EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF); Scientific Opinion on Flavouring Group Evaluation 50, Revision 1 (FGE.50Rev1): Consideration of pyrazine derivatives evaluated by JECFA (57th meeting) structurally related to pyrazine derivatives evaluated by EFSA in FGE.17Rev2 (2010)

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SCIENTIFIC OPINION

Scientific Opinion on Flavouring Group Evaluation 50, Revision 1 (FGE.50Rev1):

Consideration of pyrazine derivatives evaluated by JECFA (57th meeting) structurally related to pyrazine derivatives evaluated by EFSA in FGE.17Rev2 (2010)[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. Since the previous version of FGE.50, new *in vitro* and *in vivo* genotoxicity data on 5-methylquinoxaline [FL-no: 14.028] have been provided. The Panel concluded that these data allowed to roule out genotoxicity concerns for the substance. 5-Methylquinoxaline was then evaluated through a stepwise approach (the Procedure) 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 substance do not give rise to safety concerns at the levels of dietary intake, estimated on the basis of the MSDI approach. So in total, for all the 41 JECFA evaluated pyrazines derivatives [FL-no: 14.005, 14.006, 14.015, 14.017, 14.018, 14.019, 14.020, 14.021, 14.022, 14.024, 14.025, 14.026, 14.027, 14.028, 14.031, 14.032, 14.034, 14.035, 14.037, 14.043, 14.044, 14.049, 14.050, 14.053, 14.054, 14.055, 14.056, 14.062, 14.067, 14.069, 14.077,

1 On request from the Commission, Question No EFSA-Q-2010-00007, adopted on 25 November 2010.

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3 Acknowledgement: The Panel wishes to thank the members of the Working Groups on Flavourings for the preparation of this Opinion: wishes to thank the members of the Working Groups on Flavourings for the preparation of this Opinion: Ulla Beckman Sundh, Vibe Beltoft, Wilfried Bursch, Angelo Carere, Karl-Heinz Engel, Henrik Frandsen, Rainer Gürtler, Frances Hill, Trine Husøy, John Christian Larsen, Pia Lund, Wim Mennes, Gerard Mulder, Karin Nørby, Gerard Pascal, Iona Pratt, Gerrit Speijers, Harriet Wallin and EFSA's staff member Kim Rygaard Nielsen for the preparatory work on this scientific Opinion.

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14.082, 14.095, 14.096, 14.098, 14.100, 14.114, 14.121, 14.123, 14.142 and 14.144] evaluated in FGE.50, the Panel agrees with the JECFA conclusion, "No safety concern at estimated levels of intake as flavouring substances" based on the MSDI approach. Adequate specifications for the materials of commerce are available for all 41 flavouring substances.

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 consider the Joint FAO/WHO Expert Committee on Food Additives (the JECFA) evaluations of flavouring substances assessed since 2000, and to decide whether no further evaluation is necessary, as laid down in Commission Regulation (EC) No 1565/2000. These flavouring substances are listed in the Register, which was adopted by Commission Decision 1999/217/EC and its consecutive amendments.

The present consideration concerns 41 pyrazine derivatives evaluated by the JECFA (57th meeting) and will be considered in relation to the EFSA evaluation of 21 pyrazine derivatives evaluated in the Flavouring Group Evaluation 17, Revision 2 (FGE.17Rev2).

The Panel concluded that the 41 substances in the JECFA flavouring group of pyrazines are structurally related to the pyrazines evaluated by EFSA in FGE.17Rev2.

In the previous version of the present Flavouring Group Evaluation (FGE), the Panel concluded that it could agree in the way the application of the Procedure has been performed by the JECFA for 40 out of the 41 pyrazines derivatives. For 5-methylquinoxaline [FL-no: 14.028], the Panel concluded that in line with the conclusions for quinoxaline and the two quinoxaline derivatives (quinoxaline [FL-no: 14.147], 2-methylquinoxaline [FL-no: 14.139] and 2,3-dimethylquinoxaline [FL-no: 14.108]) in FGE.17Rev1, 5-methylquinoxaline should not be evaluated using the Procedure until adequate genotoxicity data become available. New genotoxicity data have now become available and based on these data the Panel concluded that the *in vitro* genotoxicity alert could be ruled out for 5-methylquinoxaline [FL-no: 14.028] as no genotoxic potential at gene or chromosome level was indicated.

For all 41 substances evaluated through the JECFA Procedure intake data are available for EU.

For all 41 substances 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 the evaluation.

In order to determine whether the conclusion for the 41 JECFA evaluated substances can be applied to the materials of commerce, it is necessary to consider the available specifications. Adequate specifications including complete purity criteria and identity are available for all 41 JECFA evaluated substances.

For all the 41 JECFA-evaluated pyrazines [FL-no: 14.005, 14.006, 14.015, 14.017, 14.018, 14.019, 14.020, 14.021, 14.022, 14.024, 14.025, 14.026, 14.027, 14.028, 14.031, 14.032, 14.034, 14.035, 14.037, 14.043, 14.044, 14.049, 14.050, 14.053, 14.054, 14.055, 14.056, 14.062, 14.067, 14.069, 14.077, 14.082, 14.095, 14.096, 14.098, 14.100, 14.114, 14.121, 14.123, 14.142 and 14.144] the Panel

agrees with the JECFA conclusion, "No safety concern at estimated levels of intake as flavouring substances" based on the MSDI approach.

KEY WORDS

Pyrazines, JECFA 57th meeting, pyrazine derivatives, quinoxaline derivatives, FGE.17Rev2.

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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).

Commission Regulation (EC) No 1565/2000 lays down that substances that are contained in the Register and will be classified in the future by the Joint FAO/WHO Expert Committee on Food Additives (the JECFA) so as to present no safety concern at current levels of intake will be considered by the European Food Safety Authority (EFSA), who may then decide that no further evaluation is necessary.

In the period 2000 – 2008, during its 55th, 57th, 59th, 61st, 63rd, 65th, 68th and 69th meetings, the JECFA evaluated about 1000 substances, which are in the EU Register.

TERMS OF REFERENCE

EFSA is requested to consider the JECFA evaluations of flavouring substances assessed since 2000, and to decide whether no further evaluation is necessary, as laid down in Commission Regulation (EC) No 1565/2000 (EC, 2000a). These flavouring substances are listed in the Register which was adopted by Commission Decision 1999/217 EC (EC, 1999a) and its consecutive amendments.

In addition, in letter of 1 April 2009 the Commission requested EFSA to carry out a re-evaluation of flavouring substances [FL-no: 14.028, 14.108 and 14.139] in accordance with Commission Regulation (EC) No 1565/2000:

“The European Commission requests the European Food Safety Authority to carry out a risk assessment on 5-methylquinoxaline ([FL-no: 14.028]), 2,3-dimethylquinoxaline ([FL-no: 14.108]) and 2-methylquinoxaline ([FL-no: 14.139]) in accordance with Commission Regulation (EC) No 1565/2000, if possible by the end of the evaluation programme, if not within nine month from finalisation of that programme”.

The deadline of the Terms of Reference was negotiated to 30 June 2010.

ASSESSMENT

The approach used by EFSA for safety evaluation of flavouring substances is referred to in Commission Regulation (EC) No 1565/2000 (EC, 2000a), hereafter named the “EFSA Procedure”. This Procedure is based on the Opinion of the Scientific Committee on Food (SCF, 1999a), which has been derived from the evaluation procedure developed by the Joint FAO/WHO Expert Committee on Food Additives (JECFA, 1995; JECFA, 1996a; JECFA, 1997a; JECFA, 1999b), hereafter named the “JECFA Procedure”. The Panel on Food 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 put through the EFSA Procedure.

The following issues are of special importance.

Intake

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

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

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

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

As information on use levels for the flavouring substances has not been requested by the JECFA or has not otherwise been provided to the Panel, it is not possible to estimate the daily intakes using the mTAMDI approach for the substances evaluated by the JECFA. The Panel will need information on use levels in order to finalise the evaluation.

Threshold of 1.5 Microgram/Person/Day (Step B5) Used by the JECFA

The JECFA uses the threshold of concern of 1.5 microgram/person/day as part of the evaluation procedure:

"The Committee noted that this value was based on a risk analysis of known carcinogens which involved several conservative assumptions. The use of this value was supported by additional information on developmental toxicity, neurotoxicity and immunotoxicity. In the judgement of the Committee, flavouring substances for which insufficient data are available for them to be evaluated using earlier steps in the Procedure, but for which the intake would not exceed 1.5 microgram per person per day would not be expected to present a safety concern. The Committee recommended that the Procedure for the Safety Evaluation of Flavouring Agents used at the forty-sixth meeting be amended to include the last step on the right-hand side of the original procedure ("Do the condition of use result in an intake greater than 1.5 microgram per day?")" (JECFA, 1999b).

In line with the Opinion expressed by the Scientific Committee on Food (SCF, 1999), the Panel does not make use of this threshold of 1.5 microgram per person per day.

Genotoxicity

As reflected in the Opinion of SCF (SCF, 1999a), 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.

HISTORY OF THE EVALUATION OF THE SUBSTANCES IN THE PRESENT FGE

In FGE.50, which contain a group of 41 flavouring substances consisting of pyrazine and pyrazine derivatives, the Panel concluded that for one of these substances, 5-methyl quinoxaline [FL-no: 14.028], the Procedure should not be applied until adequate genotoxicity data become available. This conclusion was in line with the Panel conclusion for three other quinoxalines evaluated in FGE.17Rev1 (EFSA, 2008r).

FGE	Opinion adopted by EFSA	Link	No. of candidate substances
FGE.50	February 2007	http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1178692189146.htm	41
FGE.50Rev1	November 2010		41

Additional genotoxicity data have now become available for [FL-no: 14.028] and the present revision of FGE.50, FGE.50Rev1 includes the evaluation of these genotoxicity data submitted by the Industry (Flavour Industry, 2009a).

Of the 41 flavouring substances considered by EFSA in FGE.50 no European production volumes were available for seven substances [FL-no: 14.025, 14.026, 14.034, 14.067, 14.069, 14.077 and 14.121]. As no European production volumes were available no MSDI could be calculated for EU and accordingly the substances could not be considered by EFSA using the Procedure. In the course of 2010, Industry provided EU production figures (EFFA, 2010a; EFFA, 2010c) for these seven substances together with similar data on approximately 100 other substances from 27 different FGEs. In order to avoid unnecessary delay, these substances were evaluated in a special FGE, FGE.96, in which EU production volumes / anticipated production volumens submitted on request by DC SANCO have been included in the evaluation (EFSA, 2010aj). The EU production volumes of these seven substances and the outcome of the evaluations have also been included in the current revision of FGE.50 (EFFA, 2010a; EFFA, 2010c).

Finally, since the previous version of FGE.50 (EFFA, 2010a), missing information have been provided on the stereoisomeric composition for two substances [FL-no: 14.037 and 14.062], identity test for one substance [FL-no: 14.024], clarification of the specific gravity for three substances [FL-no: 14.019,

14.054 and 14.062] and composition of mixtures of positional isomers [FL-no: 14.006, 14.020, 14.021, 14.025, 14.035, 14.050, 14.055, 14.067, 14.077, 14.100, 14.114 and 14.121].

1. Presentation of the Substances in the JECFA Flavouring Group

1.1. Description

1.1.1. JECFA Status

The JECFA has evaluated a group of 41 flavouring substances consisting of pyrazine and pyrazine derivatives.

1.1.2. EFSA Considerations

The Panel concluded that all the 41 substances in the JECFA flavouring group of pyrazines are structurally related to the group of 21 pyrazines evaluated by EFSA in the Flavouring Group Evaluation 17, Revision 2 (FGE.17 Rev2).

1.2. Isomers

1.2.1. Status

Two of the JECFA evaluated substances [FL-no: 14.037 and FL-no: 14.062] have a chiral centre. Further 12 substances consist of mixtures of positional isomers [FL-no: 14.006, 14.020, 14.021, 14.025, 14.035, 14.050, 14.055, 14.067, 14.077, 14.100, 14.114 and 14.121].

1.2.2. EFSA Considerations

Industry has informed that the two substances [FL-no: 14.037 and FL-no: 14.062] occur as a racemic mixture (EFFA, 2010a).

Industry has informed about the composition of the mixture of positional isomers of the 12 JECFA evaluated substances [FL-no: 14.006, 14.020, 14.021, 14.025, 14.035, 14.050, 14.055, 14.067, 14.077, 14.100, 14.114 and 14.121] (EFFA, 2010a).

1.3. Specifications

1.3.1. JECFA Status

The JECFA specifications are available for all 41 substances (JECFA, 2001c). See Table 1.

1.3.2. EFSA Considerations

The available specifications are considered adequate for all 41 JECFA evaluated substances.

2. Intake Estimations

2.1. JECFA Status

For all 41 substances evaluated through the JECFA Procedure intake data are available for EU, see Table 3.1.

2.2. EFSA Considerations

No comments.

3. Genotoxicity Data

3.1. Genotoxicity Studies – Text Taken[4] from the JECFA (JECFA, 2002a)

In vitro

2-Methylpyrazine [FL-no: 14.027], 2-ethylpyrazine [FL-no: 14.022], 2,3-dimethylpyrazine [FL-no: 14.050], 2,5-dimethylpyrazine [FL-no: 14.020], 2,6-dimethylpyrazine [FL-no: 14.021], 2,3,5-trimethylpyrazine [FL-no: 14.019], pyrazine [FL-no: 14.144]: These substances have been tested for their ability to cause reverse mutation, with uniformly negative results up to concentrations of 1000 microgram/plate in various strains of *Salmonella typhimurium* with and without an exogenous metabolic activation system from rodent liver (Stich et al., 1980; Aeschbacher et al., 1989; Lee et al., 1994a). In one of these studies, 2-methylpyrazine, 2-ethylpyrazine, 2,5-dimethylpyrazine, 2,6-dimethylpyrazine, and pyrazine were also tested for their ability to cause mitotic crossover-gene conversion in *Saccharomyces cerevisiae* and chromosomal aberrations in Chinese hamster ovary cells (Stich et al., 1980). Surviving cells in cultures of stationary phase *S. cerevisiae* strain D5 showed an increase in the percentage of aberrant colonies at test concentrations of 3300–135 000 microgram/ml; however, no increase in the number of mitotic recombinants was observed among the aberrant colonies.

Pyrazine and the other alkyl-substituted pyrazine derivatives that were tested induced significant percentages of chromosomal aberrations (breaks and exchanges) in metaphase plates in Chinese hamster ovary cells with and without metabolic activation at test concentrations of 2500–40000 microgram/ml. However, these concentrations were two to four times lower than those that were cytotoxic, and no negative controls were used to allow a demonstration that a significant increase in the incidence of aberrations had actually occurred (Stich et al., 1980).

(2, 5 or 6)-Methoxy-3-methylpyrazine [FL-no: 14.025]: A mixture of three isomers, (2, 5 or 6)-methoxy-3-methylpyrazine, did not induce reverse mutation in *S. typhimurium* strain TA98, TA100, TA1535, TA1537, or TA1538 at concentrations of 3.6 mg/plate with and without metabolic activation (Wild et al., 1983).

In vivo

(2, 5 or 6)-Methoxy-3-methylpyrazine [FL-no: 14.025]: A test for Basc mutation was performed in *Drosophila* with a concentration of 10 mmol/L (140 microgram/ml), with no mutagenic effect (Wild et al., 1983).

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

Male and female NMRI mice were treated once orally with (2, 5, or 6)-methoxy-3-methylpyrazine at a dose of 87, 174, or 248 mg/kg bw and killed, and bone-marrow smears were prepared 30 h after treatment. There was no increase in the frequency of micronuclei in polychromatic erythrocytes (Wild et al., 1983).

Conclusion on genotoxicity

The relevance of the positive results for pyrazine and certain alkylpyrazines in assays with S. cerevisiae and Chinese hamster ovary cells *in vitro* reported by Stich et al. (1980) is unclear. The studies were performed at high, nearly toxic concentrations of the weakly basic pyrazines, which may have altered cellular homeostasis. The results of studies of genotoxicity by Zajac-Kaye & Ts'o (Zajac-Kaye & Ts'o, 1984), Brusick (Brusick, 1986), Bradley et al. (Bradley et al., 1987), and Heck et al. (Heck et al., 1989), for example, indicate that agents other than the pyrazines may have caused the observed results. The positive results *in vitro* reported by Stich et al. (Stich et al., 1980) were not corroborated by the results of studies conducted *in vivo* by Wild et al. (Wild et al., 1983) with (2, 5, or 6)-methoxy-3-methylpyrazine [FL-no: 14.025].

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

3.2. Genotoxicity Studies - Text Taken[5] from EFSA FGE.17Rev2 (EFSA, 2010h)

Genotoxicity data were provided for three of the 20 candidate substances and for 11 of the 41 supporting substances. The three candidate substances are quinoxaline [FL-no: 14.147] and its derivatives 2-methylquinoxaline [FL-no: 14.139] and 2,3-dimethylquinoxaline [FL-no: 14.108]. After finalisation of the previous version of this FGE (i.e. FGE.17, Revision 1), both *in vitro* and *in vivo* genotoxicity data have become available for the supporting substance 5-methylquinoxaline [FL-no: 14.028]. This substance is a candidate flavouring substance in FGE.50. The Panel explored the option of using the genotoxicity data submitted for 5-methylquinoxaline [FL-no: 14.028] to support the evaluation of the genotoxic potential of the candidate quinoxaline derivatives in FGE.17.

Genotoxicity data on Candidate substances

In *in vitro* studies, quinoxaline [FL-no: 14.147], up to 10000 microgram/plate and 2,3-dimethylquinoxaline [FL-no: 14.108], up to 2500 microgram/plate, with and without metabolic activation, did not cause reverse mutation in various strains of *Salmonella typhimurium* (See Table IV.4). Two studies on 2-methylquinoxaline [FL-no: 14.139] are available, one study with a positive, the other with negative result in the Ames test. However, quinoxaline [FL-no: 14.147] at 250 microgram/ml culture medium and with metabolic activation was found to induce TFT-mutants in the mouse lymphoma mutagenesis assay (L5178Y $TK^{+/-}$ cells). This study was conducted in accordance with the OECD guideline 476 and therefore considered valid.

No adequate *in vivo* studies on genotoxicity of the substances are available. A study of the potential of quinoxaline [FL-no: 14.147] to induce sperm head abnormalities (Topham, 1980) did not address a genetic endpoint and the Panel considered it could not be used for evaluation of genotoxicity of this substance.

Genotoxicity data on Supporting substances

Substituted pyrazines

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

In vitro, 2-methylpyrazine [FL-no: 14.027], ethylpyrazine [FL-no: 14.022], 2,3-dimethylpyrazine [FL-no: 14.050], 2,5-dimethylpyrazine [FL-no: 14.020], 2,3-diethylpyrazine [FL-no: 14.005], 2,6-dimethylpyrazine [FL-no: 14.021], 2,3,5-trimethylpyrazine [FL-no: 14.019], pyrazine [FL-no: 14.144] and (2, 5 or 6)-methoxy-3-methylpyrazine [FL-no: 14.025] were tested for their ability to cause reverse mutation in various strains of *S. typhimurium* and consistently revealed negative results with and without metabolic activation (Table 2.2).

In one of these studies, 2-methylpyrazine, ethylpyrazine, 2,5-dimethylpyrazine, 2,6-dimethylpyrazine, and pyrazine were also tested for their potential to cause genotoxicity in *Saccharomyces cerevisiae* and chromosomal aberrations in Chinese hamster ovary cells (Table 2.2) (Stich et al., 1980). This study has strong limitations for the following reasons. The positive results were observed only in a narrow range of exceedingly high and toxic concentrations. In the case of chromosome aberrations, the concentration used exceeded the maximum level (5 mg/ml) recommended by OECD. It has been shown (Galloway, 2000) that *in vitro* chromosome breaking can occur secondary to toxicity and/or changed physiological conditions (e.g., pH, osmolarity) with compounds not able to react with DNA and negative in the Ames test and *in vivo*. The *S.cerevisiae* D5 assay for induction of "aberrant colonies" is not routinely used and has not been validated at international level due to the uncertainty on the various effects involved. Thus, the positive results reported by Stich et al. (Stich et al., 1980) are considered of limited value and not relevant for hazard and risk assessment. Furthermore, pyrazine was found negative in a wide range of concentrations both in the Salmonella assay and in the mouse lymphoma TK assay (Fung et al., 1988).

Quinoxalines

5-Methylquinoxaline [FL-no: 14.028] was examined for its mutagenic potential in *Salmonella typhimurium* strains TA100, TA1535, TA98 and TA1537, as well as in *Escherichia coli* strain WP2 uvrA. The study was conducted according to GLP and was in compliance with OECD Guideline 471. No evidence of mutagenicity was found with or without S9 metabolic activation at concentrations up to 5,000 µg/plate (Ogura & Wakamatsu, 2004).

5-methylquinoxaline was examined for its potential to induce structural chromosome aberrations in mammalian cells. The study was conducted according to GLP and was in compliance with OECD Guideline 473. The test system used was a subculture of Chinese hamster lung-derived CHL/IU cells that were exposed to the test material at concentrations of 320, 480 and 720 µg/mL without S9 mix, and 72.0, 228 and 720 µg/mL with S9 mix. The percentage of “cell productivity” (the cell number was measured and expressed as relative growth rate compared to negative control) was reported as a parameter for cytotoxicity. The Panel considered that 5-methylquinoxaline was found to induce chromosomal aberrations in cultured mammalian cells in the presence of metabolic activation. Additionally, an increased frequency of polyploid cells up to 12.5 % of the middle dose compared to 0 % in the control was observed in the presence and absence of metabolic activation at concentrations which induced only low cytotoxicity (Ajimu & Kawaguchi, 2004a)

In vivo data are available for two one of the supporting substances only, (2, 5 or 6)-Methoxy-3-methylpyrazine [FL-no: 14.025] and 5-methylquinoxaline [FL-no: 14.028].

5-Methylquinoxaline [FL-no: 14.028] was examined for its potential to induce micronucleated polychromatic erythrocytes (MNPCEs) in the bone marrow. The test material was administered daily (gavage) for two consecutive days to seven week old and six week old male SPF ICR (Crj:CD-1) mice at dosages of 125, 250 and 500 mg/kg/day (6 animals/dose). Microscopic examination of femoral bone marrow cells was conducted randomly from 5 animals. Two thousand polychromatic erythrocytes (PCE) per animal were analyzed microscopically (x1000), and the number of micronucleated polychromatic erythrocytes (MNCPE) was recorded. In order to evaluate the PCE/NCE ratio, the number of PCEs out of 200 total erythrocytes (PCEs plus NCEs) was recorded. The test was considered positive if the MNPCE frequencies in one or more treatment groups were significantly higher than that in the negative control groups. No significant increase of micronucleated

polychromatic erythrocyte frequency was observed in these treatment groups compared with the negative control group. The PCE/NCE ratio was not changed (Ajimu & Kawaguchi, 2004b). Based on the PCE/NCE ratio there is no indication that the substance reached the bone marrow, however, the Panel noted that the high dose was the maximum tolerated dose since clinical signs of toxicity have been observed after oral intake. Additionally, a doubling of this dose was lethal for two out of six animals in a preliminary test. Thus, the Panel considered it reasonable to assume that the substance was systemically available and reached the bone marrow.

For (2, 5 or 6)-methoxy-3-methylpyrazine [FL-no: 14.025] a test for Basc mutation was performed in *Drosophila* with a concentration of 10 mmol/L (140 microgram/ml) in the solutions/emulsions fed to the flies, with no mutagenic effect (Table 2.3). Secondly, male and female NMRI mice were treated once orally with 87, 174 or 248 mg/kg bw, bone-marrow smears were prepared only at one sampling time (at 30 hours) after treatment. There was no increase in the frequency of micronuclei in polychromatic erythrocytes (Table 2.3). The PCE/NCE ratio was not reported and thus, it is not clear if the test substance reached the bone marrow. However from this study, there is no evidence of genotoxic potential.

Conclusion on genotoxicity

The available data indicate that apparently there is no simple structure-activity relationship for the genotoxicity of quinoxalines, because the profile of genotoxic events *in vitro* differs for the various congeners (point mutations for [FL no: 14.139] and [FL-no: 14.147] vs chromosomal aberrations for [FL no: 14.028]). Therefore these compounds are to be evaluated based on substance-specific data for each individual quinoxaline derivative.

In vitro data indicate a genotoxic potential for quinoxaline [FL-no: 14.147] and 2-methylquinoxaline [FL-no: 14.139], for which no *in vivo* data are available. Therefore, for these two substances the Procedure cannot be applied until adequate genotoxicity data become available.

Conversely, 2,3-dimethylquinoxaline [FL-no: 14.108] is not considered genotoxic *in vitro* and hence can be evaluated through the Procedure (three negative bacterial reverse gene mutation assays which, although limited, consistently indicate lack of genotoxicity).

The Panel concluded that no genotoxic potential is indicated for 19 candidate substances, including 2,3-dimethylquinoxaline [FL-no: 14.108]. For these 19 substances, the available data do not preclude their evaluation through the Procedure.

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

3.3. EFSA Considerations

The Panel considered that no genotoxic potential at gene or chromosome level is indicated for this group of flavourings.

4. Application of the Procedure

4.1. Application of the Procedure to Pyrazine and 40 Derivatives by JECFA (JECFA, 2002b)

According to the JECFA 32 of the substances belong to structural class II and nine to structural class III using the decision tree approach presented by Cramer *et al.* (Cramer et al., 1978).

The JECFA concluded all 41 pyrazines at step A3 in the JECFA Procedure – i.e. the substances are expected to be metabolised to innocuous products (step 2) and the intakes for all substances are below the thresholds for their structural classes II and III (step A3).

In conclusion, the JECFA evaluated all 41 substances as to be of no safety concern at the estimated levels of intake as flavouring substance based on the MSDI approach.

The evaluations of the 41 substances are summarised in Table 3.1: Summary of Safety Evaluation of Pyrazine and 40 Derivatives (JECFA, 2003a).

4.2. Application of the Procedure to 21 Pyrazine Derivatives by EFSA (FGE.17Rev2) (EFSA, 2010h)

In the previous version of FGE.17, FGE.17Rev1, it was found that two of the candidate substances, quinoxaline [FL-no: 14.147] and 2-methylquinoxaline [FL-no: 14.139] showed possible genotoxic potential *in vitro*. Therefore, the Panel decided that the Procedure could not be applied to these two candidate substances nor for the structurally related 2,3-dimethylquinoxaline [FL-no: 14.108] until adequate genotoxicity data become available.

Additional genotoxicity data have now become available for the structurally related 5-methylquinoxaline [FL-no: 14.028] and in the present FGE these genotoxicity data submitted by the Industry (Flavour Industry, 2009a) have been evaluated. The available data indicate that there is no apparent structure-activity relationship for the genotoxicity of quinoxalines (Hashimoto et al., 1979): thus these compounds are to be considered individually. 2,3-Dimethylquinoxaline [FL-no: 14.108] is not genotoxic *in vitro* and can be evaluated through the Procedure; conversely, *in vitro* data indicate a genotoxic potential for quinoxaline [FL-no: 14.147] and 2-methylquinoxaline [FL-no: 14.139], for which no *in vivo* data are available. Therefore, for these two substances the Procedure cannot be applied until adequate genotoxicity data become available.

Furthermore, the candidate substance, 2,5 or 6-methoxy-3-ethylpyrazine [FL-no: 14.051] no European production figures were available and consequently no European exposure estimates could be calculated. Accordingly, the safety in use could not be assessed using the Procedure for this substance.

Of the 18 candidate substances which are evaluated using the Procedure, 16 are classified into structural class II and two substances into structural class III using the decision tree approach presented by Cramer *et al.* (Cramer et al., 1978).

Sixteen of the 18 substances were concluded at step A3 – i.e. the substances are expected to be metabolised to innocuous products (step 2) and the estimated daily intakes are below the threshold for the structural class (step A3). Two candidate substances cannot be anticipated to be metabolised to innocuous products [FL-no: 14.122 and 14.052]. Therefore these two substances are evaluated along the B-side of the Procedure scheme.

For these substance the intake is below the threshold for the structural class (step B3). For 2-isopropyl-3-methyl thiopyrazine [FL-no: 14.122] a NOAEL exists to provide an adequate margin of safety to the estimated level of intake as flavouring substance (step B4). For one substance, isopropenylpyrazine [FL-no: 14.052] or for any relevant supporting substances no valid toxicity study from which a NOAEL could be established was available. Therefore, the Panel concluded that additional toxicity data are needed for this substance.

In conclusion the Panel considered that the 17 of the 18 substances evaluated through the Procedure were of no safety concern at the estimated levels of intake based on the MSDI approach.

The stepwise evaluations of the 18 substances are summarised in Table 3.2: Summary of Safety Evaluation Applying the Procedure (EFSA, 2010h).

4.3. EFSA Considerations

In the previous version of the present FGE, FGE50, the Panel concluded that they could agree in the way the application of the Procedure has been performed by the JECFA for 40 out of the 41 pyrazines derivatives. For 5-methylquinoxaline [FL-no: 14.028], the Panel concluded that in line with the conclusions for quinoxaline and the quinoxaline derivatives in FGE17.Rev1, 5-methylquinoxaline should not be evaluated using the Procedure until adequate genotoxicity data become available. Furthermore, for seven substances [FL-no: 14.025, 14.026, 14.034, 14.067, 14.069, 14.077 and 14.121], the evaluation could not be finalised due to missing EU production volumes.

New data on *in vitro* and *in vivo* genotoxicity for 5-methylquinoxaline [FL-no: 14.028] provided by Industry, were considered in this revision. Based on these data the Panel concluded that the *in vitro* genotoxicity alert could be ruled out for 5-methylquinoxaline [FL-no: 14.028] in FGE.50 as no genotoxic potential at gene or chromosome level was indicated.

Accordingly, the Panel therefore considered that no genotoxic potential is indicated for this group of 41 pyrazine derivatives.

The JECFA concluded at their 57th meeting that 5-methylquinoxaline [FL-no: 14.028] would be metabolised to innocuous products. The Panel agrees with the JECFA conclusion "no safety concern at estimated levels of intake as flavouring substances" based on the MSDI approach.

Based on newly submitted EU production volumes for the seven JECFA evaluated substances [FL-no: 14.025, 14.026, 14.034, 14.067, 14.069, 14.077 and 14.121] the MSDIs range from 0.0012 to 2.2 microgram/*capita*/day, which are all below the threshold for their respective structural class.

For all seven substances the Panel concluded at step A3 that these substances would be of no safety concern at their estimated level of intake based on the MSDI approach (EFSA, 2010aj).

5. Conclusion

The Panel concluded that the 41 substances in the JECFA flavouring group of pyrazines are structurally related to the pyrazines evaluated by EFSA in the Flavouring Group Evaluation 17 (FGE.17Rev2).

In the previous version of the present FGE, FGE50, the Panel concluded that it could agree in the way the application of the Procedure has been performed by the JECFA for 40 out of the 41 pyrazines derivatives. For 5-methylquinoxaline [FL-no: 14.028], the Panel concluded that in line with the conclusions for quinoxaline and the two quinoxaline derivatives (quinoxaline [FL-no: 14.147], 2-methylquinoxaline [FL-no: 14.139] and 2,3-dimethylquinoxaline [FL-no: 14.108]) in FGE17.Rev1, 5-methylquinoxaline should not be evaluated using the Procedure until adequate genotoxicity data become available. New genotoxicity data have now become available and based on these data the Panel concluded that the *in vitro* genotoxicity alert could be ruled out for 5-methylquinoxaline [FL-no: 14.028] as no genotoxic potential at gene or chromosome level was indicated.

For all 41 substances evaluated through the JECFA Procedure intake data are available for EU.

For all 41 substances 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 the evaluation.

In order to determine whether the conclusion for the 41 JECFA evaluated substances can be applied to the materials of commerce, it is necessary to consider the available specifications. Adequate specifications including complete purity criteria and identity are available for all the 41 JECFA evaluated substances.

For all the 41 JECFA evaluated pyrazines [FL-no: 14.005, 14.006, 14.015, 14.017, 14.018, 14.019, 14.020, 14.021, 14.022, 14.024, 14.025, 14.026, 14.027, 14.028, 14.031, 14.032, 14.034, 14.035, 14.037, 14.043, 14.044, 14.049, 14.050, 14.053, 14.054, 14.055, 14.056, 14.062, 14.067, 14.069, 14.077, 14.082, 14.095, 14.096, 14.098, 14.100, 14.114, 14.121, 14.123, 14.142 and 14.144] the Panel agrees with the JECFA conclusion, "No safety concern at estimated levels of intake as flavouring substances" based on the MSDI approach.

TABLE 1: SPECIFICATION SUMMARY

Table 1: Specifications Summary for the JECFA Evaluated Substances in the Present Group (JECFA, 2001c)

Table 1: Specification Summary of the Substances in the JECFA Flavouring Group of Pyrazines (JECFA, 2001c)

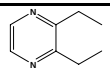
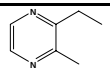
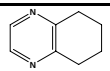
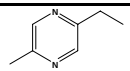
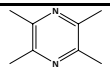
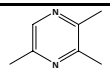
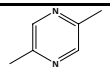
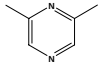
FL-no JECFA-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	EFSA comments
14.005 771	2,3-Diethylpyrazine		3136 534 15707-24-1	Liquid $C_8H_{12}N_2$ 136.20	Soluble Miscible	180 IR 97 %	1.492-1.509 0.956-0.976	
14.006 768	2-Ethyl-3-methylpyrazine		3155 548 15707-23-0	Liquid $C_7H_{10}N_2$ 122.17	Soluble Miscible	57 (13 hPa) IR 97 %	1.499-1.509 0.972-0.993	Mixture of positional isomers (2,3-; 2,5- and 2,6-isomers), 2,3- (75-85 %); 2,5- (15-25 %) and 2,6-isomers (1-2 %) (sum 97%) (EFFA, 2010a).
14.015 952	5,6,7,8-Tetrahydroquinoxaline		3321 721 34413-35-9	Solid $C_8H_{10}N_2$ 134.18	Moderately soluble Soluble	85 (4 hPa) 29-30 IR 98 %	n.a. n.a.	
14.017 770	2-Ethyl-5-methylpyrazine		3154 728 13360-64-0	Liquid $C_7H_{10}N_2$ 122.17	Soluble Miscible	79 (88 hPa) IR 95 %	1.491-1.501 0.960-0.970	
14.018 780	2,3,5,6-Tetramethylpyrazine		3237 734 1124-11-4	Solid $C_8H_{12}N_2$ 136.20	Slightly soluble Very soluble	190 85-90 IR 95 %	n.a. n.a.	
14.019 774	2,3,5-Trimethylpyrazine		3244 735 14667-55-1	Liquid $C_7H_{10}N_2$ 122.17	Soluble Miscible	171 IR 98 %	1.500-1.509 0.967-0.987	Specific gravity: depending on the quality and the producer, the SG ranges from: 0.967-0.987 (EFFA, 2010a).
14.020 766	2,5-Dimethylpyrazine		3272 2210 123-32-0	Liquid $C_6H_8N_2$ 108.14	Soluble Miscible	155 IR 98 %	1.497-1.503 0.982-1.000	Mixture of positional isomers (2,5- and 2,6-isomers), 2,5- (45-65 %) and 2,6-isomers (35-55 %) (sum 98 %) (EFFA, 2010a).
14.021 767	2,6-Dimethylpyrazine		3273 2211 108-50-9	Solid $C_6H_8N_2$ 108.14	Soluble Very soluble	154 48 IR 98 %	n.a. n.a.	Mixture of positional isomers (2,5- and 2,6-isomers), 2,5- (35-55 %) and 2,6-isomers (45-65 %) (sum 98 %) (EFFA, 2010a).

Table 1: Specification Summary of the Substances in the JECFA Flavouring Group of Pyrazines (JECFA, 2001c)

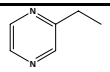
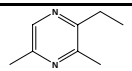
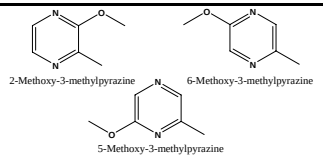
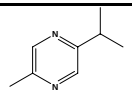
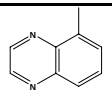
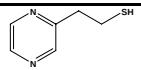
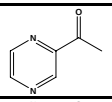
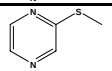
FL-no JECFA-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	EFSA comments
14.022 762	Ethylpyrazine		3281 2213 13925-00-3	Liquid $C_6H_8N_2$ 108.14	Soluble Miscible	152 IR 98 %	1.493-1.508 0.981-1.000	
14.024 776	2-Ethyl-3,5-dimethylpyrazine		3150 2245 13925-07-0	Liquid $C_8H_{12}N_2$ 136.20	Soluble Miscible	180 IR MS 95 %	1.496-1.502 0.952-0.961	
14.025 788	2,5 or 6-Methoxy-3-methylpyrazine		3183 2266 63450-30-6	Liquid $C_6H_8ON_2$ 124.14	Soluble Miscible	80-85 (13 hPa) IR 97 %	1.505-1.510 1.060-1.090	Mixture of positional isomers (2/5/6-MeO-3-Me), 2-3- (75-85 %); 6-3- (15-25 %) and 5-3- isomers (1-2 %) (sum 97 %) (EFFA, 2010a).
14.026 772	2-Isopropyl-5-methylpyrazine		3554 2268 13925-05-8	Liquid $C_8H_{12}N_2$ 136.20	Soluble Miscible	190 NMR 97 %	1.492-1.498 0.977-0.984	
14.027 761	2-Methylpyrazine		3309 2270 109-08-0	Liquid $C_5H_6N_2$ 94.12	Soluble Miscible	137 IR 98 %	1.501-1.509 1.007-1.033	
14.028 798	5-Methylquinoxaline		3203 2271 13708-12-8	Liquid $C_9H_8N_2$ 144.18	Freely soluble Very soluble	120 (20 hPa) 20 IR 98 %	1.616-1.624 1.102-1.128	
14.031 795	Pyrazineethanethiol		3230 2285 35250-53-4	Liquid $C_6H_8N_2S$ 140.21	Soluble Miscible	105-110 (26hPa) IR 97 %	1.553-1.570 1.147-1.157	
14.032 784	Acetylpyrazine		3126 2286 22047-25-2	Solid $C_6H_6ON_2$ 122.13	Slightly soluble Moderately soluble	188 74-80 IR 99 %	n.a. n.a.	
14.034 796	Pyrazinyl methyl sulfide		3231 2288 21948-70-9	Solid $C_5H_6N_2S$ 126.18	Soluble Very soluble	75 (7 hPa) 42-47 IR 99 %	n.a. n.a.	

Table 1: Specification Summary of the Substances in the JECFA Flavouring Group of Pyrazines (JECFA, 2001c)

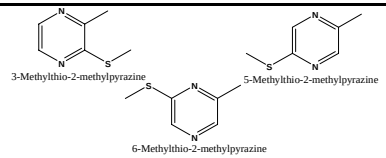
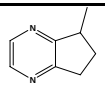
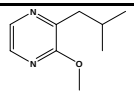
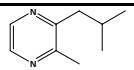
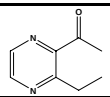
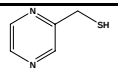
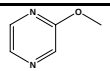
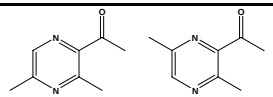
FL-no JECFA-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	EFSA comments
14.035 797	2-Methyl-3,5 or 6-methylthiopyrazine		3208 2290 67952-65-2	Liquid $C_6H_8N_2S$ 140.21	Miscible Miscible	85-87 (13 hPa) IR 99 %	1.570-1.590 1.133-1.153	Mixture of positional isomers (2-Me-3/5/6-Methylthio), 2-methyl-3-methylthio-isomer (75-85 %); main SCs: 2-5-isomer (15-25 %) and 2-6-isomer (1-2 %) (EFFA, 2010a).
14.037 781	6,7-Dihydro-5-methyl-5H-cyclopentapyrazine		3306 2314 23747-48-0	Liquid $C_8H_{10}N_2$ 134.18	Slightly soluble Miscible	200 IR 97 %	1.525-1.535 1.048-1.059	Racemate (EFFA, 2010a)
14.043 792	2-Isobutyl-3-methoxypyrazine		3132 11338 24683-00-9	Liquid $C_9H_{14}ON_2$ 166.22	Soluble Miscible	60 (3 hPa) IR 95 %	1.487-1.497 0.983-1.003	
14.044 773	2-Isobutyl-3-methylpyrazine		3133 13925-06-9	Liquid $C_9H_{14}N_2$ 150.22	Soluble Miscible	199 IR 98 %	1.488-1.498 0.936-0.942	
14.049 785	2-Acetyl-3-ethylpyrazine		3250 11293 32974-92-8	Liquid $C_8H_{10}ON_2$ 150.18	Soluble Miscible	220 IR 98 %	1.509-1.520 1.068-1.079	
14.050 765	2,3-Dimethylpyrazine		3271 11323 5910-89-4	Liquid $C_6H_8N_2$ 108.14	Soluble Miscible	156 IR 95 %	1.501-1.510 0.997-1.030	Mixture of positional isomers (2,3-; 2,5- and 2,6-isomers), 2,3- (70-85 %); 2,5- (10-25 %) and 2,6-isomers (1-2 %) (sum 95 %) (EFFA, 2010a).
14.053 794	Mercaptomethylpyrazine		3299 11502 59021-02-2	Liquid $C_5H_6N_2S$ 126.18	Soluble Miscible	94 (13 hPa) IR 98 %	1.548-1.560 1.148-1.156	
14.054 787	Methoxypyrazine		3302 11347 3149-28-8	Liquid $C_5H_6ON_2$ 110.12	Miscible Miscible	61-62 (38 hPa) IR 99 %	1.492-1.510 1.110-1.140	Specific gravity: depending on the quality and the producer, the SG ranges from: 1.110-1.140 (EFFA, 2010a).
14.055 786	2-Acetyl-3,5-dimethylpyrazine		3327 11294 54300-08-2	Liquid $C_8H_{10}ON_2$ 150.18	Soluble Miscible	70 (9 hPa) IR 97 %	1.510-1.520 1.070-1.075	Mixture of positional isomers (2-Acetyl-3,5/6-dimethyl), 3,5-dimethyl (65-70 %) and 3,6-dimethyl (25-30 %) isomers (sum 95 %) (EFFA, 2010a).

Table 1: Specification Summary of the Substances in the JECFA Flavouring Group of Pyrazines (JECFA, 2001c)

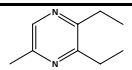
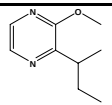
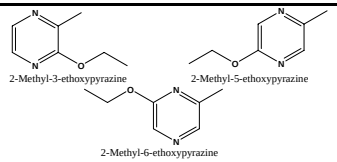
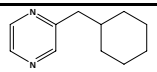
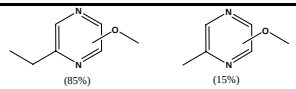
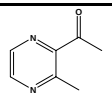
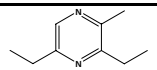
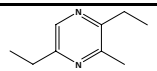
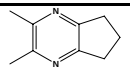
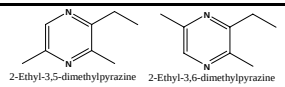
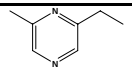
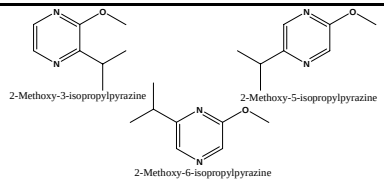
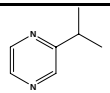
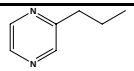
FL-no JECFA-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	EFSA comments
14.056 777	2,3-Diethyl-5-methylpyrazine		3336 11303 18138-04-0	Liquid $C_9H_{14}N_2$ 150.22	Moderately soluble Miscible	203 IR 98 %	1.493-1.505 0.938-0.957	
14.062 791	2-(sec-Butyl)-3-methoxypyrazine		3433 11300 24168-70-5	Liquid $C_9H_{14}ON_2$ 166.22	Soluble Miscible	50 (1 hPa) IR 99 %	1.478-1.498 0.976-1.002	Racemate (EFFA, 2010a). Specific gravity: depending on the quality and the producer, the SG ranges from: 0.976-1.002 (EFFA, 2010a).
14.067 793	2-Methyl-3,5 or 6-ethoxypyrazine		3569 11921 32737-14-7	Liquid $C_7H_{10}ON_2$ 138.17	Soluble Miscible	175-176 IR 97 %	1.493-1.497 1.034-1.041	Mixture of positional isomers (2-Methyl-3/5/6-ethoxy-), 2-methyl-3-ethoxypyrazine (75-85 %), 2-methyl-5-ethoxypyrazine (15-25 %) and 2-methyl-6-ethoxypyrazine (1-2 %) (sum 97 %) (EFFA, 2010a).
14.069 783	Cyclohexylmethylpyrazine		3631 28217-92-7	Liquid $C_{11}H_{16}N_2$ 176.26	Slightly soluble Miscible	100 (5 hPa) NMR 97 %	1.515-1.520 1.003-1.009	
14.077 789	2-Ethyl-(3,5 or 6)-methoxypyrazine (85%) and 2-Methyl-(3,5 or 6)-methoxypyrazine (13%)		3280 11329	Liquid	Soluble Miscible	80-95 (13 hPa) IR 99 %	1.497-1.505 1.036-1.052	Mixture of positional isomers (2-Ethyl/Methyl-(3/5/6)-methoxypyrazine: 2-Ethyl form (85 %) & 2-Methyl form (13 %), 2-3- (75-85 %); 2-5- (15-25 %) and 2-6- ethyl or methyl (1-2 %) (EFFA, 2010a).
14.082 950	2-Acetyl-3-methylpyrazine		3964 11296 23787-80-6	Liquid $C_7H_8ON_2$ 136.15	Soluble Miscible	90 (26 hPa) IR 98 %	1.521-1.523 1.105-1.114	
14.095 779	3,5-Diethyl-2-methylpyrazine		3916 11305 18138-05-1	Liquid $C_9H_{14}N_2$ 150.22	Slightly soluble Miscible	95 (18 hPa) NMR 97 %	1.492-1.502 0.944-0.954	
14.096 778	2,5-Diethyl-3-methylpyrazine		3915 11304 32736-91-7	Liquid $C_9H_{14}N_2$ 150.22	Moderately soluble Miscible	95 (18 hPa) NMR 97 %	1.4922-1.5022 0.944-0.954	

Table 1: Specification Summary of the Substances in the JECFA Flavouring Group of Pyrazines (JECFA, 2001c)

FL-no JECFA-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	EFSA comments
14.098 782	6,7-Dihydro-2,3-dimethyl-5H-cyclopentapyrazine		3917 11309 38917-62-3	Solid $C_9H_{12}N_2$ 148.21	Slightly soluble Very soluble	66 (3 hPa) 25-27 NMR 97 %	n.a. n.a.	CASrn in Register refers to 6,7-dihydro-3,5-dimethyl-5H-cyclopentapyrazine. CASrn to be changed.
14.100 775	3,(5- or 6-)-Dimethyl-2-ethylpyrazine		3149 727 55031-15-7	Liquid $C_8H_{12}N_2$ 136.2	Soluble Miscible	180 IR NMR 95 %	1.496-1.506 0.950-0.980	Mixture of positional isomers (2-Ethyl-3,5/6-dimethyl-), 2-ethyl-3,5-dimethylpyrazine (50-57 %); 2-ethyl-3,6-dimethylpyrazine (43-50 %) (EFFA, 2010a).
14.114 769	2-Ethyl-6-methylpyrazine		3919 11331 13925-03-6	Liquid $C_7H_{10}N_2$ 122.17	Soluble Miscible	80 (65 hPa) IR 95 %	1.487-1.497 0.967-0.980	Mixture of positional isomers (2-Ethyl-5/6-ethylpyrazine), 2-5- (66 %) and 2-6-isomers (33%) (sum 97 %) (EFFA, 2010a).
14.121 790	2-Isopropyl-(3,5 or 6)-methoxypyrazine		3358 11344 93905-03-4	Liquid $C_8H_{12}ON_2$ 152.2	Soluble Miscible	120-125 (26hPa) IR 97 %	1.492-1.499 1.010-1.022	Mixture of positional isomers (2-Isopropyl-3/5/6-methoxy), 2-3- (75-85 %); 2-5- (15-25 %) and 2-6-isomers (1-2 %) (sum 97 %) (EFFA, 2010a).
14.123 764	Isopropylpyrazine		3940 11343 29460-90-0	Liquid $C_7H_{10}N_2$ 122.17	Soluble Miscible	70 (26 hPa) IR 98 %	1.486-1.496 0.967-0.972	
14.142 763	Propylpyrazine		3961 11362 18138-03-9	Liquid $C_7H_{10}N_2$ 122.17	Soluble Miscible	65 (16 hPa) NMR 98 %	1.492-1.496 0.966-0.970	
14.144 951	Pyrazine		4015 11363 290-37-9	Solid $C_4H_4N_2$ 80.09	Freely soluble Very soluble	115-118 53 IR 98 %	n.a. n.a.	

1) Solubility in water, if not otherwise stated.
2) Solubility in 95 % ethanol, if not otherwise stated.
3) At 1013.25 hPa, if not otherwise stated.
4) At 20°C, if not otherwise stated.
5) At 25°C, if not otherwise stated.

TABLE 2: GENOTOXICITY DATA

Table 2.1: Genotoxicity Data (in vitro / in vivo) for 41 Pyrazines (JECFA, 2002a)

Table 2.1: Summary of Genotoxicity Data for 41 Pyrazines (JECFA, 2002a)

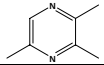
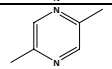
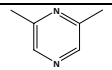
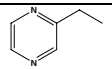
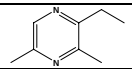
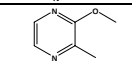
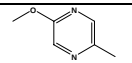
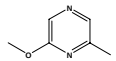
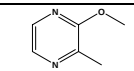
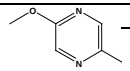
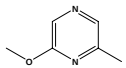
FL-no JECFA-no	EU Register name JECFA name	Structural formula	End-point	Test system	Concentration *)	Results	Reference
In vitro							
14.019 774	2,3,5-Trimethylpyrazine		Reverse mutation	*S. typhimurium* TA98, TA100, TA102	0.98–97 735 mg/plate	Negative[a]	(Aeschbacher et al., 1989)
14.020 766	2,5-Dimethylpyrazine		Reverse mutation	*S. typhimurium* TA98, TA100	Not reported	Negative[a]	(Lee et al., 1994a)
			Reverse mutation	*S. typhimurium* TA98, TA100, TA1537	12 500–200 000 mg/ plate	Negative[a]	(Stich et al., 1980)
			Mutation	*S. cerevisiae* D5	16 900–135 000 mg/ml	Positive[b]	(Stich et al., 1980)
			Chromosomal aberration	Chinese hamster ovary cells	2500–40 000 mg/ml	Positive[a]	(Stich et al., 1980)
14.021 767	2,6-Dimethylpyrazine		Reverse mutation	*S. typhimurium* TA100 *S. typhimurium* TA 98 *S. typhimurium* TA 98	86–10 800 mg/plate 2160–10 800 mg/plate 86–10 800 mg/plate	Negative[a] Positive[b] Negative[c]	(Lee et al., 1994a)
			Reverse mutation	*S. typhimurium* TA98, TA100, TA102	0.54–54 000 mg/plate	Negative[a]	(Aeschbacher et al., 1989)
			Reverse mutation	*S. typhimurium* TA98, TA100, TA1537	6300–100 000 mg/plate	Negative[a]	(Stich et al., 1980)
			Mutation	*S. cerevisiae* D5	3300–33 800 mg/ml	Positive[b]	(Stich et al., 1980)
			Chromosomal aberration	Chinese hamster ovary cells	5000–10 000 mg/ml	Positive[a]	(Stich et al., 1980)
14.022 762	Ethylpyrazine 2-Ethylpyrazine		Reverse mutation	*S. typhimurium* TA98, TA100, TA102	0.97–97 200 mg/plate	Negative[a]	(Aeschbacher et al., 1989)
			Reverse mutation	*S. typhimurium* TA98, TA100, TA1537	6300–100 000 mg/plate	Negative[a]	(Stich et al., 1980)
			Mutation	*S. cerevisiae* D5	8500–67 500 mg/ml	Positive[b]	(Stich et al., 1980)
			Chromosomal aberration	Chinese hamster ovary cells	2500–5000 mg/ml	Positive[a]	(Stich et al., 1980)
14.024 776	2-Ethyl-3,5-dimethylpyrazine 3-Ethyl-2,6-dimathylpyrazine		Reverse mutation	*S. typhimurium* TA98, TA100, TA102	0.97–97 200 mg/plate	Negative[a]	(Aeschbacher et al., 1989)
14.025 788	2,5 or 6-Methoxy-3-methylpyrazine (2 or 5 or 6)-Methoxy-3-methylpyrazine	 2-Methoxy-3-methylpyrazine  6-Methoxy-3-methylpyrazine  5-Methoxy-3-methylpyrazine	Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538	3600 mg/plate	Negative[a]	(Wild et al., 1983)
14.027 761	2-Methylpyrazine		Reverse mutation	*S. typhimurium* TA98, TA100, TA102	0.94–94,000 mg/plate	Negative[a]	(Aeschbacher et al., 1989)
			Reverse mutation	*S. typhimurium* TA98, TA100	Not reported	Negative[a]	(Lee et al., 1994a)
			Reverse mutation	*S. typhimurium* TA 98, TA100, TA1537	6300–100 000 mg/plate	Negative[a]	(Stich et al., 1980)

Table 2.1: Summary of Genotoxicity Data for 41 Pyrazines (JECFA, 2002a)

FL-no JECFA-no	EU Register name JECFA name	Structural formula	End-point	Test system	Concentration *)	Results	Reference
			Mutation	*S. cerevisiae* D5	8500–67 500 mg/ml	Positive[b]	(Stich et al., 1980)
			Chromosomal aberration	Chinese hamster ovary cells	2500–40 000 mg/ml	Positive[a]	(Stich et al., 1980)
14.050 765	2,3-Dimethylpyrazine		Reverse mutation	*S. typhimurium* TA98, TA100, TA102	0.97–97 200 mg/plate	Negative[a]	(Aeschbacher et al., 1989)
			Reverse mutation	*S. typhimurium* TA98, TA100	Not reported	Negative[a]	(Lee et al., 1994a)
14.144 951	Pyrazine		Reverse mutation	*S. typhimurium* TA98, TA100, TA102	0.64–64 000 mg/plate	Negative[a]	(Aeschbacher et al., 1989)
			Reverse mutation	*S. typhimurium* TA98, TA100	Not reported	Negative[a]	(Lee et al., 1994a)
			Reverse mutation	*S. typhimurium* TA98, TA100, TA1537	6300–100 000 mg/plate	Negative[a]	(Stich et al., 1980)
			Mutation	*S. cerevisiae* D5	7500–60 000 mg/ml	Positive[b]	(Stich et al., 1980)
			Chromosomal aberration	Chinese hamster ovary cells	2500–25 000 mg/ml	Positive[a]	(Stich et al., 1980)
In vivo							
14.025 788	2,5 or 6-Methoxy-3-methylpyrazine (2 or 5 or 6)-Methoxy-3-methylpyrazine	 2-Methoxy-3-methylpyrazine  6-Methoxy-3-methylpyra  5-Methoxy-3-methylpyrazine	Basc mutation	*Drosophila melanogaster*	10 mmol/L	Negative	(Wild et al., 1983)
			Micronucleus formation	Mouse	87, 174, or 248 mg/kg bw	Negative	(Wild et al., 1983)

*: Concentration should be in microgram not mg..A mistake in the JECFA monograph.

a: With and without metabolic activation.

b: Without metabolic activation.

c: With metabolic activation.

Table 2.2: Genotoxicity Data (in vitro) EFSA / FGE.17Rev2

Substances listed in brackets are the JECFA evaluated supporting substances in FGE.17Rev2

Table 2.2: Summary of Genotoxicity Data (*in vitro*) EFSA, FGE.17Rev2

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
(Pyrazine [14.144])	Ames test	*S. typhimurium* TA98; TA100; TA102	64000 µg/plate	Negative[1]	(Aeschbacher et al., 1989)	64000 µg/plate: highest non-bactericidal dose. Well conducted study, valid although not in accordance with OECD guideline 471: three *S. typhimurium* strains only, dose range but not individual doses reported.
	Ames test	*S. typhimurium* TA98; TA100	Not reported	Negative[1]	(Lee et al., 1994a)	Report of insufficient quality because test concentrations are not given. Reference compound within a large study, details are reported for positive compounds only.
	Ames test	*S. typhimurium* TA98; TA100; TA1537	100000 µg/plate	Negative[1]	(Stich et al., 1980)	Study with strong limitations with results of limited value.
	Ames test	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	10000 µg/ml	Negative[1, 2]	(Fung et al., 1988)	Valid study in accordancce with OECD guideline 471.
	Mutation assay	*S. cerevisiae* Strain D5	60000 µg/ml	Positive[3]	(Stich et al., 1980)	Study with strong limitations with results of limited value.
	Chromosomal Aberration assay	Chinese hamster ovary cells	10000 µg/ml 2500 µg/ml	Positive[1] Positive[1]	(Stich et al., 1980)	Study with strong limitations with results of limited value.
	Mouse lymphoma mutagenesis assay	mouse lymphocytes L5178Y $TK^{+/-}$	10000 µg/ml	Negative[1]	(Fung et al., 1988)	Study in accordancce with former OECD guideline 476 (1983); colonies were not sized and results were not confirmed in a second study as requested by the OECD guideline in force. Therefore, chromosomal aberrations effects could not be ruled out.
(2-Methylpyrazine [14.027])	Ames test	*S. typhimurium* TA98; TA100; TA102	94000 µg/plate	Negative[1]	(Aeschbacher et al., 1989)	94000 µg/plate: highest non-bactericidal dose. Well conducted study, valid although not in accordance with OECD guideline 471: three *S. typhimurium* strains only, dose range but not individual doses reported.
	Ames test	*S. typhimurium* TA98; TA100	Not reported	Negative[1]	(Lee et al., 1994a)	Report of insufficient quality because test concentrations are not given. Reference compound of a large study, details are reported for positive compounds only.
	Ames test	*S. typhimurium* TA98; TA100; TA1537	100000 µg/plate	Negative[1]	(Stich et al., 1980)	Study with strong limitations with results of limited value.
	Mutation assay	*S. cerevisiae* Strain D5	67500 µg/ml	Positive[3]	(Stich et al., 1980)	Study with strong limitations with results of limited value.
	Chromosomal Aberration assay	Chinese hamster ovary cells	40000 µg/ml 20000 µg/ml	Positive[1] Positive[1]	(Stich et al., 1980)	Study with strong limitations with results of limited value.
(Ethylpyrazine [14.022])	Ames test	*S. typhimurium* TA98; TA100; TA102	97200 µg/plate	Negative[1]	(Aeschbacher et al., 1989)	97200 µg/plate: highest non-bactericidal dose. Well conducted study, valid although not in accordance with OECD guideline 471: three *S. typhimurium* strains only, dose range but not individual doses reported.
	Ames test	*S. typhimurium* TA98; TA100; TA1537	100000 µg/plate	Negative[1]	(Stich et al., 1980)	Study with strong limitations with results of limited value.
	Mutation assay	*S. cerevisiae* Strain D5	67500 µg/ml	Positive[3]	(Stich et al., 1980)	Study with strong limitations with results of limited value.
	Chromosomal Aberration assay	Chinese hamster ovary cells	5000 µg/ml 2500 µg/ml	Positive[1] Positive[1]	(Stich et al., 1980)	Study with strong limitations with results of limited value.
(2,3-Dimethylpyrazine [14.050])	Ames test	*S. typhimurium* TA98; TA100; TA102	97200 µg/plate	Negative[1]	(Aeschbacher et al., 1989)	97200 µg/plate: highest non-bactericidal dose. Well conducted study, valid although not in accordance with OECD guideline 471: three *S. typhimurium* strains. only, dose range but not individual doses reported.
	Ames test	*S. typhimurium* TA98; TA100	NR	Negative[1]	(Lee et al., 1994a)	Report of insufficient quality because test concentrations are not given. Reference compound within a large study, details are reported for positive compounds only.

Table 2.2: Summary of Genotoxicity Data (*in vitro*) EFSA, FGE.17Rev2

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
(2,5-Dimethylpyrazine [14.020])	Ames test	*S. typhimurium* TA98; TA100; TA102	97200 µg/plate	Negative[1]	(Aeschbacher et al., 1989)	97200 µg/plate: highest non-bactericidal dose. Well conducted study, valid although not in accordance with OECD guideline 471: three *S. typhimurium* strains only, dose range but not individual doses reported.
	Ames test	*S. typhimurium* TA98; TA100	Not reported	Negative[1]	(Lee et al., 1994a)	Report of insufficient quality because test concentrations are not given. Reference compound of a large study, details are reported for positive compounds only.
	Ames test	*S. typhimurium* TA98; TA100; TA1537	200000 µg/plate	Negative[1]	(Stich et al., 1980)	Study with strong limitations with results of limited value.
	Mutation assay	*S. cerevisiae* Strain D5	135500 µg/ml	Positive[3]	(Stich et al., 1980)	Study with strong limitations with results of limited value.
	Chromosomal aberration assay	Chinese hamster ovary cells	40000 µg/ml 20000 µg/ml	Positive[1] Positive[1]	(Stich et al., 1980)	Study with strong limitations with results of limited value.
(2,6-Dimethylpyrazine [14.021])	Ames test	*S. typhimurium* TA98; TA100; TA102	54000 µg/plate	Negative[1]	(Aeschbacher et al., 1989)	54000 µg/plate: highest non-bactericidal dose. Well conducted study, valid although not in accordance with OECD guideline 471: three *S. typhimurium* strains only, dose range but not individual doses reported.
	Ames test	*S. typhimurium* TA98; TA100	10800	Negative[4]	(Lee et al., 1994a)	Well conducted study, valid although not in accordance with OECD guideline 471: two *S. typhimurium* strains only.
	Ames test	*S. typhimurium* TA98; TA100; TA1537	100000 µg/plate	Negative[1]	(Stich et al., 1980)	Study with strong limitations with results of limited value.
	Mutation assay	*S. cerevisiae* Strain D5	33800 µg/ml	Positive[3]	(Stich et al., 1980)	Study with strong limitations with results of limited value.
	Chromosomal aberration assay	Chinese hamster ovary cells	10000 µg/ml 2500 µg/ml	Positive[1] Positive[1]	(Stich et al., 1980)	Study with strong limitations with results of limited value.
(2,3-Diethylpyrazine [14.005])	Ames test	*S. typhimurium* TA98; TA100; TA102	109000 µg/plate	Negative[1]	(Aeschbacher et al., 1989)	109000 µg/plate: highest non-bactericidal dose. Well conducted study, valid although not in accordance with OECD guideline 471: three *S. typhimurium* strains only, dose range but not individual doses reported.
(2,3,5-Trimethylpyrazine [14.019])	Ames test	*S. typhimurium* TA98; TA100; TA102	97735 µg/plate	Negative[1]	(Aeschbacher et al., 1989)	97735 µg/plate: highest non-bactericidal dose. Well conducted study, valid although not in accordance with OECD guideline 471: three *S. typhimurium* strains only.
((2,5 or 6)-Methoxy-3-methylpyrazine [14.025])	Ames test	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	3600 µg/plate	Negative[1]	(Wild et al., 1983)	Well conducted study, valid although not in accordance with OECD guideline 471: test concentrations not reported.
(Pyrazinylethanethiol [14.031])	Ames test	*S. typhimurium* TA97; TA98; TA100; TA1535	NR	Negative[1]	(Zeiger and Margolin, 2000)	Well conducted study, valid although not in accordance with OECD guideline 471: report does not give test concentrations, four test concentarions.
Quinoxaline [14.147]	Ames test	*S. typhimurium* TA98; TA100	NR	Negative[3]	(Beutin et al., 1981)	TA98 ; TA100: results presented in detail, without metabolic activation. TA1535,TA1537,TA1538: results incl. metabolic activation are mentioned in text (negative), but no data given. Not in accordance with OECD guideline 471.
	Ames test	*S. typhimurium* TA98; TA100; TA102	0.35 mmol	Negative[3, 5]	(Aeschbacher et al., 1989)	0.35 mmol: highest non-bactericdial dose. Well conducted study, valid although not in accordance with OECD guideline 471: three *S. typhimurium* strains only, dose range but not individual doses reported.
	Modified Ames test	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538; G46; C3076; D3052 E. coli WP2; WP2*uvrA*$^-$	NR	Negative[3]	(McMahon et al., 1979)	Review, of limited value (concentrations tested not reported).
	Ames test (plate incorporation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	10000 µg/plate	Negative[3]	(San, 1995)	Valid study in accordancce with OECD guideline 471.

Table 2.2: Summary of Genotoxicity Data (*in vitro*) EFSA, FGE.17Rev2

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
	DNA Polymerase deficiency assay	*E. coli*	NR	Negative[3]	(Rosenkranz & Leifer, 1980)	Review, of limited value (concentrations tested not reported; without metabolic activation).
	SOS Chromosome test	*E. coli* PQ37	NR	Negative[1]	(Beutin et al., 1981)	
	Mouse lymphoma mutagenesis assay	L5178Y TK$^{+/-}$ mouse lymphocytes	(with S9) 20 – 250 (without S9) 100 – 1500 microg/ml	Positive[6] Weakly Positive[3]	(National Cancer Institute, 1998)	Valid study in accordancce with OECD guideline 476.
2-Methylquinoxaline [14.139]	Ames test	*S. typhimurium* TA98; TA100	500 µg/plate	Positive[1]	(Hashimoto et al., 1979)	Well conducted study, valid although not in accordance with OECD guideline 471: two *S. typhimurium* strains only, highest dose but not individual doses reported. Positive only in TA98 and T100 with metabolic activation.
	Ames test	*S. typhimurium* TA98; TA100; TA102	0.007 - 700 µmol/plate (equal to 0.001 – 100 mg/plate)	Negative[1, 7]	(Aeschbacher et al., 1989)	0.7 mmol: highest non-bactericidal dose. Well conducted study (valid), but not in accordance with OECD guideline 471: three *S. typhimurium* strains only, dose range but not individual doses reported.
2,3-Dimethylquinoxaline [14.108]	Ames test	*S. typhimurium* TA98; TA100; TA1535;	2500 µg/plate	Negative[6]	(Anderson and Styles, 1978)	Well conducted study, valid although not in accordance with OECD guideline 471 (with S9 metabolic activation only).
	Ames test	*S. typhimurium* TA100	NR	Negative[6]	(Epler et al., 1978)	Review, no detailed information on test conditions incl.concentration. Authors pointed out the unanswered question whether the testing of negative compounds can sensibly be terminated (in 1978).
	Ames test	*S. typhimurium* TA98; TA100	NR	Negative[1]	(Hashimoto et al., 1979)	Validity cannot be evaluated. Concentrations not reported. Results not reported in detail.
(5-Methylquinoxaline [14.028])	Reverse mutation	*S. typhimurium* TA 98, TA100, TA1535, TA1537 and *E.coli* strain WP2 uvrA	Up to 5000 microgram/plate	Negative[1]	(Ogura & Wakamatsu, 2004)	Valid. GLP-study in compliance with OECD 471 (except that no justification was provided for the use of duplicate instead of triplicate plating).
	Chromosomal aberration assay	Chinese hamster lung-derived CHL/IU cells	320, 480, 720 microgram/ml 72, 228, 720 microgram/ml	Negative[3] Positive[6]	(Ajimu & Kawaguchi, 2004a)	Valid. GLP-study mainly in compliance with OECD 473 (duration of exposure not clearly reported). The authors noted in the discussion section that cytotoxicty was observed in the form of decreased cell viability and reproductive rate. However, it is not clear if one or two parameters for cytotoxicity were measured. The percentage of "cell productivity" (the cell number was measured and expressed as relative growth rate compared to negative control) was reported. According to the authors, there was a clear evidence of cytotoxicty in the form of decreased cell viability and reproductive rate at concentrations where chromosomal aberrations were observed. However, the results presented in tables demonstrate that 30 and 66 % of cell with chromosomal aberrations were induced at the limit of exessive cytotoxicty (54 and 46% of relative growth) in the preliminary test (in which 50 cells per slide were scored) at 180 and 360 µg/mL in the presence of S9, respectively. In the main test, the percentage of cells with chromosomal aberrations in the presence of S9 was 2.0, 2.5, 6.5 and 57.5 at 0, 72, 228 and 720 µg/mL, respectively, which was accompanied by 100, 90, 85 and 46% relative growth, respectively.

NR: Not reported.

[1] With and without S9 metabolic activation.

[2] Metabolic activation was provided with both rat and hamster liver S9 mix.

[3] Without S9 metabolic activation.

[4] Results were negative in TA100 with and without S9 metabolic activation; however, in TA98 the results were negative and positive with and without S9 metabolic activation, respectively.

[5] Results were negative in TA100 with and without S9 metabolic activation. Weak results were noted in TA98 and TA102 with S9 metabolic activation. These changes may be related to the heat production products of the Maillard reaction in the presence of creatinine.

[6] With S9 metabolic activation.

[7] Weak results were noted in all strains with S9 metabolic activation. *(the number of revertants was increased up to 1.3-fold compared to control). According to the authors (Aeschbacher et al., 1989), these* changes may be related to the heat production products of the Maillard reaction in the presence of creatinine.

Table 2.3: Genotoxicity Data (in vivo) EFSA / FGE.17Rev2

Substances listed in brackets are JECFA-evaluated supporting substances in FGE.17Rev2.

Table 2.3: GENOTOXICITY (*in vivo*)

Chemical Name [FL-no]	Test System	Test Object	Route	Dose	Result	Reference	Comments
((2,5 or 6)-Methoxy-3- methylpyrazine [14.025])	Basc test	*D. melangaster*		10 mM	Negative	(Wild et al., 1983)	Limited relevance for risk assessment as the test is not in a mammalian system and the test is not used routinely.
	Micronucleus assay	Mouse		248 mg/kg	Negative	(Wild et al., 1983)	Study design does not meet the criteria of current guidelines (PCE/NCE ratio was not reported, thus it is not clear if the test substance reached the bone marrow). Not in accordance with OECD guideline 474 (1983/1997).
Quinoxaline [14.147]	Sperm head abnormality test	Mouse	I.P	2500 mg/kg	Negative	(Topham, 1980)	Sperm head abnormality test does not make use of a genetic endpoint.
(5-Methylquinoxaline [14.028])	Micronucleus assay	Mouse	Gavage	125, 250 and 500 mg/kg/day	Negative	(Ajimu & Kawaguchi, 2004b)	Valid. GLP-study mainly in compliance with OECD 474 (only 5 male mice per group instead of 5 males and 5 females),

TABLE 3: SUMMARY OF SAFETY EVALUATIONS

Table 3.1: Summary of Safety Evaluation of Pyrazine and 40 Derivatives (JECFA, 2003a)

Table 3.1: Summary of Safety Evaluation of JECFA-Evaluated Pyrazine Derivatives (JECFA, 2003a)

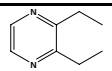
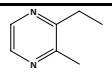
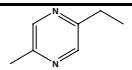
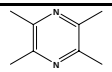
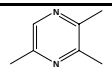
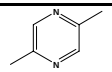
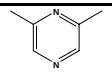
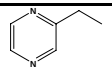
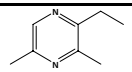
FL-no JECFA-no	EU Register name	Structural formula	EU MSDI 1) US MSDI (µg/*capita*/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	EFSA conclusion on the named compound (Procedure steps, intake estimates, NOAEL, genotoxicity)	EFSA conclusion on the material of commerce
14.005 771	2,3-Diethylpyrazine		1.6 1	Class II A3: Intake below threshold	4)	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.
14.006 768	2-Ethyl-3-methylpyrazine		72 9	Class II A3: Intake below threshold	4)	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.
14.017 770	2-Ethyl-5-methylpyrazine		4.0 1	Class II A3: Intake below threshold	4)	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.
14.018 780	2,3,5,6-Tetramethylpyrazine		6.7 19	Class II A3: Intake below threshold	4)	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.
14.019 774	2,3,5-Trimethylpyrazine		100 46	Class II A3: Intake below threshold	4)	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.
14.020 766	2,5-Dimethylpyrazine		19 8	Class II A3: Intake below threshold	4)	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.
14.021 767	2,6-Dimethylpyrazine		1.3 2	Class II A3: Intake below threshold	4)	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.
14.022 762	Ethylpyrazine		2.2 6	Class II A3: Intake below threshold	4)	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.
14.024 776	2-Ethyl-3,5-dimethylpyrazine		1.2 0.3	Class II A3: Intake below threshold	4)	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.

Table 3.1: Summary of Safety Evaluation of JECFA-Evaluated Pyrazine Derivatives (JECFA, 2003a)

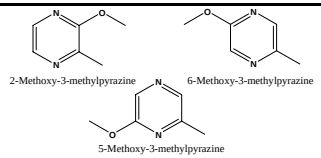
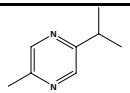
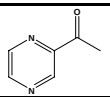
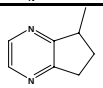
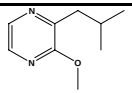
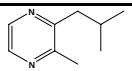
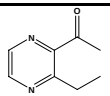
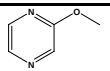
FL-no JECFA-no	EU Register name	Structural formula	EU MSDI 1) US MSDI (µg/*capita*/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	EFSA conclusion on the named compound (Procedure steps, intake estimates, NOAEL, genotoxicity)	EFSA conclusion on the material of commerce
14.025 788	2,5 or 6-Methoxy-3-methylpyrazine		2.2 15	Class II A3: Intake below threshold	4)	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.
14.026 772	2-Isopropyl-5-methylpyrazine		0.024 0.4	Class II A3: Intake below threshold	4)	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.
14.027 761	2-Methylpyrazine		17 7	Class II A3: Intake below threshold	4)	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.
14.032 784	Acetylpyrazine		12 120	Class II A3: Intake below threshold	4)	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.
14.037 781	6,7-Dihydro-5-methyl-5H-cyclopentapyrazine		3.9 4	Class II A3: Intake below threshold	4)	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.
14.043 792	2-Isobutyl-3-methoxypyrazine		1.6 1	Class II A3: Intake below threshold	4)	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.
14.044 773	2-Isobutyl-3-methylpyrazine		0.037 0.01	Class II A3: Intake below threshold	4)	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.
14.049 785	2-Acetyl-3-ethylpyrazine		0.73 0.1	Class II A3: Intake below threshold	4)	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.
14.050 765	2,3-Dimethylpyrazine		14 4	Class II A3: Intake below threshold	4)	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.
14.054 787	Methoxypyrazine		3.0 1	Class II A3: Intake below threshold	4)	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.

Table 3.1: Summary of Safety Evaluation of JECFA-Evaluated Pyrazine Derivatives (JECFA, 2003a)

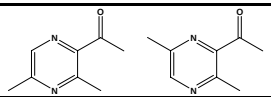
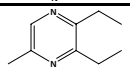
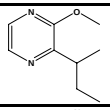
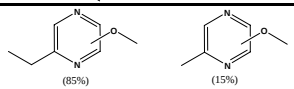
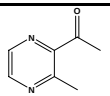
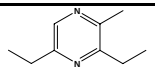
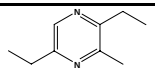
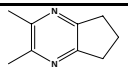
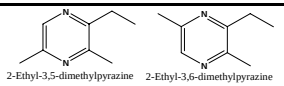
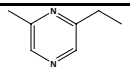
FL-no JECFA-no	EU Register name	Structural formula	EU MSDI 1) US MSDI (µg/*capita*/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	EFSA conclusion on the named compound (Procedure steps, intake estimates, NOAEL, genotoxicity)	EFSA conclusion on the material of commerce
14.055 786	2-Acetyl-3,5-dimethylpyrazine		0.97 1	Class II A3: Intake below threshold	4)	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.
14.056 777	2,3-Diethyl-5-methylpyrazine		0.11 1	Class II A3: Intake below threshold	4)	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.
14.062 791	2-(sec-Butyl)-3-methoxypyrazine		0.85 0.1	Class II A3: Intake below threshold	4)	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.
14.077 789	2-Ethyl-(3,5 or 6)-methoxypyrazine (85%) and 2-Methyl-(3,5 or 6)-methoxypyrazine (13%)		1.3 1	Class II A3: Intake below threshold	4)	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.
14.082 950	2-Acetyl-3-methylpyrazine		0.1 0.1	Class II A3: Intake below threshold	4)	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.
14.095 779	3,5-Diethyl-2-methylpyrazine		0.012 0.01	Class II A3: Intake below threshold	4)	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.
14.096 778	2,5-Diethyl-3-methylpyrazine		0.012 0.01	Class II A3: Intake below threshold	4)	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.
14.098 782	6,7-Dihydro-2,3-dimethyl-5H-cyclopentapyrazine		0.012 0.01	Class II A3: Intake below threshold	4)	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. CASrn in Register refers to 6,7-dihydro-3,5-dimethyl-5H-cyclopentapyrazine. CASrn to be changed.
14.100 775	3,(5- or 6-)-Dimethyl-2-ethylpyrazine		38 9	Class II A3: Intake below threshold	4)	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.
14.114 769	2-Ethyl-6-methylpyrazine		0.37 0.4	Class II A3: Intake below threshold	4)	No safety concern at the estimated level of intake based on the MSDI	No safety concern at the estimated level of intake based on the MSDI

Table 3.1: Summary of Safety Evaluation of JECFA-Evaluated Pyrazine Derivatives (JECFA, 2003a)

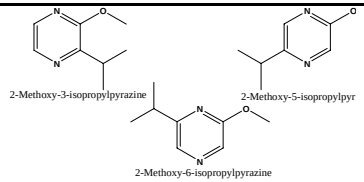
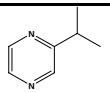
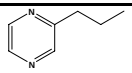
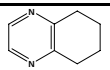
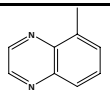
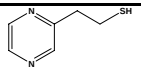
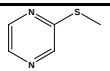
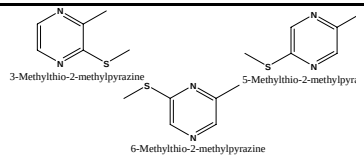
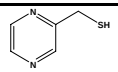
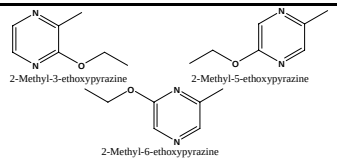
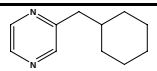
FL-no JECFA-no	EU Register name	Structural formula	EU MSDI 1) US MSDI (µg/*capita*/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	EFSA conclusion on the named compound (Procedure steps, intake estimates, NOAEL, genotoxicity)	EFSA conclusion on the material of commerce
						approach	approach.
14.121 790	2-Isopropyl-(3,5 or 6)-methoxypyrazine		0.0012 0.1	Class II A3: Intake below threshold	4)	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.
14.123 764	Isopropylpyrazine		0.12 0.1	Class II A3: Intake below threshold	4)	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.
14.142 763	Propylpyrazine		0.12 0.1	Class II A3: Intake below threshold	4)	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.
14.015 952	5,6,7,8-Tetrahydroquinoxaline		8 ND	Class III A3: Intake below threshold	4)	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.
14.028 798	5-Methylquinoxaline		22 1	Class III A3: Intake below threshold	4)	The Panel concluded based on additional data that the genotoxicity alert can be ruled out. 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.
14.031 795	Pyrazineethanethiol		0.13 1	Class III A3: Intake below threshold	4)	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.
14.034 796	Pyrazinyl methyl sulfide		0.0061 0.01	Class III A3: Intake below threshold	4)	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.
14.035 797	2-Methyl-3,5 or 6-methylthiopyrazine		6.3 13	Class III A3: Intake below threshold	4)	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.

Table 3.1: Summary of Safety Evaluation of JECFA-Evaluated Pyrazine Derivatives (JECFA, 2003a)

FL-no JECFA-no	EU Register name	Structural formula	EU MSDI 1) US MSDI (µg/*capita*/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	EFSA conclusion on the named compound (Procedure steps, intake estimates, NOAEL, genotoxicity)	EFSA conclusion on the material of commerce
14.053 794	Mercaptomethylpyrazine		0.012 0.01	Class III A3: Intake below threshold	4)	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.
14.067 793	2-Methyl-3,5 or 6-ethoxypyrazine		0.055 0.01	Class III A3: Intake below threshold	4)	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.
14.069 783	Cyclohexylmethylpyrazine		0.012 0.01	Class III A3: Intake below threshold	4)	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.
14.144 951	Pyrazine		0.024 0.2	Class III A3: Intake below threshold	4)	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.

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.

ND: not determined.

Table 3.2: Summary of Safety Evaluation Applying the Procedure (EFSA / FGE.17Rev2)

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

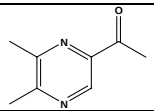
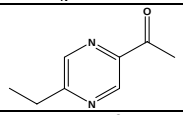
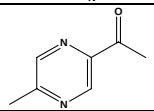
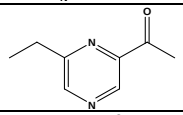
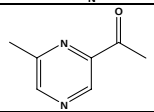
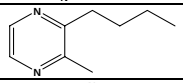
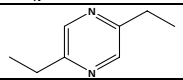
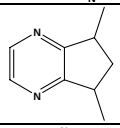
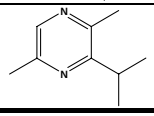
FL-no	EU Register name	Structural formula	MSDI 1) (µg/*capita*/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
14.081	5-Acetyl-2,3-dimethylpyrazine		0.012	Class II A3: Intake below threshold	4)	6)	
14.083	2-Acetyl-5-ethylpyrazine		0.012	Class II A3: Intake below threshold	4)	6)	
14.084	2-Acetyl-5-methylpyrazine		0.0024	Class II A3: Intake below threshold	4)	6)	
14.086	2-Acetyl-6-ethylpyrazine		0.0061	Class II A3: Intake below threshold	4)	6)	
14.087	2-Acetyl-6-methylpyrazine		0.028	Class II A3: Intake below threshold	4)	6)	
14.091	2-Butyl-3-methylpyrazine		0.12	Class II A3: Intake below threshold	4)	6)	
14.097	2,5-Diethylpyrazine		0.024	Class II A3: Intake below threshold	4)	6)	
14.099	6,7-Dihydro-5,7-dimethyl-5H-cyclopentapyrazine		0.032	Class II A3: Intake below threshold	4)	7)	
14.101	2,5-Dimethyl-3-isopropylpyrazine		0.018	Class II A3: Intake below threshold	4)	6)	

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

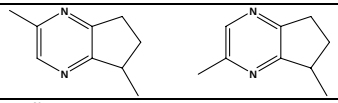
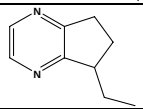
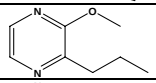
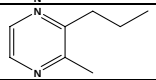
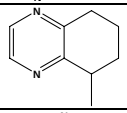
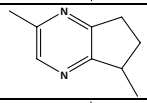
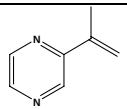
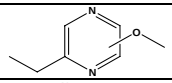
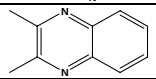
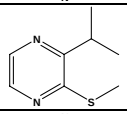
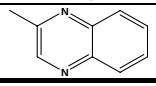
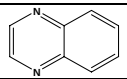
FL-no	EU Register name	Structural formula	MSDI 1) (μg/*capita*/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
14.102	5,6-Dimethyldihydrocyclopentapyrazine		0.024	Class II A3: Intake below threshold	4)	7)	
14.113	5-Ethyl-6,7-dihydro-5H-cyclopentapyrazine		0.012	Class II A3: Intake below threshold	4)	6)	
14.127	2-Methoxy-3-propylpyrazine		0.061	Class II A3: Intake below threshold	4)	6)	
14.129	2-Methyl-3-propylpyrazine		0.011	Class II A3: Intake below threshold	4)	6)	
14.148	5,6,7,8-Tetrahydro-5-methylquinoxaline		0.0073	Class II A3: Intake below threshold	4)	6)	
14.161	6,7-Dihydro-2,5-dimethyl-5H-cyclopentapyrazine		0.011	Class II A3: Intake below threshold	4)	6)	
14.052	Isopropenylpyrazine		0.012	Class II B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		
14.051	2,5 or 6-Methoxy-3-ethylpyrazine			Class II No evaluation			
14.108	2,3-Dimethylquinoxaline		0.049	Class III A3: Intake below threshold	4)	6)	
14.122	2-Isopropyl-3-methylthiopyrazine		0.061	Class III B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
14.139	2-Methylquinoxaline		0.12	Class III A3: Intake below threshold	4)	6)	

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

FL-no	EU Register name	Structural formula	MSDI 1) (µg/*capita*/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
14.147	Quinoxaline		0.12	Class III No evaluation			a)

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.

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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
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 practise
ID	Identity
Ip	Intraperitoneal
IR	Infrared spectroscopy
JECFA	The Joint FAO/WHO Expert Committee on Food Additives
MNPCE	Micronucleated polychromatic erythrocytes
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
PCE	Polychromatic erythrocyte
SCE	Sister chromatic exchange
SCF	Scientific Committee on Food

WHO World Health Organisation