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Flavouring Group Evaluation 52 (FGE.52): Consideration of hydroxy- and alkoxy-substituted benzyl derivatives evaluated by JECFA (57th meeting) structurally related to benzyl alcohols, benzaldehydes, a related acetal, benzoic acids, and related esters evaluated by EFSA in FGE.20 (2005) (Commission Regulation (EC) No 1565/2000 of 18 July 2000)

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

(Question No EFSA-Q-2008-032C)

(Adopted on 3 July 2007)

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 Scientific 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 44 hydroxy- and alkoxy-substituted benzyl derivatives evaluated by JECFA (57th 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 44 substances in the JECFA flavouring group of hydroxy- and alkoxy-substituted 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.

Further two substances were evaluated by the JECFA in this group but are not in the Register (2-methoxybenzoic acid and ethyl vanillin propylene glycol acetal) and therefore not dealt with in this consideration.

The Panel agrees with the application of the Procedure as performed by the JECFA for 43 of the 44 substances considered in this FGE. For butyl 4-hydroxybenzoate [FL-no: 09.754] additional data would be required before it can be evaluated as a flavouring substance, using the Procedure.

For eight substances [FL-no: 04.093, 08.071, 08.076, 08.092, 09.145, 09.754, 09.807 and 16.075] 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 44 substances use levels are needed to calculate the modified Theorical 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 44 JECFA evaluated substances can be applied to the materials of commerce, it is necessary to consider the available specifications:

Adequate specifications are available for 40 of the 44 JECFA evaluated substances. For four substances [FL-no: 06.132, 09.087, 09.751 and 09.763] further information on specifications are requested.

Thus, for 12 substances [FL-no: 04.093, 06.132, 08.071, 08.076, 08.092, 09.087, 09.145, 09.751, 09.754, 09.763, 09.807 and 16.075] the Panel has reservations (only USA production volumes available and/or missing data on specifications and/or isomerism/composition). For one of these 12 substances, butyl 4-hydroxybenzoate [FL-no: 09.754], the Panel concluded that additional data would be required before it can be evaluated as a flavouring substance using the Procedure. For the remaining 32 JECFA evaluated hydroxy- and alkoxy-substituted benzyl derivatives [FL-no: 02.128, 02.165, 02.213, 04.094, 05.015, 05.016, 05.017, 05.018, 05.019, 05.047, 05.055, 05.056, 05.091, 08.040, 08.043, 08.112, 09.019, 09.035, 09.058, 09.220, 09.430, 09.706, 09.713, 09.714, 09.748, 09.749, 09.750, 09.752, 09.753, 09.796, 09.811 and 09.933] the Panel agrees with the JECFA conclusion “No safety concern at estimated levels of intake as flavouring substance” based on the MSDI approach.

KEYWORDS

Hydroxy- and alkoxy-substituted benzyl derivatives, JECFA 57th meeting, FGE.20, butyl 4-hydroxybenzoate, butyl paraben.

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BACKGROUND

Regulation (EC) No 2232/96 of the European Parliament and the Council (EC, 1996) lays down a procedure for the establishment of a list of flavouring substances, the use of which will be authorised to the exclusion of all 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 55th, 57th, 59th, 61st, 63rd and 65th 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.

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 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 put 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 65th meeting considered "how to improve the identification and assessment of flavouring agents, for which the MSDI estimates may be substantially lower than the dietary exposures that would be estimated from the anticipated average use levels in foods" (JECFA, 2006c).

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

As information on use levels for the flavouring substances has not been requested by the JECFA or 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 46 flavouring substances consisting of hydroxy- and alkoxy-substituted benzyl derivatives (JECFA, 2002b). Two of these are not in the Register (2-methoxybenzoic acid and ethyl vanillin propylene glycol acetal). This consideration will therefore only deal with 44 JECFA evaluated substances. Butyl 4-hydroxybenzoate [FL-no: 09.754] has been evaluated as a flavouring substance by the JECFA at its 59th meeting where it was concluded that butyl 4-hydroxybenzoate was of no safety concern at the current intakes as a flavouring substance (JECFA, 2003a). In 2006 the JECFA has also considered butyl 4-hydroxybenzoate as a food additive and concluded that: "*The reproductive toxicity of the parabens appears to increase with increasing length of the alkyl chain, and there are specific data showing adverse reproductive effects in male rats of butyl paraben. In view of this and the fact that butyl paraben was not included in the group ADI for parabens, the Committee decided to withdraw the specifications for this substance"* (JECFA, 2007b).

1.1.2. EFSA Considerations

The Panel concluded that all the 44 substances in the JECFA flavouring group of hydroxy- and alkoxy-substituted 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).

1.2. Isomers

1.2.1. JECFA Status

The substance [FL-no: 06.132] in the group of JECFA evaluated hydroxy- and alkoxy- substituted benzyl derivatives has two chiral centres.

1.2.2. EFSA Considerations

Information is lacking about the stereoisomerism for [FL-no: 06.132].

1.3. Specifications

1.3.1. JECFA Status

JECFA specifications are available for all 44 substances (JECFA, 2001c; JECFA, 2002d). See Table 1. For one substance, p-Anisyl formate [FL-no: 09.087], the JECFA has reservations. Although a JECFA specification is available for butyl 4-hydroxybenzoate as a flavouring substance (JECFA, 2002d), the JECFA has withdrawn the specification for butyl 4-hydroxybenzoate as a food additive at its 67th meeting in 2006 (see Section 1.1.1).

1.3.2. EFSA Considerations

The available specifications are considered adequate except that information on stereoisomerism is missing for [FL-no: 06.132], see Section 1.2. For [FL-no: 09.087 and 09.751] further information on the composition is requested and for [FL-no: 09.763] an ID test is missing.

2. Intake Estimations

2.1. JECFA Status

For 36 substances evaluated through the JECFA Procedure intake data are available for the EU, see Table 3.1. For the eight remaining substances [FL-no: 04.093, 08.071, 08.076, 08.092, 09.145, 09.754, 09.807 and 16.075] production figures are only available for the USA.

2.2. EFSA Considerations

As production figures are only available for the USA for eight substances, MSDI values for the EU cannot be calculated for these [FL-no: 04.093, 08.071, 08.076, 08.092, 09.145, 09.754, 09.807 and 16.075].

3. Genotoxicity Data

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

In vitro

The hydroxy- and alkoxy-substituted benzyl derivatives were not mutagenic in standard assays for reverse mutation with plate incorporation and/or preincubation in *Salmonella typhimurium* strains TA92, TA94, TA97, TA98, TA100, TA102, TA104, TA1535, TA1537, TA1538, and TA2637, at concentrations ranging up to those that are cytotoxic or at maximum test concentrations recommended by ICH/OECD, in the absence and presence of metabolic activation (S9) (White et al., 1977; Sasaki & Endo, 1978; Douglas et al., 1980; Florin et al., 1980; Kawachi et al., 1980a; Kawachi et al., 1980b; Nestmann et al., 1980; Rapson et al., 1980); and (Kasamaki et al., 1982; Pool & Lin, 1982; Sekizawa & Shibamoto, 1982; Haworth et al., 1983; Wild et al., 1983; Ball et al., 1984; Ishidate et al., 1984; Haresaku et al., 1985; Nagabhushan & Bhide, 1985); and (Mortelmans et al., 1986; Fujita & Sasaki, 1987; Heck et al., 1989; Watanabe & Morimoto, 1989c; Dillon et al., 1992; Müller et al., 1993; King & Harnasch, 1997; Dillon et al., 1998)). An assay for mutation in *S.*

typhimurium strain TA1535/pSK1002, in which *umu* gene expression was the end-point, gave negative results with salicylaldehyde [FL-no: 05.055] (Nakamura et al., 1987). Assays for mutation or DNA repair in *Escherichia coli* strains WP2 *uvrA*, WP2s, CSH26/pYM3, CSH26/pSK1002, PQ37, and Sd-4-73 with methyl anisate [FL-no: 09.713], vanillyl alcohol [FL-no: 02.213], vanillin [FL-no: 05.018], vanillyl butyl ether [FL-no: 04.093], and piperonal [FL-no: 05.016] (Szybalski, 1958; Sekizawa & Shibamoto, 1982; Ohshima et al., 1989; Watanabe & Morimoto, 1989c; Takahashi et al., 1990), and *Saccharomyces cerevisiae* strains D3, D4, D7, and XV185-14C with veratraldehyde [FL-no: 05.017] (Nestmann & Lee, 1983) also gave negative results.

Mixed results were obtained with the hydroxy- and alkoxy-substituted benzyl derivatives in the assay for DNA repair in *Bacillus subtilis* strains H17 and M45 for *rec* mutation, both positive and negative results being reported for piperonal [FL-no: 05.016] and negative results for *para*-methoxybenzaldehyde [FL-no: 05.015], vanillin [FL-no: 05.018], ethyl vanillin [FL-no: 05.019], and methyl salicylate [FL-no: 09.749] (Oda et al., 1979; Kawachi et al., 1980a; Kawachi et al., 1980b; Sekizawa & Shibamoto, 1982). Some of the differences in the results were apparently laboratory-specific. Oda et al. (Oda et al., 1979) reported only negative results with some of the same compounds; however, the studies were reported in Japanese with English abstracts and could not be fully evaluated for methodological or other differences. It was not clear whether cytotoxicity was a factor in the results. No mutations were observed in silkworms treated with methylsalicylate [FL-no: 09.749] (Kawachi et al., 1980a; Kawachi et al., 1980b).

Both negative and positive results were obtained in assays in isolated mammalian cells with some of the hydroxy- and alkoxy-substituted benzyl derivatives. Mixed results were reported with *para*-methoxybenzaldehyde and vanillin in assays for sister chromatid exchange in several Chinese hamster cell lines and in human lymphocytes (Jansson et al., 1986; Jansson & Zech, 1987; Sasaki et al., 1987; Jansson et al., 1988). Negative results were obtained in this assay with ethyl vanillin [FL-no: 05.019], salicylaldehyde [FL-no: 05.055], and methyl salicylate [FL-no: 09.749] (Kawachi et al., 1980a; Kawachi et al., 1980b; Sasaki et al., 1987; Jansson et al., 1988). Similarly, mixed results were obtained in assays for chromosomal aberration in Chinese hamster and human cell lines with *para*-methoxybenzaldehyde [FL-no: 05.015], vanillin [FL-no: 05.018], ethyl vanillin [FL-no: 05.019], piperonal [FL-no: 05.016], and methyl salicylate [FL-no: 09.749] (Kawachi et al., 1980a; Kawachi et al., 1980b; Kasamaki et al., 1982; Ishidate et al., 1984; Kasamaki & Urasawa, 1985; Jansson & Zech, 1987). The results in the assays for sister chromatid exchange and chromosomal aberrations were generally obtained independently of the presence or absence of metabolic activation. Mixed, but mostly positive, results were obtained with veratraldehyde [FL-no: 05.017], *para*-methoxybenzaldehyde [FL-no: 05.015], and ethyl vanillin [FL-no: 05.019] in the assay for forward mutation in L5178Y mouse lymphoma cells, both with and without metabolic activation (Garberg et al., 1988; Wangenheim & Bolcsfoldi, 1988; Heck et al., 1989). Vanillin [FL-no: 05.018] and piperonal [FL-no: 05.016] were inactive in this assay (Heck et al., 1989). Vanillin weakly induced micronuclei in human Hep-G2 cells, with only a moderate response at the highest concentration tested (Sanyal et al., 1997). No unscheduled DNA synthesis was observed in rat hepatocytes exposed to veratraldehyde [FL-no: 05.017], vanillin [FL-no: 05.018], or ethyl vanillin [FL-no: 05.019] (Heck et al., 1989). Piperonal [FL-no: 05.016] caused unscheduled DNA synthesis in one test, but the finding could not be confirmed in subsequent tests (Heck et al., 1989), and the result was considered to be questionable.

para-Methoxybenzaldehyde [FL-no: 05.015] or benzaldehyde alone did not induce strand breaks in supercoiled DNA from the phage PM2, although positive results were reported with both substances

in the presence of $CuCl_2$. The finding that the effect depended on the concentration of copper suggests that DNA-damaging species are produced during redox reactions of aromatic (and aliphatic) aldehydes with $CuCl_2$ (Becker et al., 1996).

Numerous assays for anti-mutagenicity have been conducted *in vitro* with some of the hydroxy- and alkoxy-substituted benzyl derivatives, including evaluations in several sub-mammalian and mammalian cell lines. Anti-mutagenic activity was reported with *para*-methoxybenzaldehyde [FL-no: 05.015] and ethyl vanillin [FL-no: 05.019] (Ohta et al., 1986b; Imanishi et al., 1990; Ohta, 1995). Mixed results were reported with vanillin [FL-no: 05.018] (Takahashi et al., 1990; Tamai et al., 1992; Sanyal et al., 1997). Analysis of the concentrations, test organisms, and study methods did not provide an explanation for the discrepant results in these studies. No anti-mutagenic effect was observed with piperonal [FL-no: 05.016] or methyl salicylate [FL-no: 09.749] (Ohta et al., 1983; Ohta et al., 1986a; Ohta et al., 1986b).

In vivo

The hydroxy- and alkoxy-substituted benzyl derivatives were inactive in all assays *in vivo* in mammals given the compounds orally or by intraperitoneal injection at doses that were significant fractions of the reported lethal doses. Micronuclei were not induced by *para*-ethoxybenzaldehyde [FL-no: 05.056] at a dose of 1005 mg/kg bw, ethyl vanillin [FL-no: 05.019] at 1000 mg/kg bw, vanillin [FL-no: 05.018] at 500 mg/kg bw, or piperonyl acetate [FL-no: 09.220] at 620 mg/kg bw (Wild et al., 1983; Furukawa et al., 1989). Piperonal [FL-no: 05.016] administered by intraperitoneal injection at 1000 mg/kg bw caused a slight increase in the number of early fetal deaths as compared with the incidence in control mice; however, the authors reported that the result was not statistically significant, and no similar finding was reported after administration by oral gavage (Epstein et al., 1972).

In assays for sex-linked recessive lethal mutation in fruit flies (*Drosophila melanogaster*), negative results were obtained with *para*-ethoxybenzaldehyde [FL-no: 05.056], ethyl vanillin [FL-no: 05.019], and piperonyl acetate [FL-no: 09.220] after feeding at concentrations of 751, 8309, and 4855 µg/ml, respectively (Wild et al., 1983). Vanillin [FL-no: 05.018] induced an anti-mutagenic response in fruit flies, and both vanillin and *para*-methoxybenzaldehyde [FL-no: 05.015] were anti-mutagenic in mice (Imanishi et al., 1990; Sasaki et al., 1990b; de Andrade et al., 1992). The data on vanillin, including the results *in vitro*, suggest some anti-mutagenic activity, although the relevance of this finding is questionable and impossible to extrapolate to the low concentrations to which persons are likely to be exposed from its use as a flavour in food.

Conclusion on genotoxicity

The hydroxy- and alkoxy-substituted benzyl derivatives did not have mutagenic activity in bacterial or other submammalian cellular systems. Mixed results were obtained in an assay for DNA repair in bacteria and in assays for clastogenicity in isolated mammalian cells. These findings probably reflect the known activity of alcohols or aldehydes in biological systems, as they were seen both with and without metabolic activation, and cytotoxicity was often a limitation at high concentrations. Negative results were obtained in tests for genotoxicity in mice and *Drosophila in vivo*. In a 2-year study in mice, no difference in tumour incidence from that in controls was found in groups fed doses up to 900 mg/kg bw per day of butyl-*para*-hydroxybenzoate [FL-no: 09.754] (Inai et al., 1985). The Committee therefore concluded that the hydroxy- and alkoxy-substituted benzyl derivatives do not have genotoxic potential *in vivo.*

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

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

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. However, all other *in vivo* studies with benzyl acetate are negative and several of these studies, among which an UDS-test in the liver and a mouse bone marrow micronucleus test were considered to be of good quality (NTP, 1993d). Additionally, in the long term carcinogenicity studies with benzyl acetate, no carcinogenic effects were observed in mice and rats after administration via the diet (NTP, 1993d). In a previous study by NTP (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 or inassessible validity. Taking all this information into account, the Panel considered the positive result from the *in vivo* Comet assay as insufficient ground to preclude the evaluation of benzyl acetate via the Procedure.

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

Conclusion on genotoxicity:

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

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 considered that while some of the *in vitro* studies indicated equivocal weak positive or positive results, the weight of evidence from candidate and supporting substances and the *in vivo* studies do not preclude evaluation of the 44 JECFA evaluated hydroxy- and alkoxy- substituted benzyl derivatives through the Procedure.

4. Application of the Procedure

4.1. Application of the Procedure to 44 Hydroxy- and Alkoxy-substituted Benzyl Derivatives Evaluated by JECFA (JECFA, 2002a):

According to the JECFA 35 of the substances belong to structural class I and nine to structural class II using the decision tree approach presented by Cramer *et al.* (Cramer et al., 1978).

The JECFA concluded 40 of the 44 flavouring 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 the substances are below the thresholds for structural classes I and II (step A3).

The four remaining substances [FL-no: 05.016, 05.018, 05.019 and 09.749] were concluded at step A5 – i.e. the intakes are above the threshold for the structural class, the substances are not endogenous, but a NOAEL is available that can provide an adequate margin of safety to the estimated intake of the substances.

In conclusion, the JECFA evaluated all 44 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 44 substances are summarised in Table 3.1: Summary of Safety Evaluation of 44 Hydroxy- and Alkoxy-Substituted Benzyl Derivatives (JECFA, 2002b).

4.2. Application of the Procedure to 35 Benzyl Alcohols, Benzaldehydes, a Related Acetal, Benzoic Acids, and Related Esters Evaluated by EFSA (EFSA, 2006e):

Thirty-three of the flavouring substances are classified into structural class I, one is classified into structural class II and one is classified into structural class III 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 intakes for all substances are below the thresholds for structural classes I, II and III respectively (step A3).

In conclusion the Panel considered that the 35 substances evaluated through the Procedure were of no safety concern at the estimated levels of intake 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, 2006e).

4.3. EFSA Considerations

The Panel agrees with the application of the Procedure as performed by the JECFA at its 57th meeting (JECFA, 2002a) for 43 of the 44 substances in the group of hydroxy- and alkoxy-substituted benzyl derivatives.

More recent studies on butyl 4-hydroxybenzoate [FL-no: 09.754] considered in the EFSA opinion on methyl, ethyl and propyl 4-hydroxybenzoates, evaluated as food additives, have demonstrated that in juvenile rats given dietary doses of approximately 10, 100 or 1000 mg/kg body weight (bw) per day for eight weeks, effects were observed on male reproductive organs, sperm parameters or sex hormones at all doses (EFSA, 2004b; JECFA, 2007b). In juvenile mice given dietary doses of butyl 4-hydroxybenzoate of 15-1500 mg/kg bw per day for ten weeks, effects on sperm counts and serum concentrations of testosterone were observed (JECFA, 2007b). As no NOAEL could be demonstrated for these effects on male reproductive parameters in rodents the Panel concluded that additional data would be required before butyl 4-hydroxybenzoate [FL-no: 09.754] can be evaluated as a flavouring substance using the Procedure.

5. Conclusion

The Panel concluded that the 44 substances in the JECFA flavouring group of hydroxy- and alkoxy-substituted 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 two substances were evaluated by the JECFA in this group but are not in the Register (2-methoxybenzoic acid and ethyl vanillin propylene glycol acetal) and therefore not dealt with in this consideration.

The Panel agrees with the application of the Procedure as performed by the JECFA for 43 of the 44 substances considered in this FGE. For butyl 4-hydroxybenzoate [FL-no: 09.754] additional data would be required before it can be evaluated as a flavouring substance, using the Procedure.

For eight substances [FL-no: 04.093, 08.071, 08.076, 08.092, 09.145, 09.754, 09.807 and 16.075] 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 44 substances use levels are needed to calculate the mTAMDIs in order to identify those flavouring substances that need more refined exposure assessment and to finalise the evaluation.

In order to determine whether the conclusion for the 44 JECFA evaluated substances can be applied to the materials of commerce, it is necessary to consider the available specifications:

Adequate specifications are available for 40 of the 44 JECFA evaluated substances. For three substances [FL-no: 06.132, 09.087 and 09.751] further information on the composition is requested for and for one substance [FL-no: 09.763] an ID test is missing.

Thus, for 12 substances [FL-no: 04.093, 06.132, 08.071, 08.076, 08.092, 09.087, 09.145, 09.751, 09.754, 09.763, 09.807 and 16.075] the Panel has reservations (only USA production volumes

available and/or missing data on specifications and/or isomerism/composition). For one of these 12 substances, butyl 4-hydroxybenzoate [FL-no: 09.754], the Panel concluded that additional data would be required before it can be evaluated as a flavouring substance using the Procedure. For the remaining 32 JECFA evaluated hydroxy- and alkoxy-substituted benzyl derivatives [FL-no: 02.128, 02.165, 02.213, 04.094, 05.015, 05.016, 05.017, 05.018, 05.019, 05.047, 05.055, 05.056, 05.091, 08.040, 08.043, 08.112, 09.019, 09.035, 09.058, 09.220, 09.430, 09.706, 09.713, 09.714, 09.748, 09.749, 09.750, 09.752, 09.753, 09.796, 09.811 and 09.933] the Panel agrees with the JECFA conclusion "No safety concern at estimated levels of intake as flavouring substance" 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 44 Hydroxy- and Alkoxy-substituted Benzyl derivatives

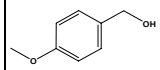
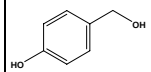
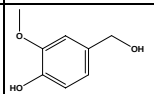
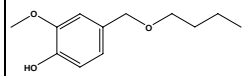
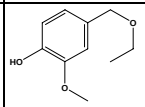
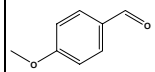
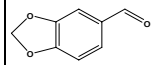
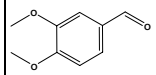
FL-no JECFA-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	EFSA comments
02.128 871	p-Anisyl alcohol		2099 66 105-13-5	Liquid $C_8H_{10}O_2$ 138.17	Insoluble Miscible	259 24-25 IR 97 %	1.540-1.547 1.107-1.115	
02.165 955	4-Hydroxybenzyl alcohol		3987 623-05-2	Solid $C_7H_8O_2$ 124.14	Slightly soluble Soluble	n.a. 110-112 IR 99 %	n.a. n.a.	
02.213 886	Vanillyl alcohol		3737 690 498-00-0	Solid $C_8H_{10}O_3$ 154.17	Soluble Soluble	n.a. 115 IR 98 %	n.a. n.a.	According to JECFA: Boiling point is "n/a (decomposes at the melting point)".
04.093 888	Butyl vanillyl ether		3796 82654-98-6	Liquid $C_{12}H_{18}O_3$ 210.27	Insoluble Miscible	241 IR 95 %	1.511-1.521 1.048-1.068	
04.094 887	Ethyl 4-hydroxy-3-methoxybenzyl ether		3815 13184-86-6	Liquid $C_{10}H_{14}O_3$ 182.22	Insoluble Miscible	212 NMR 98 %	1.528-1.532 1.106-1.113	
05.015 878	4-Methoxybenzaldehyde		2670 103 123-11-5	Liquid $C_8H_8O_2$ 136.15	Poorly soluble Miscible	248 IR 97 %	1.568-1.574 1.115-1.123	
05.016 896	Piperonal		2911 104 120-57-0	Solid $C_8H_6O_3$ 150.13	Slightly soluble Freely soluble	263 37 IR 98 %	n.a. n.a.	
05.017 877	Veratraldehyde		3109 106 120-14-9	Solid $C_9H_{10}O_3$ 166.18	Insoluble Soluble	281 42-45 IR 95 %	n.a. n.a.	

Table 1: Specification Summary of the Substances in the JECFA Flavouring Group of 44 Hydroxy- and Alkoxy-substituted Benzyl derivatives

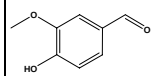
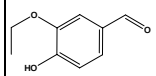
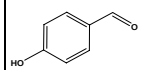
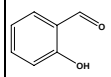
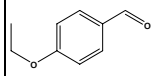
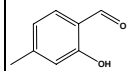
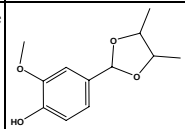
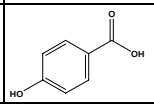
FL-no JECFA-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	EFSA comments
05.018 889	Vanillin		3107 107 121-33-5	Solid $C_8H_8O_3$ 152.15	Slightly soluble Freely soluble	285 80-81 IR 97 %	n.a. n.a.	
05.019 893	Ethyl vanillin		2464 108 121-32-4	Solid $C_9H_{10}O_3$ 166.18	Insoluble Very soluble	285 78 IR 98 %	n.a. n.a.	
05.047 956	4-Hydroxybenzaldehyde		3984 558 123-08-0	Solid $C_7H_6O_2$ 122.12	Slightly soluble Freely soluble	n.a. 116 IR 99 %	n.a. n.a.	According to JECFA: Melting point is "116° [sublimes at atmospheric pressure]".
05.055 897	Salicylaldehyde		3004 605 90-02-8	Liquid $C_7H_6O_2$ 122.12	Slightly soluble Miscible	196-197 IR 95 %	1.570-1.576 1.159-1.170	
05.056 879	4-Ethoxybenzaldehyde		2413 626 10031-82-0	Liquid $C_9H_{10}O_2$ 150.18	Poorly soluble Miscible	250 IR 97 %	1.556-1.564 1.078-1.084	According to JECFA: Boiling point is "250 (minimum)".
05.091 898	2-Hydroxy-4-methylbenzaldehyde		3697 2130 698-27-1	Solid $C_8H_8O_2$ 136.15	Insoluble Freely soluble	207 57 IR 98 %	n.a. n.a.	
06.132 960	Vanillin butan-2,3-diol acetal (mixture of stereo isomers) 6)		4023 63253-24-7	Solid $C_{12}H_{16}O_4$ 224.26	Insoluble Soluble	n.a. 48-52 IR NMR MS 95 %	n.a. n.a.	CASrn does not specify stereoisomers.
08.040 957	4-Hydroxybenzoic acid		3986 693 99-96-7	Solid $C_7H_6O_3$ 138.12	Slightly soluble Freely soluble	n.a. 213-214 IR 99 %	n.a. n.a.	

Table 1: Specification Summary of the Substances in the JECFA Flavouring Group of 44 Hydroxy- and Alkoxy-substituted Benzyl derivatives

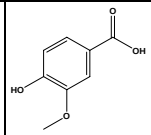
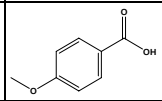
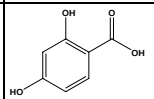
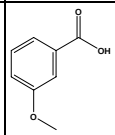
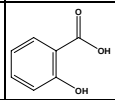
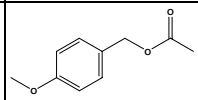
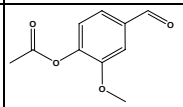
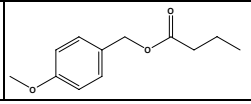
FL-no JECFA-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	EFSA comments
08.043 959	Vanillic acid		3988 697 121-34-6	Solid $C_8H_8O_4$ 168.15	Slightly soluble Soluble	n.a. 210-212 IR 99 %	n.a. n.a.	
08.071 883	p-Anisic acid		3945 10077 100-09-4	Solid $C_8H_8O_3$ 152.15	Soluble Freely soluble	275-280 184 IR 98 %	n.a. n.a.	
08.076 908	2,4-Dihydroxybenzoic acid		3798 89-86-1	Solid $C_7H_6O_4$ 154.12	Soluble Soluble	n.a. 225 IR 97 %	n.a. n.a.	According to JECFA: Melting point is "225° (decomposes, rapid heating required)".
08.092 882	3-Methoxybenzoic acid		3944 586-38-9	Solid $C_8H_8O_3$ 152.15	Soluble Freely soluble	170-172 107-109 IR 98 %	n.a. n.a.	
08.112 958	Salicylic acid		3985 10165 69-72-7	Solid $C_7H_6O_3$ 138.12	Very slightly soluble Very soluble	211 (26 hPA) 158-160 IR 99 %	n.a. n.a.	
09.019 873	p-Anisyl acetate		2098 209 104-21-2	Liquid $C_{10}H_{12}O_3$ 180.20	Insoluble Miscible	235 IR 97 %	1.511-1.517 1.104-1.111	
09.035 890	Vanillyl acetate		3108 225 881-68-5	Solid $C_{10}H_{10}O_4$ 194.19	Slightly soluble Soluble	148 (13 hPa) 77-79 IR 97 %	n.a. n.a.	
09.058 875	p-Anisyl butyrate		2100 286 6963-56-0	Liquid $C_{12}H_{16}O_3$ 208.26	Insoluble Miscible	270 IR 97 %	1.500-1.505 1.047-1.067	

Table 1: Specification Summary of the Substances in the JECFA Flavouring Group of 44 Hydroxy- and Alkoxy-substituted Benzyl derivatives

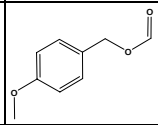
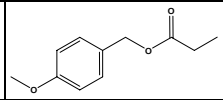
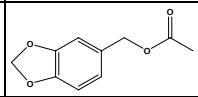
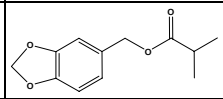
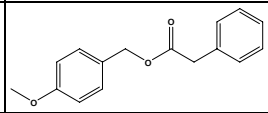
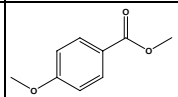
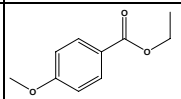
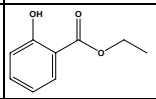
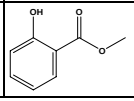
FL-no JECFA-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	EFSA comments
09.087 872	p-Anisyl formate 9)		2101 354 122-91-8	Liquid $C_9H_{10}O_3$ 166.18	Insoluble Miscible	220 IR 90 %	1.519-1.525 1.136-1.145	According to JECFA: "Minimum assay value is 90%".
09.145 874	p-Anisyl propionate		2102 426 7549-33-9	Liquid $C_{11}H_{14}O_3$ 194.23	Insoluble Miscible	100-103(0.7hPa) IR 97 %	1.505-1.510 1.070-1.086	
09.220 894	Piperonyl acetate		2912 2068 326-61-4	Liquid $C_{10}H_{10}O_4$ 194.19	Insoluble Miscible	150-151 (13hPa) IR 97 %	1.523-1.529 1.227-1.239	
09.430 895	Piperonyl isobutyrate		2913 305 5461-08-5	Liquid $C_{12}H_{14}O_4$ 222.24	Insoluble Miscible	91-92(0.007hPa) IR 97 %	1.506-1.513 1.154-1.160	
09.706 876	Anisyl phenylacetate		3740 233 102-17-0	Liquid $C_{16}H_{16}O_3$ 256.30		370 IR 97 %	1.553-1.563 1.125-1.133	
09.713 884	Methyl 4-methoxybenzoate		2679 248 121-98-2	Solid $C_9H_{10}O_3$ 166.18	Very slightly soluble Soluble	255 48 IR 97 %	n.a. n.a.	
09.714 885	Ethyl 4-methoxybenzoate		2420 249 94-30-4	Liquid $C_{10}H_{12}O_3$ 180.20	Insoluble Miscible	270 IR 97 %	1.522-1.528 1.101-1.105	
09.748 900	Ethyl salicylate		2458 432 118-61-6	Liquid $C_9H_{10}O_3$ 166.18	Slightly soluble Miscible	234 IR 98 %	1.518-1.525 1.125-1.131	
09.749 899	Methyl salicylate		2745 433 119-36-8	Liquid $C_8H_8O_3$ 152.15	Slightly soluble Miscible	222 IR 98 %	1.534-1.538 1.176-1.185	

Table 1: Specification Summary of the Substances in the JECFA Flavouring Group of 44 Hydroxy- and Alkoxy-substituted Benzyl derivatives

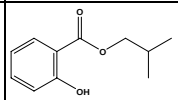
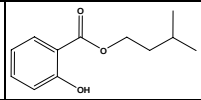
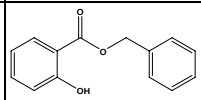
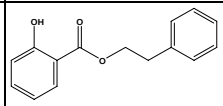
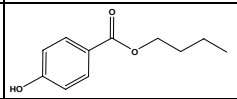
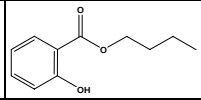
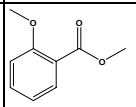
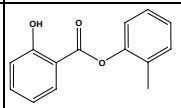
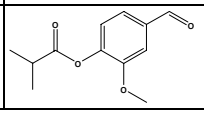
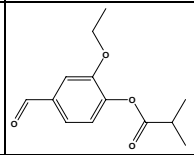
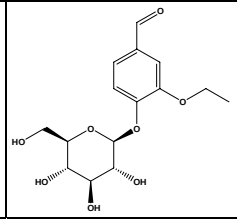
FL-no JECFA-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	EFSA comments
09.750 902	Isobutyl salicylate		2213 434 87-19-4	Liquid $C_{11}H_{14}O_3$ 194.23	Insoluble Miscible	260 IR 98 %	1.506-1.570 1.062-1.069	
09.751 903	Isopentyl salicylate 9)		2084 435 87-20-7	Liquid $C_{12}H_{16}O_3$ 208.26	Insoluble Miscible	277 IR 98 %	1.504-1.509 1.046-1.055	According to JECFA: Min. assay value is "98 (sum of isoamyl and amyl salicylate)".
09.752 904	Benzyl salicylate		2151 436 118-58-1	Liquid $C_{14}H_{12}O_3$ 228.25	Insoluble Miscible	300 24-26 IR 98 %	1.573-1.584 1.173-1.183	
09.753 905	Phenethyl salicylate		2868 437 87-22-9	Solid $C_{15}H_{14}O_3$ 242.28	Insoluble Soluble	190 (7 hPa) 44 IR 98 %	n.a. n.a.	
09.754 870	Butyl 4-hydroxybenzoate		2203 525 94-26-8	Solid $C_{11}H_{14}O_3$ 194.23	Insoluble Soluble	156-157 (5 hPa) 67-70 IR 98 %	n.a. n.a.	
09.763 901	Butyl salicylate		3650 614 2052-14-4	Liquid $C_{11}H_{14}O_3$ 194.23	Insoluble Miscible	268 98 %	1.508-1.520 1.070-1.080	ID 7).
09.796 880	Methyl 2-methoxybenzoate		2717 2192 606-45-1	Liquid $C_9H_{10}O_3$ 166.18	Very slightly soluble Miscible	246 IR 97 %	1.529-1.537 1.144-1.160	
09.807 907	o-Tolyl salicylate		3734 617-01-6	Solid $C_{14}H_{12}O_3$ 228.25	Insoluble Soluble	180 (3 hPa) NMR 99 %	1.576-1.584 1.164-1.174	
09.811 891	Vanillin isobutyrate		3754 20665-85-4	Liquid $C_{12}H_{14}O_4$ 222.24	Insoluble Miscible	130-132 (3 hPa) IR 98 %	1.522-1.526 1.110-1.136	

Table 1: Specification Summary of the Substances in the JECFA Flavouring Group of 44 Hydroxy- and Alkoxy-substituted 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
09.933 953	Ethyl vanillin isobutyrate		3837 188417-26-7	Solid $C_{13}H_{16}O_4$ 236.27	Insoluble Freely soluble	 57 IR 97 %	n.a. n.a.	
16.075 892	Ethyl vanillin beta-D-glucopyranoside		3801	Solid $C_{15}H_{20}O_8$ 328.32	Slightly soluble Slightly soluble	n.a. 199-200 NMR 99 %	n.a. n.a.	CASrn to be included in the Register: 122397-96-0. According to JECFA: Boiling point is "n/a (decomposes on heating)".

1) Solubility in water, if not otherwise stated.

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

3) At 1013.25 hPa, if not otherwise stated.

4) At 20°C, if not otherwise stated.

5) At 25°C, if not otherwise stated.

6) Stereoisomeric composition not specified.

7) ID: Missing identification test.

8) MP: Missing melting point.

9) Composition of mixture not specified.

TABLE 2: GENOTOXICITY DATA

Table 2.1: Genotoxicity Data (*in vitro / in vivo*) for 44 Hydroxy- and Alkoxy-Substituted Benzyl Derivatives (JECFA, 2002a)

Table 2.1: Summary of Genotoxicity Data of hydroxy- and alkoxy-substituted benzyl derivatives evaluated by JECFA (JECFA, 2002a)

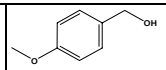
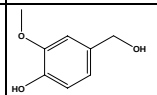
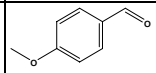
FL-no JECFA-no	EU Register name JECFA name	Structural formula	End-point	Test system	Concentration	Results	Reference
In vitro							
02.128 871	p-Anisyl alcohol Anisyl alcohol		Reverse mutation (plate incorporation)	*S. typhimurium* TA100	≥ 500 mg/plate	Negative[c]	(Ball et al., 1984)
02.213 886	Vanillyl alcohol		SOS DNA repair	*Escherichia coli* PQ37	Not reported	Positive[c]	(Ohshima et al., 1989)
05.015 878	4-Methoxybenzaldehyde p-Methoxybenzaldehyde		Reverse mutation (preincubation)	*S. typhimurium* TA92, TA1535, TA100, TA1537, TA94, TA98, TA2637	5000 mg/plate[a]	Negative[b]	(Ishidate et al., 1984)
			Reverse mutation	*S. typhimurium* TA98, TA100	≥ 500 mg/plate	Negative[b]	(Kasamaki et al., 1982)
			Chromosomal aberration	Chinese hamster fibroblasts	500 mg/ml[a]	Negative[c]	(Ishidate et al., 1984)
			Reverse mutation (preincubation)	*S. typhimurium* TA102, TA97	≥ 1000 mg/plate	Negative[b]	(Fujita & Sasaki, 1987)
			Mutation	*B. subtilis* H17, M45	22 mg/disc	Negative[c]	(Oda et al., 1979)
			Reverse mutation	*S. typhimurium* TA102	5000 mg/plate	Negative[b]	(Müller et al., 1993)
			Reverse mutation	*S. typhimurium* TA100	≥ 1000 mg/plate	Negative	(Rapson et al., 1980)
			Forward mutation	Mouse lymphoma L5178Y cells	≥ 470 mg/ml 540–780 mg/ml	Negative Positive[c]	(Wangenheim & Bolcsfoldi, 1988)
			Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537	410 mg/plate	Negative[b]	(Florin et al., 1980)
			Chromosomal aberration	Chinese hamster B241 cell line	0.0068 mg/ml	Positive[b]	(Kasamaki et al., 1982)

Table 2.1: Summary of Genotoxicity Data of hydroxy- and alkoxy-substituted benzyl derivatives evaluated by JECFA (JECFA, 2002a)

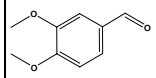
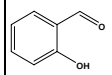
FL-no JECFA-no	EU Register name JECFA name	Structural formula	End-point	Test system	Concentration	Results	Reference
In vitro							
			Mutation	Phage PM2	1400 mg/ml	Negative	(Becker et al., 1996)
			Sister chromatid exchange	Human lymphocytes	≥ 270		
			DNA alkaline unwinding	Mouse lymphoma L5178Y/TK$^{+/-}$ cells	≥ 820 mg/ml 960–1100 mg/ml	Negative[b] Positive[b]	(Garberg et al., 1988)
			Sister chromatid exchange	Chinese hamster ovary K-1 cells	≥ 14 mg/ml	Negative	(Sasaki et al., 1987)
05.017 877	Veratraldehyde		Reverse mutation	*S. typhimurium* TA1535, TA100, TA1537, TA1538, TA98	8000 mg/plate	Negative[b]	(Nestmann et al., 1980)
			Reverse mutation	*S. typhimurium* TA1535, TA100, TA1537, TA1538, TA98	8000 mg/plate	Negative[b]	(Douglas et al., 1980)
			Mutation	*Saccharomyces cerevisiae* D7, XV185-14C	Not reported	Negative[c]	(Nestmann & Lee, 1983)
			Reverse mutation (preincubation)	*S. typhimurium* TA1535, TA98, TA100, TA97, TA1537	≥ 6666 mg/plate	Negative[b]	(Mortelmans et al., 1986)
			Reverse mutation	*S. typhimurium* TA1535, TA1537, TA1538, TA98, TA100	1000 mg/plate[a]	Negative[b]	(Heck et al., 1989)
			Forward mutation	Mouse lymphoma L5178Y cells	1400 mg/ml[a]	Positive[b]	(Heck et al., 1989)
			Reverse mutation (preincubation)	*S. typhimurium* TA100, TA102, TA104, TA1538, TA982	Not reported	Negative[b]	(Dillon et al., 1992)
			Reverse mutation (preincubation)	*S. typhimurium* TA100, TA102, TA104	33–3300 mg/plate	Negative[b]	(Dillon et al., 1998)
			Unscheduled DNA synthesis	Rat hepatocytes	100 mg/ml[a]	Negative	(Heck et al., 1989)
05.055 897	Salicylaldehyde		Mutation	*S. typhimurium* TA1535/pSK1002	110 mg/ml	Negative[b]	(Nakamura et al., 1987)
			Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537	370 mg/plate	Negative[b]	(Florin et al., 1980)
			Reverse mutation (preincubation)	*S. typhimurium* TA98, TA100	Not reported	Negative[b]	(Sasaki & Endo, 1978)
			Sister chromatid exchange	Human lymphocytes	≥ 61 mg/ml	Negative[d]	(Jansson et al., 1988)

Table 2.1: Summary of Genotoxicity Data of hydroxy- and alkoxy-substituted benzyl derivatives evaluated by JECFA (JECFA, 2002a)

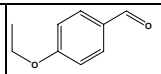
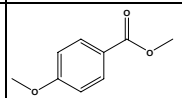
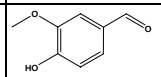
FL-no JECFA-no	EU Register name JECFA name	Structural formula	End-point	Test system	Concentration	Results	Reference
In vitro							
05.056 879	4-Ethoxybenzaldehyde p-Ethoxybenzaldehyde		Reverse mutation	*S. typhimurium* TA1535, TA100, TA1537, TA1538, TA98	3600 mg/plate	Negative[b]	(Wild et al., 1983)
09.713 884	Methyl 4-methoxybenzoate Methyl anisate		Mutation	*Escherichia coli* Sd-4-73	Not reported	Negative[c]	(Szybalski, 1958)
05.018 889	Vanillin		Reverse mutation	*S. typhimurium* TA1535, TA1537, TA1538, TA98, TA100	10 000 mg/plate[a]	Negative[b]	(Heck et al., 1989)
			Mutation	*B. subtilis* H17, M45	21 mg/disc	Negative[c]	(Oda et al., 1979)
			Chromosomal aberration	Chinese hamster fibroblasts	1000 mg/ml	Negative[c]	(Ishidate et al., 1984)
			Reverse mutation	*S. typhimurium* TA1535, TA1537, TA1538, TA98, TA100	5000 mg/plate	Negative[b]	(Pool & Lin, 1982)
			Reverse mutation (preincubation)	*S. typhimurium* TA1535, TA98, TA100, TA97, TA1537	≥ 10 000 mg/plate	Negative[b]	(Mortelmans et al., 1986)
			Mutation	*Escherichia coli* Sd-4-73	Not reported	Negative[c]	(Szybalski, 1958)
			Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538	Not reported	Negative[b]	(Nagabhushan & Bhide, 1985)
			Reverse mutation	*S. typhimurium* TA92, TA1535, TA100, TA1537, TA94, TA98, TA2637	10 000 mg/plate[a]	Negative[b]	(Ishidate et al., 1984)
			Reverse mutation	*S. typhimurium* TA100	≥ 1000 mg/plate	Negative	(Rapson et al., 1980)
			Forward mutation	Mouse lymphoma L5178Y cells	≥ 1500 mg/ml[a]	Negative[b]	(Heck et al., 1989)
			Mutation	*Escherichia coli* CSH26/pYM3, CSH26/pSK1002	≥ 15 000 mg/ml	Negative	(Takahashi et al., 1990)
			Reverse mutation	*S. typhimurium* TA98, TA100	≥ 1000 mg/plate	Negative[b]	(Kasamaki et al., 1982)
			Chromosomal aberration	Chinese hamster B241 cells	≥ 0.006 mg/ml	Negative	(Kasamaki & Urasawa, 1985)
			Sister chromatid exchange	Human lymphocytes	0–150 mg/ml	Positive	(Jansson et al., 1986)

Table 2.1: Summary of Genotoxicity Data of hydroxy- and alkoxy-substituted benzyl derivatives evaluated by JECFA (JECFA, 2002a)

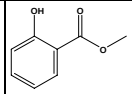
FL-no JECFA-no	EU Register name JECFA name	Structural formula	End-point	Test system	Concentration	Results	Reference
In vitro							
			Mitotic gene conversion	*S. cerevisiae*	10 000 mg/ml	Negative	(Rosin, 1984)
			Chromosomal aberration	Chinese hamster V79 lung cells	15 000–150 000 mg 300 000 mg	Negative[c] Positive[c]	(Tamai et al., 1992)
			Chromosomal aberration	Human lymphocytes	≥ 610 mg/ml	Negative	(Jansson & Zech, 1987)
			Chromosomal aberration	Chinese hamster B241 cell line	0.003 mg/ml	Negative	(Kasamaki et al., 1982)
			Sister chromatid exchange	Chinese hamster ovary K-1 cells	≥ 15 mg/ml	Negative	(Sasaki et al., 1987)
			Sister chromatid exchange	Human lymphocytes	150–300 mg/ml	Positive	(Jansson & Zech, 1987)
			Unscheduled DNA synthesis	Rat hepatocytes	500 mg/ml[a]	Negative	(Heck et al., 1989)
			SOS DNA repair	*Escherichia coli* PQ37	Not reported	Positive[c]	(Ohshima et al., 1989)
			Micronucleus formation	Human hepatoma (Hep-G2) cells	50 mg/ml 500 mg/ml	Negative Positive	(Sanyal et al., 1997)
09.749 899	Methyl salicylate		Chromosomal aberration	Hamster lung fibroblasts	Not reported	Positive[c]	(Kawachi et al., 1980a; Kawachi et al., 1980b)
			Mutation	*B. subtilis* H17, M45	23 mg/disc	Negative[c]	(Oda et al., 1979)
			Chromosomal aberration	Chinese hamster fibroblasts	250 mg/ml[a]	Negative[c]	(Ishidate et al., 1984)
			Reverse mutation	*S. typhimurium* TA92, TA1535, TA100, TA1537, TA94, TA98, TA2637	10 000 mg/plate	Negative[b]	(Ishidate et al., 1984)
			Reverse mutation (preincubation)	*S. typhimurium* TA1535, TA98, TA100, TA97, TA1537	≥ 330 mg/plate	Negative[b]	(Mortelmans et al., 1986)
			Reverse mutation	*S. typhimurium* TA100, TA98	Not reported	Negative[b]	(Kawachi et al., 1980a; Kawachi et al., 1980b)
			Mutation	*B. subtilis* H17, M45	Not reported	Negative[b]	(Kawachi et al., 1980a; Kawachi et al., 1980b)

Table 2.1: Summary of Genotoxicity Data of hydroxy- and alkoxy-substituted benzyl derivatives evaluated by JECFA (JECFA, 2002a)

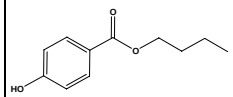
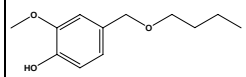
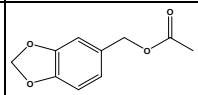
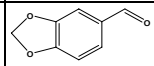
FL-no JECFA-no	EU Register name JECFA name	Structural formula	End-point	Test system	Concentration	Results	Reference
In vitro							
			Chromosomal aberration	Human embryo fibroblasts	Not reported	Negative[c]	(Kawachi et al., 1980a; Kawachi et al., 1980b)
			Sister chromatid exchange	Human embryo fibroblasts	Not reported	Negative[c]	(Kawachi et al., 1980a; Kawachi et al., 1980b)
			Mutation	Silkworm	Not reported	Negative[c]	(Kawachi et al., 1980a; Kawachi et al., 1980b)
09.754 870	Butyl 4-hydroxybenzoate		Chromosomal aberration	Chinese hamster fibroblasts	60 mg/ml[a]	Negative[b]	(Ishidate et al., 1984)
			Reverse mutation (preincubation)	S. typhimurium TA92, TA1535, TA100, TA1537, TA94, TA98, TA2637	1000 mg/platec	Negative[b]	(Ishidate et al., 1984)
			Reverse mutation	S. typhimurium TA98, TA100	< 1000 mg/plate	Negative[b]	(Haresaku et al., 1985)
04.093 888	Butyl vanillyl ether		Reverse mutation	*S. typhimurium* TA1535, TA100, TA1537, TA98	5000 mg/plate	Negative[b]	(Watanabe & Morimoto, 1989c)
			Mutation	*Escherichia coli* WP2 *uvr*A	5000 mg/plate	Negative[b]	(Watanabe & Morimoto, 1989c)
09.220 894	Piperonyl acetate		Reverse mutation (preincubation)	*S. typhimurium* TA1535, TA98, TA100, TA97, TA1537	≥ 3300 mg/plate	Negative[b]	(Mortelmans et al., 1986)
			Reverse mutation	*S. typhimurium* TA1535, TA100, TA1537, TA1538, TA98	3600 mg/plate	Negative[b]	(Wild et al., 1983)
05.016 896	Piperonal		Reverse mutation (histidine substitution)	*Escherichia coli* WP2*uvr*A*trp*$^{-}$	2400 mg	Negative[b]	(Sekizawa & Shibamoto, 1982)
			Reverse mutation	*S. typhimurium* TA1535, TA1537, TA1538, TA98, TA100	10 000 mg/plate[a]	Negative[b]	(Heck et al., 1989)
			Reverse mutation	*S. typhimurium* TA98, TA100	0.05–5000 mg/plate	Negative[b]	(Kasamaki et al., 1982)
			Reverse mutation	*S. typhimurium* TA1537, TA1538, TA98, TA100	≥ 5000 mg/plate	Negative[b]	(White et al., 1977)

Table 2.1: Summary of Genotoxicity Data of hydroxy- and alkoxy-substituted benzyl derivatives evaluated by JECFA (JECFA, 2002a)

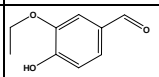
FL-no JECFA-no	EU Register name JECFA name	Structural formula	End-point	Test system	Concentration	Results	Reference
In vitro							
			Mutation	*B. subtilis* H17, M45	20 mg/disc	Negative[c]	(Oda et al., 1979)
			Reverse mutation	*S. typhimurium* TA100, TA1535, TA98, TA1537, TA1538	2400 mg	Negative[b]	(Sekizawa & Shibamoto, 1982)
			Reverse mutation (preincubation)	*S. typhimurium* TA1535, TA1537, TA98, TA100	≥ 10 000 mg/plate	Negative[b]	(Haworth et al., 1983)
			Unscheduled DNA synthesis	Rat hepatocytes	500 mg/ml	Positive	(Heck et al., 1989)
			Chromosomal aberration	Chinese hamster B241 cell line	0.075 mg/ml	Positive	(Kasamaki et al., 1982)
			Chromosomal aberration	Chinese hamster B241 cell line	≥ 0.15 mg/ml	Negative	(Kasamaki & Urasawa, 1985)
			Mutation	*B. subtilis* H17/M45	5000 mg/disc	Positive[c]	(Sekizawa & Shibamoto, 1982)
			Forward mutation	Mouse lymphoma L5178Y cells	≥ 1000 mg/ml	Negative[b]	(Heck et al., 1989)
05.019 893	Ethyl vanillin		Reverse mutation	*S. typhimurium* TA1535, TA100, TA1537, TA1538, TA98	≥ 3600 mg/plate	Negative[b]	(Wild et al., 1983)
			Mutation	*B. subtilis* H17, M45	21 mg/disc	Negative[c]	(Oda et al., 1979)
			Chromosomal aberration	Chinese hamster fibroblasts	250 mg/ml[a]	Positive[c]	(Ishidate et al., 1984)
			Reverse mutation (preincubation)	*S. typhimurium* TA1535, TA98, TA100, TA97, TA1537	≥ 8000 mg/plate	Negative[b]	(Mortelmans et al., 1986)
			Reverse mutation	*S. typhimurium* TA92, TA1535, TA100, TA1537, TA94, TA98, TA2637	10 000 mg/plate[a]	Negative[b]	(Ishidate et al., 1984)
			Forward mutation	Mouse lymphoma L5178Y cells	≥ 1000 mg/ml 800 mg/ml	Negative[d] Weakly positive[c]	(Heck et al., 1989)
			Reverse mutation (preincubation)	*S. typhimurium* TA97, TA102	≥ 1000 mg/plate	Negative[b]	(Fujita & Sasaki, 1987)
			Reverse mutation	*S. typhimurium* TA1535, TA1537, TA1538, TA98, TA100	10 000 mg/plate	Negative[b]	(Heck et al., 1989)
			Unscheduled DNA synthesis	Rat hepatocytes	200 mg/ml	Negative	(Heck et al., 1989)

Table 2.1: Summary of Genotoxicity Data of hydroxy- and alkoxy-substituted benzyl derivatives evaluated by JECFA (JECFA, 2002a)

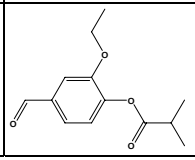
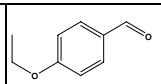
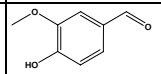
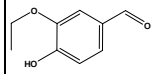
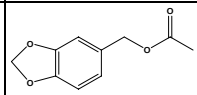
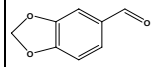
FL-no JECFA-no	EU Register name JECFA name	Structural formula	End-point	Test system	Concentration	Results	Reference
In vitro							
			Sister chromatid exchange	Human lymphocytes	≥ 330 mg/ml	Negative[c]	(Jansson et al., 1988)
			Sister chromatid exchange	Chinese hamster ovary K-1 cells	≥ 17 mg/ml	Negative	(Sasaki et al., 1987)
09.933 953	Ethyl vanillin isobutyrate		Reverse mutation	*S. typhimurium* TA1535, TA1537, TA1538, TA98, TA100	≥ 5000 mg/plate	Negative[b]	(King & Harnasch, 1997)

Table 2.1: Summary of Genotoxicity Data of hydroxy- and alkoxy-substituted 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
In vivo							
05.056 879	4-Ethoxybenzaldehyde p-Ethoxybenzaldehyde		Sex-linked recessive lethal mutation	*Drosophila melanogaster*	750 mg/ml	Negative	(Wild et al., 1983)
			Micronucleus formation	NMRI mice	≥ 1000 mg/kg bw	Negative	(Wild et al., 1983)
05.018 889	Vanillin		Micronucleus formation	Male BDF1 mice	500 mg/kg bw	Negative	(Inouye et al., 1988)
05.019 893	Ethyl vanillin		Sex-linked recessive lethal mutation	*D. melanogaster*	8300 mg/ml	Negative	(Wild et al., 1983)
			Micronucleus formation	Male BDF1 mice	Not reported	Negative	(Furukawa et al., 1989)
			Micronucleus formation	NMRI mice	1000 mg/kg bw	Negative	(Wild et al., 1983)
09.220 894	Piperonyl acetate		Sex-linked recessive lethal mutation	*D. melanogaster*	4900 mg/ml	Negative	(Wild et al., 1983)
			Micronucleus formation	NMRI mice	≥ 970 mg/kg bw	Negative	(Wild et al., 1983)
05.016 896	Piperonal		Dominant lethal mutation	ICR/Ha Swiss mice	≥ 620 mg/kg bw[e]	Negative	(Epstein et al., 1972)
			Dominant lethal mutation	ICR/Ha Swiss mice	1000 mg/kg bw[f]	Negative	(Epstein et al., 1972)

a Highest dose if result was negative; lowest active dose if result was positive.

b Without metabolic activation.

c With and without metabolic activation.

d With metabolic activation.

e Administered by intraperitoneal injection.

f Administered by oral gavage.

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

Substances listed in brackets are JECFA-evaluated substances

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

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

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

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
						evaluated. A weak positive result (i.e. 4 mm≤ D<8 mm). was reported (D=5 mm). No information on the use of metabolic activation.
	Chromosomal aberration test	Chinese hamster fibroblast cells	1000 µg /ml[4] (three concentrations, max. concentration inducing 50% cell-growth inhibition)	Negative[2]	(Ishidate et al., 1984)	Published study carried out only in the absence of metabolic activation. Thus, study is not considered valid. Cells were exposed for 24 and 48 hours. Negative response for chromosomal aberrations and polyploidization.
	Chromosomal aberration test	Chinese hamster ovary cells	50 to 5000 µg/ml	Equivocal[1]	(Anderson et al., 1990)	Published summary report including detailed results from studies on 42 compounds tested in various laboratories within the NTP in accordance with OECD guideline 473. Lowest effective dose was 4000 µg/ml with and without S9. No dose-response observed. Positive results were not reproducible in all trials. Absence of cytotoxicity reported up to the highest dose.
	Chromosomal aberration test	Chinese hamster ovary cells	50 to 5000 µg/ml	Negative[2] Weakly positive[3]	(NTP, 1989)	Valid study in accordance with OECD guideline 473. A positive result was reported only in the presence of S9 at relatively high concentrations of 4000 µg/ml in 3 of 4 tests carried out with harvest times between 12 and 18 hours. No data on cytotoxicity reported.
	Sister chromatid exchange assay	Chinese hamster ovary cells	16 to 5000 µg/ml	Weakly positive	(NTP, 1989)	Valid study in accordance with OECD guideline 479. Dose-related increase in frequency of SCE at concentrations from 500 - 1250 µg/ml (without metabolic activation) and 500 - 4000 µg/ml (with metabolic activation). No data on cytotoxicity reported. Number of chromosomes per cell reduced at 4000 µg/ml with S9.
	Sister chromatid exchange assay	Chinese hamster ovary cells	16 to 1250 µg/ml[2] 16 to 4000 µg/ml[3]	Weakly positive[1]	(Anderson et al., 1990)	Published summary report including detailed results from studies on 42 compounds tested in various laboratories within the NTP in accordance with OECD guideline 479. Significant increase (20%) in SCE only at the highest doses. No dose-response observed. No second trial using high concentrations to reproduce the positive effects performed. Absence of cytotoxicity reported up to the highest dose.
	Mammalian cell gene mutation test	Mouse lymphoma L5178Y cells	Up to 5000 µg/ml	Questionable	(McGregor et al., 1988a; Myhr et al., 1990)	Published summary report including detailed method and results from study on 72 compounds tested in various laboratories within the NTP in accordance with OECD guideline 476 (however, no colony sizing performed). Positive responses observed in some experiments at concentrations of 3500 and higher. No dose-response was observed. The highest concentration was letal in some experiments. Positive and negative responses could not be reproduced in all experiments.
	Mammalian cell gene mutation test	Mouse lymphoma L5178Y cells	150 to 5000 µg/ml	Negative[3] Positive[2]	(NTP, 1989)	Valid study in accordance with OECD guideline 476. In one of three trials without S9 a positive result (relative mutant fraction ≥1.6) was reported at 4500 µg/ml with relative total growth of 20%. The concentration of 5000 µg/ml was letal in

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

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
						this trial, whereas in another one of three trials without S9 3500 µg/ml was letal.
	Mutation assay	*E. coli* WP2 *uvr*A	Not reported	Negative	(Kuroda et al., 1984a)	Only abstract available. Methods, test concentrations and detailed results not reported.
	Cytotoxicity assay	Human alveolar tumour cells	0.5 mM	Negative	(Waters et al., 1982)	
	DNA damage assay	Human alveolar tumour cells	0.5 mM	Negative	(Waters et al., 1982)	
	DNA damage assay	Rat hepatocytes	10 mM	Negative	(Storer et al., 1996)	Cytotoxicity was reported at the highest concentration tested.
	DNA damage assay	*E. coli* P3478	50 µl/disc	Negative[1]	(Fluck et al., 1976)	
(Benzyl formate [09.077])	Rec assay	*B. subtilis* M45 (rec^-), H17 (rec^+)	20 µl/disc	Positive	(Yoo, 1986)	Study published in Japanese with English abstract. Data extracted from tables. Validity of the study cannot be evaluated. A weak positive result (i.e. 4 mm≤ D<8 mm).was reported (D=4 mm). No information on the use of metabolic activation.
	Mutation assay	*E. coli* WP2 *uvr*A	500 to 4000 µg/plate	Negative	(Yoo, 1986)	Study published in Japanese with English abstract. Data extracted from tables. Validity of the study cannot be evaluated. No information on the use of metabolic activation.
(Benzyl acetate [09.014])	Ames test (preincubation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537	10,000 µg/plate	Negative[1]	(Mortelmans et al., 1986)	
	Ames test (preincubation and plate incorporation method)	*S. typhimurium* TA98; TA100	5000 µg/plate	Negative[1]	(Schunk et al., 1986)	Cytotoxicity was observed at the three highest doses tested.
	Ames test	*S. typhimurium* TA98; TA100; TA1535; TA1537	3 µM/plate	Negative[1]	(Florin et al., 1980)	
	Rec assay	*B. subtilis* M45 (rec^-), H17 (rec^+)	21 µg/disc	Negative	(Oda et al., 1979)	Study published in Japanese without English abstract. Data extracted from tables. Validity of the study cannot be evaluated.
	Rec assay	*B. subtilis* M45 (rec^-), H17 (rec^+)	20 µl/disc	Positive	(Yoo, 1986)	Study published in Japanese with English abstract. Data extracted from tables. Validity of the study cannot be evaluated. A weak positive result (i.e. 4≤ D<8).was reported (D could not clearly be determined). No information on the use of metabolic activation.
	Mutation assay	*E. coli* WP2 *uvr*A	250 to 2000 µg/plate	Negative	(Yoo, 1986)	Study published in Japanese with English abstract. Data extracted from tables. Validity of the study cannot be evaluated. No information on the use of metabolic activation.
	Mammalian cell gene mutation test	Mouse lymphoma L5178Y cells; Human lymphoblast TK6 cells	Mouse cells 0, 250, 500, 1000 µg/ml; Human cells 0, 500, 1000, 1250, 1500 µg/ml	Negative[2] Positive[3]	(Caspary et al., 1988)	Published non-GLP study in accordance with OECD guideline 476 (except that no colony sizing was performed). Thus, the study is considered not fully valid. The lowest significantly effective doses in the presence of S9 were 500 µg/ml in mouse cells and 1500 µg/ml in human cells. Cytotoxicity was reported above 500 µg/ml with and without S9.
	Mammalian cell gene mutation test	Mouse lymphoma L5178Y cells	0-1600 µl/ml (6 concentrations)	Positive[2]	(McGregor et al., 1988a)	Published summary report including detailed method and results from study on 72 compounds tested in various laboratories within the NTP. The study was not in accordance with OECD guideline 476 (no colony sizing performed, only in the absence of metabolic activation) and

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

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
						thus not considered valid. The lowest significantly effective doses was 900 µg/ml at which the relative total growth was 50%. The highest dose was lethal. A positive response was observed in two of three experiments. No dose-response was observed.
	Mammalian cell gene mutation test	Mouse lymphoma L5178Y cells	Not reported	Negative[2] Positive[3,]	(Rudd et al., 1983)	Study carried out within a larger NTP project. Only abstract available. Validity of the study cannot be evaluated.
	Mammalian cell gene mutation test	Mouse lymphoma L5178Y TK+/- cells	Not reported	Negative[2] Inconclusive[3]	(Honma et al., 1999a)	Published collaborative study on 40 chemicals. Protocol was in accordance with OECD guideline 476, except that no colony sizing was performed. As the results are insufficiently reported, their validity cannot be evaluated. In the presence of S9 metabolic activation one laboratory achieved a statistically significant dose-dependant result, but did not induce mutations greater than three times the spontaneous response. The second laboratory did not obtain a positive response.
	Chromosomal aberration test	Chinese hamster ovary cells	160-1600 µg/ml[2]; 500-5000 µg/ml[3]	Negative[1]	(Galloway et al., 1987)	Published non-GLP study. Doses were selected based on preliminary assay. Although some details of results are not reported the study is considered valid.
	Chromosomal aberration test	Chinese hamster lung fibroblast cells	2400 µg/ml	Negative[1]	(Matsuoka et al., 1996)	Cytotoxicity was reported at the highest concentration tested.
	Sister chromatid exchange assay	Chinese hamster ovary cells	50-500 µg/ml[2]; 500-5000 µg/ml[3]	Negative[1,]	(Galloway et al., 1987)	Published non-GLP study. Doses were selected based on preliminary assay. Although some details of results are not reported the study is considered valid.
	Unscheduled DNA synthesis test	Rat hepatocytes	Not reported	Negative	(Mirsalis et al., 1983)	Only abstract available. Methods, test concentrations and detailed results not reported.
	Micronucleus test	Human lymphocytes and hepatoma cell line *Hep G2*	500 µM	Negative[1]	(Kevekordes et al., 2001)	
(Benzyl propionate [09.132])	Rec assay	*B. subtilis* M45 (rec$^-$), H17 (rec$^+$)	21 µg/disc	Negative	(Oda et al., 1979)	Study published in Japanese without English abstract. Data extracted from tables. Validity of the study cannot be evaluated.
(Benzyl benzoate [09.727])	Ames test	*S. typhimurium* TA98; TA100; TA1535; TA1537	3 µM/plate	Negative[1]	(Florin et al., 1980)	
	Ames test (preincubation and plate incorporation method)	*S. typhimurium* TA98; TA100	5000 µg/plate	Negative[1]	(Schunk et al., 1986)	Cytotoxicity was observed at the three highest doses tested.
(Benzaldehyde [05.013])	Ames test (plate incorporation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	37,500 nl/plate[4]	Negative[1]	(Heck et al., 1989)	Published non-GLP study. No information concerning a possible cytotoxic effect nor on the number of concentrations tested. The test guidelines do not require more than 5 mg/plate. Due to the lack of some important details of study design and results the validity of the study cannot be evaluated.
	Ames test (plate incorporation method)	*S. typhimurium* TA98; TA100	50 to 300 µl/plate	Negative[1]	(Rockwell & Raw, 1979)	Assay of urine samples from rats given benzaldehyde by oral gavage.
	Ames test (plate incorporation method)	*S. typhimurium* TA98; TA100	100 µl/plate	Negative[3]	(Rockwell & Raw, 1979)	Samples assayed prior to administration to rats.
	Ames test	*S. typhimurium* TA98; TA100; TA2637	2000 mg/plate	Negative[1]	(Nohmi et al., 1985)	Article published in Japanese. Data reported from English

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

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
						summary.
	Ames test	*S. typhimurium* TA98; TA100; TA1535; TA1537	3 µM/plate	Negative[1]	(Florin et al., 1980)	
	Ames test (preincubation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537	0, 10, 33, 100, 333, 1000 µg/plate	Negative[1]	(Haworth et al., 1983)	Published summary report including detailed results from studies on 250 compounds tested in various laboratories within the NTP to a large extent in accordance with OECD guideline 471.
	Ames test	*S. typhimurium* TA100; TA102; TA104	3333 µg/plate	Negative[1]	(NTP, 1990c)	
	Ames test	*S. typhimurium* TA100	1000 µg/plate	Negative	(Rapson et al., 1980)	The use of metabolic activation was not reported.
	Ames test (preincubation method)	*S. typhimurium* TA98; TA100	Not reported	Negative[1]	(Sasaki & Endo, 1978)	
	Ames test (preincubation method)	*S. typhimurium* TA100; TA102; TA104	Not reported	Negative[1]	(Dillon et al., 1992)	
	Ames test (preincubation method)	*S. typhimurium* TA100	2000 nM/	Negative[1]	(Vamvakas et al., 1989)	
	Ames test (preincubation method)	*S. typhimurium* TA97; TA102	1000 µg/plate	Negative[1]	(Fujita et al., 1992)	
	Ames test	*S. typhimurium* TA98; TA100	0.05 to 500 µg/plate	Negative[1]	(Kasamaki et al., 1982)	Published non-GLP study with insufficient report of some details of method and results. Thus, the validity of the study cannot be evaluated.
	Ames test (preincubation method)	*S. typhimurium* TA98; TA1535	5 µM/plate	Negative[1]	(Wiessler et al., 1983)	
	Ames test (preincubation method)	*S. typhimurium* TA97a; TA100; TA102; TA104	Not reported	Negative[1]	(Dillon et al., 1998)	
	Ames test (plate incorporation method)	*S. typhimurium* TA98; TA1537; TA7001; TA7002; TA7003; TA7004; TA7006; Mix of TA7001–7006 TA7005	1000 µg/ml	Negative[1] Negative[2]; Positive[3]	(Gee et al., 1998)	
	Rec assay	*B. subtilis* M45 (rec$^-$), H17 (rec$^+$)	21 µg/disc	Negative	(Oda et al., 1979)	Study published in Japanese without English abstract. Data extracted from tables. Validity of the study cannot be evaluated.
	Rec assay	*B. subtilis* M45 (rec$^-$), H17 (rec$^+$)	Not reported	Negative[2] Positive[3]	(Matsui et al., 1989)	Published non-GLP study with insufficient report of some details of method and results. Thus, the validity of the study cannot be evaluated.
	Unscheduled DNA synthesis test	Rat hepatocytes	251 nl/ml	Negative	(Heck et al., 1989)	Published non-GLP study. Some important details of study design and results are not reported. Thus, the validity of the study cannot be evaluated.
	Mammalian cell gene mutation test	Mouse lymphoma L5178Y cells	12.5 to 800 nl/ml	Negative[2] Weakly positive[3]	(Heck et al., 1989)	Published non-GLP study. Some important details of study design and results are not reported. Thus, the validity of the study cannot be evaluated. Different concentration ranges (12.5-800, 25-600, 400-600 nl/ml) were used in three independent experiments within which positive responses were observed. A 2.8 to 5.2-fold increase in mutant frequency was observed in the presence of S9.

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

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
	Mammalian cell gene mutation test	Mouse lymphoma L5178Y cells	0 to 800 µg/ml (6 concentrations)	Positive[2]	(McGregor et al., 1991)	Published summary report including detailed method and results from study on 27 compounds tested in various laboratories within the NTP in accordance with OECD guideline 476 (however, no colony sizing performed). Statistically significant increase in mutant fraction at the highest non-lethal concentration (400 µg/ml) in two experiments. Concentration of 640 and 800 µg/ml were lethal. Thus, significant increases in mutant fraction were close to toxic doses. No dose-response was observed. Since a positive response was observed without S9, no experiment was carried out with S9.
	Mammalian cell gene mutation test	Mouse lymphoma L5178Y +/- cells	600 µg/ml	Negative[2]	(Bigger & Clarke, 1991)	
	Chromosomal aberration test	Chinese hamster cells	0, 800, 1000, 1200 µg/ml	Positive[2] Weak positive[3]	(Sofuni et al., 1985)	Article published in Japanese. Data extracted from English summary and tables. Validity of the study cannot be evaluated. Cytotoxicity was observed at the two maximum concentrations tested. In the presence and in the absence of S9 a positive response was only observed at cytotoxic concentrations. Polyploidization (11%) was reported at non-cytotoxic concentrations.
	Chromosomal aberration test	Chinese hamster ovary cells	50-500 µg/ml[2]; 160-1600 µg/ml[3]	Negative[1]	(Galloway et al., 1987)	Published non-GLP study. Doses were selected based on preliminary assay. Although some details of results are not reported the study is considered valid.
	Chromosomal aberration test	Chinese hamster cell line B241	50 nM (0.0053 µg/ml)	Positive[1]	(Kasamaki et al., 1982)	Published non-GLP study of sufficient quality to be taken into account for the evaluation, although some details of method and results are not reported. Information is only given for the final concentration at which maximal frequency of aberration was observed without visible cytotoxicity in the treated cells. Dose-dependent increase of total aberrations (chromatid gaps, chromatid breaks, chromosome breaks observed, no ring or dicentric aberrations or chromatic exchanges).
	Sister chromatid exchange assay	Chinese hamster ovary cells	5-160 µg/ml[2]; 160-1600 µg/ml[3]	Positive[2] Weakly positive[3]	(Galloway et al., 1987)	Published non-GLP study. Doses were selected based on a preliminary assay. Although some deatails of results are not reported the study is considered valid. Weakly positive results with metabolic activation were observed at the highest concentration which was cytotoxic and resulted in 50% growth reduction.
	Sister chromatid exchange assay	Chinese hamster ovary cells	Up to 1000 µM (up to 106 µg/ml)	Negative[3]	(Sasaki et al., 1989)	Published non-GLP study of limited quality. Study designed to investigate the influence on spontaneous as well as on mitomycin-induced SCEs. The substance did not influence cell cycle (data not shown) and spontaneous SCEs at the concentrations used. Cytotoxicity was reported at the highest concentration tested.

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

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
	Sister chromatid exchange assay	Human lymphocytes	0-2 mM (0-212 µg/ml)	Positive[2]	(Jansson et al., 1988)	Published non-GLP study not in accordance with OECD guideline 479 (no metabolic activation). Insufficient report of important details of method and results. This study is not considered valid.
(Benzoic acid [08.021])	Ames test (plate incorporation method)	*S. typhimurium* TA98; TA100; TA1535; TA1538	2500 µg/plate	Negative[1]	(Anderson & Styles, 1978)	
	Ames test	*S. typhimurium* TA98; TA100; TA1535; TA1536	3.6 µg/plate	Negative[1]	(Cotruvo et al., 1977)	
	Ames test (preincubation method)	*S. typhimurium* TA97; TA98; TA100; TA1535; TA1537	10,000 µg/plate	Negative[1]	(Zeiger et al., 1988)	
	Ames test	*S. typhimurium* TA100	1000 µg/plate	Negative	(Rapson et al., 1980)	Cytotoxicity was reported at the highest concentration tested.
	Ames test (plate incorporation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537	1000 µg/plate	Negative[3]	(McCann et al., 1975)	
	Ames test (preincubation method)	*S. typhimurium* TA92; TA94; TA98; TA100; TA1535; TA1537	Up to 10,000 µg/plate (6 concentrations)	Negative[1]	(Ishidate et al., 1984)	Published study in accordance to OECD guideline 471. Although some details of results are not reported the study is considered valid.
	Ames test (plate incorporation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	100 µg/plate	Negative[2]	(Milvy & Garro, 1976)	
	Ames test (plate incorporation method)	*S. typhimurium* TA1535; TA1537; TA1538	0.5% (5 mg/ml)	Negative[1]	(FDA, 1975b)	
	Ames test (preincubation method)	*S. typhimurium* TA98; TA100	100 to 10000 µg/plate	Negative[1]	(Kuboyama & Fujii, 1992)	Published non-GLP study deficient in the report of some details on method and results (no single doses, no data on cytotoxicity reported), however, of sufficient quality to be taken into account in the evaluation.
	Umu mutation assay	*S. typhimurium* TA1535/ pSK1002	1607 µg/ml	Negative[1]	(Nakamura et al., 1987)	
	Rec assay (liquid method)	*B. subtilis* M45 (rec^-), H17 (rec^+)	Not reported	Positive	(Nonaka, 1989)	Only abstract available. Details on method and results not reported. Use of metabolic activation not reported. The validity of the study cannot be evaluated.
	Rec assay	*B. subtilis* M45 (rec^-), H17 (rec^+)	0 to 5000 µg/disc	Positive	(Kuboyama & Fujii, 1992)	Well conducted published non-GLP study with some minor deficiencies (no cytotoxicity data, no detailed data for different concentrations reported) of sufficient quality to be taken into account in the evaluation. A weak positive result (D>2 mm) was observed at concentrations of 4 mg/disc or more. At 5 mg/disc D=2.9 mm.
	Mutation assay	*S. cerevisiae* D3	0.18%	Negative[1]	(Cotruvo et al., 1977)	
	Mutation assay	*S. cerevisiae* D4	0.15%	Negative[1]	(FDA, 1975b)	
	Indirect DNA repair test	*E. coli* PQ37	400 µg/ml	Negative	(Glosnicka & Dziadziuszko, 1986)	Genotoxicity measured as ability to induce ß-galactosidase.
	SOS Chromotest	*E. coli* PQ37	50 µg	Negative[1]	(Kevekordes et al., 1999)	
	Chromosomal aberration test	Chinese hamster fibroblast cells	1500 µg/ml (three concentrations, max. concentration inducing 50% cell-growth inhibition)[4]	Equivocal[2]	(Ishidate et al., 1984)	Published study carried out only in the absence of metabolic activation. Thus, study is not considered valid. Cells were exposed for 24 and 48 hours. Total incidence of cells with aberrations was 8%.. Negative response for polyploidization.
	Sister chromatid exchange	Human lymphocytes	0-2 mM (0-244 µg/ml)	Negative[2]	(Jansson et al., 1988)	Published non-GLP study not in accordance with OECD

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Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
	assay					guideline 479 (no metabolic activation). Insufficient report of important details of method and results. This study is not considered valid.
	*In vitro*Micronucleus assay	Mouse lymphoma L5178Y cells	1000 µg/ml	Negative[1]	(Nesslany & Marzin, 1999)	
(Methyl benzoate [09.725])	Ames test (preincubation method)	*S. typhimurium* TA97; TA98; TA100; TA1535; TA1537	0 to 666 µg/plate (-S9); 0 to 6666 µg/plate (+S9) (6 concentrations)	Negative[1]	(Zeiger et al., 1992)	Published summary report including detailed results from NTP studies on 311 compounds in accordance with OECD guideline 471.
	Mutation assay	*E. coli* Sd-4-73	Not reported	Negative[2]	(Szybalski, 1958)	
Methyl 4-methylbenzoate [09.631]	Ames test (preincubation method)	*S. typhimurium* TA97; TA98; TA100; TA1535; TA1537;	0 to 333 µg/plate (-S9); 0 to 3333 µg/plate (+S9) (6 concentrations)	Negative[1]	(Zeiger et al., 1992)	Published summary report including detailed results from NTP studies on 311 compounds in accordance with OECD guideline 471.
(Isopentyl benzoate [09.755])	Mutation assay	*E. coli* Sd-4-73	Not reported	Negative[2]	(Szybalski, 1958)	
(4-Isopropylbenzyl alcohol [02.039])	Ames test (plate incorporation method)	*S. typhimurium* TA98; TA100	100 µl/plate	Negative[3]	(Rockwell & Raw, 1979)	
	Ames test (plate incorporation method)	*S. typhimurium* TA98; TA100	300 µl/plate	Negative[1]	(Rockwell & Raw, 1979)	Assay of urine samples from rats given isopropylbenzyl alcohol by oral gavage.
(Tolualdehydes (mixed *o, m, p*) [05.027])	Ames test (preincubation method)	*S. typhimurium* TA104	0.8 µM/plate	Negative[1]	(Marnett et al., 1985a)	
	Ames test	*S. typhimurium* TA98; TA100; TA1535; TA1537	3 µM/plate	Negative[1]	(Florin et al., 1980)	
	Ames test (plate incorporation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	18,750 µg/plate[4]	Negative[1]	(Heck et al., 1989)	Published non-GLP study. No information concerning a possible cytotoxic effect nor on the number of concentrations tested. The test guidelines do not require more than 5 mg/plate. Due to the lack of some important details of study design and results the validity of the study cannot be evaluated.
	Ames test (plate incorporation method)	*S. typhimurium* TA98; TA100; TA102	0.8 mM/plate	Negative[1]	(Aeschbacher et al., 1989)	
	Ames test (preincubation method)	*S. typhimurium* TA97; TA100; TA1535; TA1537	666 µg/plate	Negative[1]	(Zeiger et al., 1988)	
	Unscheduled DNA synthesis test	Rat hepatocytes	1000 µg/ml[4]	Negative	(Heck et al., 1989)	Published non-GLP study. No information concerning the number of concentrations tested. Due to the lack of some important details of study design and results the validity of the study cannot be evaluated.
	Mammalian cell gene mutation test	Mouse lymphoma L5178Y cells	300 µg/ml (+S9), 600 µg /ml (-S9)[4]	Negative[1]	(Heck et al., 1989)	Published non-GLP study. Some important details of study design and results are not reported. Thus, the validity of the study cannot be evaluated.
(4-Isopropylbenzaldehyde [05.022])	Ames test (plate incorporation method)	*S. typhimurium* TA98; TA100	100 µl/plate	Negative[3]	(Rockwell & Raw, 1979)	
	Ames test (plate method)incorporation	*S. typhimurium* TA98; TA100	300 µl/plate	Negative[1]	(Rockwell & Raw, 1979)	Assay of urine samples from rats given 4-isopropyl benzaldehyde (cuminaldehyde) by gavage.
	Umu test	*S. typhimurium* TA1535/ pSK1002	1 µmole/ml	Negative	(Miyazawa et al., 2000)	Results indicated that 4-isopropyl benzaldehyde (cuminaldehyde) was positive for antimutagenicty, but not genotoxic.

Table 2.2: GENOTOXICITY (*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	Up to 333 µM (up to 50 µg/ml)	Negative[2]	(Sasaki et al., 1989)	Published non-GLP study of limited quality. Study designed to investigate the influence on spontaneous as well as on mitomycin-induced SCEs. The substance did not influence cell cycle (data not shown) and spontaneous SCEs at the concentrations used. Cytotoxicity was reported at the highest concentration tested.
(4-Hydroxybenzoic acid [08.040])	Ames test (plate incorporation method)	*S. typhimurium* TA98; TA100	5000 µg/plate	Negative[2]	(Mikulasova & Bohovicova, 2000)	
	DNA Repair test	*E. coli* WP2, WP2*uvrA*, CM611; CM561	2000 µg/ml	Negative	(Mikulasova & Bohovicova, 2000)	
(Salicylic acid [08.112])	Ames test (preincubation method)	*S. typhimurium* TA98; TA100	100 to 10000 µg/plate	Negative[1]	(Kuboyama & Fujii, 1992)	Published non-GLP study deficient in the report of some details on method and results (no single doses, no data on cytotoxicity reported), however, of sufficient quality to be taken into account in the evaluation.
	Ames test (plate incorporation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537	Not reported	Negative[2]	(McCann et al., 1975)	
	Rec assay	*B. subtilis* M45 (rec^-), H17 (rec^+)	0 to 5000 µg/disc	Weakly positive	(Kuboyama & Fujii, 1992)	Well conducted published non-GLP study with some minor deficiencies (no cytotoxicity data, no detailed data for different concentrations reported) of sufficient quality to be taken into account in the evaluation. A weak positive result (D>2 mm) was observed at concentrations of 2 mg/disc or more. At 5 mg/disc D=4.7 mm.
	Mitotic recombination assay	*S. cerevisiae* D7	10,000 µg/ml	Negative[2]	(Rosin, 1984)	Published non-GLP study with insufficient report of experimental details and results. Study was carried out only in the absence of metabolic activation and is thus not considered valid. Negative response reported both at neutral and alkaline conditions.
	Mutation assay	*S. cerevisiae* rad18	Up to 0.1 mM (up to 13.8 µg/ml; 8 concentrations)	Weakly positive	(Zetterberg, 1979)	Published non-GLP study with limited report of experimental details and result. Use of metabolic activation not reported. The validity of the study cannot be evaluated. The dose level tested was clearly cytotoxic. An increase in mutant frequency was not evident until 95-99% of cells were killed.
Ethyl 4-hydroxybenzoate [09.367]	Ames test	*S. typhimurium* TA98; TA100	Not reported	Negative[1]	(Kawachi et al., 1980a)	Published summary report of unpublished extensive screening study. No details of method and results reported. Thus, the validity of the study cannot be evaluated.
	Rec assay	*B. subtilis*	Not reported	Negative[1]	(Kawachi et al., 1980a)	dito.
	Chromosomal aberration assay	Hamster lung fibroblast cells	Not reported	Positive[2] Negative[3]	(Kawachi et al., 1980a)	dito.
	Chromosomal aberration assay	Human embryo fibroblasts	Not reported	Negative[2]	(Kawachi et al., 1980a)	dito.
	Chromosomal aberration assay	Chinese hamster fibroblast cells	Up to 250 µg/ml	Positive	(Ishidate et al., 1978)	Published non-GLP study in Japanese with English summary and tabulated results. Some important details of method and results are not available. There is no information on the use of metabolic activation. The substance was tested up to the maximum dose tolerated. Thus, the validity of the study cannot be evaluated.
	Sister chromatid exchange	Human embryo fibroblasts	Not reported	Negative[2]	(Kawachi et al., 1980a)	Published summary report of unpublished extensive

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

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
	assay					screening study. No details of method and results reported. Thus, the validity of the study cannot be evaluated.
	Sister chromatid exchange assay	Human fibroblastic cells HE2144	0, 83, 166 µg/ml	Negative[2]	(Sasaki et al., 1980)	Published non-GLP study not in accordance with OECD guideline 479 (no metabolic activation). Insufficient report of important details of method and results. This study is not considered valid.
	Mutation assay	Silk worms	Not reported	Negative	(Kawachi et al., 1980a)	Published summary report of unpublished extensive screening study. Unusual protocol, no details of method and results reported. Thus, the validity of the study cannot be evaluated.
(Butyl 4-hydroxybenzoate [09.754])	Ames test	*S. typhimurium* TA98; TA100	1000 µg/plate	Negative[1]	(Haresaku et al., 1985)	
	Ames test (preincubation method)	*S. typhimurium* TA92; TA94; TA98; TA100; TA1535; TA1537; TA2637	Up to 1000 µg/plate (6 concentrations)	Negative[1]	(Ishidate et al., 1984)	Published study in accordance to OECD guideline 471. Although some details of results are not reported the study is considered valid.
	Chromosomal aberration test	Chinese hamster fibroblast cells	60 µg/ml (three concentrations, max. concentration inducing 50% cell-growth inhibition)[4]	Negative[2]	(Ishidate et al., 1984)	Published study carried out only in the absence of metabolic activation. Thus, study is not considered valid. Cells were exposed for 24 and 48 hours. Negative response for chromosomal aberrations and polyploidization.
	Ames test (plate incorporation assay)	*S. typhimurium* TA100	500 µg/plate	Negative[2]	(Ball et al., 1984)	
(Veratraldehyde [05.017])	Ames test	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA15378	8000 µg/plate	Negative[1]	(Nestmann et al., 1980)	
	Ames test	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	8000 µg/plate	Negative[1]	(Douglas et al., 1979)	
	Ames test (preincubation method)	*S. typhimurium* TA97; TA98; TA100; TA1535; TTA1537	6666 µg/plate	Negative[1]	(Mortelmans et al., 1986)	
	Ames test (plate incorporation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	1000 µg/plate[4]	Negative[1]	(Heck et al., 1989)	Published non-GLP study. No information concerning a possible cytotoxic effect nor on the number of concentrations tested. Due to the lack of some important details of study design and results the validity of the study cannot be evaluated.
	Ames test (preincubation method)	*S. typhimurium* TA100; TA102; TA104; TA982; TA1538	Not reported	Negative[1]	(Dillon et al., 1992)	
	Ames test (preincubation protocol)	*S. typhimurium* TA100; TA102; TA104	33 - 3333 µg/plate	Negative[1]	(Dillon et al., 1998)	
	Mutation assay	*S. cerevisiae* D7; XV185-14C	Not reported	Negative[2]	(Nestmann & Lee, 1983)	
	Mammalian cell gene mutation test	Mouse lymphoma L5178Y cells	250 to 1800 µg/ml	Positive[1]	(Heck et al., 1989)	Published non-GLP study. Some important details of study design and results are not reported. Thus, the validity of the study cannot be evaluated. Different concentration ranges (250, 1400-1600, 1400-1800 µg/ml) were used in three independent experiments within which positive responses were observed. A 2.3 to 6.2fold increase in the mutation frequency was observed both with and without S9.

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

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
	Ames test (plate incorporation method)	*S. typhimurium* TA98; TA100	5000 µg/plate	Negative[2]	(Mikulasova & Bohovicova, 2000)	
	DNA Repair test	*E. coli* WP2; WP2*uvrA*; CM611; CM561	2000 µg/ml	Negative	(Mikulasova & Bohovicova, 2000)	
	Unscheduled DNA synthesis test	Rat hepatocytes	100 µg/ml[4]	Negative	(Heck et al., 1989)	Published non-GLP study. No information concerning the number of concentrations tested. Due to the lack of some important details of study design and results the validity of the study cannot be evaluated.
(4-Methoxybenzaldehyde [05.015])	Ames test (preincubation method)	*S. typhimurium* TA92; TA94; TA98; TA100; TA1535; TA1537; TA2637	Up to 5000 µg/plate (6 concentrations)	Negative[1]	(Ishidate et al., 1984)	Published study in accordance to OECD guideline 471. Although some details of results are not reported the study is considered valid.
	Ames test	*S. typhimurium* TA98; TA100	0.05 to 500 µg/plate	Negative[1]	(Kasamaki et al., 1982)	Published non-GLP study with insufficient report of some details of method and results. Thus, the validity of the study cannot be evaluated.
	Ames test (preincubation method)	*S. typhimurium* TA1537	Up to 5000 µg/plate (6 concentrations)	Negative[1]	(Engelhardt, 1986)	
	Ames test	*S. typhimurium* TA98; TA100; TA1535; TA1537	408 µg/plate	Negative[1]	(Florin et al., 1980)	
	Ames test (preincubation method)	*S. typhimurium* TA97; TA102	1000 µg/plate	Negative[1]	(Fujita & Sasaki, 1987)	
	Rec assay	*B. subtilis* M45 (rec^-), H17 (rec^+)	22 µg/disc	Negative	(Oda et al., 1979)	Study published in Japanese without English abstract. Data extracted from tables. Validity of the study cannot be evaluated. No information on the use of metabolic activation.
	Ames test	*S. typhimurium* TA102	5000 µg/plate	Negative[1]	(Müller et al., 1993)	
	Ames test	*S. typhimurium* TA 100	1000 µg/plate	Negative	(Rapson et al., 1980)	
	Mutation assay	Phage PM2	1362 µg/ml	Negative	(Becker et al., 1996)	
	Chromosomal aberration test	Chinese hamster fibroblast cells	500 µg/ml (three concentrations, max. concentration inducing 50% cell-growth inhibition)[4]	Negative[2]	(Ishidate et al., 1984)	Published study carried out only in the absence of metabolic activation. Thus, study is not considered valid. Cells were exposed for 24 and 48 hours. Negative response for chromosomal aberrations and polyploidization.
	Chromosomal aberration test	Chinese hamster cell line B241	50 nM (0.0068 µg/ml)	Positive[1]	(Kasamaki et al., 1982)	Published non-GLP study of sufficient quality to be taken into account for the evaluation, although some details of method and results are not reported. Results are reported for the concentration at which maximal frequency of aberration was observed without visible cytotoxicity in the treated cells. Dose-dependent increase of total aberrations (chromatid gaps, chromatid breaks, chromosome breaks observed, ring and dicentric aberrations, chromatic exchanges).
	Mammalian cell gene mutation test	Mouse lymphoma L5178Y TK+/- cells	0-3.0 mM (0-408 µg/ml) 3.6-5.1 mM (484-691 µg/ml)	Negative[2] Positive[2]	(Wangenheim & Bolcsfoldi, 1988)	Published non-GLP study not in accordance with OECD guideline 476 (no metabolic activation, no colony sizing). Important details of method and results are insufficiently reported. This study is not considered valid.
	Ames test	*S. typhimurium* TA102	5000 µg/plate	Negative[1]	(Jung et al., 1992)	Results confirmed at three separate contract laboratories

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Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
	Sister chromatid exchange assay	Human lymphocytes	0-2 mM (0-273 µg/ml)	Positive[2]	(Jansson et al., 1988)	Published non-GLP study not in accordance with OECD guideline 479 (no metabolic activation). Insufficient report of important details of method and results. This study is not considered valid.
	Sister chromatid exchange assay	Chinese hamster ovary K1 cells	14 µg/ml	Negative	(Sasaki et al., 1987)	
	DNA alkaline unwinding assay	Mouse lymphoma L5178Y TK+/- cells	0, 4, 5, 6 mole/l (0, 544, 680, 816 µg/ml) 7, 8 mole/l (953, 1089 µg/ml)	Negative[2] Positive[2]	(Garberg et al., 1988)	Published study on 78 compounds not in accordance with standard guidelines. Test suitable for rapid screening only. Strand breaks or mutations observed only at cytotoxic concentrations.
2-Methoxybenzaldehyde [05.129]	Mutation assay	*E. coli* WP2*uvr*A, *trpE*	5000 µg/plate	Negative[2]	(Watanabe et al., 1989)	Published non-GLP study with limited report of experimental details and results. Study evaluating the enhancing effect on *N'*-nitro-*N*-nitrosoguanidine (MNNG)-induced mutagenesis in pretreated cells and not on the mutagenicity of the substance itself. Absence of an enhancing effect reported.
	Sister chromatid exchange assay	Human lymphocytes	0-0.25 mM (0-34 µg/ml)	Positive[2]	(Jansson et al., 1988)	Published non-GLP study not in accordance with OECD guideline 479 (no metabolic activation). Insufficient report of important details of method and results. This study is not considered valid.
3-Methoxybenzaldehyde [05.158]	Sister chromatid exchange assay	Human lymphocytes	0-2.0 mM (0-273 µg/ml)	Positive[2]	(Jansson et al., 1988)	dito.
	Mammalian cell gene mutation test	Mouse lymphoma L5178Y TK+/- cells	0- 2.5 mM (0- 340 µg/ml) 3 mM (408 µg/ml)	Negative[2] Positive[2]	(Wangenheim & Bolcsfoldi, 1988)	Published non-GLP study not in accordance with OECD guideline 476 (no metabolic activation, no colony sizing). Important details of method and results are insufficiently reported. This study is not considered valid.
(4-Ethoxybenzaldehyde [05.056])	Ames test	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	3600 µg/plate	Negative[2]	(Wild et al., 1983)	
(Methyl 4-methoxybenzoate [09.713])	Paper disk mutation assay	*E. coli* Sd-4-73	Not reported	Negative[2]	(Szybalski, 1958)	
Gallic acid [08.080]	Ames test (preincubation method)	*S. typhimurium* TA98; TA100	3000 µg/plate	Negative[1]	(Chen & Chung, 2000)	
	Ames test (preincubation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537	0, 100, 333, 1000, 3333, 6666 µg/plate (solvent DMSO) 0, 100, 333, 1000, 3333, 10,000 µg/plate (solvent acetone)	Negative[1] Equivocal[1]	(Haworth et al., 1983)	Published summary report including detailed results from studies on 250 compounds tested in various laboratories within the NTP to a large extent in accordance with OECD guideline 471. Results on gallic acid from two different laboratories using different solvent. A negative response was observed in both laboratories with TA98, TA1535, TA1537. A negative result was also reported with TA100 in the laboratory using DMSO as solvent. With acetone, a low-level response with a dose-related trend was found with TA100 both in the absence and in the presence of metabolic activation. The effect was reproducible in a second, not reproducible in a third experiment.
	Ames test (preincubation method)	*S. typhimurium* TA98; TA100; TA1535	5000 µg/plate	Negative[1]	(Rashid et al., 1985)	Inhibition was noted at the 5000-µg/plate dose-level; however this may have been due to toxicity. No mutagenicity was observed at the 1000-µg/plat dose-level.
	Ames test	*S. typhimurium* TA98; TA100; TA1537	15 µM/plate	Negative[1]	(Wang & Klemencic, 1979)	

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Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
	Ames test	*S. typhimurium* TA100	100 µg/plate	Weakly positive[2] Positive[3]	(Yamaguchi, 1981)	Published non-GLP. Insufficient report of important details of method and results, thus the validity of the result cannot be evaluated.
	Ames test	*S. typhimurium* TA98; TA100	Not reported	Negative[1]	(Sugimura et al., 1976)	
	Chromosomal aberration test	Chinese hamster ovary cells	50 µg/ml	Positive[1]	(Stich et al., 1981c)	Published non-GLP study. Some important details of method and results are not reported. Thus, the validity of the study cannot be evaluated. Results are reported for one concentration only which was half the dose inducing mitotic inhibition.The clastogenic activity was reported to be reduced by the addition of S9.
	Chromosomal aberration test	Chinese hamster ovary K1 cells	up to 2 mM (up to 340 µg/ml)	Negative[1]	(Tayama & Nakagawa, 2001)	Published non-GLP study. Part of the study with insufficient report of important details of method and results. The validity of the results cannot be evaluated.
	Sister chromatid exchange assay	Chinese hamster ovary K1 cells	0, 0.25, 0.5, 1.0, 1.5, 2.0 mM (0, 42.5, 85, 170, 255, 340 µg/ml)	Positive[2]	(Tayama & Nakagawa, 2001)	Published non-GLP study. Well conducted part of the study, however with insufficient report of some important details of method and results (results with metabolic activation not reported)..
	Mitotic gene conversion assay	*S. cerevisiae* D7	0, 100, 1000 µg/ml	Negative[2] Positive[2]	(Rosin, 1984)	Published non-GLP study with insufficient report of experimental details and results. Study was carried out only in the absence of metabolic activation and is thus not considered valid. Gallic acid did not induce a significant extent of gene conversions under acidic conditions. At neutral pH no convertogenic activity was reported at 100 µg/ml, however, gallic acid was considerably convertogenic at 1000 µg/ml. The presence of catalase completely inhibited the convertogenic activity.gene conversions. Under alkaline conditions (pH 10), the concentration of 100 µg/ml was reported to induce a significant ($p < 0.01$) increase of Trp^+ convertants.
(Vanillin [05.018])	Ames test (plate incorporation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	10,000 µg/plate[4]	Negative[1]	(Heck et al., 1989)	Published non-GLP study. No information concerning a possible cytotoxic effect nor on the number of concentrations tested. The test guidelines do not require more than 5 mg/plate. Due to the lack of some important details of study design and results the validity of the study cannot be evaluated.
	Ames test	*S. typhimurium* TA98; TA100; TA 1535; TA1537; TA1538	5000 µg/plate	Negative[1]	(Pool & Lin, 1982)	
	Rec assay	*B. subtilis* M45 (rec^-), H17 (rec^+)	21 µg/disc	Negative	(Oda et al., 1979)	Study published in Japanese without English abstract. Data extracted from tables. Validity of the study cannot be evaluated.
	Ames test (preincubation assay)	*S. typhimurium* TA97; TA98; TA100; TA1535; TA1537	10,000 µg/plate	Negative[1]	(Mortelmans et al., 1986)	
	Ames test	*S. typhimurium* TA98; TA100	0.05 to 1000 µg/plate	Negative[1]	(Kasamaki et al., 1982)	Published non-GLP study with insufficient report of some details of method and results. Thus, the validity of the study cannot be evaluated.

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

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
	Ames test	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	Not reported	Negative[1]	(Nagabhushan & Bhide, 1985)	
	Ames test	*S. typhimurium* TA92; TA94; TA98; TA100; TA1535; TA1537; TA2637	Up to 10,000 µg/plate (6 concentrations)	Negative[1]	(Ishidate et al., 1984)	Published study in accordance to OECD guideline 471. Although some details of results are not reported the study is considered valid.
	Ames test	*S. typhimurium* TA100	1000 µg/plate	Negative	(Rapson et al., 1980)	
	Paper disk mutation assay	*E. coli* Sd-4-73	Not reported	Negative[2]	(Szybalski, 1958)	
	Ames test (plate incorporation method)	*S. typhimurium* TA98; TA100	2500 µg/plate	Negative[2]	(Mikulasova & Bohovicova, 2000)	
	DNA Repair test	*E. coli* WP2; WP2*uvrA*; CM611; CM561	2000 µg/ml	Negative	(Mikulasova & Bohovicova, 2000)	
	Mutation assay	*E. coli* CSH26/pYM3; CSH26/pSK 1002	15,215 µg/ml	Negative	(Takahashi et al., 1990)	
	Mitotic recombination assay	*S. cerevisiae* D7	10,000 µg/ml	Negative[2]	(Rosin, 1984)	Published non-GLP study with insufficient report of experimental details and results. Study was carried out only in the absence of metabolic activation and is thus not considered valid. Negative response reported both at neutral and alkaline conditions.
	Chromosomal aberration test	Chinese hamster cell line B241	5, 20, 40 nM (0.0008, 0.003, 0.006 µg/ml)	Negative	(Kasamaki & Urasawa, 1985)	
	Chromosomal aberration test	Chinese hamster fibroblast cells	1000 µg/ml (three concentrations, max. concentration inducing 50% cell-growth inhibition)[4]	Negative[2]	(Ishidate et al., 1984)	Published study carried out only in the absence of metabolic activation. Thus, study is not considered valid. Cells were exposed for 24 and 48 hours. Negative response for chromosomal aberrations and polyploidization.
	Chromosomal aberration test	Chinese hamster V79 lung cells	15,215 -152,150 µg	Negative[2]	(Tamai et al., 1992)	
	Chromosomal aberration test	Human lymphocytes	0, 1, 2, 4 mM (0, 152, 304, 608 µg/ml)	Negative	(Jansson & Zech, 1987)	Published non-GLP study not in accordance with OECD guideline 473 (no metabolic activation). Insufficient report of important details of method and results. No information on cytotoxicity. This study is not considered valid.
	Chromosomal aberration test	Chinese hamster cell line B241	20 nM (0.003 µg/ml)	Negative[1]	(Kasamaki et al., 1982)	Published non-GLP study of sufficient quality to be taken into account for the evaluation, although some details of method and results are not reported. Results are only reported for the final concentration at which maximal frequency of aberration was observed without visible cytotoxicity in the treated cells. No significant increase increase of single types of aberrations and of total aberrations.
	Sister chromatid exchange assay	Human lymphocyte cells	0 – 1.0 mM (0 - 152 µg/ml)	Positive[2]	(Jansson et al., 1986)	Published non-GLP study not in accordance with OECD guideline 479 (no metabolic activation). This study is not considered valid. Dose-dependent effect reported. Insufficient report of important details of method and results.
	Sister chromatid exchange assay	Chinese hamster ovary K1 cells	15 µg/ml	Negative	(Sasaki et al., 1987)	
	Sister chromatid exchange assay	Human lymphocytes	0, 1, 2 mM (0, 152, 304 µg/ml)	Positive[2]	(Jansson & Zech, 1987)	Published non-GLP study not in accordance with OECD guideline 479 (no metabolic activation). Insufficient report of important details of method and results. Dose-dependent

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

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
						effect reported This study is not considered valid.
	Mutation assay	Mouse lymphoma L5178Y cells	1000 µg/ml (-S9), 1500 µg/ml (+S9)[4]	Negative[1]	(Heck et al., 1989)	Published non-GLP study. Some important details of study design and results are not reported. Thus, the validity of the study cannot be evaluated.
	Unscheduled DNA synthesis test	Rat hepatocytes	500 µg/ml[4]	Negative	(Heck et al., 1989)	Published non-GLP study. No information concerning the number of concentrations tested. Due to the lack of some important details of study design and results the validity of the study cannot be evaluated.
	Micronucleus assay	Human hepatoma (Hep-G2) cells	5, 50 µg/ml 500 µg/ml	Negative [2] Positive[2]	(Sanyal et al., 1997)	Published non-GLP study carried out only in the absence of metabolic activation. Thus, the study is not considered valid. A statistically significant increase of spontaneus micronucleus frequency was reported at the highest concentration. Low concentrations of vanillin (0.25 – 5 µg/ml) but not higher (50, 500 µg/ml) showed an inhibitory effect on micronuclei induced by heterocyclic amines.
(Vanillic acid [08.043])	Chromosomal aberration test	Chinese hamster ovary cells	25,000 µg/ml	Positive[1]	(Stich et al., 1981c)	Published non-GLP study. Some important details of method and results are not reported. Thus, the validity of the study cannot be evaluated. Data are only reported for one concentration which was half the dose inducing mitotic inhibition.The clastogenic activity was reported to be increased by the addition of S9.
	Mitotic recombination assay	*S. cerevisiae* D7	10,000 µg/ml	Negative[2]	(Rosin, 1984)	Published non-GLP study with insufficient report of experimental details and results. Study was carried out only in the absence of metabolic activation and is thus not considered valid. Negative response reported both at neutral and alkaline conditions.
4-Hydroxy-3,5-dimethoxybenzaldehyde [05.153]	Ames test	*S. typhimurium* TA100	10,000 µg/plate	Negative	(Rapson et al., 1980)	The use of metabolic activation was not reported.
4-Hydroxy-3,5- dimethoxybenzoic acid [08.087]	Ames test	*S. typhimurium* TA98; TA100; TA1535; TA1537	366 µg/plate	Negative[1]	(Florin et al., 1980)	
	Chromosomal aberration test	Chinese hamster ovary cells	3000 µg/ml	Positive[1]	(Stich et al., 1981c)	Published non-GLP study. Some important details of method and results are not reported. Thus, the validity of the study cannot be evaluated. Data are only reported for one concentration which was half the dose inducing mitotic inhibition. The clastogenic activity was reported to be reduced by the addition of S9.
	Mitotic recombination assay	*S. cerevisiae* D7	10,000 µg/ml	Negative[2]	(Rosin, 1984)	Published non-GLP study with insufficient report of experimental details and results. Study was carried out only in the absence of metabolic activation and is thus not considered valid.
(Salicylaldehyde [05.055])	Ames test	*S. typhimurium* TA98; TA100; TA1535; TA1537	366 µg/plate	Negative[1]	(Florin et al., 1980)	
	Ames test (preincubation method)	*S. typhimurium* TA98; TA100	Not reported	Negative[1]	(Sasaki & Endo, 1978)	
	Ames test	*S. typhimurium* TA98; TA100	16 µg/ml	Negative[1]	(Kono et al., 1995)	
	Mutation assay	*S. typhimurium* TA1535/ pSK1002	111 µg/ml	Negative[1]	(Nakamura et al., 1987)	

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

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
	Chromosomal aberration test	CHL/IU cells	Not reported (max. 5 mg/ml)	Positive[1]	(Kusakabe et al., 2002)	Published study in accordance to OECD guideline 473. However, some details on method and results are insufficiently reported. Thus the validity of the study cannot be evaluated. Positive result with minimum effective dose manifesting over 50% cytotoxicity at short-term treatment (6 h, less than 50% cells with chromosomal aberrations without S9, less than 20% cells with chromosomal aberrations with S9). Reduced effect at continuous treatment without S9 (24 h less than 10% cells with chromosomal aberrations). No chromosomal aberrations after 48 h treatment without S9. After 48 h treatment without S9 18% polyploid cells..
	Sister chromatid exchange assay	Human lymphocyte cells	0-0.5 mM (0-61 µg/ml)	Negative[2]	(Jansson et al., 1988)	Published non-GLP study not in accordance with OECD guideline 479 (no metabolic activation). Insufficient report of important details of method and results. This study is not considered valid.
(Methyl salicylate [09.749])	Ames test	*S. typhimurium* TA92; TA94; TA98; TA100; TA1535; TA1537; TA2637	Up to 10,000 µg/plate (6 concentrations)	Negative[1]	(Ishidate et al., 1984)	Published study in accordance to OECD guideline 471. Although some details of results are not reported the study is considered valid.
	Ames test (preincubation method)	*S. typhimurium* TA97; TA98; TA100; TA1535; TA1537	333.3 µg/plate	Negative[1]	(Mortelmans et al., 1986)	
	Ames test	*S. typhimurium* TA98; TA100	Not reported	Negative[1]	(Kawachi et al., 1980b; Kawachi et al., 1980a)	Published summary report of unpublished extensive screening study. No details of method and results reported. Thus, the validity of the study cannot be evaluated.
	Chromosomal aberration test	Hamster lung fibroblast cells	Not reported	Positive[2] Negative[3]	(Kawachi et al., 1980b; Kawachi et al., 1980a)	dito.
	Chromosomal aberration test	Chinese hamster fibroblasts	250 µg/ml[4] (three concentrations, max. concentration inducing 50% cell-growth inhibition)	Negative[2]	(Ishidate et al., 1984)	Published study carried out only in the absence of metabolic activation. Thus, study is not considered valid. Cells were exposed for 24 and 48 hours. Negative response for chromosomal aberrations and polyploidization.
	Ames test (preincubation method)	*S. typhimurium* TA98; TA100	100 to 10000 µg/plate	Positive[1]	(Kuboyama & Fujii, 1992)	Published non-GLP study deficient in the report of some details on method and results (no single doses, no data on cytotoxicity reported), however, of sufficient quality to be taken into account in the evaluation. At 100 µg/plate a positive response was observed in strain TA98 in the presence of S9 mix obtained from hamsters a negative response was observed in TA98 in the presence of S9 mix obtained from rat, mouse and guinea pig.
	Rec assay	*B. subtilis* M45 (rec^-), H17 (rec^+)	23 µg/disc	Negative	(Oda et al., 1979)	Study published in Japanese without English abstract. Data extracted from tables. Validity of the study cannot be evaluated.
	Rec assay	*B. subtilis*	Not reported	Negative[1]	(Kawachi et al., 1980b; Kawachi et al., 1980a)	Published summary report of unpublished extensive screening study. No details of method and results reported. Thus, the validity of the study cannot be evaluated.

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

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
	Rec assay	*B. subtilis* M45 (rec$^-$), H17 (rec$^+$)	0 to 5000 µg/disc	Negative	(Kuboyama & Fujii, 1992)	Well conducted published non-GLP study with some minor deficiencies (no cytotoxicity data, no detailed data for different concentrations reported), however, of sufficient quality to be taken into account in the evaluation.
	Mutation assay	Silkworm	Not reported	Negative	(Kawachi et al., 1980b; Kawachi et al., 1980a)	Published summary report of unpublished extensive screening study. Unusual protocol, no details of method and results reported. Thus, the validity of the study cannot be evaluated.
	Chromosomal aberration test	Human embryo fibroblast cells	Not reported	Negative[2]	(Kawachi et al., 1980b; Kawachi et al., 1980a)	dito.
	Sister chromatid exchange assay	Human embryo fibroblast cells	Not reported	Negative[2]	(Kawachi et al., 1980b; Kawachi et al., 1980a)	dito.
(Butyl vanillyl ether [04.093])	Ames test	*S. typhimurium* TA98; TA100; TA1535; TA1537	5000 µg/plate	Negative[1]	(Watanabe & Morimoto, 1989c)	
	Mutation assay	*E. coli* WP2 *uvr*A	5000 µg/plate	Negative[1]	(Watanabe & Morimoto, 1989c)	
(Ethyl vanillin [05.019])	Ames test	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	3600 µg/plate	Negative[1]	(Wild et al., 1983)	
	Ames test (preincubation method)	*S. typhimurium* TA97; TA98; TA100; TA1535; TA1537	8000 µg/plate	Negative[1]	(Mortelmans et al., 1986)	
	Ames test	*S. typhimurium* TA92; TA94; TA98; TA100; TA1535; TA1537; TA2637	Up to 10,000 µg/plate (six concentrations)	Negative[1]	(Ishidate et al., 1984)	Published study in accordance to OECD guideline 471. Although some details of results are not reported the study is considered valid.
	Ames test (preincubation method)	*S. typhimurium* TA97; TA102	1000 µg/plate	Negative[1]	(Fujita & Sasaki, 1987)	
	Ames test	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	10,000 µg/plate[4]	Negative[1]	(Heck et al., 1989)	Published non-GLP study. No information concerning a possible cytotoxic effect nor on the number of concentrations tested. The test guidelines do not require more than 5 mg/plate. Due to the lack of some important details of study design and results the validity of the study cannot be evaluated.
	Rec assay	*B. subtilis* M45 (rec$^-$), H17 (rec$^+$)	21 µg/disc	Negative	(Oda et al., 1979)	Study published in Japanese without English abstract. Data extracted from tables. Validity of the study cannot be evaluated.
	Chromosomal aberration test	Chinese hamster fibroblast cells	250 µg/ml (three concentrations, maximal concentration inducing 50% cell-growth inhibition)[4]	Positive[2]	(Ishidate et al., 1984)	Published study carried out only in the absence of metabolic activation. Thus, study is not considered valid. Polyploidization in 48% of cells reported at 48 hours. Negative response for chromosomal aberrations.

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

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
	Mammalian cell gene mutation test	Mouse lymphoma L5178Y cells	125-800 µg/ml	Negative[2] Weak positive[3]	(Heck et al., 1989)	Published non-GLP study. Some important details of study design and results are not reported. Thus, the validity of the study cannot be evaluated. Different concentration ranges (125-500 µg/ml, 600 µg/ml, 800 µg/ml) were used in three independent experiments within which positive responses were observed. In the presence of S9 a 2.1 to 3-fold increase in the mutant frequency was reported.
	Unscheduled DNA synthesis test	Rat hepatocytes	199 µg/ml[4]	Negative	(Heck et al., 1989)	Published non-GLP study. No information concerning the number of concentrations tested. Due to the lack of some important details of study design and results the validity of the study cannot be evaluated.
	Sister chromatid exchange assay	Human lymphocytes	0-2.0 mM (0-332 µg/ml)	Negative[2]	(Jansson et al., 1988)	Published non-GLP study not in accordance with OECD guideline 479 (no metabolic activation). Insufficient report of important details of method and results. This study is not considered valid.
	Sister chromatid exchange assay	Chinese hamster ovary K1 cells	17 µg/ml	Negative	(Sasaki et al., 1987)	
(Ethyl vanillin isobutyrate)	Ames test	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	5000 µg/plate	Negative[1]	(King & Harnasch, 1997)	
(Piperonyl acetate [09.220])	Ames test (preincubation method)	*S. typhimurium* TA97; TA98; TA100; TA1535; TA1537	3333 µg/plate	Negative[1]	(Mortelmans et al., 1986)	
	Ames test	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	3600 µg/plate	Negative[1]	(Wild et al., 1983)	
(Piperonal [05.016])	Modified Ames test	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538 *E. coli* WP2*uvr*Atrp$^-$	0, 300, 600, 1200, 2400 µg/plate	Negative[1]	(Sekizawa & Shibamoto, 1982)	Valid study in accordance with OECD guideline 471. The plate incorporation method was used -S9; the preincubation method +S9.
	Ames test (plate incorporation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	10,000 µg/plate[4]	Negative[1]	(Heck et al., 1989)	Published non-GLP study. No information concerning a possible cytotoxic effect nor on the number of concentrations tested. The test guidelines do not require more than 5 mg/plate. Due to the lack of some important details of study design and results the validity of the study cannot be evaluated.
	Ames test	*S. typhimurium* TA98; TA100	0.05 to 5000 µg/plate	Negative[1]	(Kasamaki et al., 1982)	Published non-GLP study with insufficient report of some details of method and results. Thus, the validity of the study cannot be evaluated.
	Ames test	*S. typhimurium* TA98; TA100; TA1537; TA1538	5000 µg/plate	Negative[1]	(White et al., 1977)	
	Ames test (preincubation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537	0, 10, 33, 100, 333, 1000 µg/plate	Negative[1]	(Haworth et al., 1983)	Published summary report including detailed results from studies on 250 compounds tested in various laboratories within the NTP to a large extent in accordance with OECD guideline 471.
	Rec assay	*B. subtilis* M45 (rec$^-$), H17 (rec$^+$)	20 µg/disc	Negative	(Oda et al., 1979)	Study published in Japanese without English abstract. Data extracted from tables. Validity of the study cannot be evaluated.
	Rec assay	*B. subtilis* M45 (rec$^-$), H17 (rec$^+$)	5000 µg/disc	Positive[2]	(Sekizawa & Shibamoto, 1982)	Well designed and reported study, however with some limitations with respect to results. DNA-repair tests in the

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

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
						presence of S9 were not successful (no data reported).
	Chromosomal aberration test	Chinese hamster cell line B241	50 nM (0.0075 µg/ml)	Positive[1]	(Kasamaki et al., 1982)	Published non-GLP study of sufficient quality to be taken into account for the evaluation, although some details of method and results are not reported. Data are only reported for the concentration at which maximal frequency of aberration was observed without visible cytotoxicity in the treated cells. Dose-dependent increase of total aberrations (chromatid gaps, chromatid breaks, chromosome breaks observed, no ring or dicentric aberrations or chromatic exchanges).
	Chromosomal aberration test	Chinese hamster cell line B241	0.15 µg/ml	Negative	(Kasamaki & Urasawa, 1985)	
	Mammalian cell gene mutation test	Mouse lymphoma L5178Y cells	1000 µg/ml[4]	Negative[1]	(Heck et al., 1989)	Published non-GLP study. Some important details of study design and results are not reported. Thus, the validity of the study cannot be evaluated.
	Unscheduled DNA synthesis test	Rat hepatocytes	10 to 502 µg/ml	Positive	(Heck et al., 1989)	Published non-GLP study. No information concerning the number of concentrations tested. Due to the lack of some important details of study design and results the validity of the study cannot be evaluated.

NR = not reported

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

[2] *Without S9 metabolic activation.*

[3] *With S9 metabolic activation.*

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

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

Substances listed in brackets are JECFA-evaluated substances

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

Chemical Name [FL-no]	Test System	Test Object	Route	Dose	Result	Reference	Comments
(Benzyl alcohol [02.010])	*In vivo* Sex- linked recessive lethal mutations(SLRL)	*D. melanogaster*	Diet	5000 ppm	Negative	(Foureman et al., 1994)	
	In vivo SLRL	*D. melanogaster*	Injection	8000 ppm	Negative	(Foureman et al., 1994)	
	In vivo Micronucleus test	Mouse bone marrow cells	IP injection	200 mg/kg bw	Negative	(Hayashi et al., 1988)	
	In vivo Replicative DNA synthesis test	Mouse and rat hepatocytes	Not reported	Not reported	Negative	(Yoshikawa, 1996)	Screening test for the detection of non-genotoxic hepatocarcinogens. The substance was administered once at the maximum tolerated dose or at half the maximum tolerated dose to male mice and rats. Hepatocytes were prepared after 24, 39 and 48 hours.
	In vivo Replicative DNA synthesis test	Mouse hepatocytes	Oral gavage	800 mg/kg	Negative	(Miyagawa et al., 1995)	
	In vivo Replicative DNA synthesis test	Rat hepatocytes	Oral or SC injection	600 mg/kg	Negative	(Uno et al., 1994)	
(Benzyl acetate [09.014])	*In vivo* SLRL	*D. melanogaster*	Diet	300 ppm	Negative	(NTP, 1993d; Foureman et al., 1994)	
	In vivo SLRL	*D. melanogaster*	Injection	20,000 ppm	Negative	(NTP, 1993d; Foureman et al., 1994)	
	In vivo Sister chromatid exchange assay	Mouse bone marrow cells	IP injection	1700 mg/kg bw	Negative	(NTP, 1993d)	
	In vivo Chromosomal aberration test	Mouse bone marrow cells	IP injection	0 to 1700 mg/kg bw	Negative	(NTP, 1993d)	Test substance same batch as NTP chronic bioassays. The highest dose caused toxicity and cell cycle delay. Test not fully in compliance with the OECD guideline (insufficient cells per animal studied). GLP status not stated. The study is considered of limited validity.
	In vivo Micronucleus test	Mouse bone marrow cells	3 IP injection with 24 h intervals	0, 312, 625 and 1250 mg/kg bw	Negative	(NTP, 1993d; Shelby et al., 1993)	Test substance same batch as NTP chronic bioassays. Study in compliance with OECD guideline. GLP not stated. Micronuclei were determiend at 24 h after the last dose. A dose-related decrease in PCE/NCE ratio was observed. The study is considered valid.
	In vivo Micronucleus test	Mouse erythrocytes	Dietary exposure for 13 weeks.	0 to 50,000 ppm (equal to 0 to 7900 mg/kg bw/day for males and 0 to 9400 mg/kg bw/day for females)	Negative	(NTP, 1993d)	Test substance same batch as NTP chronic bioassays. In life phase under GLP; for determination of genotoxic effects. GLP not specified. Test in compliance with OECD guideline. The test is considered valid, but of limited relevance because no chance in PCE/NCE ratio was observed.
	In vivo Unscheduled DNA	Rat hepatocytes	Oral gavage	0, 50, 200 and 1000	Negative	(Mirsalis et al., 1989)	Test substance same batch as NTP chronic bioassays.

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

Chemical Name [FL-no]	Test System	Test Object	Route	Dose	Result	Reference	Comments
	synthesis test			mg/kg bw			Test in compliance with OECD guidelines. GLP not stated. The test is considered valid.
	In vivo Unscheduled DNA synthesis test	Rat pancreatic cells	Oral gavage	1000 mg/kg bw	Negative	(Steinmetz & Mirsalis, 1984)	Only abstract available. Non guideline test. Validity cannot be assesed.
	In vivo DNA damage	Rat pancreatic cells	IP injection	0, 150, 500 and 1500 mg/kg bw	Negative	(Longnecker et al., 1990)	Alkaline elution assay. GLP status not specified. Limited number of animals/group; DNA damage monitored at 1 hr post dosing. The study is of limited validity.
	In vivo Comet assay	Mouse/ Rat	Oral	1600 mg/kg (mouse); 1200 mg/kg (rat)	Positive	(Sekihashi et al., 2002)	Non-GLP and non-guideline test; but in compliance with recommended protocols. Some important details of method and results insufficiently reported. No toxicity data reported. The administered dose was 0.5 x LD50. Sampling time was 3, 8 and 24 hours after dosing. Positive result reported in mice for stomach, colon, kidney, urinary bladder and brain, in rats for stomach, colon, liver, kidney, urinary bladder, lung. After 24 h no significant effect in mice, significant effects in rat only in lung and kidney. The study is of limited validity.
(Benzaldehyde [05.013])	*In vivo* SLRL	*D. melanogaster*	Diet	1150 ppm	Negative	(Woodruff et al., 1985)	
	In vivo SLRL	*D. melanogaster*	Injection	2500 ppm	Negative	(Woodruff et al., 1985)	
(Salicylic acid [08.112])	*In vivo* Chromosomal aberration assay	Mouse bone marrow cells	IP injection gavage	0, 50, 100, 200 mg/kg 0, 350 mg/kg	Negative Negative	(Giri et al., 1996)	Published study widely in accordance with OECD guideline 475 and well reported (except that only males were tested, only one sampling time was chosen and signs of toxicity were not reported). Oral and i.p. dose were selected to be 1/3 and 1/5 of the reported oral LD50.
	In vivo Sister chromatid exchange assay	Mouse bone marrow cells	IP injection gavage	0, 25, 50, 100 mg/kg 0, 350 mg/kg	Negative Negative	(Giri et al., 1996)	Well described published study of good quality. Oral and i.p. dose were selected to be 1/3 and 1/10 of the reported oral LD50.
Ethyl 4-hydroxybenzoate [09.367]	*In vivo* Chromosomal aberration assay	Rat bone marrow cells	Not reported	Not reported	Negative	(Kawachi et al., 1980a)	Published summary report of unpublished extensive screening study. No details of method and results reported. Thus, the validity of the study cannot be evaluated.
(4-Ethoxybenzaldehyde [05.056])	*In vivo* Basc test Micronucleus test	*D. melanogaster*	NR	751 µg/ml	Negative	(Wild et al., 1983)	Published non-GLP study. Details of study protocol reported elsewhere,. However, results sufficiently reported. Study is considered valid.
	In vivo Micronucleus test	NMRI mice	NR	1005 mg/kg bw	Negative	(Wild et al., 1983)	Published non-GLP study. Details of study protocol and results insufficiently reported. Effect on PCE/NCE ratio not reported. No positive control. Validity of the study cannot be evaluated.
Gallic acid [08.080]	*In vivo* Medium-term rat liver bioassay	Male rats initiated with IP injection of diethylnitrosamine	Not reported.	Not reported	Negative	(Shirai, 1997)	Published non-GLP study. Unusual study protocol not following OECD guidelines. Some important details of method missing and only summarized results of a large screening study reported. Thus, the validity of the study cannot be evaluated.

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

Chemical Name [FL-no]	Test System	Test Object	Route	Dose	Result	Reference	Comments
(Vanillin [05.018])	*In vivo* Micronucleus test	Male BDF1 mice	Oral gavage	500 mg/kg bw	Negative	(Inouye et al., 1988)	Published non-GLP study not in accordance with OECD guideline 474 (smaller group size, only males tested, no toxicity data reported, single dose level used, no negative control, effect on PCE/NCE ratio not reported.) Induction of micronuclei in mitomycin-treated mice was suppressed by post-treatment with vanillin due to an anticlastogenic effect. Vanillin itself did not induce micronucleated PCEs (vanillin control group without mitomycin-treatment, six sampling times from 5 to 65 h).
(Salicylaldehyde [05.055])	*In vivo* Spot test	*D. melanogaster* BINSC *D. melanogaster* Oregon-R	NR	1.05 to 1.40 ppm 0.09 to 0.35 ppm	Negative Negative	(Kono et al., 1995)	Study published in Japanese with English abstract. Data extracted from tables. Validity of the study cannot be evaluated
(Ethyl vanillin [05.019])	*In vivo* Basc test	*D. melanogaster*	NR	8309 µg/ml	Negative	(Wild et al., 1983)	Published non-GLP study. Details of study protocol reported elsewhere,. However, results sufficiently reported. Study is considered valid.
	In vivo Micronucleus test	Male BDF1 mice	IP injection	Not reported	Negative	(Furukawa et al., 1989)	*Only abstract available. Insufficient report of experimental details and result to evaluate the validity of the study*.
	In vivo Micronucleus test	NMRI mice	NR	1000 mg/kg bw	Negative	(Wild et al., 1983)	Published non-GLP study. Details of study protocol and results insufficiently reported. Effect on PCE/NCE ratio not reported. No positive control. Validity of the study cannot be evaluated.
(Piperonyl acetate [09.220])	*In vivo* Basc test	*D. melanogaster*	NR	4855 µg/ml	Negative	(Wild et al., 1983)	Published non-GLP study. Details of study protocol reported elsewhere,. However, results sufficiently reported. Study is considered valid.
	In vivo Micronucleus test	NMRI mice	NR	970 mg/kg bw	Negative	(Wild et al., 1983)	Published non-GLP study. Details of study protocol and results insufficiently reported. Effect on PCE/NCE ratio not reported. No positive control. Validity of the study cannot be evaluated.
(Piperonal [05.016])	*In vivo* Dominant lethal assay	ICR/Ha Swiss mice	IP injection	0, 124, 620 mg/kg bw	Negative	(Epstein et al., 1972)	Published non-GLP study evaluating 174 substances. Study protocol not fully in accordance with OECD guideline 478 (lower number of animals and of dose levels used, limited report of experimental observations). However, due to the large body of control data available the results are considered valid. Doses were selected in preliminary acute toxicity tests. Parameters recorded were percent pregnancy, total implants and early and late fetal deaths.
	In vivo Dominant lethal assay	ICR/Ha Swiss mice	Oral gavage	0, 1000 mg/kg bw (repeated doses on 5 successive days)	Negative	(Epstein et al., 1972)	Dito.

TABLE 3: SUMMARY OF SAFETY EVALUATION TABLES

Table 3.1: Summary of Safety Evaluation of 44 Hydroxy- and Alkoxy-Substituted Benzyl Derivatives Evaluated by JECFA (JECFA, 2002b)

Table 3.1: Summary of safety evaluation of 44 JECFA-evaluated hydroxy and alkoxy substituted benzyl derivatives (JECFA, 2002b)

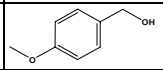
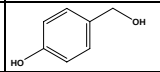
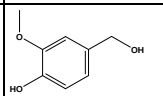
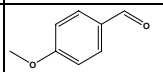
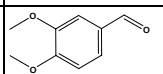
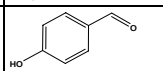
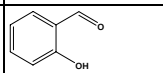
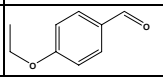
FL-no JECFA-no	EU Register name	Structural formula	EU MSDI 1) US MSDI (μg/*capita*/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	EFSA conclusion on the named compound (Procedure steps, intake estimates, NOAEL, genotoxicity)	EFSA conclusion on the material of commerce
02.128 871	p-Anisyl alcohol		130 58	Class I A3: Intake below threshold	4)	6)	6)
02.165 955	4-Hydroxybenzyl alcohol		5.2 0.06	Class I A3: Intake below threshold	4)	6)	6)
02.213 886	Vanillyl alcohol		5.4 6	Class I A3: Intake below threshold	4)	6)	6)
05.015 878	4-Methoxybenzaldehyde		370 580	Class I A3: Intake below threshold	4)	6)	6)
05.017 877	Veratraldehyde		120 55	Class I A3: Intake below threshold	4)	6)	6)
05.047 956	4-Hydroxybenzaldehyde		55 56	Class I A3: Intake below threshold	4)	6)	6)
05.055 897	Salicylaldehyde		84 16	Class I A3: Intake below threshold	4)	6)	6)
05.056 879	4-Ethoxybenzaldehyde		0.073 0.01	Class I A3: Intake below threshold	4)	6)	6)

Table 3.1: Summary of safety evaluation of 44 JECFA-evaluated hydroxy and alkoxy substituted benzyl derivatives (JECFA, 2002b)

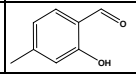
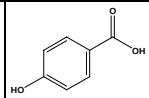
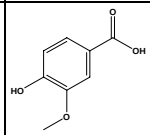
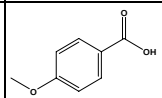
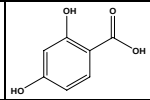
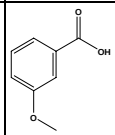
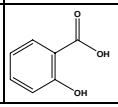
FL-no JECFA-no	EU Register name	Structural formula	EU MSDI 1) US MSDI (µg/*capita*/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	EFSA conclusion on the named compound (Procedure steps, intake estimates, NOAEL, genotoxicity)	EFSA conclusion on the material of commerce
05.091 898	2-Hydroxy-4-methylbenzaldehyde		0.61 0.3	Class I A3: Intake below threshold	4)	6)	6)
08.040 957	4-Hydroxybenzoic acid		16 17	Class I A3: Intake below threshold	4)	6)	6)
08.043 959	Vanillic acid		24 26	Class I A3: Intake below threshold	4)	6)	6)
08.071 883	p-Anisic acid		ND 0.1	Class I A3: Intake below threshold	4)	7)	7)
08.076 908	2,4-Dihydroxybenzoic acid		ND 6	Class I A3: Intake below threshold	4)	7)	7)
08.092 882	3-Methoxybenzoic acid		ND 0.01	Class I A3: Intake below threshold	4)	7)	7)
08.112 958	Salicylic acid		0.024 0.03	Class I A3: Intake below threshold	4)	6)	6)

Table 3.1: Summary of safety evaluation of 44 JECFA-evaluated hydroxy and alkoxy substituted benzyl derivatives (JECFA, 2002b)

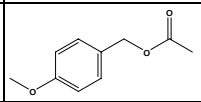
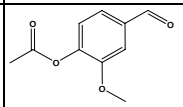
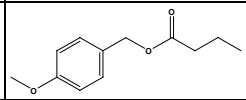
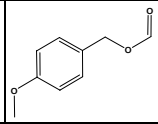
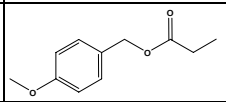
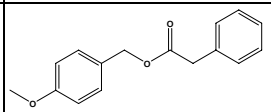
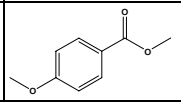
FL-no JECFA-no	EU Register name	Structural formula	EU MSDI 1) US MSDI (µg/*capita*/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	EFSA conclusion on the named compound (Procedure steps, intake estimates, NOAEL, genotoxicity)	EFSA conclusion on the material of commerce
09.019 873	p-Anisyl acetate		50 300	Class I A3: Intake below threshold	4)	6)	6)
09.035 890	Vanillyl acetate		1.8 1	Class I A3: Intake below threshold	4)	6)	6)
09.058 875	p-Anisyl butyrate		29 0.1	Class I A3: Intake below threshold	4)	6)	6)
09.087 872	p-Anisyl formate		39 24	Class I A3: Intake below threshold	4)	6)	According to JECFA: "Minimum assay value is 90%", composition of mixture to be specified.
09.145 874	p-Anisyl propionate		ND 5	Class I A3: Intake below threshold	4)	7)	7)
09.706 876	Anisyl phenylacetate		0.0024 0.1	Class I A3: Intake below threshold	4)	6)	6)
09.713 884	Methyl 4-methoxybenzoate		0.97 0.01	Class I A3: Intake below threshold	4)	6)	6)

Table 3.1: Summary of safety evaluation of 44 JECFA-evaluated hydroxy and alkoxy substituted benzyl derivatives (JECFA, 2002b)

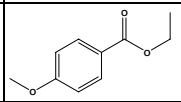
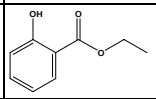
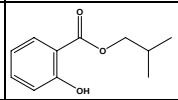
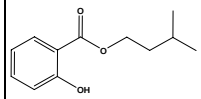
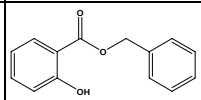
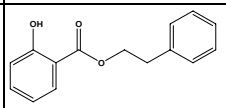
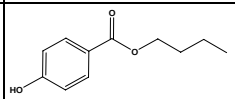
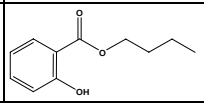
FL-no JECFA-no	EU Register name	Structural formula	EU MSDI 1) US MSDI (µg/*capita*/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	EFSA conclusion on the named compound (Procedure steps, intake estimates, NOAEL, genotoxicity)	EFSA conclusion on the material of commerce
09.714 885	Ethyl 4-methoxybenzoate		9.1 2	Class I A3: Intake below threshold	4)	6)	6)
09.748 900	Ethyl salicylate		27 1700	Class I A3: Intake below threshold	4)	6)	6)
09.750 902	Isobutyl salicylate		0.97 6	Class I A3: Intake below threshold	4)	6)	6)
09.751 903	Isopentyl salicylate		41 7	Class I A3: Intake below threshold	4)	6)	According to JECFA: Min. assay value is "98 (sum of isoamyl and amyl salicylate)", composition of mixture to be specified.
09.752 904	Benzyl salicylate		26 29	Class I A3: Intake below threshold	4)	6)	6)
09.753 905	Phenethyl salicylate		0.12 4	Class I A3: Intake below threshold	4)	6)	6)
09.754 870	Butyl 4-hydroxybenzoate		ND 0.03	Class I A3: Intake below threshold	4)	7) Additional data required	7) Additional data required.
09.763 901	Butyl salicylate		0.012 0.0007	Class I A3: Intake below threshold	4)	6)	Id test is requested.

Table 3.1: Summary of safety evaluation of 44 JECFA-evaluated hydroxy and alkoxy substituted benzyl derivatives (JECFA, 2002b)

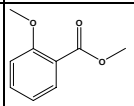
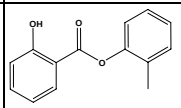
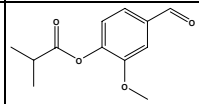
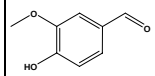
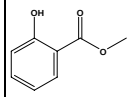
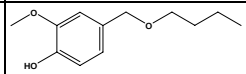
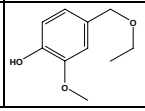
FL-no JECFA-no	EU Register name	Structural formula	EU MSDI 1) US MSDI (μg/*capita*/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	EFSA conclusion on the named compound (Procedure steps, intake estimates, NOAEL, genotoxicity)	EFSA conclusion on the material of commerce
09.796 880	Methyl 2-methoxybenzoate		49 8	Class I A3: Intake below threshold	4)	6)	6)
09.807 907	o-Tolyl salicylate		ND 30	Class I A3: Intake below threshold	4)	7)	7)
09.811 891	Vanillin isobutyrate		55 0.04	Class I A3: Intake below threshold	4)	6)	6)
05.018 889	Vanillin		47000 150000	Class I A3: Intake above threshold, A4: Not endogenous, A5: Adequate NOAEL exists	4)	6) The NOAEL of 1000 mg/kg bw/day in a 2-year study in rats is > 100 times the estimated daily intake of vanillin when used as a flavouring substance.	6)
09.749 899	Methyl salicylate		410 44000	Class I A3: Intake above threshold, A4: Not endogenous, A5: Adequate NOAEL exists	4)	6) The NOAEL of 50 mg/kg bw/day in a 2-year study in dogs is > 100 times the estimated daily intake of methyl salicylate when used as a flavouring substance.	6)
04.093 888	Butyl vanillyl ether		ND 0.1	Class II A3: Intake below threshold	4)	7)	7)
04.094 887	Ethyl 4-hydroxy-3-methoxybenzyl ether		20 22	Class II A3: Intake below threshold	4)	6)	6)

Table 3.1: Summary of safety evaluation of 44 JECFA-evaluated hydroxy and alkoxy substituted benzyl derivatives (JECFA, 2002b)

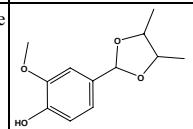
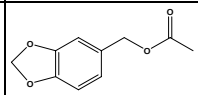
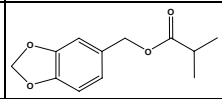
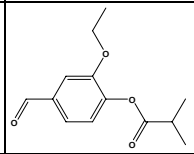
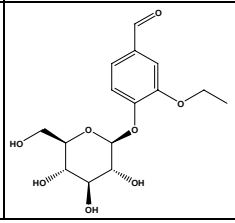
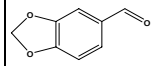
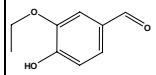
FL-no JECFA-no	EU Register name	Structural formula	EU MSDI 1) US MSDI (μg/*capita*/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [(4) or 5)]	EFSA conclusion on the named compound (Procedure steps, intake estimates, NOAEL, genotoxicity)	EFSA conclusion on the material of commerce
06.132 960	Vanillin butan-2,3-diol acetal (mixture of stereo isomers)		3.4 3	Class II A3: Intake below threshold	4)	6)	CASrn does not specify stereoisomers, stereoisomeric composition to be specified.
09.220 894	Piperonyl acetate		34 11	Class II A3: Intake below threshold	4)	6)	6)
09.430 895	Piperonyl isobutyrate		0.085 3	Class II A3: Intake below threshold	4)	6)	6)
09.933 953	Ethyl vanillin isobutyrate		0.61 ND	Class II A3: Intake below threshold	4)	6)	6)
16.075 892	Ethyl vanillin beta-D-glucopyranoside		ND 30	Class II A3: Intake below threshold	4)	7)	CASrn to be included in the Register: 122397-96-0. 7)

Table 3.1: Summary of safety evaluation of 44 JECFA-evaluated hydroxy and alkoxy substituted benzyl derivatives (JECFA, 2002b)

FL-no JECFA-no	EU Register name	Structural formula	EU MSDI 1) US MSDI (µg/*capita*/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [(4) or 5)]	EFSA conclusion on the named compound (Procedure steps, intake estimates, NOAEL, genotoxicity)	EFSA conclusion on the material of commerce
05.016 896	Piperonal		1500 3200	Class II A3: Intake above threshold, A4: Not endogenous, A5: Adequate NOAEL exists	4)	6) The NOAEL of 250 mg/kg bw/day in a 2-year study in rats is > 100 times the estimated daily intake of piperonal when used as a flavouring substance	6)
05.019 893	Ethyl vanillin		5400 43000	Class II A3: Intake above threshold, A4: Not endogenous, A5: Adequate NOAEL exists	4)	6) The NOAEL of 500 mg/kg bw/day in a 14-week study in rats is > 100 times the estimated daily intake of ethyl vanillin when used as a flavouring substance	6)

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

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

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

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

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

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

7) MSDI based on USA production figure.

ND: not determined

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

Table 3.2: Summary of Safety Evaluation Applying the Procedure of substances in FGE.20 (based on intakes calculated by the MSDI approach)

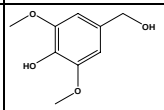
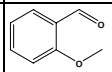
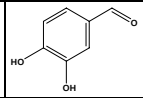
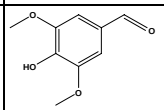
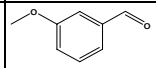
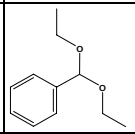
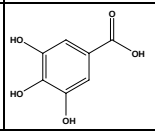
FL-no	EU Register name	Structural formula	MSDI 1) (μg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
02.164	4-Hydroxy-3,5-dimethoxybenzyl alcohol		0.037	Class I A3: Intake below threshold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

Table 3.2: Summary of Safety Evaluation Applying the Procedure of substances in FGE.20 (based on intakes calculated by the MSDI approach)

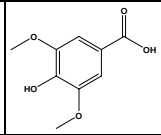
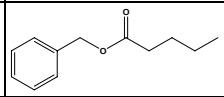
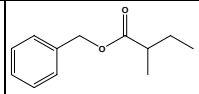
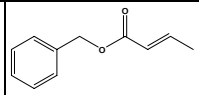
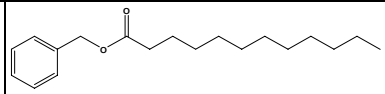
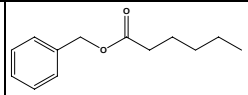
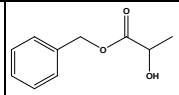
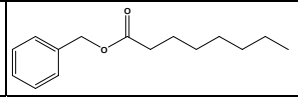
FL-no	EU Register name	Structural formula	MSDI 1) (µg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
08.087	4-Hydroxy-3,5-dimethoxybenzoic acid		1.2	Class I A3: Intake below threshold	4)	6)	
09.152	Benzyl valerate		1.7	Class I A3: Intake below threshold	4)	6)	
09.313	Benzyl 2-methylbutyrate		7.3	Class I A3: Intake below threshold	4)	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)	

Table 3.2: Summary of Safety Evaluation Applying the Procedure of substances in FGE.20 (based on intakes calculated by the MSDI approach)

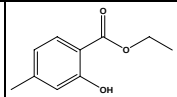
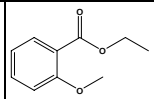
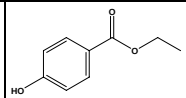
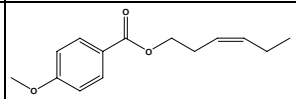
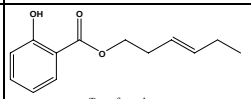
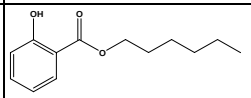
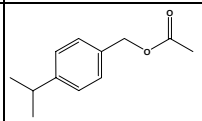
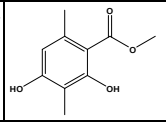
FL-no	EU Register name	Structural formula	MSDI 1) (µg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
09.362	Ethyl 2-hydroxy-4-methylbenzoate		0.0012	Class I A3: Intake below threshold	4)	6)	
09.363	Ethyl 2-methoxybenzoate		5.5	Class I A3: Intake below threshold	4)	6)	
09.367	Ethyl 4-hydroxybenzoate		10	Class I A3: Intake below threshold	4)	6)	
09.560	Hex-3(cis)-enyl anisate		0.12	Class I A3: Intake below threshold	4)	6)	
09.570	Hex-3-enyl salicylate	 Trans form shown	0.13	Class I A3: Intake below threshold	4)	7)	
09.581	Hexyl salicylate		0.018	Class I A3: Intake below threshold	4)	6)	
09.611	4-Isopropylbenzyl acetate		0.012	Class I A3: Intake below threshold	4)	6)	
09.623	Methyl 2,4-dihydroxy-3,6-dimethylbenzoate		0.012	Class I A3: Intake below threshold	4)	6)	

Table 3.2: Summary of Safety Evaluation Applying the Procedure of substances in FGE.20 (based on intakes calculated by the MSDI approach)

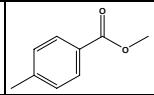
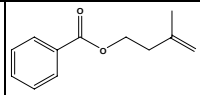
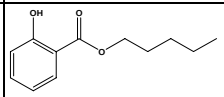
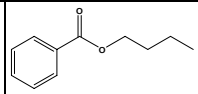
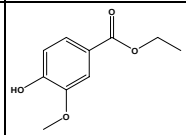
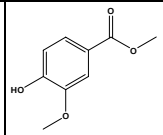
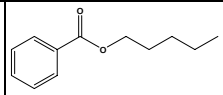
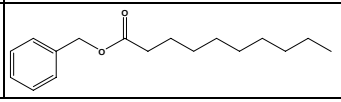
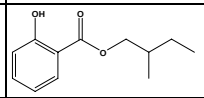
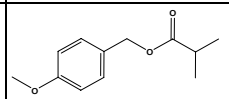
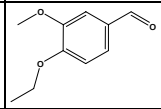
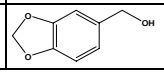
FL-no	EU Register name	Structural formula	MSDI 1) (µg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
09.631	Methyl 4-methylbenzoate		0.0012	Class I A3: Intake below threshold	4)	6)	
09.656	3-Methylbut-3-enyl benzoate		0.12	Class I A3: Intake below threshold	4)	6)	
09.762	Pentyl salicylate		0.24	Class I A3: Intake below threshold	4)	6)	
09.779	Butyl benzoate		3.7	Class I A3: Intake below threshold	4)	6)	
09.798	Ethyl vanillate		0.024	Class I A3: Intake below threshold	4)	6)	
09.799	Methyl vanillate		0.011	Class I A3: Intake below threshold	4)	6)	
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)	

Table 3.2: Summary of Safety Evaluation Applying the Procedure of substances in FGE.20 (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
09.852	2-Methylbutyl 2-hydroxybenzoate		0.011	Class I A3: Intake below threshold	4)	7)	
09.895	4-Methoxybenzyl-2-methylpropionate		0.37	Class I A3: Intake below threshold	4)	6)	
05.066	4-Ethoxy-3-methoxybenzaldehyde		1.2	Class II A3: Intake below threshold	4)	6)	
02.205	Piperonyl alcohol		0.011	Class III A3: Intake below threshold	4)	6)	

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

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

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

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

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

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

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

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

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