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EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF); Scientific Opinion on Flavouring Group Evaluation 30, Revision 1 (FGE.30Rev1): 4-Prop-1-enylphenol and 2-methoxy-4-(prop- 1enyl)phenyl 3-methylbutyrate from chemical group 17

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European Food Safety Authority

EFSA Journal 2011; 9(5):1991

SCIENTIFIC OPINION

Scientific Opinion on Flavouring Group Evaluation 30, Revision 1 (FGE.30Rev1):

4-Prop-1-enylphenol and 2-methoxy-4-(prop-1-enyl)phenyl 3-methylbutyrate from chemical group 17[1]

EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF)[2, 3]

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

The Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids of the European Food Safety Authority was requested to evaluate two flavouring substances in the Flavouring Group Evaluation 30, Revision 1, using the Procedure in Commission Regulation (EC) No 1565/2000. None of the substances were considered to have genotoxic potential. The two substances were evaluated through a stepwise approach that integrates information on structure-activity relationships, intake from current uses, toxicological threshold of concern, and available data on metabolism and toxicity. The Panel concluded that the two substances [FL-no: 04.097, 09.894] do not give rise to safety concerns at their levels of dietary intake, estimated on the basis of the MSDI approach. Besides the safety assessment of these flavouring substances, the specifications for the materials of commerce have also been considered. For [FL-no: 09.894] the composition of the stereoisomeric mixture needs to been specified.

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1 On request from the Commission, Question No EFSA-Q-2011-0050, adopted on 4 February 2011.

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SUMMARY

The European Food Safety Authority (EFSA) asked the Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (the Panel) to provide scientific advice to the Commission on the implications for human health of chemically defined flavouring substances used in or on foodstuffs in the Member States. In particular, the Panel was requested to evaluate two flavouring substances in the Flavouring Group Evaluation 30, Revision 1 (FGE.30Rev1), using the Procedure as referred to in the Commission Regulation (EC) No 1565/2000. The flavouring substances belong to chemical group 17, Annex I of the Commission Regulation (EC) No 1565/2000.

The two flavouring substances are a hydroxypropenylbenzene, 4-prop-1-enylphenol [FL-no: 04.097], and a structurally related ester, 2-methoxy-4-(prop-1-enyl)phenyl 3-methylbutyrate[FL-no: 09.894], both from chemical group 17.

Due to the presence and the position of a double bond both flavouring substances can exist as geometrical isomers. For 2-methoxy-4-(prop-1-enyl)phenyl 3-methylbutyrate the stereoisomeric composition has been specified as the E/Z mixture, but the composition of the mixture has not been given.

4-Prop-1-enylphenol and 2-methoxy-4-(prop-1-enyl)phenyl 3-methylbutyrate are classified into structural class I.

The candidate substances have been reported to occur naturally in anise oil and cider.

According to the default MSDI approach, the flavouring substances in this group have European intakes of 0.012 and 12 microgram/*capita*/day ([FL-no: 09.894 and 04.097], respectively), which are below the threshold of concern for structural class I substances of 1800 microgram/person/day.

On the basis of the reported annual production in Europe (MSDI approach) the total combined intake of the two flavouring substances and six structurally related substances from structural class I can be calculated to approximately 150 microgram/*capita*/day. This value is lower than the threshold of concern for structural class I substances of 1800 microgram/person/day.

Data available do not give rise to safety concern with respect to genotoxicity.

4-Prop-1-enylphenol and 2-methoxy-4-(prop-1-enyl)phenyl 3-methylbutyrate are expected to be metabolised to innocuous products.

It was noted that where toxicity data were available they were consistent with the conclusions in the present Flavouring Group Evaluation. The Panel considered that elicitation of tumours by the supporting substance isoeugenol in rodents is connected to a non genotoxic mechanism. Therefore, the candidate substances [FL-no: 04.097 and 09.894] can be evaluated via a threshold based approach, i.e. the Procedure.

It is considered that on the basis of the default MSDI approach the candidate substances would not give rise to safety concern at the estimated levels of intake arising from use as flavouring substances.

When the estimated intake was based on the mTAMDI approach it was 72 and 2300 microgram/person/day for 4-prop-1-enylphenol and 2-methoxy-4-(prop-1-enyl)phenyl 3-methylbutyrate, respectively. For 2-methoxy-4-(prop-1-enyl)phenyl 3-methylbutyrate this intake estimate is above the threshold of concern for structural class I substances of 1800 microgram/person/day, and therefore, more reliable exposure data are required for this substance. On the basis of such additional data, the flavouring substance should be reconsidered along the steps of the Procedure. Following this procedure additional toxicological data might become necessary. 4-Prop-1-enylphenol, for which the estimated intake is below the threshold, is also expected to be metabolised to innocuous products.

In order to determine whether the conclusion for the two flavouring 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, have been given for 4-prop-1-enylphenol [FL-no: 04.097] and 2-methoxy-4-(prop-1-enyl)phenyl 3-methylbutyrate [FL-no: 09.894], except that information on the composition of the stereoisomeric mixture has not been specified for [FL-no: 09.894]. Thus, the final evaluation of the material of commerce cannot be performed for [FL-no: 09.894], pending further information.

For 4-prop-1-enylphenol [FL-no: 04.097] the Panel concluded that it would present no safety concern at the estimated level of intake based on the MSDI approach.

KEYWORDS

Flavourings, safety, phenolic ester, propenylhydrobenzenes, FGE.30.

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BACKGROUND

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

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

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

TERMS OF REFERENCE

The European Food Safety Authority (EFSA) is requested to carry out a risk assessment on flavouring substances in the Register prior to their authorisation and inclusion in a Union List according to Commission Regulation (EC) No 1565/2000 (EC, 2000a). In addition, the Commission requested EFSA to evaluate newly notified flavouring substances, where possible, before finalising the evaluation programme.

HISTORY OF THE EVALUATION

FGE	Opinion Adopted by EFSA	Link	No. of Candidate Substances
FGE.30	January 2010	http://www.efsa.europa.eu/en/scdocs/scdoc/1787.htm	1
FGE.30Rev1	February 2011		2

The present revision of Flavouring Group Evaluation 30, Revision 1 (FGE.30Rev1), includes the assessment of one additional candidate substance [FL-no: 04.097]. No toxicity and/or metabolism data were provided for this substance. A search in open literature did not provide any further data on toxicity or metabolism.

ASSESSMENT

1. Presentation of the Substances in Flavouring Group Evaluation 30

1.1. Description

The present Flavouring Group Evaluation 30, Revision 1 (FGE.30Rev1), using the Procedure as referred to in the Commission Regulation (EC) No 1565/2000 (EC, 2000a) (The Procedure – shown in schematic form in Annex I of this FGE), deals with a hydroxypropenylbenzene, 4-prop-1-enylphenol

and a structurally related ester, 2-methoxy-4-(prop-1-enyl)phenyl 3-methylbutyrate, both from chemical group 17, Annex I of Commission Regulation (EC) No 1565/2000 (EC, 2000a).

The flavouring substances under consideration, 4-prop-1-enylphenol [FL-no: 04.097] and 2-methoxy-4-(prop-1-enyl)phenyl 3-methylbutyrate [FL-no: 09.894], as well as their FLAVIS- (FL-), Chemical Abstract Service- (CAS-), Council of Europe- (CoE-) and Flavor and Extract Manufactures Association- (FEMA-) numbers, structures and specifications, are listed in Table 1.

A summary of the safety evaluation is summarised in Table 2a.

The flavouring substances (candidate substances) are closely related structurally to six flavouring substances (supporting substances) evaluated at the 61st JECFA meeting (JECFA, 2004a) in the group of "Hydroxypropenylbenzenes". The supporting substances, with the respective structural formulas, FEMA, CoE, and CAS register numbers, evaluation status by Scientific Committee on Food (SCF), the JECFA, and by the CoE and the MSDI values, are listed in Table 3.

The hydrolysis products of the candidate ester are listed in Table 2b.

1.2. Stereoisomers

It is recognised that geometrical and optical isomers of substances may have different properties. Their flavour may be different, they may have different chemical properties resulting in possible variability in their absorption, distribution, metabolism, elimination and toxicity. Thus information must be provided on the configuration of the flavouring substance, i.e. whether it is one of the geometrical/optical isomers, or a defined mixture of stereoisomers. The available specifications of purity will be considered in order to determine whether the safety evaluation carried out for candidate substances for which stereoisomers may exist can be applied to the material of commerce. Flavouring substances with different configurations should have individual chemical names and codes (CAS number, FLAVIS number etc.).

Due to the presence and the position of a double bond both candidate substances can exist as geometrical isomers. For one of the substances [FL-no: 09.894], Industry has stated that it exists as a "mixture of isomers" (EFFA, 2010a). However, the Panel does not consider this information sufficient and requests data on the actual ratio.

1.3. Natural Occurrence in Food

2-Methoxy-4-(prop-1-enyl)phenyl 3-methylbutyrate [FL-no: 09.894] has been reported to occur in anise and 4-prop-1-enylphenol [FL-no: 04.097] has been reported to occur in cider (0.2 mg/kg) (TNO, 2000; TNO, 2010).

2. Specifications

Purity criteria for the substances have been provided by the Flavouring Industry (EFFA, 2004ae; Flavour Industry, 2007m) (Table 1).

Judged against the requirements in Annex II of Commission Regulation (EC) No 1565/2000 (EC, 2000) this information is adequate for [FL-no: 04.097]. However, information on the composition of the mixture of geometrical isomers is missing for [FL-no: 09.894] (see Section 1.2 and Table 1).

3. Intake Data

Annual production volumes of the flavouring substances as surveyed by the Industry can be used to calculate the "Maximised Survey-derived Daily Intake" (MSDI) by assuming that the production figure only represents 60 % of the use in food due to underreporting and that 10 % of the total EU population are consumers (SCF, 1999a).

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

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

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

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

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

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

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

Average per capita intake (MSDI) is estimated on the assumption that the amount added to food is consumed by 10 % of the population[4] (Eurostat, 1998). This is derived for candidate substances from estimates of annual volume of production provided by Industry and incorporates a correction factor of 0.6 to allow for incomplete reporting (60 %) in the Industry surveys (SCF, 1999a).

The annual production volumes of 2-methoxy-4-(prop-1-enyl)phenyl 3-methylbutyrate [FL-no: 09.894] and 4-prop-1-enylphenol [FL-no: 04.097] for use as flavouring substances in Europe are

[4] EU figure 375 millions. This figure relates to EU population at the time for which production data are available, and is consistent (comparable) with evaluations conducted prior to the enlargement of the EU. No production data are available for the enlarged EU.

reported to be 0.1 kg and 100 kg, respectively (EFFA, 2004ae; Flavour Industry, 2007m), and the corresponding daily per capita intakes are 0.012 microgram and 12 microgram (Table 2a).

3.2. Intake Estimated on the Basis of the Modified TAMDI (mTAMDI)

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

The assumption is that a person may consume a certain amount of flavourable foods and beverages per day.

For the present evaluation of the candidate substances, information on food categories and normal and maximum use levels[5,6,7] were submitted by the Flavour Industry (EFFA, 2004ae; EFFA, 2007a; Flavour Industry, 2007m). The candidate substances are used in flavoured food products divided into the food categories, outlined in Annex III of the Commission Regulation (EC) No 1565/2000 (EC, 2000a), as shown in Table 3.1. For the present calculation of mTAMDI, the reported normal use levels were used. In the case where different use levels were reported for different food categories the highest reported normal use level was used.

According to the Flavour Industry the normal use levels for the candidate substances are in the range of 0.1 - 20 mg/kg and the maximum use levels are in the range of 0.4 - 100 mg/kg (EFFA, 2002i; EFFA, 2004ae; Flavour Industry, 2007m; EFFA, 2007a).

The mTAMDI values for the candidate substances from structural class I are 72 and 2300 microgram/person/day for 4-prop-1-enylphenol and 2-methoxy-4-(prop-1-enyl)phenyl 3-methylbutyrate, respectively.

For detailed information on use levels and intake estimations based on the mTAMDI approach, see Section 6 and Annex II.

[5] ”Normal use” is defined as the average of reported usages and ”maximum use” is defined as the 95th percentile of reported usages (EFFA, 2002i).
[6] The normal and maximum use levels in different food categories (EC, 2000) have been extrapolated from figures derived from 12 model flavouring substances (EFFA, 2004e).
[7] The use levels from food category 5 “Confectionery” have been inserted as default values for food category 14.2 “Alcoholic beverages” for substances for which no data have been given for food category 14.2 (EFFA, 2007a).

Table 3.1 Use of Candidate Substances

Food category	Description	Flavourings used
01.0	Dairy products, excluding products of category 2	[FL-no: 04.097, 09.894]
02.0	Fats and oils, and fat emulsions (type water-in-oil)	[FL-no: 09.894]
03.0	Edible ices, including sherbet and sorbet	[FL-no: 04.097, 09.894]
04.1	Processed fruits	[FL-no: 09.894]
04.2	Processed vegetables (incl. mushrooms & fungi, roots & tubers, pulses and legumes), and nuts & seeds	None
05.0	Confectionery	[FL-no: 04.097, 09.894]
06.0	Cereals and cereal products, incl. flours & starches from roots & tubers, pulses & legumes, excluding bakery	[FL-no: 09.894]
07.0	Bakery wares	[FL-no: 04.097, 09.894]
08.0	Meat and meat products, including poultry and game	[FL-no: 09.894]
09.0	Fish and fish products, including molluscs, crustaceans and echinoderms	[FL-no: 09.894]
10.0	Eggs and egg products	None
11.0	Sweeteners, including honey	None
12.0	Salts, spices, soups, sauces, salads, protein products etc.	[FL-no: 09.894]
13.0	Foodstuffs intended for particular nutritional uses	[FL-no: 09.894]
14.1	Non-alcoholic ("soft") beverages, excl. dairy products	[FL-no: 04.097]
14.2	Alcoholic beverages, incl. alcohol-free and low-alcoholic counterparts	[FL-no: 04.097, 09.894]
15.0	Ready-to-eat savouries	[FL-no: 09.894]
16.0	Composite foods (e.g. casseroles, meat pies, mincemeat) - foods that could not be placed in categories 1 – 15	[FL-no: 09.894]

4. Absorption, Distribution, Metabolism and Elimination

The candidate substances in this FGE are 4-prop-1-enylphenol and the 3-methylbutyrate ester of the structurally related isoeugenol.

Esters of isoeugenol are anticipated to be hydrolysed *in vivo* by carboxylesterases (Heymann, 1980). Isoeugenol is rapidly absorbed from the gastrointestinal tract and metabolised principally in the liver via conjugation of the phenolic hydroxy group with sulphate or glucuronic acid. The conjugate is subsequently excreted, primarily in the urine (Badger et al., 2002b; Fuciarelli, 2001). The same metabolic pathway is anticipated for the 4-prop-1-enylphenol. The carboxylic acid resulting from the ester hydrolysis of [FL-no: 09.894] is metabolised in well-recognised biochemical pathways (Williams, 1959a).

The Panel concluded that the candidate substances could be anticipated to be metabolised to innocuous products.

For more detailed information, see Annex III.

5. Application of the Procedure for the Safety Evaluation of Flavouring Substances

The application of the Procedure is based on intakes estimated on the basis of the MSDI approach. Where the mTAMDI approach indicates that the intake of a flavouring substance might exceed its corresponding threshold of concern, a formal safety assessment is not carried out using the Procedure.

In these cases the Panel requires more precise data on use and use levels. For comparison of the intake estimations based on the MSDI approach and the mTAMDI approach, see Section 6.

For the safety evaluation of the candidate substances from chemical group 17 the Procedure as outlined in Annex I was applied, based on the MSDI approach. The stepwise evaluation of the substances is summarised in Table 2a.

Step 1

The candidate substances are classified according to the decision tree approach by Cramer et al. (Cramer et al., 1978) into structural class I.

Step 2

At the estimated level of intakes, 4-prop-1-enylphenol and 2-methoxy-4-(prop-1-enyl)phenyl 3-methylbutyrate are expected to be metabolised to innocuous products. Accordingly, the evaluation of the substances proceeds via the A-side of the Procedure scheme (Annex I).

Step A3

The estimated level of the European daily *per capita* intake (MSDI) for the candidate substances classified into structural class I is 0.012 and 12 microgram (Table 2a), which is below the threshold of concern of 1800 microgram/person/day for structural class I substances.

Accordingly, 4-prop-1-enylphenol [FL-no: 04.097] and 2-methoxy-4-(prop-1-enyl)phenyl 3-methylbutyrate [FL-no: 09.894] are not expected to be of safety concern when used as flavouring substances at their estimated levels of intake, based on the MSDI approach.

6. Comparison of the Intake Estimations Based on the MSDI Approach and the mTAMDI Approach

The estimated intakes of [FL-no: 04.097 and 09.894] from structural class I based on the mTAMDI are 72 and 2300 microgram/person/day, respectively. For [FL-no: 09.894] the mTAMDI is above the threshold of concern of 1800 microgram/person/day for structural class I. Therefore, for [FL-no: 09.894] further information is required. This would include more reliable intake data and then, if required, additional toxicological data.

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

Table 6.1 Estimated intakes based on the MSDI approach and the mTAMDI approach

FL-no	EU Register name	MSDI (µg/*capita*/day)	mTAMDI (µg/person/day)	Structural class	Threshold of concern (µg/person/day)
04.097	4-Prop-1-enylphenol	12	72	Class I	1800
09.894	2-Methoxy-4-(prop-1-enyl)phenyl 3-methylbutyrate	0.012	2300	Class I	1800

7. Considerations of Combined Intakes from Use as Flavouring Substances

Because of structural similarities of candidate and supporting substances, it can be anticipated that many of the flavourings are metabolised through the same metabolic pathways and that the metabolites may affect the same target organs. Further, in case of combined exposure to structurally related flavourings, the pathways could be overloaded. Therefore, combined intake should be considered. As flavourings not included in this FGE may also be metabolised through the same

pathways, the combined intake estimates presented here are only preliminary. Currently, the combined intake estimates are only based on MSDI exposure estimates, although it is recognised that this may lead to underestimation of exposure. After completion of all FGEs, this issue should be readdressed.

The total estimated combined daily *per capita* intake of structurally related flavourings is estimated by summing the MSDI for individual substances.

The combined intake of the two candidate substances from their use as flavouring substances is 12 micogram/capita/day which is below the threshold of concern for of 1800 microgram/person/day for a structural class I substance.

The candidate substances are structurally related to six supporting substances evaluated by the JEFCA at its 61st meeting (JECFA, 2004a). The combined intake of the six supporting substances from structural class I could be estimated to approximately 140 microgram/*capita*/day.

The total combined intake from candidate and supporting substances in Europe is approximately 150 microgram/*capita*/day, which is below the threshold of concern of a structural class I substance of 1800 microgram/person/day.

8. Toxicity

8.1. Acute Toxicity

No information on acute toxicity is available for the candidate substances. Oral LD_{50} values have been reported for four of the six supporting substances in this group and ranged from 290 to 3500 mg/kg body weight (bw) in rats and Guinea pigs.

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

8.2. Subacute, Subchronic, Chronic and Carcinogenicity Studies

No information is available for the candidate substances. Subchronic and chronic studies have been performed in rats and mice for the supporting substance isoeugenol [FL-no: 04.004] and a 29-day study has been performed in rats for the supporting substance 6-ethoxyprop-3-enylphenol [FL-no: 04.002].

Isoeugenol [FL-no: 04.004]

Rats

A subchronic oral toxicity study (NTP, 2002b) was performed in male and female rats which were administered by gavage doses of 0, 37.5, 75, 150, 300 or 600 mg isoeugenol/kg bw, 5 times per week, for 14 weeks. A treatment related effect on body weight was observed for the high dose male group only. The liver weights in female rats receiving the two highest doses, 300 and 600 mg/kg bw, were significantly increased: minimal to mild periportal hepatocellular cytoplasmic alteration occurred in all 300 and 600 mg/kg bw females. In addition, atrophy of the olfactory epithelium of the nose and olfactory nerve bundles was observed in both sexes (NTP, 2002b).

In a subsequent chronic study (NTP, 2010a) groups of 50 male and 50 female F344/N rats were administered isoeugenol in corn oil by gavage at doses of 0, 75, 150 or 300 mg/kg bw, 5 days per week, except holidays, for 105 weeks. Survival rates of the exposed male and female rats were similar to those of the vehicle controls. Mean body weights of the highest dose group male rats were 9 % greater than the vehicle controls at the end of the study (NTP, 2010a).

Two male rats in the 300 mg/kg bw/day group had rare benign or malignant thymomas, while two other males in this group had rare mammary gland carcinomas. The incidences of minimal atrophy and minimal to mild respiratory metaplasia of the olfactory epithelium were increased in 150 mg/kg bw/day males and 300 mg/kg bw/day males and females. The incidence of minimal to mild olfactory epithelial degeneration in 300 mg/kg bw/day males was also increased. The incidences of keratoacanthoma of the skin were decreased in 150 and 300 mg/kg bw/day males.

In the technical report of the NTP study (NTP, 2010a) it was concluded that "there was equivocal evidence of carcinogenic activity of isoeugenol in male F344/N rats based on increased incidences of rarely occurring thymoma and mammary gland carcinoma. There was no evidence of carcinogenic activity of isoeugenol in female F344/N rats".

Mice

In a 3-month repeated dose toxicity study (NTP, 2002b) B6C3F$_1$ mice were given isoeugenol orally by gavage at doses of 0, 37.5, 75, 150, 300 or 600 mg/kg bw, 5 days per week, for up to 14 weeks. The liver weights in the 300 and 600 mg/kg males were significantly increased. In addition, atrophy of the olfactory epithelium and nerve bundles was observed in both sexes at the 600 mg/kg dose.

The above dose-range finding study was followed by a 2 year carcinogenicity study in the same strain of mice. Male and female mice (n = 50 per experimental group) were exposed to isoeugenol in corn oil by gavage at doses of 0, 75, 150 or 300 mg/kg bw, 5 days per week, except holidays, for 104 (females) or 105 (males) weeks. Survival of 300 mg/kg bw/day males was significantly decreased compared to the vehicle controls. Mean body weights of 300 mg/kg bw/day male and female groups were less than those of vehicle controls after weeks 75 (8 %) and 64 (15 %), respectively (NTP, 2010a).

In all groups of exposed males, the incidences of hepatocellular adenoma, hepatocellular carcinoma, and hepatocellular adenoma or carcinoma (combined) were significantly greater than those in the vehicle control group; incidences of multiple hepatocellular adenoma were also significantly increased. Incidences of clear cell focus were significantly increased in 75 and 150 mg/kg bw/day male mice. There was a significant positive trend in the incidences of histocytic sarcoma in females, and this neoplasm occurred in multiple tissues. Incidences of respiratory metaplasia in the olfactory epithelium in all exposed groups, and of atrophy and hyaline droplet accumulation in all exposed groups except 75 mg/kg bw/day females, were significantly greater than those in the corresponding vehicle control groups. Incidences of minimal to marked hyperplasia of Bowman's gland were increased significantly in all exposed groups. The incidences of minimal to moderate necrosis of renal papilla and tubules were increased significantly in 300 mg/kg bw/day females. Incidences of forestomach squamous hyperplasia, inflammation and ulceration (males only) increased with increasing exposure concentration and were significant in the 300 mg/kg bw/day groups. The incidence of glandular stomach ulcers was significantly increased in the 300 mg/kg bw/day groups.

Exposure to isoeugenol resulted in non-neoplastic lesions of the nose in male and female rats and the nose, forestomach, and glandular stomach in male and female mice.

In the technical report of the NTP study (NTP, 2010a) it was concluded that "there was clear evidence of carcinogenic activity of isoeugenol in male B6C3F$_1$ mice based on increased incidences of hepatocellular adenoma, hepatocellular carcinoma and hepatocellular adenoma or carcinoma (combined). There was equivocal evidence of carcinogenic activity of isoeugenol in female B6C3F$_1$ mice based on increased incidences of histiolytic sarcoma".

The Panel noted that liver changes (slight increased liver-to-body weight ratio) were also detectable at all dose levels in male mice from the 90-day oral study and considered this effect to be consistent with the non genotoxic-mediated (see Section 8.4) liver carcinogenesis seen in the 2-year study in the same mouse strain. The Panel also noted that:

i. isoeugenol did not increase the incidence of liver cancer in other species (Fischer 344 rats), or gender (female mice)

ii. no dose-response was identified in hepatic tumour incidence in mice

iii. the $B6C3F_1$ mouse strain is known to be very sensitive to increases in liver tumours by non-genotoxic mechanisms (e.g. Phenobarbital)

iv. isoeugenol is not genotoxic (see Section 8.4).

On these grounds, the Panel considered that these experimental findings were unlikely to be relevant to humans.

In conclusion, the Panel considered that elicitation of tumours in rodents is connected to a non genotoxic mechanism. Therefore, the candidate substance can be evaluated via a threshold based approach.

Repeated dose toxicity data are summarised in Annex IV, Table IV.2.

8.3. Developmental / Reproductive Toxicity Studies

Information on developmental or reproductive toxicity is only available for the supporting substance isoeugenol.

A multigenerational reproductive toxicity study was performed in male and female Sprague-Dawley rats which were dosed with 0, 70, 230 or 700 mg of isoeugenol per kg bw per day by gavage in corn oil (EMEA, 2009). Rats from the F0 generation were mated and produced three litters (F1a, F1b and F1c). Animals from the F1c litters were first exposed to isoeugenol on postnatal day 21. On postnatal days 71 to 91, F1c animals were assigned to mating pairs and produced three litters (F2a, F2b, and F2c). The highest dose of isoeugenol, i.e. 700 mg/kg per day, caused mild reproductive toxicity (decreased number of F1 pups per litter and reduced F2 male and female pup weights).

A developmental toxicity study (NTP, 1998; George et al., 2001) was carried out in timed-pregnant CD® outbred albino Sprague-Dawley rats which were treated with 250, 500 or 1,000 mg/kg of isoeugenol by gavage in corn oil from gestational day (GD) 6 to GD 19. No prenatal mortality was detected at any dose. Average fetal body weight per litter was decreased by 7 % (male) or 9 % (female) in the 1,000 mg/kg group on gestation day 20. No other statistically significant fetal abnormalities were observed, besides an increased incidence of unossified sternebra in fetuses from the 1,000 mg/kg group.

Data on developmental and reproductive toxicity are summarised in Annex IV, Table IV.3.

8.4. Genotoxicity Studies

No data on genotoxicity are available for the candidate substances. However, there are data for three supporting substances from *in vitro* tests and for three supporting substances from *in vivo* assays.

The most relevant supporting substance is isoeugenol because it may result from hydrolysis of the candidate substance.

Negative results were obtained for isoeugenol in a battery of four standard tests (three *in vitro* and one *in vivo*), covering important genetic endpoints such as gene mutations, chromosome aberrations and unscheduled DNA synthesis (UDS). In particular, isoeugenol was unable to induce gene mutations in bacterial cells (*S. typhimurium* and *E. coli*), chromosome aberrations in Chinese hamster ovary (CHO) cells and UDS in cultured hepatocytes from male F344 rats and female $B6C3F_1$ mice. There are

conflicting results, one positive and one negative findings in two bacterial DNA repair (Rec assay) assays in *B. subtilis,* but as this bacterial DNA-repair test system is of low predictive value for genotoxicity it will not change the overall evaluation. The conflicting results of two *in vitro* sister chromatid exchanges (SCE) (one negative in CHO cells up to non cytotoxic concentrations, and one positive in human lymphocytes at higher concentrations eliciting cytotoxicity) are considered of limited relevance for the overall evaluation, taking into account the results of the other standard tests. This endpoint is known to be induced also by non-genotoxic agents (e.g.: inhibitors of DNA synthesis, tumour promoters etc.) and is generally considered less relevant than mutations at the gene or chromosome level.

In vivo, isoeugenol was negative in the Wing Spot somatic mutation/recombination test in *Drosophila*, as well as, most important, in a mouse bone marrow micronucleus assay carried out by oral gavage in male animals up to 2000 mg/kg bw, with evidence of bone marrow exposure, shown by a decreased incidence of polychromatic erythrocytes (PCEs) in the treated groups (EMEA, 2009).

Recently the NTP studied the genotoxicity of isoeugenol in association with a carcinogenicity study in rats and mice (NTP, 2010a) see Section 8.2.). All tests were negative except those of 90-day *in vivo* micronucleus results in which a three-fold increase in the frequencies of micronucleated normochromatic erythrocytes were observed in female mice at the highest dose (600 mg/kg bw/day). However, the Panel noted that this weak effect was only observed in the female mice and did not correlate with an observed carcinogenic activity. Only clearcut positive results of this type of test can be taken into account for an overall evaluation of the genotoxic potential, but this is not the case of isoeugenol. Several weaknesses were also identified in the micronucleus study design and results, such as the lack of inclusion of a positive control, the lack of historical control data and the lack of consistency in the control data sets between sexes.

The Panel was informed that two new studies had been submitted to EMEA in connection with the use of eugenol as stunning agent for fish.

These two genotoxicity studies were an *in vivo* micronucleus test in male and female CD-1 mice and an *in vivo* DNA repair (UDS) test in male and female Sprague-Dawley rats. Both studies were negative (EMEA, 2009).

The new *in vivo* micronucleus test in mice did not demonstrate any genotoxic effect of isoeugenol at oral gavage doses up to 2000 mg/kg/day and 1500 mg/kg/day in males and females, respectively. Negative results were also obtained in the *in vivo* UDS test in rats which were orally (by gavage) exposed to doses as high as 2000 mg/kg (males) and 1250 mg/kg (females) (EMEA, 2009).

Based on the weight of *in vitro* and *in vivo* evidence, the Panel concluded that isoeugenol is not genotoxic.

In vitro, the supporting substance 6-ethoxyprop-3-enylphenol [FL-no: 04.002] was negative in two Ames tests and in the rat hepatocytes UDS assay (see Table IV.4); it was positive in the mouse lymphoma assay in the presence of S9. *In vivo*, it was unable to induce gene mutations in the Sex Linked Recessive Lethal assay in *Drosophila* as well as micronuclei in mice treated intraperitoneally up to 1947 mg/kg bw. Notwithstanding the fact that the studies were carried out in 1982 and 1983 not fully in compliance with current OECD guidelines, the results can be considered sufficiently adequate for an evaluation. Overall, the data available do not raise concern for genotoxity.

In conclusion, the Panel considered that isoeugenol was not genotoxic and that the available data on the supporting substances as well as the structure of the candidate substance do not give rise to safety concern with respect to genotoxicity.

According to the NTP (NTP, 2010a) report there were clear evidence of carcinogenic activity of isoeugenol in male $B6C3F_1$ mice (hepatocellular adenoma and/or carcinoma) and equivocal evidence of carcinogenic activity of isoeugenol in female $B6C3F_1$ mice (histiocytic sarcoma) and male F344/N

rats (rare thymoma and mammary gland carcinoma). The Panel however concluded that these observations would not prevent the candidate substance from being evaluated through the Procedure, given the lack of genotoxic potential of isoeugenol.

Genotoxicity data are summarised in Annex IV, Table IV.4 and Table IV.5.

9. Conclusions

The candidate substances are 4-prop-1-enylphenol [FL-no: 04.097] and a structurally related ester, 2-methoxy-4-(prop-1-enyl)phenyl 3-methylbutyrate [FL-no: 09.894], both from chemical group 17.

Due to the presence and the position of a double bond both candidate substances can exist as geometrical isomers. For 2-methoxy-4-(prop-1-enyl)phenyl 3-methylbutyrate the stereoisomeric composition has been specified as the E/Z mixture, but the composition of the mixture has not been given.

4-Prop-1-enylphenol and 2-methoxy-4-(prop-1-enyl)phenyl 3-methylbutyrate are classified into structural class I.

The candidate substances have been reported to occur naturally in anise oil and cider.

According to the default MSDI approach, the flavouring substances in this group have European intakes of 0.012 and 12 microgram/*capita*/day ([FL-no: 09.894 and 04.097], respectively), which are below the threshold of concern for structural class I substances of 1800 microgram/person/day.

On the basis of the reported annual production in Europe (MSDI approach) the total combined intake of the two flavouring substances and six structurally related substances from structural class I can be calculated to approximately 150 microgram/*capita*/day. This value is lower than the threshold of concern for structural class I substances of 1800 microgram/person/day.

Data available do not give rise to safety concern with respect to genotoxicity.

4-Prop-1-enylphenol and 2-methoxy-4-(prop-1-enyl)phenyl 3-methylbutyrate are expected to be metabolised to innocuous products.

It was noted that where toxicity data were available they were consistent with the conclusions in the present Flavouring Group Evaluation. The Panel considered that elicitation of tumours by the supporting substance isoeugenol in rodents is connected to a non genotoxic mechanism. Therefore, the flavouring substances [FL-no: 04.097 and 09.894] can be evaluated via a threshold based approach, i.e. the Procedure.

It is considered that on the basis of the default MSDI approach the candidate substances would not give rise to safety concern at the estimated levels of intake arising from use as flavouring substances.

When the estimated intake was based on the mTAMDI approach it was 72 and 2300 microgram/person/day for 4-prop-1-enylphenol and 2-methoxy-4-(prop-1-enyl)phenyl 3-methylbutyrate, respectively. For 2-methoxy-4-(prop-1-enyl)phenyl 3-methylbutyrate this intake estimate is above the threshold of concern for structural class I substances of 1800 microgram/person/day, and therefore, more reliable exposure data are required for this substance. On the basis of such additional data, the flavouring substance should be reconsidered along the steps of the Procedure. Following this Procedure additional toxicological data might become necessary. 4-Prop-1-enylphenol, for which the estimated intake is below the threshold, is also expected to be metabolised to innocuous products.

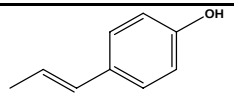
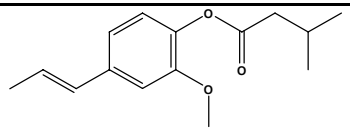
In order to determine whether the conclusion for the two flavouring substances can be applied to the materials of commerce, it is necessary to consider the available specifications. Adequate

specifications, including complete purity criteria and identity, have been given for 4-prop-1-enylphenol [FL-no: 04.097] and 2-methoxy-4-(prop-1-enyl)phenyl 3-methylbutyrate [FL-no: 09.894], except that information on the composition of the stereoisomeric mixture has not been specified for [FL-no: 09.894]. Thus, the final evaluation of the material of commerce cannot be performed for 2-methoxy-4-(prop-1-enyl)phenyl 3-methylbutyrate [FL-no: 09.894] pending further information on specifications.

For 4-prop-1-enylphenol [FL-no: 04.097] the Panel concluded that it would present no safety concern at the estimated level of intake based on the MSDI approach.

TABLE 1: SPECIFICATION SUMMARY OF THE SUBSTANCES IN THE FLAVOURING GROUP EVALUATION 30REV1

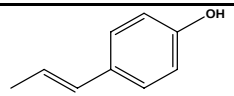
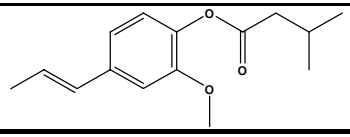
Table 1: Specification Summary of the Substances in the Flavouring Group Evaluation 30Rev1

FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	Specification comments
04.097	4-Prop-1-enylphenol		4062 539-12-8	Solid $C_9H_{10}O$ 134.18	Slightly soluble Soluble	250 94 MS 97.7%	n.a. n.a.	Minimum assay value: 97.7 % (95.2 % trans and 2.5 % cis).
09.894	2-Methoxy-4-(prop-1-enyl)phenyl 3-methylbutyrate			Solid $C_{15}H_{20}O_3$ 248.32	Practically insoluble or insoluble Freely soluble	223 81 NMR 95 %	n.a. n.a.	CASrn 61114-23-6 to be introduced in the Register (EFFA, 2004ae). The CASrn does not specify isomer. Mixture of E & Z isomers (EFFA, 2010a). Composition of mixture to be specified.

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 2A: SUMMARY OF SAFETY EVALUATION APPLYING THE PROCEDURE (BASED ON INTAKES CALCULATED BY THE MSDI APPROACH)

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

FL-no	EU Register name	Structural formula	MSDI 1) (µg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
04.097	4-Prop-1-enylphenol		12	Class I A3: Intake below threshold	4)	6)	
09.894	2-Methoxy-4-(prop-1-enyl)phenyl 3-methylbutyrate		0.012	Class I A3: Intake below threshold	4)	7)	

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 µg/person/day, Class II = 540 µg/person/day, 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.*

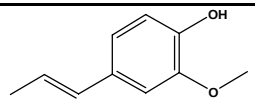
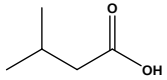
6) *No safety concern at estimated level of intake of the material of commerce meeting the specification of Table 1 (based on intake calculated by the MSDI approach).*

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 and/or information on stereoisomerism.*

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

TABLE 2B: EVALUATION STATUS OF HYDROLYSIS PRODUCTS OF CANDIDATE ESTERS

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

FL-no	EU Register name JECFA no	Structural formula	SCF status 1) JECFA status 2) CoE status 3) EFSA status	Structural class 4) Procedure path (JECFA) 5)	Comments
04.004	Isoeugenol 1260		No safety concern a) Category A b)	Class I A3: Intake below threshold	
08.008	3-Methylbutyric acid 259		Category 1 c) No safety concern d) Category A b)	Class I A3: Intake below threshold	

1) Category 1: Considered safe in use Category 2: Temporarily considered safe in use Category 3: Insufficient data to provide assurance of safety in use Category 4): Not acceptable due to evidence of toxicity.

2) No safety concern at estimated levels of intake.

3) Category A: Flavouring substance, which may be used in foodstuffs Category B: Flavouring substance which can be used provisionally in foodstuffs.

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

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

a) (JECFA, 2004a).

b) (CoE, 1992).

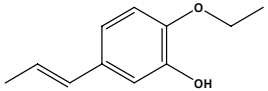
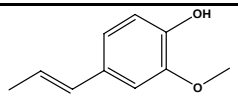
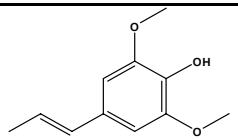
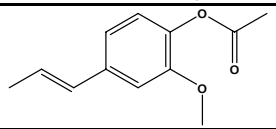
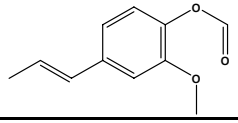
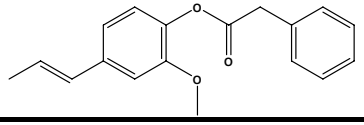
c) (SCF, 1995).

d) (JECFA, 1999b).

ND: Not detected.

TABLE 3: SUPPORTING SUBSTANCES SUMMARY

Table 3: Supporting Substances Summary

FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (µg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	EFSA Comments
04.002	6-Ethoxyprop-3-enylphenol		2922 170 94-86-0	1264 JECFA specification (JECFA, 2003b)	38	- No safety concern a) Category A b)	Registername to be changed to 6-Ethoxy-3-(prop-3-enyl)phenol. Composition of stereoisomeric mixture to be specified.
04.004	Isoeugenol		2468 172 97-54-1	1260 JECFA specification (JECFA, 2003b)	99	- No safety concern a) Category A b)	Composition of stereoisomeric mixture to be specified.
04.055	2,6-Dimethoxy-4-prop-1-enylphenol		3728 - 20675-95-0	1265 JECFA specification (JECFA, 2003b)	0.012	- No safety concern a) -	Register name to be changed to E-2,6-Dimethoxy-4-prop-1-enylphenol. Composition of stereoisomeric mixture to be specified.
09.030	2-Methoxy-4-(prop-1-enyl)phenyl acetate		2470 220 93-29-8	1262 JECFA specification (JECFA, 2003b)	0.61	- No safety concern a) Category B b)	Composition of stereoisomeric mixture to be specified.
09.089	Isoeugenyl formate		2474 356 7774-96-1	1261 JECFA specification (JECFA, 2003b)	0.012	- No safety concern a) Category B b)	Composition of stereoisomeric mixture to be specified.
09.710	Isoeugenyl phenylacetate		2477 237 120-24-1	1263 JECFA specification (JECFA, 2005b)	0.085	- No safety concern a) Category B b)	Composition of stereoisomeric mixture to be specified.

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

2) Category 1: Considered safe in use, Category 2: Temporarily considered safe in use, Category 3: Insufficient data to provide assurance of safety in use, Category 4: Not acceptable due to evidence of toxicity.

3) No safety concern at estimated levels of intake.

4) Category A: Flavouring substance, which may be used in foodstuffs, Category B: Flavouring substance which can be used provisionally in foodstuffs.

a) (JECFA, 2004a).

b) (CoE, 1992).

ND) No intake data reported.

ANNEX I: PROCEDURE FOR THE SAFETY EVALUATION

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

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

Class I contains flavourings that have simple chemical structures and efficient modes of metabolism, which would suggest a low order of oral toxicity. Class II contains flavourings that have structural features that are less innocuous, but are not suggestive of toxicity. Class III comprises flavourings that have structural features that permit no strong initial presumption of safety, or may even suggest significant toxicity (Cramer et al., 1978). The thresholds of concern for these structural classes of 1800, 540 or 90 microgram/person/day, respectively, are derived from a large database containing data on subchronic and chronic animal studies (JECFA, 1996a).

In Step 1 of the Procedure, the flavourings are assigned to one of the structural classes. The further steps address the following questions:

- can the flavourings be predicted to be metabolised to innocuous products[8] (Step 2)?
- do their exposures exceed the threshold of concern for the structural class (Step A3 and B3)?
- are the flavourings or their metabolites endogenous[9] (Step A4)?
- does a NOAEL exist on the flavourings or on structurally related substances (Step A5 and B4)?

In addition to the data provided for the flavouring substances to be evaluated (candidate substances), toxicological background information available for compounds structurally related to the candidate substances is considered (supporting substances), in order to assure that these data are consistent with the results obtained after application of the Procedure.

The Procedure is not to be applied to flavourings with existing unresolved problems of toxicity. Therefore, the right is reserved to use alternative approaches if data on specific flavourings warranted such actions.

[8] *"Innocuous metabolic products": Products that are known or readily predicted to be harmless to humans at the estimated intakes of the flavouring agent" (JECFA, 1997a).*

[9] *"Endogenous substances": Intermediary metabolites normally present in human tissues and fluids, whether free or conjugated; hormones and other substances with biochemical or physiological regulatory functions are not included (JECFA, 1997a).*

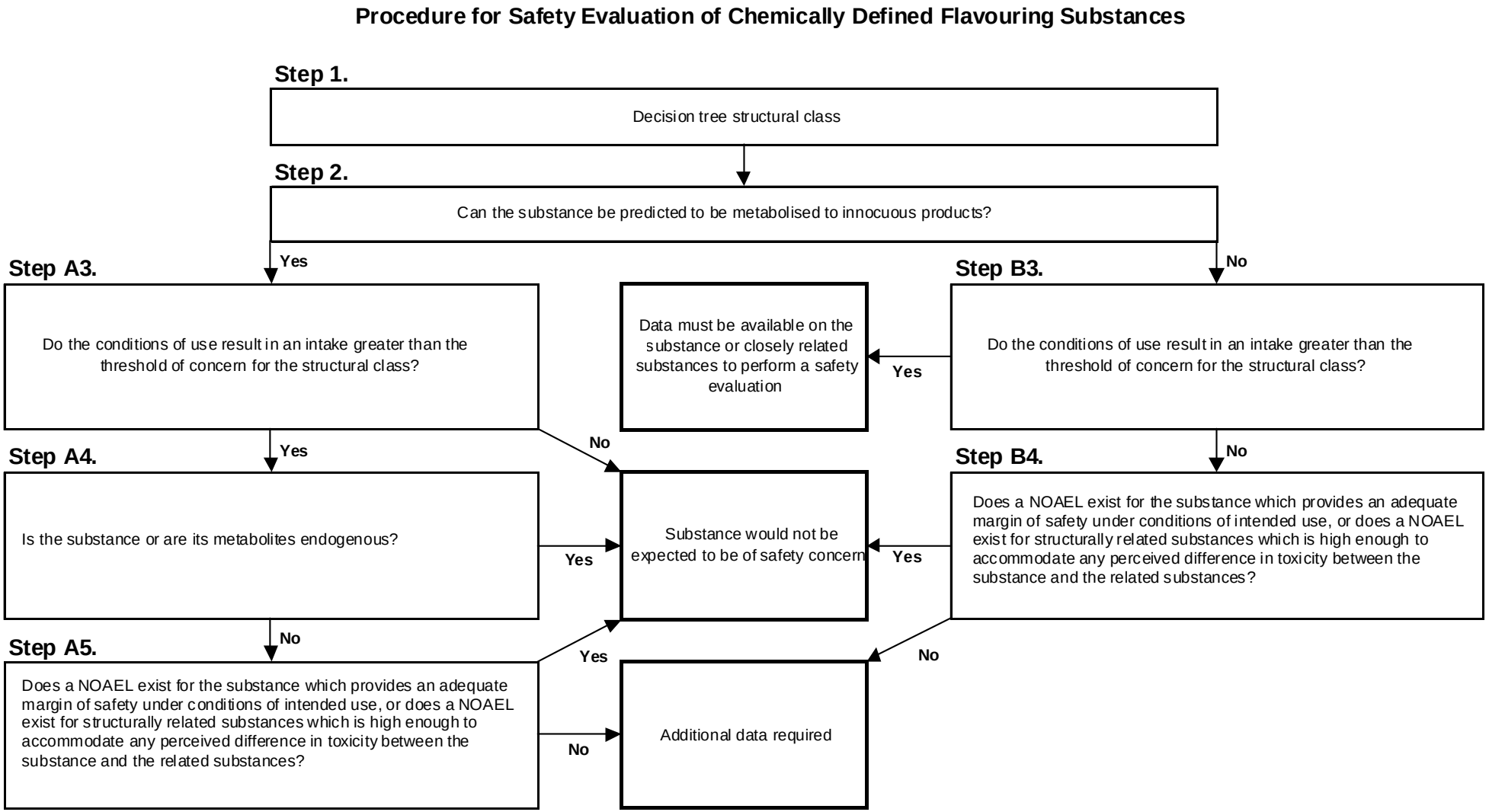


Figure I.1 *Procedure for Safety Evaluation of Chemically Defined Flavouring Substances*

ANNEX II: USE LEVELS / MTAMDI

II.1 Normal and Maximum Use Levels

For each of the 18 Food categories (Table II.1.1) in which the candidate substances are used, Flavour Industry reports a "normal use level" and a "maximum use level" (EC, 2000a). According to the Industry the "normal use" is defined as the average of reported usages and "maximum use" is defined as the 95th percentile of reported usages (EFFA, 2002i). The normal and maximum use levels in different food categories have been extrapolated from figures derived from 12 model flavouring substances (EFFA, 2004e).

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

Food category	Description
01.0	Dairy products, excluding products of category 02.0
02.0	Fats and oils, and fat emulsions (type water-in-oil)
03.0	Edible ices, including sherbet and sorbet
04.1	Processed fruit
04.2	Processed vegetables (incl. mushrooms & fungi, roots & tubers, pulses and legumes), and nuts & seeds
05.0	Confectionery
06.0	Cereals and cereal products, incl. flours & starches from roots & tubers, pulses & legumes, excluding bakery
07.0	Bakery wares
08.0	Meat and meat products, including poultry and game
09.0	Fish and fish products, including molluscs, crustaceans and echinoderms
10.0	Eggs and egg products
11.0	Sweeteners, including honey
12.0	Salts, spices, soups, sauces, salads, protein products, etc.
13.0	Foodstuffs intended for particular nutritional uses
14.1	Non-alcoholic ("soft") beverages, excl. dairy products
14.2	Alcoholic beverages, incl. alcohol-free and low-alcoholic counterparts
15.0	Ready-to-eat savouries
16.0	Composite foods (e.g. casseroles, meat pies, mincemeat) - foods that could not be placed in categories 01.0 - 15.0

The "normal and maximum use levels" are provided by Industry for the candidate substances in the present flavouring group (Table II.1.2).

Table II.1.2 Normal and Maximum use levels (mg/kg) for the candidate substances in FGE.30Rev1 (EFFA, 2004ae; EFFA, 2007a; Flavour Industry, 2007m).

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
04.097	0,15	-	0,15	-	-	0,35	-	0,2	-	-	-	-	-	-	0,1	0,15	-	-
	0,6	-	0,7	-	-	1,7	-	1	-	-	-	-	-	-	0,4	0,7	-	-
09.894	7	5	10	7	-	10	5	10	2	2	-	-	5	10	-	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	-	50	100	25

II.2 mTAMDI Calculations

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

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

Class of product category	Intake estimate (g/day)
Beverages (non-alcoholic)	324.0
Foods	133.4
Exception a: Candy, confectionery	27.0
Exception b: Condiments, seasonings	20.0
Exception c: Alcoholic beverages	20.0
Exception d: Soups, savouries	20.0
Exception e: Others, e.g. chewing gum	e.g. 2.0 (chewing gum)

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

- Beverages (SCF, 1995) correspond to food category 14.1 (EC, 2000a)
- Foods (SCF, 1995) correspond to the food categories 1, 2, 3, 4.1, 4.2, 6, 7, 8, 9, 10, 13, and/or 16 (EC, 2000a)
- Exception a (SCF, 1995) corresponds to food category 5 and 11 (EC, 2000a)
- Exception b (SCF, 1995) corresponds to food category 15 (EC, 2000a)
- Exception c (SCF, 1995) corresponds to food category 14.2 (EC, 2000a)
- Exception d (SCF, 1995) corresponds to food category 12 (EC, 2000a)
- Exception e (SCF, 1995) corresponds to others, e.g. chewing gum.

Table II.2.2 Distribution of the 18 food categories listed in Commission Regulation (EC) No 1565/2000 (EC, 2000a) into the seven SCF food categories used for TAMDI calculation (SCF, 1995)

	Food categories according to Commission Regulation 1565/2000	Distribution of the seven SCF food categories		
Key	Food category	Food	Beverages	Exceptions
01.0	Dairy products, excluding products of category 02.0	Food		
02.0	Fats and oils, and fat emulsions (type water-in-oil)	Food		
03.0	Edible ices, including sherbet and sorbet	Food		
04.1	Processed fruit	Food		
04.2	Processed vegetables (incl. mushrooms & fungi, roots & tubers, pulses and legumes), and nuts & seeds	Food		
05.0	Confectionery			Exception a
06.0	Cereals and cereal products, incl. flours & starches from roots & tubers, pulses & legumes, excluding bakery	Food		
07.0	Bakery wares	Food		
08.0	Meat and meat products, including poultry and game	Food		
09.0	Fish and fish products, including molluscs, crustaceans and echinoderms	Food		
10.0	Eggs and egg products	Food		
11.0	Sweeteners, including honey			Exception a
12.0	Salts, spices, soups, sauces, salads, protein products, etc.			Exception d
13.0	Foodstuffs intended for particular nutritional uses	Food		
14.1	Non-alcoholic ("soft") beverages, excl. dairy products		Beverages	
14.2	Alcoholic beverages, incl. alcohol-free and low-alcoholic counterparts			Exception c
15.0	Ready-to-eat savouries			Exception b
16.0	Composite foods (e.g. casseroles, meat pies, mincemeat) - foods that could not be	Food		

Table II.2.2 Distribution of the 18 food categories listed in Commission Regulation (EC) No 1565/2000 (EC, 2000a) into the seven SCF food categories used for TAMDI calculation (SCF, 1995)

Food categories according to Commission Regulation 1565/2000	Distribution of the seven SCF food categories
placed in categories 01.0 - 15.0	

The mTAMDI values (see Table II.2.3) are presented for each of the flavouring substance in the present flavouring group, for which Industry has provided use and use levels (EFFA, 2004ae; EFFA, 2007a; Flavour Industry, 2007m). The mTAMDI values are only given for the highest reported normal use levels.

TableII.2.3 Estimated intakes based on the mTAMDI approach

FL-no	EU Register name	mTAMDI (µg/person/day)	Structural class	Threshold of concern (µg/person/day)
04.097	4-Prop-1-enylphenol	72	Class I	1800
09.894	2-Methoxy-4-(prop-1-enyl)phenyl 3-methylbutyrate	2300	Class I	1800

ANNEX III: METABOLISM

Introduction

The candidate substances in this FGE are hydroxypropenylbenzene derivatives, 4-prop-1-enylphenol and the 3-methylbutyrate of the structurally related hydroxypropenylbenzene, isoeugenol.

III.1. Hydrolysis

In general, aromatic esters are hydrolysed *in vivo* through the catalytic activity of carboxylesterases (Heymann, 1980; Anders, 1989), the most important of which are the A-esterases. Carboxylesterases are found in the endoplasmic reticulum of most mammalian tissues; however, they are most abundant in hepatocytes (Anders, 1989; Graffner-Nordberg et al., 1998; Hosokawa et al., 2001).

In a study of the hydrolysis of the structurally related ester, phenyl acetate, using pig liver carboxylesterase, the K_m (substrate concentration at which half the true maximum velocity of an enzyme-catalysed reaction is achieved) and V_{max} (maximum velocity of an enzyme-catalysed reaction) values for phenyl acetate were reported to be 0.43 mmol/l and 438 mmol/min per mg protein, respectively, at a substrate (phenyl acetate) concentration of 0.2–3 mmol/l (Junge & Heymann, 1979). A second phenolic ester, *o*-tolyl acetate (*o*-methylphenyl acetate) was 60 % hydrolysed *in vitro* after incubation with pancreatin for 2 hours at 37°C (Grundschober, 1977). Phenyl 2-hydroxybenzoate (phenyl salicylate) is hydrolysed to phenol and 2-hydroxybenzoic acid in humans, as shown in a study in which one man was given one capsule containing 90 mg of phenyl salicylate per hour for 8 hours. Urine was collected for 72 hours after the first dose, in 8 hours collection periods. Analysis of total urinary phenol showed a peak concentration of 472 mg/l during the second collection period. The concentration of free urinary phenol peaked at 25 mg/l during the same period. Approximately 60 hours after the first dose, concentrations of both total and free urinary phenol returned to baseline levels (7 and 1 mg/l, respectively) (Fishbeck et al., 1975).

Recent studies have revealed that isoeugenyl acetate [FL-no: 09.030] undergoes extensive hydrolysis when incubated with rat hepatocytes or with microsomes prepared from rat liver. For example, incubation of isoeugenyl acetate (500 µmol/l) with hepatocytes (2 million cells) resulted in the complete hydrolysis of the ester to isoeugenol within 15–20 min. Hydrolytic activity was greatly enriched in the endoplasmic reticulum (i.e. microsomal fraction) of the liver. Rat blood also hydrolysed isoeugenyl acetate at a rate of 1600 nmol/ml per min (personal communication from Professor G. Sipes, University of Arizona, Tucson, Arizona, USA to the Flavor and Extract Manufacturers Association (FEMA), Washington, DC, USA; submitted to WHO by FEMA).

III.2. Absorption, Distribution and Elimination

In humans, rats and mice, orally administered hydroxypropenylbenzene derivatives are rapidly absorbed from the gastrointestinal tract and predominantly metabolized in the liver via phase II conjugation of the phenolic hydroxy (OH) group to form sulphate and glucuronic acid conjugates. These conjugates are eliminated primarily in the urine.

Male Fischer 344 rats given [14C] isoeugenol at a single oral dose of 156 mg/kg bw excreted > 85 % of the administered dose as the glucuronic acid and sulphate conjugate in the urine within 72 hours. No parent compound was detected in the blood after oral administration at any time point. Similarly, male rats given [14C] isoeugenol at a single intravenous dose of 15.6 mg/kg bw excreted 82 % of the administered dose as

the glucuronic acid and sulphate conjugates in the urine within 72 hours. Isoeugenol disappeared rapidly from the rat blood. About 12 % of the administered dose was present in the blood at the first time point, i.e. 0.017 hours, after intravenous administration, the calculated half-life ($t_{1/2}$) was 12 min, and the systemic clearance was 1.9 l/min/kg. After administration by either route, approximately 10 % of the administered dose was excreted in the faeces and < 0.1 % was recovered in expired air. Less than 0.25 % of the radiolabel remained in selected tissues (Badger et al., 2002b).

The results of toxicokinetic studies conducted with isoeugenol administered by gavage or intravenously to Fischer 344/N rats and $B6C3F_1$ mice indicate that isoeugenol undergoes extensive first-pass metabolism (Fuciarelli, 2001). Isoeugenol was detected in the plasma of rats and mice 2 min after the administration by gavage of single doses of 17 and 35 mg/kg bw to rats and mice. The time at which peak plasma concentrations (T_{max}) were attained was shown to be short, with values ranging from between 2 and 20 min in rats, and between 5 and 20 min in mice. Collectively, these data indicate that isoeugenol is rapidly absorbed from the gastrointestinal tract. However, the results indicate that isoeugenol has a low bioavailability (approximately 14 % for rats at 17 mg/kg bw and approximately 35 % for mice at 35 mg/kg bw). On the basis of the low bioavailability, the authors concluded that isoeugenol undergoes extensive first-pass metabolism before systemic distribution. Short half-lifes for both species also indicate that isoeugenol is rapidly eliminated from the systemic circulation. The high total clearance values reported for rats and mice further support the conclusion that isoeugenol is rapidly and extensively eliminated from systemic circulation after administration by gavage (Fuciarelli, 2001).

III.3. Metabolism

After absorption, orally administered hydroxypropenylbenzene derivatives are completely metabolised in humans, rats and mice. Pharmacokinetic and metabolic information on isoeugenol, isoeugenol methyl ether and related alkoxypropenylbenzene derivatives (e.g. *trans*-anethole) indicate that hydroxypropenylbenzenes primarily undergo conjugation of the phenolic OH group with sulphate or glucuronic acid, followed by excretion mainly in the urine. Dealkylation of ring alkoxy substituents and oxidation of the propenyl side-chain are minor metabolic pathways for hydroxypropenylbenzene derivatives.

Isoeugenol, which contains a free phenolic hydroxy group, is also readily conjugated with glucuronic acid and sulphate, and subsequently excreted (Williams, 1959a; Badger et al., 2002b). In male Fischer 344 rats, given [14C] isoeugenol in a single oral dose of 156 mg/kg bw or a single intravenous dose of 15.6 mg, more than 80 % of the administered dose was excreted as the glucuronic acid and sulphate conjugate in the urine within 72 hours. With both routes of administration, approximately 10 % of the administered dose was excreted in the faeces and < 0.1 % was recovered in expired air (Badger et al., 2002b).

It is important to note that in contrast to several 2-propenylbenzenes (e.g. safrole, methyleugenol, estragole or eugenol) the double bond in isoeugenol is at the 1'-carbon atom of the propenyl side chain, rather than at the 2'-position. As a result of the position of the double bond in the 2-propenylbenzenes, these substances can be subject to 1'-hydroxylation followed by sulphation. When the sulphate group is subsequently split off from the 1'-carbon, a reactive carbocation is formed which is claimed to be responsible for the genotoxicity and carcinogenicity of these 2-propenylbenzenes. This mechanism is not possible for isoeugenol or the candidate substances, due to the different position of the double bond in the propenyl chain, and therefore no concern for genotoxicity and carcinogenicity is raised from the structures of these substances.

III.4. Conclusion

In summary, the major metabolic pathway for isoeugenol and isoeugenyl esters involves rapid conjugation with sulphate or glucuronic acid, followed by excretion in the urine. The same metabolic pathway is anticipated for the 4-prop-1-enylphenol. Minor metabolic pathways involve the *O*-dealkylation of ring alkoxy substituents, or oxidation of the propenyl side-chain via omega-oxidation or epoxidation. The Panel concluded that the candidate substances could be anticipated to be metabolised to innocuous products.

ANNEX IV: TOXICITY

ACUTE TOXICITY

Oral acute toxicity data are not available for the candidate substances of the present Flavouring Group Evaluation, but for four supporting substances evaluated by the JECFA at the 61st meeting.

Table IV.1: Acute Toxicity

Chemical Name	Species	Sex	Route	LD_{50} (mg/kg bw)	Reference
(Isoeugenol [04.004])	Rat	M, F	Oral	286	(Piccirillo, 1984b)
	Rat	M, F	Oral	1560	(Jenner et al., 1964)
	Guinea Pig	M, F	Oral	1410	(Jenner et al., 1964)
(2-Methoxy-4-(prop-1-enyl)phenyl acetate [09.030])	Rat	NR	Oral	3450	(Moreno, 1973am)
(6-Ethoxyprop-3-enylphenol [04.002])	Rat	M, F	Oral	1575	(Piccirillo, 1984b)
	Rat	NR	Oral	2400	(Bär & Griepentrog, 1967)
(2,6-Dimethxy-4-Prop-1-enylphenol [04.055])	Rat	M, F	Oral	2400	(Piccirillo & Hartman, 1982)

M=Male; F=Female; NR=Not Reported.

SUBACUTE, SUBCRONIC, CHRONIC AND CARCINOGENIC TOXICITY STUDIES

Subacute / subchronic / chronic / carcinogenic toxicity data are not available for the candidate substances of the present Flavouring Group Evaluation, but for two supporting substances evaluated by the JECFA at the 61st meeting.

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

Chemical Name	Species; Sex No./Group	Route	Dose levels mg/kg bw	Duration (days)	NOAEL (mg/kg/day)	Reference	Comments
(Isoeugenol [04.004])	Mouse; M, F 20	Gavage	37.5, 75, 150, 300, 600	90	300[1]	(NTP, 2002b)	
	Rat; M, F 20	Gavage	37.5, 75, 150, 300, 600	90	F: 37.5[1] M: < 37.5[1]	(NTP, 2002b)	
	Rat; M, F 10	Gavage	1000	112	1000[2]	(Hagan et al., 1967)	
	Mouse; M, F 50	Gavage	75, 150, 300	735	75	(NTP, 2010a)	
	Rat; M, F 50	Gavage	75, 150, 300	F: 728 M: 735	F: 75 M: < 75	(NTP, 2010a)	
(6-Ethoxyprop-3-enylphenol [04.002])	Rat; M, F 20	Gavage	250, 1250, 2500	29	250	(Terrill, 1991)	

M=Male; F=Female.

1 NOEL based on limited information obtained from the National Toxicology Program Preliminary Report.

2 Study performed with either a single dose or multiple doses that produced no effect.

DEVELOPMENTAL AND REPRODUCTIVE TOXICITY STUDIES

No developmental and reproductive toxicity data are available for the candidate substances of the present Flavouring Group Evaluation. For the supporting substance isoeugenol, developmental and reproductive studies are available. The studies were not considered by the JECFA at the 61st meeting.

Table IV.3: Developmental and Reproductive Toxicity Studies

Chemical Name	Species; Sex No./Group	Route	Dose levels mg/kg bw/day	Treatment (days)	LOAEL mg/kg/day	NOAEL mg/kg bw/day	Reference	Comments
(Isoeugenol [04.004])	Rat; F 25	Gavage	250, 500 or 1,000	Gestational days (GD) 6-19	Maternal toxicity: 250 Developmental toxicity: 1,000	Developmental toxicity: 500	(NTP, 1998); (George et al., 2001)	No resorption or late fetal death. At 1,000 mg/kg decreased average fetal body weight per litter by 7 % (male) and 9 % (female) on GD20. At 1,000 mg/kg increased incidence of unossified sternebra in fetuses. At 250 mg/kg reduced body weight and gestational weight gain in dams.
	Rat; M, F 20	Gavage	70, 230, 700	Two generations		Reproductive toxicity: 230	(EMEA, 2009)	At 700 mg/kg mild reproductive toxicity (decrease in the number of F1 pups per litter and decreases in F2 male and female pup weights).

M=Male; F=Female.

GENOTOXICITY (*IN VITRO*)

In vitro mutagenicity/genotoxicity data are not available for the candidate substances of the present Flavouring Group Evaluation, but for three supporting substances evaluated by the JECFA at the 61st meeting.

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

Chemical Name	Test System	Test Object	Concentration	Result	Reference	Comments
(Isoeugenol [04.004])	Reverse Mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538	2, 20, and 200 microg/plate	Negative[1]	(Hsia et al., 1979)	Summarised by JECFA
	Reverse Mutation	*S. typhimurium* TA98 and TA100	0.05 to 100 microl/plate (54.1 to 108,200 microg/plate)	Negative[1]	(Rockwell & Raw, 1979)	Summarised by JECFA
	Reverse Mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537	3 micromol/plate (493 microg/plate)	Negative[2]	(Florin et al., 1980)	Summarised by JECFA
	Reverse Mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538	0.8 mg/plate (800 microg/plate)	Negative[2]	(Douglas et al., 1980)	Summarised by JECFA
	Reverse Mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538	60, 120, and 300 microg/plate	Negative[2]	(Sekizawa & Shibamoto, 1982)	Summarised by JECFA
	Reverse Mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538	1.0 microl/plate (1,082 microg/plate)	Negative[2]	(DeGraff, 1983c)	Summarised by JECFA
	Reverse Mutation	*S. typhimurium* TA97, TA98, TA100, TA1535, TA1537	Up to 800 microg/plate	Negative[2]	(Mortelmans et al., 1986)	Summarised by JECFA
	Reverse Mutation	*S. typhimurium* TA97 and TA102	Up to 0.5 mg/plate (500 microg/plate)	Negative[2]	(Fujita & Sasaki, 1987)	Summarised by JECFA
	Reverse Mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538	1,000 microg/plate	Negative[2]	(Heck et al., 1989)	Summarised by JECFA
	Point Mutation	*E. coli* WP2 uvrA	60, 120, and 300 microg/plate	Negative[2]	(Sekizawa & Shibamoto, 1982)	Summarised by JECFA
	DNA Repair	*B. subtillis* H 17 (rec+) and M 45 (rec-)	22.0 microg/disk	Negative	(Oda et al., 1979)	Summarised by JECFA
	DNA Repair	*B. subtillis* H 17 (rec+) and M 45 (rec-)	0.8 mg/disk (800 microg/disk)	Positive[3]	(Sekizawa & Shibamoto, 1982)	Summarised by JECFA
	Sister Chromatid Exchange	Chinese hamster ovary cells	10, 33.3, and 100 microM (1.64, 5.47, and 16.42 microg/ml)	Negative[3]	(Sasaki et al., 1989)	Summarised by JECFA
	Sister Chromatid Exchange	Human Lymphocytes	0.5 mM (82 microg/ml)	Positive	(Jansson et al., 1986)	Summarised by JECFA
	Unscheduled DNA Synthesis	Mouse Hepatocytes	Up to 1,000 microM (164.2 microg/ml)	Negative	(Burkey et al., 2000)	Summarised by JECFA
	Unscheduled DNA Synthesis	Rat Hepatocytes	Up to 1,000 microM (164.2 microg/ml)	Negative	(Burkey et al., 2000)	Summarised by JECFA
	Reverse Mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537	Up to 1,000 microg/plate	Negative[2]	(NTP, 2010a)	Summarised by JECFA
	Point Mutation	*E. coli* WP2 uvrA/pKM101	Up to 1,000 microg/plate	Negative[2]	(NTP, 2010a)	Summarised by JECFA
	Sister Chromatid Exchange	Chinese hamster ovary cells	Up to 200 microg/ml[1] Up to 500 microg/ml[3]	Negative[2]	(NTP, 2010a)	Summarised by JECFA
(Isoeugenyl phenylacetate [09.710])	Reverse Mutation	*S. typhimurium* TA1535, TA1537, TA1538, TA98 and TA100	Up to 3.6 mg/plate (3,600 microg/plate)	Negative[2]	(Wild et al., 1983)	Summarised by JECFA

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

Chemical Name	Test System	Test Object	Concentration	Result	Reference	Comments
(6-Ethoxyprop-3-enylphenol [04.002])	Reverse Mutation	*S. typhimurium* TA1535, TA1537, TA1538, TA98 and TA100	Up to 3.6 mg/plate (3,600 microg/plate)	Negative[2]	(Wild et al., 1983)	Summarised by JECFA
(6-Ethoxyprop-3-enylphenol [04.002]) cont.	Reverse Mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538	Up to 1,000 microg/plate	Negative[2]	(Jagannath, 1982)	Summarised by JECFA
	Forward Mutation	Mouse lymphoma L5178Y TK+/- cells	1.875 to 100 microg/ml	Positive[1]	(Cifone, 1983)	Summarised by JECFA
	Forward Mutation	Mouse lymphoma L5178Y TK+/- cells	7.81 to 125 microg/ml	Negative[3]	(Cifone, 1983)	Summarised by JECFA
	Unscheduled DNASynthesis	Rat Primary Hepatocytes	1.01 to 50.4 microg/ml	Negative	(Cifone, 1988b)	Summarised by JECFA

[1] *With metabolic activation.*

[2] *With and without metabolic activation.*

[3] *Without metabolic activation.*

GENOTOXICITY (*IN VIVO*)

In vivo mutagenicity/genotoxicity data are not available for the candidate substances of the present Flavouring Group Evaluation, but for two supporting substances evaluated by the JECFA at the 61st meeting. Furthermore *in vivo* data are available for the supporting substance isoeugenol, these data were not considered by the JECFA at the 61st meeting.

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

Chemical Name	Test System	Test Object	Route	Dose	Result	Reference	Comments
(Isoeugenol [04.004])	Micronucleus Induction in bone marrow (OECD No 474)	Mouse Males	Oral gavage	500, 1000, 2000 mg/kg bw one dose	M: Negative	(EMEA, 2009)	No increase in the frequency of micronucleated polychromatic erythrocytes in treated animals as compared to the negative control. Study weaknesses: Only male mice were used in this test.
	Micronucleus Induction in peripheral blood	$B6C3F_1$ Mouse Males and Females	Oral gavage	37.5, 75, 150, 300, 600 mg/kg bw for 3 months	M: Negative F: Positive	(NTP, 2010a)	Frequencies of micronucleated erythrocytes were not increased in peripheral blood of male mice exposed to isoeugenol by gavage for 3 months; however, an increasing trend and a threefold increase in the 600 mg/kg group indicate a positive result for this test in female mice. Study weaknesses: No positive control included, historical control data is lacking, lack of consistency in the control data sets between sexes, data on the ratios of micronucleated normochromatic erythrocytes per thousand normochromatic erythrocytes, together with their standard errors, appeared to be random.

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

Chemical Name	Test System	Test Object	Route	Dose	Result	Reference	Comments
(Isoeugenol [04.004]) cont.	Micronucleus Induction in bone marrow	Mouse Males and Females	Oral gavage	500, 1000, 2000 mg/kg bw (males) 500, 1000, 1500 mg/kg bw (females) (2 doses 24 hours apart)	M: Negative F: Negative	(EMEA, 2009)	No statistically significant increases in the frequency of micronucleated polychromatic erythrocytes in the treated mice as compared with the vehicle group. Some of the female vehicle control animals had an increased proportion of PCE. As a consequence of this high control value, a decrease of the proportion of PCE (%) was seen in the isoeugenol high dosed female group. The values were within the historical range.
	In vivo DNA repair (UDS) test in rat hepatocytes	Rat Males and females	Oral gavage	600, 2000 mg/kg bw (males) 600, 1250 mg/kg bw (females) (2 doses 14 hours apart)	M: Negative F: Negative	(EMEA, 2009)	No significant increases in the mean (gross) nuclear grain count or mean net nuclear grain count at any dose level compared to the vehicle control values.
	Wing spot test	*Drosophila melanogaster*		164 to 2460 mg/l	Negative*	(EMEA, 2009)	
(6-Ethoxyprop-3-enylphenol [04.002])	Sex Linked Recessive Lethal Chromosomes	*Drosophila melanogaster*	Oral	10 mM (1782 microg/ml)	Negative	(Wild et al., 1983)	
	Micronucleus Induction	Mouse	Intraperitoneally	649, 1298, and 1947 mg/kg	Negative	(Wild et al., 1983)	
(Isoeugenyl phenylacetate [09.710])	Sex Linked Recessive Lethal Chromosomes	*Drosophila melanogaster*	Oral	25 mM (7059 microg/ml)	Negative	(Wild et al., 1983)	
	Micronucleus Induction	Mouse	Intraperiotenally twice within a 24-hour period	564, 987, and 1410 mg/kg	Negative	(Wild et al., 1983)	

M=Male; F=Female.

** Using both normal bioactivation enzyme systems and increased cytochrome P450-dependent biotranformation capacity.*

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ABBREVIATIONS

ADI	Acceptable Daily Intake
BW	Body Weight
CAS	Chemical Abstract Service
CEF	Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids Chemical Abstract Service
CHO	Chinese hamster ovary (cells)
CoE	Council of Europe
DNA	Deoxyribonucleic acid
EC	European Commission
EFFA	European Food and Fragrance Authority
EFSA	The European Food Safety Authority
EMEA	The European Medicines Agency
EU	European Union
FAO	Food and Agriculture Organization of the United Nations
FEMA	Flavor and Extract Manufacturers Association
FGE	Flavouring Group Evaluation
GD	Gestational Day
FLAVIS (FL)	Flavour Information System (database)
ID	Identity
IOFI	International Organization of the Flavour Industry
IR	Infrared spectroscopy
JECFA	The Joint FAO/WHO Expert Committee on Food Additives
LD_{50}	Lethal Dose, 50%; Median lethal dose
MS	Mass spectrometry
MSDI	Maximised Survey-derived Daily Intake
mTAMDI	Modified Theoretical Added Maximum Daily Intake
NAD	Nicotinamide Adenine Dinucleotide
NADP	Nicotinamide Adenine Dinucleotide Phosphate
No	Number
NOAEL	No Observed Adverse Effect Level
NOEL	No Observed Effect Level
NTP	National Toxicology Program
OECD	Organisation for Economic Co-operation and Development
PCE	Polychromatic erythrocyte
SCE	Sister Chromatid Exchange
SCF	Scientific Committee on Food

SMART	Somatic Mutation and Recombination Test
TAMDI	Theoretical Added Maximum Daily Intake
UDS	Unscheduled DNA Synthesis
WHO	World Health Organisation