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SCIENTIFIC OPINION

Scientific Opinion on Flavouring Group Evaluation 82, Revision 1 (FGE.82Rev1): Consideration of Epoxides evaluated by the JECFA (65th meeting)[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 consider evaluations of flavouring substances assessed since 2000 by the Joint FAO/WHO Expert Committee on Food Additives (the JECFA), and to decide whether further evaluation is necessary, as laid down in Commission Regulation (EC) No 1565/2000. The present consideration concerns a group of five epoxides evaluated by the JECFA at the 65th meeting in 2005. This revision is made due to inclusion of one additional substance, beta-ionone epoxide [FL-no: 07.170], cleared for genotoxicity concern and due to additional toxicity data have become available for beta-caryophyllene epoxide [FL-no: 16.043]. Since publication of FGE.82 one substance epoxy oxophorone [FL-no: 16.051] is no longer supported for use as flavouring substances in Europe by Industry and will therefore not be considered any further. The 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. For four substances [FL-no: 16.015, 16.018, 16.040 and 16.043] the Panel agreed with the JECFA conclusion, "No safety concern at estimated levels of intake as flavouring substances" based on the MSDI approach. For one substance [FL-no: 07.170] additional toxicity data are required. Besides the safety assessment of these flavouring substances, the specifications for the materials of commerce have also been considered and for four substances, the information is adequate; but for the substance [FL-no: 07.170] further information on stereoisomerism is required.

KEY WORDS

epoxides, flavourings, food safety, JECFA 65th meeting, FGE.82

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[1] On request from the European Commission, Question No EFSA-Q-2013-00199, adopted on 21 May 2014.

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SUMMARY

Following a request from the European Commission, the EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF Panel) was asked to deliver 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 CEF Panel was requested to consider the Joint FAO/WHO Expert Committee on Food Additives (the JECFA) evaluations of flavouring substances assessed since 2000, and to decide whether no further evaluation is necessary, as laid down in Commission Regulation (EC) No 1565/2000. These flavouring substances are listed in the Register, which was adopted by Commission Decision 1999/217/EC and its consecutive amendments.

In Flavouring Group Evaluation 82, the EFSA considered five epoxides evaluated by the JECFA at its 65th meeting. Since publication of FGE.82, one substance [FL-no: 16.051] is no longer supported for use as flavouring substance in Europe by Industry and will therefore not be considered any further. The present revision is made due to inclusion of one additional substance, beta-ionone epoxide [FL-no: 07.170], cleared for genotoxicity concern in FGE.210 Revision 1 and due to additional toxicity data have become available for beta-caryophyllene epoxide [FL-no: 16.043] requested in previous version.

The Panel concluded that no structurally related substances evaluated by EFSA are available for these five epoxides in FGE.82, evaluated in the JECFA flavouring group of epoxides.

Of the further four substances evaluated by the JECFA in this group one is not in the Register and two are α,β-unsaturated aldehydes and ketones. These two will be evaluated together with other α,β -unsaturated aldehydes and ketones.

The Panel does not agree with the application of the Procedure for the five epoxides as performed by the JECFA. For the five substances [FL-no: 07.170, 16.015, 16.018, 16.040 and 16.043] it cannot be concluded that they are metabolised to innocuous substances and therefore their evaluation must proceed via the B-side of the Procedure scheme.

A 90-day study on beta-caryophyllene epoxide [FL-no: 16.043] has become available and a NOAEL of 109 mg/kg bw/day to provide adequate margin of safety is derived.

The Panel considered that four of the five substances [FL-no: 16.043, 16.015, 16.018 and 16.040] evaluated through the Procedure via the B-side of the Procedure scheme were of no safety concern at the estimated levels of intake based on the MSDI approach.

The evaluation of beta-ionone epoxide [FL-no: 07.170] cannot be finalised at step B4 of the Procedure because a NOAEL from a 90-day study is not available.

For the three substances [FL-no: 16.015, 16.018 and 16.040], evaluated through the Procedure, use levels are needed to calculate the mTAMDIs in order to identify those flavouring substances that need more refined exposure assessment and to finalise their evaluation.

In order to determine whether the conclusion for the five 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 four JECFA evaluated substances [FL-no: 16.015, 16.018, 16.040 and 16.043]. For one substances [FL-no: 07.170] information on stereoisomeric composition is not adequate.

Thus, for one substance evaluated through the Procedure [FL-no: 07.170] the Panel has reservations (data missing on stereoisomerism, need for additional toxicity data).

For the remaining substances [FL-no: 16.015, 16.018, 16.040 and 16.043] the Panel agrees with the JECFA conclusion “No safety concern at estimated levels of intake as flavouring substances based on the MSDI approach.

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BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

The use of flavourings is regulated under Regulation (EC) No 1334/2008 of the European Parliament and Council of 16 December 2008[4] on flavourings and certain food ingredients with flavouring properties for use in and on foods. On the basis of Article 9(a) of this Regulation, an evaluation and approval are required for flavouring substances.

The Union list of flavourings and source materials was established by Commission Implementing Regulation (EC) No 872/2012[5]. The list contains flavouring substances for which the scientific evaluation should be completed in accordance with Commission Regulation (EC) No 1565/2000[6].

EFSA has considered the Joint FAO/WHO Expert Committee on Food Additives (the JECFA) evaluation of the flavouring substance beta-caryophyllene epoxide [FL-no: 16.043] in the flavouring group evaluation 82 (FGE.82). The opinion was adopted on 1 April 2008.

EFSA concluded in its opinion that for beta- caryophyllene epoxide or for structurally related substances a No Observed Adverse Effect Level (NOAEL) could not be derived. Accordingly, additional toxicity data are required for this substance.

The requested information on the beta-caryophyllene epoxide has now been submitted by the European Flavour Association. The Commission asks EFSA to evaluate this new information and depending on the outcome proceed to full evaluation of the flavouring substance.

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

The European Commission requests the European Food Safety Authority to carry out a safety assessment on the flavouring substances, beta-ionone epoxide [FL-no: 07.170] and beta-caryophyllene epoxide [FL-no: 16.043] in accordance with Commission Regulation (EC) No 1565/2000.

INTERPRETATION OF THE TERMS OF REFERENCE

As additional genotoxicity data have been submitted for beta-ionone epoxide [FL-no: 07.170], the European Commission request EFSA to carry out a safety assessment in accordance with Commission Regulation (EC) No 1565/2000.

Beta-ionone epoxide [FL-no: 07.170] was first allocated to FGE.210Rev1 for evaluation with respect to genotoxicity. Based on the new genotoxicity data submitted, the Panel concluded that [FL-no: 07.170] does not give rise to concern with respect to genotoxicity and can accordingly now be evaluated through the Procedure in FGE.82Rev1.

[4] Regulation (EC) No 1334/2008 of the European Parliament and of the Council of 16 December 2008 on flavourings and certain food ingredients with flavouring properties for use in and on foods and amending Council Regulation (EEC) No 1601/91, Regulations (EC) No 2232/96 and (EC) No 110/2008 and Directive 2000/13/EC. OJ L 354, 31.12.2008, p. 34-50.

[5] Commission implementing Regulation (EU) No 872/2012 of 1 October 2012 adopting the list of flavouring substances provided for by Regulation (EC) No 2232/96 of the European Parliament and of the Council, introducing it in Annex I to Regulation (EC) No 1334/2008 of the European Parliament and of the Council and repealing Commission Regulation (EC) No 1565/2000 and Commission Decision 1999/217/EC. OJ L 267, 2.10.2012, p, 1-161.

[6] 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. OJ L 180, 19.7.2000, p. 8-16.

ASSESSMENT

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

The following issues are of special importance.

Intake

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

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

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

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 (µg)/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 µg 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 µg per day?")" (JECFA, 1999).

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 µg 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 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. History of the Evaluation of the Substances in the Present FGE

In FGE.82, which contains a group of five epoxides, the Panel concluded that epoxy oxophorone [FL-no: 16.051] should not be evaluated through the Procedure, due to concern with respect to genotoxicity. For beta-caryophyllene epoxide [FL-no: 16.043] a NOAEL could not be derived for the substance or for structurally related substances. Accordingly, additional toxicity data were required for this substance.

Industry has informed that the substance epoxy oxophorone [FL-no: 16.051] is no longer supported for use as flavouring substances in Europe (EFFA, 2009) and the substance will therefore not be considered any further.

FGE	Opinion adopted	Link	No. of substances
FGE.82	1 April 2008	http://www.efsa.europa.eu/en/efsajournal/pub/917.htm	5
FGE.82Rev1			5

The present revision of FGE.82, FGE.82Rev1, includes the consideration of one additional substance, beta-ionone epoxide [FL-no: 07.170]. This substance is an α,β-unsaturated epoxide and was originally allocated to and evaluated in FGE.210Rev1 (EFSA CEF Panel, 2014) in which it was considered not to be of concern with respect to genotoxicity.

Additional toxicity data, a 14-day range finding study and a 90-day dietary study, have now been provided for beta-caryophyllene epoxide [FL-no: 16.043] (Bauter, 2012; Bauter, 2013).

New information from Industry on missing stereoisomeric composition for [FL-no: 16.015, 16.018 and 16.040] is also included in the present revision (EFFA, 2013).

2. Presentation of the Substances in the JECFA Flavouring Group

2.1. Description

2.1.1. JECFA Status

The JECFA has at its 65th meeting in 2005 evaluated a group of nine flavouring substances consisting of epoxides.

2.1.2. EFSA Considerations

One of these is not in the Register, trans-carvone-5,6-oxide (JECFA-no: 1572). Three are α,β -unsaturated aldehydes or ketones [FL-no: 07.170, 16.044 and 16.071] and will be evaluated together with other α,β -unsaturated aldehydes and ketones. The α,β-unsaturated epoxide, beta-ionone epoxide [FL-no: 07.170] was evaluated in FGE.210Rev1 (EFSA CEF Panel, 2014) in which the substance was considered not to be of concern with respect to genotoxicity. The substance is therefore included in this revision of FGE.82. One substance [FL-no: 16.051] is no longer supported for use as flavouring substances in Europe and will therefore not be considered any further. Therefore this consideration only deals with five substances. The Panel concluded that no corresponding FGE is available.

2.2. Isomers

2.2.1. Status

All five substances [FL-no: 07.170, 16.015, 16.018, 16.040 and 16.043] in the group of JECFA evaluated epoxides have one or more chiral centres.

2.2.2. EFSA Considerations

Adequate information on isomeric composition is available for four substances. Information is lacking about the stereoisomerism for one substance [FL-no: 07.170].

2.3. Specifications

2.3.1. Status

JECFA specifications are available for all five substances (JECFA, 2005) (see Table 2).

2.3.2. EFSA Considerations

Specifications including complete purity criteria and identity are available for four substances [FL-no: 16.015, 16.018, 16.040 and 16.043]. Information about the stereoisomerism for one substance [FL-no: 07.170] is lacking. (see Section 2.2.2 and Table 3).

3. Intake Estimation

3.1. Status

For all five substances evaluated through the JECFA Procedure production volumes, based on which MSDI values can be calculated, are available for the EU (see Table 5).

3.2. EFSA Considerations

For two of the JECFA evaluated substances normal and maximum use levels have been provided by the Flavour Industry [FL-no: 07.170 and 16.043] (EFFA, 2004) (see Table 1). Based on these normal

use levels, mTAMDI figures (see Table 2) can be calculated. For definition of normal and maximum use levels and description of the method for calculation of mTAMDI consult Annex II in e.g. (EFSA, 2004).

Table 1: Table B.1. Normal and Maximum use Levels (mg/kg) available for JECFA evaluated Substances

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**
07.170	3 15	2 10	3 15	2 10	- -	4 20	2 10	5 25	1 5	1 5	- -	- -	2 10	3 15	- -	4 20	5 25	2 10
16.043	3 15	2 10	3 15	2 10	- -	4 20	2 10	5 25	1 5	1 5	- -	- -	2 10	3 15	- -	4 20	5 25	2 10
16.051	3 15	2 10	3 15	2 10	- -	4 20	2 10	5 25	1 5	1 5	- -	- -	2 10	3 15	- -	4 20	5 25	2 10

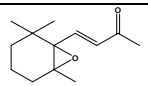
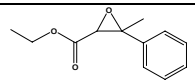
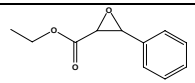
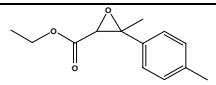
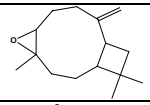
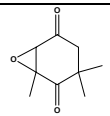
Table 2: Estimated intakes based on the MSDI- and the mTAMDI approach

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)
07.170	beta-Ionone epoxide	0.073	0.1	1000	Class III	90
16.040	Ethyl 2,3-epoxy-3-methyl-3-p-tolylpropionate	20	0.009	ND	Class III	90
16.043	beta-Caryophyllene epoxide	8	0.1	1000	Class III	90
16.051	Epoxy oxophorone	0.012	0.2	1000	Class III	90
16.015	Ethyl methylphenylglycidate	205	1840	ND	Class III	90
16.018	Ethyl 3-phenyl-2,3-epoxypropionate	97	96	ND	Class III	90

ND: No intake data available

SUMMARY OF SPECIFICATION DATA

Table 3: Specification Summary of the Substances in the JECFA Flavouring Group (JECFA, 2005)

FL-no JECFA -no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility [(a)] Solubility in ethanol [(b)]	Boiling point, °C [(c)] Melting point, °C ID test Assay minimum	Refrac. Index [(d)] Spec.gravity [(e)]	EFSA comments / Reference for specifications
07.170 1571	beta-Ionone epoxide f		4144 11202 23267-57-4	Solid $C_{13}H_{20}O_2$ 208.30	Insoluble Soluble	 48 NMR MS 95 %	n.a. n.a.	
16.015 1577	Ethyl methylphenylglycidate		2444 6002 77-83-8	Liquid $C_{12}H_{14}O_3$ 206.24	Insoluble Soluble	272-275 IR 98 %	1.504-1.513 1.086-1.096	Mixture of diastereoisomers (two cis (15-25 % each) - and two trans-forms (25-35 % each) around oxirane ring) (EFFA, 2013).
16.018 1576	Ethyl 3-phenyl-2,3-epoxypropionate		2454 11844 121-39-1	Liquid $C_{11}H_{12}O_3$ 192.21	Insoluble Soluble	96 (0.7 hPa) IR 98 %	1.516-1.521 1.120-1.125	Mixture of diastereoisomers (two cis (15-25 % each) - and two trans-forms (25-35 % each) around oxirane ring) (EFFA, 2013).
16.040 1578	Ethyl 2,3-epoxy-3-methyl-3-p-tolylpropionate		3757 11707 74367-97-8	Liquid $C_{13}H_{16}O_3$ 220.27	Insoluble Soluble	123-125 NMR 96 %	1.523-1.529 1.081-1.087 (20°)	Mixture of diastereoisomers (two cis (15-25 % each) - and two trans-forms (25-35 % each) around oxirane ring) (EFFA, 2013).
16.043 1575	beta-Caryophyllene epoxide		4085 10500 1139-30-6	Solid $C_{15}H_{24}O$ 220.36	Insoluble Soluble	 61 NMR MS 95 %	n.a. n.a.	Register name to be changed to 1R,4R,6R,10S-beta-caryophyllene epoxide.
16.051 1573	Epoxy oxophorone		 38284-11-6	Solid $C_9H_{12}O_3$ 168.19	Insoluble Soluble	 157 NMR MS 95 %	n.a. n.a.	No longer supported by Industry, DG SANCO, 2012. Racemic mixture (Me up & epoxy down or Me down and epoxy up) configuration of epoxyde up or down i.e. racemate (EFFA, 2010a).

(a): Solubility in water, if not otherwise stated.
(b): Solubility in 95 % ethanol, if not otherwise stated.
(c): At 1013.25 hPa, if not otherwise stated.
(d): At 20°C, if not otherwise stated.
(e): At 25°C, if not otherwise stated.
(f): Stereoisomeric composition not specified.

4. GENOTOXICITY DATA

4.1. Genotoxicity Studies – Text Taken[7] from the JECFA (JECFA, 2006b)

In vitro

In standard assays for reverse mutation in *Salmonella typhimurium*, ethyl methylphenylglycidate [FL-no: 16.015] and caryophyllene oxide (beta-caryophyllene epoxide) [FL-no: 16.043] were consistently non-mutagenic in strains TA98, TA100, TA1535, TA1537 and TA1538 at concentrations of up to 10,000 µg/plate, with and without metabolic activation (S9) (Richold et al., 1979; Voogd et al., 1981; Wild et al., 1983; Canter et al., 1986). Ethyl 3-phenylglycidate (ethyl-3-phenyl-2,3- epoxyproprionate) [FL-no: 16.018] gave inconsistent results in this test: at ≤ 4,000 µg/plate, it did not induce reverse mutations in *S. typhimurium* TA1535, TA1537 or TA1538 with or without metabolic activation (Wild et al., 1983; Tilch and Elias, 1984; Canter et al., 1986); however negative results were reported in several studies in TA98 incubated with ethyl 3-phenylglycidate at concentrations ≤ 4,000 µg/plate, irrespective of metabolic activation (Wild et al., 1983; Tilch and Elias, 1984; Canter et al., 1986; Wagner and Walton, 1999), whereas increased mutagenicity was found in TA98 with metabolic activation (≤ 5,000 µg/plate). In the absence of metabolic activation, the frequency of revertants was comparable to that of controls (Wagner and Walton, 1999).

Although Canter et al. (1986) reported negative results in TA100 incubated with ethyl 3-phenylglycidate at ≤ 2,200 µg/plate with and without metabolic activation, positive results were reported with and without metabolic activation in other studies (Voogd et al., 1981; Wild et al., 1983; Tilch and Elias, 1984; Wagner and Walton, 1999). In the study of Voogd et al. (1981), positive results were found only at the two highest concentrations (1,000 and 2,000 µg/ml), with and without metabolic activation. Only a weak mutagenic response was reported by Tilch and Elias (1984) and only in the presence of metabolic activation. In an assay in which the mutagenicity of a pepsin and pancreatin digest of ethyl 3-phenylglycidate (≤ 10,000 µg/plate), simulating mammalian digestion, was investigated in several strains of *S. typhimurium* including TA100, no increase in the mutagenic response was observed (Tilch and Elias, 1984).

In *Escherichia coli* PQ37, ethyl 3-phenylglycidate [FL-no: 16.018] was not mutagenic in the SOS Chromotest with or without metabolic activation (von der Hude et al., 1990a).

Ethyl 3-phenylglycidate [FL-no: 16.018] was clastogenic in Chinese hamster ovary (103 µg/mL) and V79 cells (480.5 µg/mL) with and without metabolic activation (Tilch and Elias, 1984; von der Hude et al., 1991); however, when ethyl 3-phenylglycidate was pre-treated with artificial digestive juices conducive to the formation of the corresponding diol and, subsequently, incubated with Chinese hamster ovary cells, no sister chromatid exchange was reported at concentrations ≤ 3,280 µg/mL (Tilch and Elias, 1984).

Ethyl methylphenylglycidate [FL-no: 16.015] at ≤ 160 µg/ml induced a significant increase in the number of sister chromatid exchanges when tested in the absence of rat liver S9; however, no increase in sister chromatid exchange frequency was observed in the presence of metabolic activation (≤ 500 µg/mL). Similarly, ethyl methylphenylglycidate ≤ 500 µg/ml induced chromosomal aberrations without metabolic activation. Although aberrations also occurred in the presence of S9, the results were considered equivocal owing to the lack of statistical significance in the Dunnett test (Galloway et al., 1987).

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

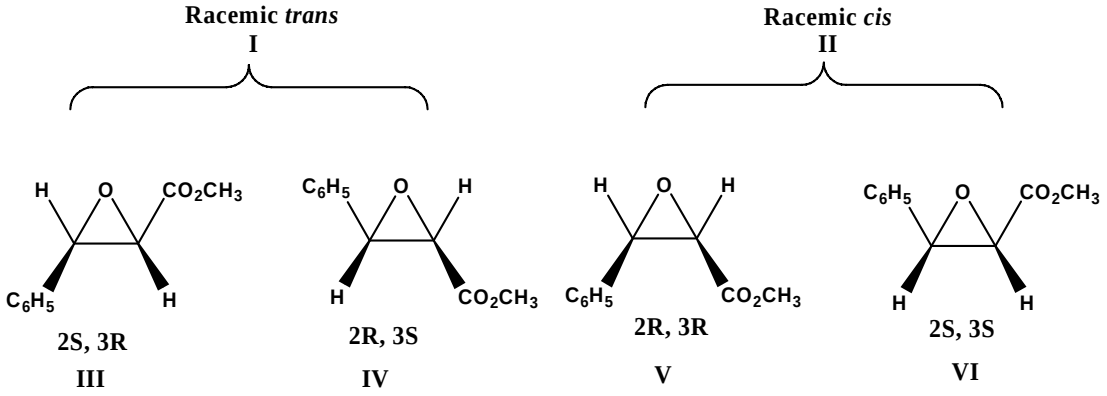


Figure 1: Structures and absolute configurations of optically active trans- and cis-methyl epoxycinnamate (3-phenyl-2,3-epoxypropanoate) and the urinary metabolites isolated from rats. (From (Rietveld et al., 1988))

In order to assess further the potential mutagenicity and genotoxicity of the epoxides, the results of a number of assays performed with structurally related glycidic and cycloaliphatic epoxide compounds were reviewed. The structurally related glycidic ester, racemic cis-methyl epoxycinnamate (II; see Figure 1) at ≤ 15,000 µg/plate was not mutagenic to *S. typhimurium* strains TA98, TA100, TA1535, TA1537 and TA1538 with or without metabolic activation in the plate incorporation test. In contrast, racemic trans-methyl epoxycinnamate (I; see Figure 1) at ≤ 15,000 µg/plate was mutagenic in *S. typhimurium* strains TA1535, TA1537, TA1538 and TA100, but not TA 98, without metabolic activation. With metabolic activation, no significant increases in mutagenic activity were observed in any of the tester strains. When the individual diastereomers of cis- (V and VI; see Figure 1) and trans-methyl epoxycinnamate (III and VI; see Figure 1) were incubated at 1,500 or 3,000 µg/plate with *S. typhimurium* TA100 in the absence of metabolic activation, the trans isomers showed the greatest mutagenicity, compound IV being the most active (IV < III < VI < V) (Rietveld et al., 1988). The authors correlated these results with the increased N-alkylating potential of trans isomers discussed above.

The potential genotoxicity of several structurally related aliphatic cyclic epoxides was also investigated in the Ames assay and SOS Chromotest. Although cyclopentane oxide and cyclohexane oxide were mutagenic in the Ames test, neither was active in the SOS Chromotest. Furthermore, cyclooctane oxide, cyclododecane oxide, (-)-2,3-epoxypinane and (+)-limonene oxide gave uniformly negative results in both assays (Basler et al., 1989). No other experimental details were provided in this abstract.

A series of cycloaliphatic epoxides was evaluated in the Ames assay with the standard battery of *S. typhimurium* strains (TA98, TA100, TA1535, TA1537 and TA1538) without metabolic activation. After incubation with cyclopentane oxide, cyclohexane oxide or norbornane oxide at 15-60 µmol/l, significant increases in the number of reverse mutations were observed only in TA100 and TA1535 in comparison with controls (Frantz and Sinsheimer, 1981). In another study, cyclohexane oxide also gave negative results in the standard Ames plate assay, but a significant increase in the number of reverse mutations was observed in *S. typhimurium* TA100 in a liquid test system (0.33 to 10 mmol/l) (Turchi et al., 1981). In a comparison of the potential mutagenicity of cycloalkane epoxides with increasingly expanding ring sizes (cyclopentane oxide, cyclohexane oxide, cycloheptane oxide, cyclooctane oxide and cyclododecane oxide) in *S. typhimurium* TA1535 and TA100 (12 µmol/plate), statistically significant mutagenic responses were obtained with cyclopentane oxide and cyclohexane oxide. Moreover, a slight but statistically significant increase in revertant frequency was observed with cycloheptane oxide, although the increase occurred in the presence of marked cytotoxicity. The mutation frequency observed with cyclooctane oxide was comparable to the spontaneous background levels. No viable colonies were detected following incubation of either *S. typhimurium* tester strain with cyclododecane oxide. Generally, therefore, the genotoxic potential of cycloaliphatic epoxides appears to be related inversely to the ring size; however, compounds with increasing ring sizes were

shown to be increasingly cytotoxic. Consequently, specially constructed base-pair mutagen detector *S. typhimurium* strains (i.e., TA92, TA1950 and TA2410), which have a normal lipopolysaccharide cell-wall coating as opposed to the more permeable coating of the TA100 and TA1535 strains, were used in an attempt to separate the mutagenic response from the confounding toxicity. The ratios of mutagenicity to relative toxicity observed after incubation of the normally coated strains with cyclohexane oxide, cyclooctane oxide and cyclododecane oxide were comparable to those observed in the more permeable strains, indicating that the increase in the mutation frequency was not due to the concomitant cytotoxicity (Frantz and Sinsheimer, 1981).

In eukaryotic V79 Chinese hamster cells, a weak but concentration-dependent increase in the occurrence of sister chromatid exchanges were observed in the presence of epoxycyclopentane and epoxycyclohexane. In contrast, V79 Chinese hamster cells incubated with epoxycyclooctane, epoxycyclododecane or (+)-limonene oxide showed no increase in sister chromatid exchange (von der Hude et al., 1991). Significant increases in mutant frequencies were reported when cyclohexane oxide (≤ 5 mmol/l) was incubated with V79 Chinese hamster cells. Cyclohexane oxide (10 mmol/l) also increased the micronucleus frequency and the number of chromosomal aberrations (bridges and lagging chromosomes) in the Chinese hamster cells (Turchi et al., 1981).

1,2-Epoxyoctane, 1,2-epoxydecane, epoxycyclooctane, epoxycyclododecane, (+)-limonene oxide, α-pinane oxide and cis-2,3-epoxysuccinic acid did not induce unscheduled DNA synthesis in primary rat hepatocytes (von der Hude et al., 1990b).

In vivo

The potential of ethyl methylphenylglycidate [FL-no: 16.015] and ethyl 3-phenylglycidate [FL-no: 16.018] to induce sex-linked recessive lethal mutations in adult *Drosophila melanogaster* was studied in the Basc test. The mutation frequency was significantly increased in flies after 3-days' exposure to 2.5 or 10 mmol/l solutions of ethyl 3-phenylglycidate (480 µg/ml) or ethyl methylphenylglycidate (2062.4 g/ml), respectively; however, the increases were statistically significant in only one of the experiments conducted with each compound and only in the first of three broods tested. Although the isolated increase in mutation frequency observed with ethyl 3-phenylglycidate did not affect the overall number of sex-linked recessive lethal mutations in brood 1, the total number of mutations in brood 1 after exposure to ethyl methylphenylglycidate was significantly greater than in controls. The authors concluded that ethyl methylphenylglycidate is only weakly mutagenic in *Drosophila* (Wild et al., 1983).

The frequencies of micronucleated bone-marrow erythrocytes obtained from groups of four male and four female NMRI mice 30 hours after administration of a single intraperitoneal dose of 619, 1237 or 1,856 mg/kg bw of ethyl methylphenylglycidate [FL-no: 16.015] or 577, 961 or 1,538 mg/kg bw of ethyl 3-phenylglycidate [FL-no: 16.018] were comparable to those in the corresponding controls (Wild et al., 1983).

Conclusion on genotoxicity

The genotoxic potential of glycidate and alicyclic epoxides was studied in several standard assays in bacteria and mammalian cells *in vitro*. In the Ames assay for reverse mutation, both caryophyllene oxide [FL-no: 16.043] and ethyl methylphenylglycidate [FL-no: 16.015] gave unequivocally negative results with and without metabolic activation in a series of standard *S. typhimurium* tester strains. Ethyl 3-phenylglycidate [FL-no: 16.018] gave positive results only in *S. typhimurium* strains TA100 and TA98 and mainly in the presence of metabolic activation; however, a digest of ethyl 3-phenylglycidate had no mutagenic potential in *S. typhimurium* TA100 and TA98 with or without metabolic activation. Negative results were reported with cis-methyl epoxycinnamate, a structurally related glycidic ester, in the standard battery of *S. typhimurium* tester strains, but trans-methyl epoxycinnamate was mutagenic in TA1535, TA1537, TA1538 and TA100, only in the absence of metabolic activation. Cyclopentane and cyclohexane oxide induced reverse mutation in *S.*

typhimurium strains TA1535 and TA100. Although, cycloaliphatic epoxides of larger ring size were generally less mutagenic activity than the smaller-ring epoxides, marked cytotoxicity was seen. Uniformly negative results were obtained in the SOS Chromotest in *E. coli*. Although clastogenic activity was reported in mammalian cell lines with most glycidate and alicyclic epoxides, increased sister chromatid exchange frequencies were observed with ethyl methylphenylglycidate only in the absence of metabolic activation. Likewise, chromosomal aberrations were reported in Chinese hamster ovary cells incubated with ethyl methylphenylglycidate or cyclohexane oxide, but the results with ethyl methylphenylglycidate were equivocal when S9 activation was incorporated into the assay. No unscheduled DNA synthesis was found with a series of epoxyalkanes and alicyclic epoxides.

In vivo, ethyl methylphenylglycidate had slight potential to induce sex-linked recessive lethal mutations in *Drosophila*, but ethyl 3-phenylglycidate showed no activity. Ethyl 3-phenylglycidate and ethyl methylphenylglycidate did not induce micronucleous formation in mice given single intraperitoneal doses.

Epoxides are naturally occurring substances that are also added to food as flavouring agents. The principal epoxide used in this way is ethyl methylphenylglycidate. Studies on the metabolism of glycidate and alicyclic (including terpene) epoxides indicate that these compounds are readily and adequately detoxicated in animals via two pathways, GSH conjugation and hydrolysis in the gastrointestinal tract or other tissues, followed by glucuronic acid or sulphate conjugation in the liver. Although glycidate and alicyclic epoxides had some genotoxic potential in standard assays *in vitro*, the results of assays for genotoxicity in mammals *in vivo* were negative. Furthermore, a number of long-term studies with dietary administration provided no evidences of carcinogenic potential. Several long-term studies with repeated doses of ethyl methylphenylglycidate showed no carcinogenicity even at intake levels that were orders of magnitude higher than the intake of epoxides added as flavouring agents. The NOEL in the 2-year bioassay of ethyl methylphenylglycidate was 35 mg/kg bw per day. This intake level is > 1100 times the daily per capita intake ("eaters only") of 0.031 mg/kg bw per day from use of ethyl methylphenylglycidate as a flavouring agent.

The known pathways of metabolic detoxication, the lack of evidence of carcinogenicity in long-term feeding studies and the lack of genotoxic potential *in vivo* indicate that it is unlikely that epoxides pose a significant genotoxic risk to humans under the conditions of their use as flavouring agents.

For a summary of *in vitro / in vivo* genotoxicity data considered by the JECFA see Table.4.

4.2. Genotoxicity Studies – Text Taken[8] from EFSA FGE.210Rev1 (EFSA CEF Panel, 2014)

Bacterial Reverse Mutation Assay

beta-Ionone epoxide [FL-no: 07.170] was tested for mutagenicity in an Ames test including four strains of *S. typhimurium* (TA98, TA100, TA1535 and TA1537) at five concentrations (5, 15, 50, 150, 500 μg/plate) in the absence and in the presence of metabolic activation (S9-mix at two different concentrations, 3 and 10 %) (Jones and Wilson, 1988). The study was performed under GLP and mainly compliant with OECD Guideline 471 (OECD, 1997), except that only four strains were used. Two independent experiments were performed and the top concentration was selected at 500 μg/plate based on toxicity in a prior range-finding test. At the concentration tested no significant toxicity was observed and no substantial increases in mutation were observed in all strains tested and in presence or absence of S9.

A more recently reported Ames study on beta-ionone-epoxide included four strains of *S. typhimurium* (TA97a, TA98, TA100 and TA1535) plus one strain of *E. coli* (WP2-uvrA-) (Kringstad, 2005). Following a range-finding assay, beta-ionone was tested in three replicates at 501, 1582 and 5000

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

µg/plate in the absence of S9-mix metabolic activation and at 158, 501 and 1582 µg/plate in the presence of metabolic activation, in a single experiment using the plate incorporation method. The top concentration (5000 µg/plate) induced significant toxicity in strain TA97a in the absence of S9-mix and also reduced the background lawn in strain TA100 in the presence and absence of S9-mix and therefore the study complies with current recommendations for the choice of concentration. There was no evidence of mutagenicity. Since there are some deviations from the OECD Guideline 471 (OECD, 1997) (only three concentrations of chemical were tested, in some cases only two concentrations could be analysed due to an excessive level of cytotoxicity and only a single experiment was performed) the test is considered of limited validity.

Mouse lymphoma thymidine kinase gene mutation assay

An assay for induction of *tk* mutations in mouse lymphoma cells (L5178Y T/K +/- 3.7.2c) was conducted on beta-ionone-epoxide [FL-no: 07.170] (Flanders, 2006). It included four hours treatment in the absence and presence of S9-mix and a 24 hours treatment in the absence of S9-mix. The concentrations were selected based on a preliminary toxicity test. The test groups included single replicates at 8 concentrations ranging from 200 to 900 µg/ml in the four hours treatment arm and from 4.1 to 520 µg/ml in the 24 hours treatment arm. The maximum concentration was limited by toxicity. The substance did not induce biologically or statistically significant increases in mutant frequency and therefore it was considered non-mutagenic in this assay. The study is compliant with OECD Guideline 476 (OECD, 1997).

For a summary of *in vitro* genotoxicity data considered by the EFSA in FGE.210Rev1, see Table 5.

4.3. EFSA Considerations

Data from *in vitro* tests are available for four substances [FL-no: 07.170, 16.015, 16.018 and 16.043]. Data from *in vivo* tests are available for two substances [FL-no: 16.015 and 16.018].

In vitro

The epoxide group and the terminal double bond in beta-caryophyllene epoxide [FL-no: 16.043] represent structural alerts for a genotoxic potential. However this substance was tested for bacterial gene mutations in a valid Ames test (TA98, TA100, TA1535, TA1537 and TA1538) where negative results were reported (Table 4).

The substance ethyl 3-phenyl-2,3-epoxypropionate [FL-no: 16.018] was positive in bacterial gene mutation tests (Ames) in strain TA100 in the majority of the available studies either in the presence of S9 or both in the absence and in the presence of S9. The effect was dose-related and occurred only in studies in which the concentration range tested reached cytotoxic levels. Negative responses were observed in strains TA1535, TA1537, TA1538 and TA98. Furthermore, ethyl 3-phenyl-2,3-epoxypropionate induced forward mutations in mammalian cells (HGPRT locus test in Chinese hamster ovary (CHO) cells) both in the absence and presence of metabolic activation and induced sister chromatid exchanges (SCE) in CHO cells in two studies, one of which had only a limited report. Ethyl 3-phenyl-2,3-epoxypropionate was negative in an SOS chromotest (Table 4).

The substance ethyl methylphenylglycidate [FL-no: 16.015] was consistently negative in the available bacterial gene mutation studies at up to cytotoxic concentrations. In contrast, ethyl methylphenylglycidate induced SCE and chromosomal aberrations in CHO cells in the absence of metabolic activation, while in the presence of S9 the observed response was negative for SCE and equivocal for chromosomal aberrations (Table 4).

In FGE.210Rev1 the concern for genotoxicity for beta-ionone epoxide [FL-no: 07.170] was ruled out (Table 5).

In vivo

In *in vivo* studies, ethyl 3-phenyl-2,3-epoxypropionate [FL-no: 16.018] did not induce mutations in *Drosophila melanogaster*, whereas ethyl methylphenylglycidate [FL-no: 16.015] was found weakly positive in the same study. For both substances a negative result was reported in a micronucleus test in the mouse bone marrow, the validity of which, however, cannot be evaluated.

The genotoxic potential of ethyl methylphenylglycidate [FL-no: 16.015] indicated by the *in vitro* studies was not substantiated by the *in vivo* micronucleous studies. Nevertheless, the available carcinogenicity study in rats (2 years) (Dunnington et al., 1981) gives no evidence for a carcinogenic activity of ethyl methylphenylglycidate. Ethyl methylphenylglycidate is structurally closely related to the candidate substances ethyl 3-phenyl-2,3-epoxypropionate [FL-no: 16.018] and ethyl 2,3-epoxy-3-methyl-3-*p*-tolylpropionate [FL-no: 16.040]. For the latter, no genotoxicity data are available. It is therefore concluded that the structural alert for genotoxicity of the two compounds as well as the evidence for a genotoxic potential from the available *in vitro* studies on ethyl 3-phenyl-2,3-epoxypropionate are outweighed by the negative findings on ethyl methylphenylglycidate.

Conclusion on genotoxicity

Data on the genotoxicity of the flavouring substances in this group are limited and the genotoxicity could not be assessed adequately for these substances. For ethyl methylphenylglycidate [FL-no: 16.015], ethyl 3-phenyl-2,3-epoxypropionate [FL-no: 16.018], ethyl 2,3-epoxy-3-methyl-3-p-tolylpropionate [FL-no: 16.040], beta-caryophyllene epoxide [FL-no: 16.043] the Panel concluded that the available data do not preclude their evaluation through the Procedure.
The data available for beta-ionone epoxide [FL-no: 07.170] demonstrated that this substance is not genotoxic.

5. 14-DAY AND 90-DAY STUDY ON BETA-CARYOPHYLLENE EPOXIDE [FL-NO: 16.043]

A 14-day range-finding dietary study was performed with 5 beta-caryophyllene epoxide [FL-no: 16.043] (Bauter, 2012). Groups (3/sex/dietary intake level) of male and female Hsd:SD® rats were fed a diet containing 0 (dietary control), 3000, 9000 and 18000 mg/kg diet of beta-caryophyllene epoxide daily. These estimated dietary levels correspond to the measured intake of 279, 789 and 1,558 mg/kg body weight (bw)/day for males and 268, 816 and 1586 mg/kg bw/day for females. Clinical observations were recorded daily and body weights and food consumption observations were made on days 0, 7 and 14. No mortality was observed throughout the course of the study and the general condition of the rats was unremarkable. The body weight gain and gross examination were comparable to controls. Based on the conditions of this 14-day study it was suggested that rats of both sexes should tolerate beta-caryophyllene epoxide at concentrations up to or greater than 18000 mg/kg diet (Bauter, 2012).

A 90-day dietary study was performed with beta-caryophyllene epoxide [FL-no: 16.043] (Bauter, 2013). The study was performed according to OECD guideline (TG 408). Four groups of rats (10/sex/dietary intake level) of male and female CRL Sprague-Dawley CD®IGS rats were fed a diet containing 0 (dietary control), 1,750, 10,500 and 21,000 mg/kg diet of beta-caryophyllene epoxide. These estimated dietary levels correspond to the measured daily intake of 0, 109, 672 and 1398 mg/kg bw, respectively for males and 0, 137, 800 and 1660 mg/kg bw, respectively for females.

Clinical observations of toxicity were performed on day 0 and weekly until sacrifice. Animals were weighed on day 0 at the start of the study and weekly thereafter. Food consumption and efficiency were measured and calculated weekly. Blood chemistry and haematology were performed on blood drawn via sublingual bleed during week 12 after overnight fast. Urine was collected during the 15 hours prior to the blood draw. At termination of the study all survivors were sacrificed and subject to full necropsy. The following tissues were weighed wet post dissection: adrenals, brain, epididymides,

heart, kidneys, liver, ovaries, testes, spleen, thymus, uterus with oviducts. The tissues were preserved for future histopathological examination according to TG 408.

No gross observations were attributed to beta-caryophyllene epoxide in the diet. A concentration-dependent increase in kidney weights for males reaching significance at the highest intake level correlated with microscopic findings both of which are most likely α2u-globulin nephropathy, a common condition in the male rat. Fine granular casts and concentration-dependent increase in volume were found upon examination of the urine. Nephropathy was indicated by tubular cytoplasmic droplets in the kidneys of all test group males. Kidney cells of affected males were reported to have necrotic nuclei and increase in eosinophilic cytoplasm. There were also increases in the number and size of hyaline droplets present in the kidneys consistent with this spontaneous nephropathy, no such droplets were seen in the female group.

The Panel concluded that because of the specificity of the phenomenon of hyaline droplet in the kidneys to male individuals of the here used rat strain, this effect was not considered relevant for humans. (Capen et al., 1999, Olson et al., 1990)

All males and females of the middle and high intake level groups were reported to show hepatocellular hypertrophy. Additionally, microscopic findings in the liver and mesenteric lymphnodes were reported at the mid and high intake levels for both sexes. Evidence of liver hypertrophy in both male and female rats was consistent with an adaptive response to the increased metabolic load resulting from exposure to the two highest dietary levels of the test substance. The presence of hepatocyte hypertrophy in both sexes, the increases in absolute and relative liver weights at the mid- and highest dietary levels, and the absence of any other significant abnormality upon histopathologic examination, mutually support the conclusion that the hepatocyte hypertrophy is the result of hepatic enzyme induction and is considered an adaptive effect. Erythrocytes were present in the sinuses of the mesenteric lymphnodes. Additionally, reduced spleen weights for males at the highest dietary level were considered related to general reductions in lymphoid system weights.

The Panel asserts that under the conditions of this 90-day dietary toxicity study and based on the toxicological endpoint of mesenteric lymph node pathology, the no-observed-adverse-effect level (NOAEL) for beta-caryophyllene epoxide in the diet is 1750 mg/kg diet for males and females, which corresponds to the calculated intake of 109 mg/kg bw/day for males and 137 mg/kg bw/day of beta-caryophyllene epoxide for females(Bauter, 2013).

5.1. EFSA Considerations

For beta-caryophyllene epoxide [FL-no: 16.043] a NOAEL of 109 mg/kg bw/day has been established and this NOAEL will be used to evaluate this substance in the procedure. No NOAEL is available for beta-ionone epoxide or for a structurally related substance.

6. APPLICATION OF THE PROCEDURE

6.1. Application of the Procedure to Aliphatic Amines and Amides Substances by the JECFA (JECFA, 2006b)

According to the JECFA all five substances belong to structural class III using the decision tree approach presented by Cramer *et al.* (Cramer et al., 1978).

According to the JECFA all five substances can be predicted to be metabolised to innocuous products, so they were evaluated by the JECFA along the A-side of the Procedure.

The JECFA concluded three epoxides [FL-no: 07.170, 16.040 and 16.043] at step A3 in the JECFA Procedure, i.e. the substances are expected to be metabolised to innocuous products (step 2) and the intakes for the substances are below the threshold for structural class III (step A3).

The two substances [FL-no: 16.015 and 16.018], for which the intakes are above the threshold, do not occur endogenously in humans. Therefore the evaluation proceeded to step A5, where the substances were considered of no safety concern at the estimated levels of intake based on a long-term study, in which a NOAEL of 35 mg/kg bw/day for ethyl methylphenylglycidate [FL-no: 16.015] provides a margin of safety of more than 8000. This NOAEL is more than 17000 times the estimated intake of the related substance, ethyl 3-phenylglycidate [FL-no: 16.018]. The JECFA therefore concluded that these flavouring substances would not present a safety concern at the estimated daily intakes.

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

The evaluations of the epoxides are summarised in Table 6.

6.2. EFSA Considerations

The Panel does not agree with the application of the Procedure for the epoxides as performed by JECFA. The five substances [FL-no: 07.170, 16.015, 16.018, 16.040 and 16.043] should be evaluated via the B-side of the Procedure scheme.

For the epoxides ethyl methylphenylglycidate [FL-no: 16.015] and ethyl 3-phenyl-2,3-epoxypropionate [FL-no: 16.018] there is substantial evidence of a genotoxic potential from the available *in vitro* and *in vivo* studies. However, for ethyl methylphenylglycidate [FL-no: 16.015] negative carcinogenicity study is available, which is considered valid by the Panel. Groups of 48 male and 48 female rats were given diet containing 0 (control), 0.02, 0.1 or 0.5 % ethyl methylphenylglycidate for 2 years. Reduced weight of female rats given 0.5 % ethyl methylphenylglycidate in the diet were observed as well as reduced glutamic-oxalacetic transaminase levels in serum for both male and females at the highest dose. A NOAEL of 0.1 % ethyl methylphenylglycidate in the diet (corresponding to 35 mg/kg bw/day for males and 60 mg/kg bw/day for females) was established (Dunnington et al., 1981). A NOAEL of 109 mg/kg bw/day was established for beta-caryophyllene epoxide [FL-no: 16.043]. A concern for genotoxicity was ruled out for beta-ionone epoxide [FL-no: 07.170]. The Panel finally consider that the negative results from the carcinogenicity study overrule the positive *in vitro* genotoxicity results and the positive sex-linked recessive lethal mutation assay for ethyl methylphenylglycidate [FL-no: 16.015] and the structurally related substances ethyl 3-phenyl-2,3-epoxypropionate [FL-no: 16.018] and ethyl 2,3-epoxy-3-methyl-3-p-tolylpropionate [FL-no: 16.040].

Accordingly, three substances [FL-no: 16.015, 16.018 and 16.040] as well as beta-caryophyllene epoxide [FL-no: 16.043] and beta-ionone epoxide [FL-no: 07.170] can be evaluated via the B-side of the Procedure scheme. All five substances are classified into structural class III. Two of the substances [FL-no: 16.015 and 16.018] have estimated European daily *per capita* intakes from use as flavourings of 205 and 97 µg/*capita*/day, which are above the threshold of concern for structural class III, while the last three substances [FL-no: 07.170, 16.040 and 16.043] have estimated intakes of 0.073, 20 and 8 µg/*capita*/day, respectively, which are below the threshold of concern for structural class III. A NOAEL of 35 mg/kg bw/day can be derived from the carcinogenicity study with ethyl methylphenylglycidate [FL-no: 16.015] in male rats, which also applies to the structurally related substances [FL-no: 16.018 and 16.040]. The combined intake of the three substances is 322 µg/*capita*/day. This provides an adequate margin of safety for the substances of approximately 7×10^3.

Additional toxicity data have now become available for beta-caryophyllene epoxide [FL-no: 16.043] and based on these new data a NOAEL of 109 mg/kg bw/day could be derived. Based on the MSDI for this substance an adequate margin of safety of approximately 8×10^5. No NOAEL is available to finalise the evaluation of beta-ionone epoxide [FL-no: 07.170].

In conclusion, the Panel considered that the substances [FL-no: 16.015, 16.018, 16.040 and 16.043] evaluated through the Procedure via the B-side of the Procedure scheme were of no safety concern at the estimated levels of intake based on the MSDI approach.

CONCLUSION

In Flavouring Group Evaluation 82, (FGE.82) the EFSA considered a group of epoxides evaluated by the JECFA at its 65th meeting.

The present revision is made due to inclusion of one additional substance, beta-ionone epoxide [FL-no: 07.170], cleared for genotoxicity concern in FGE.210 Revision 1 and due to additional toxicity data have become available for beta-caryophyllene epoxide [FL-no: 16.043]. Since publication of FGE.82 the substance [FL-no: 16.051] is no longer supported by Industry for use as flavouring substance in Europe and will therefore not be considered any further.

Therefore the present revision of FGE.82, FGE.82Rev1, considers five flavouring substances evaluated by the JECFA.

The Panel does not agree with the application of the Procedure for the five epoxides as performed by JECFA. For the five substances [FL-no: 07.170, 16.015, 16.018, 16.040 and 16.043] it cannot be concluded that they are metabolised to innocuous substances and therefore their evaluation must proceed via the B-side of the Procedure scheme.

A 90-day study on beta-caryophyllene epoxide has become available and a NOAEL of 109 mg/kg bw/day to provide adequate margin of safety is derived.

The Panel considered that four of the five substances [FL-no: 16.043, 16.015, 16.018 and 16.040] evaluated through the Procedure via the B-side of the Procedure scheme were of no safety concern at the estimated levels of intake based on the MSDI approach.

The evaluation of beta-ionone epoxide [FL-no: 07.170] cannot be finalised at step B4 of the procedure because a NOAEL from a 90-day study is not available.

For the three substances [FL-no: 16.015, 16.018 and 16.040] evaluated through the Procedure use levels are needed to calculate the mTAMDIs in order to identify those flavouring substances that need more refined exposure assessment and to finalise their evaluation.

In order to determine whether the conclusion for the five 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 four JECFA evaluated substances [FL-no: 16.015, 16.018, 16.040 and 16.043]. For one substances [FL-no: 07.170] information on stereoisomeric composition is not adequate.

Thus, for one substance evaluated through the Procedure [FL-no: 07.170] the Panel has reservations (data missing on stereoisomerism, need for additional toxicity data).

For the remaining substances [FL-no: 16.015, 16.018, 16.040 and 16.043] the Panel agrees with the JECFA conclusion “No safety concern at estimated levels of intake as flavouring substances based on the MSDI approach.

SUMMARY OF GENOTOXICITY DATA

Table 4: Summary of Genotoxicity Data (*in vitro/in vivo*) for Five Epoxides Evaluated by the JECFA (JECFA, 2006b)

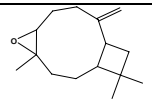
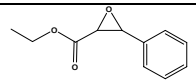
FL-no JECFA-no	EU Register name JECFA name	Structural formula	End-point	Test system	Concentration	Results	Reference
In vitro							
16.043 1575	beta-Caryophyllene epoxide		Reverse mutation	*S. typhimurium* TA1535, TA1537 and TA1538	0, 10, 100, 1,000, or 10,000 µg/plate	Negative[a,b]	(Richold et al., 1979)
			Reverse mutation	*S. typhimurium* TA1535, TA1537, TA98 and TA100	0, 10, 50, 100 or 500 µg/plate	Negative[a,c]	(Richold et al., 1979)
16.018 1576	Ethyl 3-phenyl-2,3-epoxypropionate (=Ethyl 3-phenyllycidate, EPG)		Reverse mutation	*S. typhimurium* TA98, TA100, TA1535 and TA1537	0, 10 to 500 µg/plate	Negative[d,e]	(Canter et al., 1986)
			Reverse mutation	*S. typhimurium* TA98, TA100, TA1535 and TA1537	0, 10 to 2,200 µg/plate	Negative[f,g]	(Canter et al., 1986)
			Reverse mutation	*S. typhimurium* TA98, TA100,TA1535, TA1537 and TA1538	0, 80, 400, or 2,000 µg/plate	Negative[d,h]	(Tilch and Elias, 1984)
						Negative / Positive[f,i]	
			Reverse mutation	*S. typhimurium* TA98, TA100,TA1535, TA1537 and TA1538	0, 400, 2,000, or 10,000 µg/plate[j]	Negative[a]	(Tilch and Elias, 1984)
			Reverse mutation	*S. typhimurium* TA100	0, 200, 500, 1,000, or 2,000µg/mL	Negative /Positive[a,k]	(Voogd et al., 1981)
			Reverse mutation	*S. typhimurium* TA100 and TA98	0, 25, 50, 100, 250, 500, 1,000, 2,500, or 5,000 µg/plate	Negative /Positive[d,i]	(Wagner and Walton, 1999)
						Positive[f]	
			Reverse mutation	*S. typhimurium* TA1535, TA1537, TA1538, TA98 and TA100	Up to 4,000 µg/plate	Negative /Positive[a,i]	(Wild et al., 1983)
			SOS chromotest	*Escherichia coli* PQ37	0, 0.01, 0.03, 0.1, 0.3, or 1.0 mmol/L (0, 1.9, 5.8, 19.2, 57.7, or	Negative[a]	(von der Hude et al., 1990a)

Table 4: Summary of Genotoxicity Data (*in vitro/in vivo*) for Five Epoxides Evaluated by the JECFA (JECFA, 2006b)

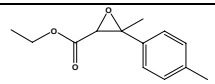
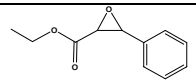
					192.2 µg/mL)		
			Sister chromatid exchange	Chinese hamster ovary-K1-BH4 cells	0 or 103 µg/mL (0.103 mg/mL)	Positive[a]	(Tilch and Elias, 1984)
			Sister chromatid exchange	Chinese hamster ovary-K1-BH4 cells	0 or 3,280 µg/mL (3.28 mg/mL)	Negative[a]	(Tilch and Elias, 1984)
			Sister chromatid exchange	Chinese hamster V79 cells	0, 0.078, 0.16, 0.32, 0.63, 1.25, or 2.5 mmol/L (0, 15, 30.8, 61.5, 121.1, 240.3, or 480.5 µg/mL)	Positive[l,m]	(von der Hude et al., 1991)
16.015 1577	Ethyl methylphenylglycidate		Reverse mutation	*S. typhimurium* TA98, TA100, TA1535 and TA1537	0, 100 to 10,000 µg/plate	Negative[a,n]	(Canter et al., 1986)
			Reverse mutation	*S. typhimurium* TA100	0, 200, 500, 1,000, 2,000, or 5,000 µg/mL	Negative[a]	(Voogd et al., 1981)
			Reverse mutation	*S. typhimurium* TA1535, TA1537, TA1538, TA98 and TA100	Up to 3,600 µg/plate	Negative[a]	(Wild et al., 1983)
			Reverse mutation	*S. typhimurium* TA1535, TA1537, TA1538, TA98 and TA100	Up to 1,200 µg/plate	Negative[a,o]	(Wild et al., 1983)
			Sister chromatid exchange	Chinese hamster ovary cells W B1	0, 16, 50, or 160 µg/mL	Positive[d]	(Galloway et al., 1987)
					0, 16, 50, 160, or 500 µg/mL	Negative[f]	
			Chromosomal aberration	Chinese hamster ovary cells W B1	0, 50, 160, or 500 µg/mL	Positive[d] Equivocal[f,p]	(Galloway et al., 1987)
In vivo							
16.018 1576	Ethyl 3-phenyl-2,3-epoxypropionate		Sex-linked recessive lethal mutation (Basc test)	*Drosophila melanogaster*	0 or 2.5 mM (480.5 µg/mL)	Negative[q]	(Wild et al., 1983)
			Micronucleus induction	NMRI Mice	0, 577, 961, or 1,538 mg/kg[r]	Negative	(Wild et al., 1983)
16.015	Ethyl		Sex-linked	*Drosophila*	0 or 10 mM (2,062.4	Weakly	(Wild et al., 1983)

Table 4: Summary of Genotoxicity Data (*in vitro/in vivo*) for Five Epoxides Evaluated by the JECFA (JECFA, 2006b)

1577	methylphenylglycidate	recessive lethal mutation (Basc test)	*melanogaster*	µg/mL)[s]	Positive	
		Micronucleus induction	NMRI Mice	0, 619, 1,237, or 1,856 mg/kg[r]	Negative	(Wild et al., 1983)

(a): With or without S9 activation.
(b): Cytotoxicity was observed at concentrations of 1,000 µg/plate and 10,000 µg/plate in S. typhimurium strains TA1535, TA1537 and TA1538.
(c): Cytotoxicity was observed at 500 µg/plate in *S. typhimurium* strains TA100 and TA98. Results for strain TA1538 were not reported with metabolic activation due to sample contamination.
(d): Without S9 activation.
(e): Cytotoxicity was observed at the highest dose in all strains tested.
(f): With S9 activation.
(g): Cytotoxicity was observed at concentrations of 1,600 and 2,200 µg/plate in all strains tested.
(h): Cytotoxicity was observed at 2,000 µg/plate in *S. typhimurium* strains TA98, TA1535 and TA1538.
(i): Only *S. typhimurium* strain TA100 showed mutagenic activity. Negative in all other strains of *S. typhimurium* tested.
(j): An in vitro pepsin and pancreatin digest of ethyl 3-phenylglycidate; performed in order to simulate mammalian digestion.
(k): Positive only at the 2 highest concentrations tested (i.e., 1,000 and 2,000 µg/mL).
(l): Precipitation and cytotoxicity reported at 2.5 mmol/L (480.5 µg/mL).
(m): Absence or presence of metabolic activation not specified.
(n): Cytotoxicity was observed at 10,000 µg/plate in *S. typhimurium* strain TA100.
(o): Pre-incubation method.
(p): For equivocal results, the P value was reported as <0.003 using the trend test; however, none of the doses were significant at $P \leq 0.05$ using the Dunnett method.
(q): Statistically significant increase in the number of SLRL mutations in only 1 out of 3 experiments and only in the 1st of 3 broods. Moreover, the cumulative number of SLRL mutations obtained in all 3 experiments for the 1st brood was not statistically different in comparison to controls.
(r): Single intraperitoneal doses.
(s): Statistically significant increase in the number of SLRL mutations in only 1 out of 4 experiments and only in the 1st of 3 broods; however, the cumulative number of SLRL mutations obtained in all 4 experiments for the 1st brood also was significantly increased in comparison to controls.

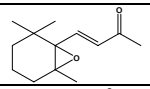
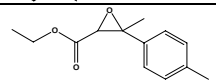
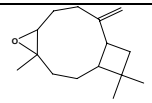
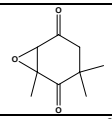
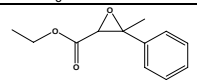
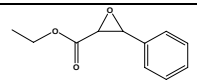
Table 5: Genotoxicity Data (*in vitro*) EFSA / FGE.210Rev1

Chemical Name FL-no	Test System *in vitro*	Test Object	Concentrations of Substance and Test Conditions	Result	Reference	Comments
beta-Ionone epoxide 07.170	Reverse Mutation	*S. typhimurium* TA98, TA100, TA1535, and TA1537	5 - 500 µg/plate (d,c)	Negative	(Jones and Wilson, 1988)	No statistically significant increase in revertant numbers was seen at any concentration, either in the presence or absence of S9-mix.
		S. typhimurium TA97a, TA98, TA100, TA1535	501, 1582 and 5000 µg/plate (a,c) 158, 501 and 1582 µg/plate (b,c)	Negative Negative	(Kringstad, 2005)	Evidence of toxicity was observed at the highest concentration in strain TA97a in the absence of S9-mix and in TA100 in the absence and presence of S9-mix. The study therefore complies with current recommendations for upper concentration limit inclusion. The study included 3 replicate plates per concentration, and was GLP compliant.
		E. coli WP2uvrA	501, 1582 and 5000 µg/plate (a,c) 158, 501 and 1582 µg/plate (b,c)	Negative Negative		
	tk Mutation Induction	Mouse Lymphoma L5178Y T/K +/- 3.7.2c cells	200 - 900 µg/mL (d,e) 4.1 - 520 µg/mL (a,f)	Negative Negative	(Flanders, 2006)	A preliminary range-finder assay was conducted to establish maximum concentrations. Top concentrations in each arm of the study induced 77, 85, and 80 % reductions in relative total growth. The study therefore complies with current recommendations.

(a): Without S9 metabolic activation.
(b): With S9 metabolic activation.
(c): Plate incorporation method.
(d): With and without S9 metabolic activation.
(e): 4-hour treatment.
(f): 24-hour treatment.

SUMMARY OF SAFETY EVALUATIONS

Table 6: Summary of Safety Evaluation by the JECFA (JECFA, 2006b)

FL-no JECFA-no	EU Register name	Structural formula	EU MSDI [(a)] US MSDI (µg/*capita*/day)	Class [(b)] Evaluation procedure path [(c)]	Outcome on the named compound [(d) or (e)]	EFSA conclusion on the named compound (Procedure steps, intake estimates, NOAEL, genotoxicity)	EFSA conclusion on the material of commerce
07.170 1571	beta-Ionone epoxide		0.073 0.1	Class III A3: Intake below threshold	d		
16.040 1578	Ethyl 2,3-epoxy-3-methyl-3-p-tolylpropionate		20 0.009	Class III A3: Intake below threshold	d	No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at the estimated level of intake based on the MSDI approach.
16.043 1575	beta-Caryophyllene epoxide		8 0.1	Class III A3: Intake below threshold	d	No safety concern at the estimated level of intake based on the MSDI approach.	Register name to be changed to 1R,4R,6R,10S-beta-caryophyllene epoxide. No safety concern at the estimated level of intake based on the MSDI approach.
16.051 1573	Epoxy oxophorone		0.012 0.2	Class III A3: Intake below threshold	d	Genotoxicity data required.	No longer supported by Industry, (DG SANCO, 2012).
16.015 1577	Ethyl methylphenylglycidate		205 1840	Class III A3: Intake above threshold, A4: Not endogenous, A5: Adequate NOAEL exists	d	No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at the estimated level of intake based on the MSDI approach.
16.018 1576	Ethyl 3-phenyl-2,3-epoxypropionate		97 96	Class III A3: Intake above threshold, A4: Not endogenous, A5: Adequate NOAEL exists	d	No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at the estimated level of intake based on the MSDI approach.

(a): 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.
(b): Thresholds of concern: Class I = 1800 µg/person/day, Class II = 540 µg/person/day, Class III = 90 µg/person/day.
(c): Procedure path A substances can be predicted to be metabolised to innocuous products. Procedure path B substances cannot.
(d): No safety concern based on intake calculated by the MSDI approach of the named compound.
(e): Data must be available on the substance or closely related substances to perform a safety evaluation.

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ABBREVIATIONS

BW	Body Weight
CAS	Chemical Abstract Service
CEF	Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
CHO	Chinese hamster ovary (cells)
CoE	Council of Europe
DNA	Deoxyribonucleic acid
EFFA	European Flavour and Fragrance Association
EFSA	The European Food Safety Authority
EPA	United States Environmental Protection Agency
EU	European Union
FAO	Food and Agriculture Organization of the United Nations
FEMA	Flavor and Extract Manufacturers Association
FGE	Flavouring Group Evaluation
FLAVIS (FL)	Flavour Information System (database)
GLP	Good laboratory practice
HPRT	Hypoxanthine Phosphoribosyl transferase
ID	Identity
IP	Intraperitoneal
IR	Infrared spectroscopy
JECFA	The Joint FAO/WHO Expert Committee on Food Additives
MNBN	Micronucleated Binucleate cells
MSDI	Maximised Survey-derived Daily Intake
mTAMDI	Modified Theoretical Added Maximum Daily Intake
NCE	Normochromatic erythrocyte
No	Number
NOAEL	No Observed Adverse Effect Level
NTP	National Toxicology Program

OECD	Organization for Economic Cooperation and Development
PCE	Polychromatic erythrocyte
RI	Replication Index
SCE	Sister chromatic exchange
SCF	Scientific Committee on Food
UDS	Unscheduled DNA Synthesis
WHO	World Health Organisation