal sulphate. Less than 5 % was demethylated (Sammons and Williams, 1941).

4-Hydroxybenzoic acid [FL-no: 08.040]

Groups of four to eight rabbits were administered doses of 100, 250, 500, 1000 or 1500 mg/kg bw 4-hydroxybenzoic acid by gavage. Urine was collected continuously and analysed for metabolites. Total urinary recovery of the test material was in the range of 84 to 104 %, with ether soluble acids comprising 64

to 75 % of the total. Glucuronic acid and sulphate conjugates were also detected in the urine at 10 to 35 % and 4 to 7 %, respectively. The levels of all the metabolites returned to background levels within 24 hours after dosing (Bray et al., 1947).

Results from four experiments showed that between 2.2 and 5.4 % was excreted in the urine within 24 hours as the corresponding hippurate of a 0.41 mmole 4-hydroxybenzoic acid dose was administered by intraperitoneal injection to female albino rats (Teuchy et al., 1971).

Other related substances

Methyl 4-hydroxybenzoate (methyl paraben)

Methyl 4-hydroxybenzoate (methylparaben) was administered to three male rabbits by oral gavage at 800 mg/kg bw as a 12 % natrium salt solution, and the 24-hours urine was analysed. Three major metabolites, 4-hydroxybenzoic acid, p-hydroxyhippuric acid and p-carboxyphenyl glucuronide, as well as two minor metabolites, p-hydroxybenzoyl glucuronide and p-carboxyphenyl sulphate, were identified (Tsukamoto and Terada, 1962).

Concluding remarks on metabolism

The esters in subgroup 1 will upon hydrolysis yield benzyl alcohol or benzoic acid along with alkyl carboxylic acids or alcohols. The metabolic fate of alkyl carboxylic acids and alcohols has been discussed in previous FGEs, and will not be discussed further in this evaluation. Benzyl alcohol, benzylaldehyde and benzoic acid have been evaluated by the JECFA (JECFA, 1996b), as has 4-isopropylbenzyl alcohol (JECFA, 2002a), which is the alcohol moiety of candidate substance [FL-no: 09.611] (4-isopropylbenzyl acetate). Benzaldehyde and the benzyl alcohols are expected to be oxidized to corresponding benzoic acids, which will be conjugated with glycine and excreted as hippuric acids.

The candidate substance [FL-no: 09.314 (benzyl crotonate)] will yield crotonic acid as acid moiety, this substance has been discussed in the previous FGE.05Rev1. In addition, crotonic acid has been evaluated by the SCF (SCF, 2002a).

The alcohol part of the candidate substance [FL-no: 09.656] (3-methylbut-3-enyl benzoate) includes a terminal double bond, a structure that has been discussed in FGE.06Rev1 and will not be further discussed in this FGE.

The candidate substance (diethoxymethyl)benzene [FL-no: 06.017] is an acetal. This substance would be expected to yield benzaldehyde and ethanol upon hydrolysis. Benzaldehyde is expected to be oxidized to benzoic acid.

Subgroup 2, hydroxy- and alkoxy-substituted benzyl derivatives, includes 13 esters of which one [FL-no: 09.895] (4-methoxybenzyl-2-methylpropionate) will yield 4-methoxy-benzyl alcohol (*p*-anisyl alcohol) [FL-no: 02.128] upon hydrolysis. This substance has been evaluated by the JECFA (JECFA, 2002a). 4-Methoxy-benzyl alcohol is expected to be excreted in the urine either unchanged or as glucuronic acid, glycine or sulphate conjugate. The same metabolic pathway is proposed for the candidate benzyl alcohol derivative [FL-no: 02.164] (4-hydroxy-3,5-dimethoxy benzylalcohol).

The remaining 12 esters in subgroup 2 will yield hydroxy- and/or alkoxy-substituted benzoic acids upon hydrolysis. The substituted benzoic acids that are hydrolysis products of candidate esters are expected to be excreted in the urine as the glucuronic acid, glycine or sulphate conjugate or at a minor extent unchanged. The same metabolic route is proposed for the candidate acid [FL-no: 08.087] (4-hydroxy-3,5-dimethoxybenzoic acid), [FL-no: 08.132] (3-hydroxybenzoic acid) and [FL-no: 08.133] (3,4-dihydroxybenzoic acid).

The candidate substance piperonyl alcohol [FL-no: 02.205] (3,4-methylenedioxybenzylalcohol) is expected to mainly undergo oxidation and conjugation of the side chain, and be excreted as a glycine conjugate. Demethylenation of the methylenedioxy moiety does seem to be only a very minor metabolic path for this compound.

For the six candidate aldehydes in subgroup 2 the main metabolic pathway is presumed to be oxidation to the corresponding acids, followed by glycine and glucuronic acid conjugation and excretion. The reduction to alcohols is a minor metabolic route and the oxidative pathway clearly dominates. To a minor extent O-demethylation followed by conjugation may occur.

The main metabolite of gallic acid (3,4,5-trihydroxy-benzoic acid) [FL-no: 08.080] is expected to be 4-O-methyl gallic acid, i.e. the product of O-methylation. Decarboxylation to pyrogallol (1,2,3-trihydroxybenzene) may occur as a very minor pathway, but no further dehydroxylation to catechol has been observed.

III.4. Summary and Conclusions

It is expected that esters in subgroup 1 and 2 will be hydrolysed *in vivo* to their component alcohols and acids. Eight of the 14 esters from subgroup 1 (benzyl derivatives) will yield benzyl alcohol which has previously been evaluated by the JECFA (JECFA, 1996b) and SCF (SCF, 2002b). One candidate ester [FL-no: 09.611] (4-isopropylbenzyl acetate) will yield 4-isopropyl benzyl alcohol. This substance has been previously evaluated by the JECFA (JECFA, 2002a). The benzyl alcohols are expected to be oxidized to corresponding benzoic acids, which will be conjugated with glycine and excreted as hippuric acids.

Of the remaining five candidate esters in subgroup 1, four are expected to yield benzoic acid and simple aliphatic alcohols upon hydrolysis [FL-no: 09.656 (3-methylbut-3-enyl benzoate); 09.779 (butyl benzoate); 09.825 (pentyl benzoate); 09.693 (prenyl benzoate)] and one ester [FL-no: 09.631 (methyl 4-methyl benzoate)] will yield 4-methyl-benzoic acid upon hydrolysis. Benzoic acid will mainly be conjugated with glycine and excreted as hippuric acid. Conjugation with glycine may be a saturable process and glucuronide conjugates increase with increasing dose.

One of the substances, [FL-no: 06.017] ((diethoxymethyl)benzene), in subgroup 1 is an acetal. This substance would be expected to yield benzaldehyde and ethanol upon hydrolysis. Benzaldehyde has been evaluated by the JECFA (JECFA, 1996b). Benzaldehyde is expected to be oxidized to benzoic acid.

Subgroup 2 (hydroxy- and alkoxy-substituted benzyl derivatives) includes 13 esters of which one [FL-no: 09.895](4-methoxybenzyl-2-methylpropionate) will yield 4-methoxybenzyl alcohol (*p*-anisyl alcohol) (supporting substance [FL-no: 02.128]) upon hydrolysis. This substance has been evaluated by the JECFA (JECFA, 2002a). 4-Methoxy-benzyl alcohol is expected to be excreted in the urine either unchanged or as a glucuronic acid, glycine or sulphate conjugate. The same metabolic pathway is proposed for the candidate benzyl alcohol derivative [FL-no: 02.164] (4-hydroxy-3,5-dimethoxybenzyl alcohol).

The remaining 12 esters in subgroup 2 [FL-no: 09.362 (ethyl 2-hydroxy-4-methylbenzoate); 09.363 (ethyl 2-methoxybenzoate); 09.367 (ethyl 4-hydroxybenzoate); 09.560 (hex-3(cis)-enyl anisate; hex-3(cis)-enyl-4-methoxybenzoate); 09.570 (hex-3-enyl salicylate; hex-3-enyl-2-hydroxybenzoate); 09.581 (hexyl salicylate; hexyl 2-hydroxybenzoate); 09.623 (methyl 2,4-dihydroxy-3,6-dimethylbenzoate); 09.696 (prenyl salicylate; 3-methyl-but-2-enyl 2-hydroxybenzoate); 09.762 (pentyl salicylate; pentyl 2-hydroxybenzoate); 09.798 (ethyl vanillate; ethyl 3-methoxy-4-hydroxybenzoate); 09.799 (methyl vanillate; methyl 3-methoxy-4-hydroxybenzoate); 09.852 (2-methylbutyl 2-hydroxybenzoate; 2-methylbutyl salicylate)] will yield hydroxy- and/or alkoxy-substituted benzoic acids upon hydrolysis. The substituted benzoic acids that are hydrolysis products of candidate esters are expected to be excreted in the urine unchanged or as the glucuronic acid,

glycine or sulphate conjugate. The same metabolic route is proposed for the candidate acids [FL-no: 08.087] (4-hydroxy-3,5-dimethoxybenzoic acid), [FL-no: 08.132 (3-hydroxybenzoic acid)] and [FL-no: 08.133] (3,4-dihydroxybenzoic acid).

The main metabolic pathway for the acetal [FL-no: 06.104 (vanillin propylene glycol acetal)], after hydrolysis to the aldehyde and for the five candidate aldehydes in subgroup 2 [FL-no: 05.066 (4-ethoxy-3-methoxybenzaldehyde); 05.129 (2-methoxybenzaldehyde); 05.142 (3,4-dihydroxybenzaldehyde); 05.153 (4-hydroxy-3,5-dimethoxybenzaldehyde); 05.158 (3-methoxybenzaldehyde)] is presumed to be oxidation to the corresponding acids, followed by conjugation and excretion. The reduction to alcohols is a minor metabolic route and the oxidative pathway dominates clearly. To a minor extent O-demethylation followed by conjugation may occur.

The candidate substance piperonyl alcohol (3,4-methylenedioxybenzyl alcohol) [FL-no: 02.205] is expected to mainly undergo oxidation and conjugation of the side chain, and be excreted as glycine conjugate. Demethylenation of the methylenedioxy moiety is a very minor metabolic path for this compound, according to published literature.

The main metabolite of gallic acid (3,4,5-trihydroxy-benzoic acid) [FL-no: 08.080] is expected to be 4-O-methyl gallic acid the product of O-methylation. Decarboxilation to pyrogallol (1,2,3-trihydroxybenzene) may occur as a very minor pathway, but no further dehydroxylation to catechol has been observed.

The substance in subgroup 3 is expected to be metabolised in a similar way to the benzaldehyde derivatives in subgroup 2. It is expected that the aldehyde group(s) will undergo oxidation to form the corresponding carboxylic acid which is likely to be conjugated and excreted. The reduction of the alcohol groups may again be a minor pathway, but some steric hindrance may occur making this less likely than for the benzaldehyde derivatives in subgroup 2.

Based on experimental evidence and general knowledge of toxicokinetics of structurally related compounds it is expected that at the reported levels of intake as flavouring substances the candidate substances in FGE.20Rev3 would be rapidly and efficiently absorbed, metabolised to innocuous products and excreted.

ANNEX IV: TOXICITY

Oral acute toxicity data are available for 13 candidate substances of the present flavouring group evaluation from chemical groups 23 and 30, and for 63 supporting substances evaluated by the JECFA at the 57th meeting (JECFA, 2002a). 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
(Benzyl alcohol [02.010])	Rabbit	NR	Oral	1040	(Graham and Kuizenga, 1945)	
	Rat	NR	Oral	2979	(Ciba-Geigy Corp., 1945)	
	Rat	NR	Oral	2080	(Graham and Kuizenga, 1945)	
	Rat	M, F	Gavage	1230	(Jenner et al., 1964)	
	Rat	M, F	Oral	1570	(Damment, 1980)	
	Rat	NR	Oral	3100	(Smyth et al., 1951a)	
	Mouse	NR	Gavage	1580	(Jenner et al., 1964)	
	Mouse	NR	Oral	1150	(Carter et al., 1958)	
(Benzyl formate [09.077])	Rat	M, F	Gavage	1.7 (1.4-2.1) ml/kg bw (1840; 1510-2270)[10]	(Shelanski and Moldovan, 1971d)	
(Benzyl acetate [09.014])	Rabbit	NR	Oral	2640	(Graham and Kuizenga, 1945)	
	Rat	M, F	Gavage	2490	(Jenner et al., 1964)	
	Rat	NR	Oral	3690	(Graham and Kuizenga, 1945)	
(Benzyl propionate [09.132])	Rat	NR	Oral	3300	(Moreno, 1973u)	
(Benzyl butyrate [09.051])	Rat	NR	Oral	1850	(Moreno, 1973v)	
	Rat	M, F	Gavage	2330	(Jenner et al., 1964)	
(Benzyl isobutyrate [09.426])	Rat	M, F	Oral	2850	(Owen, 1971)	
(Benzyl isovalerate [09.458])	Rat	NR	Oral	5000	(Moreno, 1974j)	
Benzyl dodecanoate [09.315]	Rat	NR	Oral	> 5000	(Moreno, 1975m)	
(Benzyl 2-methylcrotonate [09.494])	Rat	NR	Oral	> 5000	(Moreno, 1979d)	
(Benzyl benzoate [09.727])	Cat	NR	Oral	2240	(Graham and Kuizenga, 1945)	
	Rabbit	NR	Oral	2016	(Draize et al., 1948)	
	Rabbit	NR	Oral	1680	(Graham and Kuizenga, 1945)	
	Rabbit	NR	Oral	1800	(Lehman, 1955)	
	Guinea pig	NR	Oral	1120	(Draize et al., 1948)	
	Guinea pig	NR	Oral	1000	(Lehman, 1955)	
	Rat	NR	Oral	1904	(Draize et al., 1948)	
	Rat	NR	Oral	2800	(Graham and Kuizenga, 1945)	
	Rat	NR	Oral	1700	(Lehman, 1955)	
	Mouse	NR	Oral	1568	(Draize et al., 1948)	
	Mouse	NR	Oral	1400	(Lehman, 1955)	

TABLE IV.1: ACUTE TOXICITY

Chemical Name [FL-no]	Species	Sex	Route	LD_{50} (mg/kg bw)	Reference	Comments
(Benzyl phenylacetate [09.705])	Rat	M, F	Oral	> 5000	(Owen, 1971)	
(Benzaldehyde [05.013])	Guinea pig	M, F	Gavage	1000	(Jenner et al., 1964)	
	Rat	M, F	Gavage	1300	(Jenner et al., 1964)	
	Rat	NR	Oral	2850	(Sporn et al., 1967)	
	Rat	M, F	Gavage	1300	(Taylor et al., 1964)	
	Mouse	NR	Diet	1250	(Schafer and Bowles, 1985)	
(alpha,alpha- Dimethoxytoluene [06.003])	Rat	NR	Oral	1220	(Moreno, 1977z)	
(5-Hydroxy-2-phenyl-1,3-dioxane [06.002])	Rat	NR	Oral	3749	(Levenstein, 1974g)	
	Rat	NR	Oral	2750	(Moreno, 1980k)	
(4-Methyl-2-phenyl-1,3-dioxolane [06.032])	Rat	M, F	Gavage	3000	(Lewis and Palanker, 1979b)	
(Benzoic acid [08.021])	Mouse	NR	Oral	1250	(Schafer and Bowles, 1985)	
	Mouse	NR	Oral	1996	(Sado, 1973)	
	Mouse	NR	Gavage	1950	(Shell Oil Company, 1982)	
(Methyl benzoate [09.725])	Rabbit	NR	Oral	2170	(Graham and Kuizenga, 1945)	
	Guinea pig	NR	Gavage	4100	(Kravets-Bekker and Ivanova, 1970)	
	Rat	NR	Oral	2170	(Graham and Kuizenga, 1945)	
	Rat	M, F	Gavage	1350	(Jenner et al., 1964)	
	Rat	NR	Gavage	3500	(Kravets-Bekker and Ivanova, 1970)	
	Rat	M, F	Oral	3420	(Smyth et al., 1954)	
	Mouse	NR	Gavage	3330	(Jenner et al., 1964)	
	Mouse	NR	Gavage	3000	(Kravets-Bekker and Ivanova, 1970)	
Methyl 4-methylbenzoate [09.631]	Rat	M	Gavage	2987	(Dashiell and Hinckle, 1981)	
	Rat	NR	Oral	3300	(Moreno, 1977aa)	
(Ethyl benzoate [09.726])	Rabbit	NR	Oral	2630	(Graham and Kuizenga, 1945)	
	Rat	NR	Oral	2100	(Graham and Kuizenga, 1945)	
	Rat	M, F	Oral	6480	(Smyth et al., 1954)	
Butyl benzoate [09.779]	Mouse	M, F	Gavage	3450	(Bier, 1979)	
	Rat	M, F	Gavage	5140[1]	(Smyth et al., 1954)	
(Hexyl benzoate [09.768])	Rat	NR	Oral	12300	(Smyth et al., 1951a)	
(Isopropyl benzoate [09.770])	Rat	NR	Oral	3730	(Smyth et al., 1951a)	
(Isobutyl benzoate [09.757])	Rat	M, F	Gavage	3685	(Levenstein, 1973e)	
(Isopentyl benzoate [09.755])	Rat	NR	Oral	6330	(Weir and Wong, 1971b)	
(Hex-3-enyl benzoate [09.806])	Rat	NR	Oral	> 5000	(Moreno, 1976u)	
(4-Isopropylbenzyl alcohol [02.039])	Rat	NR	Oral	1020	(Moreno, 1973z)	

TABLE IV.1: ACUTE TOXICITY

Chemical Name [FL-no]	Species	Sex	Route	LD_{50} (mg/kg bw)	Reference	Comments
4-Isopropylbenzyl acetate [09.611]	Rat	NR	Oral	1450	(Moreno, 1978i)	
(4-Ethylbenzaldehyde [05.068])	Rat	M, F	Oral	1970	(Costello, 1984)	
(Tolualdehydes (mixed *o, m, p*) [05.027])	Rat	NR	Oral	2250	(Moreno, 1973w)	
(Tolualdehyde glyceryl acetal [06.012])	Rat	NR	Oral	3400	(Moreno, 1972i)	
(4-Isopropylbenzaldehyde [05.022])	Rat	M, F	Gavage	1390	(Jenner et al., 1964)	
(2,4-Dimethylbenzaldehyde [05.110])	Rat	M, F	Gavage	between 1750 and 5000	(deGroot et al., 1974)	Death of 3/5 male and 3/5 female rats after single dose of 5000 mg/kg bw. No death after repeated doses of 1750 mg/kg bw in 5 male and 5 female rats.
(4-Hydroxybenzaldehyde [05.047])	Rat	NR	Oral	3980	(Dow Chemical Company, 1992b)	
(4-Hydroxybenzoic acid [08.040])	Mouse	NR	Oral	2200	(Sokol, 1952)	
(Salicylic acid [08.112])	Mouse	NR	Oral	908	(Sado, 1973)	
	Rat	NR	Gavage	1050	(Hasegawa et al., 1989)	
Ethyl 4-hydroxybenzoate [09.367]	Rat	F	Gavage	4300	(CTFA, 1980b)	
	Mouse	NR	Oral	8000	(Sokol, 1952)	
	Mouse	NR	Oral	6008	(Sado, 1973)	
	Rabbit	NR[2]	Oral	5000	(Sabalitschka and Neufeld-Crzellitzer, 1954)	
	Dog	NR[2]	Oral	5000	(Sabalitschka and Neufeld-Crzellitzer, 1954)	
(Butyl 4-hydroxybenzoate [09.754])	Mouse	NR	Oral	13200	(Sado, 1973)	
	Mouse	NR	Oral	> 5000	(Sokol, 1952)	
(*p*-Anisyl alcohol [02.128])	Mouse	NR	Oral	1780	(Draize et al., 1948)	
	Rat	NR	Oral	1340	(Draize et al., 1948)	
(Anisyl formate [09.087])	Rat	NR	Oral	1770	(Levenstein, 1975j)	
(Anisyl acetate [09.019])	Rat	M, F	Oral	2250	(Weir and Wong, 1971b)	
(*p*-Anisyl propionate [09.145])	Rat	NR	Oral	3330	(Wohl, 1974d)	
(*p*-Anisyl butyrate [09.058])	Rat	NR	Oral	3400	(Moreno, 1976v)	
(Anisyl phenylacetate [09.706])	Rat	M, F	Gavage	M: 5417 F: 4641	(Reagan and Becci, 1984d)	
	Rat	NR	Oral	> 5000	(Moreno, 1977ab)	
(Veratraldehyde [05.017])	Rat	NR	Oral	2000	(Moreno, 1974k)	
	Rat	M	Oral	2040	(Field, 1979a)	
	Mouse	M	Oral	3200	(Field, 1979b)	
(4-Methoxybenzaldehyde [05.015])	Rat	NR	Oral	3210	(BASF, 1981)	
	Rat	M, F	Gavage	1510	(Jenner et al., 1964)	
	Guinea pig	M, F	Gavage	1260	(Jenner et al., 1964)	
	Rat	M, F	Gavage	1510	(Taylor et al., 1964)	
2-Methoxybenzaldehyde [05.129]	Rat	M	Gavage	2.4 –2.8 ml/kg (2705 –3156)[3]	(Field, 1979b)	
	Rat	NR	Oral	2500	(Moreno, 1977ac)	
	Mouse	M	Gavage	2.4 ml/kg (2705)[3]	(Field, 1979b)	

TABLE IV.1: ACUTE TOXICITY

Chemical Name [FL-no]	Species	Sex	Route	LD_{50} (mg/kg bw)	Reference	Comments
(4-Ethoxybenzaldehyde [05.056])	Rat	NR	Oral	2100	(Moreno, 1977ad)	
(Methyl 2-methoxybenzoate [09.796])	Rat	NR	Oral	3800	(Moreno, 1982l)	
(Methyl 4-methoxybenzoate [09.713])	Rat	NR	Oral	> 5000	(Levenstein, 1975k)	
(Ethyl 4-methoxybenzoate [09.714])	Rat	NR	Oral	2240	(Levenstein, 1975l)	
Gallic acid [08.080]	Mouse	M, F	Oral	> 5000	(Rajalakshsmi et al., 2001)	
	Rabbit	NR	Gavage	5000[4]	(Dollahite et al., 1962)	
(Vanillin [05.018])	Mouse	M	Gavage	1000	(Inouye et al., 1988)	
	Rabbit	NR	Gavage	2600	(Deichmann and Kitzmiller, 1940)	
	Rat	M, F	Gavage	1580	(Taylor et al., 1964)	
	Rat	M, F	Gavage	1580	(Jenner et al., 1964)	
	Rat	M, F	Gavage	3978[5] 3925[6]	(Lheritier, 1992)	
	Rat	M	Gavage	3830	(Monsanto Co., 1955b)	
	Rat	M, F	Oral	3300	(Monsanto Co., 1976)	
	Rat	NR	Oral	4370	(Makaruk, 1980)	
	Guinea pig	M, F	Gavage	1400	(Jenner et al., 1964)	
(Vanillin isobutyrate [09.811])	Rat	M, F	Gavage	4755	(Mallory et al., 1983)	
(Salicylaldehyde [05.055])	Rat	NR	Oral	520	(Moreno, 1977af)	
	Rat	M	Gavage	566	(Eastman Kodak Co., 1991b)	
	Mouse	M	Gavage	504	(Eastman Kodak Co., 1991b)	
(2-Hydroxy-4-methylbenzaldehyde [05.091])	Rat	M, F	Gavage	1520	(Mondino, 1982)	
	Rat	M, F	Oral	1520	(Peano and Berruto, 1982)	
(Methyl salicylate [09.749])	Mouse	M	Gavage	1390	(Ohsumi et al., 1984)	
	Rat	NR	Gavage	1250	(Giroux et al., 1954b)	
	Rat	M, F	Oral	M: 3049 F: 2642	(Hazleton Laboratories, 1982c)	
	Rat	M, F	Gavage	887	(Jenner et al., 1964)	
	Rat	NR	Oral	1220	(Nivikov et al., 1994)	
	Mouse	M	Oral	1110	(Davison et al., 1961)	
	Guinea pig	M, F	Gavage	1060	(Jenner et al., 1964)	
	Mouse	M, F	Gavage	1440	(NTP, 1984a)	
(Ethyl salicylate [09.748])	Rat	NR	Oral	1320	(Moreno, 1976x)	
(Butyl salicylate [09.763])	Rat	NR	Oral	1836	(Levenstein, 1975m)	
(Isobutyl salicylate [09.750])	Rat	NR	Oral	1560	(Moreno, 1973aa)	
(Isopentyl salicylate [09.751])	Rat	NR	Oral	4100	(Moreno, 1982m)	
	Rat	M, F	Oral	> 5000	(Hazleton Laboratories, 1982c)	
Hexyl salicylate [09.581]	Rat	NR	Oral	> 5000	(Moreno, 1975n)	
Hex-3-enyl salicylate [09.570]	Rat	NR	Oral	5000	(Moreno, 1975o)	
Prenyl salicylate [09.696]	Rat	NR	Oral	3200	(Moreno, 1978k)	

TABLE IV.1: ACUTE TOXICITY

Chemical Name [FL-no]	Species	Sex	Route	LD_{50} (mg/kg bw)	Reference	Comments
(Benzyl salicylate [09.752])	Rat	M	Gavage	2227	(Fogleman and Margolin, 1970)	
(Phenethyl salicylate [09.753])	Rat	NR	Oral	> 5000	(Moreno, 1973ab)	
(*o*-Tolyl salicylate [09.807])	Rat	M, F	Oral	1.81 ml/kg (1810)[7]	(Sterner and Chibanguza, 1983)	
(2,4-Dihydroxybenzoic acid [08.076])	Mouse	NR	Intraperitonel	> 800	(Grady et al., 1976)	Highest dose applied was not lethal.
(Ethyl 4-hydroxy-3-methoxybenzyl ether [04.094])	Rat	M, F	Oral	> 2000	(Dufour, 1994)	
(Butyl vanillyl ether [04.093])	Rat	M, F	Gavage	M: 5104 F: 4734	(Buch, 1989)	
(Ethyl vanillin [05.019])	Rat	M, F	Gavage	> 2000	(Jenner et al., 1964)	
	Rat	M	Gavage	4470	(Rhone-Poulenc Inc., 1992b)	
	Rat	M, F	Oral	3500[8]	(Monsanto Co., 1991a)	
	Rat	M, F	Oral	3500[9]	(Monsanto Co., 1991b)	
	Rabbit	NR	Gavage	2000	(Deichmann and Kitzmiller, 1940)	
(Ethyl vanillin isobutyrate [09.933])	Rat	M, F	Oral	> 2000	(Sanders and Crowther, 1997)	
(Piperonyl acetate [09.220])	Rat	NR	Oral	2100	(Moreno, 1973ac)	
(Piperonal [05.016])	Rat	M, F	Gavage	2700	(Jenner et al., 1964)	
	Rat	M, F	Gavage	2700	(Taylor et al., 1964)	
	Rat	M, F	Gavage	2700	(Hagan et al., 1965)	
Prenyl benzoate [09.693]	Rat	NR	Oral	4700	(Moreno, 1978j)	
Prenyl salicylate [09.696]	Rat	NR	Oral	3200	(Moreno, 1978k)	
3,4-Dihydroxybenzoic acid [08.133]	Mice	NR	Oral	> 800	(Grady et al., 1976)	

' NR: Not Reported

[1] Dose range-finding study.

[2] Article published in German. Data point not verified.

[3] Calculation based on a specific gravity of 1.127 g/ml.

[4] Dosed as a 10 % solution.

[5] Calculated using Bliss' method.

[6] Calculated using Litchfield and Wilcox's method.

[7] Calculation based on an assumed specific gravity of 1.0 g/ml.

[8] Administered as a 10 % solution in corn oil.

[9] Administered as a 20 % solution-suspension in corn oil.

[10] Calculated based on a specific gravity of 1.081 g/ml

Subacute / Subchronic / Chronic / Carcinogenic toxicity data are available for five candidate substances of the present flavouring group evaluation from chemical group 23 and 30, and for 18 supporting substances evaluated by the JECFA at the 46th and 57th meeting (JECFA, 1996b; JECFA, 2002a). 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
(Benzyl alcohol [02.010])	Rat; M, F 20	Gavage	0, 50, 100, 200, 400, 800 mg/kg bw/day	13 weeks	100	(NTP, 1989a)	Fully described NTP study. Reduced relative weight gain in females at 200 mg/kg bw/day and more, and at 800 mg/kg bw/day in males.
	Rat; M, F 100	Gavage	0, 200, 400 mg/kg bw/day	103 weeks	ND[7]	(NTP, 1989a)	Fully described NTP study. Survival in both dose group of females was 50 % that of controls. This was, as concluded by NTP, primarily due to an increased number of gavage-related deaths. NTP conclusion on carcinogenicity: no evidence of carcinogenic activity.
	Mouse; M, F 20	Gavage	0, 50, 100, 200, 400, 800 mg/kg bw/day	13 weeks	100	(NTP, 1989a)	Fully described NTP study. Reduced relative weight gain in females at 200 mg/kg bw/day and more, and at 400 and 800 mg/kg bw/day in males.
	Mouse; M, F 100	Gavage	0, 100, 200 mg/kg bw/day	103 weeks	200	(NTP, 1989a)	Fully described NTP study. NTP conclusion on carcinogenicity: no evidence of carcinogenic activity.
(Benzyl acetate [09.014])	Rat; M, F 20	Gavage	0, 62.5, 125, 250, 500, 1000 mg/kg bw/day	13 weeks	250	(NTP, 1986c)	Fully described NTP study. Clinical signs of toxicity in females at 500 mg/kg and in males and females at 1000 mg/kg. Decreased body weight in males at 1000 mg/kg. Deaths at highest dose (1 F/2 M). At necropsy thickened stomach walls in surviving animals (2/8 M, 4/9 F). Hippocampal necrosis in both sexes at 1000 mg/kg (8/8 M, 4/9 F).
	Rat; M 30	Oral	0, 20000, 35000, 50000 mg/kg in the diet (0, 1500, 2700, 3800 mg/kg bw/day)[25, 26]	13 weeks	ND[7]	(Abdo et al., 1998)	Published non-GLP study of good quality. Benzyl acetate caused an increase in mortality, incidence of abnormal neural behavioral signs along with astrocyte hypertrophy and neuronal necrosis in the cerebellum, hippocampus and pyriform cortex of the brain at 35000 mg/kg feed and more. Body weight was statistically significant reduced from 20000 mg/kg feed. These effects were reduced significantly by glycine but not by L-alanine.
	Rat; M, F 20	Oral	0, 3130, 6250, 12500, 25000, 50000 mg/kg in the diet (equivalent to 0, 230, 460, 900, 1750, 3900 mg/kg bw/day for males	13 weeks	460	(NTP, 1993d)	Fully described NTP study. High mortality at highest dose (9/10 F, 9/10 M). Statistically significant decreases in final body weights (over 10 %) observed at 25000 mg/kg feed. Clinical signs of intoxication at 50000 mg/kg feed. At the highest dose level degeneration and necrosis of neurons and glial cells in the cerebellum and hippocampus, renal tubular degeneration and

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
			0, 240, 480, 930, 1870, 4500 mg/kg bw/day for females)				histopathological changes in skeletal thigh muscles. Testicular tubular atrophy in a few males at 12500 mg/kg feed.
	Rat; M, F 120	Oral	0, 3000, 6000, 12000 mg/kg in the diet (equal to 0, 130, 260, 510 mg/kg bw/day in males and 0, 145, 290, 575 mg/kg bw/day in females)	103 weeks	260	(NTP, 1993d)	Fully described NTP study. Slightly reduced mean body weight and feed consumption at the highest dose. NTP conclusion on carcinogenicity: No evidence of carcinogenic activity in male and female Fischer 344/N rats.
	Rat; M, F 100	Gavage	0, 250, 500 mg/kg bw/day	103 weeks	ND[7]	(NTP, 1986c)	Fully described NTP study. No observable adverse effects on mean body weight gain and survival. NTP conclusion on carcinogenicity: Benzyl acetate increased the incidence of acinar-cell adenomas of the endocrine pancreas in male F344/N rats; the gavage vehicle may have been a contributing factor. No evidence of carcinogenic activity for female rats.
	Mouse; M, F 20	Gavage	0, 125, 250, 500, 1000, 2000 mg/kg bw/day for females and 0, 62.5, 125, 250, 500, 1000 mg/kg bw/day for males	13 weeks	500	(NTP, 1986c)	Fully described NTP study. High mortality (8/10) in females at 2000 mg/kg due to gavage error. Clinical signs of toxicity were observed at 1000 mg/kg bw/day. Hippocampal necrosis in one female at 1000 mg/kg.
	Mouse; M, F 20	Oral	0, 3130, 6250, 12500, 25000, 50000 mg/kg in the diet (equal to 0, 425, 1000, 2000, 3700, 7900 mg/kg bw/day for males and 0, 650, 1280, 2980, 4300, 9400 mg/kg bw/day for females)	13 weeks	ND[7]	(NTP, 1993d)	Fully described NTP study. Statistically significant, dose-related decreases in final body weights (over 10 %) observed in all treated animals. Hippocampal necrosis in one male and three females of highest dose group.
	Mouse; M, F 120	Oral	0, 330, 1000, 3000 mg/kg in the diet (equal to 0, 37, 112, 346 mg/kg bw/day in males and 0, 42, 132, 382 mg/kg bw/day in	103 weeks	ND[7]	(NTP, 1993d)	Fully described NTP study. Decreased mean body weights (9-13 %) in all treated mice except for females at 330 mg/kg feed (statistics not reported). Statistically significant, dose-related incidence and severity of non-neoplastic lesions of the nasal mucosa and glands in all treated animals. NTP conclusion on carcinogenicity: No evidence of carcinogenic activity in male and

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
			females)				female B6C3F$_1$ mice.
	Mouse; M, F 100	Gavage	0, 500, 1000 mg/kg bw/day	103 weeks	ND[7]	(NTP, 1986c)	Fully described NTP study. No observable adverse effects on mean body weight gain and survival. NTP conclusion on carcinogenicity: For male and female B6C3F$_1$ mice there was evidence of carcinogenicity, in that benzyl acetate caused an increased incidence of hepatocellular neoplasms particularly adenoma, and squamous cell neoplasms (papillomas) of the forestomach.
(Benzyl butyrate [09.051])	Rat; M, F 12	Oral[1]	0, 26.5 mg/kg bw/day[24]	12 weeks	26.5	(Oser, 1957)	Unpublished non-GLP study.with limited details on study protocol and results.
(Benzaldehyde [05.013])	Rat; M, F 20	Gavage	0, 50, 100, 200, 400, 800 mg/kg bw/day	13 weeks	200	(Kluwe et al., 1983; NTP, 1990c)	Fully described NTP study. High mortality in males (6/10) and death of three females in the highest group. Death of one female at 400 mg/kg. Reduced terminal body weights (26 %) in males at highest dose. Treatment-related lesions in the brain, forestomach, liver and kidney in both sexes at 800 mg/kg including necrosis and degeneration of cerebellum, necrosis of neurons of the hippocampus, hyperplasia and/or hyperkeratosis of sqamous epithelium of the forestomach, degeneration and/or necrosis of liver and kidney.
	Rat; M, F 10	Oral	0, 1% in the diet (0, 500 mg/kg bw/day)[21]	16 weeks	500[2]	(Hagan et al., 1967)	Published summary of subacute and/or chronic toxicity studies on 48 food flavourings carried out by the FDA. Validity of the results cannot be evaluated. Results not reported in detail but summarised in a table only.
	Rat; M, F 10	Oral	0, 0.1% in the diet (0, 50 mg/kg bw/day)[21]	27 - 28 weeks	50[2]	(Hagan et al., 1967)	Published summary of subacute and/or chronic toxicity studies on 48 food flavourings carried out by the FDA. Validity of the results cannot be evaluated. Results not reported in detail but summarised in a table only.
	Rat; M, F 100	Gavage	0, 200, 400 mg/kg bw/day	103 weeks	ND[7]	(NTP, 1990c)	Fully described NTP study. Significantly reduced survival of the high dose group rats after one year and significant dose-related trend to reduced survival in the treated groups of males. Body weight not affected. NTP conclusion on carcinogenicity: No evidence of carcinogenic activity for male or female F344/N rats.
	Mouse; M, F 20	Gavage	0, 75, 150, 300, 600, 1200 mg/kg bw/day	13 weeks	300	(Kluwe et al., 1983; NTP, 1990c)	Fully described NTP study. High mortality in males (9/10) and death of one female in the highest group. Reduced mean body weight at 600 mg/kg in males, but not in females. Mild to moderate renal tubular degeneration in all males at 1200 mg/kg and in one male at 600 mg/kg.

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
	Mouse; M, F	Gavage	0, 200, 400 mg/kg bw/day for males, 0, 300, 600 mg/kg bw/day for females	103 weeks	ND[7]	(NTP, 1990c)	Fully described NTP study. No significant effects on body weight and survival observed in any group. Increased incidences of .squamous cell papillomas of the forestomach in both exposure groups with dose-related increased incidences in forestomach hyperplasia. NTP conclusion on carcinogenicity: Some evidence of carcinogenic activity for male or female $B6C3F_1$ mice.
(Methyl benzoate [09.725])	Rat; NR 13	Gavage	0, 0.005, 0.05 mg/kg bw/day	6 months	0.005	(Kravets-Bekker and Ivanova, 1970)	Published non-GLP study of limited quality. Unusual study design and parameters analysed. Limited report of experimental details and results.
(Benzoic acid [08.021])	Mouse; M, F 100	Gavage	0, 80 mg/kg bw/day	3 months	ND[7]	(Shtenberg and Ignat'ev, 1970)	Published non-GLP study of limited quality. Insufficient details on methods and results provided. Reduced weight gain with normal food intake in treated animals.
	Mouse; M, F 50	oral (paste)	0, 40 mg/kg bw/day	17 months	40[2]	(Shtenberg and Ignat'ev, 1970)	Published non-GLP study of limited quality. Insufficient details on methods and results provided.
(Glyceryl tribenzoate [09.812])	Rat; M, F 30	Oral	0, 120, 600, 2600 mg/kg bw/day	90 days	600	(Carson, 1972a)	Unpublished non-GLP study carried out in accordance with OECD guideline 408. Decreased body weight gain (by 23 %) in high dose males with normal food intake.
(Propylene glycol dibenzoate [09.803])	Rat; M, F 30	Oral	0, 130, 630, 2500 mg/kg bw/day	90 days	2500[2]	(Carson, 1972b)	Unpublished non-GLP study carried out in accordance with OECD guideline 408.
(Tolualdehydes (mixed o, m, p) [05.027])	Rat; M, F 30	Oral	36 mg/kg bw/day for males, 43 mg/kg bw/day for females	90 days	36[2]	(Oser et al., 1965)	Published non-GLP study. Very limited details provided.
	Rat; M, F 30	Gavage	0, 50, 250, 500 mg/kg bw/day	13 weeks	250	(Brantom et al., 1972)	Published non-GLP study of good quality, carried out in accordance to OECD guideline 408. Decreased relative pituitary weight in females at 500 mg/kg bw/day. Reduced weight and relative weight of small intestine in all treated groups. However, this effect was not dose-related and not reproduced in a second study.
(2,4-Dimethylbenzaldehyde [05.110])	Rat; M, F 10	Gavage	0, 0.175, 1.75 mg/kg bw/day	2 weeks	1.75[2]	(deGroot et al., 1974)	Unpublished non-GLP study with limited parameters analysed and limited report of results. Increased relative liver weight in high-dose males without histopathological changes.
Ethyl 4-hydroxybenzoate [09.367]	Rat; M, F 24	Oral	0, 2, 8 % in the diet (0, 1050, 5700 mg/kg bw/day)	12 weeks	1050	(Matthews et al., 1956)	Published non-GLP study of very limited quality. Insufficient endpoints analysed. High mortality at highest dose.
	Rat; NR	Oral	0, 0.2, 1, 2 % in	25 weeks	1000	(Sado, 1973)	Published non-GLP study of limited quality.

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
	10 or 11		the diet (0, 100, 500, 1000 mg/kg bw/day)[18]				Experimental details and results insufficiently reported.
(Butyl 4-hydroxybenzoate [09.754])	Rat; NR 10 or 18[3]	Gavage	0, 0.25, 50 mg/kg bw/day	13 - 15 weeks	50[2]	(Ikeda and Yokoi, 1950)	Published non-GLP study in Japanese with English translation. Validity cannot be evaluated due to incomplete report of data.
	Rat; M, F 24	Oral	0, 2, 8 % in the diet (0, 1050, 5700 mg/kg bw/day)	12 weeks	1050	(Matthews et al., 1956)	Published non-GLP study of very limited quality. Insufficient endpoints analysed. High mortality (100 % in males) at highest dose.
	Mouse; M, F 20	Oral	0, 0.6, 1.25, 2.5, 5, 10 % in the diet (0, 900, 1850, 3750, 7500, 15000 mg/kg bw/day)	6 weeks	900	(Inai et al., 1985)	Published non-GLP study of limited quality. Experimental details and results insufficiently reported. Copy partly unreadable.
	Mouse; M, F 100	Oral	0, 0.15, 0.3, 0.6 % in the diet (0, 225, 450, 900 mg/kg bw/day)	102 weeks	900[2]	(Inai et al., 1985)	Published non-GLP study of limited quality. Experimental details and results insufficiently reported. Copy partly unreadable.
(4-Methoxybenzaldehyde [05.015])	Rat; M, F 20	Oral	0, 7.3 mg/kg bw/day	12 weeks	7.3[4, 2]	(Trubek Laboratories Inc., 1958f)	Unpublished study of poor quality with insufficient study protocol and report of data; Eugenol: JECFA evaluation; NOAEL 250 mg/kg bw/day in rat (diet), ADI 2.5 mg/kg bw.
	Rat; M, F 10	Oral	0, 1 % in the diet (0, 500 mg/kg bw/day)[21]	16 weeks	500[2]	(FDA, 1954)	Unpublished study of limited quality. Insufficient study protocol and report of data; part of screening of 50 flavouring substances.
	Rat; M, F 10	Oral	0, 0.1 % in the diet (0, 50 mg/kg bw/day)[21]	28 weeks	50[2]	(FDA, 1954)	Unpublished study of limited quality. Insufficient study protocol and report of data; part of screening of 50 flavouring substances.
	Rat; M, F 10	Oral	0, 1 % in the diet (0, 500 mg/kg bw/day)[21]	15 weeks	500[2]	(Hagan et al., 1967)	Published summary of subacute and/or chronic toxicity studies on 48 food flavourings carried out by the FDA. Validity of the results cannot be evaluated. Results not reported in detail but summarised in a table only.
	Rat; M, F 10	Oral	0, 0.1 % in the diet (0, 50 mg/kg bw/day)[21]	27 - 28 weeks	50[2]	(Hagan et al., 1967)	Published summary of subacute and/or chronic toxicity studies on 48 food flavourings carried out by the FDA. Validity of the results cannot be evaluated. Results not reported in detail but summarised in a table only.
(Methyl 2-methoxybenzoate [09.796])	Rat; M, F 10	Oral	0, 94 mg/kg bw/day	14 days	94[2]	(Van Miller and Weaver, 1987)	Unpublished GLP-study.
3,4-Dihydroxybenzaldehyde [05.142]	Rat; M, F 10	Oral	0, 1.5 % in the diet (0, 1500 mg/kg bw/day)[16]	4 weeks	1500[15]	(Shibata et al., 1990)	Published non-GLP study of limited quality. Experimental details and results insufficiently reported.

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
Gallic acid [08.080]	Rat; M, F 20	Oral	0, 0.2, 0.6, 1.7, 5 % in the diet	13 weeks	0.6 % in the diet (M: 119 F: 128)[17]	(Niho et al., 2001)	Published non-GLP study of good quality. Centrilobular liver cell hypertrophy, reflected in a significant increase in liver weight, was observed in animals of both sexes from 1.7 %.
	Rat; M 5	Oral	0, 2 % in the diet (0, 2000 mg/kg bw/day)[16]	4 weeks	2000[2, 6]	(Hirose et al., 1987)	Published non-GLP study of limited quality. Experimental details and results insufficiently reported.
	Mouse; M, F 12	Oral	0, 1000 mg/kg bw/day	28 days	1000[2]	(Rajalakshsmi et al., 2001)	Published non-GLP study of acceptable quality.
(Vanillin [05.018])	Rat; M, F 10	Oral	0, 1 % in the diet (0, 500 mg/kg bw/day)[21]	16 weeks	500[2]	(FDA, 1954)	Unpublished study of limited quality. Insufficient study protocol and report of data; part of screening of 50 flavouring substances.
	Rat; M, F 10	Oral	0, 0.1 % in the diet (0, 50 mg/kg bw/day)[21]	27 - 28 weeks	50[2]	(Hagan et al., 1967)	Published summary of subacute and/or chronic toxicity studies on 48 food flavourings carried out by the FDA. Validity of the results cannot be evaluated. Results not reported in detail but summarised in a table only.
	Rat; M 5	Oral	0, 2, 5 % in the diet (0, 1000, 2500 mg/kg bw/day)[21]	1 year	2500[2]	(Hagan et al., 1967)	Published summary of subacute and/or chronic toxicity studies on 48 food flavourings carried out by the FDA. Validity of the results cannot be evaluated. Results not reported in detail but summarised in a table only.
	Rat; NR 8	Oral	64 mg/kg bw/day	70 days	ND	(Deichmann and Kitzmiller, 1940)	Published non-GLP study with insufficient quality of experimental design and report of data. No adequate controls. No NOAEL could be derived.
	Rat; NR 8	Oral	20 mg/kg bw/day	126 days	20[2]	(Deichmann and Kitzmiller, 1940)	Published non-GLP study with insufficient quality of experimental design and report of data. No adequate controls.
	Rat; M, F 10	Oral	0, 1 % in the diet (0, 500 mg/kg bw/day)[21]	16 weeks	500[2]	(Hagan et al., 1967)	Published summary of subacute and/or chronic toxicity studies on 48 food flavourings carried out by the FDA. Validity of the results cannot be evaluated. Results not reported in detail but summarised in a table only.
	Rat; M, F 24	Oral	0, 0.5, 1, 2 % in the diet (0, 250, 500, 1000 mg/kg bw/day)[21]	2 years	1000[2]	(Hagan et al., 1967)	Published summary of subacute and/or chronic toxicity studies on 48 food flavourings carried out by the FDA. Validity of the results cannot be evaluated. Results not reported in detail but summarised in a table only.
	Rat; M, F 10	Oral	0, 0.1 % in the diet (0, 50 mg/kg bw/day)[21]	28 weeks	50[2]	(FDA, 1954)	Unpublished study of limited quality. Insufficient study protocol and report of data; part of screening of 50 flavouring substances.
	Rat; NR 12	Gavage	300 mg/kg bw twice a week	14 weeks	300[2, 5]	(Deichmann and Kitzmiller, 1940)	Published non-GLP study with insufficient quality of experimental design and report of data. No adequate controls.
	Rat; M	Oral	0, 0.1, 0.5, 1.0 %	26 weeks	437[2]	(Monsanto Co.,	Unpublished non-GLP study of limited quality.

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
	10		in the diet (0, 40, 214, 437 mg/kg bw/day)[20]			1955a)	Insufficient analysis of clinical-chemical parameters. Results of microscopic examination not reported.
	Rabbit; NR 3	Oral	240 mg/kg bw/day	56 or 126 days[19]	240[2]	(Deichmann and Kitzmiller, 1940)	Published non-GLP study with insufficient quality of experimental design and report of data. No adequate controls.
	Rabbit; NR 1	Oral	83 mg/kg bw/day for 14 days 103 mg/kg bw/day for 61 days	14 or 61 days	ND	(Deichmann and Kitzmiller, 1940)	Published non-GLP study with insufficient quality of experimental design and report of data. No adequate controls. The animal treated with 83 mg/kg bw/day died due to glycerol poisoning (solvent), the animal treated with 103 mg/kg bw/day suffered from anemia, diarrea and showed a reduced wt gain.
	Dog; M, F 2	Capsule	0, 25, 100 mg/kg bw/day	26 weeks	100	(Monsanto Co., 1955a)	Unpublished non-GLP study of limited quality. Insufficient analysis of clinical-chemical parameters. Results of microscopic examination not reported.
4-Hydroxy-3,5-dimethoxybenzoic acid [08.087]	Rat; M 5	Oral	0, 2 % in the diet (0, 2000 mg/kg bw/day) [16]	4 weeks	2000[2, 6]	(Hirose et al., 1987)	Published non-GLP study of limited quality. Experimental details and results insufficiently reported.
(Methyl salicylate [09.749])	Rat; M, F 20	Oral	0, 0.1, 1.0 % in the diet (0, 50, 500 mg/kg bw/day)[21]	17 weeks	500[2]	(Webb and Hansen, 1963)	Published non-GLP FDA-study of good quality. Preliminary study in extensive toxicological evaluation. Study protocols fully described, results not reported in detail but only summarised in text.
	Rat; M, F 6	Oral	0, 2 % in the diet (0, 1000 mg/kg bw/day)[21]	Up to 71 days	1000[7]	(Webb and Hansen, 1963)	Published non-GLP FDA-study of good quality. Supplemental study in extensive toxicological evaluation to analyse bone effects. Study protocols fully described, results not reported in detail but only summarised in text.
	Rat; NR	Oral	1.12, 2 % in the diet (560, 1000 mg/kg bw/day)[21, 23]	10 weeks	< 560	(Harrison et al., 1963)	Only abstract available. No details reported. Study carried out to further investigate the increase of cancellous bone reported by Webb & Hansen, 1963). Effects confirmed at levels of 2 % and 1.12 % but not at lower (unspecified) levels.
	Rat; M, F 10	Oral	0, 0.2, 0.36, 0.63, 1.13, 2.0 % in the diet (0, 100, 180, 320, 560, 1000 mg/kg bw/day)[21]	11 weeks	180	(Abbott and Harrison, 1978)	Unpublished non-GLP study of limited quality. Insufficient report of experimental details and results. Very limited number of analysed parameters. Decreased weight gain at 320 mg/kg, decreased weight gain and increased bone density from 560 mg/kg.
	Rat; M 5	Oral	0.6, 2.0 % in the diet (300, 1000 mg/kg bw/day)[21]	12 weeks	300	(Abbott and Harrison, 1978)	Unpublished non-GLP study of limited quality. Insufficient report of experimental details and results. Very limited number of analysed parameters. No control animals used. 100 % mortality in high dose group after 6 weeks with bone lesions. No such effects at 300 mg/kg.

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 15	Oral	0, 2.0 % in the diet (0, 1000 mg/kg bw/day)[21]	11 weeks	ND[7]	(Abbott and Harrison, 1978)	Unpublished non-GLP study of limited quality. Insufficient report of experimental details and results. Very limited number of analysed parameters. Changes in bone density and 20 % mortality in treated animals. No such effects in controls.
	Rat; M 10[8]	Oral	0, 0.6, 2.0 % in the diet (0, 300, 1000 mg/kg bw/day)[21]	6 weeks	ND[9]	(Abbott and Harrison, 1978)	Unpublished non-GLP study of limited quality. Insufficient report of experimental details and results. Very limited number of analysed parameters. Deaths occurred among the rats at the high dose ad libitum and in all members of the pair-fed groups (incl. controls).
	Rat; M, F 20	Oral	0, 0.6., 0.9, 1.2, 2.0 % in the diet (0, 300, 450, 600, 1000 mg/kg bw/day)[21]	11 weeks	450	(Abbott and Harrison, 1978)	Unpublished non-GLP study of limited quality. Insufficient report of experimental details and results. Very limited number of analysed parameters. Weekly X-ray evaluation of animals for progression of bone changes. Bone changes at 1000 mg/kg from week 2, at 600 mg/kg from week 5.
	Dog; M, F 2	Oral	50, 100, 250, 500, 800, 1200 mg/kg bw	Up to 59 days	250	(Webb and Hansen, 1963)	Published non-GLP FDA-study of good quality. Preliminary study in extensive toxicological evaluation. Study protocols fully described, results not reported in detail but only summarised in text.
	Dog; M, F 6	Oral	0, 150, 300, 500, 800 mg/kg bw/day[22]	7.5 months[10]	ND[7]	(Abbott and Harrison, 1978)	Unpublished non-GLP study of limited quality. Insufficient report of experimental details and results. Very limited number of analysed parameters. 100 % mortality at highest dose, only 2 surviving animals at 500 mg/kg. At lower doses no effects on body weight and hematological parameters. Increased liver and kidney weight at all doses but not after recovery diet. No NOEL could be derived.
	Dog; M, F 8, 12[11]	Oral	0, 50, 100, 170 mg/kg bw/day	6 months[12]	170[2]	(Abbott and Harrison, 1978)	Unpublished non-GLP study of limited quality. Insufficient report of experimental details and results. Very limited number of analysed parameters. No adverse effects at any dose.
	Rat; M, F 50	Oral	0, 0.1, 0.5, 1.0, 2.0 % in the diet (0, 50, 250, 500, 1000 mg/kg bw/day)[21]	2 years	50	(Webb and Hansen, 1963)	Published non-GLP FDA-study of good quality. Study protocols fully described, results not reported in detail but only summarised in text.
	Rat; M, F 50	Oral	0.07, 0.21 % in the diet (0, 35 100)[21]	2 years	100[2]	(Packman et al., 1961)	Only abstract available with very limited report of study details and results.
	Dog; M, F 4	Oral	0, 50,150, 350 mg/kg bw/day	2 years	50	(Webb and Hansen, 1963)	Published non-GLP FDA-study of good quality. Study protocols fully described, results not reported in detail but only summarised in text.

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
(Isopentyl salicylate [09.751])	Rat; M, F 30	Oral	0, 0.005, 0.05, 0.5 % in the diet (0, 4.7, 46, 420 mg/kg bw/day in males and 0, 4.8, 47, 480 mg/kg bw/day in females)	13 weeks	5	(Drake et al., 1975)	Published non-GLP study of good quality. Reduced body weight gain at 0.5 % associated with reduced food intake. Increased relative kidney weight without any histophathological changes at 0.05 and 0.5 % in the diet.
	Rat; M, F 10	Oral	0. 0.5 % in the diet (0, 420 mg/kg bw/day in males and 0, 480 mg/kg bw/day in females)[13]	98 days	420	(Drake et al., 1975)	Published non-GLP study of good quality. Reduced body weight gain at 0.5 % associated with reduced food intake. Only body weight gain analysed.
(Ethyl vanillin [05.019])	Rat; M 5	Oral	0, 2, 5 % in the diet (0, 1000, 2500 mg/kg bw/day)[21]	1 year	2500[2]	(Hagan et al., 1967)	Published summary of subacute and/or chronic toxicity studies on 48 food flavourings carried out by the FDA. Validity of the results cannot be evaluated. Results not reported in detail but summarised in a table only.
	Rat; NR 8	Oral	64 mg/kg bw/day	70 days	64[2]	(Deichmann and Kitzmiller, 1940)	Published non-GLP study with insufficient quality of experimental design and report of data.. No adequate controls. No NOAEL could be derived.
	Rat; NR 8	Oral	20 mg/kg bw/day	126 days	20[2]	(Deichmann and Kitzmiller, 1940)	Published non-GLP study with insufficient quality of experimental design and report of data. No adequate controls.
	Rat; NR 12	Gavage	300 mg/kg bw twice a week	14 weeks	300[2, 5]	(Deichmann and Kitzmiller, 1940)	Published non-GLP study with insufficient quality of experimental design and report of data. No adequate controls.
	Rat; M, F 40	Oral	0, 500, 1000, 2000 mg/kg bw/day	13 weeks	500	(Hooks et al., 1992)	Unpublished report summarised by the JECFA (1996a). Study has been reported to be designed in accordance with toxicological principles for the safety assessement of food additives established by the US FDA in 1982. Limited report of experimental details and results.
	Rat; M, F 24	Oral	0, 0.5, 1, 2 % in the diet (0, 250, 500, 1000 mg/kg bw/day)[21]	2 years	1000[2]	(Hagan et al., 1967)	Published summary of subacute and/or chronic toxicity studies on 48 food flavourings carried out by the FDA. Validity of the results cannot be evaluated. Results not reported in detail but summarised in a table only.
	Rabbit; NR 3	Oral	240 mg/kg bw/day	56 or 126 days[19]	240[2]	(Deichmann and Kitzmiller, 1940)	Published non-GLP study with insufficient quality of experimental design and report of data. No adequate controls.
	Rabbit; NR 1	Oral	15-49 mg/kg bw/day (15, 15, 32, 41, 49 mg/kg day for 15,	15 - 49 days	41[2]	(Deichmann and Kitzmiller, 1940)	Published non-GLP study with insufficient quality of experimental design and report of data. No adequate controls. The animal treated with 15 mg/kg bw/day for 15 days died due to glycerol

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
			31, 17, 31, 49 days, respectively)				poisoning (solvent), the animal treated with 49 mg/kg bw/day for 49 days suffered from anemia, diarrea and showed a reduced wt gain.
(Piperonal [05.016])	Rat; M, F 20	Oral	0, 16 mg/kg bw	12 weeks	16[14, 2]	(Trubek Laboratories Inc., 1958f)	Unpublished study of poor quality with insufficient study protocol and report of data; Eugenol: JECFA evaluation; NOAEL 250 mg/kg bw/day in rat (diet), ADI 2.5 mg/kg bw.
	Rat; M, F 10	Oral	0, 1 % in the diet (0, 500 mg/kg bw/day)[21]	16 weeks	500[2]	(FDA, 1954)	Unpublished study of limited quality. Insufficient study protocol and report of data; part of screening of 50 flavouring substances.
	Rat; M, F 10	Oral	0, 0.1 % in the diet (0, 50 mg/kg bw/day)[21]	28 weeks	50[2]	(FDA, 1954)	Unpublished study of limited quality. Insufficient study protocol and report of data; part of screening of 50 flavouring substances.
	Rat; M, F 10	Oral	0, 1 % in the diet (0, 500 mg/kg bw/day)[21]	15 weeks	500[2]	(Hagan et al., 1967)	Published summary of subacute and/or chronic toxicity studies on 48 food flavourings carried out by the FDA. Validity of the results cannot be evaluated. Results not reported in detail but summarised in a table only.
	Rat; M, F 10	Oral	0, 0.1 % (0, 50 mg/kg bw/day)[21]	27 - 28 weeks	50[2]	(Hagan et al., 1967)	Published summary of subacute and/or chronic toxicity studies on 48 food flavourings carried out by the FDA. Validity of the results cannot be evaluated. Results not reported in detail but summarised in a table only.
	Rat; M, F NR	Oral	0, 0.1 % in the diet (0, 50 mg/kg bw/day)[21]	28 weeks	50[2]	(Hagan et al., 1965)	Published report of subchronic and/or chronic toxicity studies on 7 food flavourings carried out by the FDA. Validity of the results cannot be evaluated. No detailed results reported.
	Rat; M, F NR	Oral	0, 1 % in the diet (0, 500 mg/kg bw/day)[21]	16 weeks	500[2]	(Hagan et al., 1965)	Published report of subchronic and/or chronic toxicity studies on 7 food flavourings carried out by the FDA. Validity of the results cannot be evaluated. No detailed results reported.
	Rat; M, F 20 -60	Oral	0, 0.1, 0.5 % in the diet (0, 50, 250 mg/kg bw/day)[21]	1.5 - 2 years	250[2]	(Bär and Griepentrog, 1967)	Published study with incomplete report of experimental details and results.
3,4-Dihydroxybenzoic acid [08.133]	Rat; M, F 10	Oral	0, 1.5 % in the diet (0, 750 mg/kg bw/day)	4 weeks	750	(Shibata et al., 1990)	Published non-GLP study of limited quality. Experimental details and results insufficiently reported.
	Rat, M 10	Oral	0, 50 mg/kg bw/day	2 weeks	50	(Guglielmi et al., 2003)	
	Rat, M 11	Oral	0, 1000 ppm (0, 50 mg/kg bw/day)	28 weeks	50	(Tanaka et al., 1993a; Tanaka et al., 1993b)	
	Rat, M 16	Oral	0, 2000 ppm (0, 100 mg/kg bw/day)	32 weeks	100	(Tanaka et al., 1994)	

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 15	Oral	0, 2000 ppm (0, 100 mg/kg bw/day)	41 weeks	100	(Hirose et al., 1995)	
	Rat, M 15	Oral	0, 1.5 % in the diet (0, 530 mg/kg bw/day)	51 weeks	< 530	(Hirose et al., 1992)	It was noted that the relative liver and kidney weight was significantly increased but this was not further evaluated.

[1] Six aromatic esters (ethyl benzoate, 0.15 ppm; isobutyl benzoate, 25 ppm; benzyl acetate, 18.7 ppm; benzyl butyrate, 25 ppm; ethyl methylphenyl glycidate, 25 ppm; and glycidate M-116, 25 ppm) were blended in the diet.

[2] This study was performed at either a single dose level or multiple dose levels that produced no adverse effects. Therefore, this dose level is the highest dose tested that produced no adverse effects.

[3] Five rats were used per group for low dose. Nine rats were used per group for high dose.

[4] Rats were fed a test mixture containing 123 ppm of eugenol, 10 ppm of 4-methoxybenzaldehyde and 22 ppm of piperonal.

[5] Compound was administered two times per week.

[6] Study evaluated histological changes in the rat forestomach.

[7] This study was performed at a single dose level or at multiple dose levels that produced adverse effects. No NOEL could be derived from that study.

[8] Two groups fed ad libitum (2.0 and 0.6 %) and one group (0.6 %) pair-fed.

[9] The 0.6 % pair-fed rats showed adverse effects; however, those fed ad libitum did not.

[10] Two animals from the 150 mg/kg/day group and three animals from the 300 mg/kg/day group were sacrificed after 6.5 months. Additionally, three animals from the 300 mg/kg/day group discontinued feeding at 6.5 months and recovered for 1.5 months, before being sacrificed.

[11] There were 8 dogs used for the 50 and 100 mg/kg bw doses and 12 dogs used for 167 mg/kg bw.

[12] All animals were fed the substance for six months and then sacrificed, with the exception of two dogs from the high dose group. These animals were fed the substance for four months, placed on control diets for two months, and then sacrificed with the other animals at six months.

[13] Pair-fed study.

[14] Rats were fed a test mixture containing 123 ppm of eugenol, 10 ppm of 4-methoxybenzaldehyde, and 22 ppm of piperonal.

[15] This study evaluated the cell proliferation in the rat forestomach at a single concentration, 1.5 %. There was a statistically significant weight reduction in the females; however, it was deemed to be associated with a palatability problem.

[16] Calculated based on a bw of 250 g and a daily food intake of 12 g.

[17] Calculated by using mean values of body weight and food intake in the 0.2 % group.

[18] Estimated based on FDA (1993). Priority-based assessment of food additives (PAFA) database. Center for food safety and applied nutrition. p. 58.

[19] One animal was treated for 56 days and two animals for 126 days.

[20] Calculated based on a final bw of 453, 444, 427g and a total consumption of test compound of 18.2, 95.0 and 186.8 mg/animal/day, respectively, in the three dose groups.

[21] Calculated based on general assumptions for bw and food intake (e.g. 1 % in diet resulting in 500 mg/kg bw/day). As estimated by JECFA (2002a).

[22] The dogs were given one-half of the dose in the morning and the other half in the afternoon for six days/week.

[23] Also lower dose levels applied, however, not reported in more detail.

[24] Benzyl butyrate administered in the diet in a mixture of six aromatic esters (ethyl benzoate 0.15 mg/kg, isobutyl benzoate 25 mg/kg, benzyl acetate 18.7 mg/kg, benzyl butyrate 25 mg/kg, ethyl methylphenylglycidate 25 mg/kg, glycidate 25 mg/kg) providing intakes of 26.5 mg benzylbutyrate/kg bw/day and a total of 126 mg/kg bw/day of the mixture.

[25] Calculated based on a final bw of 200 g and a daily intake of 15.2 g/rat, as reported for the 20000 mg/kg feed group, for all groups.

[26] Additional groups with either 50000 mg benzyl acetate/kg feed plus 27000 mg glycine/kg feed or 32000 mg L-alanine/kg feed (supplemental L-alanine and glycine were equimolar). The L-alanine group served as amino-nitrogen control.

Developmental and reproductive toxicity data are available for one candidate substance of the present flavouring group evaluation from chemical group 23 and for twelve supporting substances evaluated by the JECFA at the 57th meeting (JECFA, 2002a). Supporting substance listed in brackets.

TABLE IV.3: DEVELOPMENTAL AND REPRODUCTIVE TOXICITY STUDIES

Chemical Name	Study type Durations	Species/Sex No / group	Route	Dose levels	NOAEL (mg/kg bw/day), Including information of possible maternal toxicity	Reference	Comments
(Benzyl alcohol [02.010])	Developmental toxicity: Gestation days 6 - 15	Mouse; F 50	Gavage	0, 550 mg/kg bw/day	Maternal: 550 Foetal: 550	(JECFA, 1996a)	Unpublished study carried out by NIOSH of assumed good quality. Only summary available. In a preliminary experiment clinical signs of maternal toxicity were observed at 1320 mg/kg and reduced number of viable fetuses at 720 mg/kg.
	Developmental toxicity: Gestation days 6 - 13	Mouse; F 50	Gavage	0, 750 mg/kg bw/day	Maternal: ND[1] Foetal: ND[1]	(Hardin et al., 1987)	Published study carried out by NIOSH of assumed good quality. Increased mortality, clinical signs of maternal toxicity, reduced maternal body weight-gain and reduced pup weight in treated group.
(Benzyl acetate [09.014])	Developmental toxicity: Gestation days 6 - 15	Rat; F 20	Gavage	0, 10, 100, 500, 1000 mg/kg bw/day	Maternal: 1000 Foetal: 500	(Ishiguro et al., 1993)	Published non-GLP study. At highest dose, slight, but not significant maternal toxicity observed with slightly reduced maternal weight-gain. Also significantly reduced fetal body weight at highest dose and increased incidence of skeletal internal variations.
	Reproductive toxicity: Sperm morphology and vaginal cytology: 13 weeks	Mouse; M, F 20	Oral	0, 3130, 6250, 12500, 25000, 50000 mg/kg in the diet (equivalent to 0, 425, 1000, 2000, 3700, 7900 mg/kg bw/day for males and 0, 650, 1280, 2980, 4300, 9400 mg/kg bw/day for females)	Reproductive toxicity: Females: 3700 Males: 7900	(Morrissey et al., 1988; NTP, 1993d)	Published report of screening test (SMVCE assay) carried out at end of fully described NTP-study. Statistically significant, dose-related decreases in final body weights (over 10 %) observed in all treated animals. Mean length of estrous cycle significantly greater in high dose than in controls. No effects on male reproductive endpoints.
	Reproductive toxicity. Sperm morphology and vaginal cytology: 13 weeks	Rat; M, F 20	Oral	0, 3130, 6250, 12500, 25000, 50000 mg/kg in the diet (equivalent to 0, 230, 460, 900, 1750, 3900 mg/kg bw/day for males 0, 240, 480, 930, 1870, 4500 mg/kg bw/day for females)	Reproductive toxicity: Females: 4500 Males: 3900	(Morrissey et al., 1988; NTP, 1993d)	Published report of screening test (SMVCE assay) carried out at end of fully described NTP-study. Statistically significant decreases in final body weights (over 10 %) observed at 25000 mg/kg feed. Clinical signs of intoxication at 50000 mg/kg feed. No effects on male and female reproductive endpoints.
(Benzaldehyde [05.013])	Reproductive toxicity 32 weeks (every other day)	Rat; M, F 10	Gavage	2 mg/animal (equivalent to 5 mg/kg bw/day)	ND[1]	(Sporn et al., 1967)	Published non-GLP study of limited quality. Study in Romanian with English summary only. Reduced number of pregnant females among treated animals (no statistics presented).

TABLE IV.3: DEVELOPMENTAL AND REPRODUCTIVE TOXICITY STUDIES

Chemical Name	Study type Durations	Species/Sex No / group	Route	Dose levels	NOAEL (mg/kg bw/day), Including information of possible maternal toxicity	Reference	Comments
(Benzoic acid [08.021])	Developmental toxicity Gestation day: 9	Rat; F 7	Gavage	510 mg/kg bw/day[2]	Maternal: NR Foetal: 510	(Kimmel et al., 1971)	Published non-GLP study of limited validity due to inadequate study design. Study evaluated the influence of benzoic acid on the teratogenic effects of acetylsalicylic acid. No teratogenicity was observed with a single dose of benzoic acid. Pretreatment with benzoic acid increased the teratogenicity of acetylsalicylic acid by increasing the salicylate concentration in the embryo and serum.
	Four generation reproduction study: Continuously in diet	Rat; M, F 40	Oral	0, 0.5, 1.0 % in the diet (0, 275, 550 mg/kg bw/day)[3]	550	(Kieckebusch and Lang, 1960)	Published non-GLP study of acceptable quality. Limited male reproduction parameters analysed.
(Salicylic acid [08.112])	Developmental toxicity Gestation days: 8 - 14	Rat; F 20	Oral	0, 0.06, 0.1, 0.2, 0.4 % in the diet (0, 46.4, 77.4, 165.4, 330 mg/kg bw/day) (0, 50.7, 77.4, 165.4, 205.9 mg/kg bw/day)	Maternal: 165.4 Foetal: 77.4 Postnatal: 77.4	(Tanaka et al., 1973a)	Published non-GLP study of acceptable quality. No maternal mortality. At highest dose reduced maternal body weight, signs of clinical toxicity (salivation, piloerection), no alive fetuses in 9/15 dams, reduced litter size. External and skeletal anomalies in fetuses at 0.2 % and more. Internal anomalies at 0.4 %. Skeletal anomalies in postnatal animals at 0.4 %.
	Developmental toxicity Gestation days: 8 - 14	Rat; F 20	Gavage	0, 75, 150, 300 mg/kg bw/day	Maternal: 75 Foetal: 75 Postnatal: 75	(Tanaka et al., 1973b)	Published non-GLP study of acceptable quality. At highest dose 3 animals died, reduced maternal body weight, signs of clinical toxicity (salivation, piloerection), At 150 mg/kg and more significantly reduced uterus weight. No live fetuses at highest dose. At 150 mg/kg and more reduced litter size and fetal weight, internal, skeletal and external anomalies.
Ethyl 4-hydroxybenzoate [09.367]	Developmental toxicity Gestation days: 8 - 15	Rat; F 5 to 12	Oral	0, 0.1, 1, 10 % in the diet (0, 50, 460, 2600 mg/kg bw/day)[4]	Maternal: 460 Foetal: 2600	(Moriyama et al., 1975)	Published non-GLP study of good quality. Sufficient parameters analysed and detailed report of experimental design and results. Reduced terminal maternal body weight at high dose.
	Postnatal development Gestation days: 8 - 15	Rat; F 5 to 12 (46 to 73 fetuses nursed for 1 month)	Oral	0, 0.1, 1, 10 % in the diet (0, 50, 460, 2600 mg/kg bw/day)[4]	Maternal: NR Neonatal: 2600	(Moriyama et al., 1975)	Published non-GLP study of good quality. Sufficient parameters analysed and detailed report of experimental design and results.
	Reproductive toxicity 8 weeks	Rat; M 8	Oral	0, 0.1, 1.0 % in the diet (0, 103, 1043 mg/kg bw/day)	1043	(Oishi, 2004)	Published study of good quality. No effects on weights of reproductive organs, on sperm counts in the testes and

TABLE IV.3: DEVELOPMENTAL AND REPRODUCTIVE TOXICITY STUDIES

Chemical Name	Study type Durations	Species/Sex No / group	Route	Dose levels	NOAEL (mg/kg bw/day), Including information of possible maternal toxicity	Reference	Comments
							epididymides and sperm morphology. No effect on serum testosterone, LH, FSH
	Uterotrophic assay 3 days	Mouse; F (immature) 7-10	SC Oral	0, 100 mg/kg bw/day 0, 1000 mg/kg bw/day	100 1000	(Hossaiani et al., 2000)	Published non-GLP study of good quality. No estrogenic response observed at the dose levels tested.
	Uterotrophic assay 3 days	Rat; F (immature) 11-16	SC	0, 6, 18, 60, 180 mg/kg bw/day	60	(Lemini et al., 2003)	Published non-GLP study of good quality. Significantly increased uterine weight (wet and dry) at the highest dose (ED_{50} for uterotrophic effect: uterine wet weight 68 mg/kg, uterine dry weight 94 mg/kg).
	Uterotrophic assay 3 days	Mouse; F (immature and ovarectomized adult) 10-25	SC	0, 0.6, 6.0, 18, 60, 180 mg/kg bw/day[5]	0.6	(Lemini et al., 2003)	Published non-GLP study of good quality. Significantly increased uterine weight in immature mice at 6 mg/kg and more and in ovarectomized mice at 18 mg/kg an more (ED_{50} 74 mg/kg and 25 mg/kg, respectively).
	Uterotrophic assay 3 days	Mouse; F (adult ovarectomized) 6	SC	0, 60, 180 mg/kg bw/day	ND[1]	(Lemini et al., 2004)	Published study of good quality with full report of experimental details and results. Uterotrophic effect with estrogenic histological changes in uteri in both dose groups.
(Butyl *p*-hydroxybenzoate [09.754])	Reproductive toxicity 8 weeks	Rat; M 8	Oral	0, 0.01, 0.10, 1.0% in the diet (0, 10, 100, 1000 mg/kg bw/day)	ND[1]	(Oishi, 2001)	Published non-GLP study of good quality. Reduced cauda epididymal sperm reserve and daily sperm production in testis significantly reduced in all treated groups. Dose-dependently reduced serum testosterone concentration and absolute and relative epididymis weight (significant at 0.1 % or more).
	Reproductive toxicity 10 weeks	Mouse; M 8	Oral	0, 0.01, 0.10, 1.0% in the diet (0, 14, 146, 1500 mg/kg bw/day)	ND[1]	(Oishi, 2002)	Published non-GLP study of good quality. Absolute and relative weight of epididymides significantly increased at 1.0 %. Dose-dependent decrease of both round and elnongated spermatid counts. Significantly decreased elongated spermatid counts in all treated groups. Dose-dependant decrease of serum testosterone concentrations (significant at 1.0 %).
	Uterotrophic assay 3 days	Rat; F (ovarectomized) 5	Oral SC	4, 40 , 400, 800, 1200 mg/kg bw[6] 40, 200, 400, 600, 800, 1000, 1200 mg/kg bw/day[6]	1200 40	(Routledge et al., 1998)	Published study of good quality. No estrogenic activity found after oral administration (small, statistically insignificant increase in uterus wet weight) in immature rats. Positive response after s.c. administration. In the

TABLE IV.3: DEVELOPMENTAL AND REPRODUCTIVE TOXICITY STUDIES

Chemical Name	Study type Durations	Species/Sex No / group	Route	Dose levels	NOAEL (mg/kg bw/day), Including information of possible maternal toxicity	Reference	Comments
							same study also an *in vitro* estrogenic activity test in yeast was carried out in which butyl paraben was found to be weakly estrogenic.
	Uterotrophic assay 3 days	Mouse; F (immature) 10	SC	0, 100 mg/kg bw/day	100	(Hossaiani et al., 2000)	Published non-GLP study of good quality. No estrogenic response observed at the dose level tested.
	Uterotrophic assay 3 days	Rat; F (immature) 10	SC	0, 100, 400, 600 mg/kg bw/day	400	(Hossaiani et al., 2000)	Published non-GLP study of good quality. A weak estrogenic response with a significantly increase relative uterus weight was observed at 600 mg/kg. At 100 and 400 mg/kg the uterus wet weight was significantly increased, but not the relative uterus weight.
	Uterotrophic assay 3 days	Rat; F (immature) 11-16	SC	0, 7, 21, 70, 210 mg/kg bw/day	21	(Lemini et al., 2003)	Published non-GLP study of good quality. Significantly increased uterine wet weight at 70 mg/kg and more, and uterine dry weight at 210 mg/kg (ED_{50} for uterotrophic effect: uterine wet weight 87 mg/kg, 338 mg/kg uterine dry weight).
	Uterotrophic assay 3 days	Mouse; F (immature and ovarectomized adult) 6-16	SC	0, 0.7, 7.0, 21, 70, 210 mg/kg bw/day[5]	0.7	(Lemini et al., 2003)	Published non-GLP study of good quality. Significantly increased uterine weight in immature mice at 7 mg/kg and more and in ovarectomized mice at 21 mg/kg an more (ED_{50} 65 mg/kg and 22 mg/kg, respectively).
	Uterotrophic assay 3 days	Mouse; F (adult ovarectomized) 6	SC	0, 70, 210 mg/kg bw/day	ND[1]	(Lemini et al., 2004)	Published study of good quality with full report of experimental details and results. Uterotrophic effect with estrogenic histological changes in uteri in both dose groups.
	Developmental toxicity Gestation days: 6 - 19	Rat; F 25	Gavage	0, 10, 100, 1000 mg/kg bw/day	Maternal: 100 Foetal: 1000	(Daston, 2004)	Published study of good quality with full report of experimental details and results. Maternal food consumption and weight gain significantly decreased at highest dose.
(Methyl salicylate [09.749])	Reproductive toxicity: continuous breeding (RACB) 18 weeks[7]	Mouse; M, F 40	Gavage	0, 100, 250, 500 mg/kg bw/day	250	(NTP, 1984a)	Fully described NTP-study. Significant slight decrease in mean number of litters, number of pups per litter, mean number of pups born alive per litter and mean live pup weight at highest dose. The experiment was unable to discriminate which sex was affected in reproduction.
(Veratraldehyde [05.017])	Reproductive and	Rat; F	Gavage	0, 80, 400, 800 mg/kg bw/day	Maternal: ND[1]	(Vollmuth et al., 1990)	Unpublished study with limited report of

TABLE IV.3: DEVELOPMENTAL AND REPRODUCTIVE TOXICITY STUDIES

Chemical Name	Study type Durations	Species/Sex No / group	Route	Dose levels	NOAEL (mg/kg bw/day), Including information of possible maternal toxicity	Reference	Comments
	developmental toxicity: 1 week before mating until 4 days post parturition	10			Foetal: 800		results. Summary of study published as abstract. Validity of the study cannot be evaluated.
(Vanillin [05.018])	Reproductive and developmental toxicity: 1 week before mating until 4 days post parturition	Rat; F 10	Gavage	0, 125, 250, 500 mg/kg bw/day	Maternal: 250 Foetal: 500	(Vollmuth et al., 1990)	Unpublished study with limited report of results. Summary of study published as abstract. Validity of the study cannot be evaluated.
(2,4-Dihydroxybenzoic acid [08.076])	Teratogenicity: Gestation day 9	Rat; F 10	SC	0, 380 mg/kg bw/day	380	(Koshajki and Schulert, 1973)	Published study of limited quality. Limited report of experimental details and results. Insufficient endpoints analysed.
	Teratogenicity Gestation day 11	Rat; F Not Reported	SC	0, 428 mg/kg bw/day (plus 214 mg/kg bw/day after 2 hours)	ND[1]	(Saito et al., 1982)	Published study of limited quality. Unusual study design and limited report of experimental details and results. No effect on plasma Ca level in dams after a single dose. Reduced plasma Ca levels in dams and malformations and foetotoxicity after the additional dose. No effects on maternal reproductive parameters.
(Ethyl vanillin [05.019])	Reproductive and developmental toxicity: 1 week before mating until 4 days post parturition	Rat; F 10	Gavage	0, 200, 1000, 2000 mg/kg bw/day	Maternal: ND[1] Foetal: 2000	(Vollmuth et al., 1990)	Unpublished study with limited report of results. Summary of study published as abstract. Validity of the study cannot be evaluated.
(Piperonal [05.016])	Reproductive and developmental toxicity: 1 week before mating until 4 days post parturition	Rat; F 10	Gavage	0, 250, 500, 1000 mg/kg bw/day	Maternal: 500 Foetal: 500	(Vollmuth et al., 1990)	Unpublished study with limited report of results. Summary of study published as abstract. Validity of the study cannot be evaluated.

[1] This study was performed at a single dose level or at multiple dose levels that produced adverse effects. No NOEL could be derived from that study.

[2] Study evaluated the effects of Aspirin (acetylsalicylic acid) when administered to rats during gestation; however, two dose groups used benzoic acid (510 mg/kg) as a pretreatment and one dose group was administered only benzoic acid. Benzoic acid alone had no effect.

[3] Calculated based on the reported daily intake of 150 mg per animal, equal to 0.45 mMol per 100 g bw, in animals of the high dose group and a molecular weight of 122.12.

[4] Calculated from data on bw and food consumption presented in the article.

[5] The lowest dose was used in immature mice only.

[6] The number of dose groups was comprised from two separate experiments. In the first experiment dose levels of 40 and 400 mg/kg/day were investigated, while in the second experiment dose levels of 800 and 1200 mg/kg/day were explored.

[7] Reproductive assessment by continuous breeding (RACB) consisted of a 7-day premating phase, a 98-day cohabitation period and a 21-day segregation period.

In vitro mutagenicity/genotoxicity data are available for nine candidate substances of the present flavouring group evaluation from chemical groups 23 and 30 and for 28 supporting substances evaluated by the JECFA at the 46th and 57th meeting (JECFA, 1996b; JECFA, 2002a) and for one related substance. Supporting substances are listed in brackets.

Table IV.4: GENOTOXICITY (*in vitro*)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
(Benzyl alcohol [02.010])	Ames test (preincubation method)	*S. typhimurium* TA92; TA94; TA98; TA100; TA1535; TA1537	Up to 10,000 µg/plate (6 concentrations)	Negative[1]	(Ishidate et al., 1984)	Published study in accordance with OECD guideline 471. Although some details of results are not reported the study is considered valid.
	Ames test (plate incorporation method)	*S. typhimurium* TA100	1000 µg/plate	Negative[2]	(Ball et al., 1984)	
	Ames test (plate incorporation method)	*S. typhimurium* TA98; TA100	Not reported	Negative[2]	(Rogan et al., 1986)	
	Ames test (preincubation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537	6666 µg/plate	Negative[1]	(Mortelmans et al., 1986)	
	Ames test (plate incorporation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537	3 µmole/plate	Negative[1]	(Florin et al., 1980)	
	Ames test (plate incorporation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	50,000 µg/plate[4]	Negative[1]	(Heck et al., 1989)	Published non-GLP study. No information concerning a possible cytotoxic effect nor on the number of concentrations tested. The test guidelines do not require more than 5 mg/plate. Due to the lack of some important details of study design and results the validity of the study cannot be evaluated.
	Ames test (plate incorporation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	5 µl/plate	Negative[2]	(Milvy and Garro, 1976)	
	Ames test (plate incorporation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537	0, 100, 333, 1000, 3333, 6666 µg/plate	Negative[1]	(NTP, 1989a)	Valid study in accordance with OECD guideline 471 (except that only four strains were used). Cytotoxicity was reported at the highest concentration tested.
	Ames test (plate incorporation method)	*S. typhimurium* TA97; TA102	1000 µg/plate	Negative[1]	(Fujita et al., 1992)	
	Ames test (plate incorporation method)	*S. typhimurium* TA98; TA1535	5 µM/plate	Negative[1]	(Wiessler et al., 1983)	
	Mutation assay	*Escherichia coli* WP2 *uvr*A	1000 to 8000 µg/plate	Negative	(Yoo, 1986)	Study published in Japanese with English abstract. Data extracted from tables. Validity of the study cannot be evaluated. No information on the use of metabolic activation.
	Rec assay	*B. subtilis* M45 (rec^-), H17 (rec^+)	21 µg/disc	Negative	(Oda et al., 1979)	Study published in Japanese without English abstract. Data extracted from tables. Validity of the study cannot be evaluated.
	Rec assay	*B. subtilis* M45 (rec^-), H17 (rec^+)	10 µg/disc	Positive	(Kuroda et al., 1984b)	Study published in Japanese with English abstract. Data extracted from figure. Validity of the study cannot be evaluated. Inhibition of growth was reported.
	Rec assay	*B. subtilis* M45 (rec^-), H17 (rec^+)	20 µl/disc	Positive	(Yoo, 1986)	Study published in Japanese with English abstract. Data extracted from tables. Validity of the study cannot be evaluated. A weak positive result (i.e. 4 mm ≤ D < 8 mm). was reported (D=5 mm). No information on the use of metabolic activation.
	Chromosomal aberration test	Chinese hamster fibroblast cells	1000 µg /ml[4] (three	Negative[2]	(Ishidate et al., 1984)	Published study carried out only in the absence of

Table IV.4: GENOTOXICITY (*in vitro*)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
			concentrations, max. concentration inducing 50 % cell-growth inhibition)			metabolic activation. Thus, study is not considered valid. Cells were exposed for 24 and 48 hours. Negative response for chromosomal aberrations and polyploidization.
	Chromosomal aberration test	Chinese hamster ovary cells	50 to 5000 µg/ml	Equivocal[1]	(Anderson et al., 1990)	Published summary report including detailed results from studies on 42 compounds tested in various laboratories within the NTP in accordance with OECD guideline 473. Lowest effective dose was 4000 µg/ml with and without S9. No dose-response observed. Positive results were not reproducible in all trials. Absence of cytotoxicity reported up to the highest dose.
	Chromosomal aberration test	Chinese hamster ovary cells	50 to 5000 µg/ml	Negative[2] Positive[3]	(NTP, 1989a)	Valid study in accordance with OECD guideline 473. A positive result was reported only in the presence of S9 at relatively high concentrations of 4000 µg/ml in 3 of 4 tests carried out with harvest times between 12 and 18 hours. No data on cytotoxicity reported.
	Sister chromatid exchange assay	Chinese hamster ovary cells	16 to 5000 µg/ml	Positive	(NTP, 1989a)	Valid study in accordance with OECD guideline 479. Dose-related increase in frequency of SCE at concentrations from 500 - 1250 µg/ml (without metabolic activation) and 500 - 4000 µg/ml (with metabolic activation). No data on cytotoxicity reported. Number of chromosomes per cell reduced at 4000 µg/ml with S9.
	Sister chromatid exchange assay	Chinese hamster ovary cells	16 to 1250 µg/ml[2] 16 to 4000 µg/ml[3]	Positive[1]	(Anderson et al., 1990)	Published summary report including detailed results from studies on 42 compounds tested in various laboratories within the NTP in accordance with OECD guideline 479. Significant increase (20 %) in SCE only at the highest doses. No dose-response observed. No second trial using high concentrations to reproduce the positive effects performed. Absence of cytotoxicity reported up to the highest dose.
	Mammalian cell gene mutation test	Mouse lymphoma L5178Y cells	Up to 5000 µg/ml	Questionable	(McGregor et al., 1988a; Myhr et al., 1990)	Published summary report including detailed method and results from study on 72 compounds tested in various laboratories within the NTP in accordance with OECD guideline 476 (however, no colony sizing performed). Positive responses observed in some experiments at concentrations of 3500 and higher. No dose-response was observed. The highest concentration was letal in some experiments. Positive and negative responses could not be reproduced in all experiments.
	Mammalian cell gene mutation test	Mouse lymphoma L5178Y cells	150 to 5000 µg/ml	Negative[3] Positive[2]	(NTP, 1989a)	Valid study in accordance with OECD guideline 476. In one of three trials without S9 a positive result (relative mutant fraction ≥ 1.6) was reported at 4500 µg/ml with relative total growth of 20 %. The concentration of 5000 µg/ml was letal in this trial, whereas in another one of three trials without S9 3500 µg/ml was letal.
	Mutation assay	*E. coli* WP2 *uvr*A	Not reported	Negative	(Kuroda et al., 1984a)	Only abstract available. Methods, test concentrations and detailed results not reported.
	Cytotoxicity assay	Human alveolar tumour cells	0.5 mM	Negative	(Waters et al., 1982)	
	DNA damage assay	Human alveolar tumour cells	0.5 mM	Negative	(Waters et al., 1982)	

Table IV.4: GENOTOXICITY (*in vitro*)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
	DNA damage assay	Rat hepatocytes	10 mM	Negative	(Storer et al., 1996)	Cytotoxicity was reported at the highest concentration tested.
	DNA damage assay	*E. coli* P3478	50 µl/disc	Negative[1]	(Fluck et al., 1976)	
(Benzyl formate [09.077])	Rec assay	*B. subtilis* M45 (rec^-), H17 (rec^+)	20 µl/disc	Positive	(Yoo, 1986)	Study published in Japanese with English abstract. Data extracted from tables. Validity of the study cannot be evaluated. A weak positive result (i.e. 4 mm ≤ D < 8 mm).was reported (D = 4 mm). No information on the use of metabolic activation.
	Mutation assay	*E. coli* WP2 *uvr*A	500 to 4000 µg/plate	Negative	(Yoo, 1986)	Study published in Japanese with English abstract. Data extracted from tables. Validity of the study cannot be evaluated. No information on the use of metabolic activation.
(Benzyl acetate [09.014])	Ames test (preincubation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537	10,000 µg/plate	Negative[1]	(Mortelmans et al., 1986)	
	Ames test (preincubation and plate incorporation method)	*S. typhimurium* TA98; TA100	5000 µg/plate	Negative[1]	(Schunk et al., 1986)	Cytotoxicity was observed at the three highest doses tested.
	Ames test	*S. typhimurium* TA98; TA100; TA1535; TA1537	3 µM/plate	Negative[1]	(Florin et al., 1980)	
	Rec assay	*B. subtilis* M45 (rec^-), H17 (rec^+)	21 µg/disc	Negative	(Oda et al., 1979)	Study published in Japanese without English abstract. Data extracted from tables. Validity of the study cannot be evaluated.
	Rec assay	*B. subtilis* M45 (rec^-), H17 (rec^+)	20 µl/disc	Positive	(Yoo, 1986)	Study published in Japanese with English abstract. Data extracted from tables. Validity of the study cannot be evaluated. A weak positive result (i.e. 4 ≤ D < 8) was reported (D could not clearly be determined). No information on the use of metabolic activation.
	Mutation assay	*E. coli* WP2 *uvr*A	250 to 2000 µg/plate	Negative	(Yoo, 1986)	Study published in Japanese with English abstract. Data extracted from tables. Validity of the study cannot be evaluated. No information on the use of metabolic activation.
	Mammalian cell gene mutation test	Mouse lymphoma L5178Y cells; Human lymphoblast TK6 cells	Mouse cells 0, 250, 500, 1000 µg/ml; Human cells 0, 500, 1000, 1250, 1500 µg/ml	Negative[2] Positive[3]	(Caspary et al., 1988)	Published non-GLP study in accordance with OECD guideline 476 (except that no colony sizing was performed). Thus, the study is considered not fully valid. The lowest significantly effective doses in the presence of S9 were 500 µg/ml in mouse cells and 1500 µg/ml in human cells. Cytotoxicity was reported above 500 µg/ml with and without S9.
	Mammalian cell gene mutation test	Mouse lymphoma L5178Y cells	0-1600 µl/ml (6 concentrations)	Positive[2]	(McGregor et al., 1988a)	Published summary report including detailed method and results from study on 72 compounds tested in various laboratories within the NTP. The study was not in accordance with OECD guideline 476 (no colony sizing performed, only in the absence of metabolic activation) and thus not considered valid. The lowest significantly effective doses was 900 µg/ml at which the relative total growth was 50 %. The highest dose was lethal. A positive response was observed in two of three experiments. No dose-response was observed.
	Mammalian cell gene	Mouse lymphoma L5178Y cells	Not reported	Negative[2]	(Rudd et al., 1983)	Study carried out within a larger NTP project. Only abstract

Table IV.4: GENOTOXICITY (*in vitro*)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
	mutation test			Positive[3,]		available. Validity of the study cannot be evaluated.
	Mammalian cell gene mutation test	Mouse lymphoma L5178Y TK+/- cells	Not reported	Negative[2] Inconclusive[3]	(Honma et al., 1999a)	Published collaborative study on 40 chemicals. Protocol was in accordance with OECD guideline 476, except that no colony sizing was performed. As the results are insufficiently reported, their validity cannot be evaluated. In the presence of S9 metabolic activation one laboratory achieved a statistically significant dose-dependant result, but did not induce mutations greater than three times the spontaneous response. The second laboratory did not obtain a positive response.
	Chromosomal aberration test	Chinese hamster ovary cells	160-1600 µg/ml[2]; 500-5000 µg/ml[3]	Negative[1]	(Galloway et al., 1987a)	Published non-GLP study. Doses were selected based on preliminary assay. Although some details of results are not reported the study is considered valid.
	Chromosomal aberration test	Chinese hamster lung fibroblast cells	2400 µg/ml	Negative[1]	(Matsuoka et al., 1996)	Cytotoxicity was reported at the highest concentration tested.
	Sister chromatid exchange assay	Chinese hamster ovary cells	50-500 µg/ml[2]; 500-5000 µg/ml[3]	Negative[1,]	(Galloway et al., 1987a)	Published non-GLP study. Doses were selected based on preliminary assay. Although some details of results are not reported the study is considered valid.
	Unscheduled DNA synthesis test	Rat hepatocytes	Not reported	Negative	(Mirsalis et al., 1983)	Only abstract available. Methods, test concentrations and detailed results not reported.
	Micronucleus test	Human lymphocytes and hepatoma cell line *Hep G2*	500 µM	Negative[1]	(Kevekordes et al., 2001)	
(Benzyl propionate [09.132])	Rec assay	*B. subtilis* M45 (rec$^-$), H17 (rec$^+$)	21 µg/disc	Negative	(Oda et al., 1979)	Study published in Japanese without English abstract. Data extracted from tables. Validity of the study cannot be evaluated.
(Benzyl benzoate [09.727])	Ames test	*S. typhimurium* TA98; TA100; TA1535; TA1537	3 µM/plate	Negative[1]	(Florin et al., 1980)	
	Ames test (preincubation and plate incorporation method)	*S. typhimurium* TA98; TA100	Up to 5000 µg/plate	Negative[1]	(Schunk et al., 1986)	Cytotoxicity was observed at the three highest doses tested.
(Benzaldehyde [05.013])	Ames test (plate incorporation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	37,500 nl/plate[4]	Negative[1]	(Heck et al., 1989)	Published non-GLP study. No information concerning a possible cytotoxic effect nor on the number of concentrations tested. The test guidelines do not require more than 5 mg/plate. Due to the lack of some important details of study design and results the validity of the study cannot be evaluated.
	Ames test (plate incorporation method)	*S. typhimurium* TA98; TA100	50 to 300 µl/plate	Negative[1]	(Rockwell and Raw, 1979)	Assay of urine samples from rats given benzaldehyde by oral gavage.
	Ames test (plate incorporation method)	*S. typhimurium* TA98; TA100	100 µl/plate	Negative[3]	(Rockwell and Raw, 1979)	Samples assayed prior to administration to rats.
	Ames test	*S. typhimurium* TA98; TA100; TA2637	2000 mg/plate	Negative[1]	(Nohmi et al., 1985)	Article published in Japanese. Data reported from English summary.
	Ames test	*S. typhimurium* TA98; TA100; TA1535; TA1537	3 µM/plate	Negative[1]	(Florin et al., 1980)	
	Ames test (preincubation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537	0, 10, 33, 100, 333, 1000 µg/plate	Negative[1]	(Haworth et al., 1983)	Published summary report including detailed results from studies on 250 compounds tested in various laboratories within the NTP to a large extent in accordance with OECD guideline 471.
	Ames test	*S. typhimurium* TA100; TA102; TA104	3333 µg/plate	Negative[1]	(NTP, 1990c)	

Table IV.4: GENOTOXICITY (*in vitro*)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
	Ames test	*S. typhimurium* TA100	1000 µg/plate	Negative	(Rapson et al., 1980)	The use of metabolic activation was not reported.
	Ames test (preincubation method)	*S. typhimurium* TA98; TA100	Not reported	Negative[1]	(Sasaki and Endo, 1978)	
	Ames test (preincubation method)	*S. typhimurium* TA100; TA102; TA104	Not reported	Negative[1]	(Dillon et al., 1992)	
	Ames test (preincubation method)	*S. typhimurium* TA100	2000 nM/plate	Negative[1]	(Vamvakas et al., 1989)	
	Ames test (preincubation method)	*S. typhimurium* TA97; TA102	1000 µg/plate	Negative[1]	(Fujita et al., 1992)	
	Ames test	*S. typhimurium* TA98; TA100	0.05 to 500 µg/plate	Negative[1]	(Kasamaki et al., 1982)	Published non-GLP study with insufficient report of some details of method and results. Thus, the validity of the study cannot be evaluated.
	Ames test (preincubation method)	*S. typhimurium* TA98; TA1535	5 µM/plate	Negative[1]	(Wiessler et al., 1983)	
	Ames test (preincubation method)	*S. typhimurium* TA97a; TA100; TA102; TA104	Not reported	Negative[1]	(Dillon et al., 1998)	
	Ames test (plate incorporation method)	*S. typhimurium* TA98; TA1537; TA7001; TA7002; TA7003; TA7004; TA7006; Mix of TA7001–7006 TA7005	1000 µg/ml	Negative[1] Negative[2]; Positive[3]	(Gee et al., 1998)	
	Rec assay	*B. subtilis* M45 (rec$^-$), H17 (rec$^+$)	21 µg/disc	Negative	(Oda et al., 1979)	Study published in Japanese without English abstract. Data extracted from tables. Validity of the study cannot be evaluated.
	Rec assay	*B. subtilis* M45 (rec$^-$), H17 (rec$^+$)	Not reported	Negative[2] Positive[3]	(Matsui et al., 1989)	Published non-GLP study with insufficient report of some details of method and results. Thus, the validity of the study cannot be evaluated.
	Unscheduled DNA synthesis test	Rat hepatocytes	251 nl/ml	Negative	(Heck et al., 1989)	Published non-GLP study. Some important details of study design and results are not reported. Thus, the validity of the study cannot be evaluated.
	Mammalian cell gene mutation test	Mouse lymphoma L5178Y cells	12.5 to 800 nl/ml	Negative[2] Positive[3]	(Heck et al., 1989)	Published non-GLP study. Some important details of study design and results are not reported. Thus, the validity of the study cannot be evaluated. Different concentration ranges (12.5-800, 25-600, 400-600 nl/ml) were used in three independent experiments within which positive responses were observed. A 2.8 to 5.2-fold increase in mutant frequency was observed in the presence of S9.
	Mammalian cell gene mutation test	Mouse lymphoma L5178Y cells	0 to 800 µg/ml (6 concentrations)	Positive[2]	(McGregor et al., 1991)	Published summary report including detailed method and results from study on 27 compounds tested in various laboratories within the NTP in accordance with OECD guideline 476 (however, no colony sizing performed). Statistically significant increase in mutant fraction at the highest non-lethal concentration (400 µg/ml) in two experiments. Concentration of 640 and 800 µg/ml were lethal. Thus, significant increases in mutant fraction were close to toxic doses. No dose-response was observed. Since a positive response was observed without S9, no experiment was carried out with S9.

Table IV.4: GENOTOXICITY (*in vitro*)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
	Mammalian cell gene mutation test	Mouse lymphoma L5178Y +/- cells	600 µg/ml	Negative[2]	(Bigger and Clarke, 1991)	
	Chromosomal aberration test	Chinese hamster cells	0, 800, 1000, 1200 µg/ml	Positive[2] Weak positive[3]	(Sofuni et al., 1985)	Article published in Japanese. Data extracted from English summary and tables. Validity of the study cannot be evaluated. Cytotoxicity was observed at the two maximum concentrations tested. In the presence and in the absence of S9 a positive response was only observed at cytotoxic concentrations. Polyploidization (11 %) was reported at non-cytotoxic concentrations.
	Chromosomal aberration test	Chinese hamster ovary cells	50-500 µg/ml[2]; 160-1600 µg/ml[3]	Negative[1]	(Galloway et al., 1987a)	Published non-GLP study. Doses were selected based on preliminary assay. Although some details of results are not reported the study is considered valid.
	Chromosomal aberration test	Chinese hamster cell line B241	50 nM (0.0053 µg/ml)	Positive[1]	(Kasamaki et al., 1982)	Published non-GLP study of sufficient quality to be taken into account for the evaluation, although some details of method and results are not reported. Information is only given for the final concentration at which maximal frequency of aberration was observed without visible cytotoxicity in the treated cells. Dose-dependent increase of total aberrations (chromatid gaps, chromatid breaks, chromosome breaks observed, no ring or dicentric aberrations or chromatic exchanges).
	Sister chromatid exchange assay	Chinese hamster ovary cells	5-160 µg/ml[2]; 160-1600 µg/ml[3]	Positive[2] Positive[3]	(Galloway et al., 1987a)	Published non-GLP study. Doses were selected based on a preliminary assay. Although some deatails of results are not reported the study is considered valid. Weakly positive results with metabolic activation were observed at the highest concentration which was cytotoxic and resulted in 50 % growth reduction.
	Sister chromatid exchange assay	Chinese hamster ovary cells	Up to 1000 µM (up to 106 µg/ml)	Negative[3]	(Sasaki et al., 1989)	Published non-GLP study of limited quality. Study designed to investigate the influence on spontaneous as well as on mitomycin-induced SCEs. The substance did not influence cell cycle (data not shown) and spontaneous SCEs at the concentrations used. Cytotoxicity was reported at the highest concentration tested.
	Sister chromatid exchange assay	Human lymphocytes	0-2 mM (0-212 µg/ml)	Positive[2]	(Jansson et al., 1988)	Published non-GLP study not in accordance with OECD guideline 479 (no metabolic activation). Insufficient report of important details of method and results. This study is not considered valid.
(Benzoic acid [08.021])	Ames test (plate incorporation method)	*S. typhimurium* TA98; TA100; TA1535; TA1538	2500 µg/plate	Negative[1]	(Anderson and Styles, 1978)	
	Ames test	*S. typhimurium* TA98; TA100; TA1535; TA1536	3.6 µg/plate	Negative[1]	(Cotruvo et al., 1977)	
	Ames test (preincubation method)	*S. typhimurium* TA97; TA98; TA100; TA1535; TA1537	10,000 µg/plate	Negative[1]	(Zeiger et al., 1988)	
	Ames test	*S. typhimurium* TA100	Up to 1000 µg/plate	Negative	(Rapson et al., 1980)	Cytotoxicity was reported at the highest concentration tested.
	Ames test (plate incorporation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537	1000 µg/plate	Negative[3]	(McCann et al., 1975)	
	Ames test (preincubation	*S. typhimurium* TA92; TA94; TA98;	Up to 10,000 µg/plate (6	Negative[1]	(Ishidate et al., 1984)	Published study in accordance to OECD guideline 471.

Table IV.4: GENOTOXICITY (*in vitro*)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
	method)	TA100; TA1535; TA1537	concentrations)			Although some details of results are not reported the study is considered valid.
	Ames test (plate incorporation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	100 µg/plate	Negative[2]	(Milvy and Garro, 1976)	
	Ames test (plate incorporation method)	*S. typhimurium* TA1535; TA1537; TA1538	0.5 % (5 mg/ml)	Negative[1]	(FDA, 1975b)	
	Ames test (preincubation method)	*S. typhimurium* TA98; TA100	100 to 10000 µg/plate	Negative[1]	(Kuboyama and Fujii, 1992)	Published non-GLP study deficient in the report of some details on method and results (no single doses, no data on cytotoxicity reported), however, of sufficient quality to be taken into account in the evaluation.
	Umu mutation assay	*S. typhimurium* TA1535/ pSK1002	1607 µg/ml	Negative[1]	(Nakamura et al., 1987)	
	Rec assay (liquid method)	*B. subtilis* M45 (rec^-), H17 (rec^+)	Not reported	Positive	(Nonaka, 1989)	Only abstract available. Details on method and results not reported. Use of metabolic activation not reported. The validity of the study cannot be evaluated.
	Rec assay	*B. subtilis* M45 (rec^-), H17 (rec^+)	0 to 5000 µg/disc	Positive	(Kuboyama and Fujii, 1992)	Well conducted published non-GLP study, with some minor deficiencies (no cytotoxicity data, no detailed data for different concentrations reported), however, of sufficient quality to be taken into account in the evaluation. A weak positive result (D > 2 mm) was observed at concentrations of 4 mg/disc or more. At 5 mg/disc D = 2.9 mm.
	Mutation assay	*S. cerevisiae* D3	0.18 %	Negative[1]	(Cotruvo et al., 1977)	
	Mutation assay	*S. cerevisiae* D4	0.15 %	Negative[1]	(FDA, 1975b)	
	Indirect DNA repair test	*E. coli* PQ37	400 µg/ml	Negative	(Glosnicka and Dziadziuszko, 1986)	Genotoxicity measured as ability to induce ß-galactosidase.
	SOS Chromotest	*E. coli* PQ37	50 µg	Negative[1]	(Kevekordes et al., 1999)	
	Chromosomal aberration test	Chinese hamster fibroblast cells	1500 µg/ml (three concentrations, max. concentration inducing 50% cell-growth inhibition)[4]	Equivocal[2]	(Ishidate et al., 1984)	Published study carried out only in the absence of metabolic activation. Thus, study is not considered valid. Cells were exposed for 24 and 48 hours. Total incidence of cells with aberrations was 8 %. Negative response for polyploidization.
	Sister chromatid exchange assay	Human lymphocytes	0-2 mM (0-244 µg/ml)	Negative[2]	(Jansson et al., 1988)	Published non-GLP study not in accordance with OECD guideline 479 (no metabolic activation). Insufficient report of important details of method and results. This study is not considered valid.
	In vitro Micronucleus assay	Mouse lymphoma L5178Y cells	1000 µg/ml	Negative[1]	(Nesslany and Marzin, 1999)	
(Methyl benzoate [09.725])	Ames test (preincubation method)	*S. typhimurium* TA97; TA98; TA100; TA1535; TA1537	0 to 666 µg/plate (-S9); 0 to 6666 µg/plate (+S9) (6 concentrations)	Negative[1]	(Zeiger et al., 1992)	Published summary report including detailed results from NTP studies on 311 compounds in accordance with OECD guideline 471.
	Mutation assay	*E. coli* Sd-4-73	Not reported	Negative[2]	(Szybalski, 1958)	
Methyl 4-methylbenzoate [09.631]	Ames test (preincubation method)	*S. typhimurium* TA97; TA98; TA100; TA1535; TA1537;	0 to 333 µg/plate (-S9); 0 to 3333 µg/plate (+S9) (6 concentrations)	Negative[1]	(Zeiger et al., 1992)	Published summary report including detailed results from NTP studies on 311 compounds in accordance with OECD guideline 471.
(Isopentyl benzoate [09.755])	Mutation assay	*E. coli* Sd-4-73	Not reported	Negative[2]	(Szybalski, 1958)	
(4-Isopropylbenzyl alcohol [02.039])	Ames test (plate	*S. typhimurium* TA98; TA100	100 µl/plate	Negative[3]	(Rockwell and Raw, 1979)	

Table IV.4: GENOTOXICITY (*in vitro*)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
	incorporation method)					
	Ames test (plate incorporation method)	*S. typhimurium* TA98; TA100	300 µl/plate	Negative[1]	(Rockwell and Raw, 1979)	Assay of urine samples from rats given isopropylbenzyl alcohol by oral gavage.
(Tolualdehydes (mixed *o*, *m*, *p*) [05.027])	Ames test (preincubation method)	*S. typhimurium* TA104	0.8 µM/plate	Negative[1]	(Marnett et al., 1985a)	
	Ames test	*S. typhimurium* TA98; TA100; TA1535; TA1537	3 µM/plate	Negative[1]	(Florin et al., 1980)	
	Ames test (plate incorporation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	18,750 µg/plate[4]	Negative[1]	(Heck et al., 1989)	Published non-GLP study. No information concerning a possible cytotoxic effect nor on the number of concentrations tested. The test guidelines do not require more than 5 mg/plate. Due to the lack of some important details of study design and results the validity of the study cannot be evaluated.
	Ames test (plate incorporation method)	*S. typhimurium* TA98; TA100; TA102	0.8 mM/plate	Negative[1]	(Aeschbacher et al., 1989)	
	Ames test (preincubation method)	*S. typhimurium* TA97; TA100; TA1535; TA1537	666 µg/plate	Negative[1]	(Zeiger et al., 1988)	
	Unscheduled DNA synthesis test	Rat hepatocytes	1000 µg/ml[4]	Negative	(Heck et al., 1989)	Published non-GLP study. No information concerning the number of concentrations tested. Due to the lack of some important details of study design and results the validity of the study cannot be evaluated.
	Mammalian cell gene mutation test	Mouse lymphoma L5178Y cells	300 µg/ml (+S9), 600 µg/ml (-S9)[4]	Negative[1]	(Heck et al., 1989)	Published non-GLP study. Some important details of study design and results are not reported. Thus, the validity of the study cannot be evaluated.
(4-Isopropylbenzaldehyde [05.022])	Ames test (plate incorporation method)	*S. typhimurium* TA98; TA100	100 µl/plate	Negative[3]	(Rockwell and Raw, 1979)	
	Ames test (plate method)incorporation	*S. typhimurium* TA98; TA100	300 µl/plate	Negative[1]	(Rockwell and Raw, 1979)	Assay of urine samples from rats given 4-isopropyl benzaldehyde (cuminaldehyde) by gavage.
	Umu test	*S. typhimurium* TA1535/ pSK1002	1 µmole/ml	Negative	(Miyazawa et al., 2000)	Results indicated that 4-isopropyl benzaldehyde (cuminaldehyde) was positive for antimutagenicty, but not genotoxic.
	Sister chromatid exchange assay	Chinese hamster ovary cells	Up to 333 µM (up to 50 µg/ml)	Negative[2]	(Sasaki et al., 1989)	Published non-GLP study of limited quality. Study designed to investigate the influence on spontaneous as well as on mitomycin-induced SCEs. The substance did not influence cell cycle (data not shown) and spontaneous SCEs at the concentrations used. Cytotoxicity was reported at the highest concentration tested.
(4-Hydroxybenzoic acid [08.040])	Ames test (plate incorporation method)	*S. typhimurium* TA98; TA100	5000 µg/plate	Negative[2]	(Mikulasova and Bohovicova, 2000)	
	DNA Repair test	*E. coli* WP2, WP2*uvrA*, CM611; CM561	2000 µg/ml	Negative	(Mikulasova and Bohovicova, 2000)	
(Salicylic acid [08.112])	Ames test (preincubation method)	*S. typhimurium* TA98; TA100	100 to 10000 µg/plate	Negative[1]	(Kuboyama and Fujii, 1992)	Published non-GLP study deficient in the report of some details on method and results (no single doses, no data on cytotoxicity reported), however, of sufficient quality to be taken into account in the evaluation.
	Ames test (plate incorporation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537	Not reported	Negative[2]	(McCann et al., 1975)	

Table IV.4: GENOTOXICITY (*in vitro*)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
	Rec assay	*B. subtilis* M45 (rec^-), H17 (rec^+)	0 to 5000 µg/disc	Positive	(Kuboyama and Fujii, 1992)	Well conducted published non-GLP study, with some minor deficiencies (no cytotoxicity data, no detailed data for different concentrations reported), however, of sufficient quality to be taken into account in the evaluation. A weak positive result (D >2 mm) was observed at concentrations of 2 mg/disc or more. At 5 mg/disc D = 4.7 mm.
	Mitotic recombination assay	*S. cerevisiae* D7	10,000 µg/ml	Negative[2]	(Rosin, 1984)	Published non-GLP study with insufficient report of experimental details and results. Study was carried out only in the absence of metabolic activation and is thus not considered valid. Negative response reported both at neutral and alkaline conditions.
	Mutation assay	*S. cerevisiae* rad18	Up to 0.1 mM (up to 13.8 µg/ml; 8 concentrations)	Positive	(Zetterberg, 1979)	Published non-GLP study with limited report of experimental details and result. Use of metabolic activation not reported. The validity of the study cannot be evaluated. The dose level tested was clearly cytotoxic. An increase in mutant frequency was not evident until 95 - 99 % of cells were killed.
Ethyl 4-hydroxybenzoate [09.367]	Ames test	*S. typhimurium* TA98; TA100	Not reported	Negative[1]	(Kawachi et al., 1980a)	Published summary report of unpublished extensive screening study. No details of method and results reported. Thus, the validity of the study cannot be evaluated.
	Rec assay	*B. subtilis*	Not reported	Negative[1]	(Kawachi et al., 1980a)	Published summary report of unpublished extensive screening study. No details of method and results reported. Thus, the validity of the study cannot be evaluated.
	Chromosomal aberration assay	Hamster lung fibroblast cells	Not reported	Positive[2] Negative[3]	(Kawachi et al., 1980a)	Published summary report of unpublished extensive screening study. No details of method and results reported. Thus, the validity of the study cannot be evaluated.
	Chromosomal aberration assay	Human embryo fibroblasts	Not reported	Negative[2]	(Kawachi et al., 1980a)	Published summary report of unpublished extensive screening study. No details of method and results reported. Thus, the validity of the study cannot be evaluated.
	Chromosomal aberration assay	Chinese hamster fibroblast cells	Up to 250 µg/ml	Positive	(Ishidate et al., 1978)	Published non-GLP study in Japanese with English summary and tabulated results. Some important details of method and results are not available. There is no information on the use of metabolic activation. The substance was tested up to the maximum dose tolerated. Thus, the validity of the study cannot be evaluated.
	Sister chromatid exchange assay	Human embryo fibroblasts	Not reported	Negative[2]	(Kawachi et al., 1980a)	Published summary report of unpublished extensive screening study. No details of method and results reported. Thus, the validity of the study cannot be evaluated.
	Sister chromatid exchange assay	Human fibroblastic cells HE2144	0, 83, 166 µg/ml	Negative[2]	(Sasaki et al., 1980)	Published non-GLP study not in accordance with OECD guideline 479 (no metabolic activation). Insufficient report of important details of method and results. This study is not considered valid.
	Mutation assay	Silk worms	Not reported	Negative	(Kawachi et al., 1980a)	Published summary report of unpublished extensive screening study. Unusual protocol, no details of method and results reported. Thus, the validity of the study cannot be evaluated.
(Butyl 4-hydroxybenzoate [09.754])	Ames test	*S. typhimurium* TA98; TA100	1000 µg/plate	Negative[1]	(Haresaku et al., 1985)	

Table IV.4: GENOTOXICITY (*in vitro*)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
	Ames test (preincubation method)	*S. typhimurium* TA92; TA94; TA98; TA100; TA1535; TA1537; TA2637	Up to 1000 µg/plate (6 concentrations)	Negative[1]	(Ishidate et al., 1984)	Published study in accordance to OECD guideline 471. Although some details of results are not reported the study is considered valid.
	Chromosomal aberration test	Chinese hamster fibroblast cells	60 µg/ml (three concentrations, max. concentration inducing 50% cell-growth inhibition)[4]	Negative[2]	(Ishidate et al., 1984)	Published study carried out only in the absence of metabolic activation. Thus, study is not considered valid. Cells were exposed for 24 and 48 hours. Negative response for chromosomal aberrations and polyploidization.
	Ames test (plate incorporation assay)	*S. typhimurium* TA100	500 µg/plate	Negative[2]	(Ball et al., 1984)	
(Veratraldehyde [05.017])	Ames test	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA15378	8000 µg/plate	Negative[1]	(Nestmann et al., 1980)	
	Ames test	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	8000 µg/plate	Negative[1]	(Douglas et al., 1979)	
	Ames test (preincubation method)	*S. typhimurium* TA97; TA98; TA100; TA1535; TTA1537	6666 µg/plate	Negative[1]	(Mortelmans et al., 1986)	
	Ames test (plate incorporation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	1000 µg/plate[4]	Negative[1]	(Heck et al., 1989)	Published non-GLP study. No information concerning a possible cytotoxic effect nor on the number of concentrations tested. Due to the lack of some important details of study design and results the validity of the study cannot be evaluated.
	Ames test (preincubation method)	*S. typhimurium* TA100; TA102; TA104; TA982; TA1538	Not reported	Negative[1]	(Dillon et al., 1992)	
	Ames test (preincubation protocol)	*S. typhimurium* TA100; TA102; TA104	33 - 3333 µg/plate	Negative[1]	(Dillon et al., 1998)	
	Mutation assay	*S. cerevisiae* D7; XV185-14C	Not reported	Negative[2]	(Nestmann and Lee, 1983)	
	Mammalian cell gene mutation test	Mouse lymphoma L5178Y cells	250 to 1800 µg/ml	Positive[1]	(Heck et al., 1989)	Published non-GLP study. Some important details of study design and results are not reported. Thus, the validity of the study cannot be evaluated. Different concentration ranges (250, 1400-1600, 1400-1800 µg/ml) were used in three independent experiments within which positive responses were observed. A 2.3 to 6.2 fold increase in the mutation frequency was observed both with and without S9.
	Ames test (plate incorporation method)	*S. typhimurium* TA98; TA100	5000 µg/plate	Negative[2]	(Mikulasova and Bohovicova, 2000)	
	DNA Repair test	*E. coli* WP2; WP2*uvrA*; CM611; CM561	2000 µg/ml	Negative	(Mikulasova and Bohovicova, 2000)	
	Unscheduled DNA synthesis test	Rat hepatocytes	100 µg/ml[4]	Negative	(Heck et al., 1989)	Published non-GLP study. No information concerning the number of concentrations tested. Due to the lack of some important details of study design and results the validity of the study cannot be evaluated.
(4-Methoxybenzaldehyde [05.015])	Ames test (preincubation method)	*S. typhimurium* TA92; TA94; TA98; TA100; TA1535; TA1537; TA2637	Up to 5000 µg/plate (6 concentrations)	Negative[1]	(Ishidate et al., 1984)	Published study in accordance to OECD guideline 471. Although some details of results are not reported the study is considered valid.
	Ames test	*S. typhimurium* TA98; TA100	0.05 to 500 µg/plate	Negative[1]	(Kasamaki et al., 1982)	Published non-GLP study with insufficient report of some details of method and results. Thus, the validity of the study cannot be evaluated.
	Ames test (preincubation	*S. typhimurium* TA1537	Up to 5000 µg/plate (6	Negative[1]	(Engelhardt, 1986)	

Table IV.4: GENOTOXICITY (*in vitro*)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
	method)		concentrations)			
	Ames test	*S. typhimurium* TA98; TA100; TA1535; TA1537	408 µg/plate	Negative[1]	(Florin et al., 1980)	
	Ames test (preincubation method)	*S. typhimurium* TA97; TA102	1000 µg/plate	Negative[1]	(Fujita and Sasaki, 1987)	
	Rec assay	*B. subtilis* M45 (rec^-), H17 (rec^+)	22 µg/disc	Negative	(Oda et al., 1979)	Study published in Japanese without English abstract. Data extracted from tables. Validity of the study cannot be evaluated. No information on the use of metabolic activation.
	Ames test	*S. typhimurium* TA102	5000 µg/plate	Negative[1]	(Müller et al., 1993)	
	Ames test	*S. typhimurium* TA 100	1000 µg/plate	Negative	(Rapson et al., 1980)	
	Mutation assay	Phage PM2	1362 µg/ml	Negative	(Becker et al., 1996)	
	Chromosomal aberration test	Chinese hamster fibroblast cells	500 µg/ml (three concentrations, max. concentration inducing 50% cell-growth inhibition) [4]	Negative[2]	(Ishidate et al., 1984)	Published study carried out only in the absence of metabolic activation. Thus, study is not considered valid. Cells were exposed for 24 and 48 hours. Negative response for chromosomal aberrations and polyploidization.
	Chromosomal aberration test	Chinese hamster cell line B241	50 nM (0.0068 µg/ml)	Positive[1]	(Kasamaki et al., 1982)	Published non-GLP study of sufficient quality to be taken into account for the evaluation, although some details of method and results are not reported. Results are reported for the concentration at which maximal frequency of aberration was observed without visible cytotoxicity in the treated cells. Dose-dependent increase of total aberrations (chromatid gaps, chromatid breaks, chromosome breaks observed, ring and dicentric aberrations, chromatic exchanges).
	Mammalian cell gene mutation test	Mouse lymphoma L5178Y TK+/- cells	0 -3.0 mM (0 - 408 µg/ml) 3.6 - 5.1 mM (484 - 691 µg/ml)	Negative[2] Positive[2]	(Wangenheim and Bolcsfoldi, 1988)	Published non-GLP study not in accordance with OECD guideline 476 (no metabolic activation, no colony sizing). Important details of method and results are insufficiently reported. This study is not considered valid.
	Ames test	*S. typhimurium* TA102	5000 µg/plate	Negative[1]	(Jung et al., 1992)	Results confirmed at three separate contract laboratories
	Sister chromatid exchange assay	Human lymphocytes	0-2 mM (0-273 µg/ml)	Positive[2]	(Jansson et al., 1988)	Published non-GLP study not in accordance with OECD guideline 479 (no metabolic activation). Insufficient report of important details of method and results. This study is not considered valid.
	Sister chromatid exchange assay	Chinese hamster ovary K1 cells	14 µg/ml	Negative	(Sasaki et al., 1987)	
	DNA alkaline unwinding assay	Mouse lymphoma L5178Y TK+/- cells	0, 4, 5, 6 mole/l (0, 544, 680, 816 µg/ml) 7, 8 mole/l (953, 1089 µg/ml)	Negative[2] Positive[2]	(Garberg et al., 1988)	Published study on 78 compounds not in accordance with standard guidelines. Test suitable for rapid screening only. Strand breaks or mutations observed only at cytotoxic concentrations.
2-Methoxybenzaldehyde [05.129]	Mutation assay	*E. coli* WP2*uvr*A, *trpE*	5000 µg/plate	Negative[2]	(Watanabe et al., 1989)	Published non-GLP study with limited report of experimental details and results. Study evaluating the enhancing effect on *N'*-nitro-*N*-nitrosoguanidine (MNNG)-induced mutagenesis in pretreated cells and not on the mutagenicity of the substance itself. Absence of an enhancing effect reported.
	Sister chromatid exchange	Human lymphocytes	0 - 0.25 mM	Positive[2]	(Jansson et al., 1988)	Published non-GLP study not in accordance with OECD

Table IV.4: GENOTOXICITY (*in vitro*)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
	assay		(0 - 34 µg/ml)			guideline 479 (no metabolic activation). Insufficient report of important details of method and results. This study is not considered valid.
3-Methoxybenzaldehyde [05.158]	Sister chromatid exchange assay	Human lymphocytes	0 - 2.0 mM (0 - 273 µg/ml)	Positive[2]	(Jansson et al., 1988)	Published non-GLP study not in accordance with OECD guideline 479 (no metabolic activation). Insufficient report of important details of method and results. This study is not considered valid.
	Mammalian cell gene mutation test	Mouse lymphoma L5178Y TK+/- cells	0 - 2.5 mM (0 - 340 µg/ml) 3 mM (408 µg/ml)	Negative[2] Positive[2]	(Wangenheim and Bolcsfoldi, 1988)	Published non-GLP study not in accordance with OECD guideline 476 (no metabolic activation, no colony sizing). Important details of method and results are insufficiently reported. This study is not considered valid.
(4-Ethoxybenzaldehyde [05.056])	Ames test	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	3600 µg/plate	Negative[2]	(Wild et al., 1983)	
(Methyl 4-methoxybenzoate [09.713])	Paper disk mutation assay	*E. coli* Sd-4-73	Not reported	Negative[2]	(Szybalski, 1958)	
Gallic acid [08.080]	Ames test (preincubation method)	*S. typhimurium* TA98; TA100	3000 µg/plate	Negative[1]	(Chen and Chung, 2000)	
	Ames test (preincubation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537	0, 100, 333, 1000, 3333, 6666 µg/plate (solvent DMSO) 0, 100, 333, 1000, 3333, 10,000 µg/plate (solvent acetone)	Negative[1] Equivocal[1]	(Haworth et al., 1983)	Published summary report including detailed results from studies on 250 compounds tested in various laboratories within the NTP to a large extent in accordance with OECD guideline 471. Results on gallic acid from two different laboratories using different solvent. A negative response was observed in both laboratories with TA98, TA1535, TA1537. A negative result was also reported with TA100 in the laboratory using DMSO as solvent. With acetone, a low-level response with a dose-related trend was found with TA100 both in the absence and in the presence of metabolic activation. The effect was reproducible in a second, not reproducible in a third experiment.
	Ames test (preincubation method)	*S. typhimurium* TA98; TA100; TA1535	5000 µg/plate	Negative[1]	(Rashid et al., 1985)	Inhibition was noted at the 5000-µg/plate dose-level; however, this may have been due to toxicity. No mutagenicity was observed at the 1000-µg/plat dose-level.
	Ames test	*S. typhimurium* TA98; TA100; TA1537	15 µM/plate	Negative[1]	(Wang and Klemencic, 1979)	
	Ames test	*S. typhimurium* TA100	100 µg/plate	Positive[2] Positive[3]	(Yamaguchi, 1981)	Published non-GLP. Insufficient report of important details of method and results, thus the validity of the result cannot be evaluated.
	Ames test	*S. typhimurium* TA98; TA100	Not reported	Negative[1]	(Sugimura et al., 1976)	
	Chromosomal aberration test	Chinese hamster ovary cells	50 µg/ml	Positive[1]	(Stich et al., 1981c)	Published non-GLP study. Some important details of method and results are not reported. Thus, the validity of the study cannot be evaluated. Results are reported for one concentration only which was half the dose inducing mitotic inhibition.The clastogenic activity was reported to be reduced by the addition of S9.
	Chromosomal aberration test	Chinese hamster ovary K1 cells	Up to 2 mM (up to 340 µg/ml)	Negative[1]	(Tayama and Nakagawa, 2001)	Published non-GLP study. Part of the study with insufficient report of important details of method and results. The validity of the results cannot be evaluated.
	Sister chromatid exchange	Chinese hamster ovary K1 cells	0, 0.25, 0.5, 1.0, 1.5, 2.0	Positive[2]	(Tayama and Nakagawa,	Published non-GLP study. Well conducted part of the

Table IV.4: GENOTOXICITY (*in vitro*)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
	assay		mM (0, 42.5, 85, 170, 255, 340 µg/ml)		2001)	study, however with insufficient report of some important details of method and results (results with metabolic activation not reported).
	Mitotic gene conversion assay	*S. cerevisiae* D7	0, 100, 1000 µg/ml	Negative[2] Positive[2]	(Rosin, 1984)	Published non-GLP study with insufficient report of experimental details and results. Study was carried out only in the absence of metabolic activation and is thus not considered valid. Gallic acid did not induce a significant extent of gene conversions under acidic conditions. At neutral pH no convertogenic activity was reported at 100 µg/ml, however, gallic acid was considerably convertogenic at 1000 µg/ml. The presence of catalase completely inhibited the convertogenic activity.gene conversions. Under alkaline conditions (pH 10), the concentration of 100 µg/ml was reported to induce a significant ($p < 0.01$) increase of Trp^+ convertants.
(Vanillin [05.018])	Ames test (plate incorporation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	10,000 µg/plate[4]	Negative[1]	(Heck et al., 1989)	Published non-GLP study. No information concerning a possible cytotoxic effect nor on the number of concentrations tested. The test guidelines do not require more than 5 mg/plate. Due to the lack of some important details of study design and results the validity of the study cannot be evaluated.
	Ames test	*S. typhimurium* TA98; TA100; TA 1535; TA1537; TA1538	5000 µg/plate	Negative[1]	(Pool and Lin, 1982)	
	Rec assay	*B. subtilis* M45 (rec^-), H17 (rec^+)	21 µg/disc	Negative	(Oda et al., 1979)	Study published in Japanese without English abstract. Data extracted from tables. Validity of the study cannot be evaluated.
	Ames test (preincubation assay)	*S. typhimurium* TA97; TA98; TA100; TA1535; TA1537	10,000 µg/plate	Negative[1]	(Mortelmans et al., 1986)	
	Ames test	*S. typhimurium* TA98; TA100	0.05 to 1000 µg/plate	Negative[1]	(Kasamaki et al., 1982)	Published non-GLP study with insufficient report of some details of method and results. Thus, the validity of the study cannot be evaluated.
	Ames test	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	Not reported	Negative[1]	(Nagabhushan and Bhide, 1985)	
	Ames test	*S. typhimurium* TA92; TA94; TA98; TA100; TA1535; TA1537; TA2637	Up to 10,000 µg/plate (6 concentrations)	Negative[1]	(Ishidate et al., 1984)	Published study in accordance with OECD guideline 471. Although some details of results are not reported the study is considered valid.
	Ames test	*S. typhimurium* TA100	1000 µg/plate	Negative	(Rapson et al., 1980)	
	Paper disk mutation assay	*E. coli* Sd-4-73	Not reported	Negative[2]	(Szybalski, 1958)	
	Ames test (plate incorporation method)	*S. typhimurium* TA98; TA100	2500 µg/plate	Negative[2]	(Mikulasova and Bohovicova, 2000)	
	DNA Repair test	*E. coli* WP2; WP2*uvrA*; CM611; CM561	2000 µg/ml	Negative	(Mikulasova and Bohovicova, 2000)	
	Mutation assay	*E. coli* CSH26/pYM3; CSH26/pSK 1002	15,215 µg/ml	Negative	(Takahashi et al., 1990)	
	Mitotic recombination assay	*S. cerevisiae* D7	10,000 µg/ml	Negative[2]	(Rosin, 1984)	Published non-GLP study with insufficient report of experimental details and results. Study was carried out only in the absence of metabolic activation and is thus not considered valid. Negative response reported both at

Table IV.4: GENOTOXICITY (*in vitro*)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
						neutral and alkaline conditions.
	Chromosomal aberration test	Chinese hamster cell line B241	5, 20, 40 nM (0.0008, 0.003, 0.006 µg/ml)	Negative	(Kasamaki and Urasawa, 1985)	
	Chromosomal aberration test	Chinese hamster fibroblast cells	1000 µg/ml (three concentrations, max. concentration inducing 50% cell-growth inhibition)[4]	Negative[2]	(Ishidate et al., 1984)	Published study carried out only in the absence of metabolic activation. Thus, study is not considered valid. Cells were exposed for 24 and 48 hours. Negative response for chromosomal aberrations and polyploidization.
	Chromosomal aberration test	Chinese hamster V79 lung cells	15,215 - 152,150 µg	Negative[2]	(Tamai et al., 1992)	
	Chromosomal aberration test	Human lymphocytes	0, 1, 2, 4 mM (0, 152, 304, 608 µg/ml)	Negative	(Jansson and Zech, 1987)	Published non-GLP study not in accordance with OECD guideline 473 (no metabolic activation). Insufficient report of important details of method and results. No information on cytotoxicity. This study is not considered valid.
	Chromosomal aberration test	Chinese hamster cell line B241	20 nM (0.003 µg/ml)	Negative[1]	(Kasamaki et al., 1982)	Published non-GLP study of sufficient quality to be taken into account for the evaluation, although some details of method and results are not reported. Results are only reported for the final concentration at which maximal frequency of aberration was observed without visible cytotoxicity in the treated cells. No significant increase of single types of aberrations and of total aberrations.
	Sister chromatid exchange assay	Human lymphocyte cells	0 - 1.0 mM (0 - 152 µg/ml)	Positive[2]	(Jansson et al., 1986)	Published non-GLP study not in accordance with OECD guideline 479 (no metabolic activation). This study is not considered valid. Dose-dependent effect reported. Insufficient report of important details of method and results.
	Sister chromatid exchange assay	Chinese hamster ovary K1 cells	15 µg/ml	Negative	(Sasaki et al., 1987)	
	Sister chromatid exchange assay	Human lymphocytes	0, 1, 2 mM (0, 152, 304 µg/ml)	Positive[2]	(Jansson and Zech, 1987)	Published non-GLP study not in accordance with OECD guideline 479 (no metabolic activation). Insufficient report of important details of method and results. Dose-dependent effect reported This study is not considered valid.
	Mutation assay	Mouse lymphoma L5178Y cells	1000 µg/ml (-S9), 1500 µg/ml (+S9)[4]	Negative[1]	(Heck et al., 1989)	Published non-GLP study. Some important details of study design and results are not reported. Thus, the validity of the study cannot be evaluated.
	Unscheduled DNA synthesis test	Rat hepatocytes	500 µg/ml[4]	Negative	(Heck et al., 1989)	Published non-GLP study. No information concerning the number of concentrations tested. Due to the lack of some important details of study design and results the validity of the study cannot be evaluated.
	Micronucleus assay	Human hepatoma (Hep-G2) cells	5, 50 µg/ml 500 µg/ml	Negative [2] Positive[2]	(Sanyal et al., 1997)	Published non-GLP study carried out only in the absence of metabolic activation. Thus, the study is not considered valid. A statistically significant increase of spontaneus micronucleus frequency was reported at the highest concentration. Low concentrations of vanillin (0.25 – 5 µg/ml) but not higher (50, 500 µg/ml) showed an inhibitory effect on micronuclei induced by heterocyclic amines.
(Vanillic acid [08.043])	Chromosomal aberration test	Chinese hamster ovary cells	25,000 µg/ml	Positive[1]	(Stich et al., 1981c)	Published non-GLP study. Some important details of method and results are not reported. Thus, the validity of the study cannot be evaluated. Data are only reported for

Table IV.4: GENOTOXICITY (*in vitro*)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
						one concentration which was half the dose inducing mitotic inhibition.The clastogenic activity was reported to be increased by the addition of S9.
	Mitotic recombination assay	*S. cerevisiae* D7	10,000 µg/ml	Negative[2]	(Rosin, 1984)	Published non-GLP study with insufficient report of experimental details and results. Study was carried out only in the absence of metabolic activation and is thus not considered valid. Negative response reported both at neutral and alkaline conditions.
4-Hydroxy-3,5-dimethoxybenzaldehyde [05.153]	Ames test	*S. typhimurium* TA100	10,000 µg/plate	Negative	(Rapson et al., 1980)	The use of metabolic activation was not reported.
4-Hydroxy-3,5-dimethoxybenzoic acid [08.087]	Ames test	*S. typhimurium* TA98; TA100; TA1535; TA1537	366 µg/plate	Negative[1]	(Florin et al., 1980)	
	Chromosomal aberration test	Chinese hamster ovary cells	3000 µg/ml	Positive[1]	(Stich et al., 1981c)	Published non-GLP study. Some important details of method and results are not reported. Thus, the validity of the study cannot be evaluated. Data are only reported for one concentration which was half the dose inducing mitotic inhibition. The clastogenic activity was reported to be reduced by the addition of S9.
	Mitotic recombination assay	*S. cerevisiae* D7	10,000 µg/ml	Negative[2]	(Rosin, 1984)	Published non-GLP study with insufficient report of experimental details and results. Study was carried out only in the absence of metabolic activation and is thus not considered valid.
(Salicylaldehyde [05.055])	Ames test	*S. typhimurium* TA98; TA100; TA1535; TA1537	366 µg/plate	Negative[1]	(Florin et al., 1980)	
	Ames test (preincubation method)	*S. typhimurium* TA98; TA100	Not reported	Negative[1]	(Sasaki and Endo, 1978)	
	Ames test	*S. typhimurium* TA98; TA100	16 µg/ml	Negative[1]	(Kono et al., 1995)	
	Mutation assay	*S. typhimurium* TA1535/ pSK1002	111 µg/ml	Negative[1]	(Nakamura et al., 1987)	
	Chromosomal aberration test	CHL/IU cells	Not reported (max. 5 mg/ml)	Positive[1]	(Kusakabe et al., 2002)	Published study in accordance with OECD guideline 473. However, some details on method and results are insufficiently reported. Thus the validity of the study cannot be evaluated. Positive result with minimum effective dose manifesting over 50 % cytotoxicity at short-term treatment (6 hours, less than 50 % cells with chromosomal aberrations without S9, less than 20 % cells with chromosomal aberrations with S9). Reduced effect at continuous treatment without S9 (24 hours less than 10 % cells with chromosomal aberrations). No chromosomal aberrations after 48 hours treatment without S9. After 48 hours treatment without S9 18 % polyploid cells.
	Sister chromatid exchange assay	Human lymphocyte cells	0-0.5 mM (0-61 µg/ml)	Negative[2]	(Jansson et al., 1988)	Published non-GLP study not in accordance with OECD guideline 479 (no metabolic activation). Insufficient report of important details of method and results. This study is not considered valid.
(Methyl salicylate [09.749])	Ames test	*S. typhimurium* TA92; TA94; TA98; TA100; TA1535; TA1537; TA2637	Up to 10,000 µg/plate (6 concentrations)	Negative[1]	(Ishidate et al., 1984)	Published study in accordance with OECD guideline 471. Although some details of results are not reported the study is considered valid.
	Ames test (preincubation	*S. typhimurium* TA97; TA98; TA100;	333.3 µg/plate	Negative[1]	(Mortelmans et al., 1986)	

Table IV.4: GENOTOXICITY (*in vitro*)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
	method)	TA1535; TA1537				
	Ames test	*S. typhimurium* TA98; TA100	Not reported	Negative[1]	(Kawachi et al., 1980b; Kawachi et al., 1980a)	Published summary report of unpublished extensive screening study. No details of method and results reported. Thus, the validity of the study cannot be evaluated.
	Chromosomal aberration test	Hamster lung fibroblast cells	Not reported	Positive[2] Negative[3]	(Kawachi et al., 1980b; Kawachi et al., 1980a)	Published summary report of unpublished extensive screening study. No details of method and results reported. Thus, the validity of the study cannot be evaluated.
	Chromosomal aberration test	Chinese hamster fibroblasts	250 µg/ml[4] (three concentrations, max. concentration inducing 50% cell-growth inhibition)	Negative[2]	(Ishidate et al., 1984)	Published study carried out only in the absence of metabolic activation. Thus, study is not considered valid. Cells were exposed for 24 and 48 hours. Negative response for chromosomal aberrations and polyploidization.
	Ames test (preincubation method)	*S. typhimurium* TA98; TA100	100 to 10000 µg/plate	Positive[1]	(Kuboyama and Fujii, 1992)	Published non-GLP study deficient in the report of some details on method and results (no single doses, no data on cytotoxicity reported), however, of sufficient quality to be taken into account in the evaluation. At 100 µg/plate a positive response was observed in strain TA98 in the presence of S9 mix obtained from hamsters a negative response was observed in TA98 in the presence of S9 mix obtained from rat, mouse and guinea pig.
	Rec assay	*B. subtilis* M45 (rec^-), H17 (rec^+)	23 µg/disc	Negative	(Oda et al., 1979)	Study published in Japanese without English abstract. Data extracted from tables. Validity of the study cannot be evaluated.
	Rec assay	*B. subtilis*	Not reported	Negative[1]	(Kawachi et al., 1980b; Kawachi et al., 1980a)	Published summary report of unpublished extensive screening study. No details of method and results reported. Thus, the validity of the study cannot be evaluated.
	Rec assay	*B. subtilis* M45 (rec^-), H17 (rec^+)	0 to 5000 µg/disc	Negative	(Kuboyama and Fujii, 1992)	Well conducted published non-GLP study with some minor deficiencies (no cytotoxicity data, no detailed data for different concentrations reported), however, of sufficient quality to be taken into account in the evaluation.
	Mutation assay	Silkworm	Not reported	Negative	(Kawachi et al., 1980b; Kawachi et al., 1980a)	Published summary report of unpublished extensive screening study. Unusual protocol, no details of method and results reported. Thus, the validity of the study cannot be evaluated.
	Chromosomal aberration test	Human embryo fibroblast cells	Not reported	Negative[2]	(Kawachi et al., 1980b; Kawachi et al., 1980a)	Published summary report of unpublished extensive screening study. Unusual protocol, no details of method and results reported. Thus, the validity of the study cannot be evaluated.
	Sister chromatid exchange assay	Human embryo fibroblast cells	Not reported	Negative[2]	(Kawachi et al., 1980b; Kawachi et al., 1980a)	Published summary report of unpublished extensive screening study. Unusual protocol, no details of method and results reported. Thus, the validity of the study cannot be evaluated.
(Butyl vanillyl ether [04.093])	Ames test	*S. typhimurium* TA98; TA100; TA1535; TA1537	5000 µg/plate	Negative[1]	(Watanabe and Morimoto, 1989c)	
	Mutation assay	*E. coli* WP2 *uvr*A	5000 µg/plate	Negative[1]	(Watanabe and Morimoto, 1989c)	
(Ethyl vanillin [05.019])	Ames test	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	3600 µg/plate	Negative[1]	(Wild et al., 1983)	

Table IV.4: GENOTOXICITY (*in vitro*)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
	Ames test (preincubation method)	*S. typhimurium* TA97; TA98; TA100; TA1535; TA1537	8000 µg/plate	Negative[1]	(Mortelmans et al., 1986)	
	Ames test	*S. typhimurium* TA92; TA94; TA98; TA100; TA1535; TA1537; TA2637	Up to 10,000 µg/plate (six concentrations)	Negative[1]	(Ishidate et al., 1984)	Published study in accordance with OECD guideline 471. Although some details of results are not reported the study is considered valid.
	Ames test (preincubation method)	*S. typhimurium* TA97; TA102	1000 µg/plate	Negative[1]	(Fujita and Sasaki, 1987)	
	Ames test	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	10,000 µg/plate[4]	Negative[1]	(Heck et al., 1989)	Published non-GLP study. No information concerning a possible cytotoxic effect nor on the number of concentrations tested. The test guidelines do not require more than 5 mg/plate. Due to the lack of some important details of study design and results the validity of the study cannot be evaluated.
	Rec assay	*B. subtilis* M45 (rec^-), H17 (rec^+)	21 µg/disc	Negative	(Oda et al., 1979)	Study published in Japanese without English abstract. Data extracted from tables. Validity of the study cannot be evaluated.
	Chromosomal aberration test	Chinese hamster fibroblast cells	250 µg/ml (three concentrations, maximal concentration inducing 50 % cell-growth inhibition)[4]	Positive[2]	(Ishidate et al., 1984)	Published study carried out only in the absence of metabolic activation. Thus, study is not considered valid. Polyploidization in 48 % of cells reported at 48 hours. Negative response for chromosomal aberrations.
	Mammalian cell gene mutation test	Mouse lymphoma L5178Y cells	125-800 µg/ml	Negative[2] Weak positive[3]	(Heck et al., 1989)	Published non-GLP study. Some important details of study design and results are not reported. Thus, the validity of the study cannot be evaluated. Different concentration ranges (125-500 µg/ml, 600 µg/ml, 800 µg/ml) were used in three independent experiments within which positive responses were observed. In the presence of S9 a 2.1 to 3-fold increase in the mutant frequency was reported.
	Unscheduled DNA synthesis test	Rat hepatocytes	199 µg/ml[4]	Negative	(Heck et al., 1989)	Published non-GLP study. No information concerning the number of concentrations tested. Due to the lack of some important details of study design and results the validity of the study cannot be evaluated.
	Sister chromatid exchange assay	Human lymphocytes	0-2.0 mM (0-332 µg/ml)	Negative[2]	(Jansson et al., 1988)	Published non-GLP study not in accordance with OECD guideline 479 (no metabolic activation). Insufficient report of important details of method and results. This study is not considered valid.
	Sister chromatid exchange assay	Chinese hamster ovary K1 cells	17 µg/ml	Negative	(Sasaki et al., 1987)	
(Ethyl vanillin isobutyrate [09.933])	Ames test	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	5000 µg/plate	Negative[1]	(King and Harnasch, 1997)	
(Piperonyl acetate [09.220])	Ames test (preincubation method)	*S. typhimurium* TA97; TA98; TA100; TA1535; TA1537	3333 µg/plate	Negative[1]	(Mortelmans et al., 1986)	
	Ames test	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	3600 µg/plate	Negative[1]	(Wild et al., 1983)	
(Piperonal [05.016])	Ames test					
	Modified Ames test	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538 *E. coli* WP2*uvr*Atrp^-	0, 300, 600, 1200, 2400 µg/plate	Negative[1]	(Sekizawa and Shibamoto, 1982)	Valid study in accordance with OECD guideline 471. The plate incorporation method was used -S9; the preincubation method +S9.

Table IV.4: GENOTOXICITY (*in vitro*)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
	Ames test (plate incorporation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	10,000 µg/plate[4]	Negative[1]	(Heck et al., 1989)	Published non-GLP study. No information concerning a possible cytotoxic effect nor on the number of concentrations tested. The test guidelines do not require more than 5 mg/plate. Due to the lack of some important details of study design and results the validity of the study cannot be evaluated.
	Ames test	*S. typhimurium* TA98; TA100	0.05 to 5000 µg/plate	Negative[1]	(Kasamaki et al., 1982)	Published non-GLP study with insufficient report of some details of method and results. Thus, the validity of the study cannot be evaluated.
	Ames test	*S. typhimurium* TA98; TA100; TA1537; TA1538	5000 µg/plate	Negative[1]	(White et al., 1977)	
	Ames test (preincubation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537	0, 10, 33, 100, 333, 1000 µg/plate	Negative[1]	(Haworth et al., 1983)	Published summary report including detailed results from studies on 250 compounds tested in various laboratories within the NTP to a large extent in accordance with OECD guideline 471.
	Rec assay	*B. subtilis* M45 (rec^-), H17 (rec^+)	20 µg/disc	Negative	(Oda et al., 1979)	Study published in Japanese without English abstract. Data extracted from tables. Validity of the study cannot be evaluated.
	Rec assay	*B. subtilis* M45 (rec^-), H17 (rec^+)	5000 µg/disc	Positive[2]	(Sekizawa and Shibamoto, 1982)	Well designed and reported study, however with some limitations with respect to results. DNA-repair tests in the presence of S9 were not successful (no data reported).
	Chromosomal aberration test	Chinese hamster cell line B241	50 nM (0.0075 µg/ml)	Positive[1]	(Kasamaki et al., 1982)	Published non-GLP study of sufficient quality to be taken into account for the evaluation, although some details of method and results are not reported. Data are only reported for the concentration at which maximal frequency of aberration was observed without visible cytotoxicity in the treated cells. Dose-dependent increase of total aberrations (chromatid gaps, chromatid breaks, chromosome breaks observed, no ring or dicentric aberrations or chromatic exchanges).
	Chromosomal aberration test	Chinese hamster cell line B241	0.15 µg/ml	Negative	(Kasamaki and Urasawa, 1985)	
	Mammalian cell gene mutation test	Mouse lymphoma L5178Y cells	1000 µg/ml[4]	Negative[1]	(Heck et al., 1989)	Published non-GLP study. Some important details of study design and results are not reported. Thus, the validity of the study cannot be evaluated.
	Unscheduled DNA synthesis test	Rat hepatocytes	10 to 502 µg/ml	Positive	(Heck et al., 1989)	Published non-GLP study. No information concerning the number of concentrations tested. Due to the lack of some important details of study design and results the validity of the study cannot be evaluated.
(Vanillin 3-(l-menthoxy)propane-1,2-diol acetal [02.248]) [5]	Ames test	*S. typhimurium* TA98; TA100; TA1535; TA1537	Up to 5000 µg/plate	Negative[1]	(Kajiura, 1996b)	
	Mutation assay	*E. coli* WP2 uvrA	Up to 5000 µg/plate	Negative[1]	(Kajiura, 1996b)	
6,6'-Dihydroxy-5,5'-dimethoxy-biphenyl-3,3'-dicarbaldehyde [05.221]	Ames test (preincubation method)	*S. typhimurium* TA98; TA100; Ta102; TA1535; TA1537	0, 50, 150, 500, 1500, 5000 µg/plate	Negative[1]	(King and Harnasch, 2002c)	
	Ames test (preincubation method)	*S. typhimurium* TA98; TA100; Ta102; TA1535; TA1537	0, 15, 50, 150, 500, 1500, 5000 µg/plate	Negative[1]	(King and Harnasch, 2002c)	
3,4-Dihydroxybenzoic acid [08.133]	Sister chromatid exchange assay	Chinese hamster ovary cells		Negative[1]	(Stich et al., 1981c)	Published non-GLP study. Some important details of method and results are not reported. Thus, the validity of

Table IV.4: GENOTOXICITY (*in vitro*)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
						the study cannot be evaluated. Data are only reported for one concentration which was half the dose inducing mitotic inhibition.The clastogenic activity was reported to be increased by the addition of S9.
	Mammalian cell gene mutation test	Mouse lymphoma L5178Y cells	0, 33, 100, 333, 1000, 3333 µg/ml	Negative[2]	(McGregor et al., 1988c)	

NR = not reported

[1] With and without S9 metabolic activation.

[2] Without S9 metabolic activation.

[3] With S9 metabolic activation.

[4] Concentration listed is either the highest tested if the result was negative or the concentration at which the maximum effect was observed for positive results.

[5] Related substance.

In vivo mutagenicity/genotoxicity data are available for two candidate substances of the present flavouring group evaluation from chemical group 23 and for ten supporting substances evaluated by JECFA at the 46th and 57th meeting (JECFA, 1996b; JECFA, 2002a). 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
(Benzyl alcohol [02.010])	*In vivo* Sex- linked recessive lethal mutations(SLRL)	*D. melanogaster*	Diet	5000 ppm	Negative	(Foureman et al., 1994)	
	In vivo SLRL	*D. melanogaster*	Injection	8000 ppm	Negative	(Foureman et al., 1994)	
	In vivo Micronucleus test	Mouse bone marrow cells	IP injection	200 mg/kg bw	Negative	(Hayashi et al., 1988)	
	In vivo Replicative DNA synthesis test	Mouse and rat hepatocytes	Not reported	Not reported	Negative	(Yoshikawa, 1996)	Screening test for the detection of non-genotoxic hepatocarcinogens. The substance was administered once at the maximum tolerated dose or at half the maximum tolerated dose to male mice and rats. Hepatocytes were prepared after 24, 39 and 48 hours.
	In vivo Replicative DNA synthesis test	Mouse hepatocytes	Oral gavage	800 mg/kg	Negative	(Miyagawa et al., 1995)	
	In vivo Replicative DNA synthesis test	Rat hepatocytes	Oral or SC injection	600 mg/kg	Negative	(Uno et al., 1994)	
(Benzyl acetate [09.014])	*In vivo* SLRL	*D. melanogaster*	Diet	300 ppm	Negative	(NTP, 1993d; Foureman et al., 1994)	
	In vivo SLRL	*D. melanogaster*	Injection	20,000 ppm	Negative	(NTP, 1993d; Foureman et al., 1994)	
	In vivo Sister chromatid exchange assay	Mouse bone marrow cells	IP injection	1700 mg/kg bw	Negative	(NTP, 1993d)	
	In vivo Chromosomal aberration test	Mouse bone marrow cells	IP injection	0 to 1700 mg/kg bw	Negative	(NTP, 1993d)	Test substance same batch as NTP chronic bioassays. The highest dose caused toxicity and cell cycle delay. Test not fully in compliance with the OECD guideline (insufficient cells per animal studied). GLP status not stated. The study is considered of limited validity.
	In vivo Micronucleus test	Mouse bone marrow cells	3 IP injection with 24 h intervals	0, 312, 625 and 1250 mg/kg bw	Negative	(NTP, 1993d; Shelby et al., 1993)	Test substance same batch as NTP chronic bioassays. Study in compliance with OECD guideline. GLP not stated. Micronuclei were determiend at 24 hours after the last dose. A dose-related decrease in PCE/NCE ratio was observed. The study is considered valid.
	In vivo Micronucleus test	Mouse erythrocytes	Dietary exposure for 13 weeks.	0 to 50,000 ppm (equal to 0 to 7900 mg/kg bw/day for males and 0 to 9400 mg/kg bw/day for females)	Negative	(NTP, 1993d)	Test substance same batch as NTP chronic bioassays. In life phase under GLP; for determination of genotoxic effects. GLP not specified. Test in compliance with OECD guideline. The test is considered valid, but of limited relevance because no change in PCE/NCE ratio was observed..
	In vivo Unscheduled DNA synthesis test	Rat hepatocytes	Oral gavage	0, 50, 200 and 1000 mg/kg bw	Negative	(Mirsalis et al., 1989)	Test substance same batch as NTP chronic bioassays. Test in compliance with OECD guidelines. GLP not stated. The test is considered valid.
	In vivo Unscheduled DNA synthesis test	Rat pancreatic cells	Oral gavage	1000 mg/kg bw	Negative	(Steinmetz and Mirsalis, 1984)	Only abstract available. Non guideline test. Validity cannot be assesed.
	In vivo DNA damage	Rat pancreatic cells	IP injection	0, 150, 500 and	Negative	(Longnecker et al., 1990)	Alkaline elution assay. GLP status not specified.

Table IV.5: GENOTOXICITY (*in vivo*)

Chemical Name [FL-no]	Test System	Test Object	Route	Dose	Result	Reference	Comments
				1500 mg/kg bw			Limited number of animals/group; DNA damage monitored at 1 hour post dosing. The study is of limited validity.
	In vivo Comet assay	Mouse/ Rat	Oral	1600 mg/kg (mouse); 1200 mg/kg (rat)	Positive	(Sekihashi et al., 2002)	Non-GLP and non-guideline test; but in compliance with recommended protocols. Some important details of method and results insufficiently reported. No toxicity data reported. The administered dose was 0.5 x LD_{50}. Sampling time was 3, 8 and 24 hours after dosing. Positive result reported in mice for stomach, colon, kidney, urinary bladder and brain, in rats for stomach, colon, liver, kidney, urinary bladder, lung. After 24 hours no significant effect in mice, significant effects in rat only in lung and kidney. The study is of limited validity.
(Benzaldehyde [05.013])	*In vivo* SLRL	*D. melanogaster*	Diet	1150 ppm	Negative	(Woodruff et al., 1985)	
	In vivo SLRL	*D. melanogaster*	Injection	2500 ppm	Negative	(Woodruff et al., 1985)	
(Salicylic acid [08.112])	*In vivo* Chromosomal aberration assay	Mouse bone marrow cells	IP injection gavage	0, 50, 100, 200 mg/kg 0, 350 mg/kg	Negative Negative	(Giri et al., 1996)	Published study widely in accordance with OECD guideline 475 and well reported (except that only males were tested, only one sampling time was chosen and signs of toxicity were not reported). Oral and i.p. dose were selected to be 1/3 and 1/5 of the reported oral LD_{50}.
	In vivo Sister chromatid exchange assay	Mouse bone marrow cells	IP injection gavage	0, 25, 50, 100 mg/kg 0, 350 mg/kg	Negative Negative	(Giri et al., 1996)	Well described published study of good quality. Oral and i.p. dose were selected to be 1/3 and 1/10 of the reported oral LD_{50}.
Ethyl 4-hydroxybenzoate [09.367]	*In vivo* Chromosomal aberration assay	Rat bone marrow cells	Not reported	Not reported	Negative	(Kawachi et al., 1980a)	Published summary report of unpublished extensive screening study. No details of method and results reported. Thus, the validity of the study cannot be evaluated.
(4-Ethoxybenzaldehyde [05.056])	*In vivo* Basc test Micronucleus test	*D. melanogaster*	NR	751 µg/ml	Negative	(Wild et al., 1983)	Published non-GLP study. Details of study protocol reported elsewhere. However, results sufficiently reported. Study is considered valid.
	In vivo Micronucleus test	NMRI mice	NR	1005 mg/ kg bw	Negative	(Wild et al., 1983)	Published non-GLP study. Details of study protocol and results insufficiently reported. Effect on PCE/NCE ratio not reported. No positive control. Validity of the study cannot be evaluated.
Gallic acid [08.080]	*In vivo* Medium-term rat liver bioassay	Male rats initiated with IP injection of diethylnitrosamine	Not reported.	Not reported	Negative	(Shirai, 1997)	Published non-GLP study. Unusual study protocol not following OECD guidelines. Some important details of method missing and only summarised results of a large screening study reported. Thus, the validity of the study cannot be evaluated.
(Vanillin [05.018])	*In vivo* Micronucleus test	Male BDF_1 mice	Oral gavage	500 mg/kg bw	Negative	(Inouye et al., 1988)	Published non-GLP study not in accordance with OECD guideline 474 (smaller group size, only males tested, no toxicity data reported, single dose level used, no negative control, effect on PCE/NCE ratio not reported.) Induction of micronuclei in mitomycin-treated mice was suppressed by post-treatment with vanillin due to an anticlastogenic

Table IV.5: GENOTOXICITY (*in vivo*)

Chemical Name [FL-no]	Test System	Test Object	Route	Dose	Result	Reference	Comments
							effect. Vanillin itself did not induce micronucleated PCEs (vanillin control group without mitomycin-treatment, six sampling times from 5 to 65 hours).
(Salicylaldehyde [05.055])	*In vivo* Spot test	*D. melanogaster* BINSC *D. melanogaster* Oregon-R	NR	1.05 to 1.40 ppm 0.09 to 0.35 ppm	Negative Negative	(Kono et al., 1995)	Study published in Japanese with English abstract. Data extracted from tables. Validity of the study cannot be evaluated
(Ethyl vanillin [05.019])	*In vivo* Basc test	*D. melanogaster*	NR	8309 µg/ml	Negative	(Wild et al., 1983)	Published non-GLP study. Details of study protocol reported elsewhere,. However, results sufficiently reported. Study is considered valid.
	In vivo Micronucleus test	Male BDF_1 mice	IP injection	Not reported	Negative	(Furukawa et al., 1989)	Only abstract available. Insufficient report of experimental details and result to evaluate the validity of the study.
	In vivo Micronucleus test	NMRI mice	NR	1000 mg/kg bw	Negative	(Wild et al., 1983)	Published non-GLP study. Details of study protocol and results insufficiently reported. Effect on PCE/NCE ratio not reported. No positive control. Validity of the study cannot be evaluated.
(Piperonyl acetate [09.220])	*In vivo* Basc test	*D. melanogaster*	NR	4855 µg/ml	Negative	(Wild et al., 1983)	Published non-GLP study. Details of study protocol reported elsewhere,. However, results sufficiently reported. Study is considered valid.
	In vivo Micronucleus test	NMRI mice	NR	970 mg/kg bw	Negative	(Wild et al., 1983)	Published non-GLP study. Details of study protocol and results insufficiently reported. Effect on PCE/NCE ratio not reported. No positive control. Validity of the study cannot be evaluated.
(Piperonal [05.016])	*In vivo* Dominant lethal assay	ICR/Ha Swiss mice	IP injection	0, 124, 620 mg/kg bw	Negative	(Epstein et al., 1972)	Published non-GLP study evaluating 174 substances. Study protocol not fully in accordance with OECD guideline 478 (lower number of animals and of dose levels used, limited report of experimental observations). However, due to the large body of control data available the results are considered valid. Doses were selected in preliminary acute toxicity tests. Parameters recorded were percent pregnancy, total implants and early and late fetal deaths.
	In vivo Dominant lethal assay	ICR/Ha Swiss mice	Oral gavage	0, 1000 mg/kg bw (repeated doses on 5 successive days)	Negative	(Epstein et al., 1972)	Published non-GLP study evaluating 174 substances. Study protocol not fully in accordance with OECD guideline 478 (lower number of animals and of dose levels used, limited report of experimental observations). However, due to the large body of control data available the results are considered valid. Doses were selected in preliminary acute toxicity tests. Parameters recorded were percent pregnancy, total implants and early and late fetal deaths.

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ABBREVIATIONS

ADI	Acceptable Daily Intake
AFC	Additives, Flavourings and Food Contact Materials
AUC	Area Under Curve
CAS	Chemical Abstract Service
CEF	Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
CHO	Chinese hamster ovary (cells)
CoA	Coenzyme A
CoE	Council of Europe
DMSO	Dimethyl sulphoxide
DNA	Deoxyribonucleic acid
EC	European Commission
EFFA	European Flavour and Fragrance Association
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)
FSH	Follicle-stimulating hormone
GI	Gastroinstinal
GLP	Good Laboratory Practice
HPLC	High Performance Liquid Chromatography
ID	Identity
IP	Intraperitoneal
IV	Intravenous
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
LH	Luteinizing hormone
MNNG	*N'*-nitro-*N*-nitrosoguanidine
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
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
PAFA	Priority-based assessment of food additives
RACB	Reproductive assessment by continuous breeding
SCE	Sister Chromatid Exchange
SCF	Scientific Committee on Food
SMART	Somatic Mutation and Recombination Test
SC	Subcutaneous
TAMDI	Theoretical Added Maximum Daily Intake
TLC	Thin Layer Chromatography
UDS	Unscheduled DNA Synthesis
WHO	World Health Organisation