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SCIENTIFIC OPINION

Flavouring Group Evaluation 54, Revision 1 (FGE.54Rev1):

Consideration of benzyl derivatives evaluated by JECFA (57th meeting) structurally related to benzyl alcohols, benzaldehydes, a related acetal, benzoic acids and related esters evaluated by EFSA in FGE.20Rev1 (2009) [1]

Scientific Opinion of the Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF)

(Question No EFSA-Q-2009-00483)

ADOPTED ON 26 MARCH 2009

PANEL MEMBERS

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SUMMARY

The Scientific Panel on Food Food Contact Materials, Enzymes, Flavourings and Processing Aids (the Panel) was asked to provide scientific advice to the Commission on the implications for human health of chemically defined flavouring substances used in or on foodstuffs in the Member States. In particular, the Panel was requested to consider the Joint FAO/WHO Expert Committee on Food Additives (the JECFA) evaluations of flavouring substances assessed since 2000, and to decide whether no further evaluation is necessary, as laid down in Commission Regulation (EC) No 1565/2000. These flavouring substances are listed in the Register, which was adopted by Commission Decision 1999/217/EC and its consecutive amendments.

[1] For citation purposes: Scientific Opinion of the Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids on a request from the Commission on FGE54rev1 Consideration of benzyl derivatives evaluated by JECFA (57th meeting) structurally related to benzyl alcohols, benzaldehydes, a related acetal, benzoic acids and related esters evaluated by EFSA in FGE.20Rev1 (2009). *The EFSA Journal* (2009) 1025, 1-73.

The present consideration concerns 37 benzyl derivatives evaluated by the JECFA (57th meeting) and will be considered in relation to the European Food Safety Authority (EFSA) evaluation of 36 benzyl alcohols, benzaldehydes, a related acetal, benzoic acids and related esters evaluated in the Flavouring Group Evaluation 20, Revision 1 (FGE.20Rev1).

One of the JECFA evaluated substances, geranyl benzoate [FL-no: 09.767] may be metabolised to an alpha,beta-unsaturated aldehyde, citral. As the alpha,beta-unsaturated aldehyde and ketone structures are considered by the Panel to be structural alerts for genotoxicity (EFSA, 2008b), this substance has been given special considerations.

The remaining 36 flavouring substances have originally been considered by EFSA in the FGE.54 (EFSA, 2008al).

The genotoxicity of the alpha,beta-unsaturated substance, geranyl benzoate [FL-no: 09.767], has been considered in FGE.202. The structural alert for genotoxicity is present in the metabolite citral. The Panel concluded that the data available on citral did rule out the concern for genotoxicity and thus concluded that geranyl benzoate can be evaluated through the Procedure.

The Panel concluded that the 37 substances in the JECFA flavouring group of benzyl derivatives are structurally related to the group of benzyl alcohols, benzaldehydes, a related acetal, benzoic acids and related esters evaluated by the Panel in the FGE.20Rev1.

The Panel agrees with the way the application of the Procedure has been performed by the JECFA for all 37 substances in the group of benzyl derivatives.

However, for four substances [FL-no: 06.019, 09.294, 09.803 and 09.812] no European production figure was available and consequently no European exposure estimate could be calculated based on Maximised Survey-derived Daily Intake (MSDI). Accordingly, the safety in use in Europe could not be assessed using the Procedure for these four substances.

For all 37 substances use levels are needed to calculate the modified Theoretical Added Maximum Daily Intakes (mTAMDI) in order to identify those flavouring substances that need more refined exposure assessment and to finalise the evaluation.

In order to determine whether the conclusion for the 37 JECFA evaluated substances can be applied to the materials of commerce, it is necessary to consider the available specifications. Adequate specifications including purity and identity are available for 30 of the 37 JECFA evaluated substances. For three substances [FL-no: 06.002, 06.012 and 09.806] information on the stereoisomeric composition is lacking and for seven substances [FL-no: 05.027, 06.002, 06.012, 06.019, 09.294, 09.755 and 09.806] further information on the composition of the mixture is requested.

Thus, for nine substances [FL-no: 05.027, 06.002, 06.012, 06.019, 09.294, 09.755, 09.803, 09.806 and 09.812] the Panel has reservations (no European production volumes available, preventing them from being evaluated using the Procedure, and/or lack of information on stereoisomerism and/or further information on the composition of the mixture). For the remaining 28 of the 37 JECFA evaluated benzyl derivatives [FL-no: 02.010, 02.039, 05.013, 05.022, 05.068, 05.110, 06.003, 06.032, 08.021, 09.014, 09.051, 09.077, 09.132, 09.406, 09.426, 09.458, 09.494, 09.508, 09.705, 09.725, 09.726, 09.727, 09.757, 09.767, 09.768, 09.770, 09.771 and 09.776] the Panel agrees with the JECFA conclusion “No safety concern at estimated levels of intake as flavouring substances” based on the MSDI approach.

KEY WORDS

Benzyl derivatives, JECFA 57th meeting, benzyl alcohols, benzaldehydes, benzoic acids, FGE.20.

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BACKGROUND

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

Substances which are listed in the Register are to be evaluated according to the evaluation programme laid down in Commission Regulation (EC) No 1565/2000 (EC, 2000a), which is broadly based on the Opinion of the Scientific Committee on Food (SCF, 1999).

Commision Regulation (EC) No 1565/2000 lays down that substances that are contained in the Register and will be classified in the future by the Joint FAO/WHO Expert Committee on Food Additives (the JECFA) so as to present no safety concern at current levels of intake will be considered by the European Food Safety Authority (EFSA), who may then decide that no further evaluation is necessary.

In the period 2000 – 2007, during its 55th, 57th, 59th, 61st, 63rd, 65th and 68th meetings, the JECFA evaluated about 1000 substances which are in the EU Register.

TERMS OF REFERENCE

EFSA is requested to consider the JECFA evaluations of flavouring substances assessed since 2000, and to decide whether no further evaluation is necessary, as laid down in Commission Regulation (EC) No 1565/2000 (EC, 2000a). These flavouring substances are listed in the Register, which was adopted by Commission Decision 1999/217/EC (EC, 1999a) and its consecutive amendments.

ACKNOWLEDGEMENT

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ASSESSMENT

The approach used by EFSA for safety evaluation of flavouring substances is referred to in Commission Regulation (EC) No 1565/2000 (EC, 2000a), hereafter named the "EFSA Procedure". This Procedure is based on the Opinion of the Scientific Committee on Food (SCF, 1999), which has been derived from the evaluation procedure developed by the Joint FAO/WHO Expert Committee on Food Additives (JECFA, 1995; JECFA, 1996a; JECFA, 1997a; JECFA, 1999b), hereafter named the "JECFA Procedure". The Scientific Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (the Panel) compares the JECFA evaluation of structurally related substances with the result of a corresponding EFSA evaluation, focussing on specifications, intake estimations and toxicity data, especially genotoxicity data. The evaluations by EFSA will conclude whether the flavouring substances are of no safety concern at their estimated levels of intake, whether additional data are required or whether certain substances should not be evaluated through the EFSA Procedure.

The following issues are of special importance.

Intake

In its evaluation, the Panel as a default uses the Maximised Survey-derived Daily Intake (MSDI) approach to estimate the per capita intakes of the flavouring substances in Europe.

In its evaluation, the JECFA includes intake estimates based on the MSDI approach derived from both European and USA production figures. The highest of the two MSDI figures is used in the evaluation by the JECFA. It is noted that in several cases, only the MSDI figures from the USA were available, meaning that certain flavouring substances have been evaluated by the JECFA only on the basis of these figures. For Register substances for which this is the case the Panel will need EU production figures in order to finalise the evaluation.

When the Panel examined the information provided by the European Flavour Industry on the use levels in various foods, it appeared obvious that the MSDI approach in a number of cases would grossly underestimate the intake by regular consumers of products flavoured at the use level reported by the Industry, especially in those cases where the annual production values were reported to be small. In consequence, the Panel had reservations about the data on use and use levels provided and the intake estimates obtained by the MSDI approach. It is noted that the JECFA at its 65th meeting considered "how to improve the identification and assessment of flavouring agents, for which the MSDI estimates may be substantially lower than the dietary exposures that would be estimated from the anticipated average use levels in foods" (JECFA, 2006c).

In the absence of more accurate information that would enable the Panel to make a more realistic estimate of the intakes of the flavouring substances, the Panel has decided also to perform an estimate of the daily intakes per person using a modified Theoretical Added Maximum Daily Intake (mTAMDI) approach based on the normal use levels reported by Industry.

As information on use levels for the flavouring substances has not been requested by the JECFA or if it has not otherwise been provided to the Panel, it is not possible to estimate the daily intakes using the mTAMDI approach for the substances evaluated by the JECFA. The Panel will need information on use levels in order to finalise the evaluation.

Threshold of 1.5 Microgram/Person/Day (Step B5) Used by the JECFA

The JECFA uses the threshold of concern of 1.5 microgram/person/day as part of the evaluation procedure:

"The Committee noted that this value was based on a risk analysis of known carcinogens which involved several conservative assumptions. The use of this value was supported by additional information on developmental toxicity, neurotoxicity and immunotoxicity. In the judgement of the Committee, flavouring substances for which insufficient data are available for them to be evaluated using earlier steps in the Procedure, but for which the intake would not exceed 1.5 microgram per person per day would not be expected to present a safety concern. The Committee recommended that the Procedure for the Safety Evaluation of Flavouring Agents used at the forty-sixth meeting be amended to include the last step on the right-hand side of the original procedure ("Do the condition of use result in an intake greater than 1.5 microgram per day?") (JECFA, 1999b).

In line with the Opinion expressed by the Scientific Committee on Food (SCF, 1999), the Panel does not make use of this threshold of 1.5 microgram per person per day.

Genotoxicity

As reflected in the Opinion of SCF (SCF, 1999), the Panel has in its evaluation focussed on a possible genotoxic potential of the flavouring substances or of structurally related substances. Generally, substances for which the Panel has concluded that there is an indication of genotoxic potential *in vitro,* will not be evaluated using the EFSA Procedure until further genotoxicity data are provided. Substances for which a genotoxic potential *in vivo* has been concluded, will not be evaluated through the Procedure.

Specifications

Regarding specifications, the evaluation by the Panel could lead to a different opinion than that of the JECFA, since the Panel requests information on e.g. isomerism.

Structural Relationship

In the consideration of the JECFA evaluated substances, the Panel will examine the structural relationship and metabolism features of the substances within the flavouring group and compare this with the corresponding FGE.

HISTORY OF THE EVALUATION OF THE SUBSTANCES IN THE PRESENT FGE

At its 57th meeting the JECFA evaluated a group of 37 flavouring substances consisting of benzyl derivatives. One of the substances evaluated by the JECFA, geranyl benzoate [FL-no: 09.767], may be metabolised to an alpha,beta-unsaturated aldehyde. As the alpha,beta-unsaturated aldehyde and ketone structures are considered by the Panel to be structural alerts for genotoxicity (EFSA, 2008b), the compound has been given special considerations in the Flavouring Group Evaluation 19 (FGE.19). The remaining 36 flavouring substances have originally been considered by EFSA in the FGE.54 (EFSA, 2008al).

FGE.19 contains 360 flavouring substances from the EU Register being alpha,beta-unsaturated aldehydes or ketones and precursors which could give rise to such carbonyl substances via hydrolysis and / or oxidation (EFSA, 2008b). The alpha,beta-unsaturated carbonyls were subdivided into 28 subgroups on the basis of structural similarity (EFSA, 2008b). In an attempt to decide which of the substances could go through the Procedure, a (quantitative) structure-activity relationship

((Q)SAR) prediction of the genotoxicity of these substances was undertaken. The Panel took note of the (Q)SAR predictions by using two ISS Local Models (Benigni & Netzeva, 2007a; Benigni & Netzeva, 2007b) and four DTU-NFI MultiCASE Models (Gry et al., 2007; Nikolov et al., 2007) and the fact that there are available data on genotoxicity, *in vitro* and *in vivo*, as well as data on carcinogenicity for several substances. The Panel decided that 11 subgroups (1.1.2, 1.1.3, 1.1.4, 2.4, 2.6, 2.7, 3.1, 3.3, 4.1, 4.2 and 4.4) (EFSA, 2008b) should be further examined to determine whether evaluation through the Procedure is feasible. Corresponding to these 11 subgroups 11 FGEs were established (FGE.201, 202, 203, 210, 212, 213, 214, 216, 217, 218 and 220).

History of FGE.54:

FGE	Opinion Adopted by EFSA	Link	No of Candidate Substances
FGE.54	May 2008	http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1211902159046.htm	36
FGE.54Rev1	March 2009	http://www.efsa.europa.eu/EFSA/ScientificOpinionPublicationReport/efsa_locale-1178620753812_ScientificOpinions.htm	37

The present revision of FGE.54, FGE.54Rev1, includes the assessment of one additional substance [FL-no: 09.767] originally considered in FGE.202 (subgroup 1.1.3 in FGE.19) and for which the Panel concluded that the genotoxicity data available do not preclude its evaluation through the Procedure.

1. Presentation of the Substances in the JECFA Flavouring Group

1.1. Description

1.1.1. JECFA Status

The JECFA has evaluated a group of 37 flavouring substances consisting of benzyl derivatives.

1.1.2. EFSA Considerations

One of the JECFA evaluated substances, geranyl benzoate [FL-no: 09.767], may be metabolised to an alpha,beta-unsaturated aldehyde. As the alpha,beta-unsaturated aldehyde and ketone structures are considered by the Panel to be structural alerts for genotoxicity (EFSA, 2008b), this substance has been given special considerations in Flavouring Group Evaluation 19 (FGE.19) (EFSA, 2008b).

The remaining 36 flavouring substances have originally been considered by EFSA in the FGE.54 (EFSA, 2008al).

The genotoxicity of the alpha,beta-unsaturated substance, geranyl benzoate [FL-no: 09.767], has been considered in FGE.202 (EFSA, 2008g). The structural alert for genotoxicity is present in the metabolite citral. The Panel concluded that the data available on citral did rule out the concern for genotoxicity and thus concluded that geranyl benzoate can be evaluated through the Procedure.

The Panel concluded that the 37 substances in the JECFA flavouring group of benzyl derivatives are structurally related to the group of 36 benzyl alcohols, benzaldehydes, a related acetal, benzoic acids and related esters evaluated by the Panel in the FGE.20Rev1.

1.2. Isomers

1.2.1. JECFA Status

Six substances in the group of the JECFA evaluated benzyl derivatives have a chiral centre [FL-no: 06.002, 06.012, 06.019, 06.032, 09.771 and 09.803]. Three substances can exist as geometrical isomers [FL-no: 09.494, 09.767 and 09.806] and three substances are mixtures of positional isomers [FL-no: 05.027, 06.012 and 09.294].

1.2.2. EFSA Considerations

Information is lacking on the stereoisomerism for three substances [FL-no: 06.002, 06.012 and 09.806]. For two of the JECFA evaluated substances the configuration is given by the name [FL-no: 09.494 and 09.767] and for three substances information is lacking about the positional isomers [FL-no: 05.027, 06.012 and 09.294].

1.3. Specifications

1.3.1. JECFA status

JECFA specifications are available for all 37 substances (JECFA, 2002d) (see Table 1).

1.3.2. EFSA Considerations

The specifications are considered adequate for 30 substances. Information is missing on the stereoisomerism for three substances [FL-no: 06.002, 06.012 and 09.806]. For seven substances [FL-no: 05.027, 06.002, 06.012, 06.019, 09.294, 09.755 and 09.806], further information on the composition of the mixture is requested (see Section 1.2).

2. Intake Estimations

2.1. JECFA Status

For 33 substances evaluated through the JECFA Procedure intake data are available for the EU (see Table 3.1). For the remaining four substances [FL-no: 06.019, 09.294, 09.803 and 09.812] production figures are only available for the USA.

2.2. EFSA Considerations

As production figures are only available for the USA for four substances, MSDI values for the EU cannot be calculated for these substances [FL-no: 06.019, 09.294, 09.803 and 09.812].

3. Genotoxicity Data

3.1. Genotoxicity Studies - Text Taken[2] from the JECFA (JECFA, 2002a)

In vitro / in vivo

The results of studies of genotoxicity *in vitro* and *in vivo* with benzyl alcohol [FL-no: 02.010], benzyl acetate [FL-no: 09.014], benzaldehyde [FL-no: 05.013], and benzoic acid [FL-no: 08.021] were reviewed by the Committee at its forty-sixth meeting (Annex 1, reference 122). The Committee concluded that: "None of the four compounds was mutagenic in the Ames test, either with or without metabolic activation. The compounds all induced gene mutations in the mouse lymphoma assay at the thymidine kinase locus (benzoic acid was not tested), although the

[2] *The text is taken verbatim from the indicated reference source, but text related to substances not included in the present FGE has been removed.*

requirement for metabolic activation varied. Some weak clastogenic activity was noted in *in vitro* assays, but not in *in vivo* assays."

Further data on these four flavouring agents and the results of studies with eight other benzyl derivatives in the group [FL-no: 02.039, 05.022, 06.012, 09.077, 09.132, 09.725, 09.727 and 09.755] are presented in Table 2.1. The results of all 20 assays *in vitro* with these eight benzyl derivatives were negative, except for one for DNA repair in *Bacillus subtilis* strains H17 and M45 with benzyl formate (Yoo, 1986).

Conclusion on genotoxicity

A total of 12 benzyl derivatives in the group have been tested for genotoxicity. In view of the mainly negative results in the assays *in vitro* and the uniformly negative results in well-recognised assays *in vivo*, the Committee concluded that the group of benzyl derivatives is not genotoxic *in vivo*.

For a summary of *in vitro / in vivo* genotoxicity data considered by JECFA, see Table 2.1.

3.2. Genotoxicity Studies - Text Taken from FGE.20Rev1 (EFSA, 2009k)

In vitro

Data from *in vitro* tests are available for seven candidate substances [subgroup 1: FL-no: 09.631; subgroup 2: FL-no: 09.367, 05.129, 05.158, 08.080, 05.153 and 08.087] and for 28 supporting substances (12 from subgroup 1 and 16 from subgroup 2) and for one related substance (vanillin 3-(l-menthoxy)propane-1,2-diol acetal [FL-no: 02.248] related to subgroup 2). Data from *in vivo* tests are available for two candidate substances from subgroup 2 [FL-no: 09.367 and 08.080] and for 10 supporting substances (three from subgroup 1 and seven from subgroup 2).

All the seven candidate substances [FL-no: 09.631, 09.367, 05.129, 05.158, 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, sister chromatid exchange (SCE) and mammalian cell gene mutation assay (mouse lymphoma tests)) for most of which the validity cannot be evaluated or are known to be of very limited relevance.

The same situation was observed for the supporting substances. All the available bacterial gene mutation assays on supporting substances gave negative results. For 14 of these substances, both positive and negative results were reported in other *in vitro* test systems (Rec assay, chromosomal aberration test, 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 [FL-no: 09.104] was positive in an *in vivo* Comet assay, which may indicate a genotoxic activity at high dose levels. The study was considered of limited validity. However, all other *in vivo* studies with benzyl acetate were negative and several of these studies, among which an unscheduled DNA synthesis (UDS) test in the liver and a mouse bone marrow micronucleus test, were considered to be of good quality (NTP, 1993d). Additionally, in the long-term carcinogenicity studies with benzyl acetate, no carcinogenic effects were observed in mice and

rats after administration via the diet (NTP, 1993d). In a previous study by NTP (1986) in which this substance was administered by gavage in corn oil, concern was raised in particular about pancreatic tumours in rats, but for these tumours a confounding influence of the vehicle was suspected. In two other genotoxicity studies, specifically aiming at the determination of benzyl acetate-induced DNA damage (UDS test and alkaline elution assay) in rat pancreas, no indications of a genotoxic effect were obtained although these studies were of limited or inassessible validity. Taking all this information into account, the Panel considered the positive result from the *in vivo* Comet assay as insufficient ground to preclude the evaluation of benzyl acetate via the Procedure.

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

Conclusion on genotoxicity:

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

For a summary of *in vitro / in vivo* genotoxicity data considered by EFSA, see Table 2.2 and 2.3.

3.3. Genotoxicity and Conclusion on Genotoxicity and Carcinogenicity – Text from FGE.202 (EFSA, 2008g)

"There are ten *in vitro* studies and three *in vivo* studies available on citral [FL-no: 05.020] and on 3-methylcrotonaldehyde (3-methyl-2-butenal) [FL-no: 05.124].

3-Methylcrotonaldehyde was found mutagenic in a valid modified Ames test, i.e. the liquid suspension assay, both in the absence and to a lower extent, in the presence of metabolic activation (S9-mix), in TA100 *Salmonella typhimurium* strain (BASF, 1991b). Of doubtful relevance was a slight increase (factor 2.1) in the number of revertants observed with TA98 strain, only in the absence of S9 at the highest concentration (2500 microgram/plate). It was found negative in a valid bone marrow micronucleus assay in mice, treated orally at 175, 350 and 750 mg/kg body weight, with signs of toxicity at the highest dose, as shown by the ratio of polychromatic to normachromatic erythrocytes (BASF, 1992c). Moreover, it was found negative in a valid *in vivo* unscheduled DNA synthesis (UDS) assay, carried out on hepatocytes from rats treated orally at dose levels of 350 and 700 mg/kg body weight (BASF, 2001). In conclusion, based on the negative results in two valid *in vivo* assays (rat liver UDS and mouse bone marrow micronucleus), the positive result observed in the modified Ames test is considered of limited relevance for the overall evaluation. Therefore, for this substance, the Panel considers that genotoxicity is of no concern.

Citral was not mutagenic in several valid Ames tests (Ishidate et al., 1984; Zeiger et al., 1987; Gomes-Carneiro et al., 1998; NTP, 2003e), and it did not induce chromosome aberrations in a valid *in vitro* study with chinese hamster ovary (CHO) cells (NTP, 2003e). Moreover, it was negative in a valid *in vivo* mouse bone marrow micronucleus assay (NTP, 2003e). The positive results in an *in vitro* test for sister chromatid exchanges (SCE) (NTP, 2003e) and in inappropriate test systems like the *Rec* assay in *B. subtilis* (Yoo, 1986) and the induction of the tumour suppressor protein p53 (Duerksen-Hughes et al., 1999) are considered of limited relevance for the overall evaluation. The Panel concluded that for citral genotoxicity is not of concern.

Overall, the Panel concluded that the genotoxicity data available do not give rise to concern for the 37 substances in FGE.202 using the Procedure.

Study validation and results are presented in Table 2.4 and 2.5.

Conclusion on Genotoxicity and Carcinogenicity

Based on the available data, the Panel concluded that there would be no safety concern with respect to genotoxicity or carcinogenicity for the 37 alpha,beta-unsaturated substances presented in this FGE."

For a summary of genotoxicity data see Table 2.4: Genotoxicity data (*in vitro*) EFSA / FGE.202 and Table 2.5 Genotoxicity data (*in vivo*) EFSA / FGE.202.

3.4. EFSA Considerations

The Panel concluded that the data available do not preclude the evaluation of the 37 JECFA evaluated benzyl derivatives through the Procedure.

4. Application of the Procedure

4.1. Application of the Procedure to 37 Benzyl Derivatives by the JECFA (JECFA, 2002a):

According to the JECFA all the 37 benzyl derivatives belong to structural class I using the decision tree approach presented by Cramer et al. (1978).

The JECFA concluded 33 of the substances at step A3 in the JECFA Procedure, i.e. the substances are expected to be metabolised to innocuous products (step 2) and the intakes for all substances are below the thresholds for their structural class I (step A3).

Three substances [FL-no: 02.010, 05.013 and 09.727] were concluded at step A4, i.e. their intakes are above the threshold for the structural class but the substances are endogenous.

One substance [FL-no: 06.019] was concluded at step B4, i.e. the substance cannot be anticipated to be metabolised to innocuous products, but the intake is below threshold for the structural class and a No Observed Adverse Effect Level (NOAEL) exists to provide an adequate margin of safety to the estimated intake as flavouring substance.

In conclusion, the JECFA evaluated all 37 substances as to be of no safety concern at the estimated levels of intake as flavouring substances based on the MSDI approach.

The evaluations of the 37 benzyl derivatives are summarised in Table 3.1: Summary of Safety Evaluation of 37 Benzyl Derivatives (JECFA, 2002a).

4.2. Application of the Procedure to 36 Benzyl Derivatives by EFSA in FGE.20Rev1 (EFSA, 2009k):

Thirty-six candidate substances were evaluated in FGE.20Rev1. Thirty-three substances are classified into structural class I, two into structural class II and one substance into structural class III using the decision tree approach presented by Cramer et al. (1978).

The Panel concluded all of the 36 flavouring substances at step A3 in the EFSA Procedure, i.e. the substances are expected to be metabolised to innocuous products (step 2) and the intake for all substances is below the threshold for structural class I, II and III, respectively (step A3).

In conclusion, the Panel evaluated all 36 substances as to be of no safety concern at the estimated levels of intake as flavouring substances based on the MSDI approach.

The stepwise evaluations of the 36 substances are summarised in Table 3.2: Summary of Safety Evaluation Applying the Procedure (EFSA / FGE.20Rev1).

4.3. EFSA Considerations

The Panel agrees with the way the application of the Procedure has been performed by the JECFA for all the 37 substances in the group of benzyl derivatives performed.

However, for four substances [FL-no: 06.019, 09.294, 09.803 and 09.812] no European production figure was available and consequently no European exposure estimate could be calculated. Accordingly, the safety in use in Europe could not be assessed using the Procedure for this substance.

5. Conclusion

The JECFA has evaluated a group of 37 flavouring substances consisting of benzyl derivatives.

One of the JECFA evaluated substances, geranyl benzoate [FL-no: 09.767], may be metabolised to an alpha,beta-unsaturated aldehyde. As the alpha,beta-unsaturated aldehyde and ketone structures are considered by the Panel to be structural alerts for genotoxicity (EFSA, 2008b), this substance has been given special considerations.

The remaining 36 flavouring substances have originally been considered by EFSA in the FGE.54 (EFSA, 2008al).

The genotoxicity of the alpha,beta-unsaturated substance, geranyl benzoate [FL-no: 09.767], has been considered in FGE.202. The structural alert for genotoxicity is present in the metabolite citral. The Panel concluded that the data available on citral did rule out the concern for genotoxicity and thus concluded that geranyl benzoate can be evaluated through the Procedure.

The Panel concluded that the 37 substances in the JECFA flavouring group of benzyl derivatives are structurally related to the group of benzyl alcohols, benzaldehydes, a related acetal, benzoic acids, and related esters evaluated by the Panel in the Flavouring Group Evaluation 20 (FGE.20)

The Panel agrees with the way the application of the Procedure has been performed by the JECFA for all the 37 substances in the group of benzyl derivatives performed.

However, for four substances [FL-no: 06.019, 09.294, 09.803 and 09.812] no European production figure was available and consequently no European exposure estimate could be calculated. Accordingly, the safety in use in Europe could not be assessed using the Procedure for this substance.

For all 37 substances use levels are needed to calculate the mTAMDIs in order to identify those flavouring substances that need more refined exposure assessment and to finalise the evaluation.

In order to determine whether the conclusion for the 37 JECFA evaluated substances can be applied to the materials of commerce, it is necessary to consider the available specifications. Adequate specifications are available including purity and identity are available for 30 of the 37 JECFA evaluated substances. Information on stereoisomerism is lacking for two substances [FL-no: 06.002

and 06.012] and for seven substances [FL-no: 05.027, 06.002, 06.012, 06.019, 09.294, 09.755 and 09.806] further information on the composition of the mixture is requested.

Thus, for nine substances [FL-no: 05.027, 06.002, 06.012, 06.019, 09.294, 09.755, 09.803, 09.806 and 09.812] the Panel has reservations (no European production volumes available, preventing them from being evaluated using the Procedure, and/or lack of information on stereoisomerism and/or further information on the composition of the mixture). For the remaining 28 of the 37 JECFA evaluated benzyl derivatives [FL-no: 02.010, 02.039, 05.013, 05.022, 05.068, 05.110, 06.003, 06.032, 08.021, 09.014, 09.051, 09.077, 09.132, 09.406, 09.426, 09.458, 09.494, 09.508, 09.705, 09.725, 09.726, 09.727, 09.757, 09.767, 09.768, 09.770, 09.771 and 09.776] the Panel agrees with the JECFA conclusion "No safety concern at estimated levels of intake as flavouring substances" based on the MSDI approach.

TABLE 1: SPECIFICATION SUMMARY FOR JECFA EVALUATED SUBSTANCES IN THE PRESENT GROUP (JECFA, 2001C)

Table 1: Specification Summary of the Substances in the JECFA Flavouring Group of 37 Benzyl Derivatives (JECFA, 2001c)

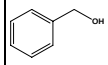
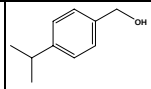
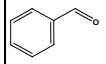
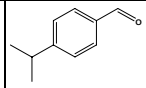
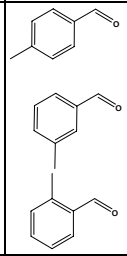
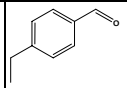
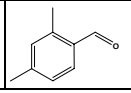
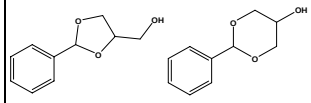
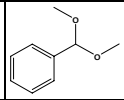
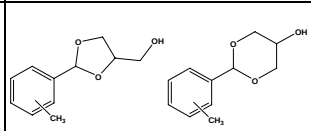
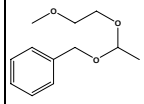
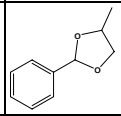
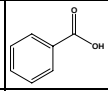
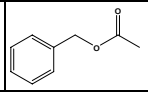
FL-no JECFA-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	EFSA Comments
02.010 25	Benzyl alcohol		2137 58 100-51-6	Liquid C_7H_8O 108.14	Slightly soluble Miscible	205 IR 98 %	1.536-1.541 1.040-1.050	
02.039 864	4-Isopropylbenzyl alcohol		2933 88 536-60-7	Liquid $C_{10}H_{14}O$ 150.22	Insoluble Miscible	248 IR 97 %	1.518-1.525 0.974-0.982	
05.013 22	Benzaldehyde		2127 101 100-52-7	Liquid C_7H_6O 106.12	Very slightly soluble Miscible	178 IR 98 %	1.544 - 1.547 1.040-1.047	
05.022 868	4-Isopropylbenzaldehyde		2341 111 122-03-2	Liquid $C_{10}H_{12}O$ 148.20	Insoluble Miscible	235-236 IR 95 %	1.527-1.534 0.973-0.981	
05.027 866	Tolualdehyde		3068 115 1334-78-7	Liquid C_8H_8O 120.15	Very slightly soluble Miscible	198 IR 95 %	1.540-1.549 1.013-1.029	CASrn In Register refers to "Incompletely Defined Substance" According to JECFA: Min. assay value is "95 (sum of o,m,p- isomers)".
05.068 865	4-Ethylbenzaldehyde		3756 705 4748-78-1	Liquid $C_9H_{10}O$ 134.18	Insoluble Miscible	220 IR 97 %	1.538-1.542 0.980-1.000	
05.110 869	2,4-Dimethylbenzaldehyde		3427 15764-16-6	Liquid $C_9H_{10}O$ 134.18	Insoluble Miscible	215 NMR 95 %	1.548-1.552 0.992-1.017	

Table 1: Specification Summary of the Substances in the JECFA Flavouring Group of 37 Benzyl Derivatives (JECFA, 2001c)

FL-no JECFA-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)	EFSA Comments
06.002 838	5-Hydroxy-2-phenyl-1,3-dioxane 6)		2129 36 1319-88-6	Liquid $C_{10}H_{12}O_3$ 180.21	Slightly soluble Miscible	185 (26 hPa) IR 98 %	1.532-1.542 1.181-1.193	CASrn in Register refers to "Incompletely Defined Substance". According to JECFA: Min. assay value is "98 (sum of isomers)".
06.003 837	alpha,alpha-Dimethoxytoluene		2128 37 1125-88-8	Liquid $C_9H_{12}O_2$ 152.20	Insoluble Miscible	198 IR 96 %	1.488-1.496 1.007-1.020	
06.012 867	Tolualdehyde glyceryl acetal 6)		3067 46 1333-09-1	Liquid $C_{11}H_{14}O_3$ 194.23	Slightly soluble Miscible	292 IR 95 %	1.527-1.537 1.148-1.158	CASrn in Register refers to "Incompletely Defined Substance" According to JECFA: Min. assay value is "95 (sum of o,m,p- isomers)".
06.019 840	1-Benzyloxy-1-(2-methoxyethoxy)ethane		2148 523 7492-39-9	Liquid $C_{12}H_{18}O_3$ 210.27	Insoluble Miscible	161-162 (13hPa) IR 98 %	1.479-1.489 1.019-1.025	Racemate. According to JECFA: Min. assay value is "98 (sum of named compound and starting materials)".
06.032 839	4-Methyl-2-phenyl-1,3-dioxolane		2130 2226 2568-25-4	Liquid $C_{10}H_{12}O_2$ 164.20	Slightly soluble Miscible	83-85 (5 hPa) IR 95 %	1.506-1.516 1.061-1.071	Racemate.
08.021 850	Benzoic acid		2131 21 65-85-0	Solid $C_7H_6O_2$ 122.12	Insoluble Very soluble	249 122 IR 99 %	n.a. n.a.	
09.014 23	Benzyl acetate		2135 204 140-11-4	Liquid $C_9H_{10}O_2$ 150.18	Very slightly soluble Miscible	215 IR 98 %	1.500 - 1.504 1.049-1.059	

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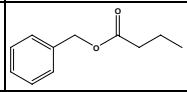
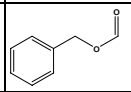
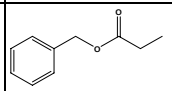
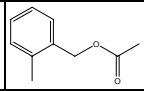
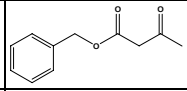
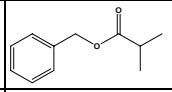
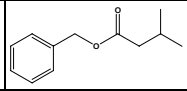
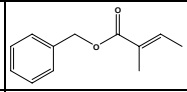
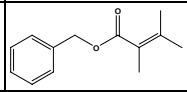
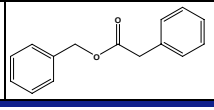
FL-no JECFA-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	EFSA Comments
09.051 843	Benzyl butyrate		2140 277 103-37-7	Liquid $C_{11}H_{14}O_2$ 178.23	Insoluble Miscible	239 IR 98 %	1.490-1.500 1.005-1.012	
09.077 841	Benzyl formate		2145 344 104-57-4	Liquid $C_8H_8O_2$ 136.15	Insoluble Miscible	202 IR 95 %	1.508-1.518 1.082-1.092	
09.132 842	Benzyl propionate		2150 413 122-63-4	Liquid $C_{10}H_{12}O_2$ 164.20	Insoluble Miscible	222 IR 98 %	1.495-1.500 1.028-1.033	
09.294 863	2-Methylbenzyl acetate		3702 17373-93-2	Liquid $C_{10}H_{12}O_2$ 164.20	Insoluble Miscible	215-222 IR 98 %	1.500-1.510 1.024-1.040	According to JECFA: Min. assay value is "98 (sum of o,m,p- isomers)".
09.406 848	Benzyl 3-oxobutyrate		2136 244 5396-89-4	Liquid $C_{11}H_{12}O_3$ 192.21	Insoluble Miscible	249 IR 98 %	1.498-1.520 1.112-1.120	
09.426 844	Benzyl isobutyrate		2141 301 103-28-6	Liquid $C_{11}H_{14}O_2$ 178.23	Insoluble Miscible	229 IR 97 %	1.488-1.492 1.000-1.006	
09.458 845	Benzyl isovalerate		2152 453 103-38-8	Liquid $C_{12}H_{16}O_2$ 192.26	Insoluble Miscible	246 IR 98 %	1.482-1.490 0.981-0.989	
09.494 846	Benzyl 2-methylcrotonate		3330 2184 37526-88-8	Liquid $C_{12}H_{14}O_2$ 190.24	Insoluble Miscible	250 IR 95 %	1.515-1.526 1.029-1.040	
09.508 847	Benzyl 2,3-dimethylcrotonate		2143 11868 7492-69-5	Liquid $C_{13}H_{16}O_2$ 204.27	Insoluble Miscible	259 NMR 97 %	1.510-1.517 1.017-1.023	
09.705 849	Benzyl phenylacetate		2149 232 102-16-9	Liquid $C_{15}H_{14}O_2$ 226.28	Insoluble Miscible	320 IR 98 %	1.552-1.560 1.094-1.100	

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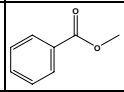
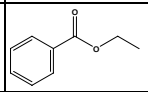
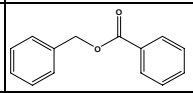
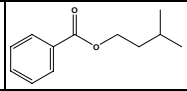
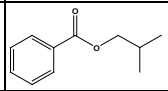
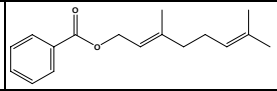
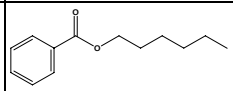
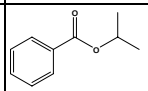
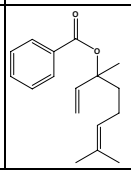
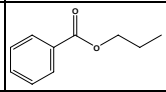
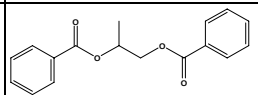
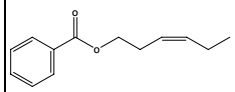
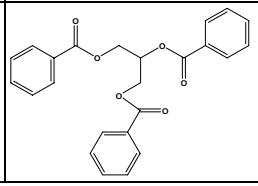
FL-no JECFA-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	EFSA Comments
09.725 851	Methyl benzoate		2683 260 93-58-3	Liquid $C_8H_8O_2$ 136.15	Insoluble Miscible	198 IR 98 %	1.513-1.520 1.082-1.089	
09.726 852	Ethyl benzoate		2422 261 93-89-0	Liquid $C_9H_{10}O_2$ 150.18	Insoluble Miscible	212 IR 98 %	1.502-1.508 1.043-1.050	
09.727 24	Benzyl benzoate		2138 262 120-51-4	Liquid $C_{14}H_{12}O_2$ 212.25	Insoluble Miscible	323-324 21 IR 99 %	1.566-1.571 1.113-1.121	
09.755 857	Isopentyl benzoate		2058 562 94-46-2	Liquid $C_{12}H_{16}O_2$ 192.26	Insoluble Miscible	261 IR 98 %	1.491-1.497 0.984-0.992	According to JECFA: Min. assay value is "98 (sum of isomers)".
09.757 856	Isobutyl benzoate		2185 567 120-50-3	Liquid $C_{11}H_{14}O_2$ 178.23	Insoluble Miscible	240 IR 98 %	1.489-1.496 0.994-0.999	
09.767 860	Geranyl benzoate		2511 639 94-48-4	Liquid $C_{17}H_{22}O_2$ 258.36		305 IR 95 %	1.514-1.521 0.980-0.990	
09.768 854	Hexyl benzoate		3691 645 6789-88-4	Liquid $C_{13}H_{18}O_2$ 206.29	Insoluble Miscible	272 IR 98 %	1.490-1.500 0.978-0.984	
09.770 855	Isopropyl benzoate		2932 652 939-48-0	Liquid $C_{10}H_{12}O_2$ 164.21	Insoluble Miscible	218 IR 98 %	1.492-1.497 1.005-1.011	
09.771 859	Linalyl benzoate		2638 654 126-64-7	Liquid $C_{17}H_{22}O_2$ 258.36	Insoluble Miscible	263 IR 95 %	1.505-1.526 0.978-0.999	Racemate.

Table 1: Specification Summary of the Substances in the JECFA Flavouring Group of 37 Benzyl Derivatives (JECFA, 2001c)

FL-no JECFA-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)	EFSA Comments
09.776 853	Propyl benzoate		2931 677 2315-68-6	Liquid $C_{10}H_{12}O_2$ 164.21	Insoluble Miscible	229 IR 98 %	1.498-1.503 1.020-1.026	
09.803 862	Propylene glycol dibenzoate		3419 10890 19224-26-1	Liquid $C_{17}H_{16}O_4$ 284.31	Insoluble Miscible	232 (16 hPa) IR 96 %	1.542-1.547 1.157-1.163	Racemate.
09.806 858	Hex-3-enyl benzoate 6)	 (Z)-isomer shown	3688 11778 25152-85-6	Liquid $C_{13}H_{16}O_2$ 204.27	Insoluble Miscible	130 (7 hPa) IR 95 %	1.503-1.514 0.995-1.004	CASrn in Register refers to (Z)-isomer. According to JECFA: Min. assay value is "95 (sum of isomers)".
09.812 861	Glyceryl tribenzoate		3398 10656 614-33-5	Solid $C_{24}H_{20}O_6$ 404.42	Insoluble Slightly soluble	n.a. 68-72 IR 95 %	n.a. n.a.	

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 2: GENOTOXICITY DATA

Table 2.1: Genotoxicity Data (*in vitro / in vivo*) for 37 Benzyl Derivatives (JECFA, 2002a)

Table 2.1: Summary of Genotoxicity Data for 37 Benzyl Derivatives evaluated by JECFA (JECFA, 2002a)

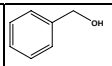
FL-no JECFA-no	EU Register name JECFA name	Structural formula	Endpoint	Test system	Concentration	Results	Reference
In vitro							
02.010	Benzyl alcohol		Reverse mutation	*S. typhimurium* TA92, TA94, TA98, TA100, TA1535, TA1537 (preincubation)	10 000 µg/plate	Negative[a]	(Ishidate et al., 1984)
			Reverse mutation	*S. typhimurium* TA100 (plate incorporation)	1000 µg/plate	Negative[b]	(Ball et al., 1984)
			Reverse mutation	*S. typhimurium* TA98, TA100 (plate incorporation)	NR	Negative[b]	(Rogan et al., 1986)
			Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537 (preincubation)	6700 µg/plate	Negative[a]	(Mortelmans et al., 1986)
			Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537 (plate incorporation)	3 µmol/plate	Negative[a]	(Florin et al., 1980)
			Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538 (plate incorporation)	50 000 µg/plate	Negative[a]	(Heck et al., 1989)
			Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538 (plate incorporation)	5 µl/plate	Negative[b]	(Milvy & Garro, 1976)
			Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537 (preincubation)	6700 µg/plate	Negative[c]	(NTP, 1989a)
			Mutation	*E. coli* WP2 *uvr*A	8 mg/plate	Negative[d]	(Yoo, 1986)
			DNA repair	*B. subtilis* H17, M45	21 µg/disc	Negative[e]	(Oda et al., 1979)
			DNA repair	*B. subtilis* H17, M45	10 µg/disk	Positive[f]	(Kuroda et al., 1984b)
			DNA repair	*B. subtilis* H17, M45	20 µl/disk	Positive[d]	(Yoo, 1986)
			Chromosomal aberration	Chinese hamster fibroblasts	1.0 mg/ml	Negative[g]	(Ishidate et al., 1984)
			Chromosomal aberration	Chinese hamster ovary cells	5000 µg/ml	Equivocal[h]	(Anderson et al., 1990)
			Chromosomal aberration	Chinese hamster ovary cells	5000 µg/ml	Positive[i]	(NTP, 1989a)
			Sister chromatid exchange	Chinese hamster ovary cells	5000 µg/ml	Positive[j]	(NTP, 1989a)
			Sister chromatid exchange	Chinese hamster ovary cells	5000 µg/ml	Positive[k]	(Anderson et al., 1990)

Table 2.1: Summary of Genotoxicity Data for 37 Benzyl Derivatives evaluated by JECFA (JECFA, 2002a)

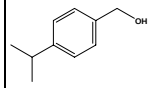
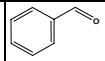
FL-no JECFA-no	EU Register name JECFA name	Structural formula	Endpoint	Test system	Concentration	Results	Reference
			Mutation	L5178Y mouse lymphoma cells	5000 µg/ml	Equivocal[l]	(McGregor et al., 1988a)
			Mutation	L5178Y mouse lymphoma cells	5000 µg/ml	Equivocal[l]	(Myhr et al., 1990)
			Mutation	L5178Y mouse lymphoma cells	4500 µg/ml	Positive[m]	(NTP, 1989a)
			Mutation	*E. coli* WP2 *uvr*A	NR	Negative[n]	(Kuroda et al., 1984b)
			Cytotoxicity	Human alveolar tumour cells	0.5 mmol/L	Negative	(Waters et al., 1982)
			DNA damage	Human alveolar tumour cells	0.5 mmol/L	Negative	(Waters et al., 1982)
			DNA damage	Rat hepatocytes	10 mmol/L	Negative[o]	(Storer et al., 1996)
			DNA damage	*E. coli* P3478	50 µl/disc	Negative[a]	(Fluck et al., 1976)
02.039	4-Isopropylbenzyl alcohol		Reverse mutation	*S. typhimurium* TA98, TA100 (plate incorporation)	100 µl/plate	Negative[p]	(Rockwell & Raw, 1979)
			Reverse mutation	*S. typhimurium* TA98, TA100 (plate incorporation)	300 µl/plate	Negative[q]	(Rockwell & Raw, 1979)
05.013	Benzaldehyde		Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538 (plate incorporation)	38 000 ug/plate	Negative[a]	(Heck et al., 1989)
			Reverse mutation	*S. typhimurium* TA98, TA100 (plate incorporation)	300 µl/plate	Negative[r]	(Rockwell & Raw, 1979)
			Reverse mutation	*S. typhimurium* TA98, TA100 (plate incorporation)	100 µl/plate	Negative[p]	(Rockwell & Raw, 1979)
			Reverse mutation[a]	*S. typhimurium* TA98, TA100, TA2637	2 mg/plate	Negative[s]	(Nohmi et al., 1985)
			Reverse mutation[a]	*S. typhimurium* TA98, TA100, TA1535, TA1537	3 µmol/plate	Negative[a]	(Florin et al., 1980)
			Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537 (preincubation)	1000 µg/plate	Negative[a]	(Haworth et al., 1983)
			Reverse mutation	*S. typhimurium* TA100, TA102, TA104	3300 µg/plate	Negative[a]	(NTP, 1990c)
			Reverse mutation[a]	*S. typhimurium* TA100	1 mg/plate	Negative[t]	(Rapson et al., 1980)
			Reverse mutation	*S. typhimurium* TA98, TA100 (preincubation)	NR	Negative[a]	(Sasaki & Endo, 1978)
			Reverse mutation	*S. typhimurium* TA100, TA102, TA104 (preincubation)	NR	Negative[a]	(Dillon et al., 1992)
			Reverse mutation	*S. typhimurium* TA100 (preincubation)	2000 nmol/plate	Negative[a]	(Vamvakas et al., 1989)
			Reverse mutation[a]	*S. typhimurium* TA98, TA100	500 µg/plate	Negative[a]	(Kasamaki et al., 1982)
			DNA repair	*B. subtilis* H17, M45	21 µg/disc	Negative[d]	(Oda et al., 1979)

Table 2.1: Summary of Genotoxicity Data for 37 Benzyl Derivatives evaluated by JECFA (JECFA, 2002a)

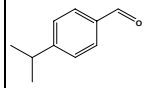
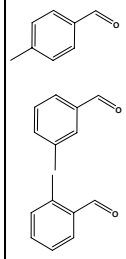
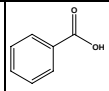
FL-no JECFA-no	EU Register name JECFA name	Structural formula	Endpoint	Test system	Concentration	Results	Reference
			DNA repair	*B. subtilis* H17, M45	NR	Positive[i]	(Matsui et al., 1989)
			Unscheduled DNA synthesis	Rat hepatocytes	250 µg/ml	Negative[b]	(Heck et al., 1989)
			Mutation	Mouse L5178Y lymphoma cells	600 µg/ml	Positive[v]	(Heck et al., 1989)
			Mutation	Mouse L5178Y lymphoma cells	800 µg/ml	Positive[w]	(McGregor et al., 1988a)
			Chromosomal aberrations	Chinese hamster cells	1.2 mg/ml	Positive[x]	(Sofuni et al., 1985)
			Chromosomal aberrations	Chinese hamster ovary cells	1600 µg/ml	Negative[a]	(Galloway et al., 1987)
			Chromosomal aberrations	Chinese hamster cells	50 nmol/L	Positive[a]	(Kasamaki et al., 1982)
			Sister chromatid exchange	Chinese hamster ovary cells	1600 µg/ml	Positive[a]	(Galloway et al., 1987)
			Sister chromatid exchange	Chinese hamster ovary cells	1000 µmol/L	Negative[y]	(Sasaki et al., 1989)
			Sister chromatid exchange	Human lymphocytes	2 mmol/L	Positive[b]	(Jansson et al., 1988)
05.022	4-Isopropylbenzaldehyde		Reverse mutation	*S. typhimurium* TA98, TA100 (plate incorporation)	100 µl/plate	Negative[p]	(Rockwell & Raw, 1979)
			Reverse mutation	*S. typhimurium* TA98, TA100 (plate incorporation)	300 µl/plate	Negative[z]	(Rockwell & Raw, 1979)
			Sister chromatid exchange	Chinese hamster ovary cells	333 µmol/L	Negative[l]	(Sasaki et al., 1989)
05.027	Tolualdehyde		Reverse mutation	*S. typhimurium* TA104 (preincubation)	0.8 µmol/plate	Negative[a]	(Marnett et al., 1985a)
			Reverse mutation[a]	*S. typhimurium* TA98, TA100, TA1535, TA1537	3 µmol/plate	Negative[a]	(Florin et al., 1980)
			Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538 (plate incorporation)	19 000 ug/plate	Negative[a]	(Heck et al., 1989)
			Reverse mutation	*S. typhimurium* TA98, TA100, TA102 (plate incorporation)	0.8 mmol/plate	Negative[a]	(Aeschbacher et al., 1989)
			Reverse mutation	*S. typhimurium* TA97, TA100, TA1535, TA1537 (preincubation)	666 µg/plate	Negative[a]	(Zeiger et al., 1988)
			Unscheduled DNA synthesis	Rat hepatocytes	1000 µg/ml	Negative[b]	(Heck et al., 1989)
			Mutation	Mouse L5178Y lymphoma cells	300 µg/ml	Negative[a]	(Heck et al., 1989)
08.021	Benzoic acid		Reverse mutation	S. typhimurium TA98, TA100, TA1535, TA1538 (plate incorporation)	2500 µg/plate	Negative[a]	(Anderson & Styles, 1978)
			Reverse mutation[a]	*S. typhimurium* TA98, TA100, TA1535, TA1536	3.6 µg/plate	Negative[a]	(Cotruvo et al., 1977)
			Reverse mutation	*S. typhimurium* TA97, TA98, TA100, TA1535, TA1537 (preincubation)	10 mg/plate	Negative[a]	(Zeiger et al., 1988)

Table 2.1: Summary of Genotoxicity Data for 37 Benzyl Derivatives evaluated by JECFA (JECFA, 2002a)

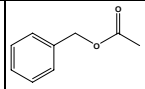
FL-no JECFA-no	EU Register name JECFA name	Structural formula	Endpoint	Test system	Concentration	Results	Reference
			Reverse mutation[a]	*S. typhimurium* TA100	1 mg/plate	Negative[t]	(Rapson et al., 1980)
			Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537 (plate incorporation)	1 mg/plate	Negative[p]	(McCann et al., 1975)
			Reverse mutation	*S. typhimurium* TA92, TA94, TA98, TA100, TA1535, TA1537 (preincubation)	10 mg/plate	Negative[a]	(Ishidate et al., 1984)
			Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538 (plate incorporation)	100 µg/plate	Negative[b]	(Milvy & Garro, 1976)
			Reverse mutation	*S. typhimurium* TA1535, TA1537, TA1538 (plate incorporation)	0.5%	Negative[a]	(FDA, 1975b)
			Mutation (*umu* gene expression)	*S. typhimurium* TA1535/ pSK1002	1.7 mg/ml	Negative[a]	(Nakamura et al., 1987)
			DNA repair	*B. subtilis* H17, H45	NR	Positive[2]	(Nonaka, 1989)
			Mutation	*Saccharomyces cerevisiae* D3	0.18%	Negative[a]	(Cotruvo et al., 1977)
			Mutation	*S. cerevisiae* D4	0.15%	Negative[a]	(FDA, 1975b)
			Indirect DNA repair (induction of *beta*-galactosidase)	*E. coli* PQ37	400 µg/ml	Negative	(Glosnicka & Dziadziuszko, 1986)
			Chromosomal aberration	Chinese hamster fibroblasts	1.5 mg/ml	Positive[3]	(Ishidate et al., 1984)
			Sister chromatid exchange	Human lymphocytes	2.0 mmol/L	Negative[b]	(Jansson et al., 1988)
09.014	Benzyl acetate		Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537 (preincubation)	10 mg/plate	Negative[a]	(Mortelmans et al., 1986)
			Reverse mutation	*S. typhimurium* TA98, TA100 (preincubation and plate incorporation)	5000 µg/plate	Negative[4]	(Schunk et al., 1986)
			Reverse mutation[a]	*S. typhimurium* TA98, TA100, TA1535, TA1537	3 µmol/plate	Negative[a]	(Florin et al., 1980)
			DNA repair	*B. subtilis* H17, M45	21 µg/disc	Negative[5]	(Oda et al., 1979)
			DNA repair	*B. subtilis* H17, M45	20 µl/disc	Positive[d]	(Yoo, 1986)
			Mutation	*E. coli* WP2 *uvr*A	2.0 mg/plate	Negative[d]	(Yoo, 1986)
			Mutation	Mouse lymphoma L5178Y cells	500 µg/ml	Positive[u]	(Caspary et al., 1988)
			Mutation	Human lymphoblast TK6 cells	1500 µg/ml	Positive[u]	(Caspary et al., 1988)
			Mutation	Mouse lymphoma L5178Y cells	1600 µL/mL	Positive[v]	(McGregor et al., 1988a)
			Mutation	Mouse lymphoma L5178Y cells	NR	Positive[u]	(Rudd et al., 1983)

Table 2.1: Summary of Genotoxicity Data for 37 Benzyl Derivatives evaluated by JECFA (JECFA, 2002a)

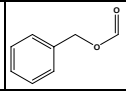
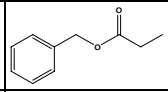
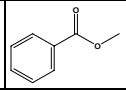
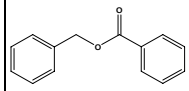
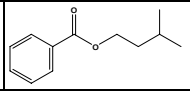
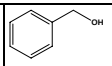
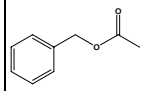
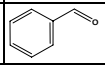
FL-no JECFA-no	EU Register name JECFA name	Structural formula	Endpoint	Test system	Concentration	Results	Reference
			Chromosomal aberration	Chinese hamster ovary cells	5000 µg/ml	Negative[a]	(Galloway et al., 1987)
			Chromosomal aberration	Chinese hamster lung fibroblasts	2.4 mg/ml	Negative[6]	(Matsuoka et al., 1996)
			Sister chromatid exchange	Chinese hamster ovary cells	5000 µg/ml	Negative[a]	(Galloway et al., 1987)
			Unscheduled DNA synthesis	Rat hepatocytes	NR	Negative[2]	(Mirsalis et al., 1983)
09.077	Benzyl formate		DNA repair	*B. subtilis* H17, M45	20 µl/disc	Positive[d]	(Yoo, 1986)
			Mutation	*E. coli* WP2 *uvr*A	4.0 mg/plate	Negative[d]	(Yoo, 1986)
09.132	Benzyl propionate		DNA repair	*B. subtilis* H17, M45	21 µg/disc	Negative[7]	(Oda et al., 1979)
09.725	Methyl benzoate		Reverse mutation	*S. typhimurium* TA97, TA98, TA100, TA1535, TA1537 (preincubation)	6700 ug/plate	Negative[a]	(Zeiger et al., 1992)
			Mutation	*E. coli* Sd-4-73	NR	Negative[b]	(Szybalski, 1958)
09.727	Benzyl benzoate		Reverse mutation[a]	*S. typhimurium* TA98, TA100, TA1535, TA1537	3 µmol/plate	Negative[a]	(Florin et al., 1980)
			Reverse mutation	*S. typhimurium* TA98, TA100 (preincubation and plate incorporation)	5000 µg/plate	Negative[4]	(Schunk et al., 1986)
09.755	Isopentyl benzoate		Mutation	*E. coli* Sd-4-73	NR	Negative[b]	(Szybalski, 1958)
In vivo							
02.010	Benzyl alcohol		Sex-linked recessive lethal mutation	*Drosophila melanogaster*	5000 mg/kg| 8000 mg/kg	Negative[8] Negative[9]	(Foureman et al., 1994)
			Micronucleus formation	Mouse bone marrow cells	200 mg/kg bw	Negative[10]	(Hayashi et al., 1988)
			Replicative DNA synthesis	Mouse hepatocytes	NR	Positive[11]	(Yoshikawa, 1996)
			Sex-linked recessive lethal mutation	*D. melanogaster*	300 mg/kg 20 000 mg/kg	Negative[8] Negative[9]	(NTP, 1993d; Foureman et al., 1994)

Table 2.1: Summary of Genotoxicity Data for 37 Benzyl Derivatives evaluated by JECFA (JECFA, 2002a)

FL-no JECFA-no	EU Register name JECFA name	Structural formula	Endpoint	Test system	Concentration	Results	Reference
			Sister chromatid exchange	Mouse bone marrow cells	1700 mg/kg bw	Negative[10]	(NTP, 1993d)
09.014	Benzyl acetate		Chromosomal aberration	Mouse bone marrow cells	1700 mg/kg bw	Negative[10]	(NTP, 1993d)
			Micronucleus formation	Mouse bone marrow cells	1300 mg/kg bw	Negative[10]	(NTP, 1993d; Shelby et al., 1993)
			Micronucleus formation	Mouse erythrocytes	50 000 mg/kg	Negative[10]	(NTP, 1993d)
			Unscheduled DNA synthesis	Rat hepatocytes	NR	Negative[12]	(Mirsalis et al., 1983)
			Unscheduled DNA synthesis	Rat hepatocytes	1000 mg/kg bw	Negative[13]	(Mirsalis et al., 1983)
			Unscheduled DNA synthesis	Rat pancreatic cells	1000 mg/kg bw	Negative[13]	(Steinmetz & Mirsalis, 1984)
			DNA damage	Rat pancreatic cells	500 mg/kg bw	Negative[14]	(Longnecker et al., 1990)
			DNA damage	Rate pancreatic cells	0.9%	Negative[15]	(Longnecker et al., 1990)
05.013	Benzaldehyde		Sex-linked recessive lethal mutation	*D. melanogaster*	1200 mg/kg 2500 mg/kg	Negative[8] Negative[9]	(Woodruff et al., 1985)

[a] *Assay performed with and without S9.*

[b] *Assay performed without S9.*

[c] *Assay performed with and without S9; cytotoxicity at highest concentration.*

[d] *Japanese article, English summary; use of S9 not reported.*

[e] *Japanese article, English summary tables.*

[f] *Japanese article, English summary tables; inhibition of growth without S9.*

[g] *Assay performed without S9; cells exposed for 48 h.*

[h] *Assay performed with and without S9; positive results not reproducible; no dose- response relationship.*

[i] *Assay performed with and without S9; positive results reported only with S9.*

[j] *Dose-response relationship at 500–1250 µg/ml without S9 and 500–4000 µg/ml with S9.*

[k] *Assay performed with and without S9; no dose-response relationship; increase at single doses.*

[l] *Positive and negative responses could not be reproduced; no dose- response relationship.*

[m] *Assay performed with and without S9; positive result only without S9.*

[n] *Abstract; methods and test concentrations not reported.*

[o] *Cytotoxicity at maximum dose .*

[p] *Assay performed with S9.*

[q] *Assay of urine samples from rats given isopropyl-benzyl alcohol by oral gavage; performed with and without S9.*

[r] *Assay of urine samples from rats given benzaldehyde by oral gavage, per- formed with and without S9.*

[s] *Japanese article, English summary; assay performed with and without S9.*

[t] *Use of S9 not reported.*

[u] *Assay performed with and without S9; positive result only with S9.*

[v] *Positive only with S9.*

[w] *Assay performed without S9; significant increase in mutant fraction at close to toxic doses.*

[x] *Japanese article, English summary; positive results without S9; weakly positive results with S9; cytotoxicity at two higher concentrations.*

[y] *Assay performed without S9; cytotoxicity at highest concentration.*

[z] *Assay of urine samples from rats given cuminaldehyde by gavage; performed with and without S9.*

[1] *Assay performed without S9; cytotoxicity at maximum concentration.*

[2] *Abstract; methods and test concentration(s) not reported.*

[3] *Total incidence of cells with aberrations, 5–9 %; performed without S9.*

[4] *Assay performed with and without S9; cytotoxicity at three higher concentrations.*

[5] *Assay performed without S9 Japanese article, English summary tables.*

[6] *Assay performed with and without S9; cytotoxicity at maximum concentration.*

[7] *Assay performed without S9 Japanese article, English summary tables.*

[8] *In feed.*

[9] *By injection.*

[10] *By intraperitoneal injection.*

[11] *Route of administration not reported.*

[12] *By oral gavage; abstract; methods and doses not reported.*

[13] *By oral gavage.*

[14] *By gavage.*

[15] *In diet.*

Table 2.2: Genotoxicity (*in vitro*) EFSA / FGE.20Rev1

Substances in brackets are the JECFA evaluated supporting substances in FGE.20Rev1.

Table 2.2: Summary of Genotoxicity Data (*in vitro)* EFSA / FGE.20Rev1

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, 1989a)	Valid study in accordance with OECD guideline 471 (except that only four strains were used). Cytotoxicity was reported at the highest concentration tested.
	Ames test (plate incorporation method)	*S. typhimurium* TA97; TA102	1000 µg/plate	Negative[1]	(Fujita et al., 1992)	
	Ames test (plate incorporation method)	*S. typhimurium* TA98; TA1535	5 µM/plate	Negative[1]	(Wiessler et al., 1983)	
	Mutation assay	*Escherichia coli* WP2 *uvr*A	1000 to 8000 µg/plate	Negative	(Yoo, 1986)	Study published in Japanese with English abstract. Data extracted from tables. Validity of the study cannot be evaluated. No information on the use of metabolic activation.
	Rec assay	*B. subtilis* M45 (rec^-), H17 (rec^+)	21 µg/disc	Negative	(Oda et al., 1979)	Study published in Japanese without English abstract. Data extracted from tables. Validity of the study cannot be evaluated.
	Rec assay	*B. subtilis* M45 (rec^-), H17 (rec^+)	10 µg/disc	Positive	(Kuroda et al., 1984b)	Study published in Japanese with English abstract. Data extracted from figure. Validity

Table 2.2: Summary of Genotoxicity Data (*in vitro*) EFSA / FGE.20Rev1

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
						of the study cannot be evaluated. Inhibition of growth was reported.
	Rec assay	*B. subtilis* M45 (rec^-), H17 (rec^+)	20 µl/disc	Positive	(Yoo, 1986)	Study published in Japanese with English abstract. Data extracted from tables. Validity of the study cannot be evaluated. A weak positive result (i.e. 4 mm≤ D<8 mm). was reported (D=5 mm). No information on the use of metabolic activation.
	Chromosomal aberration test	Chinese hamster fibroblast cells	1000 µg /ml[4] (three 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 polyploidisation.
	Chromosomal aberration test	Chinese hamster ovary cells	50 to 5000 µg/ml	Equivocal[1]	(Anderson et al., 1990)	Published summary report including detailed results from studies on 42 compounds tested in various laboratories within the NTP in accordance with OECD guideline 473. Lowest effective dose was 4000 µg/ml with and without S9. No dose-response observed. Positive results were not reproducible in all trials. Absence of cytotoxicity reported up to the highest dose.
	Chromosomal aberration test	Chinese hamster ovary cells	50 to 5000 µg/ml	Negative[2] Positive[3]	(NTP, 1989a)	Valid study in accordance with OECD guideline 473. A positive result was reported only in the presence of S9 at relatively high concentrations of 4000 µg/ml in 3 of 4 tests carried out with harvest times between 12 and 18 hours. No data on cytotoxicity reported.
	Sister chromatid exchange assay	Chinese hamster ovary cells	16 to 5000 µg/ml	Positive	(NTP, 1989a)	Valid study in accordance with OECD guideline 479. Dose-related increase in frequency of SCE at concentrations from 500 - 1250 µg/ml (without metabolic activation) and 500 - 4000 µg/ml (with metabolic activation). No data on cytotoxicity reported. Number of chromosomes per cell reduced at 4000 µg/ml with S9.
	Sister chromatid exchange assay	Chinese hamster ovary cells	16 to 1250 µg/ml[2] 16 to 4000 µg/ml[3]	Positive[1]	(Anderson et al., 1990)	Published summary report including detailed results from studies on 42 compounds tested in various laboratories within the NTP in accordance with OECD guideline 479. Significant increase (20 %) in SCE only at the highest doses. No dose-response observed. No second trial using high concentrations to reproduce the positive

Table 2.2: Summary of Genotoxicity Data (*in vitro*) EFSA / FGE.20Rev1

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
						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 colonysizing performed). Positive responses observed in some experiments at concentrations of 3500 and higher. No dose-response was observed. The highest concentration was letal in some experiments. Positive and negative responses could not be reproduced in all experiments.
	Mammalian cell gene mutation test	Mouse lymphoma L5178Y cells	150 to 5000 µg/ml	Negative[3] Positive[2]	(NTP, 1989a)	Valid study in accordance with OECD guideline 476. In one of three trials without S9 a positive result (relative mutant fraction ≥1.6) was reported at 4500 µg/ml with relative total growth of 20 %. The concentration of 5000 µg/ml was letal in this trial, whereas in another one of three trials without S9 3500 µg/ml was letal.
	Mutation assay	*E. coli* WP2 *uvr*A	Not reported	Negative	(Kuroda et al., 1984a)	Only abstract available. Methods, test concentrations and detailed results not reported.
	Cytotoxicity assay	Human alveolar tumour cells	0.5 mM	Negative	(Waters et al., 1982)	
	DNA damage assay	Human alveolar tumour cells	0.5 mM	Negative	(Waters et al., 1982)	
	DNA damage assay	Rat hepatocytes	10 mM	Negative	(Storer et al., 1996)	Cytotoxicity was reported at the highest concentration tested.
	DNA damage assay	*E. coli* P3478	50 µl/disc	Negative[1]	(Fluck et al., 1976)	
(Benzyl formate [09.077])	Rec assay	*B. subtilis* M45 (rec^-), H17 (rec^+)	20 µl/disc	Positive	(Yoo, 1986)	Study published in Japanese with English abstract. Data extracted from tables. Validity of the study cannot be evaluated. A weak positive result (i.e. 4 mm≤ D<8 mm).was reported (D=4 mm). No information on the use of metabolic activation.
	Mutation assay	*E. coli* WP2 *uvr*A	500 to 4000 µg/plate	Negative	(Yoo, 1986)	Study published in Japanese with English abstract. Data extracted from tables. Validity of the study cannot be evaluated. No information on the use of metabolic activation.
(Benzyl acetate [09.014])	Ames test (preincubation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537	10,000 µg/plate	Negative[1]	(Mortelmans et al., 1986)	
	Ames test (preincubation and plate incorporation method)	*S. typhimurium* TA98; TA100	5000 µg/plate	Negative[1]	(Schunk et al., 1986)	Cytotoxicity was observed at the three highest doses tested.
	Ames test	*S. typhimurium* TA98; TA100; TA1535; TA1537	3 µM/plate	Negative[1]	(Florin et al., 1980)	

Table 2.2: Summary of Genotoxicity Data (*in vitro)* EFSA / FGE.20Rev1

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
	Rec assay	*B. subtilis* M45 (rec$^-$), H17 (rec$^+$)	21 µg/disc	Negative	(Oda et al., 1979)	Study published in Japanese without English abstract. Data extracted from tables. Validity of the study cannot be evaluated.
	Rec assay	*B. subtilis* M45 (rec$^-$), H17 (rec$^+$)	20 µl/disc	Positive	(Yoo, 1986)	Study published in Japanese with English abstract. Data extracted from tables. Validity of the study cannot be evaluated. A weak positive result (i.e. 4≤ D<8) was reported (D could not clearly be determined). No information on the use of metabolic activation.
	Mutation assay	*E. coli* WP2 *uvr*A	250 to 2000 µg/plate	Negative	(Yoo, 1986)	Study published in Japanese with English abstract. Data extracted from tables. Validity of the study cannot be evaluated. No information on the use of metabolic activation.
	Mammalian cell gene mutation test	Mouse lymphoma L5178Y cells; Human lymphoblast TK6 cells	Mouse cells 0, 250, 500, 1000 µg/ml; Human cells 0, 500, 1000, 1250, 1500 µg/ml	Negative[2] Positive[3]	(Caspary et al., 1988)	Published non-GLP study in accordance with OECD guideline 476 (except that no colony sizing was performed). Thus, the study is considered not fully valid. The lowest significantly effective doses in the presence of S9 were 500 µg/ml in mouse cells and 1500 µg/ml in human cells. Cytotoxicity was reported above 500 µg/ml with and without S9.
	Mammalian cell gene mutation test	Mouse lymphoma L5178Y cells	0-1600 µl/ml (6 concentrations)	Positive[2]	(McGregor et al., 1988a)	Published summary report including detailed method and results from study on 72 compounds tested in various laboratories within the NTP. The study was not in accordance with OECD guideline 476 (no colony sizing performed, only in the absence of metabolic activation) and thus not considered valid. The lowest significantly effective doses was 900 µg/ml at which the relative total growth was 50 %. The highest dose was lethal. A positive response was observed in two of three experiments. No dose-response was observed.
	Mammalian cell gene 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 colonysizing was performed. As the results are insufficiently reported, their validity cannot be evaluated. In the presence of S9 metabolic activation one laboratory achieved

Table 2.2: Summary of Genotoxicity Data (*in vitro)* EFSA / FGE.20Rev1

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
						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 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., 1979)	Study published in Japanese without English abstract. Data extracted from tables. Validity of the study cannot be evaluated.
(Benzyl benzoate [09.727])	Ames test	*S. typhimurium* TA98; TA100; TA1535; TA1537	3 µM/plate	Negative[1]	(Florin et al., 1980)	
	Ames test (preincubation and plate incorporation method)	*S. typhimurium* TA98; TA100	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.
	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

Table 2.2: Summary of Genotoxicity Data (*in vitro)* EFSA / FGE.20Rev1

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
						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)	
	Ames test (plate incorporation method)	*S. typhimurium* TA98; TA1537; TA7001; TA7002; TA7003; TA7004; TA7006; Mix of TA7001–7006 TA7005	1000 µg/ml	Negative[1] Negative[2]; Positive[3]	(Gee et al., 1998)	
	Rec assay	*B. subtilis* M45 (rec$^-$), H17 (rec$^+$)	21 µg/disc	Negative	(Oda et al., 1979)	Study published in Japanese without English abstract. Data extracted from tables. Validity of the study cannot be evaluated.
	Rec assay	*B. subtilis* M45 (rec$^-$), H17 (rec$^+$)	Not reported	Negative[2] Positive[3]	(Matsui et al., 1989)	Published non-GLP study with insufficient report of some details of method and results. Thus, the validity of the study cannot be evaluated.
	Unscheduled DNA synthesis test	Rat hepatocytes	251 nl/ml	Negative	(Heck et al., 1989)	Published non-GLP study. Some important details of study design and results are not reported. Thus, the validity of the study cannot be evaluated.
	Mammalian cell gene mutation test	Mouse lymphoma L5178Y cells	12.5 to 800 nl/ml	Negative[2] Positive[3]	(Heck et al., 1989)	Published non-GLP study. Some important details of study design and results are not reported. Thus, the validity of the study cannot be evaluated. Different concentration ranges (12.5-800, 25-600, 400-600 nl/ml) were used in three independent experiments within which positive responses were observed. A 2.8 to 5.2-fold increase in mutant frequency was observed in the presence of S9.
	Mammalian cell gene mutation test	Mouse lymphoma L5178Y cells	0 to 800 µg/ml (6 concentrations)	Positive[2]	(McGregor et al., 1991)	Published summary report including detailed method and results from study on 27 compounds tested in various laboratories within the NTP in accordance with OECD guideline 476 (however, no colonysizing performed). Statistically significant increase in mutant fraction at the highest non-lethal

Table 2.2: Summary of Genotoxicity Data (*in vitro)* EFSA / FGE.20Rev1

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
						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. Polyploidisation (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).
	Sister chromatid exchange assay	Chinese hamster ovary cells	5-160 µg/ml[2]; 160-1600 µg/ml[3]	Positive[2] Positive[3]	(Galloway et al., 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

Table 2.2: Summary of Genotoxicity Data (*in vitro)* EFSA / FGE.20Rev1

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
						spontaneous SCEs at the concentrations used. Cytotoxicity was reported at the highest concentration tested.
	Sister chromatid exchange assay	Human lymphocytes	0-2 mM (0-212 µg/ml)	Positive[2]	(Jansson et al., 1988)	Published non-GLP study not in accordance with OECD guideline 479 (no metabolic activation). Insufficient report of important details of method and results. This study is not considered valid.
(Benzoic acid [08.021])	Ames test (plate incorporation method)	*S. typhimurium* TA98; TA100; TA1535; TA1538	2500 µg/plate	Negative[1]	(Anderson & 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)	

Table 2.2: Summary of Genotoxicity Data (*in vitro)* EFSA / FGE.20Rev1

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

Table 2.2: Summary of Genotoxicity Data (*in vitro*) EFSA / FGE.20Rev1

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
						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 antimutagenicity, but not genotoxic.
	Sister chromatid exchange assay	Chinese hamster ovary cells	Up to 333 µM (up to 50 µg/ml)	Negative[2]	(Sasaki et al., 1989)	Published non-GLP study of limited quality. Study designed to investigate the influence on spontaneous as well as on mitomycin-induced SCEs. The substance did not influence cell cycle (data not shown) and spontaneous SCEs at the concentrations used. Cytotoxicity was reported at the highest concentration tested.
(4-Hydroxybenzoic acid [08.040])	Ames test (plate incorporation method)	*S. typhimurium* TA98; TA100	5000 µg/plate	Negative[2]	(Mikulasova & 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	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

Table 2.2: Summary of Genotoxicity Data (*in vitro*) EFSA / FGE.20Rev1

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
						both at neutral and alkaline conditions.
	Mutation assay	*S. cerevisiae* rad18	Up to 0.1 mM (up to 13.8 µg/ml; 8 concentrations)	Positive	(Zetterberg, 1979)	Published non-GLP study with limited report of experimental details and result. Use of metabolic activation not reported. The validity of the study cannot be evaluated. The dose level tested was clearly cytotoxic. An increase in mutant frequency was not evident until 95-99 % of cells were killed.
Ethyl 4-hydroxybenzoate [09.367]	Ames test	*S. typhimurium* TA98; TA100	Not reported	Negative[1]	(Kawachi et al., 1980a)	Published summary report of unpublished extensive screening study. No details of method and results reported. Thus, the validity of the study cannot be evaluated.
	Rec assay	*B. subtilis*	Not reported	Negative[1]	(Kawachi et al., 1980a)	Published summary report of unpublished extensive screening study. No details of method and results reported. Thus, the validity of the study cannot be evaluated.
	Chromosomal aberration assay	Hamster lung fibroblast cells	Not reported	Positive[2] Negative[3]	(Kawachi et al., 1980a)	Published summary report of unpublished extensive screening study. No details of method and results reported. Thus, the validity of the study cannot be evaluated.
	Chromosomal aberration assay	Human embryo fibroblasts	Not reported	Negative[2]	(Kawachi et al., 1980a)	Published summary report of unpublished extensive screening study. No details of method and results reported. Thus, the validity of the study cannot be evaluated.
	Chromosomal aberration assay	Chinese hamster fibroblast cells	Up to 250 µg/ml	Positive	(Ishidate et al., 1978)	Published non-GLP study in Japanese with English summary and tabulated results. Some important details of method and results are not available. There is no information on the use of metabolic activation. The substance was tested up to the maximum dose tolerated. Thus, the validity of the study cannot be evaluated.
	Sister chromatid exchange assay	Human embryo fibroblasts	Not reported	Negative[2]	(Kawachi et al., 1980a)	Published summary report of unpublished extensive screening study. No details of method and results reported. Thus, the validity of the study cannot be evaluated.
	Sister chromatid exchange assay	Human fibroblastic cells HE2144	0, 83, 166 µg/ml	Negative[2]	(Sasaki et al., 1980)	Published non-GLP study not in accordance with OECD guideline 479 (no metabolic activation). Insufficient report of important details of method and results. This study is not considered valid.
	Mutation assay	Silk worms	Not reported	Negative	(Kawachi et al., 1980a)	Published summary report of unpublished extensive screening study. Unusual protocol, no details of method and results reported. Thus, the validity of the study cannot be evaluated.
(Butyl 4-hydroxybenzoate	Ames test	*S. typhimurium* TA98; TA100	1000 µg/plate	Negative[1]	(Haresaku et al., 1985)	

Table 2.2: Summary of Genotoxicity Data (*in vitro)* EFSA / FGE.20Rev1

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
[09.754])						
	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 polyploidisation.
	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 6.2-fold increase in the mutation frequency was observed both with and without S9.
	Ames test (plate incorporation method)	*S. typhimurium* TA98; TA100	5000 µg/plate	Negative[2]	(Mikulasova & Bohovicova, 2000)	
	DNA Repair test	*E. coli* WP2; WP2*uvrA*; CM611; CM561	2000 µg/ml	Negative	(Mikulasova & Bohovicova, 2000)	
	Unscheduled DNA synthesis test	Rat hepatocytes	100 µg/ml[4]	Negative	(Heck et al., 1989)	Published non-GLP study. No information concerning the number of concentrations tested.

Table 2.2: Summary of Genotoxicity Data (*in vitro)* EFSA / FGE.20Rev1

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
						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., 1979)	Study published in Japanese without English abstract. Data extracted from tables. Validity of the study cannot be evaluated. No information on the use of metabolic activation.
	Ames test	*S. typhimurium* TA102	5000 µg/plate	Negative[1]	(Müller et al., 1993)	
	Ames test	*S. typhimurium* TA 100	1000 µg/plate	Negative	(Rapson et al., 1980)	
	Mutation assay	Phage PM2	1362 µg/ml	Negative	(Becker et al., 1996)	
	Chromosomal aberration test	Chinese hamster fibroblast cells	500 µg/ml (three concentrations, max. concentration inducing 50% cell-growth inhibition)[4]	Negative[2]	(Ishidate et al., 1984)	Published study carried out only in the absence of metabolic activation. Thus, study is not considered valid. Cells were exposed for 24 and 48 hours. Negative response for chromosomal aberrations and polyploidisation.
	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

Table 2.2: Summary of Genotoxicity Data (*in vitro)* EFSA / FGE.20Rev1

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
						considered valid.
	Ames test	*S. typhimurium* TA102	5000 µg/plate	Negative[1]	(Jung et al., 1992)	Results confirmed at three separate contract laboratories.
	Sister chromatid exchange assay	Human lymphocytes	0-2 mM (0-273 µg/ml)	Positive[2]	(Jansson et al., 1988)	Published non-GLP study not in accordance with OECD guideline 479 (no metabolic activation). Insufficient report of important details of method and results. This study is not considered valid.
	Sister chromatid exchange assay	Chinese hamster ovary K1 cells	14 µg/ml	Negative	(Sasaki et al., 1987)	
	DNA alkaline unwinding assay	Mouse lymphoma L5178Y TK+/- cells	0, 4, 5, 6 mole/l (0, 544, 680, 816 µg/ml) 7, 8 mole/l (953, 1089 µg/ml)	Negative[2] Positive[2]	(Garberg et al., 1988)	Published study on 78 compounds not in accordance with standard guidelines. Test suitable for rapid screening only. Strand breaks or mutations observed only at cytotoxic concentrations.
2-Methoxybenzaldehyde [05.129]	Mutation assay	*E. coli* WP2*uvr*A, *trpE*	5000 µg/plate	Negative[2]	(Watanabe et al., 1989)	Published non-GLP study with limited report of experimental details and results. Study evaluating the enhancing effect on *N'*-nitro-*N*-nitrosoguanidine (MNNG)-induced mutagenesis in pretreated cells and not on the mutagenicity of the substance itself. Absence of an enhancing effect reported.
	Sister chromatid exchange 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)	Published non-GLP study not in accordance with OECD guideline 479 (no metabolic activation). Insufficient report of important details of method and results. This study is not considered valid.
	Mammalian cell gene mutation test	Mouse lymphoma L5178Y TK+/- cells	0 – 2.5 mM (0 – 340 µg/ml) 3 mM (408 µg/ml)	Negative[2] Positive[2]	(Wangenheim & 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

Table 2.2: Summary of Genotoxicity Data (*in vitro)* EFSA / FGE.20Rev1

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
						two different laboratories using different solvent. A negative response was observed in both laboratories with TA98, TA1535 and 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/plate 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	Positive[2] Positive[3]	(Yamaguchi, 1981)	Published non-GLP. Insufficient report of important details of method and results, thus the validity of the result cannot be evaluated.
	Ames test	*S. typhimurium* TA98; TA100	Not reported	Negative[1]	(Sugimura et al., 1976)	
	Chromosomal aberration test	Chinese hamster ovary cells	50 µg/ml	Positive[1]	(Stich et al., 1981c)	Published non-GLP study. Some important details of method and results are not reported. Thus, the validity of the study cannot be evaluated. Results are reported for one concentration only which was half the dose inducing mitotic inhibition. The clastogenic activity was reported to be reduced by the addition of S9.
	Chromosomal aberration test	Chinese hamster ovary K1 cells	up to 2 mM (up to 340 µg/ml)	Negative[1]	(Tayama & 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.

Table 2.2: Summary of Genotoxicity Data (*in vitro)* EFSA / FGE.20Rev1

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
						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., 1979)	Study published in Japanese without English abstract. Data extracted from tables. Validity of the study cannot be evaluated.
	Ames test (preincubation assay)	*S. typhimurium* TA97; TA98; TA100; TA1535; TA1537	10,000 µg/plate	Negative[1]	(Mortelmans et al., 1986)	
	Ames test	*S. typhimurium* TA98; TA100	0.05 to 1000 µg/plate	Negative[1]	(Kasamaki et al., 1982)	Published non-GLP study with insufficient report of some details of method and results. Thus, the validity of the study cannot be evaluated.
	Ames test	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	Not reported	Negative[1]	(Nagabhushan & 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)	
	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, 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	Negative[2]	(Ishidate et al., 1984)	Published study carried out only in the

Table 2.2: Summary of Genotoxicity Data (*in vitro*) EFSA / FGE.20Rev1

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
			concentrations, max. concentration inducing 50% cell-growth inhibition) [4]			absence of metabolic activation. Thus, study is not considered valid. Cells were exposed for 24 and 48 hours. Negative response for chromosomal aberrations and polyploidisation.
	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 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.
	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,

Table 2.2: Summary of Genotoxicity Data (*in vitro*) EFSA / FGE.20Rev1

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

Table 2.2: Summary of Genotoxicity Data (*in vitro*) EFSA / FGE.20Rev1

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
						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.
	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)	Published summary report of unpublished extensive screening study. No details of method and results reported. Thus, the validity of the study cannot be evaluated.
	Chromosomal aberration test	Chinese hamster fibroblasts	250 µg/ml[4] (three concentrations, max. concentration inducing 50% cell-growth inhibition)	Negative[2]	(Ishidate et al., 1984)	Published study carried out only in the absence of metabolic activation. Thus, study is not considered valid. Cells were exposed for 24 and 48 hours. Negative response for chromosomal aberrations and polyploidisation.
	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

Table 2.2: Summary of Genotoxicity Data (*in vitro)* EFSA / FGE.20Rev1

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
						presence of S9 mix obtained from rat, mouse and guinea pig.
	Rec assay	*B. subtilis* M45 (rec^-), H17 (rec^+)	23 µg/disc	Negative	(Oda et al., 1979)	Study published in Japanese without English abstract. Data extracted from tables. Validity of the study cannot be evaluated.
	Rec assay	*B. subtilis*	Not reported	Negative[1]	(Kawachi et al., 1980b; Kawachi et al., 1980a)	Published summary report of unpublished extensive screening study. No details of method and results reported. Thus, the validity of the study cannot be evaluated.
	Rec assay	*B. subtilis* M45 (rec^-), H17 (rec^+)	0 to 5000 µg/disc	Negative	(Kuboyama & Fujii, 1992)	Well conducted published non-GLP study with some minor deficiencies (no cytotoxicity data, no detailed data for different concentrations reported), however, of sufficient quality to be taken into account in the evaluation.
	Mutation assay	Silkworm	Not reported	Negative	(Kawachi et al., 1980b; Kawachi et al., 1980a)	Published summary report of unpublished extensive screening study. Unusual protocol, no details of method and results reported. Thus, the validity of the study cannot be evaluated.
	Chromosomal aberration test	Human embryo fibroblast cells	Not reported	Negative[2]	(Kawachi et al., 1980b; Kawachi et al., 1980a)	Published summary report of unpublished extensive screening study. Unusual protocol, no details of method and results reported. Thus, the validity of the study cannot be evaluated.
	Sister chromatid exchange assay	Human embryo fibroblast cells	Not reported	Negative[2]	(Kawachi et al., 1980b; Kawachi et al., 1980a)	Published summary report of unpublished extensive screening study. Unusual protocol, no details of method and results reported. Thus, the validity of the study cannot be evaluated.
(Butyl vanillyl ether [04.093])	Ames test	*S. typhimurium* TA98; TA100; TA1535; TA1537	5000 µg/plate	Negative[1]	(Watanabe & 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 method)	*S. typhimurium* TA97; TA98; TA100; TA1535; TA1537	8000 µg/plate	Negative[1]	(Mortelmans et al., 1986)	
	Ames test	*S. typhimurium* TA92; TA94; TA98; TA100; TA1535; TA1537; TA2637	Up to 10,000 µg/plate (six concentrations)	Negative[1]	(Ishidate et al., 1984)	Published study in accordance 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

Table 2.2: Summary of Genotoxicity Data (*in vitro)* EFSA / FGE.20Rev1

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
						important details of study design and results the validity of the study cannot be evaluated.
	Rec assay	*B. subtilis* M45 (rec^-), H17 (rec^+)	21 µg/disc	Negative	(Oda et al., 1979)	Study published in Japanese without English abstract. Data extracted from tables. Validity of the study cannot be evaluated.
	Chromosomal aberration test	Chinese hamster fibroblast cells	250 µg/ml (three concentrations, maximal concentration inducing 50% cell-growth inhibition) [4]	Positive[2]	(Ishidate et al., 1984)	Published study carried out only in the absence of metabolic activation. Thus, study is not considered valid. Polyploidisation in 48 % of cells reported at 48 hours. Negative response for chromosomal aberrations.
	Mammalian cell gene mutation test	Mouse lymphoma L5178Y cells	125-800 µg/ml	Negative[2] Weak positive[3]	(Heck et al., 1989)	Published non-GLP study. Some important details of study design and results are not reported. Thus, the validity of the study cannot be evaluated. Different concentration ranges (125-500 µg/ml, 600 µg/ml, 800 µg/ml) were used in three independent experiments within which positive responses were observed. In the presence of S9 a 2.1 to 3-fold increase in the mutant frequency was reported.
	Unscheduled DNA synthesis test	Rat hepatocytes	199 µg/ml[4]	Negative	(Heck et al., 1989)	Published non-GLP study. No information concerning the number of concentrations tested. Due to the lack of some important details of study design and results the validity of the study cannot be evaluated.
	Sister chromatid exchange assay	Human lymphocytes	0-2.0 mM (0-332 µg/ml)	Negative[2]	(Jansson et al., 1988)	Published non-GLP study not in accordance with OECD guideline 479 (no metabolic activation). Insufficient report of important details of method and results. This study is not considered valid.
	Sister chromatid exchange assay	Chinese hamster ovary K1 cells	17 µg/ml	Negative	(Sasaki et al., 1987)	
(Ethyl vanillin isobutyrate [09.933])	Ames test	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	5000 µg/plate	Negative[1]	(King & Harnasch, 1997)	
(Piperonyl acetate [09.220])	Ames test (preincubation method)	*S. typhimurium* TA97; TA98; TA100; TA1535; TA1537	3333 µg/plate	Negative[1]	(Mortelmans et al., 1986)	
	Ames test	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	3600 µg/plate	Negative[1]	(Wild et al., 1983)	
(Piperonal [05.016])	Ames test					
	Modified Ames test	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538 *E. coli* WP2*uvr*Atrp^-	0, 300, 600, 1200, 2400 µg/plate	Negative[1]	(Sekizawa & 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

Table 2.2: Summary of Genotoxicity Data (*in vitro)* EFSA / FGE.20Rev1

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
						more than 5 mg/plate. Due to the lack of some important details of study design and results the validity of the study cannot be evaluated.
	Ames test	*S. typhimurium* TA98; TA100	0.05 to 5000 µg/plate	Negative[1]	(Kasamaki et al., 1982)	Published non-GLP study with insufficient report of some details of method and results. Thus, the validity of the study cannot be evaluated.
	Ames test	*S. typhimurium* TA98; TA100; TA1537; TA1538	5000 µg/plate	Negative[1]	(White et al., 1977)	
	Ames test (preincubation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537	0, 10, 33, 100, 333, 1000 µg/plate	Negative[1]	(Haworth et al., 1983)	Published summary report including detailed results from studies on 250 compounds tested in various laboratories within the NTP to a large extent in accordance with OECD guideline 471.
	Rec assay	*B. subtilis* M45 (rec^-), H17 (rec^+)	20 µg/disc	Negative	(Oda et al., 1979)	Study published in Japanese without English abstract. Data extracted from tables. Validity of the study cannot be evaluated.
	Rec assay	*B. subtilis* M45 (rec^-), H17 (rec^+)	5000 µg/disc	Positive[2]	(Sekizawa & 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.
(Vanillin 3-(l-menthoxy)propane-1,2-diol acetal [02.248]) [5]	Ames test	*S. typhimurium* TA98; TA100; TA1535; TA1537	Up to 5000 µg/plate	Negative[1]	(Kajiura, 1996b)	

Table 2.2: Summary of Genotoxicity Data (*in vitro)* EFSA / FGE.20Rev1

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
	Mutation assay	*E. coli* WP2 uvrA	Up to 5000 µg/plate	Negative[1]	(Kajiura, 1996b)	

NR = not reported

[1] *With and without S9 metabolic activation.*

[2] *Without S9 metabolic activation.*

[3] *With S9 metabolic activation*

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

[5] *Related substance.*

Table 2.3: Genotoxicity (*in vivo*) EFSA / FGE.20Rev1

Substances in brackets are JECFA evaluated supporting substances in FGE.20Rev1

Table 2.3: Summary of Genotoxicity Data (*in vivo)* EFSA / FGE.20Rev1

Chemical Name [FL-no]	Test System	Test Object	Route	Dose	Result	Reference	Comments
(Benzyl alcohol [02.010])	*In vivo* Sex-linked recessive lethal mutations (SLRL)	*D. melanogaster*	Diet	5000 ppm	Negative	(Foureman et al., 1994)	
	In vivo SLRL	*D. melanogaster*	Injection	8000 ppm	Negative	(Foureman et al., 1994)	
	In vivo Micronucleus test	Mouse bone marrow cells	IP injection	200 mg/kg bw	Negative	(Hayashi et al., 1988)	
	In vivo Replicative DNA synthesis test	Mouse and rat hepatocytes	Not reported	Not reported	Negative	(Yoshikawa, 1996)	Screening test for the detection of non-genotoxic hepatocarcinogens. The substance was administered once at the maximum tolerated dose or at half the maximum tolerated dose to male mice and rats. Hepatocytes were prepared after 24, 39 and 48 hours.
	In vivo Replicative DNA synthesis test	Mouse hepatocytes	Oral gavage	800 mg/kg	Negative	(Miyagawa et al., 1995)	
	In vivo Replicative DNA synthesis test	Rat hepatocytes	Oral or SC injection	600 mg/kg	Negative	(Uno et al., 1994)	
(Benzyl acetate [09.014])	*In vivo* SLRL	*D. melanogaster*	Diet	300 ppm	Negative	(NTP, 1993d; Foureman et al., 1994)	
	In vivo SLRL	*D. melanogaster*	Injection	20,000 ppm	Negative	(NTP, 1993d; Foureman et al., 1994)	
	In vivo Sister chromatid exchange assay	Mouse bone marrow cells	IP injection	1700 mg/kg bw	Negative	(NTP, 1993d)	
	In vivo Chromosomal aberration test	Mouse bone marrow cells	IP injection	0 to 1700 mg/kg bw	Negative	(NTP, 1993d)	Test substance same batch as NTP chronic bioassays. The highest dose caused toxicity and cell cycle delay. Test not fully in compliance with the OECD guideline (insufficient cells per animal studied). GLP status not stated. The study is considered of limited validity.
	In vivo Micronucleus test	Mouse bone marrow cells	3 IP injection with 24 h intervals	0, 312, 625 and 1250 mg/kg bw	Negative	(NTP, 1993d; Shelby et al., 1993)	Test substance same batch as NTP chronic bioassays. Study in compliance with OECD guideline. GLP not stated. Micronuclei were determiend at 24 hours after the last dose. A dose-related decrease in PCE/NCE ratio was observed. The study is considered valid.
	In vivo Micronucleus test	Mouse erythrocytes	Dietary exposure for 13 weeks.	0 to 50,000 ppm (equal to 0 to 7900 mg/kg bw/day for males and 0 to 9400 mg/kg bw/day for females)	Negative	(NTP, 1993d)	Test substance same batch as NTP chronic bioassays. In life phase under GLP; for determination of genotoxic effects. GLP not specified. Test in compliance with OECD guideline. The test is considered valid, but of limited relevance because no 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.
	In vivo Unscheduled DNA	Rat pancreatic cells	Oral gavage	1000 mg/kg bw	Negative	(Steinmetz & Mirsalis, 1984)	Only abstract available. Non guideline test. Validity

Table 2.3: Summary of Genotoxicity Data (*in vivo)* EFSA / FGE.20Rev1

Chemical Name [FL-no]	Test System	Test Object	Route	Dose	Result	Reference	Comments
	synthesis test						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 monitored at 1 hours 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 hours no significant effect in mice, significant effects in rat only in lung and kidney. The study is of limited validity.
(Benzaldehyde [05.013])	*In vivo* SLRL	*D. melanogaster*	Diet	1150 ppm	Negative	(Woodruff et al., 1985)	
	In vivo SLRL	*D. melanogaster*	Injection	2500 ppm	Negative	(Woodruff et al., 1985)	
(Salicylic acid [08.112])	*In vivo* Chromosomal aberration assay	Mouse bone marrow cells	I.P. 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	I.P. 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 summarised results of a large screening study reported. Thus, the validity of the study cannot be evaluated.
(Vanillin [05.018])	*In vivo* Micronucleus test	Male 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,

Table 2.3: Summary of Genotoxicity Data (*in vivo)* EFSA / FGE.20Rev1

Chemical Name [FL-no]	Test System	Test Object	Route	Dose	Result	Reference	Comments
							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 hours).
(Salicylaldehyde [05.055])	*In vivo* Spot test	*D. melanogaster* BINSC *D. melanogaster* Oregon-R	NR	1.05 to 1.40 ppm 0.09 to 0.35 ppm	Negative Negative	(Kono et al., 1995)	Study published in Japanese with English abstract. Data extracted from tables. Validity of the study cannot be evaluated
(Ethyl vanillin [05.019])	*In vivo* Basc test	*D. melanogaster*	NR	8309 µg/ml	Negative	(Wild et al., 1983)	Published non-GLP study. Details of study protocol reported elsewhere. However, results sufficiently reported. Study is considered valid.
	In vivo Micronucleus test	Male BDF1 mice	IP injection	Not reported	Negative	(Furukawa et al., 1989)	Only abstract available. Insufficient report of experimental details and results to evaluate the validity of the study.
	In vivo Micronucleus test	NMRI mice	NR	1000 mg/kg bw	Negative	(Wild et al., 1983)	Published non-GLP study. Details of study protocol and results insufficiently reported. Effect on PCE/NCE ratio not reported. No positive control. Validity of the study cannot be evaluated.
(Piperonyl acetate [09.220])	*In vivo* Basc test	*D. melanogaster*	NR	4855 µg/ml	Negative	(Wild et al., 1983)	Published non-GLP study. Details of study protocol reported elsewhere,. However, results sufficiently reported. Study is considered valid.
	In vivo Micronucleus test	NMRI mice	NR	970 mg/kg bw	Negative	(Wild et al., 1983)	Published non-GLP study. Details of study protocol and results insufficiently reported. Effect on PCE/NCE ratio not reported. No positive control. Validity of the study cannot be evaluated.
(Piperonal [05.016])	*In vivo* Dominant lethal assay	ICR/Ha Swiss mice	IP injection	0, 124, 620 mg/kg bw	Negative	(Epstein et al., 1972)	Published non-GLP study evaluating 174 substances. Study protocol not fully in accordance with OECD guideline 478 (lower number of animals and of dose levels used, limited report of experimental observations). However, due to the large body of control data available the results are considered valid. Doses were selected in preliminary acute toxicity tests. Parameters recorded were percent pregnancy, total implants and early and late fetal deaths.
	In vivo Dominant lethal assay	ICR/Ha Swiss mice	Oral gavage	0, 1000 mg/kg bw (repeated doses on 5 successive days)	Negative	(Epstein et al., 1972)	Published non-GLP study evaluating 174 substances. Study protocol not fully in accordance with OECD guideline 478 (lower number of animals and of dose levels used, limited report of experimental observations). However, due to the large body of control data available the results are considered valid. Doses were selected in preliminary acute toxicity tests. Parameters recorded were percent pregnancy, total implants and early and late fetal deaths.

Table 2.4: Genotoxicity (*in vivo*) EFSA / FGE.202

Table 2.4: GENOTOXICITY (*in vitro*)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Reported Result	Reference	Comments
Citral [05.020]	Reverse mutation	*S. typhimurium* TA98, TA100, TA97a, TA102	5–700 µg/plate	Negative[a]	(Gomes-Carneiro et al., 1998)	Valid. Published non-GLP study containing sufficient details. Result is considered as valid.
	Reverse mutation	*S. typhimurium* TA92, TA94, TA98, TA100, TA1535, TA1537	Up to 100 µg/plate	Negative[b]	(Ishidate et al., 1984)	Valid. According to current guidelines.The study is considered valid.
	Reverse mutation	*S. typhimurium* TA100	NR	Negative[a]	(Lutz et al., 1982)	Validity cannot be evaluated. One strain only, Concentrations tested not specified. no re-run of the test; no other data on experimental results or design apart from a description of the test method.
	Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537	1–160 µg/plate	Negative[a]	(Zeiger et al., 1987) (NTP, 2003e)	Valid. Standard NTP study carried out according to US.EPA guidelines; result is considered valid.
	Mutation	E. coli WP2uvrA (trp -)	13–100 µg/plate	Negative	(Yoo, 1986)	Validity cannot be evaluated (study in Japanese).
	Sister chromatid exchange	Chinese hamster ovary cells	0.289–40.2 µg/ml	Positive[a]	(NTP, 2003e)	Valid. Standard NTP study carried out according to US EPA guidelines; result is considered valid.
	Chromosomal aberration	Chinese hamster ovary cells	12.5–60.6 µg/ml	Negative[a]	(NTP, 2003e)	Valid. Standard NTP study carried out according to US.EPA guidelines; result is considered valid.
	Chromosomal aberration	Chinese hamster fibroblast cells	Up to 30 µg/ml	Negative[c]	(Ishidate et al., 1984)	Limited validity (performed only in the presence of metabolic activation).
	Rec assay	*B. subtilis* M45 and H17	17 µg/disk	Negative	(Oda et al., 1979)	The test system used is considered inappropriate; insufficient validity.
	Rec assay	*B. subtilis* M45 and H17	0.16, 0.32, 0.63 µl/disk (142, 284, 560 µg/disk)[d] 1.25, 2.5 µl/disk (1110, 2220 µg/disk)[d]	Negative Positive	(Kuroda et al., 1984a)	Validity cannot be evaluated. Article in Japanese; with limited information in tables and abstract. Assay of limited relevance.
	Rec assay	*B. subtilis* M45 and H17	<2.5 µl/disk (<2220 µg/disk)	Positive	(Yoo, 1986)	Validity cannot be evaluated (study in Japanese). Study of limited relevance.
	Induction of tumour suppressor protein p53 (DNA damage)	Mouse fibroblast cells (NTCT 929)	10–30 µg/ml	Positive	(Duerksen-Hughes et al., 1999)	The Induction of tumor suppressor protein p53 may be considered as indicator for genotoxicity. Result is considered valid, however, it has only limited relevance.
3-methyl-2-butenal [05.124]	Ames test (preincubation)	*S. typhimurium* TA98, TA100		Positive[a]	(BASF, 1991b)	Valid. Modified Ames test: Unpublished non-GLP study, carried out in accordance with the OECD guideline no 471. The study contains sufficient details and is considered valid.

NR not reported.

[a] *With and without metabolic activation.*

[b] *With metabolic activation.*

[c] *Without metabolic activation.*

[d] *Calculated using a density of 0.888 (Merck, 1997).*

e) Validity of genotoxicity studies:

1. *Valid.*
2. *Limited validity (e.g. if certain aspects are not in accordance with OECD guidelines or current standards and / or limited documentation).*
3. *Insufficient validity (e.g. if main aspects are not in accordance with any recognised guidelines (e.g. OECD) or current standards inappropriate / not validated test system).*
4. *Validity cannot be evaluated (e.g. insufficient documentation, short abstract only, too little experimental details provided).*

Table 2.5: Genotoxicity (*in vivo*) EFSA / FGE.202

Table 2.5: GENOTOXICITY (*in vivo*)

Chemical Name [FL-no]	Test System	Test Object	Route	Dose	Result	Reference	Comments
Citral [05.020]	Micronucleus formation	Mouse bone marrow erythrocytes	Three intraperitoneal injections given at 24-h intervals; male mice only	250, 500, or 750 mg/kg bw	Negative	(NTP, 2003e)	NTP study carried out according to US-EPA guideline. Result is considered as valid.
	Micronucleus formation	Mouse peripheral blood erythrocytes	Microencapsulated citral was administered in the diet for 14 weeks	745, 1840, 3915, or 8110 mg/kg bw per day (males) 790, 1820, 3870, or 7550 mg/kg bw per day (females)	Negative Negative	(NTP, 2003e)	NTP study carried out according to a non-standard guideline; result is considered of limited validity.
3-methyl-2-butenal [05.124]	UDS	Rat hepatocytes	Oral administration	350 and 700 mg/kg body weight	Negative	(BASF, 2001)	Unpublished GLP study, carried out in accordance with OECD guideline no 486. The study is considered valid.
	Micronucleus test	Mouse bone marrow erythrocytes	Oral administration	175, 350 and 750 mg/kg body weight	Negative	(BASF, 1992c)	Unpublished GLP study, carried out in accordance with OECD guideline (1991). The study is considered valid.

Validity of genotoxicity studies:

1. *Valid.*
2. *Limited validity (e.g. if certain aspects are not in accordance with OECD guidelines or current standards and / or limited documentation).*
3. *Insufficient validity (e.g. if main aspects are not in accordance with any recognised guidelines (e.g. OECD) or current standards inappropriate / not validated test system).*
4. *Validity cannot be evaluated (e.g. insufficient documentation, short abstract only, too little experimental details provided).*

TABLE 3: SUMMARY OF SAFETY EVALUATION TABLES

Table 3.1: Summary of Safety Evaluation of 37 Benzyl Derivatives (JECFA, 2002a)

Table 3.1: Summary of Safety Evaluation of 36 JECFA Evaluated Benzyl Derivatives (JECFA, 2002a)

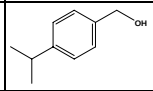
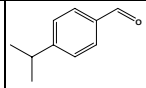
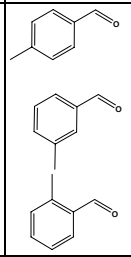
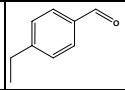
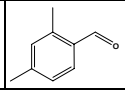
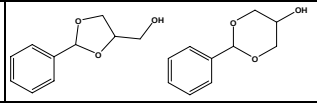
FL-no JECFA-no	EU Register name	Structural formula	EU MSDI 1) US MSDI (µg/*capita*/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [(4) or 5)]	EFSA conclusion on the named compound (Procedure steps, intake estimates, NOAEL, genotoxicity)	EFSA conclusion on the material of commerce
02.039 864	4-Isopropylbenzyl alcohol		0.24 0.3	Class I A3: Intake below threshold	4)	No safety concern at the estimated level of intake as flavouring substance based on the MSDI approach.	No safety concern at the estimated level of intake as flavouring substance based on the MSDI approach.
05.022 868	4-Isopropylbenzaldehyde		110 1	Class I A3: Intake below threshold	4)	No safety concern at the estimated level of intake as flavouring substance based on the MSDI approach.	No safety concern at the estimated level of intake as flavouring substance based on the MSDI approach.
05.027 866	Tolualdehyde		230 1100	Class I A3: Intake below threshold	4)	No safety concern at the estimated level of intake as flavouring substance based on the MSDI approach.	Composition of mixture to be specified.
05.068 865	4-Ethylbenzaldehyde		0.37 6	Class I A3: Intake below threshold	4)	No safety concern at the estimated level of intake as flavouring substance based on the MSDI approach.	No safety concern at the estimated level of intake as flavouring substance based on the MSDI approach.
05.110 869	2,4-Dimethylbenzaldehyde		0.37 0.1	Class I A3: Intake below threshold	4)	No safety concern at the estimated level of intake as flavouring substance based on the MSDI approach.	No safety concern at the estimated level of intake as flavouring substance based on the MSDI approach.
06.002 838	5-Hydroxy-2-phenyl-1,3-dioxane		13 300	Class I A3: Intake below threshold	4)	No safety concern at the estimated level of intake as flavouring substance based on the MSDI approach.	Stereoisomeric composition and composition of mixture to be specified.

Table 3.1: Summary of Safety Evaluation of 36 JECFA Evaluated Benzyl Derivatives (JECFA, 2002a)

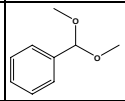
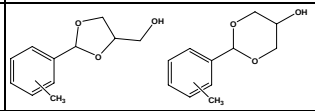
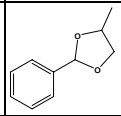
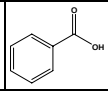
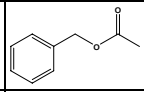
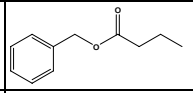
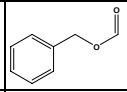
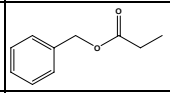
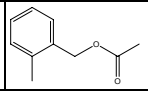
FL-no JECFA-no	EU Register name	Structural formula	EU MSDI 1) US MSDI (µg/*capita*/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [(4) or 5)]	EFSA conclusion on the named compound (Procedure steps, intake estimates, NOAEL, genotoxicity)	EFSA conclusion on the material of commerce
06.003 837	alpha,alpha-Dimethoxytoluene		0.12 0.3	Class I A3: Intake below threshold	4)	No safety concern at the estimated level of intake as flavouring substance based on the MSDI approach.	No safety concern at the estimated level of intake as flavouring substance based on the MSDI approach.
06.012 867	Tolualdehyde glyceryl acetal		0.012 1	Class I A3: Intake below threshold	4)	No safety concern at the estimated level of intake as flavouring substance based on the MSDI approach.	Stereoisomeric composition and composition of mixture to be specified.
06.032 839	4-Methyl-2-phenyl-1,3-dioxolane		0.037 110	Class I A3: Intake below threshold	4)	No safety concern at the estimated level of intake as flavouring substance based on the MSDI approach.	No safety concern at the estimated level of intake as flavouring substance based on the MSDI approach.
08.021 850	Benzoic acid		34 340	Class I A3: Intake below threshold	4)	No safety concern at the estimated level of intake as flavouring substance based on the MSDI approach.	No safety concern at the estimated level of intake as flavouring substance based on the MSDI approach.
09.014 23	Benzyl acetate		1200 850	Class I A3: Intake below threshold	4)	No safety concern at the estimated level of intake as flavouring substance based on the MSDI approach.	No safety concern at the estimated level of intake as flavouring substance based on the MSDI approach.
09.051 843	Benzyl butyrate		100 290	Class I A3: Intake below threshold	4)	No safety concern at the estimated level of intake as flavouring substance based on the MSDI approach.	No safety concern at the estimated level of intake as flavouring substance based on the MSDI approach.
09.077 841	Benzyl formate		35 17000	Class I A3: Intake below threshold	4)	No safety concern at the estimated level of intake as flavouring substance based on the MSDI approach.	No safety concern at the estimated level of intake as flavouring substance based on the MSDI approach.
09.132 842	Benzyl propionate		41 99	Class I A3: Intake below threshold	4)	No safety concern at the estimated level of intake as flavouring substance based on the MSDI approach.	No safety concern at the estimated level of intake as flavouring substance based on the MSDI approach.
09.294 863	2-Methylbenzyl acetate		ND 3	Class I A3: Intake below threshold	4)	MSDI based on USA production figure.	Composition of mixture to be specified. MSDI based on USA production figure.

Table 3.1: Summary of Safety Evaluation of 36 JECFA Evaluated Benzyl Derivatives (JECFA, 2002a)

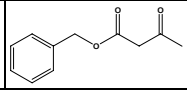
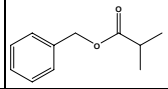
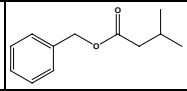
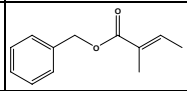
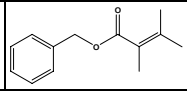
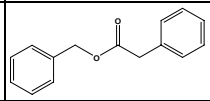
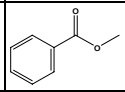
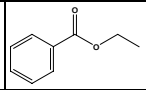
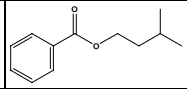
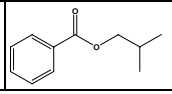
FL-no JECFA-no	EU Register name	Structural formula	EU MSDI 1) US MSDI (µg/*capita*/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [(4) or 5)]	EFSA conclusion on the named compound (Procedure steps, intake estimates, NOAEL, genotoxicity)	EFSA conclusion on the material of commerce
09.406 848	Benzyl 3-oxobutyrate		0.24 0.07	Class I A3: Intake below threshold	4)	No safety concern at the estimated level of intake as flavouring substance based on the MSDI approach.	No safety concern at the estimated level of intake as flavouring substance based on the MSDI approach.
09.426 844	Benzyl isobutyrate		13 21	Class I A3: Intake below threshold	4)	No safety concern at the estimated level of intake as flavouring substance based on the MSDI approach.	No safety concern at the estimated level of intake as flavouring substance based on the MSDI approach.
09.458 845	Benzyl isovalerate		12 19	Class I A3: Intake below threshold	4)	No safety concern at the estimated level of intake as flavouring substance based on the MSDI approach.	No safety concern at the estimated level of intake as flavouring substance based on the MSDI approach.
09.494 846	Benzyl 2-methylcrotonate		0.012 0.03	Class I A3: Intake below threshold	4)	No safety concern at the estimated level of intake as flavouring substance based on the MSDI approach.	No safety concern at the estimated level of intake as flavouring substance based on the MSDI approach.
09.508 847	Benzyl 2,3-dimethylcrotonate		0.012 1	Class I A3: Intake below threshold	4)	No safety concern at the estimated level of intake as flavouring substance based on the MSDI approach.	No safety concern at the estimated level of intake as flavouring substance based on the MSDI approach.
09.705 849	Benzyl phenylacetate		4.3 57	Class I A3: Intake below threshold	4)	No safety concern at the estimated level of intake as flavouring substance based on the MSDI approach.	No safety concern at the estimated level of intake as flavouring substance based on the MSDI approach.
09.725 851	Methyl benzoate		40 230	Class I A3: Intake below threshold	4)	No safety concern at the estimated level of intake as flavouring substance based on the MSDI approach.	No safety concern at the estimated level of intake as flavouring substance based on the MSDI approach.
09.726 852	Ethyl benzoate		96 110	Class I A3: Intake below threshold	4)	No safety concern at the estimated level of intake as flavouring substance based on the MSDI approach.	No safety concern at the estimated level of intake as flavouring substance based on the MSDI approach.
09.755 857	Isopentyl benzoate		96 33	Class I A3: Intake below threshold	4)	No safety concern at the estimated level of intake as flavouring substance based on the MSDI approach.	Composition of mixture to be specified.
09.757 856	Isobutyl benzoate		0.37 1	Class I A3: Intake below threshold	4)	No safety concern at the estimated level of intake as flavouring substance based on the MSDI approach.	No safety concern at the estimated level of intake as flavouring substance based on the MSDI approach.

Table 3.1: Summary of Safety Evaluation of 36 JECFA Evaluated Benzyl Derivatives (JECFA, 2002a)

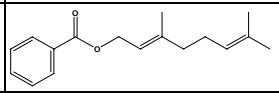
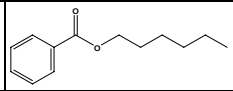
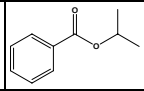
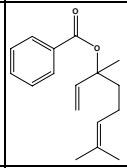
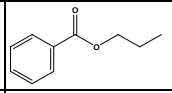
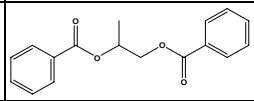
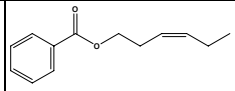
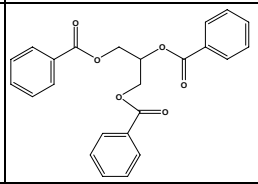
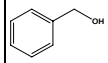
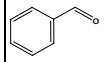
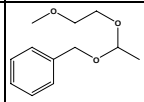
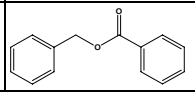
FL-no JECFA-no	EU Register name	Structural formula	EU MSDI 1) US MSDI (µg/*capita*/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [(4) or 5)]	EFSA conclusion on the named compound (Procedure steps, intake estimates, NOAEL, genotoxicity)	EFSA conclusion on the material of commerce
09.767 860	Geranyl benzoate		3.4 0.03	Class I A3: Intake below threshold	4)	No safety concern at the estimated level of intake as flavouring substance based on the MSDI approach.	No safety concern at the estimated level of intake as flavouring substance based on the MSDI approach.
09.768 854	Hexyl benzoate		320 1	Class I A3: Intake below threshold	4)	No safety concern at the estimated level of intake as flavouring substance based on the MSDI approach.	No safety concern at the estimated level of intake as flavouring substance based on the MSDI approach.
09.770 855	Isopropyl benzoate		0.0037 0.3	Class I A3: Intake below threshold	4)	No safety concern at the estimated level of intake as flavouring substance based on the MSDI approach.	No safety concern at the estimated level of intake as flavouring substance based on the MSDI approach.
09.771 859	Linalyl benzoate		8.4 2	Class I A3: Intake below threshold	4)	No safety concern at the estimated level of intake as flavouring substance based on the MSDI approach.	No safety concern at the estimated level of intake as flavouring substance based on the MSDI approach.
09.776 853	Propyl benzoate		0.012 0.3	Class I A3: Intake below threshold	4)	No safety concern at the estimated level of intake as flavouring substance based on the MSDI approach.	No safety concern at the estimated level of intake as flavouring substance based on the MSDI approach.
09.803 862	Propylene glycol dibenzoate		ND 14	Class I A3: Intake below threshold	4)	MSDI based on USA production figure.	MSDI based on USA production figure.
09.806 858	Hex-3-enyl benzoate	 (Z)-isomer shown	6.7 0.1	Class I A3: Intake below threshold	4)	No safety concern at the estimated level of intake as flavouring substance based on the MSDI approach.	Stereoisomeric composition and composition of mixture to be specified.

Table 3.1: Summary of Safety Evaluation of 36 JECFA Evaluated Benzyl Derivatives (JECFA, 2002a)

FL-no JECFA-no	EU Register name	Structural formula	EU MSDI 1) US MSDI (µg/*capita*/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	EFSA conclusion on the named compound (Procedure steps, intake estimates, NOAEL, genotoxicity)	EFSA conclusion on the material of commerce
09.812 861	Glyceryl tribenzoate		ND 49	Class I A3: Intake below threshold	4)	MSDI based on USA production figure.	MSDI based on USA production figure.
02.010 25	Benzyl alcohol		13000 37000	Class I A3: Intake above threshold, A4: Endogenous	4)	No safety concern at the estimated level of intake as flavouring substance based on the MSDI approach.	No safety concern at the estimated level of intake as flavouring substance based on the MSDI approach.
05.013 22	Benzaldehyde		7900 36000	Class I A3: Intake above threshold, A4: Endogenous	4)	No safety concern at the estimated level of intake as flavouring substance based on the MSDI approach.	No safety concern at the estimated level of intake as flavouring substance based on the MSDI approach.
06.019 840	1-Benzyloxy-1-(2-methoxyethoxy)ethane		ND 1	Class I B3: Intake below threshold, B4: Adequate NOAEL exists	4)	MSDI based on USA production figure.	Composition of mixture to be specified. MSDI based on USA production figure.
09.727 24	Benzyl benzoate		1600 4200	Class I A3: Intake above threshold, A4: Endogenous	4)	No safety concern at the estimated level of intake as flavouring substance based on the MSDI approach.	No safety concern at the estimated level of intake as flavouring substance based on the MSDI approach.

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

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

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

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

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

ND No data available.

Table 3.2: Summary of Safety Evaluation Applying the Procedure (EFSA / FGE.20Rev1)

Table 3.2: Summary of Safety Evaluation Applying the Procedure (Based on Intakes Calculated by the MSDI Approach)

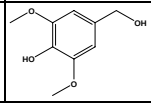
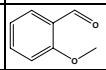
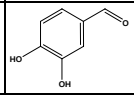
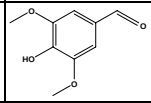
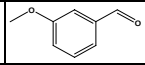
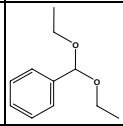
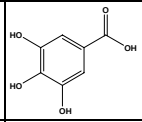
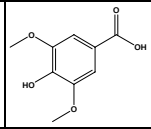
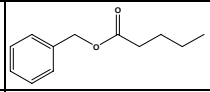
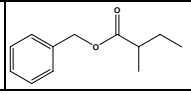
FL-no	EU Register name	Structural formula	MSDI 1) (µg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
02.164	4-Hydroxy-3,5-dimethoxybenzyl alcohol		0.037	Class I A3: Intake below threshold	4)	6)	
05.129	2-Methoxybenzaldehyde		0.16	Class I A3: Intake below threshold	4)	6)	
05.142	3,4-Dihydroxybenzaldehyde		8.5	Class I A3: Intake below threshold	4)	6)	
05.153 1878	4-Hydroxy-3,5-dimethoxybenzaldehyde		0.74	Class I A3: Intake below threshold	4)	6)	
05.158	3-Methoxybenzaldehyde		0.011	Class I A3: Intake below threshold	4)	6)	
06.017	(Diethoxymethyl)benzene		1.7	Class I A3: Intake below threshold	4)	6)	
08.080	Gallic acid		0.011	Class I A3: Intake below threshold	4)	6)	
08.087	4-Hydroxy-3,5-dimethoxybenzoic acid		1.2	Class I A3: Intake below threshold	4)	6)	
09.152	Benzyl valerate		1.7	Class I A3: Intake below threshold	4)	6)	
09.313	Benzyl 2-methylbutyrate		7.3	Class I A3: Intake below threshold	4)	6)	

Table 3.2: Summary of Safety Evaluation Applying the Procedure (Based on Intakes Calculated by the MSDI Approach)

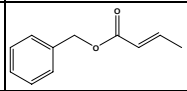
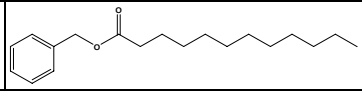
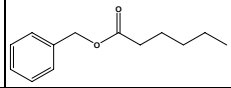
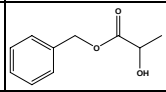
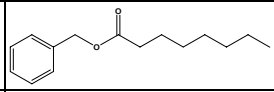
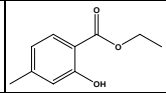
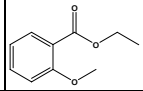
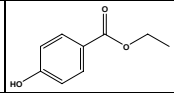
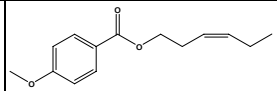
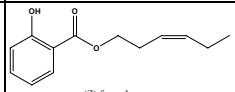
FL-no	EU Register name	Structural formula	MSDI 1) (µg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
09.314	Benzyl crotonate		0.37	Class I A3: Intake below threshold	4)	6)	
09.315	Benzyl dodecanoate		0.13	Class I A3: Intake below threshold	4)	6)	
09.316	Benzyl hexanoate		0.75	Class I A3: Intake below threshold	4)	6)	
09.317	Benzyl lactate		0.91	Class I A3: Intake below threshold	4)	6)	
09.318	Benzyl octanoate		0.12	Class I A3: Intake below threshold	4)	6)	
09.362	Ethyl 2-hydroxy-4-methylbenzoate		0.0012	Class I A3: Intake below threshold	4)	6)	
09.363	Ethyl 2-methoxybenzoate		5.5	Class I A3: Intake below threshold	4)	6)	
09.367	Ethyl 4-hydroxybenzoate		10	Class I A3: Intake below threshold	4)	6)	
09.560	Hex-3(cis)-enyl anisate		0.12	Class I A3: Intake below threshold	4)	6)	
09.570	Hex-3-enyl salicylate	 (Z)-form shown	0.13	Class I A3: Intake below threshold	4)	7)	

Table 3.2: Summary of Safety Evaluation Applying the Procedure (Based on Intakes Calculated by the MSDI Approach)

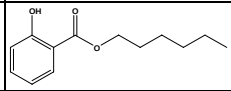
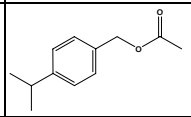
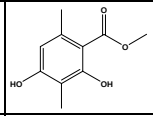
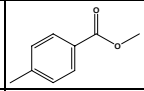
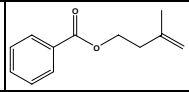
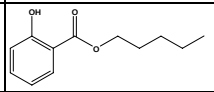
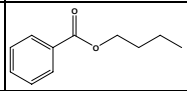
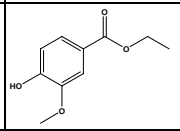
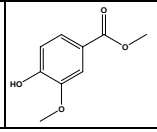
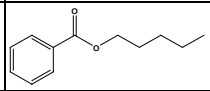
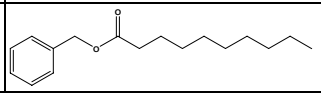
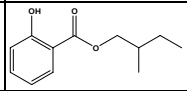
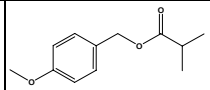
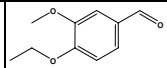
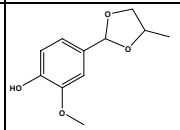
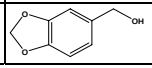
FL-no	EU Register name	Structural formula	MSDI 1) (µg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
09.581	Hexyl salicylate		0.018	Class I A3: Intake below threshold	4)	6)	
09.611	4-Isopropylbenzyl 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)	
09.799	Methyl vanillate		0.011	Class I A3: Intake below threshold	4)	6)	

Table 3.2: 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
09.825	Pentyl benzoate		1.1	Class I A3: Intake below threshold	4)	6)	
09.835	Benzyl decanoate		0.35	Class I A3: Intake below threshold	4)	6)	
09.852	2-Methylbutyl 2-hydroxybenzoate		0.011	Class I A3: Intake below threshold	4)	6)	
09.895	4-Methoxybenzyl-2-methylpropionate		0.37	Class I A3: Intake below threshold	4)	6)	
05.066	4-Ethoxy-3-methoxybenzaldehyde		1.2	Class II A3: Intake below threshold	4)	6)	
06.104 1882	Vanillin propylene glycol acetal		100	Class II A3: Intake below threshold	4)	7)	
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.

REFERENCES:

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.

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.

BASF, 1991b. Report on the study of 3-methylbuten-2-al-1 (ZST test substance no.: 88/363) in the liquid suspension assay, modified salmonella/mammalian-microsome mutagenicity test (ames preincubation test). Engelhardt, G. Project no. 41M0363/884496. September 23 1991. Unpublished report submitted by EFFA to FLAVIS Secretariat.

BASF, 1992c. Cytogenetic study *in vivo* of 3-methylbuten-2-al-1 in mice, micronucleus test,, single oral administration. Engelhardt, G. Project no. 26M0734/904515. February 19 1992. Unpublished report submitted by EFFA to FLAVIS Secretariat.

BASF, 2001. In vivo unscheduled DNA synthesis (UDS) assay with 3-methyl-2-butenal in rat hepatocytes, single oral administration. Engelhardt, G. Project no. 80M0680/004115. August 28, 2001. Unpublished report submitted by EFFA to FLAVIS Secretariat.

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.

Benigni, R., Netzeva, T., 2007a. Report on a QSAR model for prediction of genotoxicity of alpha,beta-unsaturated aldehydes in *S. typhimurium* TA 100 and its application for predictions on alpha,beta-unsaturated aldehydes in Flavouring Group Evaluation 19 (FGE.19). Unpublished report submitted by FLAVIS Secretariat to EFSA.

Benigni, R., Netzeva, T., 2007b. Report on a QSAR model for prediction of genotoxicity of alpha,beta-unsaturated ketones in *S. typhimurium* TA 100 and its application for predictions on alpha,beta-unsaturated aldehydes in Flavouring Group Evaluation 19 (FGE.19). Unpublished report submitted by FLAVIS Secretariat to EFSA.

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.

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.

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.

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.

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.

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.

Duerksen-Hughes, P.J., Yang, J., Ozcan, O., 1999. p53 induction as a genotoxic test for twenty-five chemicals undergoing *in vivo* carcinogenicity testing. Environ. Health Perspect. 107(10), 805-812.

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, 2000a. 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 180, 8-16.

EC, 2009a. Commission Decision 2009/163/EC of 26 February 2009 amending Decision 1999/217/EC as regards the register of flavouring substances used in or on foodstuffs. Official Journal of the European Union 27.2.2009, L 55, 41.

EFSA, 2008al. Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in contact with food on a request from the Commission related to Flavouring Group Evaluation 54: Consideration of benzyl derivatives evaluated by JECFA (57th meeting) structurally related to benzyl alcohols, benzaldehydes, a related acetal, benzoic acids and related esters evaluated by EFSA in FGE.20 (2005) (Commission Regulation (EC) No 1565/2000 of 18 July 2000). Adopted on 16 May 2008. EFSA-Q-2008-032E.

EFSA, 2008b. Minutes of the 26th Plenary meeting of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food, Held in Parma on 27 - 29 November 2007. Parma, 7 January 2008. [Online]. Available: http://www.efsa.europa.eu/EFSA/Event_Meeting/afc_minutes_26thplen_en.pdf

EFSA, 2008g. Opinion of the Scientific Panel on contact Materials, Enzymes, Flavourings and Processing Aids on a request from the Commission related to Flavouring Group Evaluation 202: 3-alkylated aliphatic acyclic alpha,beta-unsaturated aldehydes and precursors with and without additional double-bonds from chemical subgroup 1.1.3 of FGE.19. (Commission Regulation (EC) No 1565/2000 of 18 July 2000). Adopted on September 2008.

EFSA, 2009k. Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in contact with food on a request from the Commission related to Flavouring Group Evaluation 20, Revision 1: Benzyl alcohols, benzalaldehydes, a related acetal, benzoic acids and related esters from chemical group 23 (Commission Regulation (EC) No 1565/2000 of 18 July 2000). Adopted on 29 November 2007. EFSA-Q-2003-168B.

Engelhardt, G., 1986. Ames test (standard plate test with *Salmonella typhimurium* TA 1537) (Jan. 6, 1987) (Final 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.

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.

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.

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., Sumi, C., Sasaki, M., 1992. [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

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.

Gomes-Carneiro, M.R., Felzenszwalb, I., Paumgartten, F.J., 1998. Mutagenicity testing (+/-)-camphor, 1,8-cineole, citral, citronellal, (-)-menthol and terpineol with the Salmonella/microsome assay. Mutat. Res. 416, 129-136.

Gry, J., Beltoft, V., Benigni, R., Binderup, M.-L., Carere, A., Engel, K.-H., Gürtler, R., Jensen, G.E., Hulzebos, E., Larsen, J.C., Mennes, W., Netzeva, T., Niemelä, J., Nikolov, N., Nørby, K.K., Wedebye, E.B., 2007. Description and validation of QSAR genotoxicity models for use in evaluation of flavouring substances in Flavouring Group Evaluation 19 (FGE.19) on 360 alpha,beta-unsaturated aldehydes and ketones and precursors for these. Unpublished report submitted by FLAVIS Secretariat to EFSA.

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)

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.

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.

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.

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 Jr.. M., 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.

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, 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, 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, 2001c. Compendium of food additive specifications. Addendum 9. Joint FAO/WHO Expert Committee 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, 2002d. Compendium of food additive specifications. Addendum 10. Joint FAO/WHO Expert Committee of Food Additives 59th session. Geneva, 4-13 June 2002. FAO Food and Nutrition paper 52 Add. 10.

JECFA, 2006c. Joint FAO/WHO Expert Committee on Food Additives. Sixty-seventh meeting Rome, 20-29 June 2006, Summary and Conclusions. Issued 7 July 2006.

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.

Kajiura, Y., 1996b. Mutagenicity test of HOTACT 1MM {4-(l-menthoxymethyl)-2-(3'-methoxy-4'-hydroxyphenyl)-1,3-dioxolane}. Central Research Laboratory. Unpublished report submitted by EFFA to FLAVIS Secretariat.

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.

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.

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)

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.

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.

Lutz, D., Eder, E., Neudecker, T., Henschler, D., 1982. Structure-mutagenicity relationship in alpha,beta-unsaturated carbonylic compounds and their corresponding allylic alcohols. Mutat. Res. 93, 305-315.

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.

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.

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.

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.

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.

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.

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(3), 33-36.

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.

Nikolov, N., Jensen, G.E., Wedebye, E.B., Niemelä, J., 2007. Report on QSAR predictions of 222 alpha,beta-unsaturated aldehydes and ketones from Flavouring Group Evaluation 19 (FGE.19) on 360 alpha,beta-unsaturated aldehydes and ketones and precursors for these. Unpublished report submitted by FLAVIS Secretariat to EFSA.

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, 1989a. 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.

NTP, 2003e. NTP technical report on the toxicology and carcinogenesis studies of citral (microencapsulated) (CAS No. 5392-40-5) in F344/N rats and B6C3F1 mice (feed studies). (NTP TR 505; NIH Publication No. 01-4439). US Department of Health and Human Services, Public Health Service, National Institutes of Health, USA.

Oda, Y., Hamono, Y., Inoue, K., Yamamoto, H., Niihara, T., Kunita, N., 1979. [Mutagenicity of food flavors in bacteria]. Shokuhin. Eisei. Hen. 9, 177-181. (In Japanese)

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.

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.

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 of pH on the convertogenic activity of plant phenolics. Mutat. Res. 135, 109-113.

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.

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, 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.

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.

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.

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.

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)

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.

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 cytotoxicity and genotoxicity and results for 81 compounds. Mutat. Res. 368(2), 59-101.

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.

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

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.

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.

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.

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.

Zeiger, E., Anderson, B., Haworth, S., Lawlor, T., Mortelmans, K., Speck, W., 1987. Salmonella mutagenicity tests. 3. Results from the testing of 255 chemicals. Environ. Mol. Mutag. 9(Suppl. 9), 1-110.

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.

ABBREVIATIONS

CAS	Chemical Abstract Service
CEF	Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
CHO	Chinese hamster ovary (cells)
CoE	Council of Europe
DNA	Deoxyribonucleic acid
DTU-NFI	Danish Technical University – National Food Institute
EFSA	The European Food Safety Authority
EU	European Union
FAO	Food and Agriculture Organization of the United Nations
FEMA	Flavor and Extract Manufacturers Association
FGE	Flavouring Group Evaluation
FLAVIS (FL)	Flavour Information System (database)
GLP	Good Laboratory Practise
ID	Identity
IR	Infrared spectroscopy
ISS	Istituto Superiore di Sanita
JECFA	The Joint FAO/WHO Expert Committee on Food Additives
LD_{50}	Lethal Dose, 50%; Median lethal dose
MSDI	Maximised Survey-derived Daily Intake
mTAMDI	Modified Theoretical Added Maximum Daily Intake
NMR	Nuclear magnetic resonance
No	Number
NOAEL	No observed adverse effect level
NTP	National Toxicology Program
OECD	Organisation for Economic Co-operation and Development
PCE/NCE	Polychromatic eryhtrocyte/normochromatic erythrocyte ratio
(Q)SAR	(Quantitative) structure-activity relationship
SCE	Sister chromatid exchange
SCF	Scientific Committee on Food
SLRL	Sex-linked recessive lethal mutations

TAMDI	Theoretical Added Maximum Daily Intake
UDS	Unscheduled DNA synthesis
US EPA	United States Environmental Protection Agency
WHO	World Health Organisation