The EFSA Journal (2005) 296, 1-117

Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in contact with Food (AFC) on a request from the Commission related to

Flavouring Group Evaluation 20: Benzyl alcohols, benzaldehydes, a related acetal, benzoic acids, and related esters from chemical group 23

(Commission Regulation (EC) No 1565/2000 of 18 July 2000)

QUESTION N° EFSA-Q-2003-163

Adopted 7 December 2005

SUMMARY

The Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food is asked to advise the Commission on the implications for human health of chemically defined flavouring substances used in or on foodstuffs in the Member States. In particular, the Scientific Panel is asked to evaluate 35 flavouring substances in the Flavouring Group Evaluation FGE.20, using the procedure as referred to in the Commission Regulation EC No 1565/2000. These 35 flavouring substances belong to chemical group 23, Annex I of the Commission Regulation EC No 1565/2000.

The present Flavouring Group Evaluation deals with 35 benzyl alcohols, benzaldehydes, a related acetal, benzoic acids, and related esters.

Three of the 35 flavouring substances possess a chiral centre. In each of these cases, the substance has been presented without any indication that the commercial flavouring substance has dominance of one or the other enantiomer.

Three of the 35 substances can exist as geometrical isomers and in one of these cases, no indication has been given that one of the possible isomers has preponderance in the commercial flavouring material.

Thirty-three of the flavouring substances are classified into structural class I, one is classified into structural class II and one is classified into structural class III.

Eighteen 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 Scientific Panel as a default used the Maximised Survey-derived Daily Intakes (MSDIs) approach to estimate the *per capita* intakes of the flavouring substances in Europe. However, when the Scientific Panel examined the information provided by the European flavouring industry on the use levels in various foods, it appeared obvious that the MSDI approach in a number of cases would grossly underestimate the intake by regular consumers of products flavoured at the use level reported by the industry, especially in those cases where the annual production values were reported to be small. In consequence, the Scientific 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 Scientific Panel to make a more realistic estimate of the intakes of the flavouring substances, the Scientific 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 Scientific Panel decided not to carry out a formal safety assessment using the Procedure. In these cases the Scientific Panel requires more precise data on use and use levels.

According to the default MSDI approach, the 35 flavouring substances in this group have intakes in Europe from 0.001 to 10 microgram*/capita/*day, which are below the threshold of concern value for structural class I (1800 microgram/person/day), structural class II (540 microgram/person/day) and structural class III (90 microgram/person/day) substances.

For the substances in this group the available data do not give rise to safety concern with respect to genotoxicity and carcinogenicity.

Based on experimental evidence and general knowledge of toxicokinetics of structurally related compounds it is expected that the flavouring substances in this group would be rapidly and efficiently absorbed and metabolised to innocuous products.

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

It was considered that on the basis of the default MSDI approach these 35 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 they ranged from 1300 to 3700 microgram/person/day for the 33 flavouring substances from structural class I. Thus, 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 of the one flavouring substance [FL-no: 05.066] assigned to structural class II and of the one [FL-no: 02.205] assigned to structural class III, based on the mTAMDI are 1600 and 3700 microgram/person/day, respectively, which are above the threshold of concern for structural class II of 540 microgram/person/day and for structural class III of 90 microgram/person/day. The five substances [FL-no: 05.129, 05.142, 05.153, 05.158, and 08.080], which have mTAMDI intake estimates below the threshold of concern for structural class I, are also expected to be metabolised to innocuous products.

Thus for 30 of the 35 flavouring substances considered in this opinion the intakes, estimated on the basis of the mTAMDI, exceed the relevant threshold for their structural class, to which the flavouring substance has been assigned. Therefore, for these 30 substances more reliable exposure data are required. On the basis of such additional data, these flavouring substances should be reconsidered along the steps of the Procedure. Following this procedure additional toxicological data might become necessary.

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

Adequate specifications including complete purity criteria and identity tests for the materials of commerce have been provided for the 35 flavouring substances, except that information on stereoisomerism is missing for four of the substances. Thus, the final evaluation of the materials of commerce cannot be performed for four substances [FL-no: 09.313, 09.317, 09.570 and 09.852], pending further information.

KEYWORDS

Benzyl alcohols, benzaldehydes, benzoic acids, esters, acetals, benzyl, benzoate, aliphatic, acyclic, alicyclic, flavourings, safety

TABLE OF CONTENTS

BACKGROUND

Regulation (EC) No 2232/96 of the European Parliament and the Council (EC, 1996) lays down a procedure for the establishment of a list of flavouring substances, the use of which will be authorised to the exclusion of all others in the EU. In application of that Regulation, a register of flavouring substances used in or on foodstuffs in the Member States was adopted by Commission Decision 1999/217/EC (EC, 1999a), as last amended by Commission Decision 2005/389/EC (EC, 2005). Each flavouring substance is attributed a FLAVIS-number (FL-number) and all substances are divided into 34 chemical groups. Substances within a group should have some metabolic and biological behaviour in common.

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

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

TERMS OF REFERENCE

EFSA is requested to carry out a risk assessment on flavouring substances prior to their authorisation and inclusion in a positive list according to Commission Regulation (EC) No 1565/2000 (EC, 2000).

ASSESSMENT

1. Presentation of the substances in the Flavouring Group Evaluation 20

1.1. Description

The present Flavouring Group Evaluation, FGE.20, using the procedure as referred to in the Commission Regulation EC No 1565/2000 (EC, 2000) (The Procedure –shown in schematic form in Annex I), deals with 35 benzyl alcohols, benzaldehydes, a related acetal, benzoic acids and related esters. These flavouring substances belong to chemical group 23, Annex I of Commission Regulation (EC) No 1565/2000 (EC, 2000).

The 35 candidate substances under consideration, as well as their chemical 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 14 benzyl derivatives (subgroup 1) and 21 hydroxy- and alkoxy-substituted benzyl derivatives (subgroup 2). Subgroup 1 includes 13 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 four are benzoic acid esters [FL-no: 09.631, 09.656, 09.779 and 09.825]. Two of these candidate substances contain a double-bond in the alkyl side chain [FL-no: 09.314 and 09.656] 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], five benzaldehyde derivatives [FL-no:

05.066, 05.129, 05.142, 05.153, and 05.158], two benzoic acids [FL-no: 08.080 and 08.087] and 12 related esters [FL-no: 09.362, 09.363, 09.367, 09.560, 09.570, 09.581, 09.623, 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. Two of the esters contain a double-bond in the alkyl side chain [FL-no: 09.560 and 09.570].

The 35 flavouring substances (candidate substances) are closely related structurally to 76 flavouring substances (supporting substances) evaluated at the 46th and 57th JECFA meeting (JECFA, 1997a; JECFA, 2002b). Two further structurally related flavouring substances are also included as supporting substances in this evaluation. The supporting substances, with the respective structural formulas, FEMA, CoE, and CAS register numbers, evaluation status by Scientific committee on Food (SCF), JECFA, and by CoE and the European Maximised Survey-derived Daily Intake (MSDI) values, are listed in Table 3.

The 78 supporting substances are all based on a benzyl structure as well with 41 benzyl derivatives (subgroup 1) and 37 hydroxy- and alkoxy-substituted benzyl derivatives (subgroup 2). The 41 benzyl derivatives include two alcohols (benzyl alcohol and an alkyl derivative), one acid (benzoic acid), 11 aldehydes (benzaldehyde and ten alkyl derivatives), one ether, four acetals and 22 related esters of which ten are benzyl esters and 12 are benzoic acid esters. The 37 hydroxy- and alkoxy-substituted benzyl derivatives include three benzyl alcohols, five benzaldehydes, six benzoic acids, two related ethers and 19 related esters of which seven are benzyl esters and 12 are benzoic acid esters. Three compounds contain a double bond in the alkyl side chain.

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 variation of their absorption, distribution, metabolism, elimination and toxicity. Thus information must be provided on the configuration of the flavouring substance, i.e. whether it is one of the geometrical/optical isomers, or a defined mixture of stereoisomers. The available specifications of purity will be considered in order to determine whether the safety evaluation carried out for candidate substances for which stereoisomers may exist can be applied to the material of commerce. Flavouring substances with different configurations should have individual chemical names and codes (CAS number, FLAVIS number etc.).
Three of the 35 flavouring substances possess a chiral centre [FL-no: 09.313, 09.317, and 09.852]. In each of these cases, the substance has been presented without any indication that the commercial flavouring substance has dominance of one or the other enantiomer.
Due to the presence and the position of double bonds, three of the 35 candidate substances can exist as geometrical isomers [09.314, 09.560, and 09.570]. In one of these cases, no indication of the preponderance of either of the possible isomers in the commercial flavouring material has been given [FL-no: 09.570].

1.3. Natural occurrence in food

Eighteen out of 35 candidate substances have been reported to occur in fruit (cherry, mango, papaya, bilberry, sapodilla, cloudberry, pineapple, grape), potato, coffee, beer, rum, sherry, whisky, wine, spices, soybean, wort and pork. Quantitative data on the natural occurrence of these substances have been reported for the occurrence of 12 of these substances in food. These reports include among others:

- 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 pinapple, 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.
- 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.

Seventeen of the substances have not been reported to occur naturally in any food items according to TNO (TNO, 2000).

2. Specifications

Purity criteria for the 35 candidate substances have been provided by the flavouring industry (EFFA, 2004c).

Judged against the requirements in Annex II of Commission Regulation EC No 1565/2000 (EC, 2000), this information is adequate for the 35 substances (see Table 1). However, information on stereoisomerism is needed for four substances [FL-no: 09.313, 09.317, 09.570 and 09.852] (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 "Maximized Survey-Derived Daily Intake" (MSDI) by assuming that the production figure only represents 60 % of the use in food due to underreporting and that 10 % of the total EU population are consumers (SCF, 1999).

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

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

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

One of the alternatives is the "Theoretical Added Maximum Daily Intake" (TAMDI)-approach which is calculated on the basis of standard portions and upper use levels (SCF, 1995) for flavourable beverages and foods in general, with exceptional levels for particular foods. This method is regarded as a conservative estimate of the actual intake in most consumers because it is based on the assumption that the consumer regularly eats and drinks several food products containing the same flavouring substance at the upper use level.

One option to modify the TAMDI-approach is to base the calculation on normal rather than upper use levels of the flavouring substances. This modified approach is less conservative (e.g., it may underestimate the intake of consumers being loyal to products flavoured at the maximum use levels reported (EC, 2000). However, it is considered as a suitable tool to screen and prioritise the flavouring substances according to the need for refined intake data (EFSA, 2004a).

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

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

Average *per capita* intake (MSDI) is estimated on the assumption that the amount added to food is consumed by 10 % of the EU population[1] (Eurostat, 1998). This is derived for candidate substances from estimates of annual volume of production provided by Industry and incorporates a correction factor of 0.6 to allow for incomplete reporting (60 %) in the Industry surveys (SCF, 1999).

In the present Flavouring Group Evaluation (FGE.20) the total annual volume of production of the 35 candidate substances from use as flavouring substances in Europe has been reported to be approximately 380 kg (EFFA, 2004d). For 68 of the 78 supporting substances the total annual volume of production in Europe is approximately 660000 kg (Vanillin [FL-no: 05.018] accounts for 390000 kg) (JECFA, 2002a). The annual volumes of production in Europe for ten of the supporting substances [FL-no: 04.093, 06.019, 08.071, 08.076, 08.092, 09.145, 09.754, 09.803, 09.807 and 09.812] were not reported.

On the basis of the annual volumes of production reported for the 35 candidate substances, the MSDI values for each of these flavourings have been estimated (Table 2a).

Approximately 75 % of the total annual volume of production for the candidate substances is accounted for by five substances [FL-no: 05.142, 09.313, 09.363, 09.367 and 09.779]. The total

[1] 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.

estimated daily *per capita* intake of those candidate substances from use as flavouring substance is 35 microgram. The daily *per capita* intakes for each of the remaining substances are less than 2 microgram (Table 2a).

3.2. Estimated Daily *per Capita* Intake (TAMDI approach)

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

The assumption is that a person consumes a certain amount of flavourable foods and beverages per day (see Table II.2.1).

For the 35 candidate substances information on food categories and normal and maximum use levels[2,3] were submitted by the Flavour Industry (EFFA, 2004c).

The 35 candidate substances are used in flavoured food products divided into the food categories, outlined in Annex III of the Commission Regulation 1565/2000 (EC, 2000), as shown in table 3.1. For the present calculation of mTAMDI, the reported normal use levels were used. In the case where different use levels were reported for different food categories the highest reported normal use level was used.

[2] "Normal use" is defined as the average of reported usages and "maximum use" is defined as the 95th percentile of reported usages (EFFA, 2002i).
[3] The normal and maximum use levels in different food categories (EC, 2000) have been extrapolated from figures derived from 12 model flavouring substances (EFFA, 2004e).

Table 3.1 Use of Candidate Substances		
Food category	**Description**	**Flavourings used**
Category 1	Dairy products, excluding products of category 2	All 35
Category 2	Fats and oils, and fat emulsions (type water-in-oil)	All 35
Category 3	Edible ices, including sherbet and sorbet	All 35
Category 4.1	Processed fruits	All 35
Category 4.2	Processed vegetables (incl. mushrooms & fungi, roots & tubers, pulses and legumes), and nuts & seeds	None
Category 5	Confectionery	All 35
Category 6	Cereals and cereal products, incl. flours & starches from roots & tubers, pulses & legumes, excluding bakery	All 35
Category 7	Bakery wares	All 35 except [FL-no 05.129]
Category 8	Meat and meat products, including poultry and game	All 35
Category 9	Fish and fish products, including molluscs, crustaceans and echinoderms	All 35 except [FL-no 09.825]
Category 10	Eggs and egg products	None
Category 11	Sweeteners, including honey	None
Category 12	Salts, spices, soups, sauces, salads, protein products etc.	All 35
Category 13	Foodstuffs intended for particular nutritional uses.	All 35
Category 14.1	Non-alcoholic ("soft") beverages, excl. dairy products	All 35
Category 14.2	Alcoholic beverages, incl. alcohol-free and low-alcoholic counterparts	None
Category 15	Ready-to-eat savouries	All 35
Category 16	Composite foods (e.g. casseroles, meat pies, mincemeat) - foods that could not be placed in categories 1 – 15	All 35

According to the Flavour Industry the normal use levels for the 35 candidate substances are in the range of 1 – 20 mg/kg food, and the maximum use levels are in range of 5 – 100 mg/kg (EFFA, 2004c). For detailed information on use levels and intake estimations based on the TAMDI approach, see Annex II.

The mTAMDI values for the 33 candidate substances from structural class I range from 1300 to 3700 microgram/person/day. For the remaining two candidate substances [FL-no: 05.066 and 02.205] from structural class II and III the mTAMDI are 1600 and 3700 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 35 candidate substances are subdivided into two subgroups. Subgroup 1 includes 14 benzyl derivatives of which 13 are benzyl esters or benzoic acid esters and one is an acetal [FL-no: 06.017 ((diethoxymethyl)benzene)]. Subgroup 2 includes 21 hydroxy- and alkoxy-substituted benzyl derivatives of which nine are benzyl alcohols, benzaldehydes or benzoic acids and 12 are related esters.

It is expected that esters in subgroup 1 and 2 will be hydrolysed *in vivo* to their component alcohols and acids. Eight of the 13 esters from subgroup 1 will yield benzyl alcohol which has previously been evaluated by 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 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 four candidate esters in subgroup 1, three 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)] 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 in subgroup 1 is an acetal [FL-no: 06.017 ((diethoxymethyl)benzene)]. This substance would be expected to yield benzaldehyde and ethanol upon hydrolysis. Benzaldehyde has been evaluated by JECFA (JECFA, 1996b). Benzaldehyde is expected to be oxidized to benzoic acid.

Subgroup 2 includes 12 esters of which one [FL-no: 09.895 (4-methoxybenzyl-2-methylpropionate)] will yield 4-methoxy-benzyl alcohol (*p*-anisyl alcohol) (supporting substance [FL-no: 02.128]) upon hydrolysis. This substance has been evaluated by 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-dimethoxybenzyl alcohol)].

The remaining 11 esters in subgroup 2 [FL-no: 09.362 (ethyl 2-hydroxy-4-methyl benzoate); 09.363 (ethyl 2-methoxy benzoate); 09.367 (ethyl 4-hydroxy benzoate); 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); 09.762 (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-methylbutylsalicylate; 2-methylbutyl-2-hydroxybenzoate)] 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 conjugate. The same metabolic route is proposed for the candidate acid [FL-no: 08.087 (4-hydroxy-3,5-dimethoxybenzoic acid)].

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)] the main metabolic pathway 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-methylenedioxybenzylalcohol) [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.

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. Dehydroxylation to pyrogallol (1,2,3-trihydroxybenzene) may occur as a very minor pathway, but no further decarboxylation to catechol has been observed.

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 detailed information on metabolism and ADME 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 35 candidate substances from chemical group 23 the Procedure as outlined in Annex I was applied, based on the MSDI approach. The stepwise evaluations of the 35 substances are summarised in Table 2.

Step 1

Thirty-three of the flavouring substances are classified into structural class I, one is classified into structural class II [FL-no: 05.066] and one is classefied into structural class III [FL-no: 02.205] according to the decision tree approach presented by Cramer et al. (Cramer et al., 1978).

Step 2

At the estimated levels of intake all 35 candidate substances are expected to be metabolised to innocuous products. Accordingly, all 35 flavouring substances in the present Flavouring Group Evaluation proceed via the A-side of the Procedure scheme (Annex I).

Step A3

The estimated levels of the European daily *per capita* intake (MSDI) for the 33 candidate substances classified into structural class I, the one candidate substance classified into structural class II and the one classified into structural class III are in the range of 0.0012 to 10 micrograms (Table 2a). These intakes are below the thresholds of concern of 1800 microgram/person/day for structural class I, 540 microgram/person/day for structural class II and 90 microgram/person/day for structural class III.

Based on results of the safety evaluation sequence, the thirty-five candidate substances are not expected to be of 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 33 candidate substances in structural class I based on the mTAMDI range from 1300 to 3700 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.

The estimated intake of the one substance [FL-no: 05.066] assigned to structural class II and the one assigned to structural class III [FL-no: 02.205], based on the mTAMDI are 1600 and 3700 microgram/person/day, respectively, which is above the threshold of concern for structural class II substances of 540 microgram/person/day and for structural class III substances of 90 microgram/person/day.

For comparison of the intake estimates based on the MSDI approach and the mTAMDI approach see Table 6.1.

For 30 candidate substances further information is required. This would include more reliable intake data and then, if required, additional toxicological data.

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	3700	Class I	1800
05.129	2-Methoxybenzaldehyde	0.16	1300	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	3700	Class I	1800
08.080	Gallic acid	0.011	1600	Class I	1800
08.087	4-Hydroxy-3,5-dimethoxybenzoic acid	1.2	3000	Class I	1800
09.152	Benzyl valerate	1.7	3700	Class I	1800
09.313	Benzyl 2-methylbutyrate	7.3	3700	Class I	1800
09.314	Benzyl crotonate	0.37	3700	Class I	1800
09.315	Benzyl dodecanoate	0.13	3700	Class I	1800
09.316	Benzyl hexanoate	0.75	3700	Class I	1800
09.317	Benzyl lactate	0.91	3700	Class I	1800
09.318	Benzyl octanoate	0.12	3700	Class I	1800
09.362	Ethyl 2-hydroxy-4-methylbenzoate	0.0012	3700	Class I	1800
09.363	Ethyl 2-methoxybenzoate	5.5	3700	Class I	1800
09.367	Ethyl 4-hydroxybenzoate	10	3700	Class I	1800
09.560	Hex-3(cis)-enyl anisate	0.12	3700	Class I	1800
09.570	Hex-3-enyl salicylate	0.13	3700	Class I	1800
09.581	Hexyl salicylate	0.018	3700	Class I	1800
09.611	4-Isopropylbenzyl acetate	0.012	3700	Class I	1800
09.623	Methyl 2,4-dihydroxy-3,6-dimethylbenzoate	0.012	3700	Class I	1800
09.631	Methyl 4-methylbenzoate	0.0012	3700	Class I	1800
09.656	3-Methylbut-3-enyl benzoate	0.12	3700	Class I	1800
09.762	Pentyl salicylate	0.24	3700	Class I	1800
09.779	Butyl benzoate	3.7	3700	Class I	1800
09.798	Ethyl vanillate	0.024	3700	Class I	1800
09.799	Methyl vanillate	0.011	3700	Class I	1800
09.825	Pentyl benzoate	1.1	3700	Class I	1800
09.835	Benzyl decanoate	0.35	3700	Class I	1800
09.852	2-Methylbutyl 2-hydroxybenzoate	0.011	3700	Class I	1800
09.895	4-Methoxybenzyl-2-methylpropionate	0.37	3700	Class I	1800
05.066	4-Ethoxy-3-methoxybenzaldehyde	1.2	1600	Class II	540
02.205	Piperonyl alcohol	0.011	3700	Class III	90

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 Flavouring Group Evaluation 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, 2004d), the combined estimated daily *per capita* intake as flavourings of the 33 candidate flavouring substances assigned to structural class I is 45 microgram. This value does not exceed the threshold of concern for a substance belonging to structural class I of 1800 microgram/person/day.
The 35 candidate substances are structurally related to 76 supporting substances evaluated by JEFCA at its 46^{th} and 57^{th} meeting (JECFA, 1996b; JECFA, 2002a). Based on reported production volumes, European *per capita* intakes (MSDI) could be estimated for 66 of the 76 supporting substances. Production volumes in Europe were not reported for ten of the supporting substances [FL-no: 04.093, 06.019, 08.071, 08.076, 08.092, 09.145, 09.754, 09.803, 09.807, and 09.812].
The total combined intakes of the candidate and supporting substances are approximately 73000 and 7000 microgram/*capita*/day for structural class I and II, respectively, which exceed the threshold of concern for a compound belonging to structural class I of 1800 microgram/*capita*/day and structural class II of 540 microgram/*capita*/day. However, the supporting substances were evaluated by JECFA at the 46^{th} and 57^{th} 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 45 and 1.2 microgram/*capita*/day for structural class I and II, respectively, would not alter the JECFA conclusion based on combined intakes of approximately 73000 and 7000 microgram/*capita*/day. The Panel noted that a considerable proportion of this combined intake is accounted for by vanillin [FL-no: 05.018] and for this compound JECFA has allocated an ADI of 0 – 10 mg/kg bw (JECFA, 1967; JECFA, 2002b).

8. Toxicity

8.1. Acute Toxicity

Data are available for nine candidate substances and for 64 structurally related supporting substances evaluated by JECFA (JECFA, 2002a). The LD_{50} values are from 500 and up to more than 5000 mg/kg bw in 8 different animal species.

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

8.2. Subacute, Subchronic, Chronic and Carcinogenicity Studies

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 ten 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].

Short- and long-term toxicity data on hydroxy- and alkoxy-substituted benzyl derivatives (subgroup 2) are available for four candidate substances and eight supporting substances. The candidate substances have been tested for subacute oral toxicity [FL-no: 05.142 and 08.087] and for subchronic oral toxicity [FL-no: 08.080 and 09.367]. There are no data available on chronic toxicity and carcinogenicity for candidate substances. Valid short and long term studies are available for the supporting substances: benzyl alcohol [FL-no: 02.010], benzyl aldehyde [FL-no: 05.013] and benzyl acetate [FL-no: 09.014]. For other 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].

Benzyl derivatives

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 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. The Committee included benzyl alcohol in a previously established group ADI for benzoic acid and benzoates of 0 – 5 mg/ kg bw.

Hydroxy-/alkoxy- substituted benzyl derivatives

There are only few reliable data available for the candidate substances. NOAELs of 119 and 128 mg/kg bw/day for male and female rats, respectively, were derived for gallic acid [FL-no: 08.080] when tested for oral subchronic toxicity.

Subacute and subchronic exposure of rats to dietary concentrations of 1-2% of ethyl 4-hydroxybenzoate (syn. ethyl paraben) [FL-no: 09.367] or 3,4-dihydroxybenzaldehyde [FL-no: 05.142] (equivalent to 1000-2000 mg/kg bw/day) did not result in adverse effects. 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).

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 twelve 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 (syn. 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.

8.4. Genotoxicity Studies

Data from *in vitro* tests are available for eight 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 02.205] and 29 supporting substances (twelve from subgroup 1 and 17 from 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 ten supporting substances (three from subgroup 1 and seven from subgroup 2).

All the seven candidate substances [FL-no: 09.631, 09.367, 05.129, 05.142, 08.080, 05.153, and 08.087] tested for bacterial gene mutations gave negative results. For five candidate substances [FL-no: 09.367, 05.129, 05.158, 08.080, and 08.087] both positive and/or negative results were reported in various other *in vitro* test systems (Rec assay, chromosomal aberration test, 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 fourteen of these substances, both positive and negative results were reported in other *in vitro* test systems (Rec assay, chromosomal aberration test, 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 benzyl acetate 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 are negative and several of these studies, among which an 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 ground to preclude the evaluation of benzyl acetate via the Procedure.

Furthermore, all the studies carried out with ten 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 no safety concern with respect to genotoxicity of the substances in the present flavouring group.

Data are summarised in Annex IV, Table IV.4.

9. Conclusions

The 35 candidate substances are benzyl alcohols, benzaldehydes, a related acetal, benzoic acids and related esters. They belong to chemical group 23.

Three of the 35 flavouring substances possess a chiral centre [FL-no: 09.313, 09.317 and 09.852]. In each of these cases, the substance has been presented without any indication that the commercial flavouring substance has dominance of one or the other enantiomer.

Three of the 35 substances can exist as geometrical isomers [FL-no: 09.314, 09.560 and 09.570]. For hex-3-enyl salicylate [FL-no: 09.570] no indication has been given that one of the possible isomers has preponderance in the commercial flavouring material.

Thirty-three candidate substances are classified into structural class I. One [FL-no: 05.066] is classified into structural class II and one [FL-no: 02.205] is classified into structural class III.

Eighteen 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 35 flavouring substances in this group have intakes in Europe from 0.001 to 10 microgram/*capita*/day which are below the threshold of concern value for structural class I (1800 microgram/person/day), structural class II (540 microgram/person/day) and structural class III (90 microgram/person/day) substances.

On the basis of the reported annual production in Europe (MSDI approach), the combined intake of the 33 of the candidate substances belonging to structural class I would result in a total intake of approximately 45 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 68 of the 78 supporting substances. The total combined intakes of the candidate and supporting substances are approximately 73000 and 7000 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 data do not give rise to safety concern with respect to genotoxicity and carcinogenicity.

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.20 would be metabolized to innocuous products.

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

It is considered that on the basis of the default MSDI approach these 35 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 1300 to 3700 microgram/person/day for the 33 flavouring substances from structural class I. Thus, 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 of the two flavouring substances assigned to structural class II [FL-no: 05.066] and III [FL-no: 02.205], based on the mTAMDI, are 1600 and 3700 microgram/person/day, respectively, which are above the threshold of concern for structural class II of 540 microgram/person/day and for structural class III of 90 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 for 30 of the 35 flavouring substances considered in this opinion the intakes, estimated on the basis of the mTAMDI, exceed the relevant threshold for their structural class to which the flavouring substance has been assigned. Therefore, for these 30 substances more reliable exposure data are required. On the basis of such additional data, these flavouring substances should be reevaluated using the Procedure. Subsequently, additional toxicological data might become necessary.

In order to determine whether the conclusion for the 35 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 tests for the materials of commerce have been provided for the 35 flavouring substances, except that information on stereoisomerism is missing for four of the substances. Thus, the final evaluation of the materials of commerce cannot be performed for four substances [FL-no: 09.313, 09.317, 09.570 and 09.852], pending further information.

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

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

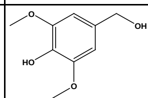
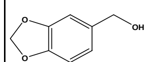
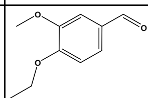
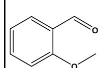
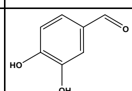
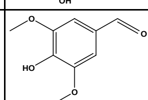
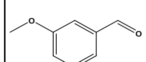
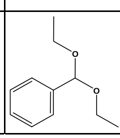
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	Specification comments
02.164	4-Hydroxy-3,5-dimethoxybenzyl alcohol		530-56-3	Solid $C_9H_{12}O_4$ 184.19	Practically insoluble or insoluble 1 ml in 1 ml	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 1 ml in 1 ml	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 1 ml in 1 ml	168 (17 hPa) 63 MS 95 %	n.a. n.a.	
05.129	2-Methoxybenzaldehyde		10350 135-02-4	Solid $C_8H_8O_2$ 136.15	Practically insoluble or insoluble 1 ml in 1 ml	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 1 ml in 1 ml	323 154 MS 98 %	n.a. n.a.	
05.153	4-Hydroxy-3,5-dimethoxybenzaldehyde		10340 134-96-3	Solid $C_9H_{10}O_4$ 182.18	Practically insoluble or insoluble 1 ml in 1 ml	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 1 ml in 1 ml	230 MS 95 %	1.549-1.555 1.116-1.122	
06.017	(Diethoxymethyl)benzene		517 774-48-1	Liquid $C_{11}H_{16}O_2$ 180.25	Practically insoluble or insoluble 1 ml in 1 ml	222 MS 95 %	1.475-1.481 0.903-0.909	

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

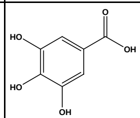
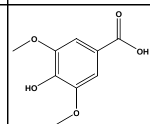
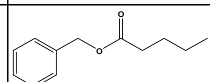
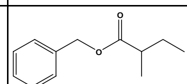
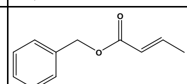
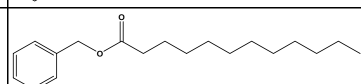
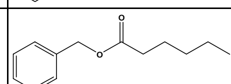
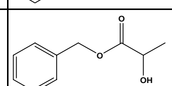
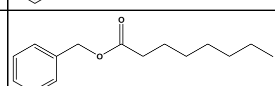
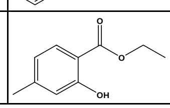
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	Specification comments
08.080	Gallic acid		10170 149-91-7	Solid $C_7H_6O_5$ 170.12	Sparingly soluble 1 ml in 1 ml	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 1 ml in 1 ml	440 206 MS 95 %	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 1 ml in 1 ml	236 MS 95 %	1.487-1.493 0.990-0.996	
09.313	Benzyl 2-methylbutyrate 6)		10523 56423-40-6	Liquid $C_{12}H_{16}O_2$ 192.26	Practically insoluble or insoluble 1 ml in 1 ml	248 MS 99 %	1.486-1.495 0.982-0.994	R/S enantiomer not specified by CAS no reported
09.314	Benzyl crotonate		65416-24-2	Liquid $C_{11}H_{12}O_2$ 176.21	Practically insoluble or insoluble 1 ml in 1 ml	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 1 ml in 1 ml	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 1 ml in 1 ml	270 MS 99 %	1.486-1.492 0.978-0.985	
09.317	Benzyl lactate 6)		2051-96-9	Liquid $C_{10}H_{12}O_3$ 180.20	Practically insoluble or insoluble 1 ml in 1 ml	134 (13 hPa) MS 95 %	1.512-1.518 1.120-1.144	R/S enantiomer not specified by CAS no reported
09.318	Benzyl octanoate		10276-85-4	Liquid $C_{15}H_{22}O_2$ 234.34	Practically insoluble or insoluble 1 ml in 1 ml	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 1 ml in 1 ml	254 MS 95 %	1.514-1.520 1.088-1.094	

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

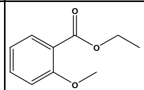
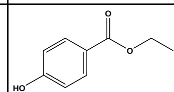
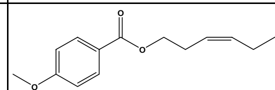
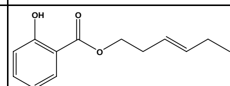
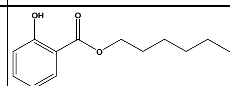
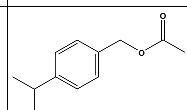
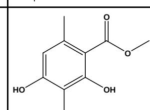
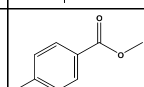
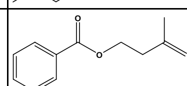
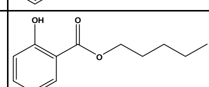
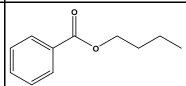
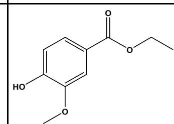
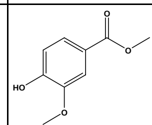
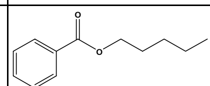
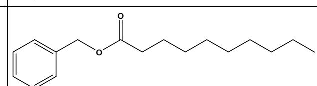
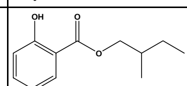
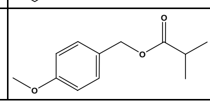
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	Specification comments
09.363	Ethyl 2-methoxybenzoate		7335-26-4	Liquid $C_{10}H_{12}O_3$ 180.20	Practically insoluble or insoluble 1 ml in 1 ml	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 1 ml in 1 ml	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 1 ml in 1 ml	363 73 NMR 95 %	n.a. n.a.	
09.570	Hex-3-enyl salicylate 6)	 Trans form shown	10685 65405-77-8	Solid $C_{13}H_{16}O_3$ 220.26	Practically insoluble or insoluble 1 ml in 1 ml	394 139 MS 98 %	1.518-1.522 1.057-1.065	CAS no reported refers to the (Z) isomer
09.581	Hexyl salicylate		10695 6259-76-3	Liquid $C_{13}H_{18}O_3$ 222.28	Practically insoluble or insoluble 1 ml in 1 ml	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 1 ml in 1 ml	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 1 ml in 1 ml	246 143 MS 95 %	n.a. n.a.	
09.631	Methyl 4-methylbenzoate		99-75-2	Solid $C_9H_{10}O_2$ 150.18	Practically insoluble or insoluble 1 ml in 1 ml	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 1 ml in 1 ml	60 (0.1 hPa) MS 95 %	1.499-1.505 0.986-0.992	
09.762	Pentyl salicylate		613 2050-08-0	Liquid $C_{12}H_{16}O_3$ 208.26	Practically insoluble or insoluble 1 ml in 1 ml	268 MS 95 %	1.533-1.539 1.062-1.068	

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

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
09.779	Butyl benzoate		740 136-60-7	Liquid $C_{11}H_{14}O_2$ 178.23	Practically insoluble or insoluble 1 ml in 1 ml	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 1 ml in 1 ml	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 1 ml in 1 ml	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 1 ml in 1 ml	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 1 ml in 1 ml	400 76 MS 95 %	n.a. n.a.	
09.852	2-Methylbutyl 2-hydroxybenzoate 6)		51115-63-0	Solid $C_{12}H_{16}O_3$ 208.26	Practically insoluble or insoluble 1 ml in 1 ml	366 117 MS 95 %	n.a. n.a.	R/S enantiomer not specified by CAS no reported
09.895	4-Methoxybenzyl-2-methylpropionate			Solid $C_{12}H_{16}O_3$ 208.26	Practically insoluble or insoluble 1 ml in 1 ml	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

6) Stereoisomeric composition not specified

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)

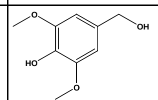
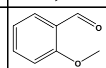
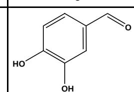
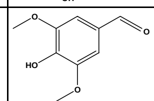
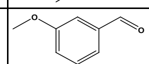
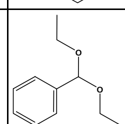
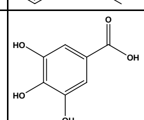
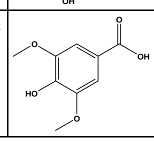
FL-no	EU Register name	Structural formula	MSDI 1) (μg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
02.164	4-Hydroxy-3,5-dimethoxybenzyl alcohol		0.037	Class I A3: Intake below threshold	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	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)	

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

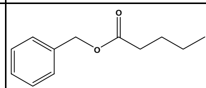
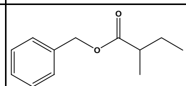
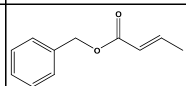
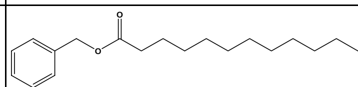
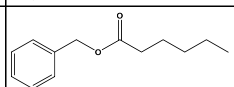
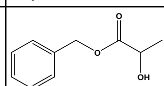
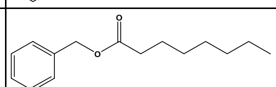
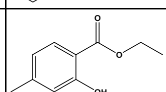
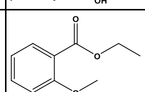
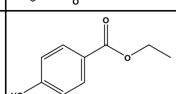
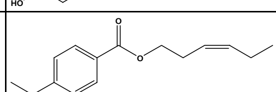
FL-no	EU Register name	Structural formula	MSDI 1) (µg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
09.152	Benzyl valerate		1.7	Class I A3: Intake below threshold	4)	6)	
09.313	Benzyl 2-methylbutyrate		7.3	Class I A3: Intake below threshold	4)	7)	
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)	7)	
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)	
09.560	Hex-3(cis)-enyl anisate		0.12	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)

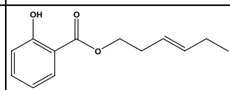
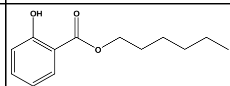
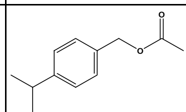
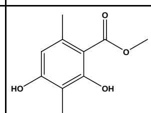
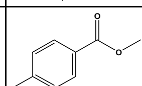
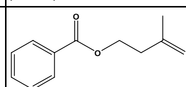
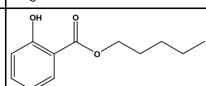
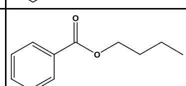
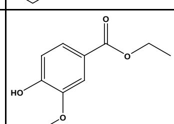
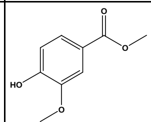
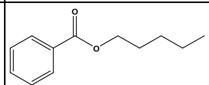
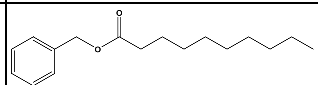
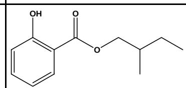
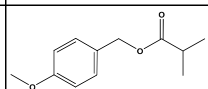
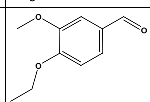
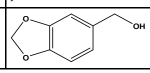
FL-no	EU Register name	Structural formula	MSDI 1) (µg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
09.570	Hex-3-enyl salicylate	 Trans form shown	0.13	Class I A3: Intake below threshold	4)	7)	
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)	
09.762	Pentyl salicylate		0.24	Class I A3: Intake below threshold	4)	6)	
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)	

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
09.799	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)	7)	
09.895	4-Methoxybenzyl-2-methylpropionate		0.37	Class I A3: Intake below threshold	4)	6)	
05.066	4-Ethoxy-3-methoxybenzaldehyde		1.2	Class II A3: Intake below threshold	4)	6)	
02.205	Piperonyl alcohol		0.011	Class III A3: Intake below threshold	4)	6)	

1) *MSDI: Amount added to food as flavour in (kg / year) x 10E9 / (0.1 x population in Europe (= 375 x 10E6) x 0.6 x 365) = µg/capita/day*
2) *Thresholds of concern: Class I = 1800, Class II = 540, Class III = 90 µg/person/day*
3) *Procedure path A substances can be predicted to be metabolised to innocuous products. Procedure path B substances cannot.*
4) *No safety concern based on intake calculated by the MSDI approach of the named compound.*
5) *Data must be available on the substance or closely related substances to perform a safety evaluation.*
6) *No safety concern at estimated level of intake of the material of commerce meeting the specification of Table 1 (based on intake calculated by the MSDI approach)*
7) *Tentatively regarded as presenting no safety concern (based on intake calculated by the MSDI approach) pending further information on the purity of the material of commerce.*
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

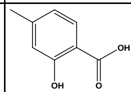
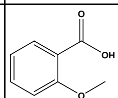
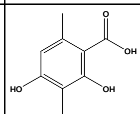
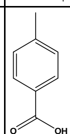
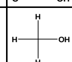
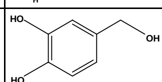
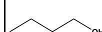
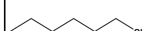
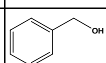
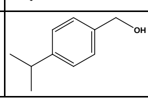
FL-no	EU Register name JECFA no	Structural formula	SCF status 1) JECFA status 2) CoE status 3)	Structural class 4) Procedure path 5)	Comments
	4-Methylsalicylic acid		Not evaluated as flavouring substance		Not in EU-Register
	o-Methoxybenzoic Acid		Evaluated as flavouring substance by JECFA (881)		Not 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 alcohok		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	
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	

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

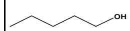
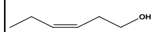
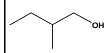
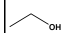
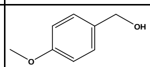
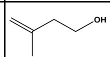
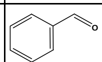
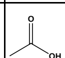
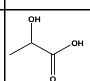
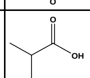
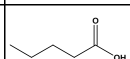
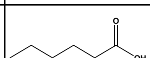
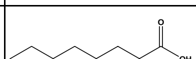
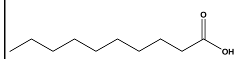
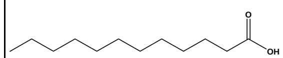
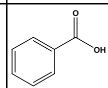
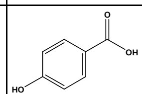
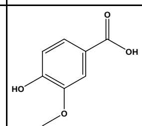
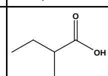
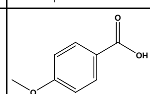
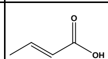
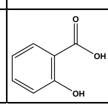
FL-no	EU Register name JECFA no	Structural formula	SCF status 1) JECFA status 2) CoE status 3)	Structural class 4) Procedure path 5)	Comments
02.040	Pentan-1-ol 88		Category 1 a) No safety concern b) Category A c)	Class I A3: Intake below threshold	
02.056	Hex-3(cis)-en-1-ol 315		Category 1 a) No safety concern e) Category A c)	Class I A3: Intake above threshold, A4: Not endogenous, A5: Adequate NOEL 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 meeting (JECFA, 1997a), the JECFA Committee concluded that ethanol posed no safety concern at its current level of intake when ethyl esters are used as flavouring agents.
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		- -	Class I A3: Intake below threshold	EFSA Opinion: FGE.06
05.013	Benzaldehyde 22		- No safety concern d) Category A c)	Class I A3: Intake above threshold, A4: Endogenous	
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	

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)	Structural class 4) Procedure path 5)	Comments
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 No evaluation	
08.040	4-Hydroxybenzoic acid 957		- No safety concern d) Category A c)	Class I A3: Intake below threshold	
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) 1371		- No safety concern i) -	No evaluation	
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, Class II = 540, Class III = 90 µg/person/day

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

a) (SCF, 1995)

b) (JECFA, 1999b)

c) (CoE, 1992)

d) (JECFA, 2002b)
e) (JECFA, 2000a)
f) (JECFA, 2004b)
g) (JECFA, 1997a)
h) (JECFA, 2002c)
i) (JECFA, 2005a)

TABLE 3: SUPPORTING SUBSTANCES SUMMARY

Table 3: Supporting Substances Summary

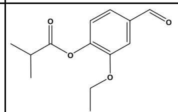
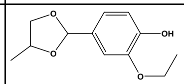
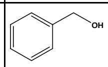
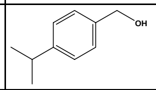
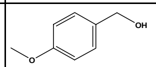
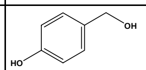
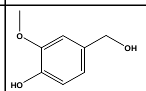
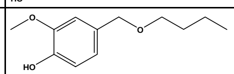
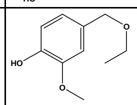
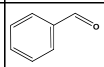
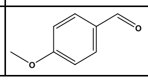
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (µg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
	Ethyl vanilin isobutyrate		 188417-26-7	953		- No safety concern a) -	Not in EU-Register
	Ethyl vanilin propylene glycol acetal		 68527-76-4	954		- No safety concern a) -	Not in EU-Register
02.010	Benzyl alcohol		2137 58 100-51-6	25 JECFA specification (JECFA, 2001c)	13000	- No safety concern a) Category A b)	
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		 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)	ND	- 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)	
05.015	4-Methoxybenzaldehyde		2670 103 123-11-5	878 JECFA specification (JECFA, 2001c)	370	- No safety concern a) Category A b)	

Table 3: Supporting Substances Summary

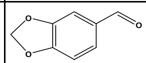
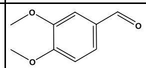
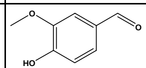
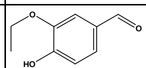
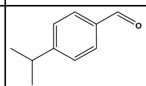
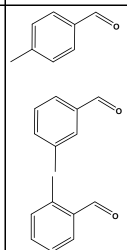
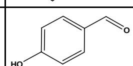
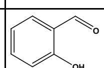
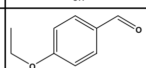
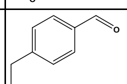
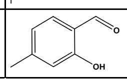
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (µg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
05.016	Piperonal		2911 104 120-57-0	896 JECFA specification (JECFA, 2001c)	1500	- No safety concern a) Category A b)	
05.017	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)	
05.019	Ethyl vanillin		2464 108 121-32-4	893 JECFA specification (JECFA, 2001c)	5400	- No safety concern a) Category A b)	
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)	
05.047	4-Hydroxybenzaldehyde		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)	

Table 3: Supporting Substances Summary

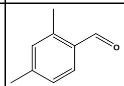
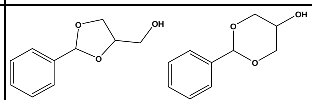
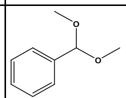
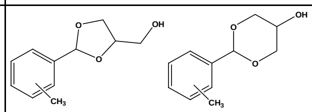
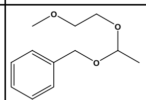
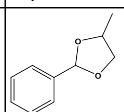
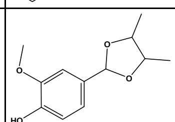
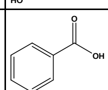
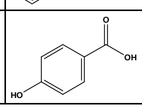
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (µg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
05.110	2,4-Dimethylbenzaldehyde		3427 15764-16-6	869 JECFA specification (JECFA, 2001c)	0.37	- No safety concern a) -	
06.002	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)	
06.003	alpha,alpha-Dimethoxytoluene		2128 37 1125-88-8	837 JECFA specification (JECFA, 2001c)	0.12	- No safety concern a) Category A b)	
06.012	Tolualdehyde glyceryl acetal		3067 46 1333-09-1	867 JECFA specification (JECFA, 2001c)	0.012	- No safety concern a) Category A b)	
06.019	1-Benzyloxy-1-(2-methoxyethoxy)ethane		2148 523 7492-39-9	840 JECFA specification (JECFA, 2001c)	ND	- No safety concern a) Category B b)	
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)	
06.132	Vanillin butan-2,3-diol acetal (mixture of stereo isomers)		4023 63253-24-7	960 JECFA specification (JECFA, 2002d)		- No safety concern a)	
08.021	Benzoic acid		2131 21 65-85-0	850 JECFA specification (JECFA, 2001c)	34	- No safety concern c) Deleted b)	
08.040	4-Hydroxybenzoic acid		 693 99-96-7	957 JECFA specification (JECFA, 2002d)	16	- No safety concern a) Category A b)	

Table 3: Supporting Substances Summary

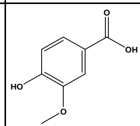
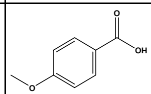
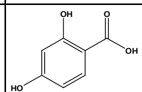
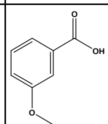
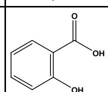
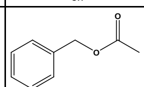
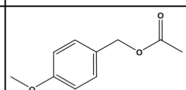
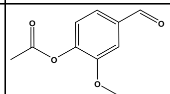
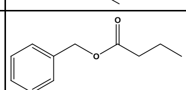
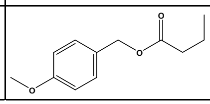
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (µg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
08.043	Vanillic acid		 697 121-34-6	959 JECFA specification (JECFA, 2002d)	24	- No safety concern a) Category A b)	
08.071	p-Anisic acid		3945 10077 100-09-4	883 JECFA specification (JECFA, 2001c)	ND	- No safety concern a) -	
08.076	2,4-Dihydroxybenzoic acid		3798 89-86-1	908 JECFA specification (JECFA, 2001c)	ND	- No safety concern a) -	
08.092	3-Methoxybenzoic acid		3944 586-38-9	882 JECFA specification (JECFA, 2001c)	ND	- No safety concern a) -	
08.112	Salicylic acid		 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)	
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)	

Table 3: Supporting Substances Summary

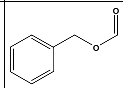
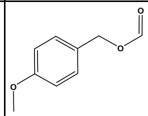
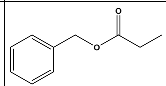
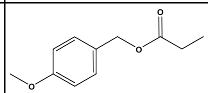
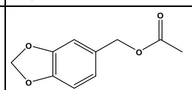
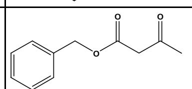
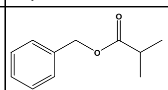
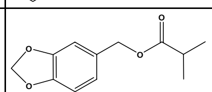
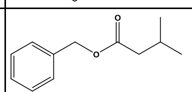
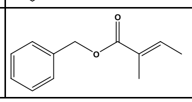
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (µg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
09.077	Benzyl formate		2145 344 104-57-4	841 JECFA specification (JECFA, 2001c)	35	- No safety concern a) Category A b)	
09.087	p-Anisyl formate		2101 354 122-91-8	872 JECFA specification (JECFA, 2002d)	39	- No safety concern a) Category B b)	
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)	ND	- 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)	

Table 3: Supporting Substances Summary

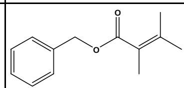
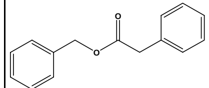
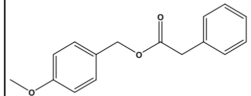
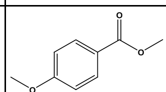
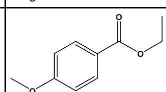
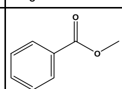
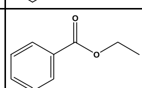
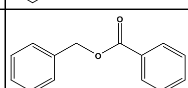
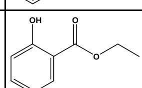
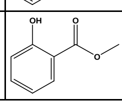
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (µg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
09.508	Benzyl 2,3-dimethylcrotonate		2143 11868 7492-69-5	847 JECFA specification (JECFA, 2002d)	0.012	- No safety concern 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)	
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)	
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)	

Table 3: Supporting Substances Summary

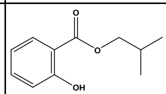
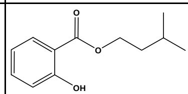
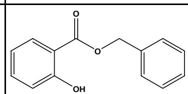
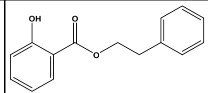
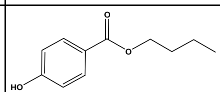
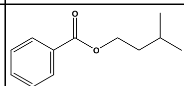
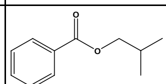
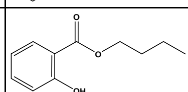
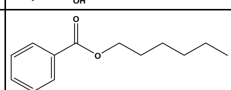
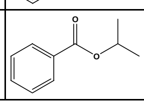
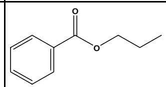
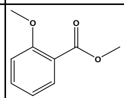
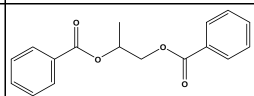
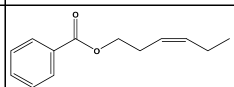
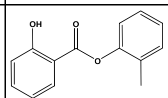
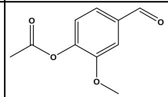
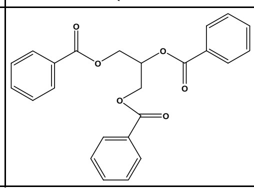
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (µg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
09.750	Isobutyl salicylate		2213 434 87-19-4	902 JECFA specification (JECFA, 2001c)	0.97	- No safety concern a) Category B b)	
09.751	Isopentyl salicylate		2084 435 87-20-7	903 JECFA specification (JECFA, 2001c)	41	- No safety concern a) Category B b)	
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)	
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)	
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)	

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
09.776	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)	ND	- No safety concern c) -	
09.806	Hex-3-enyl benzoate		3688 11778 25152-85-6	858 JECFA specification (JECFA, 2001c)	6.7	- No safety concern a) -	
09.807	o-Tolyl salicylate		3734 617-01-6	907 JECFA specification (JECFA, 2001c)	ND	- 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)	ND	- No safety concern c) -	

1) 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)

Deleted: Substances for which CoE Committee of Experts had no information as to their real use in foodstuffs and/or for which insufficient technicological and/or toxicological information was available (CoE, 1992)
ND: No data available

ANNEX I: PROCEDURE FOR THE SAFETY EVALUATION

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

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

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[4] (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[5] (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.

[4] *"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).*

[5] *"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).*

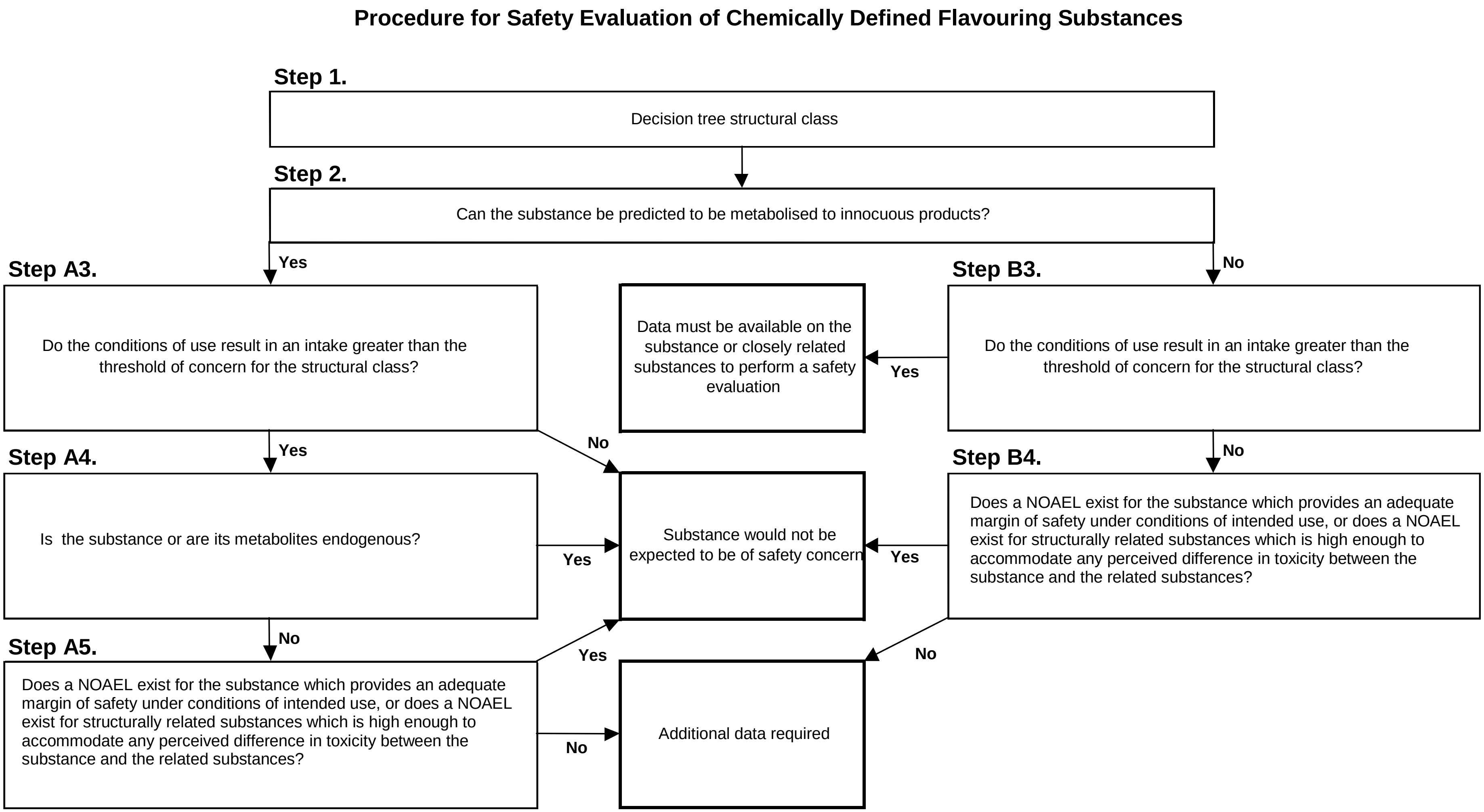


Figure I.1 *Procedure for Safety Evaluation of Chemically Defined Flavouring Substances*

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, 2000). According to the Industry the "normal use" is defined as the average of reported usages and "maximum use" is defined as the 95th percentile of reported usages (EFFA, 2002i). The normal and maximum use levels in different food categories have been extrapolated from figures derived from 12 model flavouring substances (EFFA, 2004e).

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

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 35 candidate substances in the present flavouring group (Table II.1.2).

Table II.1.2.Normal and Maximum use levels (mg/kg) for candidate substances in FGE.20 (EFFA, 2004c)

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 35	5 25	10 50	7 35	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	5 25	- -	20 100	5 25
02.205	7 35	5 25	10 50	7 35	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	5 25	- -	20 100	5 25
05.066	3 15	2 10	3 15	2 10	- -	4 20	2 10	5 25	1 5	1 5	- -	- -	2 10	3 15	2 10	- -	5 25	2 10
05.129	3 15	2 10	3 15	2 10	- -	5 25	2 10	- -	1 5	1 5	- -	- -	2 10	3 15	2 10	- -	5 25	2 10
05.142	3 15	2 10	3 15	2 10	- -	4 20	2 10	5 25	1 5	1 5	- -	- -	2 10	3 15	2 10	- -	5 25	2 10
05.153	3 15	2 10	3 15	2 10	- -	4 20	2 10	5 25	1 5	1 5	- -	- -	2 10	3 15	2 10	- -	5 25	2 10
05.158	3 15	2 10	3 15	2 10	- -	4 20	2 10	5 25	1 5	1 5	- -	- -	2 10	3 15	2 10	- -	5 25	2 10
06.017	7 35	5 25	10 50	7 35	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	5 25	- -	20 100	5 25
08.080	3 15	2 10	3 15	2 10	- -	4 20	2 10	5 25	1 5	1 5	- -	- -	2 10	3 15	2 10	- -	5 25	2 10
08.087	3 15	2 10	3 15	2 10	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	3 15	- -	15 75	5 25
09.152	7 35	5 25	10 50	7 35	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	5 25	- -	20 100	5 25
09.313	7 35	5 25	10 50	7 35	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	5 25	- -	20 100	5 25
09.314	7 35	5 25	10 50	7 35	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	5 25	- -	20 100	5 25
09.315	7 35	5 25	10 50	7 35	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	5 25	- -	20 100	5 25
09.316	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	-	20	5

	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	-	100	25
09.317	7 35	5 25	10 50	7 35	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	5 25	- -	20 100	5 25
09.318	7 35	5 25	10 50	7 35	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	5 25	- -	20 100	5 25
09.362	7 35	5 25	10 50	7 35	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	5 25	- -	20 100	5 25
09.363	7 35	5 25	10 50	7 35	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	5 25	- -	20 100	5 25
09.367	7 35	5 25	10 50	7 35	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	5 25	- -	20 100	5 25
09.560	7 35	5 25	10 50	7 35	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	5 25	- -	20 100	5 25
09.570	7 35	5 25	10 50	7 35	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	5 25	- -	20 100	5 25
09.581	7 35	5 25	10 50	7 35	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	5 25	- -	20 100	5 25
09.611	7 35	5 25	10 50	7 35	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	5 25	- -	20 100	5 25
09.623	7 35	5 25	10 50	7 35	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	5 25	- -	20 100	5 25
09.631	7 34	5 25	10 50	7 35	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	5 25	- -	20 100	5 25
09.656	7 35	5 25	10 50	7 35	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	5 25	- -	20 100	5 25
09.762	7 35	5 25	10 50	7 35	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	5 25	- -	20 100	5 25
09.779	7 35	5 25	10 50	7 35	- -	10 50	5 25	10 50	2 11	2 10	- -	- -	5 25	10 50	5 25	- -	20 100	5 25
09.798	7 35	5 25	10 50	7 35	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	5 25	- -	20 100	5 25
09.799	7 35	5 25	10 50	7 35	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	5 25	- -	20 100	5 25
09.825	7 35	5 25	10 50	7 35	- -	10 50	5 25	10 50	2 10	- -	- -	- -	5 25	10 50	5 25	- -	20 100	5 25
09.835	7 35	5 25	10 50	7 35	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	5 25	- -	20 100	5 25
09.852	7 35	5 25	10 50	7 35	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	5 25	- -	20 100	5 25
09.895	7 35	5 25	10 50	7 35	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	5 25	- -	20 100	5 25

II.2 TAMDI calculations

II.2.1 TAMDI based on SCF approach

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

Table II.2.1 Estimated amount of flavourable foods, beverages, and exceptions assumed to be consumed per person per day (SCF, 1995)

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

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

- Foods (SCF, 1995) correspond to the food categories 1, 2, 3, 4.1, 4.2, 6, 7, 8, 9, 10, 13, and/or 16 (EC, 2000)
- Exception a (SCF, 1995) corresponds to food category 5 and 11 (EC, 2000)
- Exception b (SCF, 1995) corresponds to food category 15 (EC, 2000)
- Exception c (SCF, 1995) corresponds to food category 14.2 (EC, 2000)
- Exception d (SCF, 1995) corresponds to food category 12 (EC, 2000)
- Exception e (SCF, 1995) corresponds to others, e.g. chewing gum

Table II.2.2 Distribution of the 18 food categories listed in Commission Regulation (EC) No. 1565/2000 (EC, 2000) 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	Dairy products, excluding products of category 02.0	Food		
02	Fats and oils, and fat emulsions (type water-in-oil)	Food		
03	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	Confectionery			Exception a
06	Cereals and cereal products, incl. flours & starches from roots & tubers, pulses & legumes, excluding bakery	Food		
07	Bakery wares	Food		
08	Meat and meat products, including poultry and game	Food		
09	Fish and fish products, including molluscs, crustaceans and echinoderms	Food		
10	Eggs and egg products	Food		
11	Sweeteners, including honey			Exception a
12	Salts, spices, soups, sauces, salads, protein products, etc.			Exception d
13	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	Ready-to-eat savouries			Exception b
16	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 35 flavouring substances in the present flavouring group, for which Industry has provided use and use levels (EFFA, 2004c). The mTAMDI values are only given for highest reported normal use levels (see Table II.2.3).

Table II.2.3 TAMDI (µg/*capita*/day) and MSDI (µg/*capita*/day) for substances allocated to structural class I, II and III. (Threshold of concern: 1800, 540 and 90 µg/person/day for structural class I, II and III, respectively)

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	3700	Class I	1800
05.129	2-Methoxybenzaldehyde	0.16	1300	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	3700	Class I	1800
08.080	Gallic acid	0.011	1600	Class I	1800
08.087	4-Hydroxy-3,5-dimethoxybenzoic acid	1.2	3000	Class I	1800
09.152	Benzyl valerate	1.7	3700	Class I	1800
09.313	Benzyl 2-methylbutyrate	7.3	3700	Class I	1800
09.314	Benzyl crotonate	0.37	3700	Class I	1800
09.315	Benzyl dodecanoate	0.13	3700	Class I	1800
09.316	Benzyl hexanoate	0.75	3700	Class I	1800
09.317	Benzyl lactate	0.91	3700	Class I	1800
09.318	Benzyl octanoate	0.12	3700	Class I	1800
09.362	Ethyl 2-hydroxy-4-methylbenzoate	0.0012	3700	Class I	1800
09.363	Ethyl 2-methoxybenzoate	5.5	3700	Class I	1800
09.367	Ethyl 4-hydroxybenzoate	10	3700	Class I	1800
09.560	Hex-3(cis)-enyl anisate	0.12	3700	Class I	1800

Table II.2.3 TAMDI (µg/*capita*/day) and MSDI (µg/*capita*/day) for substances allocated to structural class I, II and III. (Threshold of concern: 1800, 540 and 90 µg/person/day for structural class I, II and III, respectively)

FL-no	EU Register name	MSDI (µg/*capita*/day)	mTAMDI (µg/person/day)	Structural class	Threshold of concern (µg/person/day)
09.570	Hex-3-enyl salicylate	0.13	3700	Class I	1800
09.581	Hexyl salicylate	0.018	3700	Class I	1800
09.611	4-Isopropylbenzyl acetate	0.012	3700	Class I	1800
09.623	Methyl 2,4-dihydroxy-3,6-dimethylbenzoate	0.012	3700	Class I	1800
09.631	Methyl 4-methylbenzoate	0.0012	3700	Class I	1800
09.656	3-Methylbut-3-enyl benzoate	0.12	3700	Class I	1800
09.762	Pentyl salicylate	0.24	3700	Class I	1800
09.779	Butyl benzoate	3.7	3700	Class I	1800
09.798	Ethyl vanillate	0.024	3700	Class I	1800
09.799	Methyl vanillate	0.011	3700	Class I	1800
09.825	Pentyl benzoate	1.1	3700	Class I	1800
09.835	Benzyl decanoate	0.35	3700	Class I	1800
09.852	2-Methylbutyl 2-hydroxybenzoate	0.011	3700	Class I	1800
09.895	4-Methoxybenzyl-2-methylpropionate	0.37	3700	Class I	1800
05.066	4-Ethoxy-3-methoxybenzaldehyde	1.2	1600	Class II	540
02.205	Piperonyl alcohol	0.011	3700	Class III	90

ANNEX III: METABOLISM

III.1. General Information

The flavouring group consists of 35 substances out of which 14 are benzyl derivatives (subgroup 1) and 22 are hydroxy- and alkoxy-substituted benzyl derivatives (subgroup 2).

Subgroup 1 includes 13 alkyl esters of which eight contain benzylalcohol 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 isopropyl-benzyl alcohol as alcohol moiety [FL-no: 09.611 (4-isopropylbenzyl acetate)]. Four of the esters contain benzoic acid in the acid moiety [FL-no: 09.631 (methyl-4-methyl benzoate); 09.656 (3-methylbut-3-enyl 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-methyl benzoate)]. Two compounds contain a double bond in an alkyl chain [FL-no: 09.314 (benzyl crotonate); 09.656 (3-methylbut-3-enyl benzoate)].

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

Five substances are derivatives of benzylaldehyde [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)].

Two are derivatives of benzoic acid [FL-no: 08.080 (gallic acid; 3,4,5-trihydroxybenzoic acid); 08.087 (4-hydroxy-3,5-dimethoxybenzoic acid)].

The remaining twelve 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 eleven 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; syn. hex-3(cis)-enyl-4-methoxybenzoate); 09.570 (hex-3-enyl salicylate; syn. hex-3-enyl 2-hydroxybenzoate); 09.581 (hexyl salicylate; syn. hexyl 2-hydroxybenzoate); 09.623 (methyl 2,4-dihydroxy-3,6-dimethylbenzoate); 09.762 (pentyl salicylate; syn. pentyl 2-hydroxybenzoate); 09.798 (ethyl vanillate; syn. ethyl 3-methoxy-4-hydroxybenzoate); 09.799 (methyl vanillate; syn. methyl 3-methoxy-4-hydroxybenzoate); 09.852 (2-methylbutyl 2-hydroxybenzoate; syn.2-methylbutyl salicylate)].

Two of the esters in subgroup 2 contain a double bond in an alkyl chain [FL-no: 09.560 (hex-3(cis)-enyl anisate; hex-3(cis)-enyl-4-methoxybenzoate); 09.570 (hex-3-enyl salicylate].

III.2. Absorption, distribution and elimination

Subgroup 1 (benzyl derivatives)

Candidate substances from subgroup 1

There are no studies submitted on the candidate chemicals 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 plasma of pre-term babies were almost ten times higher than in full-term newborns. In 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 human 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 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-^{14}C]-benzoic acid/kg bw to rats (orally) and mice (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 >95% of benzoic acid is absorbed, metabolized 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 5 mg ^{14}C-benzyl acetate in the tail vein and three mice were similarly injected 10 mg. For single oral dose studies groups of three rats were given a single oral dose of 5, 50, or 500 mg/kg bw and groups of three mice were given a single oral dose of 10, 100, or 1000 mg/kg bw in corn oil by gavage. For 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 HPLC. After administration of radioactive compound, rats and mice were housed in metabolism cages and urine and faeces were collected during 24 h. 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 h, 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 h after dosing. The major metabolite of benzyl acetate in urine from 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 were 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 of the 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 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 6-hour interval thereafter; urine and faeces samples were taken at 12, 24, and 48 hours after treatment; selected organs removed from the animals and the remaining carcass 48 hours after treatment. Only the 12-hour 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 by the GI tract, then metabolized and rapidly and almost quantitatively 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 & Scheline, 1984).

Gallic acid (syn. 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 a maximum concentration at 15 minutes in portal blood. The concentration was halved by 30 minutes and gallic acid had almost disappeared after 6 h. The metabolite 4-O-methyl gallic acid also reached peak values within 15 minutes, and then decreased slowly. In inferior vena cava gallic acid and its metabolite were detected in approx. equal proportions and both reached peak values at 30 minutes after oral administration and decreased gradually until 6 h 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 (syn. ethyl paraben) [FL-no: 09.367]

Ethyl paraben [FL-no: 09.367] was given orally in capsules at 1 g/kg bw to groups of three fasted dogs and blood and urine were analysed at frequent predetermined intervals until 48 h after dosing. Metabolites were detectable in the blood up to 24 hours post-ingestion. Recovery as urine metabolites were 66% of the administered dose at 48 h. Also, dogs were administered a 100 mg/kg bw dose of ethyl paraben intravenously and then killed to determine 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-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 & Gourdon, 1963).

The ^{14}C-labelled ethyl 4-hydroxybenzoatewas 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 the 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 was 90% of the given dose, and metabolite excretion occurred mainly within 24 h. No unchanged compound was detected in the urine (Klungsoeyr & Scheline, 1984).

Veratraldehyde (syn. 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 urine mainly as the corresponding acid and its conjugates (Scheline, 1972).

Vanillin (syn. 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 h, mainly as glucuronide and sulphate conjugates although vanillic acid was also excreted as free acid and as glycine conjugate. After 48 h 94% of the dose excreted as different metabolites (Strand & Sheline, 1975).

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

4-Hydroxybenzoic acid [FL-no: 08.040]

Groups of four to eight rabbits were administered doses of 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, metabolized 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 methoxy-substituted benzyl derivatives in subgroup 2 are expected to be rapidly absorbed, metabolized 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 chemicals 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 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% 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 degrees 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 (t½) 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), the ethyl ester 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 & 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 h incubation. Benzyl cinnamate and methyl phenylacetate were 80 and 70% hydrolysed, respectively (Leegwater & 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 (syn. 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 h (Grundschober, 1977).

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

An *in vitro* assay demonstrated that ethylparaben is efficiently hydrolysed by 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 h 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 or 50 mg/kg bw by the intravenous 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).

Supporting substances from subgroup 2

Methyl-2-hydroxybenzoate (methylsalicylate) [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).

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 as for some supporting and candidate substances in subgroup 2.

It is expected that esters in subgroup 1 and 2 will be hydrolysed *in vivo*. Seven of the candidate substances in subgroup 1 [FL-no: 09.313 (benzyl 2-methylbutyrate; 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.

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

Three 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); 09.779 (butyl benzoate); 09.825 (pentyl benzoate)]. One ester [FL-no: 09.631 (methyl-4-methyl benzoate)] 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 twelve esters in subgroup 2 one [FL-no: 09.895 (4-methoxybenzyl-2-methyl propionate)] will yield 4-methoxybenzyl alcohol (*p*-anisyl alcohol) [FL-no: 02.128] upon hydrolysis. The remaining eleven esters in subgroup 2 [FL-no: 09.362 (ethyl-2-hydroxy-4-methyl benzoate); 09.363 (ethyl-2-methoxy benzoate); 09.367 (ethyl-4-hydroxy benzoate); 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); 09.762 (pentyl salicylate); 09.798 (ethyl vanillate); 09.799 (methyl vanillate); 09.852 (2-methylbutyl-2-hydroxybenzoate)] will yield alkoxy- and/or hydroxy 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 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 dosages 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 (disulfiram) 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 groups of 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 h after the 2^{nd}, the 8^{th} and the 13^{th} 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 benzyl mercapturic acid after the 8^{th} dose, all groups showed increased urinary levels of the after 13^{th} doses. An increase in dose from 400 to 1000 mg/kg bw/d resulted in a 7- to 8-fold increase in benzyl mercapturic acid excretion. The amount of benzylmercapturic acid excreted in urine collected for 24 h 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 & Potvin, 1987).

In the rabbit 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 benzyl mercapturic acid (Laham et al., 1988).

4-Isopropylbenzaldehyde (syn. 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 where reduction 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 (syn. 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, >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 , and 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 biologic 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 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 doses 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 h. 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 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 benzylmercapturic acid (1.0 - 1.9%) excreted in urine were not significantly affected by dose or vehicle (Chidgey & 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 benzylmercapturic 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 h. Co-administration of benzyl acetate with pyrazole, an inhibitor of alcohol dehydrogenase, caused an 11-fold increase in the excretion of benzylmercapturic 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 benzylmercapturic 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 aolone. Taken together, these results suggest that the formation of benzylmercapturic 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 months 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 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 months 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 months 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 lib 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 min rat plasma samples and the 5 min 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 h after gavage with the peak benzoic acid concentrations being much higher (about ten- to twenty-fold) than the peak hippuric acid concentrations. Plasma concentrations of benzoic acid in the dosed feed studies were much lower (more than hundred-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 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 & Levy, 1969).

After administration of oral doses of 40, 80, and 160 mg/kg bw of sodium benzoate to humans, the mean plasma 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 & 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) (supporting substance; [FL-no: 05.016]) was studied in male Wistar rats. Piperonyl alcohol dissolved in propylene glycol was administered by oral gavage at a dose level 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 h. Piperonyl glycine was identified as the major metabolite (70 ± 5%; 24-hour 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 cathecolderivatives 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 & 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-hour interval thereafter. Urine and faeces samples were taken at 12, 24, and 48 hours after treatment. The 12-hour 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 minimize their entrance to the lipid components of the microsomal enzymes so that no extensive demethylenation would occur (Kamienski & 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 per 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 per rat as intraperitoneal injection the results were similar to those obtained when the substance was given per os, 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-methylpyrogallol. 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-methylpyrogallol, 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, neither pyrogallol or 2-O-pyrogallol were detected in these urines. A study of the ability of rat intestinal contents to metabolise gallic acid showed that these were 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 h in medium containing ceacal contents. Solutions together with appropriate standards were then examined by thin-layer chromatography. Gallic acid [FL-no: 08.080] gave rise to pyrogallol which was present in 5 out of 8 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 is shown as the mean of 4-6 animals.) Blood samples were taken from portal vein and 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. 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 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 h. The only metabolite detected in blood and urine was identified as 4-O-methyl gallic acid. 4-O-Methyl gallic acid reached also peak concentration in portal vein after 15 minutes, and then decreased slowly. In the inferior vena cava both gallic acid and 4-O-methyl gallic acid both reached peak concentration 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 portal vein. 4-O-Methyl gallic acid, but not gallic acid was found in 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 inferior vena cava. Gallic acid was also found in urine at a higher concentration than in 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 thin layer chromatography 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).

<u>Ethyl 4-hydroxybenzoate (ethyl paraben) [FL-no: 09.367]</u>

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 ethylparaben 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 & 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-hour intervals, total faeces were collected at 72-hours, and all samples were assayed for total radioactivity. The radioactive metabolites present in the 24-hour 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 four animals as follows: 24-hour urine, 85.8%; 48-hour urine, 3.6%; 72-hour urine, 0.8%; 72-hour faeces, 5.8%. The 24-hour 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 (syn. ethyl paraben) [FL-no: 09.367] and supporting substances 4-hydroxy benzoic acid [FL-no 08.040] and butyl-4-hydroxybenzoate (syn. butyl paraben) [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 glucuronide acid conjugate. The test material was mainly excreted within 24 hours after dosing. The low rate of recovery seen in 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. There was no observation of O-demethylation (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 hour 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 h, mainly as glucuronide and sulphate conjugates although vanillic acid was also excreted as free acid and as glycine conjugate. After 48 h 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 also were excreted in appreciable amounts in the bile. Bile collected for 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 & Sheline, 1975).

In Sprague-Dawley albino rats 100 mg vanillin [FL-no: 05.018]/ kg bw was given by intraperitoneal injection and 24-hour urine was collected and analysed. The main urinary metabolite was conjugated vanillic acid which accounted for 41% of the administered dose, and 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, hewever the importance of the minor reductive pathway may be magnified by inhibiting the oxidative process, as was achieved by administration of disulfiram in the study (Wong & Sourkes, 1966).

An experiment was conducted with the aim to determine whether man is capable of oxidizing 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 & Wirtzfeld, 1964).

<u>Veratraldehyde (syn. 3,4-dimethoxybenzaldehyde) [FL-no: 05.017]</u>

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, 1946). Presumably, veratric acid may enter 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).

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

In rabbits, 96% of a single oral dose of 400 mg 4-hydroxybenzaldehyde/kg bw 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 2-hydroxybenzaldehyde (salicylaldehyde)/kg bw 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 salicylaldehyde/kg bw administered to two groups of four rabbits was excreted unchanged or as the glucuronic acid and sulphate conjugates with the major part 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 dosage 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% conjugated, as glucuronide conjugate or ethereal sulphate. A small amount, less than 5% was demethylated (Sammons & Williams, 1941).

4-Hydroxybenzoic acid [FL-no: 08.040]

In a study, groups of four to eight rabbits were administered doses of 100, 250, 500, 1000, or 1500 mg 4-hydroxybenzoic acid/kg bw 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 for 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 administered by intraperitoneal injection to female albino rats (Teuchy et al., 1971).

Other related substances

Methyl-4-hydroxybenzoate (methylparaben)

Methyl-4-hydroxybenzoate (methylparaben) was administered to three male rabbits by oral gavage at 800 mg/kg bw as a 12% Na salt solution, and the 24-hour 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 & Terada, 1962).

Concluding remarks on metabolism

The substances in subgroup 1 are esters that upon hydrolysis will yield simple benzyl alcohol or benzoic acid upon hydrolysis along with simple alkyl carboxylic acids or alcohols. The metabolic fate of simple 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 JECFA (JECFA, 1996b), as has 4-isopropylbenzyl alcohol which is the alcohol moiety of candidate substance [FL-no: 09.611 (4-isopropylbenzyl acetate)] (JECFA, 2002a). 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 previous FGE.05. 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.06 and will not be further discussed in this FGE.

Subgroup 2, alkoxy- and hydroxy-substituted benzyl derivatives, includes twelve 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 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 eleven esters in subgroup 2 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 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)].

The candidate substance piperonyl alcohol (3,4-methylenedioxybenzylalcohol) [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 does seem to be only a very minor metabolic path for this compound.

For the five 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. Dehydroxylation to pyrogallol (1,2,3-trihydroxybenzene) may occur as a very minor pathway, but no further dehydroxylation to catechol has been observed.

III.4 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 thirteen esters from subgroup 1 (benzyl derivatives) will yield benzyl alcohol which has previously been evaluated by 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 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 four candidate esters in subgroup 1, three 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)] 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 in subgroup 1 is an acetal [FL-no: 06.017 ((diethoxymethyl)benzene)]. This substance would be expected to yield benzaldehyde and ethanol upon hydrolysis. Benzaldehyde has been evaluated by JECFA (JECFA, 1996b). Benzaldehyde is expected to be oxidized to benzoic acid.

Subgroup 2 (alkoxy- and hydroxy-substituted benzyl derivatives) includes twelve esters of which one [FL-no: 09.895 (4-methoxybenzyl-2-methylpropionate)] will yield 4-methoxy-benzyl alcohol (*p*-anisyl alcohol) (supporting substance [FL-no: 02.128]) upon hydrolysis. This substance has been evaluated by 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-dimethoxybenzyl alcohol)].

The remaining eleven esters in subgroup 2 [FL-no: 09.362 (ethyl 2-hydroxy-4-methyl benzoate); 09.363 (ethyl 2-methoxy benzoate); 09.367 (ethyl 4-hydroxy benzoate); 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); 09.762 (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 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 conjugate. The same metabolic route is proposed for the candidate acid [FL-no: 08.087 (4-hydroxy-3,5-dimethoxybenzoic acid)].

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)] the main metabolic pathway 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-methylenedioxybenzylalcohol) [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. Dehydroxylation to pyrogallol (1,2,3-trihydroxybenzene) may occur as a very minor pathway, but no further dehydroxylation to catechol has been observed.

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.20 would be rapidly and efficiently absorbed, metabolized to innocuous products and excreted.

ANNEX IV: TOXICITY

Oral acute toxicity data are available for 9 candidate substances of the present flavouring group evaluation from chemical group 23, and for 66 supporting substances evaluated by JECFA at the 57th meeting. The supporting substances are listed in brackets.

TABLE IV.1: ACUTE TOXICITY

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 & Kuizenga, 1945)	
	Rat	NR	Oral	2979	(Ciba-Geigy Corp., 1945)	
	Rat	NR	Oral	2080	(Graham & 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 & Moldovan, 1971d)	
(Benzyl acetate [09.014])	Rabbit	NR	Oral	2640	(Graham & Kuizenga, 1945)	
	Rat	M, F	Gavage	2490	(Jenner et al., 1964)	
	Rat	NR	Oral	3690	(Graham & 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 & Kuizenga, 1945)	
	Rabbit	NR	Oral	2016	(Draize et al., 1948)	
	Rabbit	NR	Oral	1680	(Graham & 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 & Kuizenga, 1945)	
	Rat	NR	Oral	1700	(Lehman, 1955)	
	Mouse	NR	Oral	1568	(Draize et al., 1948)	
	Mouse	NR	Oral	1400	(Lehman, 1955)	
(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)	

Table IV.1: ACUTE TOXICITY

Chemical Name [FL-no]	Species	Sex	Route	LD_{50} (mg/kg bw)	Reference	Comments
	Mouse	NR	Diet	1250	(Schafer & 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 & Palanker, 1979b)	
(Benzoic acid [08.021])	Mouse	NR	Oral	1250	(Schafer & 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 & Kuizenga, 1945)	
	Guinea pig	NR	Gavage	4100	(Kravets-Bekker & Ivanova, 1970)	
	Rat	NR	Oral	2170	(Graham & Kuizenga, 1945)	
	Rat	M, F	Gavage	1350	(Jenner et al., 1964)	
	Rat	NR	Gavage	3500	(Kravets-Bekker & 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 & Ivanova, 1970)	
Methyl 4-methylbenzoate [09.631]	Rat	M	Gavage	2987	(Dashiell & Hinckle, 1981)	
	Rat	NR	Oral	3300	(Moreno, 1977aa)	
(Ethyl benzoate [09.726])	Rabbit	NR	Oral	2630	(Graham & Kuizenga, 1945)	
	Rat	NR	Oral	2100	(Graham & 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 & 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)	
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 & Neufeld-Crzellitzer, 1954)	
	Dog	NR[2]	Oral	5000	(Sabalitschka & Neufeld-Crzellitzer, 1954)	

Table IV.1: ACUTE TOXICITY

Chemical Name [FL-no]	Species	Sex	Route	LD_{50} (mg/kg bw)	Reference	Comments
(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 & 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 & 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)	
(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 & 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)	
(Vanillin [05.018]) continued	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 & 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	(Givaudan Corporation, 1982)	

Table IV.1: ACUTE TOXICITY

Chemical Name [FL-no]	Species	Sex	Route	LD_{50} (mg/kg bw)	Reference	Comments
				F: 2642		
	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, 1984)	
(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	(Givaudan Corporation, 1982)	
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)	
(Benzyl salicylate [09.752])	Rat	M	Gavage	2227	(Fogleman & 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 & 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	(Rhône-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 & Kitzmiller, 1940)	
(Ethyl vanillin isobutyrate)	Rat	M, F	Oral	> 2000	(Sanders & Crowther, 1997)	
(Ethyl vanillin β-*d*-glucopyranoside)	Rat	M, F	Oral	> 5000	(Cerven, 1990)	
(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)	

[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 four candidate substances of the present flavouring group evaluation from chemical group 23 and for 18 supporting substances evaluated by JECFA at the 46th and 57th meeting. The supporting substances are listed in brackets.

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

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

Chemical Name	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, 1989)	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, 1989)	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, 1989)	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, 1989)	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.

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

Chemical Name	Species; Sex No./Group	Route	Dose levels	Duration	NOAEL (mg/kg bw/day)	Reference	Comments
(Benzyl acetate [09.014]) continued	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)	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 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 females)	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 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.

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

Chemical Name	Species; Sex No./Group	Route	Dose levels	Duration	NOAEL (mg/kg bw/day)	Reference	Comments
(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 summarized 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 summarized 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 mg7kg.
	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 B6C3F1 mice.
(Methyl benzoate [09.725])	Rat; NR 13	Gavage	0, 0.005, 0.05 mg/kg bw/day	6 months	0.005	(Kravets-Bekker & 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 & 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.
(Benzoic acid [08.021]) continued	Mouse; M, F 50	oral (paste)	0, 40 mg/kg bw/day	17 months	40[2]	(Shtenberg & 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

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

Chemical Name	Species; Sex No./Group	Route	Dose levels	Duration	NOAEL (mg/kg bw/day)	Reference	Comments
							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 10 or 11	Oral	0, 0.2, 1, 2% in the diet (0, 100, 500, 1000 mg/kg bw/day)[18]	25 weeks	1000	(Sado, 1973)	Published non-GLP study of limited quality. 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 & 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
(*4*-Methoxybenzaldehyde [05.015]) continued	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 summarized 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 summarized in a table only.

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

Chemical Name	Species; Sex No./Group	Route	Dose levels	Duration	NOAEL (mg/kg bw/day)	Reference	Comments
(Methyl 2-methoxybenzoate [09.796])	Rat; M, F 10	Oral	0, 94 mg/kg bw/day	14 days	94[2]	(Van Miller & 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.
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 summarized 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 summarized in a table only.
	Rat; NR 8	Oral	64 mg/kg bw/day	70 days	ND	(Deichmann & 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 & 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 summarized 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 summarized in a table only.
(Vanillin [05.018]) continued	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 & Kitzmiller, 1940)	Published non-GLP study with insufficient quality of experimental design and report of data. No adequate controls.
	Rat; M 10	Oral	0, 0.1, 0.5, 1.0% in the diet (0, 40, 214, 437 mg/kg bw/day)[20]	26 weeks	437[2]	(Monsanto Co., 1955a)	Unpublished non-GLP study of limited quality. 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 & Kitzmiller, 1940)	Published non-GLP study with insufficient quality of experimental design and report of data. No adequate controls.
	Rabbit; NR	Oral	83 mg/kg bw/day for 14 days	14 or 61 days	ND	(Deichmann & Kitzmiller,	Published non-GLP study with insufficient quality of

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

Chemical Name	Species; Sex No./Group	Route	Dose levels	Duration	NOAEL (mg/kg bw/day)	Reference	Comments
	1		103 mg/kg bw/day for 61 days			1940)	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 & 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 summarized 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 & 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 summarized 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 & 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 & 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.
(Methyl salicylate [09.749]) continued	Rat; M, F 15	Oral	0, 2.0% in the diet (0, 1000 mg/kg bw/day)[21]	11 weeks	ND[7]	(Abbott & 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 & 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 & 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

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

Chemical Name	Species; Sex No./Group	Route	Dose levels	Duration	NOAEL (mg/kg bw/day)	Reference	Comments
							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 & 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 summarized in text.
	Dog; M, F 6	Oral	0, 150, 300, 500, 800 mg/kg bw/day[22]	7.5 months[10]	ND[7]	(Abbott & 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[12]	Oral	0, 50, 100, 170 mg/kg bw/day	6 months[12]	170[2]	(Abbott & 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 & Hansen, 1963)	Published non-GLP FDA-study of good quality. Study protocols fully described, results not reported in detail but only summarized 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 & Hansen, 1963)	Published non-GLP FDA-study of good quality. Study protocols fully described, results not reported in detail but only summarized in text.
(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.
(Isopentyl salicylate [09.751]) continued	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 summarized in a table only.
	Rat; NR 8	Oral	64 mg/kg bw/day	70 days	64[2]	(Deichmann & 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 & 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 & 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 summarized by 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.

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

Chemical Name	Species; Sex No./Group	Route	Dose levels	Duration	NOAEL (mg/kg bw/day)	Reference	Comments
							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 summarized in a table only.
	Rabbit; NR 3	Oral	240 mg/kg bw/day	56 or 126 days[19]	240[2]	(Deichmann & 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, 31, 17, 31, 49 days, respectively)	15 - 49 days	41[2]	(Deichmann & 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 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.
(Piperonal [05.016]) continued	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 summarized 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 summarized 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 & Griepentrog, 1967)	Published study with incomplete report of experimental details and results.

[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 benzylacetate/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 JECFA at the 57th meeting. Supporting substance listed in brackets.

TABLE IV.3: DEVELOPMENTAL AND REPRODUCTIVE TOXICITY STUDIES

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 maternel 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 matermal 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).
(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.

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 maternel toxicity	Reference	Comments
							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 & 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.
Ethyl-4-hydroxybenzoate [09.367] continued	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 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)	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

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 maternel toxicity	Reference	Comments
		11-16					and dry) at the highest dose (ED50 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 (ED50 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 (signif. 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%).
(Butyl *p*-hydroxybenzoate [09.754]) continued	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 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.

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 maternel toxicity	Reference	Comments
	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 (ED50 for uterotrophic effect: uterine wet weight 87mg/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 (ED50 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, 1984)	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 developmental toxicity: 1 week before mating until 4 days post parturition	Rat; F 10	Gavage	0, 80, 400, 800 mg/kg bw/day	Maternal: ND[1] Foetal: 800	(Vollmuth et al., 1990)	Unpublished study with limited report of 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 & 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	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

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 maternel toxicity	Reference	Comments
	before mating until 4 days post parturition						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 secondr 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 group 23 and for 29 supporting substances evaluated by JECFA at the 46th and 57th meeting. Supporting substances are listed in brackets.

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

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 to 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 & Garro, 1976)	
	Ames test (plate incorporation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537	0, 100, 333, 1000, 3333, 6666 µg/plate	Negative[1]	(NTP, 1989)	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., 1978)	Study published in Japanese without English abstract. Data extracted from tables. Validity of the study cannot be evaluated.
(Benzyl alcohol [02.010]) continued	Rec assay	*B. subtilis* M45 (rec^-), H17 (rec^+)	10 µg/disc	Weakly 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	Weakly 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 concentrations, max.	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

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

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
			concentration inducing 50% cell-growth inhibition)			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] Weakly positive[3]	(NTP, 1989)	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	Weakly positive	(NTP, 1989)	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]	Weakly 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.
(Benzyl alcohol [02.010]) continued	Mammalian cell gene mutation test	Mouse lymphoma L5178Y cells	150 to 5000 µg/ml	Negative[3] Positive[2]	(NTP, 1989)	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)	
	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

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

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
						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., 1978)	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.
(Benzyl acetate [09.014]) continued	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 mutation test	Mouse lymphoma L5178Y cells	Not reported	Negative[2] Positive[3,]	(Rudd et al., 1983)	Study carried out within a larger NTP project. Only abstract 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., 1987)	Published non-GLP study. Doses were selected based on preliminary assay. Although some details of results are not

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

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
						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., 1987)	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., 1978)	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	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 & Raw, 1979)	Assay of urine samples from rats given benzaldehyde by oral gavage.
(Benzaldehyde [05.013]) continued	Ames test (plate incorporation method)	*S. typhimurium* TA98; TA100	100 µl/plate	Negative[3]	(Rockwell & 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)	
	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 & 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/	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)	

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; 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., 1978)	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] Weakly 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.
(Benzaldehyde [05.013]) continued	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.
	Mammalian cell gene mutation test	Mouse lymphoma L5178Y +/- cells	600 µg/ml	Negative[2]	(Bigger & 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., 1987)	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).

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

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
	Sister chromatid exchange assay	Chinese hamster ovary cells	5-160 µg/ml[2]; 160-1600 µg/ml[3]	Positive[2] Weakly positive[3]	(Galloway et al., 1987)	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.
(Benzaldehyde [05.013]) continued	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 & 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	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 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 to OECD guideline 471. 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 & 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 & 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 & Fujii, 1992)	Well conducted published non-GLP study with some minor deficiencies (no cytotoxicity data, no detailed data for different concentrations reported) 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 & Dziadziuszko, 1986)	Genotoxicity measured as ability to induce ß-galactosidase.
	SOS Chromotest	*E. coli* PQ37	50 µg	Negative[1]	(Kevekordes et al., 1999)	

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

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
	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.
(Benzoic acid [08.021]) continued	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 & 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 incorporation method)	*S. typhimurium* TA98; TA100	100 µl/plate	Negative[3]	(Rockwell & Raw, 1979)	
	Ames test (plate incorporation method)	*S. typhimurium* TA98; TA100	300 µl/plate	Negative[1]	(Rockwell & 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 & Raw, 1979)	
	Ames test (plate method)incorporation	*S. typhimurium* TA98; TA100	300 µl/plate	Negative[1]	(Rockwell & 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.

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

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
(4-Isopropylbenzaldehyde [05.022]) continued	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 & Bohovicova, 2000)	
	DNA Repair test	*E. coli* WP2, WP2*uvrA*, CM611; CM561	2000 µg/ml	Negative	(Mikulasova & Bohovicova, 2000)	
(Salicylic acid [08.112])	Ames test (preincubation method)	*S. typhimurium* TA98; TA100	100 to 10000 µg/plate	Negative[1]	(Kuboyama & 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)	
	Rec assay	*B. subtilis* M45 (rec$^-$), H17 (rec$^+$)	0 to 5000 µg/disc	Weakly positive	(Kuboyama & Fujii, 1992)	Well conducted published non-GLP study with some minor deficiencies (no cytotoxicity data, no detailed data for different concentrations reported) 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)	Weakly 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)	dito.
	Chromosomal aberration assay	Hamster lung fibroblast cells	Not reported	Positive[2] Negative[3]	(Kawachi et al., 1980a)	dito.
	Chromosomal aberration assay	Human embryo fibroblasts	Not reported	Negative[2]	(Kawachi et al., 1980a)	dito.
	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.

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

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
Ethyl 4-hydroxybenzoate [09.367] continued	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)	
	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 & 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 6.2fold 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 & Bohovicova, 2000)	
	DNA Repair test	*E. coli* WP2; WP2*uvrA*; CM611; CM561	2000 µg/ml	Negative	(Mikulasova & Bohovicova, 2000)	

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

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
(Veratraldehyde [05.017]) continued	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 method)	*S. typhimurium* TA1537	Up to 5000 µg/plate (6 concentrations)	Negative[1]	(Engelhardt, 1986)	
	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 & Sasaki, 1987)	
	Rec assay	*B. subtilis* M45 (rec^-), H17 (rec^+)	22 µg/disc	Negative	(Oda et al., 1978)	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 & 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)	

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

Chemical Name [FL-no]	**Test System**	**Test Object**	**Concentration**	**Result**	**Reference**	**Comments**
(4-Methoxybenzaldehyde [05.015]) continued	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 assay	Human lymphocytes	0- 0.25 mM (0-34 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.
3-Methoxybenzaldehyde [05.158]	Sister chromatid exchange assay	Human lymphocytes	0-2.0 mM (0-273 µg/ml)	Positive[2]	(Jansson et al., 1988)	dito.
	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 & 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 & 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 & Klemencic, 1979)	
	Ames test	*S. typhimurium* TA100	100 µg/plate	Weakly 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)	

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

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
Gallic acid [08.080] continued	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 & 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 assay	Chinese hamster ovary K1 cells	0, 0.25, 0.5, 1.0, 1.5, 2.0 mM (0, 42.5, 85, 170, 255, 340 µg/ml)	Positive[2]	(Tayama & Nakagawa, 2001)	Published non-GLP study. Well conducted part of the 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 & Lin, 1982)	
	Rec assay	*B. subtilis* M45 (rec^-), H17 (rec^+)	21 µg/disc	Negative	(Oda et al., 1978)	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 & 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 to 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)	
(Vanillin [05.018]) continued	Ames test (plate incorporation method)	*S. typhimurium* TA98; TA100	2500 µg/plate	Negative[2]	(Mikulasova & Bohovicova, 2000)	
	DNA Repair test	*E. coli* WP2; WP2*uvrA*; CM611; CM561	2000 µg/ml	Negative	(Mikulasova & Bohovicova,	

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

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

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

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
(Vanillin [05.018]) continued	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 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 & 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 to 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 h, 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 h less than 10% cells with chromosomal aberrations). No chromosomal aberrations after 48 h treatment without S9. After 48 h treatment without S9 18% polyploid cells..

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

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
(Salicylaldehyde [05.055]) continued	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 to OECD guideline 471. Although some details of results are not reported the study is considered valid.
	Ames test (preincubation method)	*S. typhimurium* TA97; TA98; TA100; TA1535; TA1537	333.3 µg/plate	Negative[1]	(Mortelmans et al., 1986)	
	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)	dito.
	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 & 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., 1978)	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 & 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)	dito.
	Sister chromatid exchange assay	Human embryo fibroblast cells	Not reported	Negative[2]	(Kawachi et al., 1980b; Kawachi et al., 1980a)	dito.
(Butyl vanillyl ether [04.093])	Ames test	*S. typhimurium* TA98; TA100; TA1535; TA1537	5000 µg/plate	Negative[1]	(Watanabe & Morimoto, 1989c)	
	Mutation assay	*E. coli* WP2 *uvr*A	5000 µg/plate	Negative[1]	(Watanabe & Morimoto, 1989c)	
(Ethyl vanillin [05.019])	Ames test	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	3600 µg/plate	Negative[1]	(Wild et al., 1983)	
	Ames test (preincubation	*S. typhimurium* TA97; TA98; TA100;	8000 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* 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 to 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 & 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., 1978)	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)	Ames test	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	5000 µg/plate	Negative[1]	(King & 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)	

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

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
(Piperonal [05.016)]	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 & Shibamoto, 1982)	Valid study in accordance with OECD guideline 471. The plate incorporation method was used -S9; the preincubation method +S9.
	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., 1978)	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 & 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 & 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.

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.

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. Supporting substances are listed in brackets.

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

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 h 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 chance 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.
(Benzyl acetate [09.014]) continued	*In vivo* Unscheduled DNA synthesis test	Rat pancreatic cells	Oral gavage	1000 mg/kg bw	Negative	(Steinmetz & 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 1500 mg/kg bw	Negative	(Longnecker et al., 1990)	Alkaline elution assay. GLP status not specified. Limited number of animals/group; DNA damage

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

Chemical Name [FL-no]	Test System	Test Object	Route	Dose	Result	Reference	Comments
							monitored at 1 hr 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 LD50. 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 h 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 LD50.
	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 LD50.
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 summarized results of a large screening study reported. Thus, the validity of the study cannot be evaluated.
(Vanillin [05.018])	*In vivo* Micronucleus test	Male BDF1 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 effect. Vanillin itself did not induce micronucleated PCEs (vanillin control group without mitomycin-treatment, six sampling times from 5 to 65 h).
(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

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

Chemical Name [FL-no]	Test System	Test Object	Route	Dose	Result	Reference	Comments
							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 BDF1 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)	Dito.

REFERENCES:

Abbott, D.D., Harrisson, J.W.E., 1978. Methyl salicylate: Studies of osseous changes inthe rat, reproduction in the rat and mouse, and liver and kidney effects in the dog. LaWall and Harrison Research Laboratories, Inc. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Abdo, K.M., Huff, J.E., Haseman, J.K., Boorman, G.A., Eustis, S.L., Matthews, H.B., Burka, L.T., Prejean, J.D., Thompson, R.B., 1985. Benzyl acetate carcinogenicity, metabolism and disposition in Fischer 344 rats and B6C3F1 mice. Toxicology 37, 159-170.

Abdo, K.M., Wenk, M.L., Harry, G.J., Mahler, J., Goehl, T.J., Irwin, R.D., 1998. Glycine modulates the toxicity of benzyl acetate in F344 rats. Toxicol. Pathol. 26(3), 395-402.

Aeschbacher, H.U., Wolleb, U., Loliger, J., Spadone, J.C., Liardon, R., 1989. Contribution of coffee aroma constituents to the mutagenicity of coffee. Food Chem. Toxicol. 27(4), 227-232.

Amsel, L.P., Levy, G., 1969. Drug biotransformation interactions in man. II: A pharmacokinetic study of the simultaneous conjugation of benzoic and salicylic acids with glycine. J. Pharm. Sci. 58(3), 321.

Anderson, D., Styles, J.A., 1978. An evaluation of 6-short-term tests for detecting organic chemical carcinogens. Appendix 2. The bacterial mutation test. Br. J. Cancer 37, 924-930.

Anderson, B.E., Zeiger, E., Shelby, M.D., Resnick, M.A., Gulati, D.K., Ivett, J.L., Loveday, K.S., 1990. Chromosome aberration and sister chromatid exchange test results with 42 chemicals. Environ. Mol. Mutag. 16(Suppl. 18), 55-137.

Ball, J., Foxall-VanAken, S., Jensen, T.E., 1984. Mutagenicity studies of p-substituted benzyl derivatives in the ames salmonella plate-incorporation assay. Mutat. Res. 138, 145-151.

Bär, F., Griepentrog, F., 1967. Die Situation in der gesundheitlichen Beurteilung der Aromatisierungsmittel für Lebensmittel. Med. Ernähr. 8, 244-251.

BASF, 1981. [Acute toxicity studies on p-methoxybenzaldehyde]. Unpublished report submited by EFFA to FLAVIS Secretariat. (In German)

Becker, T.W., Kriger, G., Witte, I., 1996. DNA single and double strand breaks induced by aliphatic and aromatic aldehydes in combination with copper (II). Free Radical Res. 24(5), 325-332.

Bier, C.B., 1979. Acute oral toxicity in mice administered butyl benzoate with cover letter dated 04/28/92. Bio Research Labs. EPA Doc 88-920002167, microfiche no. OTS0539230. Date 3/05/79. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Bigger, C.A.H., Clarke, J.J., 1991. Test for chemical induction of mutation in mammalian cells in culture the L5178Y TK+/- mouse lymphoma assay (final report) with cover letter dated 112691 (sanitized). Microbiological Accosiates Inc. EPA Doc 86-920000497S, microfiche no. OTS0533786. Date 7/24/91. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Booth, A.N., Masri, M.S., Robbins, D.J., Emerson, O.H., Jones, F.T., DeEds, F., 1959. The metabolic fate of gallic acid and related compounds. J. Biol. Chem. 234(11), 3014-3016.

Brantom, P.G., Gaunt, I.F., Grasso, P., Lansdown, A.B.G., 1972. Short-term toxicity of tolualdehyde in rats. Food Cosmet. Toxicol. 10, 637-647.

Bray, H.G., Ryman, B.E., Thorpe, W.V., 1947. The fate of certain organic acids and amides in the rabbit. 2. p-Hydroxybenzoic acid and its amide. Biochem. J. 41, 212-218.

Bray, H.G., Ryman, B.E., Thorpe, W.V., 1948. The fate of certain organic acids and amides in the rabbit. 5. o- And m-hydroxybenzoic acids and amides. Biochem. J. 43, 561-567.

Bray, H.G., Thorpe, W.V., White, K., 1952b. Kinetic studies of the metabolism of foreign organic compounds. 5. A mathematical model expressing the metabolic fate of phenols, benzoic acids and their precursors. Biochem. J. 52, 423-430.

Bridges, J.W., French, M.R., Smith, R.L., Williams, R.T., 1970. The fate of benzoic acid in various species. Biochem. J. 118, 47-51.

Buch, S.A., 1989. Vanillyl alcohol n-butyl ether acute oral toxicity in the rat. Life Science Research. LSR report no. 80/TAG005/438. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Carson, S., 1972a. 90-Day studies with glyceryl tribenzoate in rats. Food and Drug Research Laboratories, Inc. Lab. no. 0732. April 10, 1972. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Carson, S., 1972b. 90-Day studies with propylene glycol dibenzoate in rats. Food and Drug Research Laboratories, Inc. Lab. no. 2-0732. APril 18, 1972. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Carter, D.V., Charlton, P.T., Fenton, A.H., Housley, J.R., Lessel, B., 1958. The preparation and the antibacterial and antifungal properties of some substituted benzyl alcohols. J. Pharm. Pharmacol. 10, T149-T159.

Caspary, W.J., Langenbach, R., Penman, B.W., Crespi, C., Myhr, B.C., Mitchell, A.D., 1988. The mutagenic activity of selected compounds at the TK locus rodent vs. human cells. Mutat. Res. 196, 61-81.

Cerven, D.R., 1990. Ethyl vanillin glucoside DA-5-70. Single dose oral toxicity in rats/LD50 in rats. MB Research Laboratories, Inc. Project ID MB 90-9977 A. Date 8/29/90. Unpublished report submitted by EEFA to FLAVIS Secretariat.

Chang, S.H., Chun, B.C., Lee, W.J., Christiani, D.C., 2000. Urinary excretion of hippuric acid after consumption of non-alcoholic beverages. Int. J. Occup. Environ. Health 6(3), 238-242.

Chen, S.C., Chung, K.T., 2000. Mutagenicity and antimutagenicity studies of tannic acid and its related compounds. Food Chem. Toxicol. 38(1), 1-5.

Chidgey, M.A.J., Caldwell, J., 1986. Studies on benzyl acetate. I. Effect of dose size and vehicle on the plasma pharmacokinetics and metabolism of [methylene-14C] benzyl acetate in the rat. Food Chem. Toxicol. 24, 1257-1265.

Chidgey, M.A.J., Kennedy, J.F., Caldwell, J., 1986. Studies on benzyl acetate. II. Use of specific metabolic inhibitors to define the pathway leading to the formation of benzylmercaptic acid in the rat. Food Chem. Toxicol. 24, 1267-1272.

Ciba-Geigy Corp., 1945. Initial submission: Acute oral LD50 in the rat of TK 12186 (final report) with cover letter dated 11/25/91. Ciba-Geigy Ltd. EPA Doc 88-920000203, microfiche no OTS0534655. Unpublished report submitted by EFFA to FLAVIS Secretariat.

CoE, 1992. Flavouring substances and natural sources of flavourings. 4th Ed. vol. I. Chemically defined flavouring substances. Council of Europe, partial agreement in the social and public health field. Strasbourg.

Costello, B.A., 1984. Acute oral toxicity LD50 - rats. Aldehyde E. Biosearch Incorporated. Project no. 84-4171A. Date 7/19/84. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Cotruvo, J.A., Simmon, V.F., Spanggord, R.J., 1977. Investigation of mutagenic effects of products of ozonation reactions in water. Ann. N.Y. Acad. Sci. 298, 124-140.

Cramer, G.M., Ford, R.A., Hall, R.L., 1978. Estimation of toxic hazard - a decision tree approach. Food Cosmet. Toxicol. 16(3), 255-276.

CTFA, 1980b. CIR safety data test summary: Acute oral toxicity. Ethylparaben. Company test code OT26/212, OT26/213. Unpublished data submitted by EFFA to FLAVIS Secretariat.

Damment, S.J.P., 1980. Acute oral toxicity study (LD50) in the rat. Benzyl alcohol. Hazleton Laboratories Ltd. Report no. 2181-110/288. February 1980. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Dashiell, O.L., Hinckle, L., 1981. Initial submission: Oral LD50 test in rats with cover letter dated 081092. Haskell Labs. EPA Doc 88-
920009096, microfiche no. OTS0546379. Date 08/03/81. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Daston, G.P., 2004. Developmental toxicity evaluation of butylparaben in Sprague-Dawley rats. Birth Defects Res. 71 part B, 296-302.

Davison, C., Zimmerman, E.F., Smith, P.K., 1961. On the metabolism and toxicity of methyl salicylate. J. Pharm. Exp. Ther. 132(1), 207-211.

deGroot, A.P., Spanjers, M.T., van der Heijden, C.A., 1974. Acute and sub-acute oral toxicity studies in rats with five flavour compounds. Central Institute for Nutrition and Food Researh. Report no. R 4284. January 1974. Unpublished report submitted by EFFA to SCF.

Deichmann, W., Kitzmiller, K.V., 1940. On the toxicity of vanillin and ethyl vanillin for rabbits and rats. J. Am. Pharm. Assoc. 29, 425-428.

Derache, R., Gourdon, J., 1963. Metabolism of a food preservative: p-hydroxybenzoic acid and its esters. Food Cosmet. Toxicol. 1, 189-195.

Dillon, D.M., McGregor, D.B., Combes, R.D., Zeiger, E., 1992. Detection of mutagenicity in Salmonella of some aldehydes and peroxides. Environ. Mol. Mutag. 19(Suppl. 20), 15.

Dillon, D., Combes, R., Zeiger, E., 1998. The effectiveness of Salmonella strains TA100, TA102 and TA104 for detecting mutagenicity of some aldehydes and peroxides. Mutagenesis 13(1), 19-26.

Dirscherl, W., Wirtzfeld, A., 1964. Vanillic acid in human urine, its isolation, determination and origin. Hoppe-Seyler's Z. Physiol. Chem. 336, 81-90.

Dollahite, J.W., Pigeon, R.F., Camp, B.J., 1962. The toxicity of gallic acid, pyrogallol, tannic acid, and Quercus havardi in the rabbit. Am. J. Vet. Res. 23, 1264-1267.

Douglas, G.R., Nestmann, E.R., Betts, J.L., Mueller, J.C., Lee, E.G.H., Stich, H.F., San, R.H.C., Brouzesm, R.J.P., Chmelauskasm, A.L., Paavilam, D.H., Walden, C.C., 1979. Mutagenic activity in pulp mill effluents. In: Jolley, R.L., Brungs, W.A., Cumming, R.B., Jacobs, V.A. (Eds.). Water Chlorination, Environmental Impact and Health Effects. vol. 3. Ann Arbor Science, Michigan, pp. 865-880.

Dow Chemical Company, 1992b. Results of range finding toxicological tests on p-hydroxy benzaldehyde. Biochemical Research Laboratory. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Draize, J.H., Alvarez, E., Whitesell, M.F., Woodward, G., Hagan, E.C., Nelson, A.A., 1948. Toxicological investigations of compounds proposed for use as insect repellents. J. Pharmacol. Exp. Ther. 93, 26-39.

Drake, J.J.P., Gaunt, I.F., Butterworth, K.R., Hooson, J., Hardy, J., Gangolli, S.D., 1975. Short-term toxicity of isoamyl salicylate in rats. Food Cosmet. Toxicol. 13, 185-193.

Dufour, 1994. Acute oral toxicity in the rat of the aromatic substance. Evic-Ceba. Study report T 263/4511 - Laboratoire de Recherche et d'Experimentation. June 30, 1994. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Eastman Kodak Co., 1991b. Letter from Eastman Kodak Company to U.S. EPA submitting enclosed toxicity and hazard summary and toxicity report on salicylaldehyde with attachments. EPA Doc 86-920000075, microfiche no. OTS0533438. Date 10/22/91. Unpublished report submitted by EFFA to FLAVIS Secretariat.

EC, 1996. Regulation No 2232/96 of the European Parliament and of the Council of 28 October 1996. Official Journal of the European Communities 23.11.1996, L 299, 1-4.

EC, 1999a. Commission Decision 1999/217/EC of 23 February 1999 adopting a register of flavouring substances used in or on foodstuffs. Official Journal of the European Communities 27.3.1999, L 84, 1-137.

EC, 2000. Commission Regulation No 1565/2000 of 18 July 2000 laying down the measures necessary for the adoption of an evaluation programme in application of Regulation (EC) no. 2232/96. Official Journal of the European Communities 19.7.2000, L 84, 8-16.

EC, 2002b. Commission Regulation No 622/2002 of 11 April 2002 establishing deadlines for the submission of information for the evaluation of chemically defined flavouring substances. Official Journal of the European Communities 12.4.2002, L 95, 10-11.

EC, 2005. Commission Decision 1999/217/EC of 18 May 2005 amending Commission Decision as regards the register of flavouring substances. Official Journal of the European Union 21.5.2004, L 128, 73-76.

EFFA, 2002i. Letter from EFFA to Dr. Joern Gry, Danish Veterinary and Food Administration. Dated 31 October 2002. Re.: Second group of questions. FLAVIS/8.26.

EFFA, 2004c. Submission 2003-7. Flavouring group evaluation of 37 flavouring substances (candidate chemicals) of the chemical group 23 (annex I of 1565/2000/EC) structurally related to benzyl derivatives [JECFA/WHO FAS 48/57] used as flavouring substances. 20 November 2003. FLAVIS/8.32.

EFFA, 2004d. Submission 2003-7. Flavouring group evaluation of 37 flavouring substances (candidate chemicals) of the chemical group 23 (annex I of 1565/2000/EC) structurally related to benzyl derivatives [JECFA/WHO FAS 48/57] used as flavouring substances. 20 November 2003. FLAVIS/8.32. European inquiry on volume of use. IOFI, International Organization of the Flavor Industry, 1995. Private communication to FEMA. Unpublished report submitted by EFFA to SCF.

EFFA, 2004e. Intake - Collection and collation of usage data for flavouring substances. Letter from Dan Dils, EFFA to Torben Hallas-Møller, EFSA. May 31, 2004.

EFSA, 2004a. Minutes of the 7th Plenary meeting on the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food, Held in Brussels on 12-13 July 2004. Annex. Brussels, 28 September 2004. [Online]. Available: http://www.efsa.eu.int/science/afc/afc_meetings/502_en.html

EFSA, 2004b. Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Material in Contact with Food on a Request from the Commission related to para hydroxybenzoates (E214-219). The EFSA Journal 83, 1-26.

Engelhardt, G., 1986. Ames test (standard plate test with Salmonella typhimurium TA 1537) (Jan. 6, 1987) (Fianl report) with cover letter dated 121691. BASF AG. EPA Doc 86-920000679, microfiche no. OTS0535562. Date 1/06/87. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Epstein, S.S., Arnold, E., Andrea, J., Bass, W., Bishop, Y., 1972. Detection of chemical mutagens by the dominant lethal assay in the mouse. Toxicol. Appl. Pharmacol. 23, 288-325.

Eurostat, 1998. Total population. Cited in Eurostat, 2004. The EU population, Total population. Available: http://europa.eu.int/comm/eurostat/newcronos/queen/display.do?screen=detail&language=en&product=YES&root=YES/yearlies/c/ca/caa/caa10000. August 13, 2004.

FDA, 1954. Pathological changes in rats from feeding of various flavoring agents, 1% in diet, for 16 weeks, or at 0,1% of diet for 28 weeks. Food and Drug Administration. Unpublished report submitted by EFFA to SCF.

FDA, 1975b. Mutagenic evaluation of compound FDA 73-70, benzoic acid, certified A.C.S. Litton Bionetics, Incorporated. LBI project 2468, PB-245 500. 30 May, 1975. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Field, W.E., 1979a. In: Eastman Kodak Co., 1991. Letter from Eastman Kodak Company to U.S. EPA submitting enclosed material safety data sheet, toxicity report and information on 3,4-dimethoxybenzaldehyde with attachments. EPA Doc 86-920000083, microfiche no. OTS0533446. Date 10/22/91. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Field, W.E., 1979b. In: Eastman Kodak Co., 1991. Letter to USEPA submitting enclosed material safety data sheet, toxicity & health hazard summary and toxicity reports on 2-methoxybenzaldehyde w-attachments. EPA Doc 86-920000059, microfiche no. OTS0533625. Date 10/10/91. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Florin, I., Rutberg, L., Curvall, M., Enzell, C.R., 1980. Screening of tobacco smoke constituents for mutagenicity using the Ames' test. Toxicology 18, 219-232.

Fluck, E.R., Poirier, L.A., Ruelius, H.W., 1976. Evaluation of a DNA polymerasedeficient mutant of E. coli for the rapid detection of carcinogens. Chem. -Biol. Interact. 15, 219-231.

Fogleman, R.W., Margolin, S., 1970. Oral LD50 test - rats. Benzyl salicylate, benzophenone, isobornyl acetate, bergamot oil - furocoumarin free, musk ketone, musk xylol, benzoin. Date 7/1/70. Unpublished data submitted by EFFA to FLAVIS Secretariat.

Foureman, P., Mason, J.M., Valencia, R., Zimmering, S., 1994. Chemical mutagenesis testing in Drosophila. X. Results of 70 coded chemicals tested for the National Toxicology Program. Environ. Mol. Mutag. 23, 208-227.

Fujita, H., Sasaki, M., 1987. [Mutagenicity test of food additives with Salmonella typhimurium TA97 and TA102]. Ann. Rep. Tokyo Metrop. Res. Lab. Public Health 38, 423-430. (In Japanese)

Fujita, H., Aoki, N., Sasaki, M., 1994. [Mutagenicity test of food additives with Salmonella typhimurium TA97 and TA102]. Ann. Rep. Tokyo Metrop. Res. Lab. Public Health 43, 219-227. (In Japanese)

Furukawa, A., Ohuchida, A., Wierzba, K., 1989. In vivo mutagenicity tests on polyploid inducers. Environ. Mol. Mutagen. 14(15), 63-64.

Galloway, S.M., Armstrong, M.J., Reuben, C., Colman, S., Brown, B., Cannon, C., Bloom, A.D., Nakamura, F., Ahmed, M., Duk, S., Rimpo, J., Margolin, B.H., Resnick, M.A., Anderson, B., Zeiger, E., 1987. Chromosome aberrations and sister chromatid exchanges in Chinese hamster ovary cells: evaluations of 108 chemicals. Environ. Mol. Mutag. 10(Suppl. 10), 1-175.

Garberg, P., Aakerblom, E.-L., Bolcsfoldi, G., 1988. Evaluation of a genotoxicity test measuring DNA-strand breaks in mouse lymphoma cells by alkaline unwinding and hydroxyapatite elution. Mutat. Res. 203(3), 155-176.

Gee, P., Sommers, C.H., Melick, A.S., Gidrol, X.M., Todd, M.D., Burris, R.B., Nelson, M.E., Klemm, R.C., Zeiger, E., 1998. Comparison of responses of base-specific Salmonella tester strains with the traditional strains for identifying mutagens: The results of a validation study. Mutat. Res. 412(2), 115-130.

Giri, A.K., Adhikari, N., Khan, K.A., 1996. Comparative genotoxicity of six salicylic acid derivatives in bone marrow cells of mice. Mutat. Res. 370(1), 1-9

Giroux, J., Granger, R., Monnier, P., 1954b. Comparative toxicity of methyl diethylacetylsalicylate and methyl salycilate. Soc. Pharm. Montpellier 14(4), 383-390.

Givaudan Corporation, 1982. Acute toxicity studies on allyl cyclohexanepropionate, 6-isopropylquinoline, allyl phenoxyacetate, allyl hexanoate, isoamyl salicylate, 6-acetyl-1,1,2,4,4,7-hexamethyltetraline and methylsalicylate in rats. Hazleton Laboratories Deutchland GMPH. Project no. 161/100. Unpublished data submitted by EFFA to FLAVIS Secretariat.

Glosnicka, R., Dziadziuszko, H., 1986. Mutagenic action of styrene and its metabolites. II. Genotoxic activity of styrene, styrene oxide, styrene glycol and benzoic acid tested with the SOS Chromotest. Bull. Inst. Mar. Trop. Med. Gdynia 37(3-4), 295-301.

Grady, R.W., Graziano, J.H., Akers, H.A., Cerami, A., 1976. The development of new iron-chelating drugs. J. Pharmacol. Exp. Ther. 196, 478-485.

Graham, B.E., Kuizenga, M.H., 1945. Toxicity studies on benzyl benzoate and related benzyl compounds. J. Pharmacol. Exp. Ther. 84(4), 358-362.

Grundschober, F., 1977. Toxicological assessment of flavouring esters. Toxicology 8, 387-390.

Hagan, E.C., Jenner, P.M., Jones, W.I., Fitzhugh, O.G., Long, E.L., Brouwer, J.G., Webb, W.K., 1965. Toxic properties of compounds related to safrole. Toxicol. Appl. Pharmacol. 7, 18-24.

Hagan, E.C., Hansen, W.H., Fitzhugh, O.G., Jenner, P.M., Jones, W.I., Taylor, J.M., Long, E.L., Nelson, A.A., Brouwer, J.B., 1967. Food flavourings and compounds of related structure. II. Subacute and chronic toxicity. Food Cosmet. Toxicol. 5(2), 141-157.

Hardin, B.D., Schuler, R.L., Burg, J.B., Booth, G.M., Hazelden, K.P., MacKenzie, K.M., Piccirillo, V.J., Smith, K.N., 1987. Evaluation of 60 chemicals in a preliminary developmental toxicity test. Teratog. Carcinog. Mutag. 7, 29-48.

Haresaku, M., Nabeshima, J., Ishigaki, K., Hashimoto, N., Tovoda, Y., 1985. Mutagenicity study (Ames' test) of toothpaste ingredients. J. Soc. Cosmet. Chem. 19(2), 100-104. (In Japanese)

Harrisson, J.W.E., Abbott, D.D., Packman, E.W., 1963. Salicylates and other hydroxybenzoates: Effect upon osseous tissue of young rats. Fed. Proc. 22(2), Part 1, 554.

Hasegawa, R., Nakaji, Y., Kurokawa, Y., Tobe, M., 1989. Acute toxicity tests on 113 environmental chemicals. Sci. Rep. Res. Inst. Tohoku Univ. 36, 10-16.

Haworth, S., Lawlor, T., Mortelmans, K., Speck, W., Zeiger, E., 1983. Salmonella mutagenicity test results for 250 chemicals. Environ. Mutag. Suppl. 1, 3-142.

Hayashi, M., Kishi, M., Sofuni, T., Ishidate Jr., M., 1988. Micronucleus tests in mice on 39 food additives and eight miscellaneous chemicals. Food Chem. Toxicol. 26(6), 487-500.

Heck, J.D., Vollmuth, T.A., Cifone, M.A., Jagannath, D.R., Myhr, B., Curren, R.D., 1989. An evaluation of food flavoring ingredients in a genetic toxicity screening battery. Toxicologist 9(1), 257-272.

Heim, F., Leuschner, F., Wunderlich, G., 1957. Metabolism of p-hydroxybenzoic acid ethyl ester. Klin. Wochenschr. 35, 823-825. (In German)

Heymann, E., 1980. Carboxylesterases and amidases. In: Jakoby, W.B. (Ed.). Enzymatic basis of detoxication. 2nd Ed. Academic Press, New York, pp. 291-323.

Hirose, M., Masuda, A., Imaida, K., Kagawa, M., Tsuda, H., Ito, N., 1987. Induction of forestomach lesions in rats by oral administrations of naturally occurring antioxidants for 4 weeks. Jap. J. Cancer. Res. 78, 317-321.

Honma, M., Hayashi, M., Shimada, H., Tanaka, N., Wakuri, S., Awogi, T., Yamamoto, K.I., Kodani, N-U., Nishi, Y., Nakadate, M., Sofuni, T., 1999a. Evaluation of the mouse lymphoma tk assay (microwell method) as an alternative to the in vitro chromosomal aberration test. Mutagenesis 14(1), 5-22.

Hooks, W.N., Kirk, S.J., Smith, H.L., Crook, D., Gibson, W.A., Gregson, R.L., Gopinath, C., Anderson, A., Dawe, S.I., 1992. Ethyl vanillin toxicity to rats by repeated dietary administration. Cited in JECFA Joint FAO/WHO Expert Committee on Food Additives (1996) Toxicological evaluation of certain food additives. Prepared by the 44th meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA). International Programme on Chemical Safety. World Health Organization, Geneva.

Hossaiani, A., Larsen, R.-R. and Larsen, J.C., 2000. Lack of oestrogenic effects of food preservatives (parabens) in uterotrophic assays. Food Chem. Toxicol. 38, 319-323.

Ikeda, Y., Yokoi, Y., 1950. Studies on the influence of ethyl rhodanacetate, an antifungal additive of soy sauce. Bull. Natl. Hyg. Lab. 67, 79-106.

Inai, K., Aoki, Y., Akamizu, H., Eto, R., Nishida, T., Tokuoka, S., 1985. Tumorigenicity study of butyl and isobutyl p-hydroxybenzoates administered orally to mice. Food Chem. Toxicol. 23(6), 575-578.

Inouye, T., Sasaki, Y.F., Imanishi, H., Watanebe, M., Ohta, T., Shirasu, Y., 1988. Suppression of mitomycin C-induced micronuclei in mouse bone marrow cells by post-treatment with vanillin. Mutat. Res. 202, 93-95.

IOFI, 1995. European inquiry on volume of use. IOFI, International Organization of the Flavor Industry, 1995.

Ishida, T., Toyota, M., Asakawa, Y., 1989b. Terpenoid biotransformation in mammals. V. Metabolism of (+)-citronellal,(+-) 7-hydroxycitronellal, citral, (-)-perillaldehyde, (-)-myrtenal, cuminaldehyde, thujone, and (+-)- carvone in rabbits. Xenobiotica 19(8), 843-855.

Ishidate, M., Hayashi, M., Sawada, M., Matsuoka, A., Yoshikawa, K, Ono, M., Nakadate, M., 1978. Cytotoxicity test on medical drugs. Chromosome aberration tests with Chinese hamster cells in vitro. Bull. Natl. Inst. Hyg. Sci. 96, 55-61. (In Japanese)

Ishidate, M.Jr., Sofuni, T., Yoshikawa, K., Hayashi, M., Nohmi, T., Sawada, M., Matsuoka, A., 1984. Primary mutagenicity screening of food additives currently used in Japan. Food Chem. Toxicol. 22(8), 623-636.

Ishiguro, S., Miyamoto, A., Obi, T., Nishio, A., 1993. Teratological studies on benzyl acetate in pregnant rats. Bull. of the Faculty of Agric., Kagoshima University, 43, 25-31.

Jansson, T., Zech, L., 1987. Effects of vanillin on sister-chromatid exchanges and chromosome aberrations in human lymphocytes. Mutat. Res. 190, 221-224.

Jansson, T., Curvall, M., Hedin, A., Enzell, C., 1986. In vitro studies of biological effects of cigarette smoke condensate. II. Induction of sister-chromatid in human lymphocytes by weakly acidic, semivolatile constituents. Mutat. Res. 169, 129-139.

Jansson, T., Curvall, M., Hedin, A., Enzell, C., 1988. In vitro studies of the biological effects of cigarette smoke condensate. III. Induction of SCE by some phenolic and related constituents derived from cigarette smoke. Mutat. Res. 206, 17-24.

JECFA, 1967. 10. Report: "Toxicological evaluation of certain food additives". 10th report of the joint FAO/WHO Expert Committee on the Food Additives. Geneva 1967, no. 373.

JECFA, 1995. Evaluation of certain food additives and contaminants. Forty-fourth Meeting of the Joint FAO/WHO Expert Committee on Food Additives. WHO Technical Report Series, no. 859. Geneva.

JECFA, 1996a. Toxicological evaluation of certain food additives. The forty-fourth meeting of the Joint FAO/WHO Expert Committee on Food Additives and contaminants. WHO Food Additives Series: 35. IPCS, WHO, Geneva.

JECFA, 1996b. Toxicological evaluation of certain food additives. Forty-sixth meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA). Food Additives Series: 37. IPCS, WHO, Geneva.

JECFA, 1997a. Evaluation of certain food additives and contaminants. Forty-sixth report of the Joint FAO/WHO Expert Committee on Food Additives. Geneva, 6-15 February 1996. WHO Technical Report Series, no. 868. Geneva.

JECFA, 1999b. Evaluation of certain food additives and contaminants. Forty-ninth report of the Joint FAO/WHO Expert Committee on Food Additives. Rome, 17-26 June 1997. WHO Technical Report Series, no. 884. Geneva.

JECFA, 2000a. Evaluation of certain food additives. Fifty-first meeting of the Joint FAO/WHO Expert Committee on Food Additives. Geneva, 9-18 June 1998. WHO Technical Report Series, no. 891. Geneva.

JECFA, 2001c. Compendium of food additive specifications. Addendum 9. Joint FAO/WHO Expert Commitee of Food Additives 57th session. Rome, 5-14 June 2001. FAO Food and Nutrition paper 52 Add. 9.

JECFA, 2002a. Safety evaluation of certain food additives and contaminants. Fifty-seventh meeting of the Joint FAO/WHO Expert Committee on Food Additives, WHO Food Additives Series: 48. IPCS, WHO, Geneva.

JECFA, 2002b. Evaluation of certain food additives and contaminants. Fifty-seventh report of the Joint FAO/WHO Expert Committee on Food Additives. WHO Technical Report Series, no. 909. Geneva, 5-14 June 2001.

JECFA, 2002c. Evaluation of certain food additives. Fifty-ninth report of the Joint FAO/WHO Expert Committee on Food Additives. WHO Technical Report Series, no. 913. Geneva, 4-13 June 2002.

JECFA, 2002d. Compendium of food additive specifications. Addendum 10. Joint FAO/WHO Expert Commitee of Food Additives 59th session. Geneva, 4-13 June 2002. FAO Food and Nutrition paper 52 Add. 10.

JECFA, 2004b. Safety evaluation of certain food additives and contaminants. Sixty-first meeting of the Joint FAO/WHO Expert Committee on Food Additives, WHO Food Additives Series: 52. IPCS, WHO, Geneva.

JECFA, 2005a. Safety evaluation of certain food additives and contaminants. Sixty-third meeting of the Joint FAO/WHO Expert Committee on Food Additives, WHO Food Additives Series: XX. IPCS, WHO, Geneva. In press.

Jenner P.M., Hagan E.C., Taylor J.M., Cook E.L., Fitzhugh O.G., 1964. Food flavorings and compounds of related structure. I. Acute oral toxicity. Food Cosmet. Toxicol. 2, 327-343.

Jones, P.S., Thigpen, D., Morrison, J.L., Richardson, A.P., 1956. p-Hydroxybenzoic esters as preservatives. III The physiological disposition of p-hydroxybenzoic acid and its esters. J. Am. Pharm. Assoc. Sci. Ed. 45, 268-273.

Jung, R., Engelhart, G., Herbolt, B., Jaeckh, R., Mueller, W., 1992. Collaborative study of mutagenicity with Salmonella typhimurium TA102. Mutat. Res. 278(4), 265-270.

Kamienski, F.X., Casida, J.E., 1970. Importance of demethylenation in the metabolism in vivo and in vitro of methylenedioxyphenyl synergists and related compounds in mammals. Biochem. Pharmacol. 19(1), 91-112.

Kasamaki, A., Urasawa, S., 1985. Transforming potency of flavoring agents in chinese hamster cells. J. Toxicol. Sci. 10, 177-185.

Kasamaki, A., Takahashi, H., Tsumura, N., Niwa, J., Fujita, T., Urasawa, S., 1982. Genotoxicity of flavoring agents. Mutat. Res. 105, 387-392.

Kawachi, T., Yahagi, T., Kada, T., Tazima, Y., Ishidate, M., Sasaki, M., Sugiyama, T., 1980a. Cooperative programme on short-term assays for carcinogenicity in Japan. IARC Sci. Publ. 27, 323-330.

Kawachi, T., Komatsu, T., Kada, T., Ishidate, M., Sasaki, T., Sugiyama, T., Tazima, Y., 1980b. Results of recent studies on the relevance of various short-term screening tests in Japan. Appl. Methods Oncol. 3, 253-267.

Kevekordes, S., Mersch-Sundermann, V., Burghaus, C.M., Spielberger, J., Schmeiser, H.H., Arlt, V.M., Dunkelberg, H., 1999. SOS induction of selected naturally occurring substances in Escherichia coli (SOS Chromotest). Mutat. Res. 445(1), 81-91.

Kevekordes, S., Spielberger, J., Burghaus, C.M., Birkenkamp, P., Zietz, B., Paufler, P., Diez, M., Bolten, C. and Dunkelberg, H., 2001. Micronucleus formation in human lymphocytes and in the metabolically competent human hepatoma cell line Hep-G2: results with 15 naturally occurring substances. Anticancer Res. 21(1A), 461-469.

Kieckebusch, W., Lang, K., 1960. Die Verträglichkeit der Benzoesäure im chronischen Fütterungsversuch. Arzneim.-Forsch./Drug Res. 10, 1001-1003.

Kimmel, C.A., Wilson, J.G., Schumacher, H.J., 1971. Studies on metabolism and identification of the causative agent in aspirin teratogenesis in rats. Teratology 4, 15-24.

King, M.T., Harnasch, D., 1997. Mutagenicity study of ethyl vanillin isobutyrate in the Salmonella typhimurium/mammalian microsome reverse mutation assay (Ames-test). Freiburger Labor für Mutagenitätsprüfung. Project no. AM02397N. April 25, 1997. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Klungsoeyr, J., Scheline, R.R., 1984. Metabolism of piperonal and piperonyl alcohol in the rat with special reference to the scission of the methylenedioxy group. Acta Pharm. Suec. 21(1), 67-72.

Kluwe, W.M., Montgomery, C.A., Giles, H.D., Prejean, J.D., 1983. Encephalopathy in rats and nephropathy in rats after subchronic oral exposure to benzaldehyde. Food Chem. Toxicol. 21(3), 245-250.

Kono, M., Yoshida, Y., Itaya, Y., Shimobo, K., Yoshikawa, K., Terashita, T., Shishiyama, J., 1995. Antimicrobial activity and mutagenicity of allyl isothiocyanates and several essential oils from spices. Mem. Fac. Agri. Kinki Univ. 28, 11-19. (In Japanese)

Koshakji, P.R., Schulert, A.R., 1973. Biochemical mechanisms of salicylate teratology in the rat. Biochem. Pharmacol. 22, 407-416.

Kravets-Bekker, A.A., Ivanova, O.P., 1970. Toxicological characteristics of methyl benzoate and potassium benzoate. Faktory Vneshn. Sredy Ikh Znach. Zdorov'ya Naseleniya (2), 125-129.

Kubota, K., Ishizaki, T., 1991. Dose-dependent pharmacokinetics of benzoic acid following oral administration of sodium benzoate to humans. Eur. J. Clin. Pharmacol. 41, 363-368.

Kubota, K., Horai, Y., Kushida, K., Ishizaki, T., 1988. Determination of benzoic acid in human plasma and urine by high-performance liquid chromatography. J. Chromatogr. 425, 67-75.

Kuboyama, N., Fujii, A., 1992. Mutagenicity of analgesics, their derivatives, and anti-inflammatory drugs with S-9 mix of several animal species. J. Nihon Univ. Sch. Dent., 34(3), 183-195.

Kuroda, K., Tanaka, S., Yu, Y.S., Ishibashi, T., 1984a. [Rec-assay of food additives]. Nippon. Koshu. Eisei. Zasshi 31(6), 277-281. (In Japanese)

Kuroda, K., Yoo, Y.S., Ishibashi, T., 1984b. Antimutagenic activity of food additives. Mutat. Res. 130(5), 369.

Kusakabe, H., Yamakage, K., Wakuri, S., Sasaki, K., Nakagawa, Y., Watanabe, M., Hayashi, M., Sofuni, T., Ono, H., Tanaka, N., 2002. Relevance of chemical structure and cytotoxicity to the induction of chromosome aberrations based on the testing results of 98 high production volume industrial chemicals. Mutat. Res. 517, 187-198.

Laham, S., Potvin, M., 1987. Biological conversion of benzaldehyde to benzylmercaptic acid in the Sprague-Dawley rat. Drug Chem. Toxicol. 10(4&3), 209-225.

Laham, S., Potvin, M., Robinet, M., 1988. Metabolism of benzaldehyde in New Zealand white rabbits. Chemosphere 17(3), 517-524.

LeBel, M., Ferron, L., Masson, M., Pichette, J., Carrier, C., 1988. Benzyl alcohol metabolism and elimination in neonates. Dev. Pharmacol. Ther. 11, 347-356.

Leegwater, D.C., Straten, S., 1974a. In vitro study of the hydrolysis of twenty-six organic esters by pancreatin. Central Institute for Nutrition and Food Research. Report no. R 4319. Project no. 8.33.01. February, 1974.

Lehman, A.J., 1955. Insect Repellents. Assoc. Food Drug Officials U.S., Quart. Bull. 19, 87-99.

Lemini, C., Jaimez, R., Avila, M.E., Franco, Y., Lerrea, F., Lemus, A.N., 2003. In vivo and in vitro estrogen bioactivities af alkyl parabens. Toxicol. Ind. Health 19, 69-79.

Lemini, C., Hernández, A., Jaimez, R., Franco, Y., Avila, M.E., Castell, A., 2004. Morphometric analysis of mice uteri treated with the preservatives mathyl, ethyl, propyl and butylparaben. Toxicol. Ind. Health 20, 123-132.

Levenstein, I., 1973e. To determine the oral LD50, in rats, of the test material as submitted. Phenoxy ethyl propionate, assay no. 30974. February 14, 1973. Tepyl acetate, assay no. 30976. February 1, 1973. Isobutyl benzoate, assay no. 30967. January 10, 1973. Iso cyclo citral, assay no. 30968. January 9, 1973. Muguol, assay no. 30972. February 2, 1973. Leberco Laboratories. Unpublished data submitted by EFFA to FLAVIS Secretariat.

Levenstein, I., 1974g. Acute oral toxicity (rat - 5 gms./kg. body weight dose). Dermal toxicity (rabbit - 5 gms./kg. body weight dose). Benzal glyceryl acetal. Leberco Laboratories. Assay no. 41766. March 18, 1974. Unpublished data submitted by EFFA to FLAVIS Secretariat.

Levenstein, I., 1975j. Acute oral toxicity (rats - 5 gms./kg. body weight dose). Dermal toxicity (rabbits - 5 gms./kg. body weight dose). Anisyl formate. Leberco Laboratories. Assay no. 53277. May 19, 1975. Unpublished data submitted by EFFA to FLAVIS Secretariat.

Levenstein, I., 1975k. Acute oral toxicity (rats - 5 gms./kg. body weight dose). Dermal toxicity (rabbits - 5 gms./kg. body weight dose). Methyl anisate. Leberco Laboratories. Assay no. 53300. May 16, 1975. Unpublished data submitted by EFFA to FLAVIS Secretariat.

Levenstein, I., 1975l. Acute oral toxicity (rats - 5 gms./kg. body weight dose). Dermal toxicity (rabbits - 5 gms./kg. body weight dose). Ethyl anisate. Leberco Laboratories. Assay no. 53283. May 19, 1975. Unpublished data submitted by EFFA to FLAVIS Secretariat.

Levenstein, I., 1975m. Acute oral toxicity (rats - 5 gms./kg. body weight dose). Dermal toxicity (rabbits - 5 gms./kg. body weight dose). n-Butyl salicylate. Leberco Laboratories. Assay no. 53279. May 19, 1975. Unpublished data submitted by EFFA to FLAVIS Secretariat.

Lewis, C.A., Palanker, A.L., 1979b. Acute oral toxicity (rat). Acute dermal toxicity (rabbit). Oral LD50 (rat). Benzaldehyde propylene glycol acetal. Consumer Product Testing. Experiment ref. no. 79104-19. May 31, 1979. Unpublished data submitted by EFFA to FLAVIS Seceratriat.

Lheritier, M., 1992. Test to evaluate the acute toxicity following a single oral administration (LD 50) in the rat with cover letter dated 05/07/92 (sanitized). Submitting organization: confidential, Contractor: Hazleton France. EPA Doc. 86-920000929S, microfiche no. OTS0536271. Date 3/04/92. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Longnecker, D.S., Roebuck, B.D., Curphey, T.J., MacMillan, D.L., 1990. Evaluation of promotion of pancreatic carcinogenesis in rats by benzyl acetate. Food Chem. Toxicol. 29(10), 665-668.

Makaruk, M.I., 1980. On the toxicity of vanillin. Gig. Sanit. 6, 78-80. (In Russian)

Mallory, V.T., Naismith, R.W., Matthews, R.J., 1983. Acute oral toxicity study in rats (14 day) with a pharmacotoxic screen. Pharmakon Research International, Inc. September 22, 1983. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Marnett, L.J., Hurd, H.K., Hollstein, M.C., Levin, D.E., Esterbauer, H., Ames, B.N., 1985a. Naturally-occurring carbonyl compounds are mutagens in Salmonella tester strain TA104. Mutat. Res. 148, 25-34.

Matsui, S., Yamamoto, R., Yamada, H., 1989. The Bacillus Subtilis/Microsome rec-assay for the detection of DNA damaging substances which may occur in chlorinated and ozonated waters. Water Sci. Technol. 21, 875-887.

Matsuoka, A., Yamakage, K., Kusakabe, H., Wakuri, S., Asakura, M., Noguchi, T., Sugiyama, T., Shimada, H., Nakayama, S., Kasahara, Y., Takahashi, Y., Miura, K.F., Hatanaka, M., Ishidate, M., Morita, T., Watanabe, K., Hara, M., Odawara, K., Tanaka, N., Hayashi, M., Sofuni, T., 1996. Re-evaluation of chromosomal aberration induction on nine mouse lymphoma assay 'unique positive' NTP carcinogens. Mutat. Res. 369, 243-252.

Matthews, C., Davidson, J., Bauer, E., Morrison, J.L., Richardson, A.P., 1956. p-Hydroxybenzoic acid esters as preservatives. II. Acute and chronic toxicity in dogs, rats and mice. J. Am. Pharm. Assoc. 45, 260-267.

McCann, J., Choi, E., Yamasaki, E., Ames, B.N., 1975. Detection of carcinogens as mutagens in the Salmonella/microsome test: Assay of 300 chemicals. Proc. Nat. Acad. Sci. USA, 72(12), 5135-5139.

McCloskey, S.E., Gershanik, J.J., Lertora, J.J.L., White, L., George, W.J., 1986. Toxicity of benzyl alcohol in adult and neonatal mice. J. Pharm. Sci. 75(7), 702-705.

McGregor, D.B., Brown, A., Cattanach, P., Edwards, I., McBride, D., Riach, C., Caspary, W.J., 1988a. Responses of the L5178Y tk+/tk- mouse lymphona cell forward mutation assay: III. 72 coded chemicals. Environ. Mol. Mutag. 12, 85-153.

McGregor, D.B., Brown, A.G., Howgate, S., McBride, D., Riach, C., Caspary, W.J., 1991. Responses of the L5178Y mouse lymphona cell forward mutation assay. Environ. Mol. Mutag. 17, 196-219.

McMahon, T.F., Diliberto, J.J., Birnbaum, L.S., 1989. Age-related changes in the disposition of benzyl acetate. Drug Metab. Disposition 17(5), 506-512.

Mikulasova, M., Bohovicova, I., 2000. Genotoxic effect of vanillin derivatives. Biologia (Bratislava) 55(3), 229-234.

Milvy, P., Garro, A.J., 1976. Mutagenic activity of styrene oxide (1,2-epoxyethylbenzene), a presumed styrene metabolite. Mutat. Res. 40(1), 15-18.

Mirsalis, J., Tyson, K., Beck, J., Loh, E., Steinmetz, K., Contreras, C., Austere, L., Martin, S., Spalding, J., 1983. Induction of unscheduled DNA synthesis (UDS) in hepatocytes following in vitro and in vivo treatment. Environ. Mol. Mutag. 5(3), 482.

Mirsalis, J.C., Tyson, C.K., Steinmetz, K.L., Loh, E.K., Hamilton, C.M., Bakke, J.P., Spalding, J.W., 1989. Measurement of unscheduled DNA synthesis and S-phase synthesis in rodent hepatocytes following in vivo treatment: Testing of 24 compounds. Environ. Mol. Mutag. 14, 155-164.

Miyagawa, M., Takasawa, H., Sugiyama, A., Inoue, Y., Murata, T., Uno, Y., Yoshikawa, K., 1995. The in vivo-in vitro replicative DNA synthesis (RDS) test with hepatocytes prepared from male B6C3F1 mice as an early prediction assay for putative nongenotoxic (Ames-negative) mouse hepatocarcinogens. Mutat. Res. 343, 157-183.

Miyazawa, M., Okuno, Y., Nakamura, S., Kosaka, H., 2000. Suppression of the furylfuramide-induced SOS response by monoterpenoids with a p-menthane skeleton using the Salmonella typhimurium TA1535/pSK1002 umu test. J. Agric. Food Chem. 48(11), 5440-5443.

Mondino, A., 1982. Acute toxicity study. Species: Charles River CD rats. Administration route: oral. 2-Hydroxy-4-methylbenzaldehyde. Istituto Di Richerche Biomediche - "Antoine Marxer" S.p.A. April 16, 1982. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Monsanto Co., 1955a. Chronic feeding in rats and chronic oral administration in dogs of vanillin (final report) with cover letter dated 11/21/91. EPA Doc 86-920000145, microfiche no. OTS0534351. Date 10/07/55. Unpublished data submitted by EFFA to FLAVIS Secretariat.

Monsanto Co., 1955b. Acute oral administration of vanillin to rats (final report) with cover letter dated 11/21/91. EPA Doc 86-920000147, microfiche no. OTS0534353. Date 2/15/55. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Monsanto Co., 1976. Final report of several studies on vanillin with cover letter dated 112191. EPA Doc 86-920000159, microfiche no. OTS0534364. Date 8/13/76. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Monsanto Co., 1991a. Final report of several tests with ethavan with cover letter dated 112191. EPA Doc 86-920000149, microfiche no. OTS0534355. Date 8/17/76. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Monsanto Co., 1991b. Final report on several tests with ethavan with cover letter dated 112191. EPA Doc 86-920000150, microfiche no. OTS0534311. Date 6/15/77. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Moreno, O.M., 1972i. Acute oral toxicity in rats. Tolualdehyde glyceryl acetal. Toxicological Resources. Project no. 817-72. May 5, 1975. Unpublished data submitted by EFFA to FLAVIS Secretariat.

Moreno, O.M., 1973aa. Acute oral toxicity in rats. Dermal toxicity in rabbits. Isobutyl salicylate. MB Research Laboratories, Inc. Project no. MB 73-145. July 18, 1973. Unpublished data submitted by EFFA to FLAVIS Secretariat.

Moreno, O.M., 1973ab. Acute oral toxicity in rats. Dermal toxicity in rabbits. Phenyl ethyl salicylate. MB Research Laboratories, Inc. Project no. MB 72-27. Date 2/23/73. Unpublished data submitted by EFFA to FLAVIS Secretariat.

Moreno, O.M., 1973ac. Acute oral toxicity in rats. Dermal toxicity in rabbits. Piperonyl acetate. MB Research Laboratories, Inc. Project no. MB 73-95. June 14, 1973. Unpublished data submitted by EFFA to FLAVIS Secretariat.

Moreno, O.M., 1973u. Acute oral toxicity in rats. Dermal toxicity in rabbits. Benzyl propionate. MB Research Laboratories, Inc. Project no. MB 79-139. July 19, 1973. Unpublished data submitted by EFFA to FLAVIS Secretariat.

Moreno, O.M., 1973v. Acute oral toxicity in rats. Acute dermal toxicity in rabbits. Benzyl butyrate. MB Research Laboratories, Inc. Project 72-7. Date 2/1/73. Unpublished data submitted by EFFA to FLAVIS Secretariat.

Moreno, O.M., 1973w. Acute oral toxicity in rats. Dermal toxicity in rabbits. Tolyl aldehyde. MB Research Laboratories, Inc. Project no. MB 73-204. July 23, 1973. Unpublished data submitted by EFFA to FLAVIS Secretariat.

Moreno, O.M., 1973z. Acute oral toxicity in rats. Dermal toxicity in rabbits. Cuminyl alcohol. MB Research Laboratories, Inc. Project no. MB 73-233. September 20, 1973. Unpublished data submitted by EFFA to FLAVIS Secretariat.

Moreno, O.M., 1974j. Acute oral toxicity in rats. Dermal toxicity in rabbits. Benzyl isovalerate. MB Research Laboratories, Inc. Project no. MB 73-433. January 23, 1974. Unpublished data submitted by EFFA to FLAVIS Secretariat.

Moreno, O.M., 1974k. Acute oral toxicity in rats. Dermal toxicity in rabbits. Veratraldehyde. MB Research Laboratories, Inc. Project no. MB 74-611. August 26, 1974. Unpublished data submitted by EFFA to FLAVIS Secretariat.

Moreno, O.M., 1975m. Acute oral toxicity in rats. Dermal toxicity in rabbits. Benzyl laurate. MB Research Laboratories, Inc. Project no. MB 75-755. April 9, 1975. Unpublished data submitted by EFFA to FLAVIS Secretariat.

Moreno, O.M., 1975n. Acute oral toxicity in rats. Dermal toxicity in rabbits. Hexyl salicylate. MB Research Laboratories, Inc. Project no. MB 75-724. January 31, 1975. Unpublished data submitted by EFFA to FLAVIS Secretariat.

Moreno, O.M., 1975o. Acute oral toxicity in rats. Dermal toxicity in rabbits. cis-3-Hexyl salicylate. MB Research Laboratories, Inc. Project no. MB 75-727. February 3, 1975. Unpublished data submitted by EFFA to FLAVIS Secretariat.

Moreno, O.M., 1976u. Acute oral toxicity in rats. Dermal toxicity in rabbits. cis-3-Hexenyl benzoate. MB Research Laboratories, Inc. Project no. MB 76-1032. March 13, 1976. Unpublished data submitted by EFFA to FLAVIS Secretariat.

Moreno, O.M., 1976v. Acute oral toxicity in rats. Dermal toxicity in rabbits. Anisyl n-butyrate. MB Research Laboratories, Inc. Project no. MB 75-922. January 5, 1976. Unpublished data submitted by EFFA to FLAVIS Secretariat.

Moreno, O.M., 1976x. Acute oral toxicity in rats. Dermal toxicity in rabbits. Ethyl salicylate. MB Research Laboratories, Inc. Project no. MB 76-1112. May 12, 1976. Unpublished data submitted by EFFA to FLAVIS Secretariat.

Moreno, O.M., 1977aa. Acute oral toxicity in rats. Dermal toxicity in rabbits. Methyl p-toluate. MB Research Laboratories, Inc. Project no. MB 77-1752. August 22, 1977. Unpublished data submitted by EFFA to FLAVIS Secretariat.

Moreno, O.M., 1977ab. Acute oral toxicity in rats. Dermal toxicity in rabbits. Anisyl phenyl acetate. MB Research Laboratories, Inc. Project no. MB 77-1939. October 7, 1977. Unpublished data submitted by EFFA to FLAVIS Secretariat.

Moreno, O.M., 1977ac. Acute oral toxicity in rats. Dermal toxicity in rabbits. ortho-Anisaldehyde. MB Research Laboratories, Inc. Project no. MB 77-1542. April 8, 1977. Unpublished data submitted by EFFA to FLAVIS Secretariat.

Moreno, O.M., 1977ad. Acute oral toxicity in rats. Dermal toxicity in rabbits. Ethoxy benzaldehyde. MB Research Laboratories, Inc. Project no. MB 77-155. September 30, 1977. Unpublished data submitted by EFFA to FLAVIS Secretariat.

Moreno, O.M., 1977af. Acute oral toxicity in rats. Dermal toxicity in rabbits. Salicylaldehyde. MB Research Laboratories, Inc. Project no. MB 76-1452. January 27, 1977. Unpublished data submitted by EFFA to FLAVIS Secretariat.

Moreno, O.M., 1977z. Acute oral toxicity in rats. Dermal toxicity in rabbits. Benzaldehyde dimethyl acetal. MB Research Laboratories, Inc. Project no. MB 76-1443. January 31, 1977. Unpublished data submitted by EFFA to FLAVIS Secretariat.

Moreno, O.M., 1978i. Acute oral toxicity in rats. Acute dermal toxicity in rabbits. Cuminyl acetate. MB Research Laboratories, Inc. Project no. MB 78-2939. Date 9/30/78. Unpublished data submitted by EFFA to FLAVIS Secretariat.

Moreno, O.M., 1978k. Acute oral toxicity in rats. Acute dermal toxicity in rabbits. Prenyl salicylate. MB Research Laboratories, Inc. Project no. MB 78-2643. Date 5/05/78. Unpublished data submitted by EFFA to FLAVIS Secretariat.

Moreno, O.M., 1979d. Acute oral toxicity in rats. Acute dermal toxicity in rabbits. Benzyl tiglate. MB Research Laboratories, Inc. Project no. MB 78-3419. Date 3/22/79. Unpublished data submitted by EFFA to FLAVIS Secretariat.

Moreno, O.M., 1980k. Oral toxicity in rats. Dermal toxicity in rabbits. Benzyl glyceryl acetal. MB Research Laboratories, Inc. Project no. MB 80-4427. Date 5/28/80. Unpublished data submitted by EFFA to FLAVIS Secretariat.

Moreno, O.M., 1980l. Oral toxicity in rats. Dermal toxicity in rabbits. Methyl ortho methoxy B. MB Research Laboratories, Inc. Project no. MB 31-5683. Date 2/22/82. Unpublished data submitted by EFFA to FLAVIS Secretariat.

Moreno, O.M., 1982m. Oral toxicity in rats. Dermal toxicity in rabbits. Amyl salicylate. MB Research Laboratories, Inc. Project no. MB 82-5829. Date 4/30/82. Unpublished data submitted by EFFA to FLAVIS Secretariat.

Morgareidge, K., 1962a. In vitro digestion of four acetals. Food and Drug Research Laboratories, Inc. Lab. no. 83179. August 7, 1962. Unpublished report submitted by EFFA to SCF.

Moriyama, I., Hiraoka, K., Yamaguchi, R., 1975. Teratogenic effects of food addictive ethyl-p-hydroxy benzoate studied in pregnant rats. Acta Obst. Gynaec. Jap. 22(2), 94-106.

Morrissey, R.E., Schwetz, B.A., Lamb IV, J.C., Ross, M.D., Teague, J.L., Morris, R.W., 1988. Evaluation of rodent sperm, vaginal cytology, and reproductive organ weight data from National Toxicology Program 13-week studies. Fundam. Appl. Toxicol. 11, 343-358.

Mortelmans, K., Haworth, S., Lawlor, T., Speck, W., Tainer, B., Zeiger, E., 1986. Salmonella mutagenicity tests II. Results from the testing of 270 chemicals. Environ. Mol. Mutag. 8(Suppl. 7), 1-119.

Müller, W., Engelhart, G., Herbold, B., Jäckh, R., Jung, R., 1993. Evaluation of mutagenicity testing with Salmonella typhimurium TA102 in three different laboratories. Environ. Health Perspec. Suppl. 101(Suppl. 3), 33-36.

Myhr, B., McGregor, D., Bowers, L., Riach, C., Brown, A.G., Edwards, I., McBride, D., Martin, R., Caspary, W.J., 1990. L5178Y mouse lymphoma cell mutation assay results with 41 compounds. Environ. Mol. Mutag. 16 Suppl. 18, 138-167.

Nagabhushan, M., Bhide, S.V., 1985. Mutagenicity of chili extract and capsaicin in short-term tests. Environ. Mutag. 7, 881-888.

Nakamura, S.I., Oda, Y., Shimada, T., Oki, I., Sugimoto, K., 1987. SOS-inducing activity of chemical carcinogens and mutagens in Salmonella typhimurium TA1535/pSK1002: examination with 151 chemicals. Mutat. Res. 192, 239-246.

Nesslany, F., Marzin, D., 1999. A micromethod for the in vitro micronucleus assay. Mutagenesis 14(4), 403-410.

Nestmann, E.R., Lee, E.G.H., 1983. Mutagenicity of constituents of pulp and paper mill effluent in growing cells of Saccharomyces cerevisiae. Mutat. Res. 119, 273-280.

Nestmann, E.R., Lee, E.G., Matula, T.I., Douglas, G.R., Mueller, J.C., 1980. Mutagenicity of constituents identified in pulp and paper mill effluents using the Salmonella/mammalian-microsome assay. Mutat. Res. 79, 203-212.

Nielsen, N.M., Bundgaard, H., 1987. Prodrugs as drugs delivery systems. 68. Chemical and plasma-catalyzed hydrolysis of various esters of benzoic acid: A reference system for designing prodrug esters of carboxylic acid agents. Int. J. Pharm. 39, 75-85.

Niho, N., Shibutani, M., Tamura, T., Toyoda, K., Uneyama, C., Takahashi, N., Hirose, M., 2001. Subchronic toxicity study of gallic acid by oral administration in F344 rats. Food Chem. Toxicol. 39(11), 1063-1070.

Nivikov, S.M., Melnikova, N.N., Neryueva, V.V., Semenovykh, L.N., Murkova, M.V., 1994. Hygienic standardization of methyl salicylate and isoamyl salicylate in ambient air of populated places. Gig. Sanit. 1, 4-5.

Nohmi, T., Miyata, R., Yoshikawa, K., Ishidate, M., 1985. [Mutagenicity tests on organic chemical contaminants in city water and related compounds. I. Bacterial mutagenicity tests]. Bull. Natl. Inst. Hyg. Sci. (Eisei Shikenjo Hokoku) 103(60), 60-64. (In Chinese)

Nonaka, M., 1989. DNA repair tests on food additives. Environ. Mol. Mutag. 14(Suppl.15), 143.

NTP, 1984. Methyl salicylate: Reproduction and fertility assessment in CD-1 mice when administered by gavage. November 1984. NTP 85-022.

NTP, 1986c. NTP technical report on the toxicology and carcinogenesis studies of benzyl acetate (CAS no. 140-11-4) in F344/N rats and B6C3F1 mice (gavage studies). August 1986. NTP-TR 250. NIH Publication no. 86-2506.

NTP, 1989. NTP technical report on the toxicology and carcinogenesis studies of benzyl alcohol (CAS no. 100-51-6) in F344/N rats and B6C3F1 mice (gavage studies). June 1989. NTP-TR 343. NIH Publication no. 89-2599.

NTP, 1990c. Toxicology and carcinogenesis studies of benzaldehyde (CAS no. 100-52-7) in F344/N rats and B6C3F1 mice. (gavage studies). March 1990. NTP-TR 378. NIH Publication no. 90-2833.

NTP, 1993d. NTP technical report on the toxicology and carcinogenesis studies of benzyl acetate (CAS. no. 140-11-4) in F344/N rats and B6C3F1 mice (feed studies). September 1993. NTP-TR 431. NIH Publication no. 93-3162.

Nutley, B.P., 1990. Investigations into the metabolism of cinnamic acid, cinnamyl alcohol, and cinnamaldehyde in relation to their safety evaluation. A thesis submitted for the degree of Doctor of Philosophy in the University of London, Department of Pharmacology.

Oda, Y., Hamono, Y., Inoue, K., Yamamoto, H., Niihara, T., Kunita, N., 1978. [Mutagenicity of food flavors in bacteria]. Shokuhin. Eisei. Hen. 9, 177-181. (In Japanese)

Ohsumi, T., Kuroki, K., Kimura, T., Murakami, Y., 1984. A study on acute toxicities of essential oils used in endodontic treatment. J. Kyushu Dent. Soc. 38(6), 1064-1071. (In Japanese)

Oishi, S., 2001. Effects of butylparaben on the male reproductive system in rats. Toxicol. Ind. Health. 17(1), 31-39.

Oishi, S., 2002. Effects of butyl paraben on the male reproductive system in mice. Arch. Toxicol. 76(7), 423-429.

Oishi, S., 2004. Lack of spermatotoxic effects of methyl and ethyl esters of p-hydroxybenzoic acid in rats. Fodd Chem. Toxicol. 42, 1845-1849.

Oser, B.L., Carson, S., Oser, M., 1965. Toxicological tests on flavouring matters. Food Cosmet. Toxicol. 3(4), 563-569.

Oser, B.L., 1957. Toxicological screening of components of food flavors Class V. aromatic esters. Food Research Laboratories, Inc. Lab. no. 73800. June 5, 1957. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Owen, G., 1971. Acute oral toxicity investigation in rats. Benzyl isobutyrate, benzyl phenyl acetate. Research Institute for Fragrance and Material, Inc. June 28, 1971. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Packman, E.W., Abbott, D.D., Wagner, B.M., Harrisson, W.E., 1961. Chronic toxicity of oil sweet birch (methyl salicylate). Pharmacologist 3(1), 62.

Peano, S., Berruto, G., 1982. Acute toxicity study. Species: Charles River CD rats. Administration route: oral. Aldehyde 4-methylsalicylique. Istituto Di Recherche Biomediche - "Antoine Marxer" S.p.A. Ref. no.125b. April 16, 1982. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Phillips, J.C., Topp, C.S., Gangolli, S.D., 1978. The metabolism of ethyl and n-propyl-phydroxybenzoate ("parabens") in male cats. Toxicol. Lett. 2(4), 237-242.

Pool, B.L., Lin, P.Z., 1982. Mutagenicity testing in the Salmonella typhimurium assay of phenolic compounds and phenolic fractions obtained from smokehouse condensates. Food Chem. Toxicol. 20, 383-391.

Rajalakshmi, K., Devaraj, H., Niranjali Devaraj, S., 2001. Assessment of the no-observedadverse-effect level (NOAEL) of gallic acid in mice. Food Chem. Toxicol. 39(9), 919-922.

Rapson, W.H., Nazar, M.A., Butzky, V.V., 1980. Mutagenicity produced by aqueous chlorination of organic compounds. Bull. Environ. Contam. Toxicol. 24, 590-596.

Rashid, K.A., Baldwin, I.T., Babish, J.G., Schultz, J.C., Mumma, R.O., 1985. Mutagenicity tests with gallic-acid and tannic-acid in the Salmonella-typhimurium mammalian microsome assay. J. Environ. Sci. Health B20(2), 153-165.

Reagan, E.L., Becci, P.J., 1984d. Acute oral LD50 study of anisyl-phenyl acetate in Sprague-Dawley rats (amended report). Food and Drug Research Laboratories, Inc. Study no. 8009D. October 24, 1984. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Rhône-Poulenc, Inc., 1992b. Initial submission: Acute oral administration of ethavan in rats with cover letter dated 102792. EPA Doc 88-920009660, microfiche no. OTS0571317. Date 02/15/55. Unpublished data submitted by EFFA to FLAVIS Secretariat.

Rockwell, P., Raw, I., 1979. A mutagenic screening of various herbs, spices and food additives. Nutr. Cancer 1(4), 10-15.

Rogan, E.G., Cavalieri, E.L., Walker, B.A., Balasubramanian, R., Wislocki, P.G., Roth, R.W., Saugier, R.K., 1986. Mutagenicity of benzylic acetate, sulfates, and bromides of polycyclic aromatic hydrocarbons. Chem. Biol. Interact. 58(3), 253-275.

Rosin, M.P., 1984. The influence pf pH on the convertogenic activity of plant phenolics. Mutat. Res. 135, 109-113.

Routledge, E.J., Parker, J., Odum, J., Ashby, J., Sumpter, J.P., 1998. Some alkyl hydroxy benzoate preservative (Parabens) are estrogenic. Toxicol. Appl. Pharmacol. 153(1), 12-19.

Rudd, C.J., Mitchell, A.D., Spalding, J., 1983. L5178Y mouse lymphoma cell mutagenesis assay of coded chemicals incorporating analyses of the colony size distributions. Environ. Mutag. 5(3), 419.

Sabalitschka, T., Neufeld-Crzellitzer, R., 1954. Zum Verhalten der p-Oxybenxoesäureester im menschlichen Körper. Arzneim.-Forsch. 4, 575-579. (In German)

Sado, I., 1973. Synergistic toxicity of officially permitted food preservatives. Nippon Eiseigaku Zasshi 28(5), 463-476.

Saito, H., Yokoyama, A., Takeno, S., Sakai, T., Ueno, K., Masumura, H., Kitagawa, H., 1982. Fetal toxicity and hypocalcemia induced by acetylsalicylic acid analogues. Res. Commun. Chem. Pathol. Pharmacol. 38(2), 209-220.

Sammons, H.G., Williams, R.T., 1941. 131. Studies in detoxication. 12. The metabolism of vanillin and vanillic acid in the rabbit. The identification of glucurovallin and glucurovanillic acid. Biochem. J. 35, 1175-1189.

Sanders, A., Crowther, J.M., 1997. Acute oral toxicity (limit test) in the rat. Ethyl vanillin isobutyrate. SPL project no. 012/231. 22 April, 1997. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Sanyal, R., Darroudi, F., Parzefall, W., Nagao, M., Knasmüller, S., 1997. Inhibition of the genotoxic effects of heterocyclic amines in human derived hepatoma cells by dietary bioantimutagens. Mutagenesis 12(4), 297-303.

Sasaki, Y., Endo, R., 1978. Mutagenicity of aldehydes in Salmonella. Mutat. Res. 54, 251-252.

Sasaki, M., Sugimura, K., Yoshida, M.A., Abe, S., 1980. Cytogenetic effects of 60 chemicals on cultured human and Chinese hamster cells. Kromosomo 20, 574-584.

Sasaki, Y.F., Imanishi, H., Ohta, T., Shirasu, Y., 1987. Effects of antimutagenic flavourings on SCEs induced by chemical mutagens in cultured Chinese hamster cells. Mutat. Res. 189, 313-318.

Sasaki, Y.F., Imanishi, H., Ohta, T., Yasuhiko, S., 1989. Modifying effects of components of plant essence on the induction of sister-chromatid exchanges in cultured Chinese hamster ovary cells. Mutat. Res. 226, 103-110.

SCF, 1995. Scientific Committee for Food. First annual report on chemically defined flavouring substances. May 1995, 2nd draft prepared by the SCF Working Group on Flavouring Substances (Submitted by the SCF Secretariat, 17 May 1995). CS/FLAV/FL/140-Rev.2. Annex 6 to Document III/5611/95, European Commission, Directorate-General III, Industry.

SCF, 1999. Opinion on a programme for the evaluation of flavouring substances (expressed on 2 December 1999). Scientific Committee on Food. SCF/CS/FLAV/TASK/11 Final 6/12/1999. Annex I the minutes of the 119th Plenary meeting. European Commission, Health & Consumer Protection Directorate-General.

SCF, 2002a. Opinion of the Scientific Committee on Food on the 19th additional list of monomers and additives for food contact materials (expressed on 26 September 2002). (4.4'(1,3,6,8-tetrahydro-1,3,6,8-tetraoxobenzo[lmn][3,8]phenanthroline-2,7-diyl)bisbenzoic acid, diethyl ester), crotonic acid, ethylene carbonate. SCF/CS/PM/GEN/M90 Final. 3 October, 2002. European Commission, Health & Consumer Protection Directorate-General.

SCF, 2002b. Opinion of the Scientific Committee on Food on benzyl alcohol (expressed on 26 September 2002). Scientific Committee on Food. SCF/CS/ADD/FLAV/78 Final. 17 Sept, 2002. European Commission, Health & Consumer Protection Directorate-General.

Schafer, E.W., Bowles, W.A., 1985. The acute oral toxicity and repellency of 933 chemicals to house and deer mice. Arch. Environ. Contam. Toxicol. 14, 111-129.

Scheline, R.R., 1966a. The decarboxylation of some phenolic acids in the rat. Acta Pharmacol. Toxicol. 24, 275-285.

Scheline, R.R., 1966b. Decarboxylation and demethylation of some phenolic benzoic acid derivatives by rat caecal contents. J. Pharm. Pharmacol. 18, 664-669.

Scheline, R.R., 1972. The metabolism of some aromatic aldehydes and alcohols by the rat intestinal microflora. Xenobiotica 2(3), 227-236.

Schunk, H.H., Shibamoto, T., Tan, H.K., Wei, C-I., 1986. Biological and chemical studies on photochemical products obtained from euronol, benzyl acetate and benzyl benzoate. In: Lawrence, B.M., Mookherjee B.D., Willis B.J. (Eds.) Flavors and Fragrances: A World Perspective. Proceedings of the 10th International Congress of Essential Oils, Fragrance and Flavors, Washington, DC, USA, 16-29 November 1986. 1045-1068.

Sekihashi, K., Yamamoto, A., Matsumura, Y., Ueno, S., Watanabe-Akanuma, M., Kassie, F., Knasmüller, S., Tsuda, S., Sasaki, Y.F., 2002. Comparative investigation of multiple organs of mice and rats in the comet assay. Mutat. Res. 517(1-2), 53-75.

Sekizawa, J., Shibamoto, T., 1982. Genotoxicity of safrole-related chemicals in microbial test systems. Mutat. Res. 101, 127-140.

Shelanski, M.V., Moldovan, M., 1971d. Acute oral toxicity study. Benzyl formate. Food and Drug Research Laboratories, Inc. IBL no. 30357-F. 26 November 1971. Unpublished data submitted by EFFA to FLAVIS Secretariat.

Shelby, M.D., Erexson, G.L., Hook, G.J., Tice, R.R., 1993. Evaluation of a three-exposure mouse bone marrow micronucleus protocol: Results with 49 chemicals. Environ. Mol. Mutag. 21(2), 160-179.

Shell Oil Company, 1982. Toxicity data on benzoic acid. EPA Doc 88-8200380, microfiche no. OTS0505458. May 26, 1982. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Shibata, M-A., Hirose, M., Yamada, M., Tatematsu, M., Uwagawa, S., Ito, N., 1990. Epithelial cell proliferation in rat forestomach and glandular stomach mucosa induced by catechol and analogous dihydroxybenzenes. Carcinogenesis 11(6), 997-1000.

Shirai, T., 1997. A medium-term rat liver bioassay as a rapid in vitro test for carcinogenic potential: A historical review of model development and summary of results from 291 tests. Toxicol. Pathol. 25(5), 453-460.

Shtenberg, A.J., Ignat'ev, A.D., 1970. Toxicological evaluation of some combinations of food preservatives. Food Cosmet. Toxicol. 8, 369-380.

Smyth Jr., H.F., Carpenter, C.P., Weil, C.S., 1951a. Range finding toxicity data: List IV. Arch. Ind. Hyg. Occup. Med. J. 4, 119-122.

Smyth Jr., H.F., Carpenter, C.P., Weil, C.S., Pozzani, U.C., 1954. Range-finding toxicity data: List V. Arch. Ind. Hyg. Occup. Med. 10, 61-68.

Sofuni, T., Hayashi, M., Matsuoka, A., Sawada, M., Hatanaka, M., Ishidate Jr., M., 1985. Mutagenicity tests on organic chemical contaminants in city water and related compounds. II. Chromosome aberration tests in cultured mammalian cells. Eisei Shikenjo Hokoku 103, 64-75. (In Japanese)

Sokol, H., 1952. Recent developments in the preservation of pharmaceuticals. Drug Stand. 20, 89-106.

Sporn, A., Dinu, I., Stanciu, V., 1967. [Investigations on the toxicity of benzaldehyde]. Igena 16(1), 23-24. (In Rumanian)

Steinmetz, K., Mirsalis, J., 1984. Measurement of DNA repair in primary cultures of rat pancreatic cells following in vivo treatment. Environ. Mutag. 6(3), 446.

Sterner, W., Chibanguza, G., 1983. Acute oral toxicity in rats. ortho-Cresylsalicylate. Forschungs GmbH. Project no. 1-4-9-83. March, 1983. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Stich, H.F., Rosin, M.P., Wu, C.H., Powrie, W.D., 1981c. The action of transition metals on the genotoxicity of simple phenols, phenolic acids and cinnamic acids. Cancer Lett.14(3), 251-260.

Storer, R.D., McKelvey, T.W., Kraynak, A.R., Elia, M.C., Barnum, J.E., Harmon, L.S., Nichols, W.W., DeLuca, J.G., 1996. Revalidation of the in vitro alkaline elution/rat hepatocyte assay for DNA damage: improved criteria for assessment of cytotoxiciity and genotoxicity and results for 81 compounds. Mutat. Res. 368(2), 59-101.

Strand, L.P., Scheline, R.R., 1975. Metabolism of vanillin and isovanillin in the rat. Xenobiotica 5(1), 49-63.

Sugimura, T., Sato, S., Nagao, M., Yahagi, T., Matsushima, T., Seino, Y., Takeuchi, M., Kawachi, T., 1976. Overlapping of carcinogens and mutagens. In: Magee, P.N., Takayama, S., Sugimura, T., Matsushima, T. (Eds.). Proceedings of the International Symposium of the Princess Takamatsu Cancer Research Fund, Tokyo, 1975. Fundamentals In Cancer Prevention. vol. 6. University Par Press, Baltimore, pp. 191-215.

Szybalski, W., 1958. Special microbiological systems. II. Observations on chemical mutagenesis in microorganisms. Ann. N.Y. Acad. Sci. 76, 475-489.

Takahashi, K., Sekiguchi, M., Kawazoe, Y., 1990. Effects of vanillin and o-vanillin on induction of DNA-repair networks: modulation of mutagenesis in Escherichia coli. Mutat. Res. 230, 127-134.

Tamai, K., Tezuka, H., Kuroda, Y., 1992. Different modifications by vanillin in cytotoxicity and genetic changes induced by EMS and H2O2 in cultured Chinese hamster cells. Mutat. Res. 268, 231-237.

Tanaka, S., Kawashima, K., Nakura, S., Nagao, S., Kuwamura, T., Takanaka, A., Omori, Y., 1973a. Studies on teratogenicity of food additives. Teratogenic effect of dietary salicylic acid in rats. J. Food Hyg. Soc. 14(6), 549-557.

Tanaka, S., Kawashima, K., Nakaura, S., Nagao, S., Kuwamura, T., Takanaka, A., Omori, Y., 1973b. Studies on the teratogenic effects of salicylic acid and aspirin in rats as related to fetal distribution. Cong. Anom. 13, 73-84.

Tayama, S., Nakagawa, Y., 2001. Cytogenetic effects of propyl gallate in CHO-K1 cells. Mutat. Res. 498, 117-127.

Taylor, J.M., Jenner, P.M., Jones, W.I., 1964. A comparison of the toxicity of some allyl, propenyl and propyl compounds in the rat. Toxicol. Appl. Pharmacol. 6, 378-387.

Teuchy, H., Quatacker, G., Wolf, G., Van Sumere, C.F., 1971. Quantitative investigation of the hippuric acid formation in the rat after administration of some possible aromatic and hydroaromatic precursors. Arch. Intern. Physiol. Biochem. 79, 573-587.

TNO, 2000. Volatile Compounds in Food - VCF Database. TNO Nutrition and Food Research Institute. Boelens Aroma Chemical Information Service BACIS, Zeist, The Netherlands.

Trubek Laboratories, Inc., 1958f. Toxicolgical screening of eugenol, p-methoxybenzaldehyde and piperonal in rats. Class IX. Aromatic aldehydes. Food and Drug Research Laboratories, Inc. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Tsukamoto, H., Terada, S., 1962. Metabolism of drugs. XXVI. Metabolic fate of phydroxybenzoic acid and its derivatives in rabbit. Chem. Pharm. Bull. (Tokyo), 10(2), 86-90.

Uno, Y., Takasawa, H., Miyagawa, M., Inoue, Y., Murata, T., Yoshikawa, K., 1994. An in vivo-in vitro replicative DNA synthesis (RDS) test using rat hepatocytes as an early prediction assay for nongenotoxic hepatocarcinogens screening of 22 known positives and 25 noncarcinogens. Mutat. Res. 320, 189-205.

Vamvakas, S., Dekant, W., Anders, M.W., 1989. Mutagenicity of benzyl S-haloalkyl and S-haloalkenyl sulfides in the Ames test. Biochem. Pharmacol. 38(6), 935-939.

Van Miller, J.P., Weaver, E.V., 1987. Fourteen-day dietary minimum toxicity screen (MTS) in albino rats. Bushy Run Research Center. Project report 50-526. August 10, 1987. Unpublished report submitted by EFFA to SCF.

Vollmuth, T.A., Bennet, M.B., Hoberman, A.M., Christian, M.S., 1990. An evaluation of food flavoring ingredients using an in vivo reproductive and development toxicity screening test. Teratology 41, 597-598.

Wang, C.Y., Klemencic, J.M., 1979. Mutagenicity and carcinogenicity of polyhydric phenols. Am. Assoc. Cancer Res. 20, 117.

Wangenheim, J., Bolcsfoldi, G., 1988. Mouse lymphoma L5178Y thymidine kinase locus assay of 50 compounds. Mutagenesis 3(3), 193-205.

Watanabe, S., Morimota, Y., 1989c. Mutagenicity test (Salmonella, Escherichia coli /microsome). Vanillyl alcohol n-butyl ether. Central Research Laboratory. November 9, 1989. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Watanabe, K., Ohta, T., Shirasu, Y., 1989. Enhancement and inhibition of mutation by ovanillin in Escherichia coli. Mutat. Res. 218, 105-109.

Waters, R., Mirzayans, R., Meredith, J., Mallalah, G., Danford, N., Parry, J.M., 1982. Correlations in mammalian cells between types of DNA damage, rates of DNA repair and the biological consequence. Prog. Mutat. Res. 4, 247-259.

Webb, W.K., Hansen, W.H., 1963. Chronic and subacute toxicology and pathology of methyl salicylate in dogs, rats and rabbits. Toxicol. Appl. Pharmacol. 5, 576-587.

Weir, R.J., Wong, L.C.K., 1971b. Acute oral toxicity studies - rats. Acute dermal toxicity studies - rabbits. Primary skin irritation - rabbits. Anise oil, olibanum, anisyl acetate, phenyl propyl alcohol, amyl benzoate, nerolin. Bionetics Research Laboratories. BRL project no. 2221. August 25, 1971. Unpublished report submitted by EFFA to FLAVIS Secretariat.

White, T.J., Goodman, D., Shulgin, A.T., Castagnoli Jr., N., Lee, R., Petrakis, N.I., 1977. Mutagenic activity of some centrally active aromatic amines in Salmonella typhimurium. Mutat. Res. 56, 199-202.

Wiessler, M., Romruen, K., Pool, B.L., 1983. Biological activity of benzylating N-nitroso compounds. Models of activated N-nitrosomethylbenzylamine. Carcinogenesis 4(7), 867-871

Wild, D., King, M.T., Gocke, E., Eckhard, K., 1983. Study of artificial flavouring substances for mutagenicity in the Salmonella/microsome, BASC and micronucleus tests. Food Chem. Toxicol. 21(6), 707-719.

Wohl, A.J., 1974d. Acute oral toxicity (rat - 5 gms/kg body weight dose). Dermal toxicity (rabbit - 5 gms/kg body weight dose). Anisyl propionate. April 2, 1974. Unpublished data submitted by EFFA to SCF.

Wong, K.P., Sourkes, T.L., 1966. Metabolism of vanillin and related substances in the rat. Can. J. Biochem. 44(5), 635-644.

Woodruff, R.C., Mason, J.M., Valencia, R., Zimmering, S., 1985. Chemical mutagenesis testing in Drosophila. V. Results of 53 coded compounds tested for the National Toxicology Program. Environ. Mutag. 7, 677-702.

Yamaguchi, T., 1981. Mutagenicity of low molecular substances in various superoxide generating systems. Agric. Biol. Chem. 45(1), 327-330.

Yoo, Y.S., 1986. Mutagenic and antimutagenic activities of flavoring agents used in foodstuffs. Osaka City Med. J. 34(3-4), 267-288. (In Japanese)

Yoshikawa, K., 1996. Anomalous nonidentity between Salmonella genotoxicants and rodent carcinogens: Nongenotoxic carcinogens and genotoxic noncarcinogens. Environ. Health Perspect. 104(1), 40-46.

Yuan, J.H., Goehl, T.J., Abdo, K., Clark, J., Espinosa, O., Bugge, C., Garcia, D., 1995. Effects of gavage versus dosed feed administration on the toxicokinetics of benzyl acetate in rats and mice. Food Chem. Toxicol. 33(2), 151-158.

Zeiger, E., Anderson, B., Haworth, S., Lawlor, T., Mortelmans, K., 1988. Salmonella mutagenicity tests: IV. Results from the testing of 300 chemicals. Environ. Mol. Mutag. 11(Suppl. 12), 1-158.

Zeiger, E., Anderson, B., Haworth, S., Lawlor, T., Mortelmans, K., 1992. Salmonella mutagenicity tests: V. Results from the testing of 311 chemicals. Environ. Mol. Mutag. 19(21), 2-141.

Zetterberg, G., 1979. Mechanism of the lethal and mutagenic effects of phenoxyacetic acids in Saccharomyces cerevisiae. Mutat. Res. 60, 291-300.

Zong, L., Inoue, M., Nose, M., Kojima, K., Sakaguchi, N., Isuzugawa, K., Takeda, T., Ogihara, Y., 1999. Metabolic fate of gallic acid orally administered to rats. Biol. Pharm. Bull. 22(3), 326-329.

SCIENTIFIC PANEL MEMBERS

Robert Anton, Sue Barlow, Dimitrios Boskou, Laurence Castle, Riccardo Crebelli, Wolfgang Dekant, Karl-Heinz Engel, Stephen Forsythe, Werner Grunow, Marina Heinonen, John Chr. Larsen, Catherine Leclercq, Wim Mennes, Maria Rosaria Milana, Iona Pratt, Ivonne Rietjens, Kettil Svensson, Paul Tobback, Fidel Toldrá.

ACKNOWLEDGEMENT

The Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food wishes to thank Judith Amberg-Müller (rapporteur), Ulla Beckman Sundh, Jørn Gry, Harriet Wallin, Vibe Beltoft, and Krestine Greve, Karin Nørby and Trine Klein Reffstrup for their contribution to the draft opinion.