European Food Safety Authority

The EFSA Journal (2009) ON-965, 1-53

Flavouring Group Evaluation 60 (FGE.60)[1]:

Consideration of eugenol and related hydroxyallylbenzene derivatives evaluated by JECFA (65th meeting) structurally related to ring-substituted phenolic substances evaluated by EFSA in FGE.22 (2006)

Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food (AFC)

(Question EFSA-2008-32L)

Adopted on 1 April 2008

SUMMARY

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

The present consideration concerns eugenol and six related hydroxyallylbenzene derivatives evaluated by the JECFA (65th meeting) and will be considered in relation to the European Food Safety Authority (EFSA) evaluation of 23 ring-substituted phenolic substances evaluated in the Flavouring Group Evaluation 22 (FGE.22).

[1] For citation purposes: Scientific Opinion of the Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food on a request from Commission on Consideration of eugenol and related hydroxyallylbenzene derivatives evaluated by JECFA (65th meeting) structurally related to ring-substituted phenolic substances evaluated by EFSA in FGE.22 (2006). *The EFSA Journal* (2009) 1 of 53.

The Panel concluded that the seven substances in the JECFA flavouring group of eugenol and related hydroxyallylbenzene derivatives are structurally related to the phenolic substances evaluated by EFSA in FGE.22.

The Panel agrees with the application of the Procedure as performed by the JECFA. As the EU (Maximised Survey-derived Daily Intake) MSDI for eugenol [FL-no: 04.003] is below the threshold of the structural class, it can be concluded to be of no safety concern at step A3 in the Procedure scheme. Moreover, a NOAEL of 300 mg/kg bw has been identified.

For three of the substances [FL-no: 04.058, 04.096 and 09.878] considered in this FGE use levels are available corresponding to modified Theoretical Added Maximum Daily Intake (mTAMDI) values of 360, 420 and 2300 microgram/person/day, respectively. For one of these substances [FL-no: 09.878] the mTAMDI exceeds the threshold for the structural class I. For the remaining four substances [FL-no: 04.003, 09.020, 09.088 and 09.766] 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 seven JECFA evaluated substances can be applied to the materials of commerce, it is necessary to consider the available specifications:

Adequate specifications including complete purity criteria and identity tests are available for six of the seven JECFA evaluated substances. For one substance [FL-no: 09.088] information on the composition of mixture is requested.

Thus, for one substance [FL-no: 09.088] the Panel has reservations (composition of mixture is requested). For the remaining six substances [FL-no: 04.003, 04.058, 04.096, 09.020, 09.766 and 09.878] the Panel agrees with the JECFA conclusion "No safety concern at estimated levels of intake as flavouring substances" based on the MSDI approach.

KEYWORDS

Eugenol, hydroxyallylbenzene derivatives, JECFA, 65th meeting, ring-substituted phenolic substances, FGE.22, hydroxypropenylbenzene derivatives.

FGE.22:

http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1178620772628.htm

JECFA evaluation:
http://whqlibdoc.who.int/publications/2006/9241660562_part2_c_eng.pdf

TABLE OF CONTENTS

BACKGROUND

Regulation (EC) No 2232/96 of the European Parliament and the Council (EC, 1996) lays down a Procedure for the establishment of a list of flavouring substances, the use of which will be authorised to the exclusion of all 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, 2000a), which is broadly based on the Opinion of the Scientific Committee on Food (SCF, 1999).

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

The following issues are of special importance.

Intake

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

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

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

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

As information on use levels for the flavouring substances has not been requested by the JECFA or 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 seven flavouring substances consisting of eugenol and related hydroxyallylbenzene derivatives.

1.1.2. EFSA Considerations

The Panel concluded that the substances in this group are related to the phenolic substances evaluated by EFSA in FGE.22 with several substances having free phenolic groups in the 2- or 4-position to an allyl group and many in addition also have alkoxy group substituents. Similar to the eugenol derivatives, the substances considered in FGE.22 all have a free phenolic group or are phenolic esters which will release phenols upon hydrolysis. Therefore, the substances in FGE.22 are considered as supporting substances for the eugenol derivatives evaluated by the JECFA.

The Panel also considered whether safrole, estragole and methyleugenol could be used as supporting substances but these substances do not have a free phenolic hydroxyl group, which makes them more susceptible to oxidation in the propenyl chain, hence in contrast to eugenol, they do not readily conjugate with glucuronic acid or sulphate and are not excreted in the urine. Therefore safrole, estragole and methyleugenol are not used as supporting substances.

Furthermore, it was questioned if isoeugenol (a prop-1-enylbenzene) could be used as supporting substance for eugenol (a prop-2-en-1-ylbenzene). Prop-2-en-1-ylbenzenes such as safrole are metabolically activated by 1-hydroxylation followed by esterification to ultimately carcinogenic

metabolites. 3-Hydroxylation of prop-1-enylbenzenes such as isosafrole, in contrast, gives non-carcinogenic metabolites that undergo further side chain oxidation (Phillips, 1994). Therefore, the Panel concluded that isoeugenol should not be used as a supporting substance.

1.2. Isomers

1.2.1. JECFA Status

None of the seven Register substances in the group of eugenol and related hydroxyallylbenzene derivatives have a chiral centre or can exist as geometrical isomers.

1.2.2. EFSA Considerations

No comments.

1.3. Specifications

1.3.1. JECFA Status

JECFA specifications are available for all seven substances (JECFA, 2005d) (see Table 1).

1.3.2. EFSA Considerations

No comments.

2. Intake Estimations

2.1. JECFA Status

For all seven substances evaluated through the JECFA Procedure intake data are available (see Table 3.1).

2.2. EFSA Considerations

For three of the seven JECFA evaluated substances use levels have been provided by Flavour Industry [FL-no: 04.058, 04.096 and 09.878] (EC, 2000a; EFFA, 2005g; EFFA, 2007a) (see Table 2.2.1) Based on these use levels mTAMDI figures can be calculated (EFSA, 2004d) (see Table 2.2.2).

Table 2.2.1 Normal and Maximum use levels (mg/kg) available for JECFA evaluated substances in FGE.60

FL-no	Food Categories Normal use levels (mg/kg) Maximum use levels (mg/kg)																	
	01.0	02.0	03.0	04.1	04.2	05.0	06.0	07.0	08.0	09.0	10.0	11.0	12.0	13.0	14.1	14.2	15.0	16.0
04.058	0,5 2,5	0,2 1	0,5 2,5	0,4 2	- -	1 5	0,2 1	2 10	0,2 1	0,2 1	- -	- -	0,3 1,5	0,5 2,5	- -	1 5	2 10	0,4 2
04.096	0,5 2,5	0,2 1	0,5 2,5	0,4 2	- -	1 5	0,2 1	2 10	0,2 1	0,2 1	- -	- -	0,3 1,5	0,5 2,5	0,2 1	1 5	2 10	0,4 2
09.878	7 35	5 25	10 50	7 35	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	- -	10 50	20 100	5 25

Table 2.2.2 Estimated intakes based on the MSDI and the mTAMDI approach

FL-no	EU Register name	MSDI – EU (µg/*capita*/day)	MSDI – USA (µg/*capita*/day)	mTAMDI (µg/person/day)	Structural class	Threshold of concern (µg/person/day)
04.058	4-Allylphenol	0.073	0.6	360	Class I	1800
04.096	2-Methoxy-6-(2-propenyl)phenol	0.12	0.2	420	Class I	1800
09.020	Eugenyl acetate	19	90		Class I	1800
09.088	4-Eugenyl formate	0.012	0.06		Class I	1800
09.766	Eugenyl benzoate	0.0024	0.9		Class I	1800
09.878	Eugenyl isovalerate	0.37	0.5	2300	Class I	1800
04.003	Eugenol	950	3364		Class I	1800

3. Genotoxicity and Toxicity Data

3.1. Genotoxicity Studies – Text Taken from the JECFA (JECFA, 2007a)

In vitro

In standard assays for mutagenicity in *Salmonella typhimurium*, eugenol [FL-no: 04.003] was not mutagenic in strains TA92, TA94, TA97, TA98, TA100, TA102, TA1530, TA1531, TA1532, TA1535, TA1537, TA1538 and TA1964 at concentrations ≤ 107,000 µg/plate (about 652 µmol/plate), with or without metabolic activation (Dorange et al. 1977; Green & Savage, 1978; Rockwell & Raw, 1979; Swanson et al., 1979; Douglas et al., 1980; Eder et al., 1980; Florin et al., 1980; Nestmann et al., 1980; Rapson et al., 1980; Yoshimura et al., 1981; Eder et al., 1982b; Pool & Lin, 1982); and (Sekizawa & Shibamoto, 1982; To et al., 1982; Haworth et al., 1983; NTP, 1983a; Ishidate et al., 1984; Orstavik & Hongslo, 1985; Amonkar et al., 1986); and (Miller et al., 1986; Tennant et al., 1987; Schiestl et al., 1989; Azizan & Blevins, 1995; Sukumaran & Kuttan, 1995)). Eugenol was mutagenic in *S. typhimurium* strain TA1535 but only when 3'-phosphoadenosine-5'-phosphosulfate was included in the metabolic activation mix. Eugenol did not show a concentration-dependent increase in mutagenic activity. The authors suggested that the result was an artefact of the experimental design, as mutation was not reported as the number of revertants per survivor (induced mutation frequency) but only as the number of revertants per plate (To et al., 1982).

Eugenol 2',3'-epoxide was not mutagenic in *S. typhimurium* strains TA1537, TA1538 and TA98 but showed mutagenic potential in strains TA1535 (Dorange et al. 1977; Swanson et al., 1979) and TA100 (Dorange et al. 1977).

In a study designed to investigate the mutagenicity of the metabolites of eugenol, Sprague-Dawley rats were given a single dose of 0.5 ml of eugenol (about 2140 mg/kg bw) by gavage, and their urine was collected for 24 h. Before being administered to rats, samples of 0.05-100 µl (53.5-107,000 µg/plate) were each assayed for reverse mutation potential in *S. typhimurium* strains TA98 and TA100 in the presence of metabolic activation. Negative results were obtained. To assess the genotoxic potential of urinary metabolites, the urine was assayed directly or extracted with ether

after dilution in a phosphate buffer and treatment with P-glucuronidase to hydrolyse glucuronide conjugates. The 24-h urine samples (500 µl), ether extracts of the urine and aqueous fractions of the extracts were then incubated separately with *S. typhimurium* strains TA98 and TA100 with S9 activation. The urinary solutions isolated from rats given 0.5 ml of eugenol did not show evidence of mutagenicity in either TA98 or TA100 (Rockwell & Raw, 1979).

No mutagenic potential was reported when 60, 120, 300 or 600 µg/plate (0.37, 0.74, 1.8 or 3.6 µmol/plate) eugenol was incubated with *Escherichia coli* WP2 uvrA (trp-) (Sekizawa & Shibamoto, 1982). Rec assays in *Bacillus subtilis* M45 (rec-) and H17 (rec+) performed with 400 µg/disc (2.4 µmol/disc) in the absence of S9 activation gave a positive result (Sekizawa & Shibamoto, 1982); however, DNA repair assays with ≤ l00000 µg/disc (609 µmol/disc), under standard incubation conditions, gave uniformly negative results (Oda et al., 1979; Yoshimura et al., 1981). With an inhibition zone difference between rec- and rec+ cells of 6.9 mm, (Sekizawa & Shibamoto, 1982) reported positive results, observed as evidence of preferential lethality of eugenol for M45 rec- cells. The authors noted, however, that as the eugenol sample used was somewhat oily it did not diffuse effectively in the aqueous agar layer. Therefore, the H17 red cells (which had a doubling time of 48 min, to be compared with 75 min for M45 rec-) might have grown before the sample diffused effectively, which would have resulted in smaller inhibition zones than in the M45 rec- cells.

In an assay for forward mutation in mouse lymphoma cells, eugenol was mutagenic in L5178Y cells at concentrations of 21.3-128 µg/ml (0.13-0.78 mmol/l) (Tennant et al., 1987; Myhr & Caspary, 1991). The concentrations used, however, were highly cytotoxic. In the two trials conducted by Myhr and Caspary (1991), a negative correlation was found between mutation frequency and relative total growth. In the first trial, the relative total growth was 16-28% at a eugenol concentration of 80 nl/ml and 5-11% at a concentration of 120 nl/ml. In the second trial, the relative total growth was 5-30% at a concentration of 40 nl/ml and 4% at 60 nl/ml. The mutation frequency increased by 4.2- and 2.2-fold at the highest doses tested in the first and second trials, respectively. According to Myhr and Caspary (1991), the interaction between eugenol and the cells was not well controlled, and additional studies are necessary to characterize the dose-response relation better.

Assays for sister chromatid exchange in mammalian cells conducted with eugenol gave equivocal results (NTP, 1983a; Jansson et al., 1986; Fukuda, 1987; Galloway et al., 1987; Tennant et al., 1987). In human peripheral lymphocytes, no sister chromatid exchange was induced at eugenol concentrations ≤ 82.1 µg/ml (0.5 mmol/l) (Jansson et al., 1986). When 0.3-10 µg/ml [0.00003-0.001% (v/v)] of eugenol were incubated with Syrian hamster embryo cells, a significant increase ($p < 0.001$) in sister chromatid exchange was reported but at less than twice the level found in negative controls (Fukuda, 1987). Weak induction of sister chromatid exchange was reported in Chinese hamster ovary cells incubated with 11-123 µg/ml (0.07-0.75 mmol/l) of eugenol in the absence of metabolic activation, and positive results were observed after incubation with 273-326 µg/ml (1.7-2.0 mmol/l) of eugenol in the presence of metabolic activation. The authors commented that the increase in sister chromatid exchange observed with and without metabolic activation occurred at doses that caused severe cell cycle delay (Galloway et al., 1987). Sister chromatid exchange was induced in Chinese hamster ovary cells incubated with 75-326 µg/ml (0.5-2.0 mmol/l) of eugenol in the absence and presence of metabolic activation (NTP, 1983a; Tennant et al., 1987).

Equivocal results were reported in standard assays for chromosomal aberration (Stich et al., 1981c; NTP, 1983a; Ishidate et al., 1984; Galloway et al., 1987; Tennant et al., 1987; Bean et al., 1992; Bean & Galloway, 1993). In a study designed to investigate the optimal sampling time for detecting chromosomal aberrations in Chinese hamster ovary cells, significant increases in chromosomal aberrations were reported at eugenol concentrations of 131-263 µg/ml (0.8-1.6 mmol/l) in the presence of metabolic activation (Bean et al., 1992). In the first of two trials, when cells were harvested 15 and 24.5 h after the beginning of treatment, cell viability was 57% and 37% that of controls, respectively, after incubation with 0.8 mmol/l of eugenol, and 53% and 43% that of controls after incubation with 1.2 mmol/l of eugenol. The percentage of aberrant cells increased to a maximum of 25.5% on harvesting 15 h after incubation with 0.8 mmol/l of eugenol. In the second trial, the aberration yield shifted with the cytotoxicity, as concentrations of 1.2-1.6 mmol/l of eugenol were as cytotoxic as 0.8 mmol/l in the first trial. The maximal increase in the percentage of aberrant cells (26.5%) was found at harvest of cells 17 h after incubation with 1.6 mmol/l of eugenol.

In a subsequent study with a similar purpose, Chinese hamster ovary cells incubated with 197, 230 or 263 µg/ml (1.2, 1.4 or 1.6 mmol/l) of eugenol for 3 h and harvested 20 or 44 h after the beginning of treatment showed significant increases in chromosomal aberrations. Severe cell cycle delay was observed 20 h after incubation with 263 µg/ml (1.6 mmol/l) of eugenol, and the chromosomal aberrations were accompanied by a reduction in cell count to about 50% that of controls (Bean & Galloway, 1993).

Chromosomal aberrations occurred when Chinese hamster fibroblast cells were incubated for 48 h with ≤ 125 µg/ml (0.77 mmol/l) of eugenol; however, negative results were reported after only 24 h of incubation. The dose at which structural aberrations were detected in 20% of metaphases was 14.8 mglml (Ishidate et al., 1984). In another study, no chromosomal aberrations were found in Chinese hamster ovary cells incubated with 50 or 100 µg/ml (0.31 or 0.61 mmol/l) of eugenol; however, a slight increase (2.0%) in the incidence of chromosomal aberrations was obtained at the highest dose, 200 µg/ml (1.22 mmol/l) when compared with controls (0.8%) (Stich et al., 1981c). Negative results were reported with eugenol in a standard assay for chromosomal aberrations without S9 activation at concentrations ≤ 300 µg/ml (1.8 mmol/l), which was a toxic concentration. in the presence of S9 activation, weakly positive results were found at concentrations of 201-324 µg/ml (1.2-2.0 mmol/l) (Galloway et al., 1987). Induction of chromosomal aberrations was reported in Chinese hamster ovary cells exposed to eugenol at concentrations of 300-324 µg/ml (1.8-2.0 mmol/l) in the presence of metabolic activation (NTP, 1983a; Tennant et al., 1987). Hepatocytes isolated from male Fischer 344 rats and female $B6C3F_1$ mice showed no unscheduled DNA synthesis after incubation with 0.01642, 0.1642, 1.642, 16.42 or 164.2 µg/ml (0.1, 1, 10, 100 or 1000 µmol/l) of eugenol. The LC_{50} values for eugenol in this study were 49.3 µg/ml (300 µmol/l) for rat hepatocytes and 32.8 µg/ml (200 µmol/l) for mouse hepatocytes; no unscheduled DNA synthesis induction was observed at these doses (Burkey et al., 2000). In a similar study, no unscheduled DNA synthesis was reported in rat hepatocytes incubated in the presence of 0.1642-164.2 µg/ml (10^{-6}-10^{-3} mol/l) of eugenol. Cytotoxicity occurred, as indicated by increased activity of lactate dehydrogenase, at 82.1 µg/ml (5×10^{-4} mol/l) (Howes et al., 1990). No unscheduled DNA synthesis was reported when hepatocytes from male Sprague-Dawley rats were exposed for 20 h to 16, 41 or 82 µg/ml (0.1, 0.25 or 0.5 mmol/l) of eugenol (Allavena et al., 1992). Fukuda (1987),

however, reported positive results with 0.3-1 µg/ml [0.00003-0.0001% (v/v)] in an assay for unscheduled DNA synthesis in Syrian hamster embryo cells in the presence of metabolic activation.

When 1, 2.5, 5, 10 or 15 µg/ml of eugenyl acetate [FL-no: 09.020] were incubated with isolated rat hepatocytes, no unscheduled DNA synthesis was reported. The compound was cytotoxic at a concentration of 33 µg/ml, as evidenced by lactate dehydrogenase leakage (San & Reece, 2003).

In vivo

In a standard assay for micronucleus induction *in vivo*, groups of eight male CF1 mice were given 100, 400 or 600 mg/kg bw of eugenol [FL-no: 04.003] by intraperitoneal injection. The lowest dose did not induce micronucleated polychromatic erythrocytes in the bone marrow of the mice, but the intermediate and high doses had significantly increased the frequency ($p < 0.01$) by 30 h after treatment. In groups of eight male CF1 mice given 400 mg/kg bw of eugenol by intraperitoneal injection and killed 24, 30 or 48 h later, significant increases ($p < 0.01$) in the frequency of micronucleated polychromatic erythrocytes were reported, regardless of the time of sacrifice (Ellahueñe et al., 1994).

Groups of 12 adult male Swiss-Webster mice received 148 or 740 mg/kg bw of eugenol by intraperitoneal injection or 14,794 mg/kg bw by oral intubation. Bone marrow isolated from the femur of each animal showed a significant increase in micronucleated polychromatic erythrocytes over that in saline controls; however, intraperitoneal administration induced a higher frequency (18.5-20.5%) than oral administration (5.7%), with a considerably lower concentration of test material (Woolverton et al., 1986).

Studies in various strains of mice (Hayashi et al., 1984; Shelby et al., 1993; Rompelberg et al., 1995) and rats (Maura et al., 1989; Allavena et al., 1992; Rompelberg et al., 1996a) and in human lymphocytes (Rompelberg et al., 1996b) showed no evidence of mutagenicity. In male ddY mice given eugenol at 100, 200, 400 or 800 mg/kg bw by intraperitoneal injection, no increase in the frequency of micronucleated polychromatic erythrocytes was reported (Hayashi et al., 1984). Male CD-l mice fed a diet containing 0.4% eugenol daily (average intake, about 680 mgl kg bw per day) for 15 days showed no increase in the incidence of micronuclei in bone marrow (Rompelberg et al., 1995). No micronuclei were induced when male $B6C3F_1$ mice received 150, 300 or 600 mg/kg bw per day by intraperitoneal injection for 3 consecutive days (Shelby et al., 1993).

Male albino Sprague-Dawley rats showed no induction of micronuclei in bone marrow after administration of 1340 or 2680 mg/kg bw of eugenol by gavage by three protocols. The first involved administering 1340 mg/kg bw of eugenol 20 h after a partial hepatectomy, followed by sacrifice at 48 h, while the second protocol involved administering 2680 mg/kg bw of eugenol 30 and 6 h before sacrifice. In the third protocol, 1340 mg/kg bw of eugenol were administered, followed by sacrifice at 2 h. No positive dose-dependent relation was observed with these protocols. Administration of 1340 or 2680 mg/kg bw by gavage did not induce DNA fragmentation in primary cultures of rat hepatocytes 4 and 20 h after seeding (Allavena et al., 1992).

Groups of four female Sprague-Dawley rats received 335, 670 or 1340 mg/kg bw of eugenol orally in an aqueous suspension, half of the dose being administered 30 h before sacrifice and the second half 6 h before sacrifice. No micronucleated polychromatic erythrocytes were induced in bone marrow. At 1340 mg/kg bw, eugenol did not induce DNA fragmentation in the liver or kidney after

2, 24 or 48 h of treatment. Furthermore, administration of 1.5, 5 or 10 mmol/kg bw (164.2, 821.0 and 1642 mg/kg bw, respectively) of eugenol by gavage did not significantly increase the mutation frequency or induce DNA fragmentation in granuloma cells. The authors proposed that the mutagenic and clastogenic activity of eugenol observed in mammalian cells *in vitro* might be due to the absence of the necessary detoxifying enzymes that exist *in vivo* (Maura et al., 1989).

In a similar study, male Wistar rats were given 0, 500 or l000 mg/kg bw per day of eugenol in corn oil suspension by gavage for 10 days. No significant increase in the occurrence of bone-marrow micronucleated polychromatic erythrocytes was reported at either dose when compared with controls. Under similar conditions, primary hepatocyte cultures obtained from male Wistar rats given 500 mg/kg bw per day of eugenol orally for 10 days showed no unscheduled DNA synthesis. The authors did not report results for unscheduled DNA synthesis in hepatocytes of rats given l000 mg/kg bw eugenol (Rompelberg et al., 1996a).

In a 7-day, placebo-controlled, cross-over study with a 1-week washout period, 10 healthy male volunteers received a placebo or 150 mg of eugenol daily in the form of a capsule (50 mg/capsule) taken orally 3 times a day. On the basis of the body weights of the volunteers (68-88 kg), this dose provided about 1.7-2.2 mg/kg bw per day of eugenol. Aliquots of blood were collected on days 8 and 22 of the study and analysed for micronucleus induction and chromosomal aberrations. Eugenol did not increase the background level of micronuclei or chromosomal aberrations in human lymphocytes (Rompelberg et al., 1996b).

Conclusion on genotoxicity

One representative agent of this group, eugenol [FL-no: 04.003], gave consistently negative results in assays for reverse mutation in various strains of *S. typhimurium* and *E. coli.* Generally, negative results were also found for DNA repair in *B. subtilis* M45 (rec-) and H17 (rec+) cells; the one positive finding for DNA repair occurred at a concentration of 400 µg/disc, while a similar study with higher concentrations (≤ 100000 µg/disc) yielded negative results. In assays for unscheduled DNA synthesis in rat and mouse hepatocytes *in vitro*, no genotoxic activity was observed at concentrations ≤ 164.2 µg/ml of eugenol and ≤ 15 µg/ml of eugenyl acetate; however, one positive result was reported with eugenol in Syrian hamster embryo cells at concentrations ≤ 1 µg/ml. Assays in mammalian cells *in vitro* in which mutagenicity was found (forward mutation in mouse lymphoma cells, sister chromatid exchange and chromosomal aberrations) were performed at concentrations that resulted in cytotoxicity or severe cell cycle delay. Mammalian cells in culture might not have the metabolic pathways of detoxication available to counter such toxicity. Assays for mutagenicity (micronuclei, chromosomal aberrations and mutation) and genotoxicity (unscheduled DNA synthesis and DNA fragmentation) *in vivo* generally gave negative results, even at very high doses of eugenol (≤ 800 mg/kg bw by intraperitoneal injection, ≤ 2680 mg/kg bw orally). The two reports of micronucleus induction involved doses as high as 740 mg/kg bw given by intraperitoneal injection and 14794 mg/kg bw given orally. The available results indicate that eugenol and other hydroxypropylbenzene derivatives are unlikely to pose a significant mutagenic or genotoxic risk to humans under the intended conditions of their use as flavouring agents.

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

3.2. Genotoxicity Studies - Text Taken from EFSA (EFSA, 2007m)

Data from *in vitro* tests are available for 12 candidate [FL-no: 04.020, 04.021, 04.065, 04.066, 04.070, 04.076, 04.077, 04.080, 04.095, 07.142, 07.164 and 07.243] and 18 supporting substances. Data from *in vivo* tests are available for one candidate [FL-no: 04.077] and six supporting substances. Most studies are of limited or insufficient quality or are inadequately reported, thus for some of the studies the validity of the results could not be evaluated.

Positive results were observed with three candidate substances [FL-no: 04.077, 04.080 and 07.142].

4-Methoxyphenol [FL-no: 04.077] did not induce gene mutations in bacteria (Haworth et al., 1983). In a gene mutation assay in mammalian cells (MLTK assay) a positive result was observed for 4-methoxyphenol without metabolic activation and a negative result with metabolic activation using an S9 homogenate (Rogers-Back, 1986). In the test without metabolic activation an increase in the percentage of small colonies was noted indicating a potential for chromosomal aberrations. 4-Methoxyphenol induced chromosomal aberrations in Chinese hamster ovary (CHO) cells in the presence and absence of metabolic activation (Putman, 1986). 4-Methoxyphenol did not induce sister chromatid exchanges (SCE) in human lymphocytes (Jansson et al., 1988); however, the study was of limited quality. Since 4-methoxyphenol did not induce chromosomal aberration *in vivo* in rat bone marrow cells after oral administration (Esber, 1986) the results observed *in vitro* with 4-methoxyphenol were considered to be of no concern.

3,4-Methylenedioxyphenol [FL-no: 04.080] was reported to be negative in a bacterial mutagenicity assay in the presence and absence of metabolic activation while a positive result was reported in a gene mutation assay in mammalian cells (MLTK assay) both in the presence and absence of metabolic activation (Longfellow, 1985/1986). However, this information was only available as a very short abstract and the study reports were not available for evaluation. *In vivo* studies were not available for this candidate substance.

Acetovanillone [FL-no: 07.142] was positive in a yeast assay without metabolic activation (Nestmann & Lee, 1983). This result is not considered to preclude the substance to be evaluated through the Procedure. The substance was negative in bacterial mutagenicity assays in the presence and absence of metabolic activation (Nestmann et al., 1980; Xu et al., 1984). However, reporting of the bacterial assays and the quality of data were insufficient and the validity of the results could not be evaluated.

With the candidate substances 2-ethylphenol [FL-no: 04.070] and 2,4-dimethylphenol [FL-no: 04.066] negative results were observed in bacterial gene mutation assays (Pool & Lin, 1982; Zeiger et al., 1992; Mortelmans et al., 1986). All other results observed in several assays with these two and seven further candidate substances for which data were available were negative. However, these data were of limited or insufficient quality and the validity of the studies could not be evaluated.

With supporting substances positive and negative results were obtained in *in vitro* tests.

2-Methylphenol [FL-no: 04.027], 3-methylphenol [FL-no: 04.026], 4-methylphenol [FL-no: 04.028], 2-methoxyphenol [FL-no: 04.005], and 2,6-dimethoxyphenol [FL-no: 04.036] did not induce gene mutations in bacterial assays of acceptable quality (Pool & Lin, 1982; Haworth et al.,

1983). The validity of a positive result observed with 2-methylphenol [FL-no: 04.027] in bacteria (Claxton, 1985) cannot be evaluated.

2,6-Dimethylphenol [FL-no: 04.042] induced chromosomal aberrations in mammalian cells in the presence of S9 while the result was negative in the absence of metabolic activation (Völkner, 1994). The *in vitro* genotoxic potential of 2,6-dimethylphenol does not give rise to concern with respect to other alkylated phenols in this FGE, as they are alkyl substituted in either *m*- or *p*-positions. Phenols, substituted in *m*- or *p*-posititon are expeted to be metabolised differently from 2,6-dimethylphenol.

2-Methoxyphenol [FL-no: 04.005], 2-methoxy-4-methylphenol [FL-no: 04.007], 2-methylphenol [FL-no: 04.027] and a mixture of 2-methylphenol [FL-no: 04.027], 3-methylphenol [FL-no: 04.026] and 4-methylphenol [FL-no: 04.028] induced SCE in human lymphocytes or CHO cells (Jansson et al., 1986) [FL-no: 04.005]; (Jansson et al., 1988) [FL-no: 04.007]; (Galloway & Brusick, 1981) [FL-no: 04.027]; (Galloway & Brusick, 1980) [mixture]). In most cases the effects were observed in the presence and absence of metabolic activation.

The mixture of 2-methylphenol [FL-no: 04.027], 3-methylphenol [FL-no: 04.026] and 4-methylphenol [FL-no: 04.028] resulted in an equivocal response in a unscheduled DNA synthesis (UDS) assay (Myhr & Brusick, 1980), while induction of UDS was observed with 4-methylphenol [FL-no: 04.028] in another *in vitro* study (Crowley & Margard, 1978).

All other results observed in several *in vitro* assays with these and the remaining supporting substances were negative; however, these data were of limited or insufficient quality and the validity of the studies could not be evaluated.

With the supporting substances 2-methylphenol [FL-no: 04.027], 3-methylphenol [FL-no: 04.026] and 4-methylphenol [FL-no: 04.028] negative results were obtained in *in vivo* SCE assays (Cheng & Kligerman, 1984). However, these data were of limited quality. 3-Methylphenol [FL-no: 04.026] did not induce chromosomal aberrations in mice (Ivett et al., 1989). However, the validity of the result could not be evaluated as the study was inadequately reported. 2-Methylphenol [FL-no: 04.027] and carvacrol [FL-no: 04.031] did not induce mutations in *Drosophila* (Sernau, 1989; Kono et al., 1995).

Overall, the available genotoxicity data on the supporting substances would not preclude evaluation of the candidate substances through the Procedure. One of the candidate substances, 3,4-methylenedioxyphenol [FL-no: 04.080], was reported to have genotoxic potential *in vitro*. *In vivo* studies were not available for this candidate substance. Therefore, the Panel decided that the Procedure could not be applied to this candidate substance until adequate genotoxicity data become available.

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

3.3. Additional Genotoxicity Studies

The following studies on eugenol [FL-no: 04.003] were not considered by the JECFA or by EFSA in 2006 (EFSA, 2007m).

Treatment of myeloperoxidase containing HL-60 cells with eugenol produced a dose-dependent formation of three DNA adducts as detected with 32P-postlabelling. Incubation of HL-60 cells with a combination of eugenol and H_2O_2 increased the levels of DNA adducts by 14-fold, which suggests peroxidase activation in adduct formation. The DNA adducts formed in HL-60 cells treated with eugenol were the same as those formed in peroxidase activation. In addition to adduct formation, peroxidase activation of eugenol also produced an increase (2-3-fold) of oxidative DNA damage (8-OH-2'-deoxyguanosine). Reaction of eugenol quinone methide with either DNA or deoxyguanosine-3'-phosphate produced two principal adducts (2 and 4). DNA adduct 2 formed by eugenol quinone methide was identical to DNA 2 adduct formed by eugenol in HL-60 cells. This suggests that eugenol quinone methide is one of the reactive intermediates leading to DNA adduct formation. These results demonstrated that eugenol can be activated to form both DNA adducts and oxidative DNA base damage in *vitro* (Bodell et al., 1998).

Dietary administration (0.4 %, w/w) of eugenol for 58 days in male transgenic mice (Muta-TM-Mouse) provided no evidence for antimutagenic or antigenotoxic potential of eugenol *in vivo*. In particular, eugenol did not affect either the mutations or the DNA adducts induced by benzo-a-pyrene, the last effects revealed by 32P-postlabelling analysis of liver DNA. Eugenol was not mutagenic itself; however, one spot indicative of possible DNA adduct formation was detected. This result is in contrast with earlier *in vivo* studies, showing that eugenol was unable to induce DNA adduct formation in the liver of mice (Randerath et al., 1984; Phillips, 1990). In particular, Phillips showed that eugenol, in contrast to safrole, was unable to form DNA adducts in $B6C3F_1$ male mice by 32P-postlabelling with a limit of detection of 1 adduct in 10 billion nucleotides (Rompelberg et al., 1996c).

3.4. EFSA Considerations on Genotoxicity

There are several positive genotoxicity studies with eugenol *in vitro*, which indicate that this substance (and consequently all substances in this FGE) may induce genotoxicity. In particular, the data presently available have shown the following genotoxic profile for eugenol: no induction of gene mutations in bacterial cells, prevalence of chromosomal effects *in vitro*, preferential induction of small colonies in the mouse lymphoma assay *in vitro* assay, increase of 8-OH-deoxyguanosine in cultured mammalian cells in the presence of peroxidase activation, induction of DNA adducts *in vitro*, but not *in vivo*. *In vivo* eugenol produced contrasting results in the bone marrow micronucleus assay when administered intraperitoneally to different mouse strains, while uniformly negative results were obtained in rats and mice following oral administration, with the exception of a single study at a dose exceeding the maximum recommended dose level. Dietary administration of eugenol did not induce gene mutations in the liver of transgenic mice. The candidate substances in this group are conjugated with glucuronic acid or sulphate very efficiently, and these pathways are not easily saturated at the expected levels of intake resulting from their use as flavouring substances. At high dose levels reactive metabolites (quinones, catechols, quinone methides) may be formed. However, the formation of these metabolites is not expected to overwhelm the detoxication capacity through conjugation with sulphate, glucuronic acid or, in particular, glutathione, when the substances are used as flavouring substances. Overall, the genotoxic profile of eugenol suggests a clastogenic effect *in vitro* with no evidence for genotoxicity *in vivo* by the oral route.

3.5. EFSA Considerations on Long-Term Toxicity and Carcinogenicity of Eugenol

Long-term toxicity

In 1982 the JECFA established an acceptable daily intake (ADI) of 0–2.5 mg/kg body weight (bw) per day for eugenol (JECFA, 1982a) on the basis of the results of a 19-week study in rats (10 males and 10 females per group) fed diets containing 0, 0.1 and 1.0 % (0, 1000 or 10000 mg/kg) eugenol for 19 weeks without any adverse effect on growth rate, haematology, organ weights and histology of major tissues (Hagan et al., 1967). The results of the lifetime feeding study in rats provided additional information for evaluating the acceptable daily intake for man.

In a two-year study male rats were given 3000 or 6000 mg/kg and female rats were given 6000 or 12,500 mg/kg of eugenol in the diet for 103 weeks, equivalent to intakes of 150 or 300 mg/kg bw/day for males and 300 or 625 mg/kg bw/day in females. The only effect seen was an on body weight at 625 mg/kg bw/day in females, and 300 mg/kg was a NOAEL (NTP, 1983b). At their 65th meeting (JECFA, 2006b), the JECFA used the NOEL of 300 mg/kg/day to maintain their previous ADI of 0–2.5 mg/kg bw/day for eugenol.

Carcinogenicity

Eugenol has been tested for carcinogenicity in long-term studies in rats and mice (Miller et al., 1983; NTP, 1983b), in addition to some special carcinogenicity studies (skin painting and promotion studies).

Eugenol was not carcinogenic in a limited dietary study with groups of 30 CD-1 mice, in a limited gavage study in 55 male and 59 female CD-1 mice and after intraperitoneal injection in 52 male CD-1 mice (Miller et al., 1983).

In B6C3F$_1$ mice the incidences of hepatocellular tumours (carcinomas, adenomas) were 14/50, 39/45 and 19/49 in the controls, low-dose and high-dose males. The corresponding incidences in females were 2/50, 7/49 and 9/49. In the males, the low-dose but not the high-dose, gave a statistically significant increase in hepatocellular tumours (NTP, 1983b).

In the rat study endometrial stromal polyps of the uterus were found in increased incidence in female rats. The incidence was 6/40, 6/50 and 16/50 in the controls, low and high doses, respectively. Incidence of alveolar-bronchiolar adenomas of the lung in males was 0/40, 5/49 and 2/50 in the controls, low-dose and high-dose, respectively. The historical incidence of this tumour in male F-344 control rats at the performing laboratory is 6/299 (2 %). No statistically significant increase in this tumour was observed in high-dose males or in any of the female groups. C-cell adenomas of the thyroid gland were observed at the following incidence in females: 3/40, 11/49 and 2/50 in the controls, low-dose and high-dose, respectively. The increase was statistically significant in the low-dose animals. There was no increased incidence of this tumour at either dose in the males. The NTP concluded eugenol was not carcinogenic to rats (NTP, 1983b).

Having reviewed the available subchronic and chronic studies on eugenol, the Panel concluded also that eugenol is of relatively low long-term toxicity, the target organ being the liver, at higher doses. A NOAEL for non-carcinogenic effects of 300 mg/kg bw/day could be derived.

There are currently three separate carcinogenicity experiments on eugenol available. The two experiments by Miller et al., (1983) in which they compared the hepatocarcinogenicity of eugenol

and a number of related hydroxyallylbenzenes (among which the carcinogenic safrole) showed in both experiments that eugenol was not carcinogenic. Safrole on the other hand, was clearly carcinogenic. In one of these experiments the compounds were administered orally, in the other intraperitoneally.

The NTP study showed that in the rat no carcinogenic effect was observed (NTP, 1983b). In the mouse strain, which showed a background level of hepatocellular tumors, the observed changes in the incidence of hepatocellular tumors are consistent with this background level. Therefore, this study is also considered negative.

Based on these data the Panel concluded that eugenol is not carcinogenic.

4. Application of the Procedure

4.1. Application of the Procedure to Eugenol and Six Related Hydroxyallylbenzene Derivatives by the JECFA (JECFA, 2007a):

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

The JECFA concluded six of the seven 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 six substances are below the threshold for structural class I (step A3).

One substance, eugenol [FL-no: 04.003], was concluded at step A5, i.e. the intake is above the threshold for the structural class, the substance is not endogenous, but a NOAEL is available that can provide an adequate margin of safety to the MSDI value for the substance. For this substance the intake used in the JECFA evaluation was based on USA MSDI (worst case), which is 3364 microgram/capita/day – if the intake is based on EU figures (950 microgram/capita/day) the intake would be below the threshold for the structural class and the substance would have been concluded at step A3 to be of no safety concern.

In conclusion, the JECFA evaluated all seven 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 eugenol and the six related hydroxyallylbenzene derivatives are summarised in Table 3.1: Summary of Safety Evaluation of Eugenol and Related Hydroxyallylbenzene Derivatives (JECFA, 2007a).

4.2. Application of the Procedure to 23 Ring Substituted Phenolic Substances by EFSA (EFSA, 2007m)

Twenty-three flavouring substances were evaluated in FGE.22. One of the candidate substances, 3,4-methylenedioxyphenol [FL-no: 04.080], showed genotoxic potential *in vitro*. Therefore, the Panel concluded that the Procedure could not be applied to this candidate substance until adequate genotoxicity data become available.

Nineteen of the 22 substances, to which the Procedure could be applied, are classified into structural class I and three into structural class II using the decision tree approach presented by Cramer et al. (1978).

The 22 substances, evaluated through the Procedure, in FGE.22 are assumed to be well absorbed. The substances are readily conjugated with sulphate or glucuronic acid at the hydroxy group, followed by rapid excretion usually via urine. The phenol esters will be hydrolysed, probably in the intestinal lumen by pancreatic enzymes to the corresponding phenols and formic and acetic acid. Sulphation and glucuronidation of phenols have been found in many species including humans, but species differences in efficacy of the two routes have been reported. These pathways are not easily saturated at the expected levels of intake resulting from the use as flavouring substances. At high dose levels reactive metabolites (quinones, catechols, quinone methides) may be formed. However, the formation of these metabolites is not expected to overwhelm the detoxication capacity through conjugation with sulphate, glucuronic acid or in particular glutathione, when the substances are used as flavouring substances. Thus, for the flavouring substances in FGE.22, it is concluded that the substances in this group may be expected to be metabolised to innocuous substances at the estimated levels of intake, based on the MSDI approach.

The 22 substances were concluded at step A3, i.e. the substances are expected to be metabolised to innocuous products (step 2) and the estimated daily intakes are below the thresholds for the structural classes I and II (step A3).

In conclusion, the Panel considered that the 22 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 22 substances are summarised in Table 3.2: Summary of Safety Evaluation Applying the Procedure (EFSA / FGE.22).

4.3. EFSA Considerations

There are several genotoxicity studies with eugenol *in vitro*, which indicate that eugenol (and consequently all substances in this FGE) may induce genotoxicity. The *in vitro* genotoxic profile suggests a possible role of oxidative DNA damage, with limited evidence for genotoxicity *in vivo*.

Taking into account the lack of carcinogenicity for eugenol, the Panel concluded that the available data on genotoxicity do not preclude that eugenol, and related hydroxyallylbenzene derivatives, are evaluated using the Procedure.

Accordingly the Panel agrees with the application of the Procedure as performed by the JECFA.

5. Conclusion

The Panel concluded that the seven substances in the JECFA flavouring group of eugenol and related hydroxyallylbenzene derivatives are structurally related to the phenolic substances evaluated by EFSA in FGE.22.

The Panel agrees with the application of the Procedure as performed by the JECFA. As the EU MSDI for eugenol [FL-no: 04.003] is below the threshold of the structural class it can already be

concluded to be of no safety concern at step A3 in the Procedure scheme. Moreover, a NOAEL of 300 mg/kg bw has been identified.

For three of the substances considered in this FGE use levels are available [FL-no: 04.058, 04.096 and 09.878] corresponding to mTAMDI values of 360, 420 and 2300 microgram/person/day, respectively. For one of these substances [FL-no: 09.878] the mTAMDI exceeds the threshold for the structural class. For the remaining four substances [FL-no: 04.003, 09.020, 09.088 and 09.766] 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 seven JECFA evaluated substances can be applied to the materials of commerce, it is necessary to consider the available specifications:

Adequate specifications including complete purity criteria and identity tests are available for six of the seven JECFA evaluated substances. For one substance [FL-no: 09.088] information on the composition of mixture is requested.
Thus, for one substance [FL-no: 09.088] the Panel has reservations (composition of mixture is requested). For the remaining six substances [FL-no: 04.003, 04.058, 04.096, 09.020, 09.766 and 09.878] the Panel agrees with the JECFA conclusion "No safety concern at estimated levels of intake as flavouring substances" based on the MSDI approach.

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

Table 1: Specification Summary of the Substances in the JECFA Flavouring Group of Eugenol and Six Related Hydroxyallylbenzene Derivatives

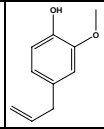
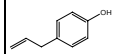
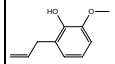
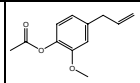
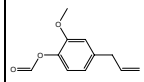
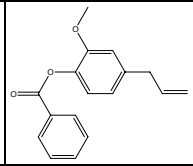
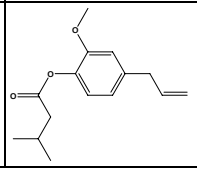
FL-no JECFA-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys. form Mol. formula Mol. weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec. gravity 5)	EFSA comments
04.003 1529	Eugenol		2467 171 97-53-0	Liquid $C_{10}H_{12}O_2$ 164.20	Slightly soluble Soluble	256 IR 98 %	1.540-1.542 1.064-1.070	
04.058 1527	4-Allylphenol		4075 11218 501-92-8	Liquid $C_9H_{10}O$ 134.18	Insoluble Soluble	235 16 NMR 95 %	1.542-1.548 1.017-1.023	
04.096 1528	2-Methoxy-6-(2-propenyl)phenol		579-60-2	Liquid $C_{10}H_{12}O_2$ 164.20	Slightly soluble Soluble	119-121 (16hPa) NMR 98 %	1.535-1.541 1.065-1.071	
09.020 1531	Eugenyl acetate		2469 210 93-28-7	Solid $C_{12}H_{14}O_3$ 206.24	Insoluble Soluble	282 25 IR 98 %	n.a. n.a.	
09.088 1530	4-Eugenyl formate		2473 355 10031-96-6	Liquid $C_{11}H_{12}O_3$ 192.21	Insoluble Soluble	270 NMR 94 %	1.524-1.526 1.115-1.125	According to JECFA: Min. assay value is "94 %" and secondary components "eugenol". Register name to be changed to Eugenyl formate.

Table 1: Specification Summary of the Substances in the JECFA Flavouring Group of Eugenol and Six Related Hydroxyallylbenzene 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
09.766 1533	Eugenyl benzoate		2471 636 531-26-0	Solid $C_{17}H_{16}O_3$ 268.31	Insoluble Soluble	n.a. 69-70 NMR 97 %	n.a. n.a.	
09.878 1532	Eugenyl isovalerate		4118 61114-24-7	Solid $C_{15}H_{20}O_3$ 248.32	Insoluble Soluble	222 85 NMR 99 %	n.a. n.a.	

1) *Solubility in water, if not otherwise stated.*

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

3) *At 1013.25 hPa, if not otherwise stated.*

4) *At 20°C, if not otherwise stated.*

5) *At 25°C, if not otherwise stated.*

TABLE 2: GENOTOXICITY DATA

Table 2.1: Genotoxicity Data (*in vitro / in vivo*) for Eugenol and Six Related Hydroxyallylbenzene Derivatives (JECFA, 2007a)

Table 2.1: Summary of Genotoxicity Data for Eugenol and Six Related Hydroxyallylbenzene Derivatives (JECFA, 2007a)

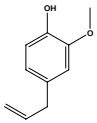
FL-no JECFA-no	EU Register name JECFA name	Structural formula	End-point	Test system	Concentration	Results	Reference
In vitro							
04.003 1529	Eugenol		Reverse Mutation	*Salmonella typhimurium* TA1530, TA1531, TA1532, and TA1964	0.02 or 0.2 M (3284 or 32841 µg/ml)[1]	Negative[2]	(Green & Savage, 1978)
			Reverse Mutation	*S. typhimurium* TA1530, TA1531, TA1532, and TA1964	1 to 5 mg/plate (1000 to 5000 µg/plate)	Negative[3]	(Green & Savage, 1978)
			Reverse Mutation	*S. typhimurium* TA98, TA100, TA1535, TA1538, and TA1537	2000 µg/plate	Negative[4]	(Nestmann et al., 1980)
			Reverse Mutation	*S. typhimurium* TA92, TA94, TA98, TA100, TA1535, and TA1537	Up to 2000 µg/plate	Negative[2,5]	(Ishidate et al., 1984)
			Reverse Mutation	*S. typhimurium* TA98, TA100, TA1535, and TA1537	3 µmol/plate (492.6 µg/plate)[1]	Negative[4,6]	(Florin et al., 1980)
			Reverse Mutation	*S. typhimurium* TA98, TA100, TA1535, TA1538, and TA1537	32.84 µg/plate	Negative[3,6]	(Douglas et al., 1979)
			Reverse Mutation	*S. typhimurium* TA98, TA100, TA1535, and TA1537	333 µg/plate	Negative[4,7]	(Tennant et al., 1987)
			Reverse Mutation	*S. typhimurium* TA98, TA100, TA1535, TA1538, and TA1537	60, 120, 300 or 600 µg/plate	Negative[3,8] Negative[2,5,8]	(Sekizawa & Shibamoto, 1982)
			Reverse Mutation	*S. typhimurium* TA97, TA98, TA100, and TA104	3000 µg/plate	Negative[4,6,9]	(Miller et al., 1986)
			Reverse Mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537, and TA1538	Not provided	Negative[4,10]	(Eder et al., 1982b)
			Reverse Mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537, and TA1538	0.01 to 3 µl per 2 ml incubation volume (5 to 1605 µg/ml)[11]	Negative[4,8,10]	(Eder et al., 1980)

Table 2.1: Summary of Genotoxicity Data for Eugenol and Six Related Hydroxyallylbenzene Derivatives (JECFA, 2007a)

FL-no JECFA-no	EU Register name JECFA name	Structural formula	End-point	Test system	Concentration	Results	Reference
			Reverse Mutation	*S. typhimurium* TA100	0.1 to 1000 µg/plate	Negative	(Rapson et al., 1980)
			Reverse Mutation	*S. typhimurium* TA98 and TA100	2500 µg/ml	Negative[4]	(Orstavik & Hongslo, 1985)
			Reverse Mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537, and TA1538	10, 150, 300, 600 or 1200 µg/plate	Negative[4,12]	(Amonkar et al., 1986)
			Reverse Mutation	*S. typhimurium* TA98, TA100, TA1535, TA1538, and TA1537	2000 µg/plate	Negative[4]	(Douglas et al., 1980)
			Reverse Mutation	*S. typhimurium* TA100, TA102 and TA1535	50 to 400 µg/plate	Negative[4]	(Sukumaran & Kuttan, 1995)
			Reverse Mutation	*S. typhimurium* TA98 and TA100	Up to 30 µmoles/plate (4926 µg/plate)	Negative[4,13]	(Swanson et al., 1979)
			Reverse Mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537 and TA1538	0.5, 5, 50, 500 or 5000 µg/plate	Negative[4,14]	(Pool & Lin, 1982)
			Reverse Mutation	*S. typhimurium* TA98, TA100, TA1535 and TA1537	3.3 to 333.3 µg/plate	Negative[4,14,15]	(Haworth et al., 1983)
			Reverse Mutation	*S. typhimurium* TA98 and TA100	0.05 to 100 µl (53.5 to 107000 µg/plate)	Negative[2]	(Rockwell & Raw, 1979)
			Reverse Mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537 and TA1538	0.5 to 500 µg/plate	Negative[4,16,17]	(To et al., 1982)
			Reverse Mutation	*S. typhimurium* TA1535	0.5 to 500 µg/plate	Positive[2,18] Negative[3]	(To et al., 1982)
			Reverse Mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537 and TA1538	5 to 500 µg/plate	Negative[4,5]	(Yoshimura et al., 1981)
			Reverse Mutation	*S. typhimurium* TA97, TA98 and TA100	1000 µg/ml	Negative[4,5]	(Azizan & Blevins, 1995)
			Reverse Mutation	*S. typhimurium* TA97, TA98, TA100 and TA102	0.25 to 6 µM/plate (41 to 985 µg/plate)	Negative[4,5,19]	(Schiestl et al., 1989)
			Reverse Mutation	*S. typhimurium* TA98, TA100, TA1535 and TA1537	3.3 to 333.3 µg/plate	Negative[4,5]	(NTP, 1983a)
			Reverse Mutation	*Escherichia coli* WP2 uvrA (trp-)	60, 120, 300 or 600 µg/plate	Negative[3,8] Negative[2,5,8]	(Sekizawa & Shibamoto, 1982)
			DNA Repair	*Bacillus subtilis* M45 (rec-) and H17 (rec+)	1 to 100 mg/disk (1000 to 100 000 µg/disk)	Negative	(Yoshimura et al., 1981)
			DNA Repair	*B. subtilis* M45 (rec-) and H17 (rec+)	400 µg/disk	Positive[3]	(Sekizawa & Shibamoto, 1982)

Table 2.1: Summary of Genotoxicity Data for Eugenol and Six Related Hydroxyallylbenzene Derivatives (JECFA, 2007a)

FL-no JECFA-no	EU Register name JECFA name	Structural formula	End-point	Test system	Concentration	Results	Reference
			DNA Repair	*B. subtilis* M45 (rec-) and H17 (rec+)	21 µg/disk	Negative	(Oda et al., 1979)
			Forward Mutation	L5178Y Mouse Lymphoma cells	21.3 µg/ml	Positive[3,7]	(Tennant et al., 1987)
			Forward Mutation	L5178Y Mouse Lymphoma cells	20 to 120 nl/ml (21 to 128 µg/ml)	Positive[3,20]	(Myhr & Caspary, 1991)
			Sister Chromatid Exchange	Peripheral Human lymphocytes	0 to 0.5 mM (0 to 82 µg/ml)	Negative	(Jansson et al., 1986)
			Sister Chromatid Exchange	Chinese Hamster Ovary cells	273 to 326 µg/ml	Positive[2,21]	(Galloway et al., 1987)
			Sister Chromatid Exchange	Chinese Hamster Ovary cells	11 to 123 µg/ml	Weakly positive[3,22]	(Galloway et al., 1987)
			Sister Chromatid Exchange	Syrian Hamster Embryo cells	0.00003 to 0.001% v/v (0.3 to 10 µg/ml)	Weakly positive[22]	(Fukuda, 1987)
			Sister Chromatid Exchange	Chinese Hamster Ovary cells	75 to 326 µg/ml	Positive[4]	(NTP, 1983a)
			Sister Chromatid Exchange	Chinese Hamster Ovary cells	75 µg/ml	Positive[4,7]	(Tennant et al., 1987)
			Chromosomal Aberrations	Chinese Hamster Fibroblast cells	125 µg/ml	Negative[23] Positive[24]	(Ishidate et al., 1984)
			Chromosomal Aberrations	Chinese Hamster Ovary cells	198 to 300 µg/ml	Negative[3,8]	(Galloway et al., 1987)
			Chromosomal Aberrations	Chinese Hamster Ovary cells	201 to 324 µg/ml	Weakly positive[2,25]	(Galloway et al., 1987)
			Chromosomal Aberrations	Chinese Hamster Ovary cells	50, 100 or 200 µg/ml	Negative[26]	(Stich et al., 1981c)
			Chromosomal Aberrations	Chinese Hamster Ovary cells	0.8 to 1.6 mM (131 to 263 µg/ml)	Positive[2,27]	(Bean et al., 1992)
			Chromosomal Aberrations	Chinese Hamster Ovary cells	1.2, 1.4 or 1.6 mM (197, 230 or 263 µg/ml)	Positive[2,8,28]	(Bean & Galloway, 1993)
			Chromosomal Aberrations	Chinese Hamster Ovary cells	198 to 324 µg/ml	Positive[2]	(NTP, 1983a)
			Chromosomal Aberrations	Chinese Hamster Ovary cells	300 µg/ml	Positive[2,7]	(Tennant et al., 1987)

Table 2.1: Summary of Genotoxicity Data for Eugenol and Six Related Hydroxyallylbenzene Derivatives (JECFA, 2007a)

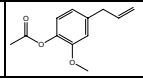
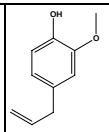
FL-no JECFA-no	EU Register name JECFA name	Structural formula	End-point	Test system	Concentration	Results	Reference
			Unscheduled DNA Synthesis	Male Fischer 344 rats (hepatocytes)	0.1 to 1,000 µM (0.01642 to 164.2 µg/ml)	Negative	(Burkey et al., 2000)
			Unscheduled DNA Synthesis	Female B6C3F$_1$ mice (hepatocytes)	0.1 to 1,000 µM (0.01642 to 164.2 µg/ml)	Negative	(Burkey et al., 2000)
			Unscheduled DNA Synthesis	Male Fischer 344 rats (hepatocytes)	10^{-6} to 10^{-3} M (0.1642 to 164.2 µg/ml)	Negative[29]	(Howes et al., 1990)
			Unscheduled DNA Synthesis	Syrian Hamster Embryo cells	0.00003 to 0.0001% v/v (0.3 to 1 µg/ml)	Positive[2]	(Fukuda, 1987)
			Unscheduled DNA Synthesis	Male Sprague-Dawley rats (hepatocytes)	0.1, 0.25, or 0.5 mM (16, 41 or 82 µg/ml)	Negative	(Allavena et al., 1992)
09.020 1531	Eugenyl acetate		Unscheduled DNA Synthesis	Male Sprague-Dawley rats (hepatocytes)	1, 2.5, 5, 10 or 15 µg/ml	Negative[30]	(San & Reece, 2003)
In vivo							
04.003 1529	Eugenol		Micronucleus Induction	Male Swiss CD-1 mice (bone marrow)	680 mg/kg bw/day for 15 days	Negative[31]	(Rompelberg et al., 1995)
			Micronucleus Induction	Male B6C3F$_1$ mice (bone marrow)	150, 300 or 600 mg/kg bw/day for 3 days	Negative[32]	(Shelby et al., 1993)
			Micronucleus Induction	Human lymphocytes	150 mg/day for 7 days (1.7 to 2.2 mg/kg bw/day)[33]	Negative[34]	(Rompelberg et al., 1996b)
			Micronucleus Induction	Male CF1 mice (bone marrow)	400 mg/kg bw	Positive[32,35]	(Ellahueñe et al., 1994)
			Micronucleus Induction	Male Sprague-Dawley rats (bone marrow)	1340 or 2680 mg/kg bw	Negative[36]	(Allavena et al., 1992)
			Micronucleus Induction	Male CF1 mice (bone marrow)	100, 400 or 600 mg/kg bw	Positive[32,37,38]	(Ellahueñe et al., 1994)
			Micronucleus Induction	Male Sprague-Dawley rats (liver and bone marrow)	1340 mg/kg bw	Negative[36,39]	(Allavena et al., 1992)
			Micronucleus Induction	Male Wistar rats (bone marrow)	500 or 1000 mg/kg bw per day for 10 days	Negative[34,40]	(Rompelberg et al., 1996a)
			Micronucleus Induction	Female Sprague-Dawley rats (bone marrow)	335, 670 or 1340 mg/kg bw	Negative[36]	(Maura et al., 1989)

Table 2.1: Summary of Genotoxicity Data for Eugenol and Six Related Hydroxyallylbenzene Derivatives (JECFA, 2007a)

FL-no JECFA-no	EU Register name JECFA name	Structural formula	End-point	Test system	Concentration	Results	Reference
			Micronucleus Induction	Male ddY mice (bone marrow)	100, 200, 400 or 800 mg/kg mw	Negative[32]	(Hayashi et al., 1984)
			Micronucleus Induction	Male Swiss Webster mice (bone marrow)	148[32], 740[32] or 14794[34] mg/kg bw	Positive	(Woolverton et al., 1986)
			Unscheduled DNA Synthesis	Male Sprague-Dawley rats (hepatocytes)	1340 or 2680 mg/kg bw	Negative[36]	(Allavena et al., 1992)
			Unscheduled DNA Synthesis	Male Wistar rats (hepatocytes)	500 or 1000 mg/kg bw per day for 10 days	Negative[36,41]	(Rompelberg et al., 1996b)
			DNA fragmentation	Female Sprague-Dawley rats (granuloma cells)	1, 5 or 10 mmol/kg bw (164, 821, or 1642 mg/kg bw)[1]	Negative[36]	(Maura et al., 1989)
			DNA fragmentation	Female Sprague-Dawley rats (liver and kidney)	1340 mg/kg bw	Negative[36]	(Maura et al., 1989)
			DNA fragmentation	Male Sprague-Dawley rats (hepatocytes)	1340 or 2680 mg/kg bw	Negative[36,42]	(Allavena et al., 1992)
			Chromosomal aberration	Human lymphocytes	150 mg/day for 7 days (1.7 – 2.2 mg/kg bw per day)[33]	Negative[34]	(Rompelberg et al., 1996b)
			Mutation	Female Sprague-Dawley rats (granuloma cells)	1, 5 or 10 mmol/kg bw (164, 821, or 1642 mg/kg bw)[1]	Negative[36]	(Maura et al., 1989)

[1] *Calculated from relative molecular mass for eugenol = 164.203.*

[2] *With metabolic activation.*

[3] *Without metabolic activation.*

[4] *With and without metabolic activation.*

[5] *Pre-incubation method.*

[6] *Spot test.*

[7] *Lowest dose that gave positive results or highest dose that gave negative results.*

[8] *Cytotoxicity observed at highest dose.*

[9] *Toxic to strains TA97, TA100 and TA104 at 3000 µg/plate.*

[10] *Modified liquid suspension test system.*

[11] *Calculated from density of eugenol = 1.07 g/ml.*

[12] *Toxic to all strains at 1200 µg/plate.*

[13] *Cytotoxicity observed at 5 µmoles/plate (821 µg/plate).*

[14] *Toxic to all strains at 5000 µg/plate.*

[15] *Cytotoxicity observed at 333.3 µg/plate in strains TA100 and TA1537.*

[16] *Statistically significant in strain TA98 at 500 µg/plate.*

[17] *Statistically significant in strain TA1537 at 10, 50, 150 and 500 µg/plate; at least twice the number of revertants per plate as in negative control only in first of three determinations.*

[18] *3'-Phosphoadenosine-5'-phosphosulphate included in metabolic activation mix.*

[19] *No effects with or without metabolic activation in strains TA97 and TA100; weak effects in strains TA98 and TA102.*

[20] *Cytotoxicity observed at 64 and 128 µg/ml.*

[21] *Increases at doses that caused severe cell cycle delay.*

[22] *Statistical significant, but induced level not twice in the control.*

[23] *At 24 h; dose is highest non-cytotoxic dose used.*

[24] *At 48 h; dose is that at which maximum effect obtained.*

[25] *In the first trial, cells fixed at about 10.5 h showed a small increase in chromosomal aberrations at the highest dose; in the second trial (with a harvesting time of 20 h), clear increase observed.*

[26] *Slight increase in the incidence at 200 µg/ml (2.0 %) relative to controls (0.8 %).*

[27] *In the first trial, with harvesting at 15.0 and 24.5 h after treatment initiation, cell viability was 57 % and 37 % that of controls after incubation with 0.8 mmol/l of eugenol, and 53 % and 43 % that of controls after incubation with 1.2 mmol/l of eugenol. The percentage of aberrant cells increased to a maximum of 25.5 % at harvesting 15 h after incubation with 0.8 mmol/l of eugenol. In the second trial, the aberration yield shifted with the cytotoxicity, as concentrations of 1.2 to 1.6 mmol/l of eugenol was as cytotoxic as 0.8 mmol/l in the first trial. Maximum percentage of aberrant cells (26.5 %) on harvesting 17 h after incubation with 1.6 mmol/l of eugenol.*

[28] *Cells harvested 20-21 h or 42-44 hs after beginning of 3h treatment with eugenol. Severe cell cycle delay observed 20 h after incubation with 1.6 mmol/l of eugenol, and the chromosomal aberrations accompanied by a reduction in cell counts to about 50 % that of controls.*

[29] *Cytotoxicity observed at 500 µmol/l.*

[30] *Cytotoxicity observed at 10 and 15 µg/ml.*

[31] *Administered in diet..*

[32] *Administered by intraperitoneal injection.*

[33] *Range of doses calculated on basis of the body weight range of the volunteers: 68 to 88 kg.*

[34] *Administered orally.*

[35] *Sampling 24, 30, or 48 h after intraperitoneal injection.*

[36] *Administered by gavage.*

[37] *Sampled once, 30 h after intraperitoneal injection.*

[38] *Significant increases at 400 and 600 mg/kg bw of eugenol.*

[39] *Rats underwent two-thirds hepatectomy 20 h before eugenol was administered.*

[40] *Slight, not statistically significant increase in percentage of polychromatic erythrocytes at 1000 mg/kg bw per day.*

[41] *Hepatocytes from rats pre-treated with 500 mg/kg bw of eugenol and then exposed in vitro to dimethyl sulphoxide had significantly lower mean net nuclear grain count than control hepatocytes (dimethyl sulphoxide); no results for 1000 mg/kg bw eugenol plus dimethyl sulphoxide.*

[42] *Observed 4 and 20 h after seeding.*

Table 2.2: Genotoxicity (*in vitro*) for Ring Substituted Phenolic Substances (EFSA, 2007m)

Table 2.2: GENOTOXICITY (*in vitro*)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
(2-Methylphenol [04.027])	Ames assay	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	2.5 µl/plate (26,200µg/plate)	Negative[1, 2]	(Douglas et al., 1980)	[7]
	Ames assay (preincubation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	324 µg/plate	Negative[1, 2]	(Florin et al., 1980)	[7] Insufficient quality. Not in accordance with OECD guideline 471. Inadequate study design (Spot test).
	Ames assay	*S. typhimurium* TA98; TA100; TA1535; TA1537	5 concentrations from 1 to 100 µg/plate	Negative[1, 2]	(Haworth et al., 1983)	[7] Acceptable quality. Published summary report including detailed results from studies on 250 chemicals tested in various laboratories within the NTP. In accordance with OECD guideline 471 (1983).
	Ames assay	*S. typhimurium* TA98; TA100	5 µg/plate	Negative[1, 2]	(Massey et al., 1994)	[7]
	Ames assay	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	Up to 2600 µg/plate (range not reported)	Negative[1, 2, 3]	(Nestmann et al., 1980)	[7] Insufficient quality as main details of method and results were not reported. Additionally, the test was not repeated.
	Ames assay	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	4 concentrations from 5 to 5000 µg/plate	Negative[1, 2]	(Pool & Lin, 1982)	[7] Acceptable quality.
	Ames assay (preincubation assay)	*S. typhimurium* TA98; TA100; TA1535; TA1537	1000 µg/plate	Negative[1, 2]	(Canter, 1981)	[7]
	Ames assay	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	500 µg/plate	Negative[1, 2]	(Nuodex Inc., 1980a)	[7]
	Ames assay	*S. typhimurium* TA98; TA100	Not reported	Negative[4] Positive[5]	(Claxton, 1985)	[7] Result cannot be evaluated since it was reported only as a very short summary in table format. The paper was on methodological aspects of the assay and not specifically on this compound.
	Sister chromatid exchange	Human lymphocytes	5 concentrations up to 0.5 mM (54 µg/ml)	Negative	(Jansson et al., 1986; Jansson et al., 1988)	[7] Limited quality (selection of maximum concentration not justified and experiment not repeated).
	Sister chromatid exchange	Human fibroblasts	86.5 - 865 µg/ml without S9	Equivocal	(Cheng & Kligerman, 1984)	[7] Limited quality. Only the highest concentration resulted in a result statistically significantly different from control (1.2-fold increase only). A second experiment was not performed.

Table 2.2: GENOTOXICITY (*in vitro*)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
	Sister chromatid exchange	Chinese Hamster ovary cells	4 concentrations from 12.5 to 75 nl/ml (78.6 µg/ml) without S9, 11 concentrations from 1.56 to 700 nl/ml (733 µg/ml) with S9	Positive[1] Positive[2]	(Galloway & Brusick, 1981)	[17] Acceptable quality. Statistically significant dose-related increase (up to two-fold).
	Sister chromatid exchange	Chinese hamster ovary cells	Up to 500 nl/ml (524 µg/ml)[4]	Positive[1, 2]	(Galloway & Brusick, 1980)	[17] Acceptable quality but limited relevance because the test material was a mixture of *p*-cresol, *m*-cresol and *o*-cresol (33 1/3 % each).
	Unscheduled DNA synthesis	Rat primary hepatocytes	7 concentrations from 0.5 to 50 nl/ml (52.4 µg/ml)[4]	Equivocal	(Myhr & Brusick, 1980)	[17] Acceptable quality but limited relevance because the test material was a mixture of *p*-cresol, *m*-cresol and *o*-cresol (33 1/3 % each). Response was not dose-related. A slight response was observed at concentrations up to 5.0 nl/ml, while UDS was not observed at concentrations from 10 to 50 nl/ml..
	DNA Repair assay	*E. coli* W3110	5000 µg/ml	Negative[1, 2]	(Pepper Hamilton and Scheetz, 1980)	[17] Test substance included 60 % *o*-cresol.
	DNA repair assay	*E. coli*	5000 µg/ml	Negative[1, 2]	(Nuodex Inc., 1980b)	[17]
(3-Methylphenol [04.026])	Ames assay	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	2000 µg/plate	Negative[1, 2]	(Douglas et al., 1980)	[17]
	Ames assay (preincubation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	324 µg/plate	Negative[1, 2]	(Florin et al., 1980)	[17] Insufficient quality. Not in accordance with OECD guideline 471. Inadequate study design (Spot test).
	Ames assay	*S. typhimurium* TA98; TA100; TA1535; TA1537	5 concentrations from 3.3 to 333 µg/plate	Negative[1, 2]	(Haworth et al., 1983)	[17] Acceptable quality. Published summary report including detailed results from studies on 250 chemicals tested in various laboratories within the NTP. In accordance with OECD guideline 471 (1983).
	Ames assay	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	Up to 2000 µg/plate (range not reported)	Negative[1, 2, 3]	(Nestmann et al., 1980)	[17] Insufficient quality as main details of method and results were not reported. Additionally, the test was not repeated.
	Ames assay	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	5 concentrations from 0.5 to 5000 µg/plate	Negative[1, 2]	(Pool & Lin, 1982)	[17] Acceptable quality.

Table 2.2: GENOTOXICITY (*in vitro*)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
	Ames assay (preincubation assay)	*S. typhimurium* TA98; TA100; TA1535; TA1537	3333 µg/plate	Negative[1, 2]	(Canter, 1981)	[17]
	Sister chromatid exchange	Human lymphocytes	5 concentrations up to 1 mmol/L (108 µg/ml)	Negative	(Jansson et al., 1986); (Jansson et al., 1988)	[17] Limited quality (selection of maximum concentration not justified and experiment not repeated).
	Sister chromatid exchange	Human fibroblasts	865 µg/ml	Negative	(Cheng & Kligerman, 1984)	[17]
	Sister chromatid exchange	Chinese hamster ovary cells	Up to 500 nl/ml (524 µg/ml)[4]	Positive[1, 2]	(Galloway & Brusick, 1980)	[17] Acceptable quality but limited relevance because the test material was a mixture of *p*-cresol, *m*-cresol and *o*-cresol (33 1/3 % each).
	Unscheduled DNA synthesis	Rat primary hepatocytes	10 µg/ml	Negative	(Cifone, 1988a)	[17]
	Unscheduled DNA synthesis	Rat primary hepatocytes	7 concentrations from 0.5 to 50 nl/ml (51.7 µg/ml)[4]	Equivocal	(Myhr & Brusick, 1980)	[17] Limited relevance because the test material was a mixture of *p*-cresol, *m*-cresol and *o*-cresol (33 1/3 % each). Response was not dose-related. A slight response was observed at concentrations up to 5.0 nl/ml, while UDS was not observed at concentrations from 10 to 50 nl/ml..

Table 2.2: GENOTOXICITY (*in vitro*)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
(4-Methylphenol [04.028])	Ames assay	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	1000 µg/plate	Negative[1, 2]	(Douglas et al., 1980)	[17]
	Ames assay (preincubation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	324 µg/plate	Negative[1, 2]	(Florin et al., 1980)	[17] Insufficient quality. Not in accordance with OECD guideline 471. Inadequate study design (Spot test).
	Ames assay	*S. typhimurium* TA98; TA100; TA1535; TA1537	5 concentrations from 3.3 to 333 µg/plate	Negative[1, 2]	(Haworth et al., 1983)	[17] Acceptable quality. Published summary report including detailed results from studies on 250 chemicals tested in various laboratories within the NTP. In accordance with OECD guideline 471 (1983).
	Ames assay	*S. typhimurium* TA98; TA100	5 µg/plate	Negative[1, 2]	(Massey et al., 1994)	[17]
	Ames assay	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	Up to 1000 µg/plate (range not reported)	Negative[1, 2, 3]	(Nestmann et al., 1980)	[17] Insufficient quality as main details of method and results were not reported. Additionally, the test was not repeated.
	Ames assay	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	5 concentrations from 0.5 to 5000 µg/plate	Negative[1, 2]	(Pool & Lin, 1982)	[17] Acceptable quality.
	Ames assay (preincubation assay)	*S. typhimurium* TA98; TA100; TA1535; TA1537	1000 µg/plate	Negative[1, 2]	(Canter, 1981)	[17]
	Ames assay	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	1 µl/plate (1030 µg/plate)	Negative[1, 2]	(Crowley & Margard, 1978)	[17]
	Sister chromatid exchange	Human lymphocytes	5 concentrations up to 0.5 mmol/L (54 µg/ml)	Negative	(Jansson et al., 1986)	[17] Limited quality (selection of maximum concentration not justified and experiment not repeated).
	Sister chromatid exchange	Human fibroblasts	865 µg/ml	Negative	(Cheng & Kligerman, 1984)	[17]
	Sister chromatid exchange	Chinese hamster ovary cells	Up to 500 nl/ml (524 µg/ml)[4]	Positive[1, 2]	(Galloway & Brusick, 1980)	[17] Acceptable quality but limited relevance because the test material was a mixture of *p*-cresol, *m*-cresol and *o*-cresol (33 1/3 % each).
	Unscheduled DNA synthesis	Human lymphocytes	25 µM (2.7 µg/ml)	Negative	(Daugherty & Franks, 1986)	[17] Not relevant since only an inhibition of UV-induced UDS was measured.. Additionally, a result is reported only for one concentration (resulting in inhibition by 30 %) and a negative

Table 2.2: GENOTOXICITY (*in vitro*)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
						control was not included.
	Unscheduled DNA synthesis	Rat primary hepatocytes	7 concentrations from 0.5 to 50 nl/ml (51.5 µg/ml)	Equivocal	(Myhr & Brusick, 1980)	[17] Acceptable quality but limited relevance because the test material was a mixture of *p*-cresol, *m*-cresol and *o*-cresol (33 1/3 % each). Response was not dose-related. A slight response was observed at concentrations up to 5.0 nl/ml, while UDS was not observed at concentrations from 10 to 50 nl/ml.
	Unscheduled DNA synthesis	WI-38 human embryonic lung fibroblast cells	Not unambiguously reported	Positive	(Crowley & Margard, 1978)	[17] Unpublished study report of limited quality because concentrations were not unambiguously reported and only 3 concentrations have been tested. However, the result was reproducible. Liquid scintillation counting.
2-Ethylphenol [04.070]	Ames assay (preincubation method)	*S. typhimurium* TA97; TA98; TA100; TA1535	5 doses from 0.01 to 10 mg/plate	Negative[1, 2]	(Zeiger et al., 1992)	Acceptable quality.
	Ames assay (preincubation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537	3 µmol/plate (367 µg/plate)	Negative[1, 2]	(Florin et al., 1980)	Insufficient quality. Not in accordance with OECD guideline 471. Inadequate study design (Spot test).
	Sister chromatid exchange	Human lymphocytes	5 concentrations up to 0.25 mmol/L (30.5 µg/ml)	Negative[6]	(Jansson et al., 1986)	Limited quality (selection of maximum concentration not justified and experiment not repeated).
3-Ethylphenol [04.021]	Ames assay (preincubation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537	3 µmol/plate (366 µg/plate)	Negative[1, 2]	(Florin et al., 1980)	Insufficient quality. Not in accordance with OECD guideline 471. Inadequate study design (Spot test).
	Sister chromatid exchange	Human lymphocytes	5 concentrations up to 0.25 mmol/L (30.5 µg/ml)	Negative[6]	(Jansson et al., 1986)	Limited quality (selection of maximum concentration not justified and experiment not repeated).
(4-Ethylphenol [04.022])	Ames assay (preincubation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	367 µg/plate	Negative[1, 2]	(Florin et al., 1980)	[17] Insufficient quality. Not in accordance with OECD guideline 471. Inadequate study design (Spot test).
	Ames assay	*S. typhimurium* TA98; TA100	Not reported	Negative[1, 2]	(Epler et al., 1979)	[17] Insufficient quality. Not in accordance with OECD guideline 471. Only two strains tested. Results not reported in detail.
	Sister chromatid exchange	Human lymphocytes	5 concentrations up to 0.25 mmol/L (27 µg/ml)	Negative	(Jansson et al., 1986)	[17] Limited quality (selection of maximum concentration not justified and experiment not repeated).
(4-(1,1-Dimethyl)ethyl phenol	Ames assay	*S. typhimurium*	2000 µg/plate	Negative[1, 2]	(Dean et al., 1985)	[17]

Table 2.2: GENOTOXICITY (*in vitro*)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
[04.064])		TA98; TA100; TA1535; TA1537; TA1538				Insufficiently reported. Validity cannot be evaluated as the results were not reported in detail.
	Mutation assay	*E. coli* WP2 and WP2 *uvrA*	2000 µg/plate	Negative[1, 2]	(Dean et al., 1985)	[17] Insufficiently reported. Validity cannot be evaluated as main details of the method (e.g. concentration range tested) were not reported. Additionally, the result was not reported in detail.
	Mutation assay	*S. cerevisiae* JD1	2000 µg/plate	Negative[1, 2]	(Dean et al., 1985)	[17] Insufficiently reported. Validity cannot be evaluated as main details of the method (e.g. concentration range tested) were not reported. Additionally, the result was not reported in detail.
	Chromosomal aberration assay	Rat liver cell RL_1, RL_2	Not specifically indicated[7]	Negative	(Dean et al., 1985)	[17] Insufficiently reported. Validity cannot be evaluated as main details of the method (e.g. concentrations tested) were not reported. Additionally, the result was not reported in detail.
	Chromosome aberration assay	Chinese hamster lung cells	Not reported	Negative[8]	(Kusakabe et al., 2002)	[17]
	Mouse lymphoma assay	L5178Y *tk* +/- mouse lymphoma cells	80 µg/ml	Negative	(Honma et al., 1999b)	[17]
2,3-Dimethylphenol [04.065]	Ames assay	*S. typhimurium* TA98; TA100	Not reported	Negative[1, 2]	(Epler et al., 1979)	Insufficient quality. Not in accordance with OECD guideline 471. Only two strains tested. Results not reported in detail.
	Sister chromatid exchange	Human lymphocytes	5 concentrations up to 0.5 mmol/L (61 µg/ml)	Negative[6]	(Jansson et al., 1986)	Limited quality (selection of maximum concentration not justified and experiment not repeated).
2,4-Dimethylphenol [04.066]	Ames assay (preincubation method)	*S. typhimurium* TA97; TA98; TA100; TA1535; TA1537	0, 0.33, 1, 3.3, 10, 33 µg/plate	Negative[1, 2]	(Mortelmans et al., 1986)	Acceptable quality.
	Ames assay (preincubation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537	3 µmol/plate[9] (366 µg/plate)	Negative[1, 2]	(Florin et al., 1980)	Insufficient quality. Not in accordance with OECD guideline 471. Inadequate study design (Spot test).
	Ames assay	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	5 concentrations from 0.5 to 5000 µg/plate[10]	Negative[1, 2]	(Pool & Lin, 1982)	Acceptable quality.
	Sister chromatid exchange	Human lymphocytes	5 concentrations up to 0.1 mmol/L (12 µg/ml)	Negative[6]	(Jansson et al., 1986)	Limited quality (selection of maximum concentration not justified and experiment not repeated).

Table 2.2: GENOTOXICITY (*in vitro*)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
(2,5-Dimethylphenol [04.019])	Ames assay (preincubation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	367 µg/plate	Negative[1, 2]	(Florin et al., 1980)	[17] Insufficient quality. Not in accordance with OECD guideline 471. Inadequate study design (Spot test).
	Ames assay	*S. typhimurium* TA98; TA100	Not reported	Negative[1, 2]	(Epler et al., 1979)	[17] Insufficient quality. Not in accordance with OECD guideline 471. Only two strains tested. Results not reported in detail.
(2,6-Dimethylphenol [04.042])	Ames assay (preincubation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	367 µg/plate	Negative[1, 2]	(Florin et al., 1980)	[17] Insufficient quality. Not in accordance with OECD guideline 471. Inadequate study design (Spot test).
	Ames assay (preincubation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	5 mg/plate (5000 µg/plate)	Negative[1, 2]	(Schechtman et al., 1980)	[17]
	Chromosome aberration assay	Chinese hamster V79 cells	3 concentrations from 10 to 100 µg/ml (without S9) and 5 concentrations from 30 to 600 µg/ml (with S9)	Negative[1] Positive[2]	(Völkner, 1994)	[17] Acceptable quality. This GLP-study was in accordance with OECD guideline 473 (1983). A final report was not available and the draft was not signed. However, the results and conclusions available as draft report are considered valid.
	Sister chromatid exchange	Human lymphocytes	5 concentrations up to 0.25 mmol/L (31 µg/ml)	Negative	(Jansson et al., 1986)	[17] Limited quality (selection of maximum concentration not justified and experiment not repeated).
(3,4-Dimethylphenol [04.048])	Ames assay (preincubation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	367 µg/plate	Negative[1, 2]	(Florin et al., 1980)	[17] Insufficient quality. Not in accordance with OECD guideline 471. Inadequate study design (Spot test).
	Ames assay	*S. typhimurium* TA98; TA100	Not reported	Negative[1, 2]	(Epler et al., 1979)	[17] Insufficient quality. Not in accordance with OECD guideline 471. Only two strains tested. Results not reported in detail.
3,5-Dimethylphenol [04.020]	Ames assay (plate incorporation, preincubation, spot test, and treat-and-plate methods)	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538; *E. coli* WP2; WP2*uvrA*	6 concentrations from 125 to 4000 µg/plate	Negative[1, 2]	(Dean et al., 1985)	Insufficiently reported. Validity cannot be evaluated as the results were not reported in detail.
	Mitotic gene conversion assay	*S. cerevisiae* JD1	Not reported	Negative[1, 2]	(Dean et al., 1985)	Insufficiently reported. Validity cannot be evaluated as main details of the method (e.g. concentration range tested) were not reported. Additionally, the result was not reported in

Table 2.2: GENOTOXICITY (*in vitro*)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
						detail.
	Chromosome aberration assay	Rat liver cells RL_4	3 concentrations from 0.125 to 0.5 of GI_{50} (50% growth inhibition). Values in µg/ml or µmol/ml not reported.	Negative[1, 2]	(Dean et al., 1985)	Insufficiently reported. Validity cannot be evaluated as main details of the method (e.g. concentrations tested) were not reported. Additionally, the result was not reported in detail.
2,4,6-Trimethylphenol [04.095]	Ames assay (preincubation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537	3 µmol/plate[10] (409 µg/plate)	Negative[1, 2]	(Florin et al., 1980)	Insufficient quality. Not in accordance with OECD guideline 471. Inadequate study design (Spot test).
	Ames assay	*S. typhimurium* TA98; TA100	Not reported	Negative[1, 2]	(Epler et al., 1979)	Insufficient quality. Not in accordance with OECD guideline 471. Only two strains tested. Results not reported in detail.
(Thymol [04.006])	Ames assay	*S. typhimurium* TA97; TA98; TA100	1000 µg/ml	Negative[1, 2]	(Azizan & Blevins, 1995)	[17]
	Ames assay (preincubation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	451 µg/plate	Negative[1, 2]	(Florin et al., 1980)	[17] Insufficient quality. Not in accordance with OECD guideline 471. Inadequate study design (Spot test).
	Ames assay (preincubation method)	*S. typhimurium* TA97; TA98; TA100	Not reported	Negative[1, 2]	(Azizan & Blevins, 1995)	[17]
	Sister chromatid exchange	SHE cells	5 concentrations from 0.3 to 30 µg/ml	Equivocal	(Fukuda, 1987)	[17] Validity cannot be evaluated since the study was published in Japanese (e.g. presence or absence of S9 is not clear). However, the results reported in a table were not dose-related.
	Unscheduled DNA synthesis	SHE cells	4 concentrations from 0.3 to 10 µg/ml	Equivocal	(Fukuda, 1987)	[17] Validity cannot be fully evaluated since the study was published in Japanese (e.g. presence or absence of S9 is not clear). However, the results reported in a table were not dose-related.
(Carvacrol [04.031])	Ames assay	*S. typhimurium* TA98; TA100	2 concentrations (8 and 16 ppm)	Negative[1, 2]	(Kono et al., 1995)	[17] Not in accordance with OECD guideline 471 (only two strains used and only two concentrations tested). In Japanese with a short summary in English.
	Ames assay (plate incorporation assay)	*S. typhimurium* TA98; TA100	3 concentrations from 0.6 to 2.5 µmol/plate	Negative[1, 2]	(Stammati et al., 1999)	[17] This study was not in accordance with OECD guideline 471 (only two strains used and only 3 concentrations tested).
	Bacterial DNA repair test	*E. coli* WP2 *trpE65*; CM8781	4 concentrations from 2.5 to 6 µmol/paper disk	Positive	(Stammati et al., 1999)	[17] Effects were measured as inhibition zones. This

Table 2.2: GENOTOXICITY (*in vitro*)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
		trpE65; uvrA155, recA56, lexA				assay is considered to be of minor relevance. Positive results from such assays may be interpreted as an indication of a genotoxic potential which needs to be clarified by other assays.
	SOS Chromotest	*E. coli* PQ37	4 concentrations (not unambiguously reported)	Negative	(Stammati et al., 1999)	[17] Concentrations not unambiguously reported, only without S9 tested. This assay is considered to be of minor relevance. Positive results from such assays may be interpreted as an indication of a genotoxic potential which needs to be clarified by other assays.
(4-Vinylphenol [04.057])	Sister chromatid exchange	Human lymphocytes	5 concentrations up to 0.1 mmol/L (12 µg/ml)	Negative	(Jansson et al., 1988)	[17] Limited quality (selection of maximum concentration not justified and experiment not repeated).
(2-Methoxyphenol [04.005])	Ames assay	*S. typhimurium* TA98; TA100; TA102	111,726 µg/plate	Negative[1, 2]	(Aeschbacher et al., 1989)	[17]
	Ames assay	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	16,000 µg/plate	Negative[1, 2]	(Douglas et al., 1980)	[17]
	Ames assay	*S. typhimurium* TA98; TA100; TA1535; TA1537	5 concentrations from 333 to 11,740 µg/plate in one experiment and 5 concentrations from 33 to 3333 µg/plate in two further experiments performed in another laboratory	Negative[1, 2]	(Haworth et al., 1983)	[17] Acceptable quality. Published summary report including detailed results from studies on 250 chemicals tested in various laboratories within the NTP. Three experiments performed in two laboratories. In accordance with OECD guideline 471 (1983).
	Ames assay	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	Up to 16,000 µg/plate (range not reported)	Negative[1, 2]	(Nestmann et al., 1980)	[17] Insufficient quality as main details of method and results were not reported. Additionally, the test was not repeated.
	Ames assay	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	5 conentrations from 0.5 to 5000 µg/plate	Negative[1, 2]	(Pool & Lin, 1982)	[17] Acceptable quality.
	Sister chromatid exchange	Human lymphocytes	5 concentration up to 0.5 mmol/L (62 µg/ml)	Positive	(Jansson et al., 1988)	[17] Acceptable quality. Only the highest concentration resulted in a statistically significant increase. The effect was very waek but reproducible.
3-Methoxyphenol [04.076]	Ames assay (preincubation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537	30 µmol/plate (3724 µg/plate)	Negative[1, 2]	(Florin et al., 1980)	Insufficient quality. Not in accordance with OECD guideline 471. Inadequate study design (Spot test).

Table 2.2: GENOTOXICITY (*in vitro*)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
	Sister chromatid exchange	Human lymphocytes	5 concentrations up to 1 mmol/L (124 µg/ml)	Negative[6]	(Jansson et al., 1986)	Limited quality (selection of maximum concentration not justified and experiment not repeated).
4-Methoxyphenol [04.077]	Ames assay (preincubation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537	5 concentrations from 3.3 to 167 µg/plate in the first experiment and 5 concentrations from 100 to 5000 µg/plate in the second experiment performed in another laboratory	Negative[2,]	(Haworth et al., 1983)	Acceptable quality. Published summary report including detailed results from studies on 250 chemicals tested in various laboratories within the NTP. Experiments performed in two laboratories. In accordance with OECD guideline 471 (1983).
	Ames assay (preincubation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537	30 µmol/plate (3724 µg/plate)	Negative[1, 2]	(Florin et al., 1980)	Not in accordance with OECD guideline 471. Inadequate study design (Spot test).
	Ames assay (plate incorporation method)	*S. typhimurium* TA100; TA1530	Up to 4 µmol/plate	Negative[1, 2, 12]	(Bartsch et al., 1980)	As only two strains were used the quality of the study must be considered insufficient for the purpose of this Flavouring Group Evaluation Validity cannot be evaluated as details of the result were not reported.
	Mouse lymphoma assay	Mouse L5178Y TK +/- lymphocytes	27 to 2000 µg/ml (without S9) 1.3 to 100 µg/ml (with S9)	Positive[1] Negative[2]	(Rogers-Back, 1986)	The validity of this unpublished report cannot fully be evaluated since all pages in table format are lacking.
	Sister chromatid exchange	Human lymphocytes	5 concentrations up to 0.05 mmol/L (6.2 µg/ml)	Negative[6]	(Jansson et al., 1986)	Limited quality (selection of maximum concentration not justified and experiment not repeated).
	Chromosome aberration assay	Chinese hamster ovary cells	954, 1269, and 1692 µg/ml (each in the presence and absence of S9)	Positive[1, 2]	(Putman, 1986)	The validity of this unpublished report cannot fully be evaluated since all pages in table format are lacking.
	Unscheduled DNA synthesis	Human lymphocytes	25 µM (3.1 µg/ml)	Equivocal	(Daugherty & Franks, 1986)	Not relevant since only an inhibition of UV-induced UDS was measured. Additionally, a result is reported only for one concentration (resulting in inhibition by 30 %) and a negative control was not included.
(2-Methoxy-4-methylphenol [04.007])	Sister chromatid exchange	Human lymphocytes	5 concentrations up to 1 mmol/L (138 µg/ml)	Positive	(Jansson et al., 1988)	[17] Acceptable quality. The effect was weak (twofold increase) but dose-related and statistically significant.
(4-Ethylguaiacol [04.008])	Sister chromatid exchange	Human lymphocytes	5 concentration up to 1 mmol/L (152 µg/ml)	Negative	(Jansson et al., 1988)	[17] Limited quality (selection of maximum concentration not justified and experiment not

Table 2.2: GENOTOXICITY (*in vitro*)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
						repeated).
(2,6-Dimethoxyphenol [04.036])	Ames assay	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	16,000 µg/plate	Negative	(Douglas et al., 1980)	[17]
	Ames assay (preincubation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	463 µg/plate	Negative[1, 2]	(Florin et al., 1980)	[17] Insufficient quality. Not in accordance with OECD guideline 471. Inadequate study design (Spot test).
	Ames assay	*S. typhimurium* TA98; TA100; TA1535; TA1537	1000 µg/ml	Negative[1, 2]	(McMahon et al., 1979)	[17]
	Ames assay	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	5 concentrations from 0.5 to 5000 µg/plate	Negative[1, 2]	(Pool & Lin, 1982)	[17] Acceptable quality.
	Mutation assay	*E. coli*	1000 µg/ml	Negative[1, 2]	(McMahon et al., 1979)	[17]
	Sister chromatid exchange	Human lymphocytes	4 concentrations up to 0.5 mmol/L (77 µg/ml)	Negative	(Jansson et al., 1986)	[17] Limited quality (selection of maximum concentration not justified and experiment not repeated).
4-Hydroxy-3,5-dimethoxyacetophenone [07.164]	Ames assay (plate incorporation assay)	*S. typhimurium* TA97; TA98; TA100; TA102	6 concentrations from 10 to 4000 µg/plate	Negative[1, 2]	(Pfuhler et al., 1995)	Limited quality. Strain TA 1535 was not used although recommended by OECD 471 (1983 and 1997) which may be acceptable but the test was not repeated.
	Ames assay	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	Up to 1 mg/plate (range not reported)	Negative[1, 2]	(Nestmann et al., 1980)	Insufficient quality as main details of method and results were not reported. Additionally, the test was not repeated.
	Mutagenicity assay	*S. cerevisiae* D7; XV185-14C	Not reported	Negative[1]	(Nestmann & Lee, 1983)	Insufficient quality. Details of concentrations and results not reported.
	Sister chromatid exchange	Human peripheral lymphocytes	4 concentrations from 3.3 to 100 µg/ml	Negative[1, 2]	(Pfuhler et al., 1995)	Limited quality as the test was not repeated in an independent experiment. Otherwise in accordance with OECD 479 (1986).
(2-Methoxy-4-vinylphenol [04.009])	Sister chromatid exchange	Human lymphocytes	5 concentrations up to 0.5 mmol/L (75 µg/ml)	Equivocal	(Jansson et al., 1988)	[17] Limited quality (selection of maximum concentration not justified and experiment not repeated). Weak effect (only the highest concentration resulted in a twofold increase of SCE frequency which was statistically significant but was not repeated in a second experiment).

Table 2.2: GENOTOXICITY (*in vitro*)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
3,4- Methylenedioxyphenol [04.080]	Ames assay (preincubation method)	*S. typhimurium* TA98; TA100; TA102	4 concentrations from 1 to 10 µM/plate (1381 µg/plate)	(Not applicable)[13]	(Kaur & Saini, 2000)	Limited relevance. Antimutagenic activity was investigated only. The substance was tested only in combination with mutagens.
	Ames Assay (plate incorporation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	33 - 3333 µg/plate	Negative[1, 2, 14]	(Longfellow, 1985/1986)	Validity cannot be evaluated. The information was generated from the Chemical Carcinogenesis Research Information System database. Details of methods and results were not available.
	Mouse lymphoma assay	Mouse L5178Y TK +/- lymphocytes	25 - 215 µg/ml	Positive[1, 2]	(Longfellow, 1985/1986)	Validity cannot be evaluated. The information was generated from the CCRIS database. Details of methods and results were not available.
(2-Hydroxyacetophenone [07.124])	Ames assay (preincubation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	408 µg/plate	Negative[1, 2]	(Florin et al., 1980)	[17] Insufficient quality. Not in accordance with OECD guideline 471. Inadequate study design (Spot test).
4- Hydroxy acetophenone [07.243]	Ames assay (preincubation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537	30 µmol/plate (4085 µg/plate)	Negative[1, 2]	(Florin et al., 1980)	Insufficient quality. Not in accordance with OECD guideline 471. Inadequate study design (Spot test).
Acetovanillone [07.142]	Ames assay (preincubation and plate incorporation methods)	*S. typhimurium* TA98; TA100	Not reported	Negative[1, 2]	(Xu et al., 1984)	Insufficient quality. Not in accordance with OECD guideline 471. Only two strains used. Concentration range not reported. Details of results not reported.
	Ames assay	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	Up to 1 mg/plate (range not reported)	Negative[1, 2]	(Nestmann et al., 1980)	Insufficient quality as main details of method and results were not reported. Additionally, the test was not repeated.
	Mutagenicity assay	*S. cerevisiae* D7; *S. cerevisiae* XV185-14C	6 concentrations from 100 to 1000 µg/ml	Negative[15] Positive[15]	(Nestmann & Lee, 1983)	Tested only without S9, however the positive results reported seem to be reliable.
(Vanillyl acetone [07.005])	Ames assay (plate incorporation method)	*S. typhimurium* TA98; TA100	1000 µg/plate	Negative[2, 16]	(Mikulasova & Bohovicova, 2000)	[17]
	DNA Repair test	*E. coli* WP2, WP2*uvrA*, CM611; CM561	2000 µg/ml	Negative	(Mikulasova & Bohovicova, 2000)	[17]

GI = Growth inhibition.

IP = Intraperitoneal.

1) Without metabolic activation.

2) With metabolic activation.

3) Presumably non-mutagenic but solubility did not allow testing in amounts that result in lethality.

4) Negative results in TA100, with and without S9 metabolic activation.

5) Positive results in TA98, with and without metabolic activation..

6) The use of metabolic activation was not reported.

7) The concentrations selected for this assay corresponded to 0.5, 0.25, and 0.125 of the concentration causing 50 % growth inhibition (this concentration was not specified) as determined from a cytotoxicity assay.

8) Test substance was negative in a short-term assay without S9 metabolic activation and in a long-term assay (48 hrs) with and without S9 metabolic activation. The test substance gave positive results in the short-term assay with S9 metabolic activation.

9) Tested quantitatively with TA100. Substance was cytotoxic at 30 µmol/plate.

10) 5000 µg/plate resulted in cytotoxicity which was defined as a thinning of the background lawn.

11) Tested quantitatively with TA98. Substance was cytotoxic at 30 µmol/plate.

12) The presentation of the result in the publication obviously led the petitioner to the interpretion that the substance was positive in TA1530 but this is not correct. From the footnotes of the publication it becomes clear that the substance was tested in TA100 and TA1530 and that the result was negative. However, as only two strains were used the quality of the study must be considered insufficient for the purpose of this Flavouring Group Evaluation.

13) Antimutagenicity study. Sesamol greatly reduced the mutagenic effects of t-BOOH.

14) Test with both rat and mouse S-9 metabolic activation.

15) Negative response for gene conversion (strain D7) and a positive response for reversion (strain XV185-14C).

16) Dose level was the highest non-toxic dose level examined. At 2500 µg/ml cytotoxicity was observed.

17) Summarised by JECFA, 55th meeting (JECFA, 2001b).

Table 2.3: Genotoxicity (*in vivo*) for Ring Substituted Phenolic Substances (EFSA, 2007m)

Table 2.3: GENOTOXICITY (*in vivo*)

Chemical Name [FL-no]	Test System	Test Object	Route	Dose	Result	Reference	Comments
(2-Methylphenol [04.027])	*In vivo* Sister chromatid exchange	Mouse bone marrow cells, alveolar macrophages, and regenerating liver cells	IP injection	0, 200 mg/kg	Negative	(Cheng & Kligerman, 1984)	[1] Limited quality since only two animals were used and only 20 metaphases were analysed for each cell type from each animal. Only one dose tested.
	In vivo Sex-linked recessive lethal test	*D. melanogaster*	Oral	0, 100, 500, 1000 µg/ml	Negative	(Sernau, 1989)	[1] Acceptable quality. GLP study generally in accordance with OECD 477 (1984).
(3-Methylphenol [04.026])	*In vivo* Sister chromatid exchange	Mouse bone marrow cells, alveolar macrophages, and regenerating liver cells	IP injection	0, 200 mg/kg	Negative	(Cheng & Kligerman, 1984)	[1] Limited quality since only three animals were used and only 20 metaphases were analysed for each cell type from each animal. Only one dose tested.
	In vivo Chromosome aberration assay	Mouse bone marrow	Oral (gavage)	0, 96, 320, 960 mg/kg	Negative	(Ivett et al., 1989)	[1] GLP study in accordance with OECD guideline 475 (1984). However, the validity of the result

Table 2.3: GENOTOXICITY (*in vivo*)

Chemical Name [FL-no]	Test System	Test Object	Route	Dose	Result	Reference	Comments
							cannot be evaluated as all pages with results in table format are lacking.
(4-Methylphenol [04.028])	*In vivo* Sister chromatid exchange	Mouse bone marrow cells, alveolar macrophages, and regenerating liver cells	IP injection	0, 75 mg/kg	Negative	(Cheng & Kligerman, 1984)	[1] Limited quality since only three animals were used and only 20 metaphases were analysed for each cell type from each animal. Only one dose tested.
(Carvacrol [04.031])	*In vivo* Spot test	*D. melanogaster* BINSC; Oregon-R		1.40 ppm; 0.35 ppm	Negative	(Kono et al., 1995)	[1] Validity cannot be evaluated. Publication is in Japanese with a short summary in English. Results reported only for two doses in table format. Not clear if control groups were treated concomitantly.
4-Methoxyphenol [04.077]	*In vivo* Chromosome aberration assay	Rat	Oral (gavage)	0, 100, 333, 1000 mg/kg bw	Negative	(Esber, 1986)	The study design was in accordance with OECD guideline 475 (1984). The study was incompletely reported, however, the study report contained sufficient details to conclude that the outcome of the study is negative.

1) Summarised by JECFA, 55th meeting (JECFA, 2001b)

TABLE 3: SUMMARY OF SAFETY EVALUATION TABLES

Table 3.1: Summary of Safety Evaluation of Eugenol and Six Related Hydroxyallylbenzene Derivatives (JECFA, 2007a)

Table 3.1: Summary of Safety Evaluation of Eugenol and Six Related Hydroxyallylbenzene Derivatives (JECFA, 2007a)

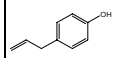
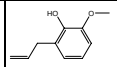
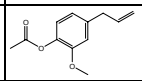
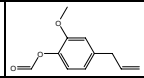
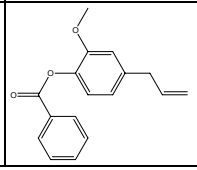
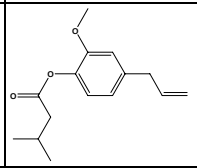
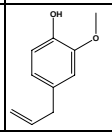
FL-no JECFA-no	EU Register name	Structural formula	EU MSDI 1) US MSDI (µg/*capita*/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	EFSA conclusion on the named compound (Procedure steps, intake estimates, NOAEL, genotoxicity)	EFSA conclusion on the material of commerce
04.058 1527	4-Allylphenol		0.073 0.6	Class I A3: Intake below threshold	4)	No safety concern at estimated levels of intake based on the MSDI approach.	No safety concern at estimated levels of intake based on the MSDI approach.
04.096 1528	2-Methoxy-6-(2-propenyl)phenol		0.12 0.2	Class I A3: Intake below threshold	4)	No safety concern at estimated levels of intake based on the MSDI approach.	No safety concern at estimated levels of intake based on the MSDI approach.
09.020 1531	Eugenyl acetate		19 90	Class I A3: Intake below threshold	4)	No safety concern at estimated levels of intake based on the MSDI approach.	No safety concern at estimated levels of intake based on the MSDI approach.
09.088 1530	4-Eugenyl formate		0.012 0.06	Class I A3: Intake below threshold	4)	No safety concern at estimated levels of intake based on the MSDI approach.	Composition of mixture to be specified.
09.766 1533	Eugenyl benzoate		0.0024 0.9	Class I A3: Intake below threshold	4)	No safety concern at estimated levels of intake based on the MSDI approach.	No safety concern at estimated levels of intake based on the MSDI approach.

Table 3.1: Summary of Safety Evaluation of Eugenol and Six Related Hydroxyallylbenzene Derivatives (JECFA, 2007a)

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
09.878 1532	Eugenyl isovalerate		0.37 0.5	Class I A3: Intake below threshold	4)	No safety concern at estimated levels of intake based on the MSDI approach.	No safety concern at estimated levels of intake based on the MSDI approach.
04.003 1529	Eugenol		950 3364	Class I A3: Intake above threshold, A4: Not endogenous, A5: Adequate NOAEL exists	4)	No safety concern at estimated levels of intake based on the MSDI approach.	No safety concern at estimated levels of intake based on the MSDI approach.

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

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

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

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

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

Table 3.2: Summary of Safety Evaluation Applying the Procedure (EFSA / FGE.22)

Table 3.2: Summary of Safety Evaluation Applying the Procedure of substances in FGE.22 (based on intakes calculated by the MSDI approach)

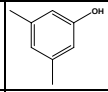
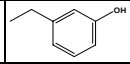
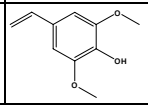
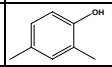
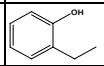
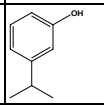
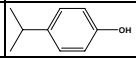
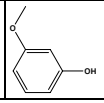
FL-no	EU Register name	Structural formula	MSDI 1) (µg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
04.020	3,5-Dimethylphenol		0.037	Class I A3: Intake below threshold	4)	6)	
04.021	3-Ethylphenol		0.073	Class I A3: Intake below threshold	4)	6)	
04.061	2,6-Dimethoxy-4-vinylphenol		1.2	Class I A3: Intake below threshold	4)	6)	
04.065	2,3-Dimethylphenol		0.013	Class I A3: Intake below threshold	4)	6)	
04.066	2,4-Dimethylphenol		0.011	Class I A3: Intake below threshold	4)	6)	
04.070	2-Ethylphenol		0.037	Class I A3: Intake below threshold	4)	6)	
04.072	3-Isopropylphenol		0.0012	Class I A3: Intake below threshold	4)	6)	
04.073	4-Isopropylphenol		0.24	Class I A3: Intake below threshold	4)	6)	
04.076	3-Methoxyphenol		0.011	Class I A3: Intake below threshold	4)	6)	

Table 3.2: Summary of Safety Evaluation Applying the Procedure of substances in FGE.22 (based on intakes calculated by the MSDI approach)

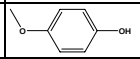
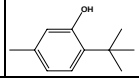
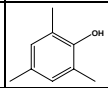
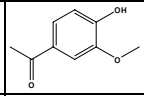
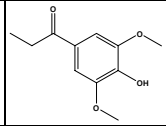
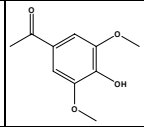
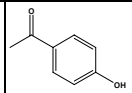
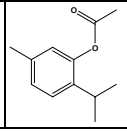
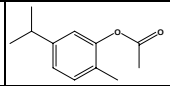
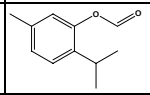
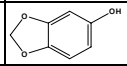
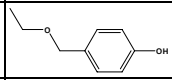
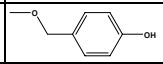
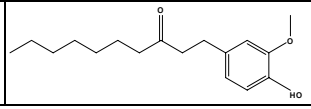
FL-no	EU Register name	Structural formula	MSDI 1) (µg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
04.077	4-Methoxyphenol		0.12	Class I A3: Intake below threshold	4)	6)	
04.078	5-Methyl-2-(tert-butyl)phenol		0.061	Class I A3: Intake below threshold	4)	6)	
04.095	2,4,6-Trimethylphenol		0.0097	Class I A3: Intake below threshold	4)	6)	
07.142	Acetovanillone		2.2	Class I A3: Intake below threshold	4)	6)	
07.154	1-(3,5-Dimethoxy-4-hydroxyphenyl)propan-1-one		0.026	Class I A3: Intake below threshold	4)	6)	
07.164	4-Hydroxy-3,5-dimethoxyacetophenone		0.24	Class I A3: Intake below threshold	4)	6)	
07.243	4-Hydroxyacetophenone		0.016	Class I A3: Intake below threshold	4)	6)	
09.253	2-Isopropyl-5-methylphenyl acetate		1.1	Class I A3: Intake below threshold	4)	6)	

Table 3.2: Summary of Safety Evaluation Applying the Procedure of substances in FGE.22 (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
09.337	Carvacryl acetate		0.61	Class I A3: Intake below threshold	4)	6)	
09.893	2-Isopropyl-5-methylphenyl formate		0.52	Class I A3: Intake below threshold	4)	6)	
04.080	3,4-Methylenedioxyphenol		1.7	Class I No evaluation			a)
04.091	Ethyl 4-hydroxybenzyl ether		0.0012	Class II A3: Intake below threshold	4)	6)	
04.092	4-Hydroxybenzyl methyl ether		0.61	Class II A3: Intake below threshold	4)	6)	
07.234	5-Paradol		0.012	Class II 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.

a) Evaluation deferred pending further genotoxicity data..

REFERENCES:

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

Allavena, A., Martelli, A., Robbiano, L., Brarnbilla G., 1992. Evaluation in a battery of in vivo assays of four *in vitro* genotoxins proved to be noncarcinogens in rodents. Teratog. Carcinog. Mutag. 12, 31-41.

Amonkar, A.J., Nagabhushan, M., D'Souza, A.V., Bhide, S.V., 1986. Hydroxychavicol: a new phenolic antimutagen from betel leaf. Food Chem. Toxicol. 24, 1321-1324.

Azizan, A., Blevins, R.D., 1995. Mutagenicity and antimutagenicity testing of six chemicals associated with the pungent properties of specific spices as revealed by the ames *salmonella*/microsomal assay. Arch. Environ. Contam. Toxicol. 28, 248-258.

Bartsch, H., Malaveille, C., Camus, A.M., Martel-Planche, G., Brun, G., Hautefeuille, A., Sabadie, N., Barbin, A., Kuroki, T., Drevon, C., Piccoli, C., Montesano, R., 1980. Validation and comparative studies on 180 chemicals with *S. typhimurium* strains and V79 Chinese hamster cells in the presence of various metabolizing systems. Mutat. Res. 76, 1-50.

Bean, C.L., Galloway, S.M., 1993. Evaluation of the need for a late harvest time in the assay for chromosome aberrations in Chinese hamster ovary cells. Mutat. Res. 292, 3-16.

Bean, C.L., Armstrong, M.J., Galloway, S.M., 1992. Effect of sampling time on chromosome aberration yield for 7 chemicals in Chinese hamster ovary cells. Mutat. Res. 65, 31-44.

Bodell, W.J., Ye, Q., Pathak, D.N., Pongracz, K., 1998. Oxidation of eugenol to form DNA adducts and 8-hydroxy-2′-deoxyguanosine: Role of quinone methide derivative in DNA adduct formation. Carcinogenesis. 19(3), 437-443.

Burkey, J.L., Sauer, J.-M,, McQueen, C.A., Sipes, I.G., 2000. Cytotoxicity and genotoxicity of methyleugenol and related congeners-a mechanism of activation for methyleugenol. Mutat. Res. 453, 25-33.

Canter, D.A., 1981. Letter from Dept of Health and Human services to U S EPA regarding Salmonella assays performed on cresols, with attachments. EPA Doc. 40-8160101, microfiche no. OTS0517549. Date 2/10/81. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Cheng, M., Kligerman, A.D., 1984. Evaluation of the genotoxicity of cresols using sisterchromatid exchange (SCE). Mutat. Res. 137(1), 51-55.

Cifone, M.A., 1988a. Mutagenicity test on meta-cresol in a rat primary hepatocyte unscheduled DNA Synthesis assay with cover letter dated 07/06/88. Chemical Manufacturers Association. EPA Doc. 40-8860250, microfiche no. OTS0517692. Date 6/28/88. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Claxton, L.D., 1985. Assessment of bacterial mutagenicity methods for volatile and semivolatile compounds and mixtures. Environ. Int. 11, 375-383.

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

Crowley, J.P., Margard, W., 1978. Determination of mutagenic/carcinogenic and cytotoxic potential of four chemical compounds (summary report). Sherwin Williams Co. EPA Doc. 40-7860090, microfiche no. OTS0517540. Date 10/31/78. Selected pages. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Daugherty, J.P., Franks, H., 1986. Effect of monocyclic derivatives on DNA repair in human lymphocytes. Res. Commun. Chem. Pathol. Pharmacol. 54,133-136.

Dean, B.J., Brooks, T.M., Hodson-Walker, G., Hutson, D.H., 1985. Genetic toxicology testing of 41 industrial chemicals. Mutat. Res. 153, 57-77.

Dorange, J.-L., Delaforge, M., Janiaud, P. Padieu, P., 1977. Mutagenicity of the metabolites of the epoxide diol pathway of safrole and analogs. Study on salmonella typhimurium. Soc. Biol. Dijon, 171, 1041-1048. (In Frence).

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

Douglas, G.R., Nestmann, E.R., Betts, J.L., Mueller, J.C., Lee, E.G.H., Stich, H.F.,San, H.C., Brouzes, R.J.P., Chmelauskas, A.L., Paavila, H.D., Walden, C.C., 1980. Mutagenic activity in pulp mill effluents. In: Jolley, R. L., Brungs, W.A., Cumming, R.B., Jacobs, V.A., (eds.). Water Chlorination: Environmental Impact and Health Effects. vol. 3. Ann Arbor Science Publishers Inc., Ann Arbor, MI, pp. 865-880.

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

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

EC, 2000a. Commission Regulation No 1565/2000 of 18 July 2000 laying down the measures necessary for the adoption of an evaluation programme in application of Regulation (EC) No 2232/96. Official Journal of the European Communities 19.7.2000, L 180, 8-16.

EC, 2006. Commission Decision 2006/252/EC of 27 March 2006 amending Decision 1999/217/EC as regards the register of flavouring substances used in or on foodstuffs. Official Journal of the European Union 29.3.2006, L 91, 48.

Eder, E., Neudecker, T., Lutz, D., Henschler, D., 1980. Mutagenic potential of allyl and allylic compounds. Structure-activity relationship as determined by alkylating and direct *in vitro* mutagenic properties. Biochem. Pharmacol. 29, 993-998.

Eder, E., Neudecker, T., Lutz, D., Henschler, D., 1982b. Correlation of alkylating and mutagenic activities of allyl and allylic compounds: Standard alkylation test vs. kinetic investigation. Chem. -Biol. Interact. 38, 303-315.

EFFA, 2005g. Submission 2004/18. Flavouring group evaluation of 3 flavouring substance (candidate chemical) of the chemical group 18 (annex I of 1565/2000/EC) eugenol and structurally related substances used as flavouring substances. 14 February 2005. FLAVIS/8.44. Unpublished report submitted by EFFA to FLAVIS Secretariat.

EFFA, 2007a. E-mail from Jan Demyttenaere, EFFA to Flavis Secretariat, National Foodinstitute, Technical University of Denmark. Dated 8 February 2007. RE: FLAVIS submissions - use levels for Category 14.2 - Alcoholic beverages FLAVIS/8.70.

EFSA, 2004d. Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in contact with food on a request from the Commission related to Flavouring Group Evaluation 3: Acetals of branched- and straight-chain aliphatic saturated primary alcohols and branched- and straight-chain saturated aldehydes, and an orthoester of formic acid, from chemical groups 1 and 2 (Commission Regulation (EC) No 1565/2000 of 18 July 2000). Adopted on 7 October 2004. EFSA-Q-2003-146.

EFSA, 2007m. Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in contact with food on a request from the Commission related to Flavouring Group Evaluation 22: Ring-substituted phenolic substances from chemical groups 21 and 25 (Commission Regulation (EC) No 1565/2000 of 18 July 2000). Adopted on 27 September 2006. EFSA-Q-2003-165.

Ellahueñe, M.F., Perez-Alzola, L.P., Orellana-Vandebenito, M., Muñoz, C., Lafuente-Valdebenito, N., 1994. Genotoxic evaluation of eugenol using the bone marrow micronucleus assay. Mutat. Res. 320, 175-180.

Epler, J.L., Rao, T.K., Guerin, M.R., 1979. Evaluation of feasibility of mutagenic testing of shale oil products and effluents. Environ. Health Perspect. 30, 179-184.

Esber, H.J., 1986. In vivo cytogenetics study in rats - compound W1188.01 with cover letter dated 08/17/92. Proctor & Gamble Co. EPA Doc. 88-920007233, microfiche no. OTS0545546. Date 7/09/86. Unpublished report submitted by EFFA to FLAVIS Secretariat.

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

Fukuda, S., 1987. Assessment of the carcinogenic hazard of 6 substances used in dental practices. I.Morphological transformations, DNA damage and SCE in cultured Syrian hamster embryo cells induced by camphor, eugenol, thymol, EDTA, benzalkonium chloride and benzethonium chloride. Shigaku 74(6), 1365-1383. (In Japanese)

Galloway, S.M., Brusick, D.J., 1980. Mutagenicity evaluation of sample containing 33 1/3% each ortho-, meta-, and para-cresol in sister chromatid exchange assay with Chinese hamster ovary (CHO) cells. Final report. Cresol Task Force. EPA Doc. FYI-OTS-0780-0079, microfiche no. OTS0000079-0. Date 060180. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Galloway, S.M., Brusick, D.J., 1981. Sister chromatid exchange assay, Ames assay, mouse lymphoma forward mutation assay, and cell transformation on o-cresol. Pepper, Hamilton & Scheetz. EPA Doc. 40-8160079, microfiche no. OTS0517531. Date 5/01/81. Selected pages. Unpublished report submitted by EFFA to FLAVIS Secretariat.

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

Green, N.R., Savage, J.R., 1978. Screening of safrole, eugenol, their ninhydrin positive metabolites and selected secondary amines for potential mutagenicity. Mutat. Res. 57, 115-121.

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

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

Hayashi, M., Sofuni, T., Ishidate, M., Jr, 1984. A pilot experiment for the micronucleus test. The multi-sampling at multi-dose levels method. Mutat. Res. 141, 165-169.

Hayashi, M., Sofuni, T., Ishidate Jr., M., 1984. A pilot experiment for the micronucleus test. The multi-sampling at multi-dose levels method. Mutat. Res. 141(2), 165-169.

Honma, M., Zhang, L.-S., Sakamoto, H., Ozaki, M., Takeshita, K., Momose, M., Hayashi, M., Sufuni, T., 1999. The need for long-term treatment in the mouse lymphoma assay. Mutagenesis 14(1), 23-29.

Howes, A.J., Chan, V.S.W., Caldwell, J., 1990. Structure-specificity of the genotoxicity of some naturally occurring alkenylbenzenes determined by the unscheduled DNA synthesis in rat hepatocytes. Food Chem. Toxicol. 28(8), 537-542.

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

Ivett, J.L., Brown, B.M., Rodgers, C., Anderson, B.E., Resmick, M.A., Zeiger, E., 1989. Chromosomal aberrations and sister chromatid exchange tests in chinese hamster ovary cells in vitro. IV. Results with 15 chemicals. Environ. Mol. Mutag. 14, 165-187.

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

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

JECFA, 1982a. 26. Report: Twenty-sixth Meeting of the Joint FAO/WHO Expert Committee on Food Additives; Report: WHO Technical Report Series, no. 683.

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

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

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

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

JECFA, 2001b. Safety evaluation of certain food additives and contaminants. Fifty-fifth meeting of the Joint FAO/WHO Expert Committee on Food Additives, WHO Food Additives Series: 46. IPCS, WHO, Geneva.

JECFA, 2005d. Compendium of food additive specifications. Addendum 13. Joint FAO/WHO Expert Commitee of Food Additives 65th session. Geneva, 7-16 June 2005. FAO Food and Nutrition paper 52 Add. 13.

JECFA, 2006b. Evaluation of certain food additives. Sixty-fifth report of the Joint FAO/WHO Expert Committee on Food Additives. WHO Technical Report Series, no. 934. Geneva, 7-16 June 2005.

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

JECFA, 2007a. Safety evaluation of certain food additives and contaminants. Sixty-fifth meeting of the Joint FAO/WHO Expert Committee on Food Additives, WHO Food Additives Series: 56. IPCS, WHO, Geneva.

Kaur, I.P., Saini, A., 2000. Sesamol exhibits antimutagenic activity against oxygen species mediated mutagenicity. Mutat. Res. 470(1), 71-76.

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

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

Longfellow, D., 1985/1986. Mutagenicity studies. Sesamol. Short-term test program sponsored by the Division of Cancer Etiology, National Cancer Institute. As cited in Chemical Carcinogenesis Research Information System (CCRIS), a database of the National Library of Medicine's TOXNET system (http://toxnet.nlm.nih.gov) on July 1, 2004.

Massey, I.J., Aitken, M.D., Ball, L.M., Heck, P.E., 1994. Mutagenicity screening of reaction products from the enzyme-catalyzed oxidation of phenolic pollutants. Environ. Toxicol. Chem. 13(11), 1743-1752.

Maura, A., Pino, A., Ricci, R., 1989. Negative evidence *in vivo* of DNA-damaging, mutagenic and chromosomal effects of eugenol. Mutat. Res. 227, 125-129.

McMahon, R.E., Cline, J.C., Thompson, C.Z., 1979. Assay of 855 test chemicals in ten tester strains using a new modification of the ames test for bacterial mutagens. Cancer Res. 39, 682-693.

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

Miller, E.C., Swanson, A.B., Phillips, D.H., Fletcher, T.L., Liem, A., Miller, J.A., 1983. Structure-activity studies of the carcinogenicities in the mouse and rat of some naturally occuring and synthetic alkenylbenzene derivatives related to safrole + estragole. Cancer Res. 43, 1124-1134.

Miller, E.G., Washington, V.H., Bowles, W.H., Zimmermann, E.R., 1986. Mutagenic potential of some chemical components of dental materials. Dental Mater. 2, 163-165.

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

Myhr, B.C., Brusick, D.J., 1980. Evaluation of ortho-,meta-,and para-cresol 33 1/3% each in primary rat hepatocyte unscheduled DNA synthesis assay, draft report. Cresol Task Force. EPA Doc. FYI-OTS-0980-0079, microfiche no. OTS0000079-0. Date 080180. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Myhr, B.C., Caspary, W.J., 1991. Chemical mutagenesis at the thymidine kinase locus in L5178Y mouse lymphoma cells: Results for 31 coded compounds in the national toxicology program. Environ. Mol. Mutag. 18, 51-83.

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

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

NTP, 1983a. Carcinogenesis studies of allyl isovalerate in F344/N rats and B6C3F1 mice. TR no. 253.

NTP, 1983b. Toxicology and carcinogenesis studies of eugenol (CAS no. 97-53-0) in F344/N rats and B6C3F1 mice (feed studies). NTP-TR 223. http://ntp.niehs.nih.gov/ntp/htdocs/LT_rpts/tr223.pdf.

Nuodex Inc., 1980a. Mutagenicity studies ON R-1044. EPA Doc. 878211352, microfiche no. OTS0206261. Date 032780. Selected pages. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Nuodex Inc., 1980b. Evaluation of R-1044 in the E. coli DNA repair - suspension assay. EPA Doc. 878211353, microfiche no. OTS0206261. Date 032780. Selected pages. Unpublished report submitted by EFFA to FLAVIS Secretariat.

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

Orstavik, D., Hongslo, J.K., 1985. Mutagenicity of endodontic sealers. Biomaterials. 6, 129-132.

Pepper Hamilton and Scheetz, 1980. Evaluation of R-1044 In the E. coli DNA repair suspension assay with cover
letter to EPA dated 10/11/83. EPA Doc. 40-8360166, microfiche no. OTS0507480. Date 032780. Selected pages. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Pfuhler, S., Stehrer-Schmid, P., Dorsch, W., Wagner, H., Wolf, H.U., 1995. Investigation of genotoxic effects of the anti-asthmatic and anti-inflammatory drugs apocyninand acetosyringenin in the *Salmonella typhimurium* mutagenicity assay and the SCE-test with human lymphocytes. Phytomedicine 1(4), 319-322.

Phillips, D.H., 1994. DNA adducts derived from safrole, estragole and related compounds, and from benzene and its metabolites. In: IARC Scientific Publications No. 125. Lyon, International Agency for research on Cancer, 1994. p. 131

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

Putman, D.L., 1986. Initial submission: *In vitro* cytogenicity study with 4-methoxyphenol in Chinese Hamster Ovary (CHO) Cells (final report) with cover letter dated 08/17/92. Proctor & Gamble Co. EPA Doc. 88-920007112, microfiche no. OTS0545451. Date 8/06/86. Unpublished report submitted by EFFA to FLAVIS Secretariat.

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

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

Rogers-Back, A., 1986. Initial submission: L5178Y: Mouse lymphoma assay with cover letter dated 08/17/92. Proctor & Gamble Company. EPA Doc. 88-920007210, microfiche no. OTS0545512. Date 8/13/86. Selected pages. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Rompelberg, C.J.M., Stenhuis, W.H., de Vogel, N., van Osenbruggen, V., Schouten, A., Verhagen H., 1995. Antimutagenicity of eugenol in the rodent bone marrow micronucleus test. Mutat. Res, 346, 69-75.

Rompelberg, C.J.M., Evertz, S.J.C.J., Bruijntjes-Rozier, G.C.D.M., van den Heuvel, P.D., Verhagen, H., 1996a. Effect of eugenol on the genotoxicity of established mutagens in the liver. Food Chem. Toxicol. 34, 33-42.

Rompelberg, C.J.M., Vogels, J.T.W.E., de Vogel N., Bruijntjes-Rozier, G.C.D.M., Stenhuis, W.H., Bogaards, J.J.P., Verhagen, H., 1996b. Effect of short-term dietary administration of eugenol in humans. Hum. Exp. Toxicol, 15, 129-135.

Rompelberg, C.J.M., Steenwinkel, M-J.S.T., van Asten, J.G., van Delfit, J.H.M., Baan, R.A., Verhagen, H., 1996c. Effect of eugenol on the mutagenicity of benzo[a]pyrene and the formation of benzo[a]pyrene-DNA adducts in the lambda-lacZ-transgenic mouse. Mutat. Res. 369, 87-96.

San, R.H.C., Reece, J.D., 2003. Unscheduled DNA synthesis in mammalian cells *in vitro*. Study No. AA51FW-FZ, AA52CV.380.BTL. BioReliance, Rockville, Maryland, USA. Unpublished report to the Research Institute for Fragrance Materials, Woodcliff Lake, New Jersey, USA. Submitted to WHO by the Flavor and Extract Manufacturers Association of the United States, Washington DC, USA.

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

Schechtman, L.M., Curren, R.D., Parmar, A.S., Sinsky, P.M., 1980. Activity of T1570 in the *Salmonella*/microsomal assay for bacterial mutagenicity with attachments and cover letter dated 11/21/91. EPA Doc. 86-920000183, microfiche no. OTS0534388. Date 5/13/80. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Schiestl, R.H., Chan, W.S., Gietz, R.D., Mehta, R.D., Hast, P.J., 1989. Safrole, eugenol and methyleugenol induce intrachromosomal recombination in yeast. Mutat. Res. 224, 427-436.

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

Sernau, D., 1989. Mutagenicity tests on ortho- and para-cresol: drosophila melanogaster sex-linked recessive lethal test (final report) with attachments and cover letter dated 03/21/89. Chemical Manufacturers Association. EPA Doc. 40-8960320, microfiche no. OTS0529221. Date 2/22/89. pp. 1-33. Unpublished report submitted by EFFA to FLAVIS Secretariat.

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

Stammati, A., Bonsi, P., Zucco, F., Moezelaar, R., Alakomi, H.L., von Wright, A., 1999. Toxicity of selected plant volatiles in microbial and mammalian short-term assays. Food Chem. Toxicol. 37(8), 813-823.

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

Sukumaran, K., Kuttan, R., 1995. Inhibition of tobacco-induced mutagenesis by eugenol and plant extracts. Mutat. Res. 343, 25-30.

Swanson, A.B., Chambliss, D.D., Blomquist, J.C., Miller, E.C., Miller, J.A., 1979. The mutagenicities of safrole, estragole, eugenol, trans-anethole and some of their known or possible metabolites for *Salmonella typhimurium* mutants. Mutat. Res. 60, 143-153.

Tennant, R.W., Margolin, B.H., Shelby, M.D., Zeiger, E., Haseman, J.K., Spalding, J., Caspary, W., Resnick, M., Stasiewicz, S., Anderson, B., Minor, R., 1987. Prediction of chemical carcinogenicity in rodents from *in vitro* genetic toxicity assays. Science 236, 933-941.

To, L.P., Hunt, T.P., Andersen, M.E., 1982. Mutagenicity of trans-anethole, estragole, eugenol, and safrole in the Ames *Salmonella typhimurium* assay. Bull. Environ. Contam. Toxicol. 28, 647-654.

Völkner, W., 1994. Support: Letter from General Elec Co. to US EPA re: Chromosome aberration assay in Chinese hamster V79 cells In vitro with 2, 6-dimethylphenol with attachments and cover letter dated 06/01/94. General Elec Co. EPA Doc. 8EHQ-0694-1027, microfiche no. OTS0527745-2. Date 06/01/94. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Woolverton, C., Fotos, P,, Mokas, M., Mermigas, M., 1986. Evaluation of eugenol for mutagenicity by the mouse micronucleus test. Oral Pathol. 15, 450-453.

Xu, J., Whong, W.-Z., Ong, T.-M., 1984. Validation of the *Salmonella* (SV50)-arabinoseresistant forward mutation assay system with 26 compounds. Mutat. Res. 130(2), 79-86.

Yoshimura, H., Nakamura, M., Koeda, T., 1981. Mutagenicity-screening of anesthetics for fishes. Mutat. Res. 90, 119-124.

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

SCIENTIFIC PANEL MEMBERS

Fernando Aguilar, Herman Nybro Autrup, Susan Barlow, Laurence Castle, Riccardo Crebelli, Wolfgang Dekant, Karl-Heinz Engel, Nathalie Gontard, David Michael Gott, Sandro Grilli, Rainer Gürtler, John Christian Larsen, Jean-Charles Leblanc, Catherine Leclercq, F. Xavier Malcata, Wim Mennes, Maria Rosaria Milana, Iona Pratt, Ivonne Magdalena Catharina Maria Rietjens, Paul P. Tobback, Fidel Toldrá.

ACKNOWLEDGEMENT

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