

## **Flavouring Group Evaluation 54 (FGE.54)<sup>1</sup>**

**Consideration of benzyl derivatives evaluated by JECFA (57<sup>th</sup> meeting) structurally related to benzyl alcohols, benzaldehydes, a related acetal, benzoic acids and related esters evaluated by EFSA in FGE.20 (2005)**

**Scientific Opinion of the Panel on Food Additives,**

**Flavourings, Processing Aids and Materials in Contact with Food**

**(EFSA-Q-2008-032E)**

**Adopted on 16 May 2008**

### **PANEL MEMBERS**

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### **SUMMARY**

The Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food (the Panel) is asked to advise the Commission on the implications for human health of chemically defined flavouring substances used in or on foodstuffs in the Member States. In particular, the Panel is 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.

The present consideration concerns 36 benzyl derivatives evaluated by the JECFA (57<sup>th</sup> meeting) and will be considered in relation to the European Food Safety Authority (EFSA) evaluation of 35 benzyl alcohols, benzaldehydes, a related acetal, benzoic acids and related esters evaluated in the Flavouring Group Evaluation 20 (FGE.20).

The Panel concluded that the 36 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 EFSA in the FGE.20.

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Further one substance, geranyl benzoate [FL-no: 09.767], was evaluated by the JECFA, which may be metabolised to an alpha, beta-unsaturated aldehyde and will be evaluated together with other alpha,beta-unsaturated aldehydes and ketones.

The Panel agrees with the application of the Procedure as performed by the JECFA for the 36 substances considered in this FGE.

For four substances [FL-no: 06.019, 09.294, 09.803 and 09.812], the JECFA evaluation is only based on Maximised Survey-derived Daily Intake (MSDI) values derived from production figures from the USA. EU production figures are needed in order to finalise the evaluation of these substances.

For all 36 substances use levels are needed to calculate the modified Theoretical Added Maximum Daily Intake (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 36 JECFA evaluated substances can be applied to the materials of commerce, it is necessary to consider the available specifications:

Adequate specifications, including complete purity criteria and identity, are available for 26 of the 36 JECFA evaluated substances. For the remaining ten substances, eight [FL-no: 05.027, 06.002, 06.012, 06.019, 06.032, 09.771, 09.803 and 09.806] lack information on the isomeric composition 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 is requested.

Thus, for eleven substances [FL-no: 05.027, 06.002, 06.012, 06.019, 06.032, 09.294, 09.771, 09.755, 09.803, 09.806 and 09.812] the Panel has reservations (only USA production volumes available and/or missing data on isomerism/composition). For the remaining 25 of the 36 JECFA evaluated benzyl derivatives [FL-no: 02.010, 02.039, 05.013, 05.022, 05.068, 05.110, 06.003, 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, 09.768, 09.770 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.

## KEYWORDS

Benzyl derivatives, JECFA 57<sup>th</sup> meeting, benzyl alcohols, benzaldehydes, benzoic acids, FGE.20.

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## **BACKGROUND**

Commission 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 2006/252/EC (EC, 2006). Each flavouring substance is attributed a FLAVIS-number (FL-number) and all substances are divided into 34 chemical groups. Substances within a group should have some metabolic and biological behaviour in common.

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

Commission 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 – 2006, during its 55<sup>th</sup>, 57<sup>th</sup>, 59<sup>th</sup>, 61<sup>st</sup>, 63<sup>rd</sup> and 65<sup>th</sup> meetings, the JECFA evaluated about 900 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, 2000). These flavouring substances are listed in the Register, which was adopted by Commission Decision 1999/217/EC (EC, 1999a) and its consecutive amendments.

## **ACKNOWLEDGEMENTS**

The Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food wishes to thank Vibe Beltoft, Frederikke Bentzen, Jørn Gry, Karin Nørby and Trine Klein Reffstrup for their contribution to the draft opinion.

## ASSESSMENT

The approach used by EFSA for safety evaluation of flavouring substances is referred to in Commission Regulation (EC) No 1565/2000 (EC, 2000), 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 Additives, Flavourings, Processing Aids and Materials in Contact with Food (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 Flavouring Industry on the use levels in various foods, it appeared obvious that the MSDI approach in a number of cases would grossly underestimate the intake by regular consumers of products flavoured at the use level reported by the Industry, especially in those cases where the annual production values were reported to be small. In consequence, the Panel had reservations about the data on use and use levels provided and the intake estimates obtained by the MSDI approach. It is noted that the JECFA, at its 65<sup>th</sup> 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 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.

## **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. One of the substances, geranyl benzoate [FL-no: 09.767], may be metabolised to an alpha, beta-unsaturated aldehyde. This substance will be considered together with other alpha, beta-unsaturated aldehydes and ketones. This consideration will therefore only deal with 36 JECFA evaluated substances.

#### **1.1.2. EFSA Considerations**

The Panel concluded that the 36 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).

## 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]. Two substances can exist as geometrical isomers [FL-no: 09.494 and 09.806] and three substances can exist as mixtures of positional isomers [FL-no: 05.027, 06.002 and 06.012].

### 1.2.2. EFSA Considerations

For one of the JECFA evaluated substances the configuration is given by the name [FL-no: 09.494], for the remaining eight substances information is lacking about the stereoisomerism/positional isomers [FL-no: 05.027, 06.002, 06.012, 06.019, 06.032, 09.771, 09.803 and 09.806].

## 1.3. Specifications

### 1.3.1. JECFA status

JECFA specifications are available for all 36 substances (JECFA, 2002d). See Table 1.

### 1.3.2. EFSA Considerations

The available specifications are considered adequate except that information on isomerism is lacking for [FL-no: 05.027, 06.002, 06.012, 06.019, 06.032, 09.771, 09.803 and 09.806], see Section 1.2, and further information on the composition of seven substances [FL-no: 05.027, 06.002, 06.012, 06.019, 09.294, 09.755 and 09.806] is requested.

## 2. INTAKE ESTIMATIONS

### 2.1. JECFA Status

For 32 substances evaluated through the JECFA Procedure intake data are available for the EU, see Table 3.1. For the remaining four substances 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 [FL-no: 06.019, 09.294, 09.803 and 09.812].

## 3. GENOTOXICITY DATA

### 3.1. Genotoxicity Studies - Text Taken 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 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 EFSA (EFSA, 2006e)**

### *In vitro*

Data from *in vitro* tests are available for eight candidate substances [FL-no: 09.631, 09.367, 05.129, 05.158, 08.080, 05.153, 08.087 and 02.205] and 29 supporting substances. Data from *in vivo* tests are available for two candidate substances [FL-no: 09.367 and 08.080] and for ten supporting substances.

All the seven candidate substances [FL-no: 05.129, 05.142, 05.153, 08.080, 08.087, 09.367 and 09.631] tested for bacterial gene mutations gave negative results. For five candidate substances [FL-no: 05.129, 05.158, 08.080, 08.087 and 09.367,] both positive and/or negative results were reported in various other *in vitro* test systems (Rec assay, chromosomal aberration test, SCE and mammalian cell gene mutation assay (mouse lymphoma tests)) for most of which the validity cannot be evaluated or are known to be of very limited relevance.

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

### *In vivo*

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

The Panel noted that benzyl acetate was positive in an *in vivo* Comet assay, which may indicate a genotoxic activity at high dose levels. The study was considered of limited validity. Further on, all other *in vivo* studies with benzyl acetate are 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 (NTP, 1986c) 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 validity. Taking all this information into account, the Panel considered the positive result from the *in vivo* Comet assay as insufficient ground to preclude the evaluation of benzyl acetate via the Procedure.

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

#### *Conclusion on genotoxicity*

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

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

### **3.3. EFSA Considerations**

The Panel concluded that the data available do not preclude evaluation of the 36 JECFA evaluated benzyl derivatives through the Procedure.

## **4. APPLICATION OF THE PROCEDURE**

### **4.1. Application of the Procedure to 36 Benzyl Derivatives by the JECFA (JECFA, 2002a):**

According to the JECFA all the 36 benzyl derivatives belong to structural class I using the decision tree approach presented by Cramer et al. (Cramer et al., 1978).

The JECFA concluded 32 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 NOAEL exists to provide an adequate margin of safety to the estimated intake as flavouring substance.

In conclusion, the JECFA 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 evaluations of the 36 benzyl derivatives are summarised in Table 3.1: Summary of Safety Evaluation of 36 Benzyl Derivatives (JECFA, 2002a).

### **4.2. Application of the Procedure to 35 Benzyl Derivatives by EFSA (EFSA, 2006e):**

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

The Panel concluded all of the 35 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 35 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 35 substances are summarised in Table 3.2: Summary of Safety Evaluation Applying the Procedure (EFSA/FGE.20).

### 4.3. EFSA Considerations

The Panel agrees with the application of the Procedure as performed by the JECFA for the 36 substances in the group of benzyl derivatives performed.

## CONCLUSIONS

The Panel concluded that the 36 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 EFSA in the Flavouring Group Evaluation 20 (FGE.20).

Further one substance, geranyl benzoate [FL-no: 09.767], was evaluated by the JECFA, which may be metabolised to an alpha,beta-unsaturated aldehyde and will be evaluated together with other alpha,beta-unsaturated aldehydes and ketones.

The Panel agrees with the application of the Procedure as performed by the JECFA for the 36 substances considered in this FGE.

For four substances [FL-no: 06.019, 09.294, 09.803 and 09.812] the JECFA evaluation is only based on MSDI values derived from production figures from the USA. EU production figures are needed in order to finalise the evaluation of these substances.

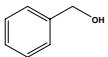
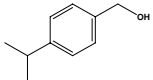
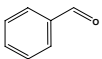
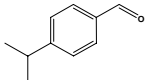
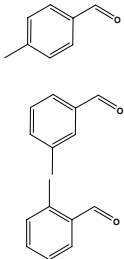
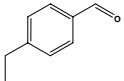
For all 36 substances evaluated through the Procedure use levels are needed to calculate the mTAMDI in order to identify those flavouring substances that need more refined exposure assessment and to finalise the evaluation.

In order to determine whether the conclusion for the 36 JECFA evaluated substances can be applied to the materials of commerce, it is necessary to consider the available specifications:

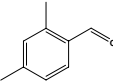
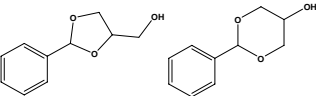
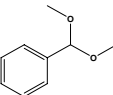
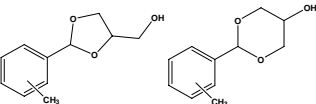
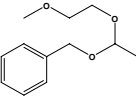
Adequate specifications including complete purity criteria and identity are available for 26 of the 36 JECFA evaluated substances. For eight substances [FL-no: 05.027, 06.002, 06.012, 06.019, 06.032, 09.771, 09.803 and 09.806] information on the isomeric composition is lacking and for seven substances [FL-no: 05.027, 06.002, 06.012, 06.019, 09.294, 09.755 and 09.806] further information on the composition is requested.

Thus, for eleven substances [FL-no: 05.027, 06.002, 06.012, 06.019, 06.032, 09.294, 09.771, 09.755, 09.803, 09.806 and 09.812] the Panel has reservations (only USA production volumes available and/or missing data on isomerism/composition). For the remaining 25 of the 36 JECFA evaluated benzyl derivatives [FL-no: 02.010, 02.039, 05.013, 05.022, 05.068, 05.110, 06.003, 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, 09.768, 09.770 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.

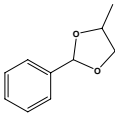
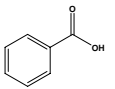
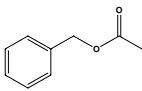
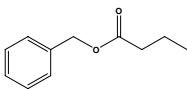
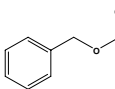
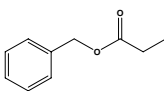
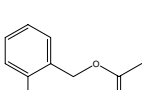
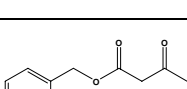
**TABLE 1: SPECIFICATION SUMMARY FOR JECFA EVALUATED SUBSTANCES IN THE PRESENT GROUP**

Table 1: Specification Summary of the Substances in the JECFA Flavouring Group of 36 Benzyl Derivatives								
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 <sub>7</sub> H <sub>8</sub> O 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 <sub>10</sub> H <sub>14</sub> 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 <sub>7</sub> H <sub>6</sub> O 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 <sub>10</sub> H <sub>12</sub> O 148.20	Insoluble Miscible	235-236  IR 95 %	1.527-1.534 0.973-0.981	
05.027 866	Tolualdehyde 7)		3068 115 1334-78-7	Liquid C <sub>8</sub> H <sub>8</sub> O 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 <sub>9</sub> H <sub>10</sub> O 134.18	Insoluble Miscible	220  IR 97 %	1.538-1.542 0.980-1.000	

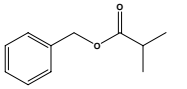
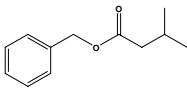
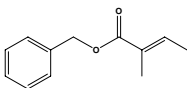
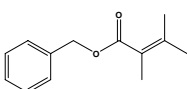
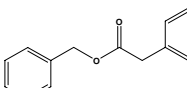
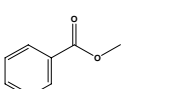
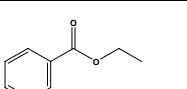
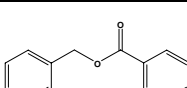
**Table 1: Specification Summary of the Substances in the JECFA Flavouring Group of 36 Benzyl Derivatives**

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
05.110 869	2,4-Dimethylbenzaldehyde		3427 15764-16-6	Liquid C <sub>9</sub> H <sub>10</sub> O 134.18	Insoluble Miscible	215 NMR 95 %	1.548-1.552 0.992-1.017	
06.002 838	5-Hydroxy-2-phenyl-1,3-dioxane 6) 7)		2129 36 1319-88-6	Liquid C <sub>10</sub> H <sub>12</sub> O <sub>3</sub> 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 <sub>9</sub> H <sub>12</sub> O <sub>2</sub> 152.20	Insoluble Miscible	198 IR 96 %	1.488-1.496 1.007-1.020	
06.012 867	Tolualdehyde glyceryl acetal 6) 7)		3067 46 1333-09-1	Liquid C <sub>11</sub> H <sub>14</sub> O <sub>3</sub> 194.23	Slightly soluble Miscible	292 IR 95 %	1.527-1.537 1.148-1.158	CASrn in Register, "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 6) 7)		2148 523 7492-39-9	Liquid C <sub>12</sub> H <sub>18</sub> O <sub>3</sub> 210.27	Insoluble Miscible	161-162 (13hPa) IR 98 %	1.479-1.489 1.019-1.025	CASrn in Register refers to the racemate According to JECFA: Min. assay value is "98 (sum of named compound and starting materials)".

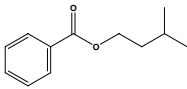
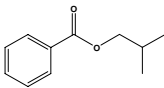
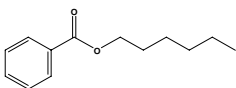
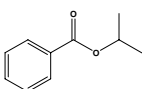
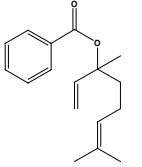
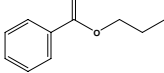
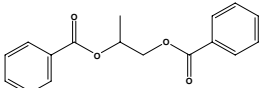
**Table 1: Specification Summary of the Substances in the JECFA Flavouring Group of 36 Benzyl Derivatives**

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
06.032 839	4-Methyl-2-phenyl-1,3-dioxolane 6)		2130 2226 2568-25-4	Liquid C <sub>10</sub> H <sub>12</sub> O <sub>2</sub> 164.20	Slightly soluble Miscible	83-85 (5 hPa) IR 95 %	1.506-1.516 1.061-1.071	CASrn in Register refers to racemate.
08.021 850	Benzoic acid		2131 21 65-85-0	Solid C <sub>7</sub> H <sub>6</sub> O <sub>2</sub> 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 <sub>9</sub> H <sub>10</sub> O <sub>2</sub> 150.18	Very slightly soluble Miscible	215 IR 98 %	1.500 - 1.504 1.049-1.059	
09.051 843	Benzyl butyrate		2140 277 103-37-7	Liquid C <sub>11</sub> H <sub>14</sub> O <sub>2</sub> 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 <sub>8</sub> H <sub>8</sub> O <sub>2</sub> 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 <sub>10</sub> H <sub>12</sub> O <sub>2</sub> 164.20	Insoluble Miscible	222 IR 98 %	1.495-1.500 1.028-1.033	
09.294 863	2-Methylbenzyl acetate 7)		3702 17373-93-2	Liquid C <sub>10</sub> H <sub>12</sub> O <sub>2</sub> 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 <sub>11</sub> H <sub>12</sub> O <sub>3</sub> 192.21	Insoluble Miscible	249 IR 98 %	1.498-1.520 1.112-1.120	

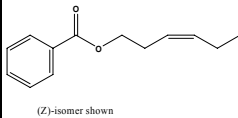
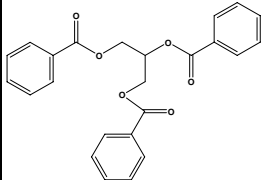
**Table 1: Specification Summary of the Substances in the JECFA Flavouring Group of 36 Benzyl Derivatives**

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
09.426 844	Benzyl isobutyrate		2141 301 103-28-6	Liquid C <sub>11</sub> H <sub>14</sub> O <sub>2</sub> 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 <sub>12</sub> H <sub>16</sub> O <sub>2</sub> 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 <sub>12</sub> H <sub>14</sub> O <sub>2</sub> 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 <sub>13</sub> H <sub>16</sub> O <sub>2</sub> 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 <sub>15</sub> H <sub>14</sub> O <sub>2</sub> 226.28	Insoluble Miscible	320  IR 98 %	1.552-1.560 1.094-1.100	
09.725 851	Methyl benzoate		2683 260 93-58-3	Liquid C <sub>8</sub> H <sub>8</sub> O <sub>2</sub> 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 <sub>9</sub> H <sub>10</sub> O <sub>2</sub> 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 <sub>14</sub> H <sub>12</sub> O <sub>2</sub> 212.25	Insoluble Miscible	323-324 21 IR 99 %	1.566-1.571 1.113-1.121	

**Table 1: Specification Summary of the Substances in the JECFA Flavouring Group of 36 Benzyl Derivatives**

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
09.755 857	Isopentyl benzoate 7)		2058 562 94-46-2	Liquid C <sub>12</sub> H <sub>16</sub> O <sub>2</sub> 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 <sub>11</sub> H <sub>14</sub> O <sub>2</sub> 178.23	Insoluble Miscible	240  IR 98 %	1.489-1.496 0.994-0.999	
09.768 854	Hexyl benzoate		3691 645 6789-88-4	Liquid C <sub>13</sub> H <sub>18</sub> O <sub>2</sub> 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 <sub>10</sub> H <sub>12</sub> O <sub>2</sub> 164.21	Insoluble Miscible	218  IR 98 %	1.492-1.497 1.005-1.011	
09.771 859	Linalyl benzoate 6)		2638 654 126-64-7	Liquid C <sub>17</sub> H <sub>22</sub> O <sub>2</sub> 258.36	Insoluble Miscible	263  IR 95 %	1.505-1.526 0.978-0.999	CASrn in Register refers to the racemate.
09.776 853	Propyl benzoate		2931 677 2315-68-6	Liquid C <sub>10</sub> H <sub>12</sub> O <sub>2</sub> 164.21	Insoluble Miscible	229  IR 98 %	1.498-1.503 1.020-1.026	
09.803 862	Propylene glycol dibenzoate 6)		3419 10890 19224-26-1	Liquid C <sub>17</sub> H <sub>16</sub> O <sub>4</sub> 284.31	Insoluble Miscible	232 (16 hPa)  IR 96 %	1.542-1.547 1.157-1.163	CASrn in Register refers to the racemate.

**Table 1: Specification Summary of the Substances in the JECFA Flavouring Group of 36 Benzyl Derivatives**

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
09.806 858	Hex-3-enyl benzoate 6) 7)	 (Z)-isomer shown	3688 11778 25152-85-6	Liquid C <sub>13</sub> H <sub>16</sub> O <sub>2</sub> 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 <sub>24</sub> H <sub>20</sub> O <sub>6</sub> 404.42	Insoluble Slightly soluble	n.a. 68-72 IR 95 %	n.a. n.a.	

<sup>1)</sup> Solubility in water, if not otherwise stated.

<sup>2)</sup> Solubility in 95% ethanol, if not otherwise stated.

<sup>3)</sup> At 1013.25 hPa, if not otherwise stated.

<sup>4)</sup> At 20°C, if not otherwise stated.

<sup>5)</sup> At 25°C, if not otherwise stated.

<sup>6)</sup> Stereoisomeric composition not specified.

<sup>7)</sup> Composition of mixture not specified.



**TABLE 2: GENOTOXICITY DATA**

**TABLE 2.1: GENOTOXICITY DATA (*IN VITRO/IN VIVO*) FOR 36 BENZYL DERIVATIVES (JECFA, 2002A)**

Table 2.1: Summary of genotoxicity data for 36 Benzyl Derivatives evaluated by JECFA (JECFA, 2002a)

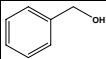
FL-no JECFA-no	EU Register name JECFA name	Structural formula	End-point	Test system	Concentration	Results	Reference
<i>In vitro</i>							
02.010	Benzyl alcohol		Reverse mutation	<i>S. typhimurium</i> TA92, TA94, TA98, TA100, TA1535, TA1537 (preincubation)	10 000 µg/plate	Negative <sup>a</sup>	(Ishidate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

Table 2.1: Summary of genotoxicity data for 36 Benzyl Derivatives evaluated by JECFA (JECFA, 2002a)

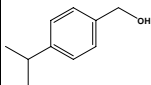
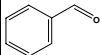
FL-no JECFA-no	EU Register name JECFA name	Structural formula	End-point	Test system	Concentration	Results	Reference
			Chromosomal aberration	Chinese hamster ovary cells	5000 µg/ml	Positive <sup>i</sup>	(NTP, 1989)
			Sister chromatid exchange	Chinese hamster ovary cells	5000 µg/ml	Positive <sup>j</sup>	(NTP, 1989)
			Sister chromatid exchange	Chinese hamster ovary cells	5000 µg/ml	Positive <sup>k</sup>	(Anderson et al., 1990)
			Mutation	L5178Y mouse lymphoma cells	5000 µg/ml	Equivocal <sup>l</sup>	(McGregor et al., 1988a)
			Mutation	L5178Y mouse lymphoma cells	5000 µg/ml	Equivocal <sup>l</sup>	(Myhr et al., 1990)
			Mutation	L5178Y mouse lympho- ma cells	4500 µg/ml	Positive <sup>m</sup>	(NTP, 1989)
			Mutation	<i>E. coli</i> WP2 <i>uvrA</i>	NR	Negative <sup>n</sup>	(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 <sup>o</sup>	(Storer et al., 1996)
DNA damage	<i>E. coli</i> P3478	50 µl/disc	Negative <sup>a</sup>	(Fluck et al., 1976)			
02.039	4-Isopropylbenzyl alcohol		Reverse mutation	<i>S. typhimurium</i> TA98, TA100 (plate incorporation)	100 µl/plate	Negative <sup>p</sup>	(Rockwell & Raw, 1979)
			Reverse mutation	<i>S. typhimurium</i> TA98, TA100 (plate incorporation)	300 µl/plate	Negative <sup>q</sup>	(Rockwell & Raw, 1979)
05.013	Benzaldehyde		Reverse mutation	<i>S. typhimurium</i> TA98, TA100, TA1535, TA1537, TA1538 (plate incorporation)	38 000 ug/plate	Negative <sup>a</sup>	(Heck et al., 1989)
			Reverse mutation	<i>S. typhimurium</i> TA98, TA100 (plate incorporation)	300 µl/plate	Negative <sup>r</sup>	(Rockwell & Raw, 1979)
			Reverse mutation	<i>S. typhimurium</i> TA98, TA100 (plate incorporation)	100 µl/plate	Negative <sup>p</sup>	(Rockwell & Raw, 1979)
			Reverse mutation <sup>a</sup>	<i>S. typhimurium</i> TA98, TA100, TA2637	2 mg/plate	Negative <sup>s</sup>	(Nohmi et al., 1985)
			Reverse mutation <sup>a</sup>	<i>S. typhimurium</i> TA98, TA100, TA1535, TA1537	3 µmol/plate	Negative <sup>a</sup>	(Florin et al., 1980)

Table 2.1: Summary of genotoxicity data for 36 Benzyl Derivatives evaluated by JECFA (JECFA, 2002a)

FL-no JECFA-no	EU Register name JECFA name	Structural formula	End-point	Test system	Concentration	Results	Reference
			Reverse mutation	<i>S. typhimurium</i> TA98, TA100, TA1535, TA1537 (preincubation)	1000 µg/plate	Negative <sup>a</sup>	(Haworth et al., 1983)
			Reverse mutation	<i>S. typhimurium</i> TA100, TA102, TA104	3300 µg/plate	Negative <sup>a</sup>	(NTP, 1990c)
			Reverse mutation <sup>a</sup>	<i>S. typhimurium</i> TA100	1 mg/plate	Negative <sup>l</sup>	(Rapson et al., 1980)
			Reverse mutation	<i>S. typhimurium</i> TA98, TA100 (preincubation)	NR	Negative <sup>a</sup>	(Sasaki & Endo, 1978)
			Reverse mutation	<i>S. typhimurium</i> TA100, TA102, TA104 (preincubation)	NR	Negative <sup>a</sup>	(Dillon et al., 1992)
			Reverse mutation	<i>S. typhimurium</i> TA100 (preincubation)	2000 nmol/plate	Negative <sup>a</sup>	(Vamvakas et al., 1989)
			Reverse mutation <sup>a</sup>	<i>S. typhimurium</i> TA98, TA100	500 µg/plate	Negative <sup>a</sup>	(Kasamaki et al., 1982)
			DNA repair	<i>B. subtilis</i> H17, M45	21 µg/disc	Negative <sup>d</sup>	(Oda et al., 1979)
			DNA repair	<i>B. subtilis</i> H17, M45	NR	Positive <sup>i</sup>	(Matsui et al., 1989)
			Unscheduled DNA synthesis	Rat hepatocytes	250 µg/ml	Negative <sup>b</sup>	(Heck et al., 1989)
			Mutation	Mouse L5178Y lymphoma cells	600 µg/ml	Positive <sup>v</sup>	(Heck et al., 1989)
			Mutation	Mouse L5178Y lymphoma cells	800 µg/ml	Positive <sup>w</sup>	(McGregor et al., 1988a)
			Chromosomal aberrations	Chinese hamster cells	1.2 mg/ml	Positive <sup>x</sup>	(Sofuni et al., 1985)
			Chromosomal aberrations	Chinese hamster ovary cells	1600 µg/ml	Negative <sup>a</sup>	(Galloway et al., 1987)
			Chromosomal aberrations	Chinese hamster cells	50 nmol/L	Positive <sup>a</sup>	(Kasamaki et al., 1982)
			Sister chromatid exchange	Chinese hamster ovary cells	1600 µg/ml	Positive <sup>a</sup>	(Galloway et al., 1987)
			Sister chromatid exchange	Chinese hamster ovary cells	1000 µmol/L	Negative <sup>y</sup>	(Sasaki et al., 1989)
			Sister chromatid exchange	Human lymphocytes	2 mmol/L	Positive <sup>b</sup>	(Jansson et al., 1988)

Table 2.1: Summary of genotoxicity data for 36 Benzyl Derivatives evaluated by JECFA (JECFA, 2002a)

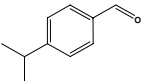
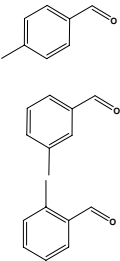
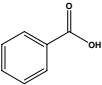
FL-no JECFA-no	EU Register name JECFA name	Structural formula	End-point	Test system	Concentration	Results	Reference
05.022	4-Isopropylbenzaldehyde		Reverse mutation	<i>S. typhimurium</i> TA98, TA100 (plate incorporation)	100 µl/plate	Negative <sup>p</sup>	(Rockwell & Raw, 1979)
			Reverse mutation	<i>S. typhimurium</i> TA98, TA100 (plate incorporation)	300 µl/plate	Negative <sup>z</sup>	(Rockwell & Raw, 1979)
			Sister chromatid exchange	Chinese hamster ovary cells	333 µmol/L	Negative <sup>l</sup>	(Sasaki et al., 1989)
05.027	Tolualdehyde		Reverse mutation	<i>S. typhimurium</i> TA104 (preincubation)	0.8 µmol/plate	Negative <sup>a</sup>	(Marnett et al., 1985a)
			Reverse mutation <sup>a</sup>	<i>S. typhimurium</i> TA98, TA100, TA1535, TA1537	3 µmol/plate	Negative <sup>a</sup>	(Florin et al., 1980)
			Reverse mutation	<i>S. typhimurium</i> TA98, TA100, TA1535, TA1537, TA1538 (plate incorporation)	19 000 µg/plate	Negative <sup>a</sup>	(Heck et al., 1989)
			Reverse mutation	<i>S. typhimurium</i> TA98, TA100, TA102 (plate incorporation)	0.8 mmol/plate	Negative <sup>a</sup>	(Aeschbacher et al., 1989)
			Reverse mutation	<i>S. typhimurium</i> TA97, TA100, TA1535, TA1537 (preincubation)	666 µg/plate	Negative <sup>a</sup>	(Zeiger et al., 1988)
			Unscheduled DNA synthesis	Rat hepatocytes	1000 µg/ml	Negative <sup>b</sup>	(Heck et al., 1989)
			Mutation	Mouse L5178Y lymphoma cells	300 µg/ml	Negative <sup>a</sup>	(Heck et al., 1989)
08.021	Benzoic acid		Reverse mutation	<i>S. typhimurium</i> TA98, TA100, TA1535, TA1538 (plate incorporation)	2500 µg/plate	Negative <sup>a</sup>	(Anderson & Styles, 1978)
			Reverse mutation <sup>a</sup>	<i>S. typhimurium</i> TA98, TA100, TA1535, TA1536	3.6 µg/plate	Negative <sup>a</sup>	(Cotruvo et al., 1977)
			Reverse mutation	<i>S. typhimurium</i> TA97, TA98, TA100, TA1535, TA1537 (preincubation)	10 mg/plate	Negative <sup>a</sup>	(Zeiger et al., 1988)
			Reverse mutation <sup>a</sup>	<i>S. typhimurium</i> TA100	1 mg/plate	Negative <sup>l</sup>	(Rapson et al., 1980)
			Reverse mutation	<i>S. typhimurium</i> TA98, TA100, TA1535, TA1537 (plate incorporation)	1 mg/plate	Negative <sup>p</sup>	(McCann et al., 1975)
			Reverse mutation	<i>S. typhimurium</i> TA92, TA94, TA98, TA100, TA1535, TA1537 (preincubation)	10 mg/plate	Negative <sup>a</sup>	(Ishidate et al., 1984)
			Reverse mutation	<i>S. typhimurium</i> TA98, TA100, TA1535, TA1537, TA1538 (plate incorporation)	100 µg/plate	Negative <sup>b</sup>	(Milvy & Garro, 1976)
			Reverse mutation	<i>S. typhimurium</i> TA1535, TA1537, TA1538 (plate incorporation)	0.5%	Negative <sup>a</sup>	(FDA, 1975b)

Table 2.1: Summary of genotoxicity data for 36 Benzyl Derivatives evaluated by JECFA (JECFA, 2002a)

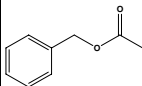
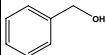
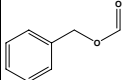
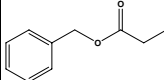
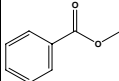
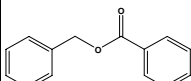
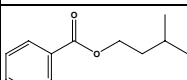
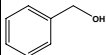

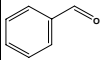
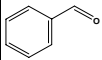
FL-no JECFA-no	EU Register name JECFA name	Structural formula	End-point	Test system	Concentration	Results	Reference
			Mutation ( <i>umu</i> gene expression)	<i>S. typhimurium</i> TA1535/ pSK1002	1.7 mg/ml	Negative <sup>a</sup>	(Nakamura et al., 1987)
			DNA repair	<i>B. subtilis</i> H17, H45	NR	Positive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

Table 2.1: Summary of genotoxicity data for 36 Benzyl Derivatives evaluated by JECFA (JECFA, 2002a)

FL-no JECFA-no	EU Register name JECFA name	Structural formula	End-point	Test system	Concentration	Results	Reference
			Chromosomal aberration	Chinese hamster lung fibroblasts	2.4 mg/ml	Negative <sup>6</sup>	(Matsuoka et al., 1996)
			Sister chromatid exchange	Chinese hamster ovary cells	5000 µg/ml	Negative <sup>a</sup>	(Galloway et al., 1987)
			Unscheduled DNA synthesis	Rat hepatocytes	NR	Negative <sup>2</sup>	(Mirsalis et al., 1983)
09.077	Benzyl formate		DNA repair	<i>B. subtilis</i> H17, M45	20 µl/disc	Positive <sup>d</sup>	(Yoo, 1986)
			Mutation	<i>E. coli</i> WP2 <i>uvrA</i>	4.0 mg/plate	Negative <sup>d</sup>	(Yoo, 1986)
09.132	Benzyl propionate		DNA repair	<i>B. subtilis</i> H17, M45	21 µg/disc	Negative <sup>7</sup>	(Oda et al., 1979)
09.725	Methyl benzoate		Reverse mutation	<i>S. typhimurium</i> TA97, TA98, TA100, TA1535, TA1537 (preincubation)	6700 ug/plate	Negative <sup>a</sup>	(Zeiger et al., 1992)
			Mutation	<i>E. coli</i> Sd-4-73	NR	Negative <sup>b</sup>	(Szybalski, 1958)
09.727	Benzyl benzoate		Reverse mutation <sup>a</sup>	<i>S. typhimurium</i> TA98, TA100, TA1535, TA1537	3 µmol/plate	Negative <sup>a</sup>	(Florin et al., 1980)
			Reverse mutation	<i>S. typhimurium</i> TA98, TA100 (preincubation and plate incorporation)	5000 µg/plate	Negative <sup>4</sup>	(Schunk et al., 1986)
09.755	Isopentyl benzoate		Mutation	<i>E. coli</i> Sd-4-73	NR	Negative <sup>b</sup>	(Szybalski, 1958)
<i>In vivo</i>							
02.010	Benzyl alcohol		Sex-linked recessive lethal mutation	<i>Drosophila melanogaster</i>	5000 mg/kg  8000 mg/kg	Negative <sup>8</sup> Negative <sup>9</sup>	(Fouremant et al., 1994)
			Micronucleus formation	Mouse bone marrow cells	200 mg/kg bw	Negative <sup>10</sup>	(Hayashi et al., 1988)
			Replicative DNA synthesis	Mouse hepatocytes	NR	Positive <sup>11</sup>	(Yoshikawa, 1996)
			Sex-linked recessive lethal mutation	<i>D. melanogaster</i>	300 mg/kg 20 000 mg/kg	Negative <sup>8</sup> Negative <sup>9</sup>	(NTP, 1993d; Fouremant et al., 1994)
			Sister chromatid exchange	Mouse bone marrow cells	1700 mg/kg bw	Negative <sup>10</sup>	(NTP, 1993d)
09.014	Benzyl acetate		Chromosomal aberration	Mouse bone marrow cells	1700 mg/kg bw	Negative <sup>10</sup>	(NTP, 1993d)

**Table 2.1: Summary of genotoxicity data for 36 Benzyl Derivatives evaluated by JECFA (JECFA, 2002a)**

FL-no JECFA-no	EU Register name JECFA name	Structural formula	End-point	Test system	Concentration	Results	Reference
			Micronucleus formation	Mouse bone marrow cells	1300 mg/kg bw	Negative <sup>10</sup>	(NTP, 1993d; Shelby et al., 1993)
			Micronucleus formation	Mouse erythrocytes	50 000 mg/kg	Negative <sup>10</sup>	(NTP, 1993d)
			Unscheduled DNA synthesis	Rat hepatocytes	NR	Negative <sup>12</sup>	(Mirsalis et al., 1983)
			Unscheduled DNA synthesis	Rat hepatocytes	1000 mg/kg bw	Negative <sup>13</sup>	(Mirsalis et al., 1983)
			Unscheduled DNA synthesis	Rat pancreatic cells	1000 mg/kg bw	Negative <sup>13</sup>	(Steinmetz & Mirsalis, 1984)
			DNA damage	Rat pancreatic cells	500 mg/kg bw	Negative <sup>14</sup>	(Longnecker et al., 1990)
			DNA damage	Rat pancreatic cells	0.9%	Negative <sup>15</sup>	(Longnecker et al., 1990)
05.013	Benzaldehyde		Sex-linked recessive lethal mutation	<i>D. melanogaster</i>	1200 mg/kg 2500 mg/kg	Negative <sup>8</sup> Negative <sup>9</sup>	(Woodruff et al., 1985)

<sup>a</sup> Assay performed with and without S9.

<sup>b</sup> Assay performed without S9.

<sup>c</sup> Assay performed with and without S9; cytotoxicity at highest concentration.

<sup>d</sup> Japanese article, English summary; use of S9 not reported.

<sup>e</sup> Japanese article, English summary tables.

<sup>f</sup> Japanese article, English summary tables; inhibition of growth without S9.

<sup>g</sup> Assay performed without S9; cells exposed for 48 h.

<sup>h</sup> Assay performed with and without S9; positive results not reproducible; no dose-response relationship.

<sup>i</sup> Assay performed with and without S9; positive results reported only with S9.

<sup>j</sup> Dose-response relationship at 500–1250 µg/ml without S9 and 500–4000 µg/ml with S9.

<sup>k</sup> Assay performed with and without S9; no dose-response relationship; increase at single doses.

<sup>l</sup> Positive and negative responses could not be reproduced; no dose-response relationship.

<sup>m</sup> Assay performed with and without S9; positive result only without S9.

<sup>n</sup> Abstract; methods and test concentrations not reported.

<sup>o</sup> Cytotoxicity at maximum dose.

<sup>p</sup> Assay performed with S9.

<sup>q</sup> Assay of urine samples from rats given isopropyl-benzyl alcohol by oral gavage; performed with and without S9.

<sup>r</sup> Assay of urine samples from rats given benzaldehyde by oral gavage, performed with and without S9.

<sup>s</sup> Japanese article, English summary; assay performed with and without S9.

<sup>t</sup> Use of S9 not reported.

<sup>u</sup> Assay performed with and without S9; positive result only with S9.

- <sup>v</sup> Positive only with S9.
- <sup>w</sup> Assay performed without S9; significant increase in mutant fraction at close to toxic doses.
- <sup>x</sup> Japanese article, English summary; positive results without S9; weakly positive results with S9; cytotoxicity at two higher concentrations.
- <sup>y</sup> Assay performed without S9; cytotoxicity at highest concentration.
- <sup>z</sup> Assay of urine samples from rats given cuminaldehyde by gavage; performed with and without S9.
- <sup>1</sup> Assay performed without S9; cytotoxicity at maximum concentration.
- <sup>2</sup> Abstract; methods and test concentration(s) not reported.
- <sup>3</sup> Total incidence of cells with aberrations, 5–9%; performed without S9.
- <sup>4</sup> Assay performed with and without S9; cytotoxicity at three higher concentrations.
- <sup>5</sup> Assay performed without S9 Japanese article, English summary tables.
- <sup>6</sup> Assay performed with and without S9; cytotoxicity at maximum concentration.
- <sup>7</sup> Assay performed without S9 Japanese article, English summary tables.
- <sup>8</sup> In feed.
- <sup>9</sup> By injection.
- <sup>10</sup> By intraperitoneal injection.
- <sup>11</sup> Route of administration not reported.
- <sup>12</sup> By oral gavage; abstract; methods and doses not reported.
- <sup>13</sup> By oral gavage.
- <sup>14</sup> By gavage.
- <sup>15</sup> In diet.



**TABLE 2.2: GENOTOXICITY (*IN VITRO*) EFSA/FGE.20**

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

Table 2.2: Summary of Genotoxicity Data ( <i>in vitro</i> ) EFSA/FGE.20						
Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
(Benzyl alcohol [02.010])	Ames test (preincubation method)	<i>S. typhimurium</i> TA92; TA94; TA98; TA100; TA1535; TA1537	Up to 10,000 µg/plate (6 concentrations)	Negative <sup>1</sup>	(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)	<i>S. typhimurium</i> TA100	1000 µg/plate	Negative <sup>2</sup>	(Ball et al., 1984)	
	Ames test (plate incorporation method)	<i>S. typhimurium</i> TA98; TA100	Not reported	Negative <sup>2</sup>	(Rogan et al., 1986)	
	Ames test (preincubation method)	<i>S. typhimurium</i> TA98; TA100; TA1535; TA1537	6666 µg/plate	Negative <sup>1</sup>	(Mortelmans et al., 1986)	
	Ames test (plate incorporation method)	<i>S. typhimurium</i> TA98; TA100; TA1535; TA1537	3 µmole/plate	Negative <sup>1</sup>	(Florin et al., 1980)	
	Ames test (plate incorporation method)	<i>S. typhimurium</i> TA98; TA100; TA1535; TA1537; TA1538	50,000 µg/plate <sup>4</sup>	Negative <sup>1</sup>	(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)	<i>S. typhimurium</i> TA98; TA100; TA1535; TA1537; TA1538	5 µl/plate	Negative <sup>2</sup>	(Milvy & Garro, 1976)	
	Ames test (plate incorporation method)	<i>S. typhimurium</i> TA98; TA100; TA1535; TA1537	0, 100, 333, 1000, 3333, 6666 µg/plate	Negative <sup>1</sup>	(NTP, 1989)	Valid study in accordance with OECD guideline 471 (except that only four strains were used). Cytotoxicity was reported at the highest concentration tested.
	Ames test (plate incorporation method)	<i>S. typhimurium</i> TA97; TA102	1000 µg/plate	Negative <sup>1</sup>	(Fujita et al., 1992)	
	Ames test (plate incorporation method)	<i>S. typhimurium</i> TA98; TA1535	5 µM/plate	Negative <sup>1</sup>	(Wiessler et al., 1983)	
	Mutation assay	<i>Escherichia coli</i> WP2 <i>uvrA</i>	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	<i>B. subtilis</i> M45 (rec <sup>-</sup> ), H17 (rec <sup>+</sup> )	21 µg/disc	Negative	(Oda et al., 1979)	Study published in Japanese without English abstract. Data extracted from tables. Validity of

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

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
						the study cannot be evaluated.
	Rec assay	<i>B. subtilis</i> M45 (rec <sup>-</sup> ), H17 (rec <sup>+</sup> )	10 µg/disc	Weakly positive	(Kuroda et al., 1984b)	Study published in Japanese with English abstract. Data extracted from figure. Validity of the study cannot be evaluated. Inhibition of growth was reported.
	Rec assay	<i>B. subtilis</i> M45 (rec <sup>-</sup> ), H17 (rec <sup>+</sup> )	20 µl/disc	Weakly positive	(Yoo, 1986)	Study published in Japanese with English abstract. Data extracted from tables. Validity of the study cannot be evaluated. A weak positive result (i.e. 4 mm ≤ D < 8 mm). was reported (D=5 mm). No information on the use of metabolic activation.
	Chromosomal aberration test	Chinese hamster fibroblast cells	1000 µg /ml <sup>4</sup> (three concentrations, max. concentration inducing 50% cell-growth inhibition)	Negative <sup>2</sup>	(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 <sup>1</sup>	(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 <sup>2</sup> Weakly positive <sup>3</sup>	(NTP, 1989)	Valid study in accordance with OECD guideline 473. A positive result was reported only in the presence of S9 at relatively high concentrations of 4000 µg/ml in 3 of 4 tests carried out with harvest times between 12 and 18 hours. No data on cytotoxicity reported.
	Sister chromatid exchange assay	Chinese hamster ovary cells	16 to 5000 µg/ml	Weakly positive	(NTP, 1989)	Valid study in accordance with OECD guideline 479. Dose-related increase in frequency of SCE at concentrations from 500 - 1250 µg/ml (without metabolic activation) and 500 - 4000 µg/ml (with metabolic activation). No data on cytotoxicity reported. Number of chromosomes per cell reduced at 4000 µg/ml with S9.

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

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
	Sister chromatid exchange assay	Chinese hamster ovary cells	16 to 1250 µg/ml <sup>2</sup> 16 to 4000 µg/ml <sup>3</sup>	Weakly positive <sup>1</sup>	(Anderson et al., 1990)	Published summary report including detailed results from studies on 42 compounds tested in various laboratories within the NTP in accordance with OECD guideline 479. Significant increase (20%) in SCE only at the highest doses. No dose-response observed. No second trial using high concentrations to reproduce the positive effects performed. Absence of cytotoxicity reported up to the highest dose.
	Mammalian cell gene mutation test	Mouse lymphoma L5178Y cells	Up to 5000 µg/ml	Questionable	(McGregor et al., 1988a; Myhr et al., 1990)	Published summary report including detailed method and results from study on 72 compounds tested in various laboratories within the NTP in accordance with OECD guideline 476 (however, no colony sizing performed). Positive responses observed in some experiments at concentrations of 3500 and higher. No dose-response was observed. The highest concentration was lethal 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 <sup>3</sup> Positive <sup>2</sup>	(NTP, 1989)	Valid study in accordance with OECD guideline 476. In one of three trials without S9 a positive result (relative mutant fraction ≥1.6) was reported at 4500 µg/ml with relative total growth of 20%. The concentration of 5000 µg/ml was lethal in this trial, whereas in another one of three trials without S9 3500 µg/ml was lethal.
	Mutation assay	<i>E. coli</i> WP2 <i>uvrA</i>	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	<i>E. coli</i> P3478	50 µl/disc	Negative <sup>1</sup>	(Fluck et al., 1976)	
(Benzyl formate [09.077])	Rec assay	<i>B. subtilis</i> M45 (rec <sup>-</sup> ), H17 (rec <sup>+</sup> )	20 µl/disc	Positive	(Yoo, 1986)	Study published in Japanese with English abstract. Data extracted from tables. Validity of

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

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
						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	<i>E. coli</i> WP2 <i>uvrA</i>	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)	<i>S. typhimurium</i> TA98; TA100; TA1535; TA1537	10,000 µg/plate	Negative <sup>1</sup>	(Mortelmans et al., 1986)	
	Ames test (preincubation and plate incorporation method)	<i>S. typhimurium</i> TA98; TA100	5000 µg/plate	Negative <sup>1</sup>	(Schunk et al., 1986)	Cytotoxicity was observed at the three highest doses tested.
	Ames test	<i>S. typhimurium</i> TA98; TA100; TA1535; TA1537	3 µM/plate	Negative <sup>1</sup>	(Florin et al., 1980)	
	Rec assay	<i>B. subtilis</i> M45 (rec <sup>-</sup> ), H17 (rec <sup>+</sup> )	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	<i>B. subtilis</i> M45 (rec <sup>-</sup> ), H17 (rec <sup>+</sup> )	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	<i>E. coli</i> WP2 <i>uvrA</i>	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 <sup>2</sup> Positive <sup>3</sup>	(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 <sup>2</sup>	(McGregor et al., 1988a)	Published summary report including detailed method and results from study on 72 compounds tested in various laboratories

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

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
						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 <sup>2</sup> Positive <sup>3</sup>	(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 <sup>2</sup> Inconclusive <sup>3</sup>	(Honma et al., 1999a)	Published collaborative study on 40 chemicals. Protocol was in accordance with OECD guideline 476, except that no colony sizing was performed. As the results are insufficiently reported, their validity cannot be evaluated. In the presence of S9 metabolic activation one laboratory achieved a statistically significant dose-dependant result, but did not induce mutations greater than three times the spontaneous response. The second laboratory did not obtain a positive response.
	Chromosomal aberration test	Chinese hamster ovary cells	160-1600 µg/ml <sup>2</sup> ; 500-5000 µg/ml <sup>3</sup>	Negative <sup>1</sup>	(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 <sup>1</sup>	(Matsuoka et al., 1996)	Cytotoxicity was reported at the highest concentration tested.
	Sister chromatid exchange assay	Chinese hamster ovary cells	50-500 µg/ml <sup>2</sup> ; 500-5000 µg/ml <sup>3</sup>	Negative <sup>1</sup>	(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 <i>Hep G2</i>	500 µM	Negative <sup>1</sup>	(Kevekordes et al., 2001)	

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

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
(Benzyl propionate [09.132])	Rec assay	<i>B. subtilis</i> M45 (rec <sup>-</sup> ), H17 (rec <sup>+</sup> )	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	<i>S. typhimurium</i> TA98; TA100; TA1535; TA1537	3 µM/plate	Negative <sup>1</sup>	(Florin et al., 1980)	
	Ames test (preincubation and plate incorporation method)	<i>S. typhimurium</i> TA98; TA100	5000 µg/plate	Negative <sup>1</sup>	(Schunk et al., 1986)	Cytotoxicity was observed at the three highest doses tested.
(Benzaldehyde [05.013])	Ames test (plate incorporation method)	<i>S. typhimurium</i> TA98; TA100; TA1535; TA1537; TA1538	37,500 nl/plate <sup>4</sup>	Negative <sup>1</sup>	(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)	<i>S. typhimurium</i> TA98; TA100	50 to 300 µl/plate	Negative <sup>1</sup>	(Rockwell & Raw, 1979)	Assay of urine samples from rats given benzaldehyde by oral gavage.
	Ames test (plate incorporation method)	<i>S. typhimurium</i> TA98; TA100	100 µl/plate	Negative <sup>3</sup>	(Rockwell & Raw, 1979)	Samples assayed prior to administration to rats.
	Ames test	<i>S. typhimurium</i> TA98; TA100; TA2637	2000 mg/plate	Negative <sup>1</sup>	(Nohmi et al., 1985)	Article published in Japanese. Data reported from English summary.
	Ames test	<i>S. typhimurium</i> TA98; TA100; TA1535; TA1537	3 µM/plate	Negative <sup>1</sup>	(Florin et al., 1980)	
	Ames test (preincubation method)	<i>S. typhimurium</i> TA98; TA100; TA1535; TA1537	0, 10, 33, 100, 333, 1000 µg/plate	Negative <sup>1</sup>	(Haworth et al., 1983)	Published summary report including detailed results from studies on 250 compounds tested in various laboratories within the NTP to a large extent in accordance with OECD guideline 471.
	Ames test	<i>S. typhimurium</i> TA100; TA102; TA104	3333 µg/plate	Negative <sup>1</sup>	(NTP, 1990c)	
	Ames test	<i>S. typhimurium</i> TA100	1000 µg/plate	Negative	(Rapson et al., 1980)	The use of metabolic activation was not reported.
	Ames test (preincubation method)	<i>S. typhimurium</i> TA98; TA100	Not reported	Negative <sup>1</sup>	(Sasaki & Endo, 1978)	
	Ames test (preincubation method)	<i>S. typhimurium</i> TA100; TA102; TA104	Not reported	Negative <sup>1</sup>	(Dillon et al., 1992)	
	Ames test (preincubation method)	<i>S. typhimurium</i> TA100	2000 nM/	Negative <sup>1</sup>	(Vamvakas et al., 1989)	
	Ames test	<i>S. typhimurium</i> TA97; TA102	1000 µg/plate	Negative <sup>1</sup>	(Fujita et al., 1992)	

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

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
	(preincubation method)					
	Ames test	<i>S. typhimurium</i> TA98; TA100	0.05 to 500 µg/plate	Negative <sup>1</sup>	(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)	<i>S. typhimurium</i> TA98; TA1535	5 µM/plate	Negative <sup>1</sup>	(Wiessler et al., 1983)	
	Ames test (preincubation method)	<i>S. typhimurium</i> TA97a; TA100; TA102; TA104	Not reported	Negative <sup>1</sup>	(Dillon et al., 1998)	
	Ames test (plate incorporation method)	<i>S. typhimurium</i> TA98; TA1537; TA7001; TA7002; TA7003; TA7004; TA7006; Mix of TA7001–7006 TA7005	1000 µg/ml	Negative <sup>1</sup>  Negative <sup>2</sup> ; Positive <sup>3</sup>	(Gee et al., 1998)	
	Rec assay	<i>B. subtilis</i> M45 (rec <sup>-</sup> ), H17 (rec <sup>+</sup> )	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	<i>B. subtilis</i> M45 (rec <sup>-</sup> ), H17 (rec <sup>+</sup> )	Not reported	Negative <sup>2</sup> Positive <sup>3</sup>	(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 <sup>2</sup> Weakly positive <sup>3</sup>	(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 <sup>2</sup>	(McGregor et al., 1991)	Published summary report including detailed method and results from study on 27 compounds tested in various laboratories within the NTP in accordance with OECD guideline 476 (however, no colony sizing

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

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
						performed). Statistically significant increase in mutant fraction at the highest non-lethal concentration (400 µg/ml) in two experiments. Concentration of 640 and 800 µg/ml were lethal. Thus, significant increases in mutant fraction were close to toxic doses. No dose-response was observed. Since a positive response was observed without S9, no experiment was carried out with S9.
	Mammalian cell gene mutation test	Mouse lymphoma L5178Y +/- cells	600 µg/ml	Negative <sup>2</sup>	(Bigger & Clarke, 1991)	
	Chromosomal aberration test	Chinese hamster cells	0, 800, 1000, 1200 µg/ml	Positive <sup>2</sup> Weak positive <sup>3</sup>	(Sofuni et al., 1985)	Article published in Japanese. Data extracted from English summary and tables. Validity of the study cannot be evaluated. Cytotoxicity was observed at the two maximum concentrations tested. In the presence and in the absence of S9 a positive response was only observed at cytotoxic concentrations. Polyploidization (11%) was reported at non-cytotoxic concentrations.
	Chromosomal aberration test	Chinese hamster ovary cells	50-500 µg/ml <sup>2</sup> ; 160-1600 µg/ml <sup>3</sup>	Negative <sup>1</sup>	(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 <sup>1</sup>	(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 <sup>2</sup> ; 160-1600 µg/ml <sup>3</sup>	Positive <sup>2</sup> Weakly positive <sup>3</sup>	(Galloway et al., 1987)	Published non-GLP study. Doses were selected based on a preliminary assay. Although some details of results are not reported the study is considered valid. Weakly positive results with



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

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
						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 <sup>3</sup>	(Sasaki et al., 1989)	Published non-GLP study of limited quality. Study designed to investigate the influence on spontaneous as well as on mitomycin-induced SCEs. The substance did not influence cell cycle (data not shown) and spontaneous SCEs at the concentrations used. Cytotoxicity was reported at the highest concentration tested.
	Sister chromatid exchange assay	Human lymphocytes	0-2 mM (0-212 µg/ml)	Positive <sup>2</sup>	(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)	<i>S. typhimurium</i> TA98; TA100; TA1535; TA1538	2500 µg/plate	Negative <sup>1</sup>	(Anderson & Styles, 1978)	
	Ames test	<i>S. typhimurium</i> TA98; TA100; TA1535; TA1536	3.6 µg/plate	Negative <sup>1</sup>	(Cotruvo et al., 1977)	
	Ames test (preincubation method)	<i>S. typhimurium</i> TA97; TA98; TA100; TA1535; TA1537	10,000 µg/plate	Negative <sup>1</sup>	(Zeiger et al., 1988)	
	Ames test	<i>S. typhimurium</i> TA100	1000 µg/plate	Negative	(Rapson et al., 1980)	Cytotoxicity was reported at the highest concentration tested.
	Ames test (plate incorporation method)	<i>S. typhimurium</i> TA98; TA100; TA1535; TA1537	1000 µg/plate	Negative <sup>3</sup>	(McCann et al., 1975)	
	Ames test (preincubation method)	<i>S. typhimurium</i> TA92; TA94; TA98; TA100; TA1535; TA1537	Up to 10,000 µg/plate (6 concentrations)	Negative <sup>1</sup>	(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)	<i>S. typhimurium</i> TA98; TA100; TA1535; TA1537; TA1538	100 µg/plate	Negative <sup>2</sup>	(Milvy & Garro, 1976)	
	Ames test (plate incorporation method)	<i>S. typhimurium</i> TA1535; TA1537; TA1538	0.5% (5 mg/ml)	Negative <sup>1</sup>	(FDA, 1975b)	
	Ames test (preincubation method)	<i>S. typhimurium</i> TA98; TA100	100 to 10000 µg/plate	Negative <sup>1</sup>	(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.
	<i>Umu</i> mutation assay	<i>S. typhimurium</i> TA1535/pSK1002	1607 µg/ml	Negative <sup>1</sup>	(Nakamura et al., 1987)	

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

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
	Rec assay (liquid method)	<i>B. subtilis</i> M45 (rec <sup>-</sup> ), H17 (rec <sup>+</sup> )	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	<i>B. subtilis</i> M45 (rec <sup>-</sup> ), H17 (rec <sup>+</sup> )	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	<i>S. cerevisiae</i> D3	0.18%	Negative <sup>1</sup>	(Cotruvo et al., 1977)	
	Mutation assay	<i>S. cerevisiae</i> D4	0.15%	Negative <sup>1</sup>	(FDA, 1975b)	
	Indirect DNA repair test	<i>E. coli</i> PQ37	400 µg/ml	Negative	(Glosnicka & Dziadziuszko, 1986)	Genotoxicity measured as ability to induce β-galactosidase.
	SOS Chromotest	<i>E. coli</i> PQ37	50 µg	Negative <sup>1</sup>	(Kevekordes et al., 1999)	
	Chromosomal aberration test	Chinese hamster fibroblast cells	1500 µg/ml (three concentrations, max. concentration inducing 50% cell-growth inhibition) <sup>4</sup>	Equivocal <sup>2</sup>	(Ishidate et al., 1984)	Published study carried out only in the absence of metabolic activation. Thus, study is not considered valid. Cells were exposed for 24 and 48 hours. Total incidence of cells with aberrations was 8%. Negative response for polyploidization.
	Sister chromatid exchange assay	Human lymphocytes	0-2 mM (0-244 µg/ml)	Negative <sup>2</sup>	(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.
	<i>In vitro</i> Micronucleus assay	Mouse lymphoma L5178Y cells	1000 µg/ml	Negative <sup>1</sup>	(Nesslany & Marzin, 1999)	
(Methyl benzoate [09.725])	Ames test (preincubation method)	<i>S. typhimurium</i> TA97; TA98; TA100; TA1535; TA1537	0 to 666 µg/plate (-S9); 0 to 6666 µg/plate (+S9) (6 concentrations)	Negative <sup>1</sup>	(Zeiger et al., 1992)	Published summary report including detailed results from NTP studies on 311 compounds in accordance with OECD guideline 471.
	Mutation assay	<i>E. coli</i> Sd-4-73	Not reported	Negative <sup>2</sup>	(Szybalski, 1958)	
Methyl 4-methylbenzoate [09.631]	Ames test (preincubation method)	<i>S. typhimurium</i> TA97; TA98; TA100; TA1535; TA1537;	0 to 333 µg/plate (-S9); 0 to 3333	Negative <sup>1</sup>	(Zeiger et al., 1992)	Published summary report including detailed results from NTP studies on 311 compounds in

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

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
			µg/plate (+S9) (6 concentrations)			accordance with OECD guideline 471.
(Isopentyl benzoate [09.755])	Mutation assay	<i>E. coli</i> Sd-4-73	Not reported	Negative <sup>2</sup>	(Szybalski, 1958)	
(4-Isopropylbenzyl alcohol [02.039])	Ames test (plate incorporation method)	<i>S. typhimurium</i> TA98; TA100	100 µl/plate	Negative <sup>3</sup>	(Rockwell & Raw, 1979)	
	Ames test (plate incorporation method)	<i>S. typhimurium</i> TA98; TA100	300 µl/plate	Negative <sup>1</sup>	(Rockwell & Raw, 1979)	Assay of urine samples from rats given isopropylbenzyl alcohol by oral gavage.
(Tolualdehydes (mixed <i>o, m, p</i> ) [05.027])	Ames test (preincubation method)	<i>S. typhimurium</i> TA104	0.8 µM/plate	Negative <sup>1</sup>	(Marnett et al., 1985a)	
	Ames test	<i>S. typhimurium</i> TA98; TA100; TA1535; TA1537	3 µM/plate	Negative <sup>1</sup>	(Florin et al., 1980)	
	Ames test (plate incorporation method)	<i>S. typhimurium</i> TA98; TA100; TA1535; TA1537; TA1538	18,750 µg/plate <sup>4</sup>	Negative <sup>1</sup>	(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)	<i>S. typhimurium</i> TA98; TA100; TA102	0.8 mM/plate	Negative <sup>1</sup>	(Aeschbacher et al., 1989)	
	Ames test (preincubation method)	<i>S. typhimurium</i> TA97; TA100; TA1535; TA1537	666 µg/plate	Negative <sup>1</sup>	(Zeiger et al., 1988)	
	Unscheduled DNA synthesis test	Rat hepatocytes	1000 µg/ml <sup>4</sup>	Negative	(Heck et al., 1989)	Published non-GLP study. No information concerning the number of concentrations tested. Due to the lack of some important details of study design and results the validity of the study cannot be evaluated.
	Mammalian cell gene mutation test	Mouse lymphoma L5178Y cells	300 µg/ml (+S9), 600 µg/ml (-S9) <sup>4</sup>	Negative <sup>1</sup>	(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)	<i>S. typhimurium</i> TA98; TA100	100 µl/plate	Negative <sup>3</sup>	(Rockwell & Raw, 1979)	
	Ames test (plate method)incorporation	<i>S. typhimurium</i> TA98; TA100	300 µl/plate	Negative <sup>1</sup>	(Rockwell & Raw, 1979)	Assay of urine samples from rats given 4-isopropyl benzaldehyde (cuminaldehyde) by gavage.
	<i>Umu</i> test	<i>S. typhimurium</i> TA1535/	1 µmole/ml	Negative	(Miyazawa et al.,	Results indicated that 4-isopropyl

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

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
		pSK1002			2000)	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 <sup>2</sup>	(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)	<i>S. typhimurium</i> TA98; TA100	5000 µg/plate	Negative <sup>2</sup>	(Mikulasova & Bohovicova, 2000)	
	DNA Repair test	<i>E. coli</i> WP2, WP2uvrA, CM611; CM561	2000 µg/ml	Negative	(Mikulasova & Bohovicova, 2000)	
(Salicylic acid [08.112])	Ames test (preincubation method)	<i>S. typhimurium</i> TA98; TA100	100 to 10000 µg/plate	Negative <sup>1</sup>	(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)	<i>S. typhimurium</i> TA98; TA100; TA1535; TA1537	Not reported	Negative <sup>2</sup>	(McCann et al., 1975)	
	Rec assay	<i>B. subtilis</i> M45 (rec <sup>-</sup> ), H17 (rec <sup>+</sup> )	0 to 5000 µg/disc	Weakly positive	(Kuboyama & Fujii, 1992)	Well conducted published non-GLP study with some minor deficiencies (no cytotoxicity data, no detailed data for different concentrations reported) of sufficient quality to be taken into account in the evaluation. A weak positive result (D>2 mm) was observed at concentrations of 2 mg/disc or more. At 5 mg/disc D=4.7 mm.
	Mitotic recombination assay	<i>S. cerevisiae</i> D7	10,000 µg/ml	Negative <sup>2</sup>	(Rosin, 1984)	Published non-GLP study with insufficient report of experimental details and results. Study was carried out only in the absence of metabolic activation and is thus not considered valid. Negative response reported both at neutral and alkaline conditions.
	Mutation assay	<i>S. cerevisiae</i> rad18	Up to 0.1 mM (up to 13.8 µg/ml; 8 concentrations)	Weakly positive	(Zetterberg, 1979)	Published non-GLP study with limited report of experimental details and result. Use of metabolic activation not reported. The validity of the study cannot be evaluated. The dose level tested was clearly cytotoxic. An increase

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

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
						in mutant frequency was not evident until 95-99% of cells were killed.
Ethyl 4-hydroxybenzoate [09.367]	Ames test	<i>S. typhimurium</i> TA98; TA100	Not reported	Negative <sup>1</sup>	(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	<i>B. subtilis</i>	Not reported	Negative <sup>1</sup>	(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 <sup>2</sup> Negative <sup>3</sup>	(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 <sup>2</sup>	(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 <sup>2</sup>	(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 <sup>2</sup>	(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	<i>S. typhimurium</i> TA98; TA100	1000 µg/plate	Negative <sup>1</sup>	(Haresaku et al.,	

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

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
[09.754])					(1985)	
	Ames test (preincubation method)	<i>S. typhimurium</i> TA92; TA94; TA98; TA100; TA1535; TA1537; TA2637	Up to 1000 µg/plate (6 concentrations)	Negative <sup>1</sup>	(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) <sup>4</sup>	Negative <sup>2</sup>	(Ishidate et al., 1984)	Published study carried out only in the absence of metabolic activation. Thus, study is not considered valid. Cells were exposed for 24 and 48 hours. Negative response for chromosomal aberrations and polyploidization.
	Ames test (plate incorporation assay)	<i>S. typhimurium</i> TA100	500 µg/plate	Negative <sup>2</sup>	(Ball et al., 1984)	
(Veratraldehyde [05.017])	Ames test	<i>S. typhimurium</i> TA98; TA100; TA1535; TA1537; TA15378	8000 µg/plate	Negative <sup>1</sup>	(Nestmann et al., 1980)	
	Ames test	<i>S. typhimurium</i> TA98; TA100; TA1535; TA1537; TA1538	8000 µg/plate	Negative <sup>1</sup>	(Douglas et al., 1979)	
	Ames test (preincubation method)	<i>S. typhimurium</i> TA97; TA98; TA100; TA1535; TTA1537	6666 µg/plate	Negative <sup>1</sup>	(Mortelmans et al., 1986)	
	Ames test (plate incorporation method)	<i>S. typhimurium</i> TA98; TA100; TA1535; TA1537; TA1538	1000 µg/plate <sup>4</sup>	Negative <sup>1</sup>	(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)	<i>S. typhimurium</i> TA100; TA102; TA104; TA982; TA1538	Not reported	Negative <sup>1</sup>	(Dillon et al., 1992)	
	Ames test (preincubation protocol)	<i>S. typhimurium</i> TA100; TA102; TA104	33 - 3333 µg/plate	Negative <sup>1</sup>	(Dillon et al., 1998)	
	Mutation assay	<i>S. cerevisiae</i> D7; XV185-14C	Not reported	Negative <sup>2</sup>	(Nestmann & Lee, 1983)	
	Mammalian cell gene mutation test	Mouse lymphoma L5178Y cells	250 to 1800 µg/ml	Positive <sup>1</sup>	(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.

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

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
	Ames test (plate incorporation method)	<i>S. typhimurium</i> TA98; TA100	5000 µg/plate	Negative <sup>2</sup>	(Mikulasova & Bohovicova, 2000)	
	DNA Repair test	<i>E. coli</i> WP2; WP2uvrA; CM611; CM561	2000 µg/ml	Negative	(Mikulasova & Bohovicova, 2000)	
	Unscheduled DNA synthesis test	Rat hepatocytes	100 µg/ml <sup>4</sup>	Negative	(Heck et al., 1989)	Published non-GLP study. No information concerning the number of concentrations tested. Due to the lack of some important details of study design and results the validity of the study cannot be evaluated.
(4-Methoxybenzaldehyde [05.015])	Ames test (preincubation method)	<i>S. typhimurium</i> TA92; TA94; TA98; TA100; TA1535; TA1537; TA2637	Up to 5000 µg/plate (6 concentrations)	Negative <sup>1</sup>	(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	<i>S. typhimurium</i> TA98; TA100	0.05 to 500 µg/plate	Negative <sup>1</sup>	(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)	<i>S. typhimurium</i> TA1537	Up to 5000 µg/plate (6 concentrations)	Negative <sup>1</sup>	(Engelhardt, 1986)	
	Ames test	<i>S. typhimurium</i> TA98; TA100; TA1535; TA1537	408 µg/plate	Negative <sup>1</sup>	(Florin et al., 1980)	
	Ames test (preincubation method)	<i>S. typhimurium</i> TA97; TA102	1000 µg/plate	Negative <sup>1</sup>	(Fujita & Sasaki, 1987)	
	Rec assay	<i>B. subtilis</i> M45 (rec <sup>-</sup> ), H17 (rec <sup>+</sup> )	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	<i>S. typhimurium</i> TA102	5000 µg/plate	Negative <sup>1</sup>	(Müller et al., 1993)	
	Ames test	<i>S. typhimurium</i> 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) <sup>4</sup>	Negative <sup>2</sup>	(Ishidate et al., 1984)	Published study carried out only in the absence of metabolic activation. Thus, study is not considered valid. Cells were exposed for 24 and 48 hours. Negative response for chromosomal aberrations and polyploidization.
	Chromosomal aberration test	Chinese hamster cell line B241	50 nM (0.0068 µg/ml)	Positive <sup>1</sup>	(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

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

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
						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 <sup>2</sup> Positive <sup>2</sup>	(Wangenheim & Bolcsfoldi, 1988)	Published non-GLP study not in accordance with OECD guideline 476 (no metabolic activation, no colony sizing). Important details of method and results are insufficiently reported. This study is not considered valid.
	Ames test	<i>S. typhimurium</i> TA102	5000 µg/plate	Negative <sup>1</sup>	(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 <sup>2</sup>	(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 <sup>2</sup> Positive <sup>2</sup>	(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	<i>E. coli</i> WP2uvrA, <i>trpE</i>	5000 µg/plate	Negative <sup>2</sup>	(Watanabe et al., 1989)	Published non-GLP study with limited report of experimental details and results. Study evaluating the enhancing effect on <i>N'</i> -nitro- <i>N</i> -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 <sup>2</sup>	(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	Sister chromatid	Human lymphocytes	0-2.0 mM (0-273	Positive <sup>2</sup>	(Jansson et al., 1988)	ditto.



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

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
[05.158]	exchange assay		µg/ml)			
	Mammalian cell gene mutation test	Mouse lymphoma L5178Y TK+/- cells	0- 2.5 mM (0- 340 µg/ml)  3 mM (408 µg/ml)	Negative <sup>2</sup>  Positive <sup>2</sup>	(Wangenheim & Bolesfoldi, 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	<i>S. typhimurium</i> TA98; TA100; TA1535; TA1537; TA1538	3600 µg/plate	Negative <sup>2</sup>	(Wild et al., 1983)	
(Methyl 4-methoxybenzoate [09.713])	Paper disk mutation assay	<i>E. coli</i> Sd-4-73	Not reported	Negative <sup>2</sup>	(Szybalski, 1958)	
Gallic acid [08.080]	Ames test (preincubation method)	<i>S. typhimurium</i> TA98; TA100	3000 µg/plate	Negative <sup>1</sup>	(Chen & Chung, 2000)	
	Ames test (preincubation method)	<i>S. typhimurium</i> 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 <sup>1</sup>  Equivocal <sup>1</sup>	(Haworth et al., 1983)	Published summary report including detailed results from studies on 250 compounds tested in various laboratories within the NTP to a large extent in accordance with OECD guideline 471. Results on gallic acid from two different laboratories using different solvent. A negative response was observed in both laboratories with TA98, TA1535 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)	<i>S. typhimurium</i> TA98; TA100; TA1535	5000 µg/plate	Negative <sup>1</sup>	(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	<i>S. typhimurium</i> TA98; TA100; TA1537	15 µM/plate	Negative <sup>1</sup>	(Wang & Klemencic, 1979)	
	Ames test	<i>S. typhimurium</i> TA100	100 µg/plate	Weakly positive <sup>2</sup> Positive <sup>3</sup>	(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	<i>S. typhimurium</i> TA98; TA100	Not reported	Negative <sup>1</sup>	(Sugimura et al., 1976)	

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

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
	Chromosomal aberration test	Chinese hamster ovary cells	50 µg/ml	Positive <sup>1</sup>	(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 <sup>1</sup>	(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 <sup>2</sup>	(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	<i>S. cerevisiae</i> D7	0, 100, 1000 µg/ml	Negative <sup>2</sup> Positive <sup>2</sup>	(Rosin, 1984)	Published non-GLP study with insufficient report of experimental details and results. Study was carried out only in the absence of metabolic activation and is thus not considered valid. Gallic acid did not induce a significant extent of gene conversions under acidic conditions. At neutral pH no convertogenic activity was reported at 100 µg/ml, however, gallic acid was considerably convertogenic at 1000 µg/ml. The presence of catalase completely inhibited the convertogenic activity.gene conversions. Under alkaline conditions (pH 10), the concentration of 100 µg/ml was reported to induce a significant (p <0.01) increase of Trp <sup>+</sup> convertants.
(Vanillin [05.018])	Ames test (plate incorporation method)	<i>S. typhimurium</i> TA98; TA100; TA1535; TA1537; TA1538	10,000 µg/plate <sup>4</sup>	Negative <sup>1</sup>	(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	<i>S. typhimurium</i> TA98; TA100;	5000 µg/plate	Negative <sup>1</sup>	(Pool & Lin, 1982)	

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

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
		TA 1535; TA1537; TA1538				
	Rec assay	<i>B. subtilis</i> M45 (rec <sup>-</sup> ), H17 (rec <sup>+</sup> )	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)	<i>S. typhimurium</i> TA97; TA98; TA100; TA1535; TA1537	10,000 µg/plate	Negative <sup>1</sup>	(Mortelmans et al., 1986)	
	Ames test	<i>S. typhimurium</i> TA98; TA100	0.05 to 1000 µg/plate	Negative <sup>1</sup>	(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	<i>S. typhimurium</i> TA98; TA100; TA1535; TA1537; TA1538	Not reported	Negative <sup>1</sup>	(Nagabhushan & Bhide, 1985)	
	Ames test	<i>S. typhimurium</i> TA92; TA94; TA98; TA100; TA1535; TA1537; TA2637	Up to 10,000 µg/plate (6 concentrations)	Negative <sup>1</sup>	(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	<i>S. typhimurium</i> TA100	1000 µg/plate	Negative	(Rapson et al., 1980)	
	Paper disk mutation assay	<i>E. coli</i> Sd-4-73	Not reported	Negative <sup>2</sup>	(Szybalski, 1958)	
	Ames test (plate incorporation method)	<i>S. typhimurium</i> TA98; TA100	2500 µg/plate	Negative <sup>2</sup>	(Mikulasova & Bohovicova, 2000)	
	DNA Repair test	<i>E. coli</i> WP2; WP2uvrA; CM611; CM561	2000 µg/ml	Negative	(Mikulasova & Bohovicova, 2000)	
	Mutation assay	<i>E. coli</i> CSH26/pYM3; CSH26/pSK 1002	15,215 µg/ml	Negative	(Takahashi et al., 1990)	
	Mitotic recombination assay	<i>S. cerevisiae</i> D7	10,000 µg/ml	Negative <sup>2</sup>	(Rosin, 1984)	Published non-GLP study with insufficient report of experimental details and results. Study was carried out only in the absence of metabolic activation and is thus not considered valid. Negative response reported both at neutral and alkaline conditions.
	Chromosomal aberration test	Chinese hamster cell line B241	5, 20, 40 nM (0.0008, 0.003, 0.006 µg/ml)	Negative	(Kasamaki & Urasawa, 1985)	
	Chromosomal aberration test	Chinese hamster fibroblast cells	1000 µg/ml (three concentrations, max. concentration inducing 50% cell-growth inhibition) <sup>4</sup>	Negative <sup>2</sup>	(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	Chinese hamster V79 lung cells	15,215 -152,150 µg	Negative <sup>2</sup>	(Tamai et al., 1992)	

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

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
	aberration test					
	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 <sup>1</sup>	(Kasamaki et al., 1982)	Published non-GLP study of sufficient quality to be taken into account for the evaluation, although some details of method and results are not reported. Results are only reported for the final concentration at which maximal frequency of aberration was observed without visible cytotoxicity in the treated cells. No significant increase increase of single types of aberrations and of total aberrations.
	Sister chromatid exchange assay	Human lymphocyte cells	0-1.0 mM (0 - 152 µg/ml)	Positive <sup>2</sup>	(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 <sup>2</sup>	(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) <sup>4</sup>	Negative <sup>1</sup>	(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 <sup>4</sup>	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

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

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
						study cannot be evaluated.
	Micronucleus assay	Human hepatoma (Hep-G2) cells	5, 50 µg/ml 500 µg/ml	Negative <sup>2</sup> Positive <sup>2</sup>	(Sanyal et al., 1997)	Published non-GLP study carried out only in the absence of metabolic activation. Thus, the study is not considered valid. A statistically significant increase of spontaneous 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 <sup>1</sup>	(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	<i>S. cerevisiae</i> D7	10,000 µg/ml	Negative <sup>2</sup>	(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	<i>S. typhimurium</i> 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	<i>S. typhimurium</i> TA98; TA100; TA1535; TA1537	366 µg/plate	Negative <sup>1</sup>	(Florin et al., 1980)	
	Chromosomal aberration test	Chinese hamster ovary cells	3000 µg/ml	Positive <sup>1</sup>	(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	<i>S. cerevisiae</i> D7	10,000 µg/ml	Negative <sup>2</sup>	(Rosin, 1984)	Published non-GLP study with insufficient report of experimental details and results. Study was carried out only in the absence of

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

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
						metabolic activation and is thus not considered valid.
(Salicylaldehyde [05.055])	Ames test	<i>S. typhimurium</i> TA98; TA100; TA1535; TA1537	366 µg/plate	Negative <sup>1</sup>	(Florin et al., 1980)	
	Ames test (preincubation method)	<i>S. typhimurium</i> TA98; TA100	Not reported	Negative <sup>1</sup>	(Sasaki & Endo, 1978)	
	Ames test	<i>S. typhimurium</i> TA98; TA100	16 µg/ml	Negative <sup>1</sup>	(Kono et al., 1995)	
	Mutation assay	<i>S. typhimurium</i> TA1535/ pSK1002	111 µg/ml	Negative <sup>1</sup>	(Nakamura et al., 1987)	
	Chromosomal aberration test	CHL/IU cells	Not reported (max. 5 mg/ml)	Positive <sup>1</sup>	(Kusakabe et al., 2002)	Published study in accordance to OECD guideline 473. However, some details on method and results are insufficiently reported. Thus the validity of the study cannot be evaluated. Positive result with minimum effective dose manifesting over 50% cytotoxicity at short-term treatment (6 h, less than 50% cells with chromosomal aberrations without S9, less than 20% cells with chromosomal aberrations with S9). Reduced effect at continuous treatment without S9 (24 h less than 10% cells with chromosomal aberrations). No chromosomal aberrations after 48 h treatment without S9. After 48 h treatment without S9 18% polyploid cells.
	Sister chromatid exchange assay	Human lymphocyte cells	0-0.5 mM (0-61 µg/ml)	Negative <sup>2</sup>	(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	<i>S. typhimurium</i> TA92; TA94; TA98; TA100; TA1535; TA1537; TA2637	Up to 10,000 µg/plate (6 concentrations)	Negative <sup>1</sup>	(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)	<i>S. typhimurium</i> TA97; TA98; TA100; TA1535; TA1537	333.3 µg/plate	Negative <sup>1</sup>	(Mortelmans et al., 1986)	
	Ames test	<i>S. typhimurium</i> TA98; TA100	Not reported	Negative <sup>1</sup>	(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	Hamster lung fibroblast cells	Not reported	Positive <sup>2</sup>	(Kawachi et al.,	ditto.

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

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
	aberration test			Negative <sup>3</sup>	1980b; Kawachi et al., 1980a)	
	Chromosomal aberration test	Chinese hamster fibroblasts	250 µg/ml <sup>4</sup> (three concentrations, max. concentration inducing 50% cell-growth inhibition)	Negative <sup>2</sup>	(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)	<i>S. typhimurium</i> TA98; TA100	100 to 10000 µg/plate	Positive <sup>1</sup>	(Kuboyama & Fujii, 1992)	Published non-GLP study deficient in the report of some details on method and results (no single doses, no data on cytotoxicity reported), however, of sufficient quality to be taken into account in the evaluation. At 100 µg/plate a positive response was observed in strain TA98 in the presence of S9 mix obtained from hamsters a negative response was observed in TA98 in the presence of S9 mix obtained from rat, mouse and guinea pig.
	Rec assay	<i>B. subtilis</i> M45 (rec <sup>-</sup> ), H17 (rec <sup>+</sup> )	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	<i>B. subtilis</i>	Not reported	Negative <sup>1</sup>	(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	<i>B. subtilis</i> M45 (rec <sup>-</sup> ), H17 (rec <sup>+</sup> )	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 <sup>2</sup>	(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 <sup>2</sup>	(Kawachi et al., 1980b; Kawachi et	Published summary report of unpublished extensive screening study. Unusual protocol,

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

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
					al., 1980a)	no details of method and results reported. Thus, the validity of the study cannot be evaluated.
(Butyl vanillyl ether [04.093])	Ames test	<i>S. typhimurium</i> TA98; TA100; TA1535; TA1537	5000 µg/plate	Negative <sup>1</sup>	(Watanabe & Morimoto, 1989c)	
	Mutation assay	<i>E. coli</i> WP2 <i>uvrA</i>	5000 µg/plate	Negative <sup>1</sup>	(Watanabe & Morimoto, 1989c)	
(Ethyl vanillin [05.019])	Ames test	<i>S. typhimurium</i> TA98; TA100; TA1535; TA1537; TA1538	3600 µg/plate	Negative <sup>1</sup>	(Wild et al., 1983)	
	Ames test (preincubation method)	<i>S. typhimurium</i> TA97; TA98; TA100; TA1535; TA1537	8000 µg/plate	Negative <sup>1</sup>	(Mortelmans et al., 1986)	
	Ames test	<i>S. typhimurium</i> TA92; TA94; TA98; TA100; TA1535; TA1537; TA2637	Up to 10,000 µg/plate (six concentrations)	Negative <sup>1</sup>	(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)	<i>S. typhimurium</i> TA97; TA102	1000 µg/plate	Negative <sup>1</sup>	(Fujita & Sasaki, 1987)	
	Ames test	<i>S. typhimurium</i> TA98; TA100; TA1535; TA1537; TA1538	10,000 µg/plate <sup>4</sup>	Negative <sup>1</sup>	(Heck et al., 1989)	Published non-GLP study. No information concerning a possible cytotoxic effect nor on the number of concentrations tested. The test guidelines do not require more than 5 mg/plate. Due to the lack of some important details of study design and results the validity of the study cannot be evaluated.
	Rec assay	<i>B. subtilis</i> M45 (rec <sup>-</sup> ), H17 (rec <sup>+</sup> )	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) <sup>4</sup>	Positive <sup>2</sup>	(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 <sup>2</sup> Weak positive <sup>3</sup>	(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



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

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
						mutant frequency was reported.
	Unscheduled DNA synthesis test	Rat hepatocytes	199 µg/ml <sup>4</sup>	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 <sup>2</sup>	(Jansson et al., 1988)	Published non-GLP study not in accordance with OECD guideline 479 (no metabolic activation). Insufficient report of important details of method and results. This study is not considered valid.
	Sister chromatid exchange assay	Chinese hamster ovary K1 cells	17 µg/ml	Negative	(Sasaki et al., 1987)	
(Ethyl vanillin isobutyrate)	Ames test	<i>S. typhimurium</i> TA98; TA100; TA1535; TA1537; TA1538	5000 µg/plate	Negative <sup>1</sup>	(King & Harnasch, 1997)	
(Piperonyl acetate [09.220])	Ames test (preincubation method)	<i>S. typhimurium</i> TA97; TA98; TA100; TA1535; TA1537	3333 µg/plate	Negative <sup>1</sup>	(Mortelmans et al., 1986)	
	Ames test	<i>S. typhimurium</i> TA98; TA100; TA1535; TA1537; TA1538	3600 µg/plate	Negative <sup>1</sup>	(Wild et al., 1983)	
(Piperonal [05.016])	Modified Ames test	<i>S. typhimurium</i> TA98; TA100; TA1535; TA1537; TA1538 <i>E. coli</i> WP2uvrAtrp <sup>-</sup>	0, 300, 600, 1200, 2400 µg/plate	Negative <sup>1</sup>	(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)	<i>S. typhimurium</i> TA98; TA100; TA1535; TA1537; TA1538	10,000 µg/plate <sup>4</sup>	Negative <sup>1</sup>	(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	<i>S. typhimurium</i> TA98; TA100	0.05 to 5000 µg/plate	Negative <sup>1</sup>	(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	<i>S. typhimurium</i> TA98; TA100; TA1537; TA1538	5000 µg/plate	Negative <sup>1</sup>	(White et al., 1977)	
	Ames test (preincubation method)	<i>S. typhimurium</i> TA98; TA100; TA1535; TA1537	0, 10, 33, 100, 333, 1000 µg/plate	Negative <sup>1</sup>	(Haworth et al., 1983)	Published summary report including detailed results from studies on 250 compounds tested in various laboratories within the NTP to a

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

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
						large extent in accordance with OECD guideline 471.
	Rec assay	<i>B. subtilis</i> M45 (rec <sup>-</sup> ), H17 (rec <sup>+</sup> )	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	<i>B. subtilis</i> M45 (rec <sup>-</sup> ), H17 (rec <sup>+</sup> )	5000 µg/disc	Positive <sup>2</sup>	(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 <sup>1</sup>	(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 <sup>4</sup>	Negative <sup>1</sup>	(Heck et al., 1989)	Published non-GLP study. Some important details of study design and results are not reported. Thus, the validity of the study cannot be evaluated.
	Unscheduled DNA synthesis test	Rat hepatocytes	10 to 502 µg/ml	Positive	(Heck et al., 1989)	Published non-GLP study. No information concerning the number of concentrations tested. Due to the lack of some important details of study design and results the validity of the study cannot be evaluated.

NR = not reported

1 With and without S9 metabolic activation.

2 Without S9 metabolic activation.

3 With S9 metabolic activation.

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

**TABLE 2.3: GENOTOXICITY (IN VIVO) EFSA/FGE.20**

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

Table 2.3: Summary of Genotoxicity Data (in vivo) EFSA / FGE.20							
Chemical Name [FL-no]	Test System	Test Object	Route	Dose	Result	Reference	Comments
(Benzyl alcohol [02.010])	<i>In vivo</i> Sex- linked recessive lethal mutations (SLRL)	<i>D. melanogaster</i>	Diet	5000 ppm	Negative	(Foureman et al., 1994)	
	<i>In vivo</i> SLRL	<i>D. melanogaster</i>	Injection	8000 ppm	Negative	(Foureman et al., 1994)	
	<i>In vivo</i> Micronucleus test	Mouse bone marrow cells	IP injection	200 mg/kg bw	Negative	(Hayashi et al., 1988)	
	<i>In vivo</i> 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.
	<i>In vivo</i> Replicative DNA synthesis test	Mouse hepatocytes	Oral gavage	800 mg/kg	Negative	(Miyagawa et al., 1995)	
	<i>In vivo</i> Replicative DNA synthesis test	Rat hepatocytes	Oral or SC injection	600 mg/kg	Negative	(Uno et al., 1994)	
(Benzyl acetate [09.014])	<i>In vivo</i> SLRL	<i>D. melanogaster</i>	Diet	300 ppm	Negative	(NTP, 1993d; Foureman et al., 1994)	
	<i>In vivo</i> SLRL	<i>D. melanogaster</i>	Injection	20,000 ppm	Negative	(NTP, 1993d; Foureman et al., 1994)	
	<i>In vivo</i> Sister chromatid exchange assay	Mouse bone marrow cells	IP injection	1700 mg/kg bw	Negative	(NTP, 1993d)	
	<i>In vivo</i> 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.
	<i>In vivo</i> Micronucleus test	Mouse bone marrow cells	3 IP injection with 24 h	0, 312, 625 and 1250 mg/kg	Negative	(NTP, 1993d; Shelby et al., 1993)	Test substance same batch as NTP chronic bioassays. Study in compliance with

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

Chemical Name [FL-no]	Test System	Test Object	Route	Dose	Result	Reference	Comments
			intervals	bw			OECD guideline. GLP not stated. Micronuclei were determined at 24 h after the last dose. A dose-related decrease in PCE/NCE ratio was observed. The study is considered valid.
	<i>In vivo</i> Micronucleus test	Mouse erythrocytes	Dietary exposure for 13 weeks	0 to 50,000 ppm (equal to 0 to 7900 mg/kg bw/day for males and 0 to 9400 mg/kg bw/day for females)	Negative	(NTP, 1993d)	Test substance same batch as NTP chronic bioassays. In life phase under GLP; for determination of genotoxic effects. GLP not specified. Test in compliance with OECD guideline. The test is considered valid, but of limited relevance because no change in PCE/NCE ratio was observed.
	<i>In vivo</i> 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.
	<i>In vivo</i> Unscheduled DNA synthesis test	Rat pancreatic cells	Oral gavage	1000 mg/kg bw	Negative	(Steinmetz & Mirsalis, 1984)	Only abstract available. Non guideline test. Validity cannot be assessed.
	<i>In vivo</i> 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 hr post dosing. The study is of limited validity.
	<i>In vivo</i> Comet assay	Mouse/ Rat	Oral	1600 mg/kg (mouse); 1200 mg/kg (rat)	Positive	(Sekihashi et al., 2002)	Non-GLP and non-guideline test; but in compliance with recommended protocols. Some important details of method and results insufficiently reported. No toxicity data reported. The administered dose was 0.5 x LD50. Sampling time was 3, 8 and 24 hours after dosing. Positive result reported in mice for stomach, colon, kidney, urinary bladder and brain, in rats for stomach, colon, liver, kidney, urinary bladder, lung. After 24 h no significant effect in mice, significant effects in rat only in lung and kidney. The study is of limited validity.
(Benzaldehyde [05.013])	<i>In vivo</i> SLRL	<i>D. melanogaster</i>	Diet	1150 ppm	Negative	(Woodruff et al., 1985)	

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

Chemical Name [FL-no]	Test System	Test Object	Route	Dose	Result	Reference	Comments
	<i>In vivo</i> SLRL	<i>D. melanogaster</i>	Injection	2500 ppm	Negative	(Woodruff et al., 1985)	
(Salicylic acid [08.112])	<i>In vivo</i> Chromosomal aberration assay	Mouse bone marrow cells	IP injection gavage	0, 50, 100, 200 mg/kg 0, 350 mg/kg	Negative Negative	(Giri et al., 1996)	Published study widely in accordance with OECD guideline 475 and well reported (except that only males were tested, only one sampling time was chosen and signs of toxicity were not reported). Oral and i.p. dose were selected to be 1/3 and 1/5 of the reported oral LD50.
	<i>In vivo</i> 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]	<i>In vivo</i> 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])	<i>In vivo</i> Basc test Micronucleus test	<i>D. melanogaster</i>	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.
	<i>In vivo</i> 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]	<i>In vivo</i> Medium-term rat liver bioassay	Male rats initiated with i.p. 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])	<i>In vivo</i> Micronucleus test	Male BDF1 mice	Oral gavage	500 mg/kg bw	Negative	(Inouye et al., 1988)	Published non-GLP study not in accordance with OECD guideline 474 (smaller group size, only males tested, no toxicity data reported, single dose level used, no negative control, effect on PCE/NCE ratio not reported). Induction of micronuclei in mitomycin-treated mice was suppressed by post-treatment with vanillin due to an anticlastogenic effect.

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

Chemical Name [FL-no]	Test System	Test Object	Route	Dose	Result	Reference	Comments
							Vanillin itself did not induce micronucleated PCEs (vanillin control group without mitomycin-treatment, six sampling times from 5 to 65 h).
(Salicylaldehyde [05.055])	<i>In vivo</i> Spot test	<i>D. melanogaster</i> BINS <i>D. melanogaster</i> 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])	<i>In vivo</i> Basc test	<i>D. melanogaster</i>	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.
	<i>In vivo</i> 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.
	<i>In vivo</i> 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])	<i>In vivo</i> Basc test	<i>D. melanogaster</i>	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.
	<i>In vivo</i> 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])	<i>In vivo</i> 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

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

Chemical Name [FL-no]	Test System	Test Object	Route	Dose	Result	Reference	Comments
							late fetal deaths.
	<i>In vivo</i> 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 3: SUMMARY OF SAFETY EVALUATION TABLES**

**TABLE 3.1: SUMMARY OF SAFETY EVALUATION OF 36 BENZYL DERIVATIVES (JECFA, 2002A)**

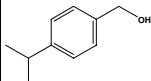
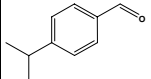
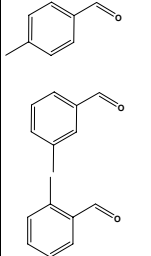
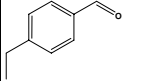
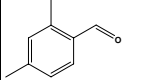
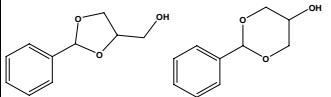
Table 3.1: Summary of Safety Evaluation of 36 JECFA Evaluated Benzyl Derivatives (JECFA, 2002a)							
FL-no JECFA-no	EU Register name	Structural formula	EU MSDI 1) US MSDI ( $\mu\text{g}/\text{capita}/\text{day}$ )	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	EFSA conclusion 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)	6)	6)
05.022 868	4-Isopropylbenzaldehyde		110 1	Class I A3: Intake below threshold	4)	6)	6)
05.027 866	Tolualdehyde		230 1100	Class I A3: Intake below threshold	4)	6)	Composition of mixture to be specified.
05.068 865	4-Ethylbenzaldehyde		0.37 6	Class I A3: Intake below threshold	4)	6)	6)
05.110 869	2,4-Dimethylbenzaldehyde		0.37 0.1	Class I A3: Intake below threshold	4)	6)	6)
06.002 838	5-Hydroxy-2-phenyl-1,3-dioxane		13 300	Class I A3: Intake below threshold	4)	6)	Stereoisomeric composition and composition of mixture to be specified.



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

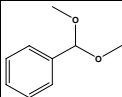
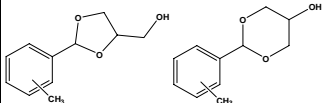
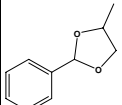
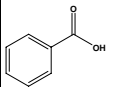
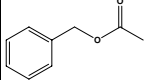
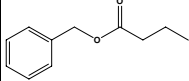
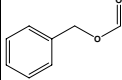
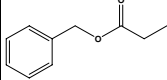
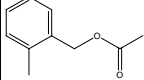
FL-no JECFA-no	EU Register name	Structural formula	EU MSDI 1) US MSDI ( $\mu\text{g}/\text{capita}/\text{day}$ )	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	EFSA conclusion on the named compound (Procedure steps, intake estimates, NOAEL, genotoxicity)	EFSA conclusion on the material of commerce
06.003 837	alpha,alpha-Dimethoxytoluene		0.12 0.3	Class I A3: Intake below threshold	4)	6)	6)
06.012 867	Tolualdehyde glyceryl acetal		0.012 1	Class I A3: Intake below threshold	4)	6)	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)	6)	Stereoisomeric composition to be specified.
08.021 850	Benzoic acid		34 340	Class I A3: Intake below threshold	4)	6)	6)
09.014 23	Benzyl acetate		1200 850	Class I A3: Intake below threshold	4)	6)	6)
09.051 843	Benzyl butyrate		100 290	Class I A3: Intake below threshold	4)	6)	6)
09.077 841	Benzyl formate		35 17000	Class I A3: Intake below threshold	4)	6)	6)
09.132 842	Benzyl propionate		41 99	Class I A3: Intake below threshold	4)	6)	6)
09.294 863	2-Methylbenzyl acetate		ND 3	Class I A3: Intake below threshold	4)	7)	7) Composition of mixture to be specified.

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

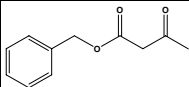
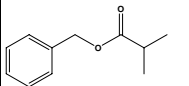
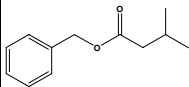
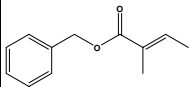
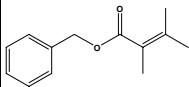
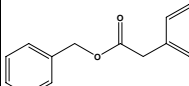
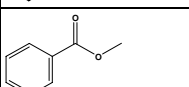
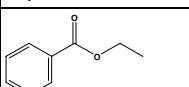
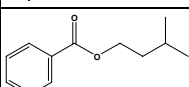
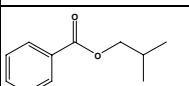
FL-no JECFA-no	EU Register name	Structural formula	EU MSDI 1) US MSDI ( $\mu\text{g}/\text{capita}/\text{day}$ )	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	EFSA conclusion on the named compound (Procedure steps, intake estimates, NOAEL, genotoxicity)	EFSA conclusion on the material of commerce
09.406 848	Benzyl 3-oxobutyrate		0.24 0.07	Class I A3: Intake below threshold	4)	6)	6)
09.426 844	Benzyl isobutyrate		13 21	Class I A3: Intake below threshold	4)	6)	6)
09.458 845	Benzyl isovalerate		12 19	Class I A3: Intake below threshold	4)	6)	6)
09.494 846	Benzyl 2-methylcrotonate		0.012 0.03	Class I A3: Intake below threshold	4)	6)	6)
09.508 847	Benzyl 2,3- dimethylcrotonate		0.012 1	Class I A3: Intake below threshold	4)	6)	6)
09.705 849	Benzyl phenylacetate		4.3 57	Class I A3: Intake below threshold	4)	6)	6)
09.725 851	Methyl benzoate		40 230	Class I A3: Intake below threshold	4)	6)	6)
09.726 852	Ethyl benzoate		96 110	Class I A3: Intake below threshold	4)	6)	6)
09.755 857	Isopentyl benzoate		96 33	Class I A3: Intake below threshold	4)	6)	Composition of mixture to be specified.
09.757 856	Isobutyl benzoate		0.37 1	Class I A3: Intake below threshold	4)	6)	6)

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

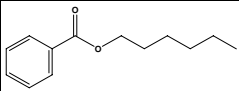
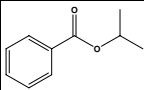
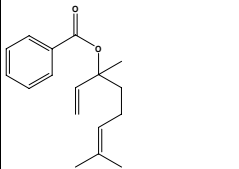
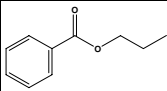
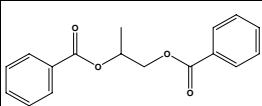
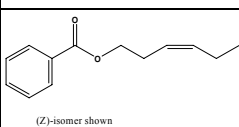
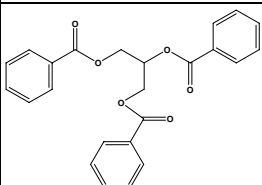
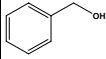
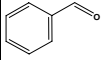
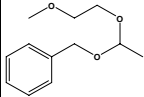
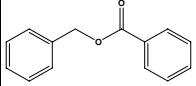
FL-no JECFA-no	EU Register name	Structural formula	EU MSDI 1) US MSDI ( $\mu\text{g}/\text{capita}/\text{day}$ )	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	EFSA conclusion on the named compound (Procedure steps, intake estimates, NOAEL, genotoxicity)	EFSA conclusion on the material of commerce
09.768 854	Hexyl benzoate		320 1	Class I A3: Intake below threshold	4)	6)	6)
09.770 855	Isopropyl benzoate		0.0037 0.3	Class I A3: Intake below threshold	4)	6)	6)
09.771 859	Linalyl benzoate		8.4 2	Class I A3: Intake below threshold	4)	6)	Stereoisomeric composition to be specified.
09.776 853	Propyl benzoate		0.012 0.3	Class I A3: Intake below threshold	4)	6)	6)
09.803 862	Propylene glycol dibenzoate		ND 14	Class I A3: Intake below threshold	4)	7)	7) Stereoisomeric composition to be specified.
09.806 858	Hex-3-enyl benzoate	 <small>(Z)-isomer shown</small>	6.7 0.1	Class I A3: Intake below threshold	4)	6)	Stereoisomeric composition to be specified.
09.812 861	Glyceryl tribenzoate		ND 49	Class I A3: Intake below threshold	4)	7)	7)

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 ( $\mu\text{g}/\text{capita}/\text{day}$ )	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
02.010 25	Benzyl alcohol		13000 37000	Class I A3: Intake above threshold, A4: Endogenous	4)	6)	6)
05.013 22	Benzaldehyde		7900 36000	Class I A3: Intake above threshold, A4: Endogenous	4)	6)	6)
06.019 840	1-Benzyloxy-1-(2-methoxyethoxy)ethane		ND 1	Class I B3: Intake below threshold, B4: Adequate NOAEL exists	4)	7)	7) Stereoisomeric composition and composition of mixture to be specified.
09.727 24	Benzyl benzoate		1600 4200	Class I A3: Intake above threshold, A4: Endogenous	4)	6)	6)

<sup>1)</sup> EU MSDI: Amount added to food as flavour in (kg / year)  $\times 10E9$  / (0.1 x population in Europe (= 375 x 10E6) x 0.6 x 365) =  $\mu\text{g}/\text{capita}/\text{day}$ .

<sup>2)</sup> Thresholds of concern: Class I = 1800, Class II = 540, Class III = 90  $\mu\text{g}/\text{person}/\text{day}$ .

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

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

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

<sup>6)</sup> No safety concern at estimated level of intake as flavouring substance based on the MSDI approach.

<sup>7)</sup> MSDI based on USA production figure.

ND No data available.

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

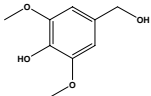
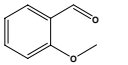
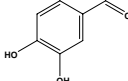
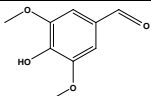
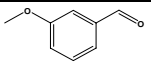
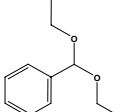
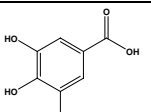
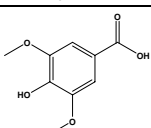
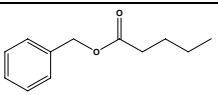
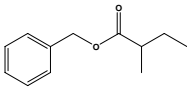
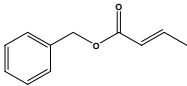
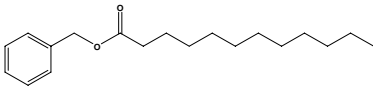
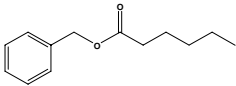
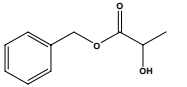
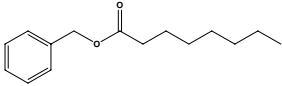
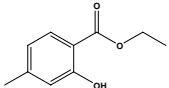
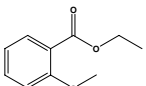
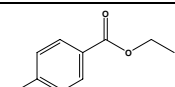
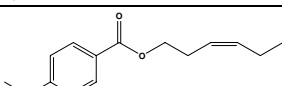
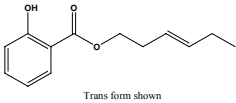
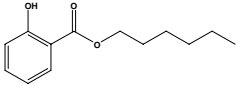
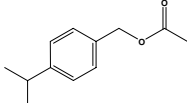
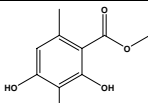
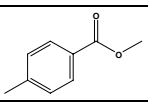
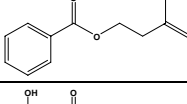
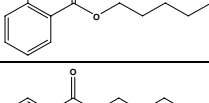
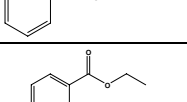
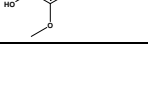
Table 3.2: Summary of Safety Evaluation Applying the Procedure (based on intakes calculated by the MSDI approach)							
FL-no	EU Register name	Structural formula	MSDI 1) (µg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	Outcome on the material of commerce [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	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)	

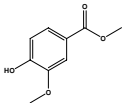
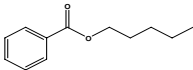
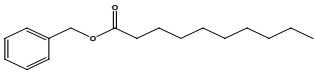
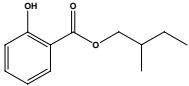
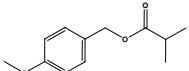
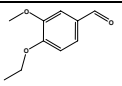
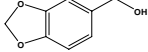
Table 3.2: Summary of Safety Evaluation Applying the Procedure (based on intakes calculated by the MSDI approach)

FL-no	EU Register name	Structural formula	MSDI 1) ( $\mu\text{g}/\text{capita}/\text{day}$ )	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
09.313	Benzyl 2-methylbutyrate		7.3	Class I A3: Intake below threshold	4)	7)	
09.314	Benzyl crotonate		0.37	Class I A3: Intake below threshold	4)	6)	
09.315	Benzyl dodecanoate		0.13	Class I A3: Intake below threshold	4)	6)	
09.316	Benzyl hexanoate		0.75	Class I A3: Intake below threshold	4)	6)	
09.317	Benzyl lactate		0.91	Class I A3: Intake below threshold	4)	7)	
09.318	Benzyl octanoate		0.12	Class I A3: Intake below threshold	4)	6)	
09.362	Ethyl 2-hydroxy-4-methylbenzoate		0.0012	Class I A3: Intake below threshold	4)	6)	
09.363	Ethyl 2-methoxybenzoate		5.5	Class I A3: Intake below threshold	4)	6)	
09.367	Ethyl 4-hydroxybenzoate		10	Class I A3: Intake below threshold	4)	6)	
09.560	Hex-3(cis)-enyl anisate		0.12	Class I A3: Intake below threshold	4)	6)	

**Table 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
09.570	Hex-3-enyl salicylate	 <small>Trans form shown</small>	0.13	Class I A3: Intake below threshold	4)	7)	
09.581	Hexyl salicylate		0.018	Class I A3: Intake below threshold	4)	6)	
09.611	4-Isopropylbenzyl acetate		0.012	Class I A3: Intake below threshold	4)	6)	
09.623	Methyl 2,4-dihydroxy-3,6-dimethylbenzoate		0.012	Class I A3: Intake below threshold	4)	6)	
09.631	Methyl 4-methylbenzoate		0.0012	Class I A3: Intake below threshold	4)	6)	
09.656	3-Methylbut-3-enyl benzoate		0.12	Class I A3: Intake below threshold	4)	6)	
09.762	Pentyl salicylate		0.24	Class I A3: Intake below threshold	4)	6)	
09.779	Butyl benzoate		3.7	Class I A3: Intake below threshold	4)	6)	
09.798	Ethyl vanillate		0.024	Class I A3: Intake below threshold	4)	6)	

**Table 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
09.799	Methyl vanillate		0.011	Class I A3: Intake below threshold	4)	6)	
09.825	Pentyl benzoate		1.1	Class I A3: Intake below threshold	4)	6)	
09.835	Benzyl decanoate		0.35	Class I A3: Intake below threshold	4)	6)	
09.852	2-Methylbutyl 2-hydroxybenzoate		0.011	Class I A3: Intake below threshold	4)	7)	
09.895	4-Methoxybenzyl-2-methylpropionate		0.37	Class I A3: Intake below threshold	4)	6)	
05.066	4-Ethoxy-3-methoxybenzaldehyde		1.2	Class II A3: Intake below threshold	4)	6)	
02.205	Piperonyl alcohol		0.011	Class III A3: Intake below threshold	4)	6)	

<sup>1)</sup> 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.

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

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

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

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

<sup>6)</sup> 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).

<sup>7)</sup> 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.

<sup>8)</sup> No conclusion can be drawn due to lack of information on the purity of the material of commerce



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