EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF); Scientific Opinion on Flavouring Group Evaluation 17, Revision 3 (FGE.17Rev3): Pyrazine derivatives from chemical group 24

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SCIENTIFIC OPINION

Scientific Opinion on Flavouring Group Evaluation 17, Revision 3 (FGE.17Rev3):

Pyrazine derivatives from chemical group 24[1]

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

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

The Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids of the European Food Safety Authority was requested to evaluate 28 flavouring substances in the Flavouring Group Evaluation 17, including seven additional substances considered in this Revision 3, using the Procedure in Commission Regulation (EC) No 1565/2000. From the *in vitro* data available, genotoxic potential is indicated for the flavouring substances quinoxaline [FL-no: 14.147] and 2-methylquinoxaline [FL-no: 14.139]. Therefore, the Panel decided that the Procedure could not be applied to these two substances, until adequate data showing absence of genotoxicity are provided. For one substance [FL-no: 14.051] no intake data are available preventing it from being evaluated through the Procedure. The remaining 25 substances were evaluated through a stepwise approach that integrates information on structure-activity relationships, intake from current uses, toxicological threshold of concern, and available data on metabolism and toxicity. The Panel concluded that 24 substances [FL-no: 14.057, 14.081, 14.083, 14.084, 14.086, 14.087, 14.091, 14.097, 14.099, 14.101, 14.102, 14.108, 14.109, 14.111, 14.112, 14.113, 14.122, 14.126, 14.127, 14.128, 14.129, 14.148, 14.161 and 14.170] do not give rise to safety concerns at their levels of dietary intake, estimated on the basis of the MSDI approach. For the remaining substance [FL-no: 14.052], additional toxicity data are required. Besides the safety assessment of these flavouring substances, the specifications for the materials of commerce have also been considered and for one substance [FL-no: 14.102], the composition of mixture has not been specified sufficiently.

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1 On request from the Commission, Question No EFSA-Q-2010-01556, EFSA-Q-2011-01139, EFSA-Q-2011-01140, EFSA-Q-2011-01141, EFSA-Q-2011-01142, EFSA-Q-2011-01143, EFSA-Q-2011-01144, adopted on 24 November 2011.

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SUMMARY

The European Food Safety Authority (EFSA) asked the Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (the Panel) to provide scientific advice to the Commission on the implications for human health of chemically defined flavouring substances used in or on foodstuffs in the Member States. In particular, the Panel was requested to evaluate 28 flavouring substances in the Flavouring Group Evaluation 17, Revision 3 (FGE.17Rev3), using the Procedure as referred to in the Commission Regulation (EC) No 1565/2000. These 28 flavouring substances belong to chemical group 24 of Annex I of the Commission Regulation (EC) No 1565/2000.

The present Flavouring Group Evaluation (FGE) deals with 28 pyrazine derivatives. Three of these derivatives are quinoxalines.

Five flavouring substances possess one or two chiral centres. For all five substances, the stereoisomeric composition has been specified.

Twenty flavouring substances are classified into structural class II and eight are classified into structural class III.

Twenty-four of the flavouring substances in the present group have been reported to occur naturally in a wide range of food items.

In its evaluation, the Panel as a default used the "Maximised Survey-derived Daily Intakes" (MSDIs) approach to estimate the *per capita* intakes of the flavouring substances in Europe. However, when the Panel examined the information provided by the European Flavouring Industry on the use levels in various foods, it appeared obvious that the MSDI approach in a number of cases would grossly underestimate the intake by regular consumers of products flavoured at the use level reported by 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.

In the absence of more precise 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. In those cases where the mTAMDI approach indicated that the intake of a flavouring substance might exceed its corresponding threshold of concern, the Panel decided not to carry out a formal safety assessment using the Procedure. In these cases the Panel requires more precise data on use and use levels.

According to the default MSDI approach, the flavouring substances in this group, which are evaluated through the Procedure and for which Industry has provided intake data, have intakes in Europe from 0.0024 to 12 microgram*/capita/*day, which are below the threshold of concern value for both structural class II (540 microgram/person/day) and structural class III (90 microgram/person/day) substances. For one candidate substance [FL-no: 14.051], no intake data are available, preventing it from being evaluated through the Procedure.

From the *in vitro* data available, a genotoxic potential is indicated for quinoxaline [FL-no: 14.147] and 2-methylquinoxaline [FL-no: 14.139]. Therefore, the Panel decided that the Procedure could not be applied to these two substances until adequate genotoxicity data become available.

No genotoxic potential at gene or chromosome level is indicated for 2,3-dimethylquinoxaline [FL-no: 14.108] and the remaining alkyl- and cycloalkyl-substituted pyrazines, which allows these substances to be evaluated through the Procedure.

So, in total, 25 substances are evaluated through the Procedure, based on the MSDI approach Appendix I).

Twenty-two of the alkyl- and cycloalkyl-substituted pyrazines and one of the alkyl-substituted quinoxalines may be expected to be metabolised to innocuous products [FL-no: 14.051, 14.057, 14.081, 14.083, 14.084, 14.086, 14.087, 14.091, 14.097, 14.099, 14.101, 14.102, 14.108, 14.109, 14.111, 14.112, 14.113, 14.126, 14.127, 14.129, 14.148, 14.161 and 14.170]. Regarding the remaining five substances, they cannot be anticipated to be metabolised to innocuous products. Two sulphur-containing flavouring substance [FL-no: 14.122 and 14.128], may be converted to a reactive free thiol. It can also not be assumed that quinoxaline [FL-no: 14.147] and its derivative 2-methylquinoxaline [FL-no: 14.139] are metabolised to innocuous products. The fifth substance [FL-no: 14.052] has a terminal double bond in conjugation with a heterocyclic aromatic ring, which may be epoxidised giving rise to reactive metabolites.

It was noted, that where toxicity data were available they were consistent with the conclusions in the present FGE.

It was considered that on the basis of the default MSDI approach, the 24 of the 25 flavouring substances evaluated through the Procedure would not give rise to safety concerns at the estimated levels of intake arising from their use as flavouring substances. No valid toxicity study from which a NOAEL could be established was available for isopropenylpyrazine [FL-no: 14.052] or for any relevant supporting substance. Therefore, the Panel concluded that additional toxicity data are needed for this substance.

When the estimated intakes, of the substances evaluated through the Procedure, were based on the mTAMDI approach they ranged from 190 to 1100 microgram/person/day for the 17 flavouring substances from structural class II for which data have been provided. These intakes were below the threshold of concern for structural class II of 540 microgram/person/day, except for one substance [FL-no: 14.170]. The estimated intake of the two flavouring substances [FL-no: 14.108 and 14.122] assigned to structural class III for which data have been provided, and evaluated through the Procedure, are 190 and 270 microgram/person/day, respectively, which are above the threshold of concern for structural class III of 90 microgram/person/day. The 16 substances, which have mTAMDI intake estimates below the threshold of concern for structural class II, are also expected to be metabolised to innocuous products. Thus, for three flavouring substances [FL-no: 14.108, 14.122 and 14.170] the intakes estimated on the basis of the mTAMDI, exceed the relevant threshold for their structural class, to which the flavouring substance has been assigned. For seven substances [FL-no: 14.051, 14.057, 14.109, 14.111, 14.112, 14.126 and 14.128], no use levels were provided. Therefore, for these 10 substances more reliable exposure data are required. On the basis of such additional data, the flavouring substances should be re-evaluated using the Procedure. Subsequently, additional toxicity data might become necessary.

Thus, in conclusion, 25 of the 28 flavouring substances were evaluated through the Procedure (based on MSDI approach), as two flavouring substances, quinoxaline [FL-no: 14.147] and 2-methylquinoxaline [FL-no: 14.139] could not be evaluated through the Procedure until adequate genotoxicity data become available and one substance [FL-no: 14.051] was presented with no intake data and therefore could not be evaluated through the Procedure.

In order to determine whether the conclusion for the 25 flavouring substances which have been evaluated using the Procedure (MSDI approach) can be applied to the materials of commerce, it is necessary to consider the available specifications. Adequate specifications including purity criteria and identity for the materials of commerce have been provided for 24 flavouring substances evaluated through the Procedure. For the substance [FL-no: 14.102], the final evaluation of the materials of commerce cannot be performed pending further information on composition of mixture of positional isomers.

For one substance [FL-no: 14.052] evaluated through the Procedure, additional toxicity data are required.

For the remaining 23 substances [FL-no: 14.057, 14.081, 14.083, 14.084, 14.086, 14.087, 14.091, 14.097, 14.099, 14.101, 14.108, 14.109, 14.111, 14.112, 14.113, 14.122, 14.126, 14.127, 14.128, 14.129, 14.148, 14.161 and 14.170] the Panel concluded that they would present no safety concern at the level of intake estimated on the basis of the MSDI approach.

KEYWORDS

Flavourings, pyrazine, quinoxaline, food safety, FGE.17, FGE.50Rev1.

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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 2008/163/EC (EC, 2009a). Each flavouring substance is attributed a FLAVIS-number (FL-number) and all substances are divided into 34 chemical groups. Substances within a group should have some metabolic and biological behaviour in common.

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

The FGE is revised to include substances for which data were submitted after the deadline as laid down in Commission Regulation (EC) No 622/2002 and to take into account additional information that has been made available since the previous Opinion on this FGE.

The Revision also includes newly notified substances belonging to the same chemical groups evaluated in this FGE.

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

HISTORY OF THE EVALUATION

In previous revisions of the present FGE, it was found that two of the candidate substances, quinoxaline [FL-no: 14.147] and 2-methylquinoxaline [FL-no: 14.139] showed a possible genotoxic potential *in vitro* and accordingly 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 became available.

Additional genotoxicity data have become available for the structurally related supporting substance 5-methylquinoxaline [FL-no: 14.028] (evaluated in FGE.50) and in FGE.17Rev2 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). Therefore these compounds [FL-no; 14.139 and 14.147] are to be evaluated based on substance-specific data for each individual quinoxaline derivative.

The revision 2 of FGE.17 also included the evaluation of one new substance [FL-no: 14.051]. [FL-no: 14.051] is one isomer of the mixture of the structural isomers of [FL-no: 14.077] (see Table 1 and Table 3, respectively). The Panel decided that the evaluation of [FL-no: 14.077] could not cover [FL-no: 14.051] and accordingly the evaluation of [FL-no: 14.051] was included in FGE.17Rev2. No European production figure for this substance is available.

FGE	Opinion adopted by EFSA	Link	No. of candidate substances
FGE.17	7 December 2005	http://www.efsa.eu.int/science/afc/afc_opinions/1291_en.html	18
FGE.17Rev1	30 January 2008	http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1211902101869.htm	20
FGE.17Rev2	23 November 2010	http://www.efsa.europa.eu/en/efsajournal/pub/1920.htm	21

FGE	Opinion adopted by EFSA	Link	No. of candidate substances
FGE.17Rev3	23 November 2011		28

The present revision of FGE.17, FGE.17Rev3, includes the assessment of seven additional candidate substances [FL-no: 14.057, 14.109, 14.111, 14.112, 14.126, 14.128 and 14.170]. No toxicity or metabolism data were provided for the new substances. A search in open literature provided no further data on toxicity or metabolism for the substances.

Furthermore, new information from Industry on missing stereoisomeric composition for [FL-no: 14.099 and 14.102] received after publication of the last revision is included in the present revision (EFFA, 2011a).

TERMS OF REFERENCE

The European Food Safety Authority (EFSA) is requested to carry out a risk assessment on flavouring substances in the Register (Commission decision 1999/217/EC), according to Commission Regulation (EC) No 1565/2000 (EC, 2000a), prior to their authorisation and inclusion in the Union list (Regulation (EC) No 1334/2008). In addition, the Commission requested EFSA to evaluate newly notified flavouring substances, where possible, before finalising the evaluation programme. The evaluation programme was finalised at the end of 2009.

In a 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. This re-evaluation was made in FGE.17Rev2.

After the finalisation of the evaluation programme, in their letter of the 30th July 2010, the Commission requested EFSA to carry out an evaluation of the flavouring substance 5-ethyl 2,3-dimethyl pyrazine [FL-no: 14.170], also according to Commission Regulation (EC) No 1565/2000 (EC, 2000a).

In addition, in letter of 22 September 2011, the Commission has asked EFSA to reflect newly requested information on specifications in the revisions of FGEs.

ASSESSMENT

1. Presentation of the Substances in Flavouring Group Evaluation 17, Revision 3

1.1. Description

The present revision of Flavouring Group Evaluation 17, FGE.17Rev3, using the Procedure as referred to in the Commission Regulation (EC) No 1565/2000 (EC, 2000a) (The Procedure – shown in schematic form in Annex I of this FGE), deals with 28 pyrazine derivatives (candidate substances), which belong to chemical group 24 of Annex I of Commission Regulation (EC) No 1565/2000 (EC, 2000a).

The candidate substances under consideration, as well as their chemical Register names, FLAVIS- (FL-), Chemical Abstract Service- (CAS-), Council of Europe- (CoE-) and Flavor and Extract Manufacturers Association- (FEMA-) numbers, structure and specifications, are listed in Table 1.

All the candidate substances contain a pyrazine moiety. In 20 substances [FL-no: 14.051, 14.052, 14.057, 14.081, 14.083, 14.084, 14.086, 14.087, 14.091, 14.097, 14.101, 14.109, 14.111, 14.112,

14.122, 14.126, 14.127, 14.128, 14.129 and 14.170] only one heterocyclic ring is present. In five candidate substances a pyrazine ring is fused with either cyclopentane [FL-no: 14.099, 14.102, 14.113 and 14.161] or with cyclohexane [FL-no: 14.148]. All of these 25 substances have different ring substituents. In 16 substances, the substituents are simple alkyl chains and/or ketones. In nine substances, the substituents are, next to alkyl chains, either a methoxy- ([FL-no: 14.051, 14.057, 14.112, 14.126 and 14.127], an ethoxy- [FL-no: 14.109], a thiomethyl- [FL-no: 14.122 and 14.128]) or an isopropenyl residue [FL-no: 14.052]. In the remaining three candidate substances the pyrazine ring is fused with benzene giving quinoxalines. In two of the quinoxalines [FL-no: 14.108 and 14.139] the pyrazine ring also bears one or two methyl substituents; in the third no substituents are present (quinoxaline; [FL-no: 14.147]).

A summary of the outcome of the safety evaluation of the candidate substances is listed in Table 2.

The candidate substances are structurally related to 41 flavouring substances (supporting substances) evaluated at the 57th JECFA meeting in the group "Pyrazine Derivatives" (JECFA, 2002b). The name and structures of the 41 supporting substances are listed in Table 3, together with their evaluation status (JECFA. 2002b; SCF,1992).

1.2. Stereoisomers

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

Five of the flavouring substances possess one or two chiral centres [FL-no: 14.099, 14.102, 14.113, 14.148 and 14.161]. For all five substances, the stereoisomeric composition has been specified.

1.3. Natural Occurrence in Food

Twenty-four of the candidate substances have been reported to occur in beef, chicken, cocoa, coffee, green tea, fruit juice, beer, potato, pork, whisky, sherry, nuts, peanut, roasted sesame seed, peas, malt or wild rice. Quantitative data on the natural occurrence in food have been reported for 10 of these substances (TNO, 2000; TNO, 2011).

These reports are:

- Isopropenylpyrazine [FL-no: 14.052]: 0.11 mg/kg in malt.
- 2-Isopropyl-3-methoxypyrazine [FL-no: 14.057]: up to 0.09 mg/kg in coffee, up to 0.006 mg/kg in wine, up to 0.0001 mg/kg in potato and 0.00001 mg/kg in peas.
- 2-Acetyl-5-methylpyrazine [FL-no: 14.084]: up to 0.3 mg/kg in coffee.
- 2-Acetyl-6-methylpyrazine [FL-no: 14.087]: up to 0.55 mg/kg in coffee.
- 2-Butyl-3-methylpyrazine [FL-no: 14.091]: 1 mg/kg in cocoa.
- 2,5-Diethylpyrazine [FL-no: 14.097]: up to 0.1 mg/kg in coffee, and 0.02 mg/kg in peanut.

- 3-Ethyl-2,5-dimethylpyrazine [FL-no: 14.111]: up to 2.8 mg/kg in cocoa, up to 2.2 mg/kg in coffee, up to 12.5 mg/kg in pork, 2.2 mg/kg in potato chips, up to 0.4 mg/kg in malt, up to 0.3 mg/kg in shrimps and soybean, 0.2 mg/kg in tea, 0.2 mg/kg in rum, 0.1 mg/kg in peanut, up to 0.02 mg/kg in beer, up to 0.013 mg/kg in maize and beef,

- Quinoxaline [FL-no: 14.147]: up to 0.1 mg/kg in malt.

- 6,7-Dihydro-2,5-dimethyl-5H-cyclopentapyrazine [FL-no: 14.161]: up to 5.1 mg/kg in coffee, and up to 0.0001 mg/kg in pork.

- 5-Ethyl-2,3-dimethyl pyrazine [FL-no: 14.170]: 10 mg/kg in coffee, up to 4 mg/kg in coconut, 0.3 mg/kg in potato chips, up to 0.2 mg/kg in cocoa and up to 0.01 mg/kg in malt.

According to TNO, four of the candidate substances, 2-, 5- or 6-methoxy-3-ethylpyrazine, 2-ethoxy-3-methylpyrazine 2-isopropyl-3-methylthiopyrazine and 2-methyl-3-methylthiopyrazine [FL-no: 14.051,14.109 14.122 and 14.128], have not been reported to occur naturally in any food items (TNO, 2000; TNO, 2009; TNO, 2011).

2. Specifications

Purity criteria for 27 of the candidate substances have been provided by the Flavouring Industry (EFFA, 2003q; EFFA, 2007e; EFFA, 2007f; Flavour Industry, 2010m; EFFA, 2011e). For one substance [FL-no: 14.051] no purity criteria has been provided.

Judged against the requirements in Annex II of Commission Regulation (EC) No 1565/2000 (EC, 2000a), this information is adequate for the 27 of the candidate substances. Specifications are lacking for [FL-no: 14.051].

However, according to Industry (EFFA, 2007e; EFFA, 2011a) [FL-no: 14.102] covers a mixture of 2,5-dimethyl-6,7-dihydro-5H-cyclopentapyrazine (60 - 100 %) and 3,5-dimethyl-6,7-dihydro-5H-cyclopentapyrazine (30 - 60 %). The composition of this mixture has to be further specified (Table 1).

3. Intake Data

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

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

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

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

One of the alternatives is the "Theoretical Added Maximum Daily Intake" (TAMDI) approach, which is calculated on the basis of standard portions and upper use levels (SCF, 1995) for flavourable

beverages and foods in general, with exceptional levels for particular foods. This method is regarded as a conservative estimate of the actual intake by most consumers because it is based on the assumption that the consumer regularly eats and drinks several food products containing the same flavouring substance at the upper use level.

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

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

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

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

In the FGE.17Rev3 the total annual volume of production for 27 candidate substances from use as flavouring substances in Europe has been reported to be approximately 130 kg (EFFA, 2003r; EFFA, 2007e; EFFA, 2007f; Flavour Industry, 2010m; EFFA, 2011e). For one substance [FL-no: 14.051] no annual volumes of production in Europe have been submitted. For the 41 supporting substances the total annual volume of production is approximately 2700 kg. About 65 % of the total annual volume of production in Europe is accounted for by 2,3,5-trimethylpyrazine [FL-no: 14.019], 2-ethyl-3-methylpyrazine [FL-no: 14.006] and 3,(5- or 6-)-dimethyl-2-ethylpyrazine [FL-no: 14.100] (JECFA, 2002b).

On the basis of the annual volumes of production reported for the candidate substances, the daily *per capita* intakes for each of these flavourings have been estimated (Table 2). Approximately 70 % of the total annual volume of production for the candidate substances (EFFA, 2003r; EFFA, 2011e) is accounted for by the candidate substance 2-methoxy-3-methylpyrazine [FL-no: 14.126]. The estimated daily *per capita* intake of this candidate substance from use as flavouring substances is 12 microgram, and below 2.1 microgram for each of the remaining candidate substances (Table 2).

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

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

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

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

For the present evaluation of the candidate substances information on food categories and normal and maximum use levels[5,6,7] were submitted by the Flavour Industry for 21 substances (EFFA, 2003q; EFFA, 2007a; EFFA, 2007e; EFFA, 2007f; Flavour Industry, 2010m). No information on use levels have been submitted for [FL-no: 14.051, 14.057, 14.109, 14.111, 14.112, 14.126 and 14.128]. The 21 candidate substances are used in flavoured food products divided into the food categories, outlined in Annex III of the Commission Regulation (EC) No 1565/2000 (EC, 2000a), as shown in Table 3.1. For the present calculation of mTAMDI, the reported normal use levels were used. In the case where different use levels were reported for different food categories the highest reported normal use level was used.

Table 3.1 Use of Candidate Substances in Various Food Categories for 21 Candidate Substances for which Data on Use have been provided.

Food category	Description	Flavourings used*
01.0	Dairy products, excluding products of category 2	All
02.0	Fats and oils, and fat emulsions (type water-in-oil)	All except [FL-no:14.170]
03.0	Edible ices, including sherbet and sorbet	All
04.1	Processed fruits	All except [FL-no:14.170]
04.2	Processed vegetables (incl. mushrooms & fungi, roots & tubers, pulses and legumes), and nuts & seeds	None
05.0	Confectionery	All
06.0	Cereals and cereal products, incl. flours & starches from roots & tubers, pulses & legumes, excluding bakery	All except [FL-no:14.170]
07.0	Bakery wares	All except [FL-no: 14.099 & 14.101]
08.0	Meat and meat products, including poultry and game	All
09.0	Fish and fish products, including molluscs, crustaceans and echinoderms	All except [FL-no:14.170]
10.0	Eggs and egg products	None
11.0	Sweeteners, including honey	None
12.0	Salts, spices, soups, sauces, salads, protein products etc.	All except [FL-no:14.170]
13.0	Foodstuffs intended for particular nutritional uses	All except [FL-no:14.170]
14.1	Non-alcoholic ("soft") beverages, excl. dairy products	All
14.2	Alcoholic beverages, incl. alcohol-free and low-alcoholic counterparts	All
15.0	Ready-to-eat savouries	All except [FL-no:14.170]
16.0	Composite foods (e.g. casseroles, meat pies, mincemeat) - foods that could not be placed in categories 1 – 15	All except [FL-no:14.170]

* No use levels have been submitted for [FL-no: 14.051, 14.057, 14.109, 14.111, 14.112, 14.126 and 14.128].

According to the Flavour Industry the normal use levels for the candidate substances for which intake data are available are in the range of 0.1 - 3.13 mg/kg food, and the maximum use levels are in the range of 0.4 - 10 mg/kg (EFFA, 2003q; EFFA, 2007a; EFFA, 2007e; EFFA, 2007f; Flavour Industry, 2010m) Table II.1.2, Appendix II.

[5] "Normal use" is defined as the average of reported usages and "maximum use" is defined as the 95th percentile of reported usages (EFFA, 2002i).

[6] The normal and maximum use levels in different food categories (EC, 2000) have been extrapolated from figures derived from 12 model flavouring substances (EFFA, 2004e).

[7] The use levels from food category 5 "Confectionery" have been inserted as default values for food category 14.2 "Alcoholic beverages" for substances for which no data have been given for food category 14.2 (EFFA, 2007a).

The mTAMDI values for the 17 candidate substances from structural class II (see Section 5) for which use levels are available range from 190 to 1100 microgram/person/day. For the remaining four candidate substances from structural class III the mTAMDI range from 190 to 270 microgram/person/day.

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

4. Absorption, Distribution, Metabolism and Elimination

A more detailed description of the metabolism of the 28 candidate substances in this FGE is given in Annex III.

This group of flavouring substances consists of candidate substances, all containing a pyrazine ring.

In 20 substances [FL-no: 14.051, 14.052, 14.057, 14.081, 14.083, 14.084, 14.086, 14.087, 14.091, 14.097, 14.101, 14.109, 14.111, 14.112, 14.122, 14.126, 14.127, 14.128, 14.129 and 14.170] only one heterocyclic ring is present. In five candidate substances a pyrazine ring is fused with either cyclopentane [FL-no: 14.099, 14.102, 14.113 and 14.161] or with cyclohexane [FL-no: 14.148]. All of these 25 substances have different substituents on the rings. In 16 of them, the substituents are simple alkyl chains and / or ketones. In nine, the substituents are, next to alkyl chains, either a methoxy- ([FL-no: 14.051, 14.057, 14.112, 14.126 and 14.127], an ethoxy- [FL-no: 14.109], a thiomethyl- [FL-no: 14.122 and 14.128]) or an isopropenyl residue [FL-no: 14.052].

In the remaining three candidate substances the pyrazine ring is fused with benzene giving quinoxalines. In two of these substances [FL-no: 14.139 and 14.108] the pyrazine ring also bears one or two methyl substituents and in the third no substituents are present (quinoxaline [FL-no 14.147]).

A group with 41 related supporting substances has been evaluated by the JECFA (JECFA, 2002a).

Very little data on absorption, distribution and elimination of the candidate or supporting flavouring substances are available. The available data indicate that the weak basic heterocyclic substances in this group may be well absorbed, mainly from the intestinal lumen, and may be rapidly excreted.

Limited information has been submitted to describe the metabolism of the pyrazines and alkyl-, aryl- or alicyclic-substituted pyrazines in this group of flavouring substances. Almost all data available come from one paper (Hawksworth and Scheline, 1975) and a few review papers (Beedham, 1985; Beedham, 1988; Parkinson, 1996a). Additional information provided in other papers is supportive of the metabolic conversions that have been described, but of little quantitative relevance as they concern substances that are widely different from the candidate substances in this group and the supporting ones evaluated by the JECFA.

Pyrazines with a simple alkyl substituent (e.g. [FL-no: 14.097]) may be expected to be oxidised at the side chain to give the corresponding carboxylic acid. If such oxidations are not possible, e.g. due to steric hindrance, hydroxylation of the pyrazine ring may occur [FL-no: 14.091, 14.101, 14.111, 14.129 and 14.170]. The bicyclic pyrazine derivatives with an additional alicyclic or aryl ring substituent [FL-no: 14.099, 14.102, 14.108, 14.113, 14.139, 14.147, 14.148 and 14.161] may be better substrates for ring hydroxylation, which seems to be carried out preferably by molybdenum hydroxylases. The candidate substances bearing a ketone ring substituent [FL-no: 14.081, 14.083, 14.084, 14.086 and 14.087] may be reduced at the carbonyl in the side chain to give the corresponding alcohol. The five monocyclic pyrazine derivatives with a methoxy side chain [FL-no: 14.051, 14.057, 14.112, 14.126 and 14.127] may be expected to be metabolised via both ring hydroxylation and O-demethylation of the methoxy side chain. Accordingly, the monocyclic pyrazine derivatives with an ethoxy side chain [FL-no: 14.109] may also be expected to be metabolised via both ring hydroxylation and O-

deethylation of the ethoxy side chain. With the resulting products of any of these flavouring substances, conjugation with glycine, sulphate or glucuronide may occur. In none of the studies, N-oxidation or N-methylation, which would lead to the formation of bioactive metabolites, has been observed. This is in agreement with the reactive properties of the heterocyclic nitrogen in pyrazine moieties.

Two candidate substances in this group, 2-isopropyl-3-methylthiopyrazine [FL-no: 14.122] and 2-methyl-3-methylthiopyrazine [FL-no: 14.128], are thioethers, which may be detoxified by formation of a sulphoxide and subsequently a sulphone, which are both stable and usually rapidly excreted. Alternatively, they may also be bioactivated via S-demethylation, resulting in the formation of a reactive free thiol. No data were provided to show that either route predominates. For that reason, it cannot be anticipated that these sulphur-containing pyrazine derivatives are metabolised to innocuous products. Neither can it be assumed that quinoxaline [FL-no: 14.147] and its derivative 2-methylquinoxaline [FL-no: 14.139] are metabolised to innocuous products. One of the candidate substances, isopropenylpyrazine [FL-no: 14.052] has a terminal double-bond (in conjugation with a heterocyclic aromatic ring) and it may be anticipated to be epoxidised, thereby giving rise to reactive metabolites (as for alkenes with terminal double bonds). It can therefore not be concluded that isopropenylpyrazine can be metabolised to innocuous products.

Based on the available data, the following substances in this group [FL-no: 14.051, 14.057, 14.081, 14.083, 14.084, 14.086, 14.087, 14.091, 14.097, 14.099, 14.101, 14.102, 14.108, 14.109, 14.111, 14.112, 14.113, 14.126, 14.127, 14.129, 14.148, 14.161 and 14.170] may be expected to be metabolised to innocuous products. Three substances [FL-no: 14.122, 14.128 and 14.052] cannot be anticipated to be metabolised to innocuous products. For the remaining two quinoxalines [FL-no: 14.139 and 14.147] a concern for genotoxicity has been identified (see also Section 8.4).

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

The application of the Procedure is based on intakes estimated on the basis of the MSDI approach. Where the mTAMDI approach indicates that the intake of a flavouring substance might exceed its corresponding threshold of concern, a formal safety assessment is not carried out using the Procedure. In these cases the Panel requires more precise data on use and use levels. For comparison of the intake estimations based on the MSDI approach and the mTAMDI approach, see Section 6.

In previous versions of the present FGE, 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 to the structurally related 2,3-dimethylquinoxaline [FL-no: 14.108] until adequate genotoxicity data become available.

Additional genotoxicity data have been submitted by the Industry (Flavour Industry, 2009a) for the structurally related 5-methylquinoxaline [FL-no: 14.028] and these genotoxicity data have been evaluated in FGE.17Rev2. 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.

For one 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.

The safety evaluation of the remaining 25 candidate substances to be evaluated using the Procedure, as outlined in Annex I, was applied, based on the MSDI approach. The stepwise evaluations of the substances are summarised in Table 2.

Step 1

According to the decision tree approach, presented by Cramer et al., 19 candidate substances for which the Procedure could be applied are classified into structural class II. The six remaining substances, for which the Procedure could be applied, are classified into structural class III (Cramer et al., 1978).

Step 2

Three candidate substances cannot be anticipated to be metabolised to innocuous products: two sulphur-containing flavouring substances [FL-no: 14.122 and 14.128], which may be converted to a reactive free thiol and one substance with a terminal double bond [FL-no: 14.052] (in conjugation with a heterocyclic aromatic ring), which may be epoxidised giving rise to reactive metabolites.

Therefore these three substances are evaluated along the B-side of the Procedure.

Based on the available data, 22 substances [FL-no: 14.057, 14.081, 14.083, 14.084, 14.086, 14.087, 14.091, 14.097, 14.099, 14.101, 14.102, 14.108, 14.109, 14.111, 14.112, 14.113, 14.126, 14.127, 14.129, 14.148 14.161 and 14.170] may be expected to be metabolised to innocuous products. Therefore, these 22 flavouring substances are evaluated along the A-side of the Procedure (Annex I).

Step A3

Eighteen candidate substances evaluated along the A-side of the Procedure scheme are classified into structural class II and four into structural class III. The estimated daily *per capita* intakes from use as flavouring substances range from 0.0024 to 12 microgram. These intakes are below the threshold of concern of 540 and 90 microgram/person/day for structural class II and III substances, respectively.

Based on the results of the safety evaluation sequence of the Procedure, these 22 candidate substances proceeding *via* the A-side of the Procedure scheme do not pose a safety concern when used as flavouring substances at the estimated levels of intake, based on the MSDI approach.

Step B3

The level of intake of the candidate substance isopropenylpyrazine [FL-no: 14.052] was estimated to be 0.012 microgram/*capita*/day, which is below the threshold of concern of 540 microgram/person/day for structural class II substances.

The levels of intake of the candidate substances, 2-isopropyl-3-methyl thiopyrazine [FL-no: 14.122] and 2-methyl-3-methylthiopyrazine [FL-no: 14.128] were estimated to be 0.061 and 0.68 microgram/*capita*/day, respectively, which is below the threshold of concern of 90 microgram/person/day for structural class III substances.

Accordingly, these candidate substances proceed to step B4 of the Procedure.

Step B4

No valid toxicity study from which a NOAEL could be established was available for isopropenylpyrazine [FL-no: 14.052] or for any relevant supporting substance. Therefore, the Panel concluded that additional toxicity data are needed for this substance.

Ninety days oral feeding studies in rats are available for two supporting substances [FL-no: 14.031 and 14.034] structurally related to the candidate substances 2-isopropyl-3-methylthiopyrazine [FL-no:

14.122] and 2-methyl-3-methylthiopyrazine [FL-no: 14.128]. Although the studies are not performed in accordance with modern guidelines (see Section 8.2), the studies have been considered adequate for deriving a No Observed Adverse Effect Level (NOAEL) for each, which are 16.3 and 1.63 mg/kg body weight (bw) for [FL-no: 14.031 and 14.034], respectively. The combined daily *per capita* intake of 0.74 microgram for the two candidate substances [FL-no: 14.122 and 14.128] corresponds to 0.012 microgram/kg bw/day at a body weight of 60 kg. Thus, a margin of safety of 1.4 x 10^5 using the NOAEL of 1.63 can be calculated. 2-Isopropyl-3-methylthiopyrazine [FL-no: 14.122] and 2-methyl-3-methylthiopyrazine [FL-no: 14.128] are accordingly not expected to be of safety concern at their estimated levels of intake.

Based on results of the safety evaluation sequence, 24 of the 25 candidate substances, for which the Procedure could be applied, are not expected to be of safety concern when used as flavouring substances at the estimated levels of intake, based on the MSDI approach. For the candidate substance isopropenylpyrazine [FL-no: 14.052], additional toxicity data is needed.

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

For the candidate substances [FL-no: 14.051, 14.057, 14.109, 14.111, 14.112, 14.126 and 14.128] no use levels were provided. Use levels for these seven substances are required.

Based on the mTAMDI approach, the estimated intakes for the 16 of the 17 candidate substances in structural class II range from 190 to 400 microgram/person/day, which is below the threshold of concern of 540 microgram/person/day for structural class II substances. For one substance [FL-no: 14.170] the mTAMDI is 1100 microgram/person/day, which is above the threshold.

The estimated intakes of the four substances [FL-no: 14.108, 14.122, 14.139 and 14.147] assigned to structural class III, based on the mTAMDI, range from 190 to 270 microgram/person/day, which are all above the threshold of concern for structural class III of 90 microgram/person/day.

For the candidate substances [FL-no: 14.108, 14.122 and 14.170] further information is needed. This would include more reliable intake data and then, if required, additional toxicological data.

For the candidate substances [FL-no: 14.139 and 14.147], additional genotoxicity data are required before they can be evaluated using the Procedure, based on the MSDI approach. Subsequently, more reliable intake data (mTAMDI) may be required.

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

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

FL-no	EU Register name	MSDI (µg/*capita*/day)	mTAMDI (µg/person/day)	Structural class	Threshold of concern (µg/person/day)
14.057	2-Isopropyl-3-methoxypyrazine	0.085		Class II	540
14.081	5-Acetyl-2,3-dimethylpyrazine	0.012	270	Class II	540
14.083	2-Acetyl-5-ethylpyrazine	0.012	270	Class II	540
14.084	2-Acetyl-5-methylpyrazine	0.0024	270	Class II	540
14.086	2-Acetyl-6-ethylpyrazine	0.0061	270	Class II	540
14.087	2-Acetyl-6-methylpyrazine	0.028	270	Class II	540
14.091	2-Butyl-3-methylpyrazine	0.12	270	Class II	540
14.097	2,5-Diethylpyrazine	0.024	270	Class II	540
14.099	6,7-Dihydro-5,7-dimethyl-5H-cyclopentapyrazine	0.032	190	Class II	540
14.101	2,5-Dimethyl-3-isopropylpyrazine	0.018	190	Class II	540
14.102	5,6-Dimethyldihydrocyclopentapyrazine	0.1	270	Class II	540
14.111	3-Ethyl-2,5-dimethylpyrazine	2.1		Class II	540
14.113	5-Ethyl-6,7-dihydro-5H-cyclopentapyrazine	0.012	270	Class II	540
14.127	2-Methoxy-3-propylpyrazine	0.061	270	Class II	540

14.129	2-Methyl-3-propylpyrazine	0.011	400	Class II	540
14.148	5,6,7,8-Tetrahydro-5-methylquinoxaline	0.0073	270	Class II	540
14.161	6,7-Dihydro-2,5-dimethyl-5H-cyclopentapyrazine	0.011	400	Class II	540
14.170	5-Ethyl-2,3-dimethyl pyrazine	0.12	1100	Class II	540
14.052	Isopropenylpyrazine	0.012	400	Class II	540
14.051	2,5 or 6-Methoxy-3-ethylpyrazine			Class II	540
14.108	2,3-Dimethylquinoxaline	0.049	190	Class III	90
14.109	2-Ethoxy-3-methylpyrazine	0.012		Class III	90
14.112	2-Ethyl-3-methoxypyrazine	0.17		Class III	90
14.126	2-Methoxy-3-methylpyrazine	12		Class III	90
14.122	2-Isopropyl-3-methylthiopyrazine	0.061	270	Class III	90
14.128	2-Methyl-3-methylthiopyrazine	0.68		Class III	90
14.139	2-Methylquinoxaline	0.12	270	Class III	90
14.147	Quinoxaline	0.12	270	Class III	90

7. Considerations of Combined Intakes from Use as Flavouring Substances

Because of structural similarities of candidate and supporting substances, it can be anticipated that many of the flavourings are metabolised through the same metabolic pathways and that the metabolites may affect the same target organs. Further, in case of combined exposure to structurally related flavourings, the pathways could be overloaded. Therefore, combined intake should be considered. As flavourings not included in this FGE may also be metabolised through the same pathways, the combined intake estimates presented here are only preliminary. Currently, the combined intake estimates are only based on MSDI exposure estimates, although it is recognised that this may lead to underestimation of exposure. After completion of all FGEs, this issue should be readdressed.

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

As two of the candidate substances, quinoxaline [FL-no: 14.147] and 2-methylquinoxaline [FL-no: 14.139] show possible genotoxic potential *in vitro*, the substances are not taken through the Procedure. As no intake data are available for 2,5 or 6-methoxy-3-ethylpyrazine [FL-no: 14.051] the substance is not taken through the Procedure. These three substances are therefore not included in the calculation of the combined intake of the candidate substances evaluated in FGE.17Rev3.

On the basis of the reported annual production volumes in Europe (EFFA, 2003r; EFFA, 2007e; EFFA, 2007f; Flavour Industry, 2010m; EFFA, 2011e) the combined estimated daily *per capita* intake as flavourings of the 19 candidate flavouring substances assigned to structural class II is 2.7 microgram, and of the six substances assigned to structural class III, 13.2 microgram. These values do not exceed the thresholds of concern for substances belonging to structural class II and III of 540 and 90 microgram/person/day, respectively.

The candidate substances are structurally related to 41 supporting substances evaluated by the JECFA at its 57th meeting (JECFA, 2002b). Based on reported production volumes, European *per capita* intakes (MSDI) could be estimated for the 41 supporting substances.

The total combined intake of the 19 candidate substances and 32 supporting substances from structural class II is approximately 310 microgram/*capita*/day, which is below the threshold of concern for a compound belonging to structural class II of 540 microgram/person/day. The total combined intake of the six candidate substances from structural class III, and nine supporting substances from structural class III is approximately 50 microgram/*capita*/day, which is also below the threshold of concern for a compound belonging to structural class III of 90 microgram/person/day.

8. Toxicity

8.1. Acute Toxicity

Acute oral LD_{50} values are available on 18 of the 41 supporting substances. The acute oral LD_{50} in rats and mice range from 200 to more than 4000 mg/kg bw indicating a low level of oral toxicity in these species.

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

8.2. Subacute, Subchronic, Chronic and Carcinogenicity Studies

No data are available on subacute or subchronic toxicity for the candidate substances. Data on subchronic toxicity are available on 17 of the 41 supporting substances. Most of the studies were performed at single dose levels and all studies were performed in rats. The No Observed Adverse Effect Level (NOAEL) of the substances were in the range from 0.44 mg/kg bw to 55 mg/kg body weight (bw).

Toxicity studies on three supporting substances used for deriving the NOAELs used in the Procedure are briefly reported in the following.

Pyrazinyl methyl sulphide [FL-no: 14.034] and pyrazinylethanethiol [FL-no: 14.031]

The supporting substances pyrazinyl methyl sulphide [FL-no: 14.034] and pyrazinylethanethiol [FL-no: 14.031] were administered in the feed to male and female rats (16 animals/sex) for 13 weeks at doses of 1.63 mg/kg bw/day and 16.30 mg/kg bw/day, respectively. After 90 days, all animals were killed, subjected to detailed necropsy examinations, and liver and kidney weights were measured. A wide range of tissues and organs from each animal were preserved and histopathological examinations were performed on major organs and tissues. The authors stated that no major differences were observed between groups of treated and control animals, based on measurements of growth, food intake, haematological parameters, blood urea determinations, organ weights and gross and histopathologic examinations. However, no numeric data were reported. The only levels tested (1.63 and 16.30 mg/kg bw/day) have been taken as NOAELs for pyrazinyl methyl sulphide [FL-no: 14.034] and pyrazinylethanethiol [FL-no: 14.031], respectively (Posternak et al., 1975).

5-Methylquinoxaline [FL-no: 14.028]

The supporting substance 5-methylquinoxaline [FL-no: 14.028] was administered in the feed to male and female rats (16 animals/sex) for 90 days at a single dose of 17.1 mg/kg bw/day. After 7 weeks and after 13 weeks, 50 % of the animals were killed, and liver and kidney weights were measured and gross and histological examinations were carried out on a wide range of organs. The authors stated that no major differences were observed between groups of treated and control animals, based on measurements of growth, food intake, haematological parameters, blood urea determinations, organ weights and gross and histopathologic examinations. However, no numeric data were reported. The only level tested (17.1 mg/kg bw/day) has been taken as NOAEL for 5-methylquinoxaline [FL-no: 14.028] (Posternak et al., 1969)

No studies are available on chronic toxicity or on carcinogenicity for either candidate or supporting substances.

The repeated dose toxicity are summarised in Annex IV, Table IV.2.

8.3. Developmental / Reproductive Toxicity Studies

Data are available for four of the supporting substances 2,3-dimethylpyrazine, 2,5-dimethylpyrazine, 2,6-dimethylpyrazine, 2,3,5,6-tetramethylpyrazine. 2,5-Dimethylpyrazine [FL-no: 14.020] has been reported to occur naturally in the urine of female mice at concentrations of 0.25 µg/l. A study of a mixture of 2,5-dimethylpyrazine with five naturally occurring ketones and esters was reported to delay puberty in juvenile female mice, but the compound responsible for this effect was not identified (Novotny et al., 1986). The effect of isomers of dimethylpyrazine on reproductive organs in male and female rats has been studied. (Yamada et al., 1992; Yamada et al., 1993; Yamada et al., 1994). The effects on reproductive organ parameters have been described after subcutaneous (s.c.) injection of 2,5-dimethylpyrazine [FL-no: 14.020] at doses > 30 mg/kg bw, which can be considered as the NOAEL. Also 2,6-methylpyrazine [FL-no: 14.021]) showed some effects, but to a lesser extent while, 2,3- dimethylpyrazine [FL-no: 14.050] showed no effect. However, the relevance of s.c. route of administration is limited, considering the use of the chemical as a flavouring substance. In addition, as recently reviewed, neither the mechanism(s) nor the relevance for the human reproductive system have been clarified (Koyama, 2004). In any case, the NOAEL of 30 mg/kg bw is orders of magnitude higher than the predicted level of exposure as a flavouring substance, according to the MSDI approach. A study on a fourth supporting substance, 2,3,5,6-tetramethylpyrazine [FL-no: 14.018], showed no adverse effects on several reproductive parameters after oral administration (Vollmuth et al., 1997)

Developmental/reproductive toxicity data are summarised in Annex IV, Table IV.3.

8.4. Genotoxicity Studies

Genotoxicity data were provided for three of the candidate substances and for 11 of the 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].

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* (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 candidate 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

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], 2,6-dimethylpyrazine [FL-no: 14.021], 2,3-diethylpyrazine [FL-no: 14.005], 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 IV.4).

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 IV.4) (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 TA98, TA100, TA1535 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 and 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 and Kawaguchi, 2004a).

In vivo data are available for two structurally related substances only, (2, 5 or 6)-methoxy-3-methylpyrazine [FL-no: 14.025 (mixture of three structural isomers)] 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 and 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 IV.5). 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 IV.5). 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 the supporting substance [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, for the supporting substance 5-methylquinoxaline [FL-no: 14.028] a negative Micronucleus assay is available.

The candidate substance 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 26 candidate substances, including 2,3-dimethylquinoxaline [FL-no: 14.108]. For these substances, the available data do not preclude their evaluation through the Procedure.

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

9. Conclusions

This group of flavouring substances consists of 28 candidate substances, all of which contain a pyrazine moiety. In 20 substances [FL-no: 14.051, 14.052, 14.057, 14.081, 14.083, 14.084, 14.086, 14.087, 14.091, 14.097, 14.101, 14.109, 14.111, 14.112, 14.122, 14.126, 14.127, 14.128, 14.129 and 14.170] only one heterocyclic ring is present. In five candidate substances a pyrazine ring is fused with either cyclopentane [FL-no: 14.099, 14.102, 14.113 and 14.161] or with cyclohexane [FL-no: 14.148]. All of these 25 substances have different ring substituents. In 16 of them, the substituents are simple alkyl chains and/or ketones. In nine, the substituents are, next to alkyl chains, either a methoxy- ([FL-no: 14.051 14.057, 14.112, 14.126 and 14.127], an ethoxy- [FL-no: 14.109], a thiomethyl- [FL-no: 14.122 and 14.128]) or an isopropenyl residue [FL-no: 14.052]. In the remaining three candidate substances the pyrazine ring is fused with benzene giving quinoxalines. In two of the quinoxalines [FL-no 14.139 and 14.108] the pyrazine ring also bears one or two methyl substituents; in the third no substituents are present (quinoxaline [FL-no: 14.147]).

For the five flavouring substances, which possess one or two chiral centres [FL-no: 14.099, 14.102, 14.113, 14.148 and 14.161], the stereoisomeric composition has been specified.

Twenty candidate substances are classified into structural class II and eight are classified into structural class III.

Twenty-four flavouring substances in the present group have been reported to occur naturally in a wide range of food items.

According to the default MSDI approach, the flavouring substances in this group, which are evaluated through the Procedure and for which Industry has provided intake data, have intakes in Europe from 0.0024 to 12 microgram/*capita*/day. These values are below the threshold of concern value for both structural class II (540 microgram/person/day) and structural class III (90 microgram/person/day) substances. For one candidate substance [FL-no: 14.051] no intake data are available preventing it from being evaluated through the Procedure.

The available data indicate that apparently there is no simple structure-activity relationship for the genotoxicity of quinoxalines, of which three are evaluated in this FGE, 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 the supporting substance [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, for the supporting substance 5-methylquinoxaline [FL-no: 14.028] a negative Micronucleus assay is available.

The candidate substance 2,3-dimethylquinoxaline [FL-no: 14.108] is not considered genotoxic *in vitro* and hence can be evaluated through the Procedure. No genotoxic potential at gene or chromosome level is indicated for the remaining alkyl- and cycloalkyl-substituted pyrazines. Therefore these substances may also be evaluated through the Procedure.

So, in total, 25 substances are evaluated through the Procedure, based on the MSDI approach Appendix I).

Twenty-two of the alkyl- and cycloalkyl-substituted pyrazines and one of the alkyl-substituted quinoxalines may be expected to be metabolised to innocuous products [FL-no: 14.051, 14.057, 14.081, 14.083, 14.084, 14.086, 14.087, 14.091, 14.097, 14.099, 14.101, 14.102, 14.108, 14.109, 14.111, 14.112, 14.113, 14.126, 14.127, 14.129, 14.148, 14.161 and 14.170]. Regarding the remaining five substances, they cannot be anticipated to be metabolised to innocuous products. Two sulphur-containing flavouring substances [FL-no: 14.122 and 14.128], may be converted to a reactive free thiol. Neither can it be assumed that quinoxaline [FL-no: 14.147] and its derivative [FL-no: 14.139] are metabolised to innocuous products. One substance with a terminal double bond [FL-no: 14.052] (in conjugation with a heterocyclic aromatic ring), may be epoxidised giving rise to reactive metabolites.

Where toxicity data were available for substances evaluated using the Procedure, they were consistent with the conclusions in the present FGE.

It is considered that on the basis of the default MSDI approach, the 24 of the 25 candidate substances evaluated through the Procedure would not give rise to safety concerns at the estimated levels of intake arising from their use as flavouring substances. No valid toxicity study from which a NOAEL could be established was available for isopropenylpyrazine [FL-no: 14.052] or for any relevant supporting substance. Therefore, the Panel concluded that additional toxicity data are needed for this substance.

When the estimated intakes, of the substances evaluated through the Procedure, were based on the mTAMDI approach they ranged from 190 to 1100 microgram/person/day for the flavouring substances from structural class II for which data have been provided. The intakes for 16 substances were below the threshold of concern for structural class II of 540 microgram/person/day. These 16 substances are also expected to be metabolised to innocuous products. For one substance [FL-no:

14.170] the mTAMDI is above the threshold. The estimated intakes (mTAMDI) of the two flavouring substances [FL-no: 14.108 and 14.122] assigned to structural class III, for which use levels have been submitted and which were evaluated through the Procedure, are 190 and 270 microgram/person/day, respectively, which are above the threshold of concern for structural class III of 90 microgram/person/day. Thus, for three flavouring substances [FL-no: 14.108, 14.122 and 14.170] the intakes estimated on the basis of the mTAMDI, exceed the relevant threshold for their structural class, to which the flavouring substance has been assigned. For seven substances [FL-no: 14.051, 14.057, 14.109, 14.111, 14.112, 14.126 and 14.128], no use levels were provided. Therefore, for these 10 substances more reliable exposure data (mTAMDI) are required. On the basis of such additional data, the flavouring substances should be re-evaluated using the Procedure. Subsequently, additional toxicity data might become necessary.

Thus, in conclusion, only 25 of the 28 candidate substances were evaluated through the Procedure, based on the MSDI approach, as two flavouring substances, quinoxaline [FL-no: 14.147] and 2-methylquinoxaline [FL-no: 14.139] could not be evaluated through the Procedure until adequate genotoxicity data become available and one substance, 2- ,5- or 6-methoxy-3-ethylpyrazine [FL-no: 14.051], was presented with no intake data and therefore cannot not be evaluated through the Procedure.

In order to determine whether the conclusion for the 25 candidate substances which have been evaluated using the Procedure (MSDI approach) can be applied to the materials of commerce, it is necessary to consider the available specifications. Adequate specifications including complete purity criteria and identity for the materials of commerce have been provided for 24 flavouring substances evaluated through the Procedure. For one substance [FL-no: 14.102], the composition of mixture has not been specified sufficiently. So, the final evaluation of the materials of commerce cannot be performed for one substance, 5,6-dimethyldihydrocyclopentapyrazine [FL-no: 14.102], pending further information on composition of mixture.

For one substance [FL-no: 14.052] evaluated through the Procedure, additional toxicity data are required.

For the remaining 23 substances [FL-no: 14.057, 14.081, 14.083, 14.084, 14.086, 14.087, 14.091, 14.097, 14.099, 14.101, 14.108, 14.109, 14.111, 14.112, 14.113, 14.122, 14.126, 14.127, 14.128, 14.129, 14.148 14.161 and 14.170], the Panel concluded that they would present no safety concern at the level of intake estimated on the basis of the MSDI approach.

TABLE 1: SPECIFICATION SUMMARY OF THE SUBSTANCES IN FGE.17REV3

Table 1: Specification Summary of the Substances in the Flavouring Group Evaluation 17, Revision 3

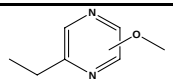
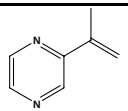
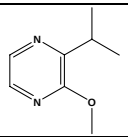
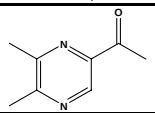
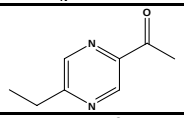
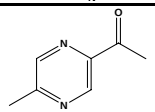
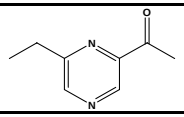
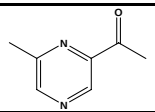
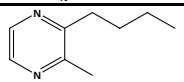
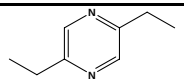
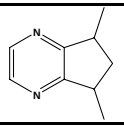
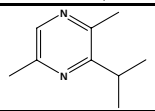
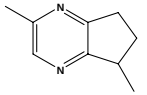
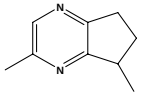
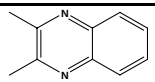
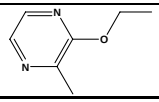
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	Specification comments
14.051	2,5 or 6-Methoxy-3-ethylpyrazine		3280 11329 68739-00-4	 124.14				AV 6), BP 7), ID 8), MP 9), PF 10), RI 11), SG 12), SE 13), SW 14) Registername to be changed to mixture of 2-, 5- or 6-methoxy-3-ethylpyrazine.
14.052	Isopropenylpyrazine		3296 11341 38713-41-6	Liquid $C_7H_8N_2$ 120.16	Practically insoluble or insoluble Soluble	73-75 (23 hPa) NMR 99 %	1.480-1.486 0.964-0.968	
14.057	2-Isopropyl-3-methoxypyrazine		3358 11344 25773-40-4	Liquid $C_8H_{12}N_2O$ 152.20	Soluble Soluble	120-125 (26 hPa MS 97 %	1.492-1.499 1.010-1.022	
14.081	5-Acetyl-2,3-dimethylpyrazine		 54300-10-6	Solid $C_8H_{10}N_2O$ 150.18	Soluble Freely soluble	308 151 MS 95 %	n.a. n.a.	
14.083	2-Acetyl-5-ethylpyrazine		 43108-58-3	Solid $C_8H_{10}N_2O$ 150.18	Soluble Freely soluble	303 138 NMR 95 %	n.a. n.a.	
14.084	2-Acetyl-5-methylpyrazine		 11297 22047-27-4	Solid $C_7H_8N_2O$ 136.15	Soluble Freely soluble	80 (11 hPa) 56 MS 95 %	n.a. n.a.	
14.086	2-Acetyl-6-ethylpyrazine		 11295 34413-34-8	Solid $C_8H_{10}N_2O$ 150.18	Soluble Freely soluble	302 137 NMR 95 %	n.a. n.a.	

Table 1: Specification Summary of the Substances in the Flavouring Group Evaluation 17, Revision 3

FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	Specification comments
14.087	2-Acetyl-6-methylpyrazine		11298 22047-26-3	Solid $C_7H_8N_2O$ 136.15	Soluble Freely soluble	280 33 MS 95 %	n.a. n.a.	
14.091	2-Butyl-3-methylpyrazine		15987-00-5	Solid $C_9H_{14}N_2$ 150.22	Slightly soluble Freely soluble	84 (12 hPa) 100 MS 95 %	n.a. n.a.	
14.097	2,5-Diethylpyrazine		11306 13238-84-1	Solid $C_8H_{12}N_2$ 136.20	Slightly soluble Freely soluble	187 88 MS 95 %	n.a. n.a.	
14.099	6,7-Dihydro-5,7-dimethyl-5H-cyclopentapyrazine		41330-21-6	Solid $C_9H_{12}N_2$ 148.21	Slightly soluble Freely soluble	84 (13 hPa) 113 MS 95 %	n.a. n.a.	Meso-form (RS- = SR–form) equal 50 % and RR- and SS-forms 25 % each (EFFA, 2011a).
14.101	2,5-Dimethyl-3-isopropylpyrazine		11318 40790-20-3	Solid $C_9H_{14}N_2$ 150.22	Slightly soluble Freely soluble	276 97 NMR 95 %	n.a. n.a.	
14.102	5,6-Dimethyldihydrocyclopentapyrazine	 	38917-61-2	Liquid $C_9H_{12}N_2$ 148.21	Slightly soluble Freely soluble	230-231 MS 98 %	1.524-1.532 1.020-1.030	Change name to: 2,5-dimethyl-6,7-dihydro-5H-cyclopentapyrazine. Racemate. Covers mix of 2,5-dimethyl-6,7-dihydro-5H-cyclopentapyrazine (60 - 100 %) & 3,5-dimethyl-6,7-dihydro-5H-cyclopentapyrazine (30 - 60 %) (EFFA, 2011a). Composition of mixture to be specified.
14.108	2,3-Dimethylquinoxaline		2379-55-7	Solid $C_{10}H_{10}N_2$ 158.20	Slightly soluble Freely soluble	128 (2 hPa) 106 MS 95 %	n.a. n.a.	
14.109	2-Ethoxy-3-methylpyrazine		3569 11325 32737-14-7	Liquid $C_7H_{10}N_2O$ 138.17	Soluble Soluble	176 MS 97 %	1.493-1.497 1.034-1.041	

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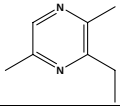
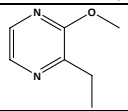
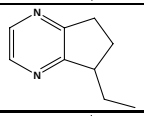
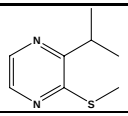
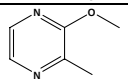
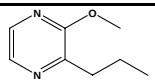
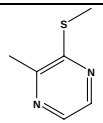
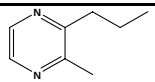
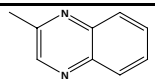
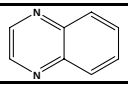
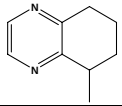
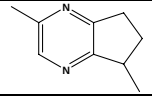
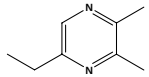
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	Specification comments
14.111	3-Ethyl-2,5-dimethylpyrazine		3149 2246 13360-65-1	Liquid $C_8H_{12}N_2$ 136.19	Soluble Soluble	176 MS 97 %	1.493-1.497 1.034-1.041	Registername to be changed to 2,5-Dimethyl-3-ethylpyrazine.
14.112	2-Ethyl-3-methoxypyrazine		3280 11329 25680-58-4	Liquid $C_7H_{10}N_2O$ 132.14	Soluble Soluble	95 (1,3 hPa) MS 99 %	1.497-1.505 1.036-1.052	
14.113	5-Ethyl-6,7-dihydro-5H-cyclopentapyrazine		52517-53-0	Solid $C_9H_{12}N_2$ 148.21	Slightly soluble Freely soluble	278 117 NMR 95 %	n.a. n.a.	Racemate (EFFA, 2007m).
14.122	2-Isopropyl-3-methylthiopyrazine		11342 67952-59-4	Solid $C_8H_{12}N_2S$ 168.28	Slightly soluble Freely soluble	317 108 MS 95 %	n.a. n.a.	
14.126	2-Methoxy-3-methylpyrazine		3183 2266 2847-30-5	Liquid $C_6H_8N_2O$ 124.14	Soluble Soluble	85 (1.3 hPa) MS 97 %	1.505-1.510 1.060-1.090	
14.127	2-Methoxy-3-propylpyrazine		25680-57-3	Solid $C_8H_{12}N_2O$ 152.20	Slightly soluble Freely soluble	271 111 MS 95 %	n.a. n.a.	
14.128	2-Methyl-3-methylthiopyrazine		2882-20-4	Liquid $C_6H_8N_2S$ 140.21	Soluble Soluble	87 (1.3 hPa) MS 99 %	1.570-1.590 1.133-1.153	
14.129	2-Methyl-3-propylpyrazine		15986-80-8	Liquid $C_8H_{12}N_2$ 136.20	Practically insoluble or insoluble Freely soluble	190 MS 95 %	1.495-1.501 0.978-0.984	
14.139	2-Methylquinoxaline		7251-61-8	Solid $C_9H_8N_2$ 144.18	Slightly soluble Freely soluble	245 132 MS 95 %	n.a. n.a.	
14.147	Quinoxaline		11365 91-19-0	Solid $C_8H_6N_2$ 130.15	Slightly soluble Freely soluble	225 30 MS	n.a. n.a.	

Table 1: Specification Summary of the Substances in the Flavouring Group Evaluation 17, Revision 3

FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	Specification comments
						95 %		
14.148	5,6,7,8-Tetrahydro-5-methylquinoxaline		52517-54-1	Solid $C_9H_{12}N_2$ 148.21	Slightly soluble Freely soluble	72 (4 hPa) 114 MS 95 %	n.a. n.a.	Racemate (EFFA, 2007m).
14.161	6,7-Dihydro-2,5-dimethyl-5H-cyclopentapyrazine		4702 11310 38917-61-2	Solid $C_9H_{12}N_2$ 148.21	Practically insoluble or insoluble Freely soluble	91 (16 hPa) 110 MS 95 %	n.a. n.a.	Racemate (EFFA, 2007m).
14.170	5-Ethyl-2,3-dimethyl pyrazine		4434 15707-34-3	Liquid $C_8H_{12}N_2$ 136.19	Slightly soluble Soluble	191 NMR MS 98 %	1.4982 0.964	

1) Solubility in water, if not otherwise stated.
2) Solubility in 95 % ethanol, if not otherwise stated.
3) At 1013.25 hPa, if not otherwise stated.
4) At 20°C, if not otherwise stated.
5) At 25°C, if not otherwise stated.
6) AV: Missing minimum assay value.
7) BP: Missing boiling point.
8) ID: Missing identification test.
9) MP: Missing melting point.
10) PF: Missing data on physical form.
11) RI: Missing refractive index.
12) SG: Missing specific gravity.
13) SE: Missing data on solubility in ethanol.
14) SW: Missing data on solubility.

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

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

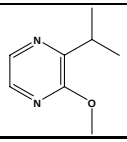
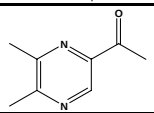
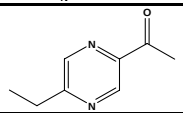
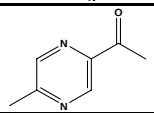
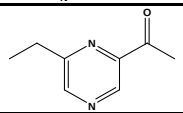
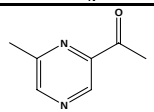
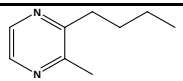
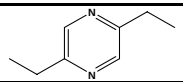
FL-no	EU Register name	Structural formula	MSDI 1) (µg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
14.057	2-Isopropyl-3-methoxypyrazine		0.085	Class II A3: Intake below threshold	4)	6)	
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)	

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

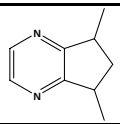
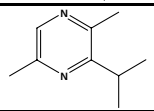
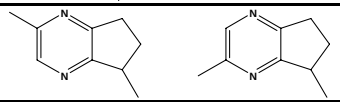
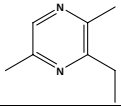
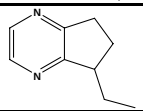
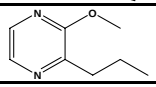
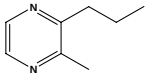
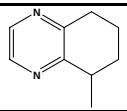
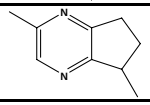
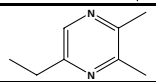
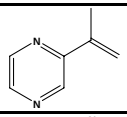
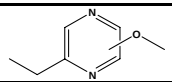
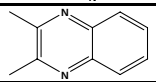
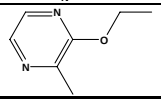
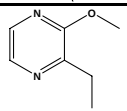
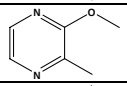
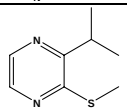
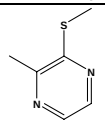
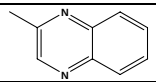
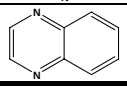
FL-no	EU Register name	Structural formula	MSDI 1) (µg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
14.099	6,7-Dihydro-5,7-dimethyl-5H-cyclopentapyrazine		0.032	Class II A3: Intake below threshold	4)	6)	
14.101	2,5-Dimethyl-3-isopropylpyrazine		0.018	Class II A3: Intake below threshold	4)	6)	
14.102	5,6-Dimethyldihydrocyclopentapyrazine		0.1	Class II A3: Intake below threshold	4)	7)	
14.111	3-Ethyl-2,5-dimethylpyrazine		2.1	Class II A3: Intake below threshold	4)	6)	
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)	
14.170	5-Ethyl-2,3-dimethyl pyrazine		0.12	Class II A3: Intake below threshold	4)	6)	

Table 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
14.052	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			a)
14.108	2,3-Dimethylquinoxaline		0.049	Class III A3: Intake below threshold	4)	6)	
14.109	2-Ethoxy-3-methylpyrazine		0.012	Class III A3: Intake below threshold	4)	6)	
14.112	2-Ethyl-3-methoxypyrazine		0.17	Class III A3: Intake below threshold	4)	6)	
14.126	2-Methoxy-3-methylpyrazine		12	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)	
14.128	2-Methyl-3-methylthiopyrazine		0.68	Class III B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
14.139	2-Methylquinoxaline		0.12	Class III No evaluation			b)
14.147	Quinoxaline		0.12	Class III No evaluation			c)

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

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

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

4) No safety concern based on intake calculated by the MSDI approach of the named compound.
5) Data must be available on the substance or closely related substances to perform a safety evaluation.
6) No safety concern at estimated level of intake 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.
a) No production volume for EU available.
b) Additional genotoxicity data required.
c) Additional genotoxicity data required.

TABLE 3: SUPPORTING SUBSTANCES SUMMARY

Table 3: Supporting Substances Summary

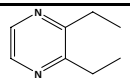
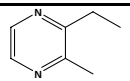
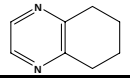
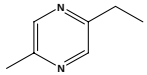
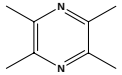
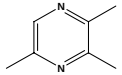
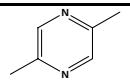
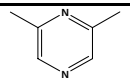
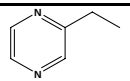
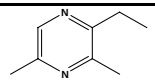
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (µg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
14.005	2,3-Diethylpyrazine		3136 534 15707-24-1	771 JECFA specification (JECFA, 2001c)	1.6	No safety concern a) Category B b)	
14.006	2-Ethyl-3-methylpyrazine		3155 548 15707-23-0	768 JECFA specification (JECFA, 2001c).	72	No safety concern a) Category B b)	
14.015	5,6,7,8-Tetrahydroquinoxaline		3321 721 34413-35-9	952 JECFA specification (JECFA, 2001c)	8	No safety concern a) Category B b)	
14.017	2-Ethyl-5-methylpyrazine		3154 728 13360-64-0	770 JECFA specification (JECFA, 2001c)	4.0	No safety concern a) Category B b)	
14.018	2,3,5,6-Tetramethylpyrazine		3237 734 1124-11-4	780 JECFA specification (JECFA, 2001c)	6.7	No safety concern a) Category B b)	
14.019	2,3,5-Trimethylpyrazine		3244 735 14667-55-1	774 JECFA specification (JECFA, 2001c)	100	No safety concern a) Category B b)	
14.020	2,5-Dimethylpyrazine		3272 2210 123-32-0	766 JECFA specification (JECFA, 2001c)	19	No safety concern a) Category B b)	
14.021	2,6-Dimethylpyrazine		3273 2211 108-50-9	767 JECFA specification (JECFA, 2001c)	1.3	No safety concern a) Category B b)	
14.022	Ethylpyrazine		3281 2213 13925-00-3	762 JECFA specification (JECFA, 2001c)	2.2	No safety concern a) Category B b)	
14.024	2-Ethyl-3,5-dimethylpyrazine		3150 2245 13925-07-0	776 JECFA specification (JECFA, 2001c)	1.2	No safety concern a)	

Table 3: Supporting Substances Summary

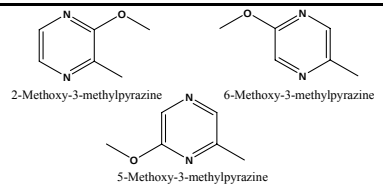
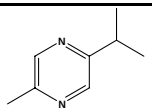
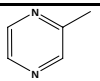
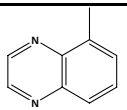
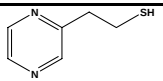
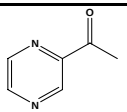
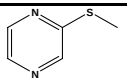
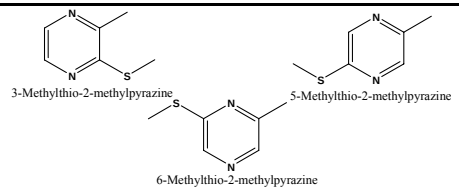
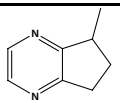
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (µg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
14.025	2,5 or 6-Methoxy-3-methylpyrazine		3183 2266 63450-30-6	788 JECFA specification (JECFA, 2001c)	2.2	No safety concern a) Category B b)	
14.026	2-Isopropyl-5-methylpyrazine		3554 2268 13925-05-8	772 JECFA specification (JECFA, 2001c)	0.024	No safety concern a) Category B b)	
14.027	2-Methylpyrazine		3309 2270 109-08-0	761 JECFA specification (JECFA, 2001c)	17	No safety concern a) Category B b)	
14.028	5-Methylquinoxaline		3203 2271 13708-12-8	798 JECFA specification (JECFA, 2001c)	22	No safety concern a) Category B b)	
14.031	Pyrazineethanethiol		3230 2285 35250-53-4	795 JECFA specification (JECFA, 2001c)	0.13	No safety concern a) Category B b)	
14.032	Acetylpyrazine		3126 2286 22047-25-2	784 JECFA specification (JECFA, 2001c)	12	No safety concern a) Category B b)	
14.034	Pyrazinyl methyl sulfide		3231 2288 21948-70-9	796 JECFA specification (JECFA, 2001c)	0.0061	No safety concern a) Category B b)	
14.035	2-Methyl-3,5 or 6-methylthiopyrazine		3208 2290 67952-65-2	797 JECFA specification (JECFA, 2001c)	6.3	No safety concern a) Category B b)	
14.037	6,7-Dihydro-5-methyl-5H-cyclopentapyrazine		3306 2314 23747-48-0	781 JECFA specification (JECFA, 2001c)	3.9	No safety concern a) Category B b)	

Table 3: Supporting Substances Summary

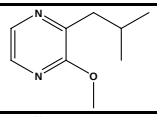
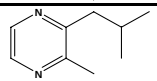
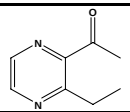
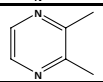
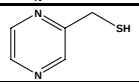
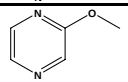
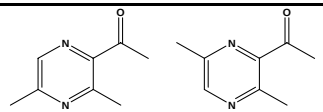
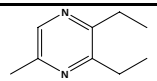
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (µg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
14.043	2-Isobutyl-3-methoxypyrazine		3132 11338 24683-00-9	792 JECFA specification (JECFA, 2001c)	1.6	No safety concern a)	
14.044	2-Isobutyl-3-methylpyrazine		3133 13925-06-9	773 JECFA specification (JECFA, 2001c)	0.037	No safety concern a)	
14.049	2-Acetyl-3-ethylpyrazine		3250 11293 32974-92-8	785 JECFA specification (JECFA, 2001c)	0.73	No safety concern a)	
14.050	2,3-Dimethylpyrazine		3271 11323 5910-89-4	765 JECFA specification (JECFA, 2001c)	14	No safety concern a)	
14.053	Mercaptomethylpyrazine		3299 11502 59021-02-2	794 JECFA specification (JECFA, 2001c)	0.012	No safety concern a)	
14.054	Methoxypyrazine		3302 11347 3149-28-8	787 JECFA specification (JECFA, 2001c)	3.0	No safety concern a)	
14.055	2-Acetyl-3,5-dimethylpyrazine		3327 11294 54300-08-2	786 JECFA specification (JECFA, 2001c)	0.97	No safety concern a)	The JECFA evaluated 2-acetyl-3,(5 or 6)-dimethylpyrazine (mixture of two substancs with individual CASrn for each substance). Register CASrn to be changed/deleted.
14.056	2,3-Diethyl-5-methylpyrazine		3336 11303 18138-04-0	777 JECFA specification (JECFA, 2001c)	0.11	No safety concern a)	

Table 3: Supporting Substances Summary

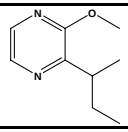
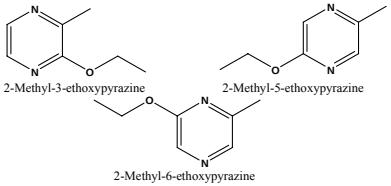
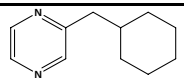
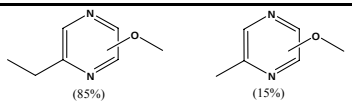
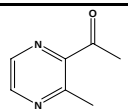
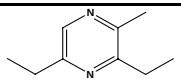
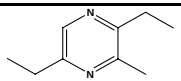
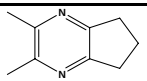
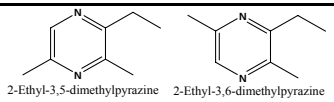
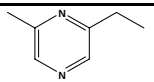
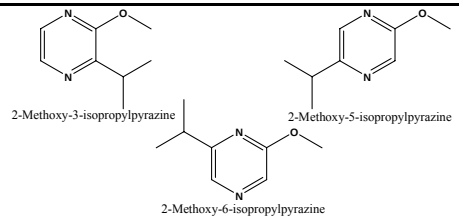
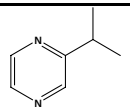
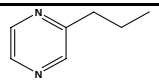
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (µg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
14.062	2-(sec-Butyl)-3-methoxypyrazine		3433 11300 24168-70-5	791 JECFA specification (JECFA, 2001c)	0.85	No safety concern a)	
14.067	2-Methyl-3,5 or 6-ethoxypyrazine		3569 11921 32737-14-7	793 JECFA specification (JECFA, 2001c)	0.055	No safety concern a)	The JECFA evaluated 2-methyl-3(or 5 or 6)-ethoxypyrazine (mixture of three substancs with individual CASrn for each substance). Register CASrn to be changed/deleted.
14.069	Cyclohexylmethylpyrazine		3631 28217-92-7	783 JECFA specification (JECFA, 2001c)	0.012	No safety concern a)	
14.077	2-Ethyl-(3,5 or 6)-methoxypyrazine (85%) and 2-Methyl-(3,5 or 6)-methoxypyrazine (13%)		3280 11329	789 JECFA specification (JECFA, 2001c)	1.3	No safety concern a)	
14.082	2-Acetyl-3-methylpyrazine		3964 11296 23787-80-6	950 JECFA specification (JECFA, 2001c)	0.1	No safety concern a)	
14.095	3,5-Diethyl-2-methylpyrazine		3916 11305 18138-05-1	779 JECFA specification (JECFA, 2001c)	0.012	No safety concern a)	
14.096	2,5-Diethyl-3-methylpyrazine		3915 11304 32736-91-7	778 JECFA specification (JECFA, 2001c)	0.012	No safety concern a)	
14.098	6,7-Dihydro-2,3-dimethyl-5H-cyclopentapyrazine		3917 11309 38917-62-3	782 JECFA specification (JECFA, 2001c)	0.012	No safety concern a)	
14.100	3,(5- or 6-)-Dimethyl-2-ethylpyrazine		3149 727 55031-15-7	775 JECFA specification (JECFA, 2001c)	38	No safety concern a)	JECFA CASrn: (13360-65-1, 13925-07-0).
14.114	2-Ethyl-6-methylpyrazine		3919 11331 13925-03-6	769 JECFA specification (JECFA, 2001c)	0.37	No safety concern a)	

Table 3: Supporting Substances Summary

FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (µg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
14.121	2-Isopropyl-(3,5 or 6)-methoxypyrazine		3358 11344 93905-03-4	790 JECFA specification (JECFA, 2001c)	0.0012	No safety concern a)	
14.123	Isopropylpyrazine		3940 11343 29460-90-0	764 JECFA specification (JECFA, 2001c)	0.12	No safety concern a)	
14.142	Propylpyrazine		3961 11362 18138-03-9	763 JECFA specification (JECFA, 2001c)	0.12	No safety concern a)	
14.144	Pyrazine		4015 11363 290-37-9	951 JECFA specification (JECFA, 2001c)	0.024	No safety concern a)	

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

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

3) No safety concern at estimated levels of intake.

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

a) (JECFA, 2002b).

b) (CoE, 1992).

ND) No intake data reported.

ANNEX I: PROCEDURE FOR THE SAFETY EVALUATION

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

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

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

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

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

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

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

[8] "Innocuous metabolic products": Products that are known or readily predicted to be harmless to humans at the estimated intakes of the flavouring agent" (JECFA, 1997a).
[9] "Endogenous substances": Intermediary metabolites normally present in human tissues and fluids, whether free or conjugated; hormones and other substances with biochemical or physiological regulatory functions are not included (JECFA, 1997a).

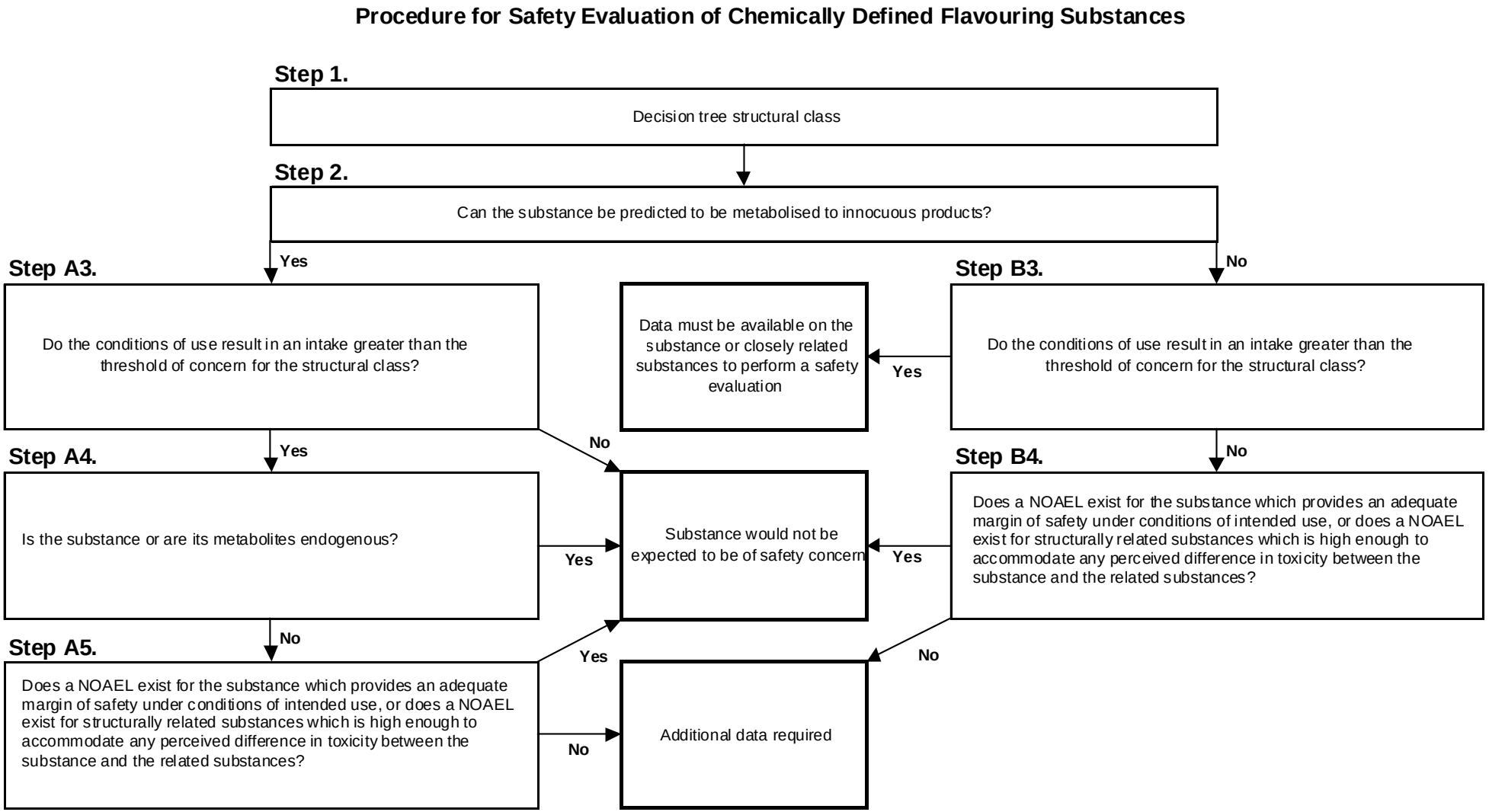


Figure I.1 *Procedure for Safety Evaluation of Chemically Defined Flavouring Substances*

ANNEX II: USE LEVELS / MTAMDI

II.1 NORMAL AND MAXIMUM USE LEVELS

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

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

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

The "normal and maximum use levels" are provided by Industry for 21 of the candidate substances in the present flavouring group (EFFA, 2003q; EFFA, 2007a; EFFA, 2007e; EFFA, 2007f; Flavour Industry, 2010m) (Table II.1.2).

Table II.1.2 Normal and Maximum use levels (mg/kg) for the candidate substances in FGE.17Rev3 (EFFA, 2003q; EFFA, 2007a; EFFA, 2007e; EFFA, 2007f; Flavour Industry, 2010m).

FL-no	Food Categories Normal use levels (mg/kg) Maximum use levels (mg/kg)																	
	01.0	02.0	03.0	04.1	04.2	05.0	06.0	07.0	08.0	09.0	10.0	11.0	12.0	13.0	14.1	14.2	15.0	16.0
14.052	0,4	0,1	0,4	0,4	-	1	0,2	2	0,2	0,2	-	-	0,1	0,2	0,2	1	1	0,2
	2	0,5	2	2	-	5	1	10	1	1	-	-	0,5	1	1	5	5	1
14.081	0,4	0,1	0,4	0,4	-	1	0,2	1	0,1	0,1	-	-	0,1	0,2	0,2	1	1	0,2
	2	0,5	2	2	-	5	1	5	0,4	0,4	-	-	0,5	1	1	5	5	1
14.083	0,4	0,1	0,4	0,4	-	1	0,2	1	0,1	0,1	-	-	0,1	0,2	0,2	1	1	0,2
	2	0,5	2	2	-	5	1	5	0,5	0,5	-	-	0,5	1	1	5	5	1
14.084	0,4	0,1	0,4	0,4	-	1	0,2	1	0,1	0,1	-	-	0,1	0,2	0,2	1	1	0,2
	2	0,5	2	2	-	5	1	5	0,5	0,5	-	-	0,5	1	1	5	5	1
14.086	0,4	0,1	0,4	0,4	-	1	0,2	1	0,1	0,1	-	-	0,1	0,2	0,2	1	1	0,2
	2	0,5	2	2	-	5	1	5	0,4	0,4	-	-	0,5	1	1	5	5	1
14.087	0,4	0,1	0,4	0,4	-	1	0,2	1	0,1	0,1	-	-	0,1	0,2	0,2	1	1	0,2
	2	0,5	2	2	-	5	1	5	0,4	0,4	-	-	0,5	1	1	5	5	1
14.091	0,4	0,1	0,4	0,4	-	1	0,2	1	0,1	0,1	-	-	0,1	0,2	0,2	1	1	0,2
	2	0,5	2	2	-	5	1	5	0,4	0,4	-	-	0,5	1	1	5	5	1
14.097	0,4	0,1	0,4	0,4	-	1	0,2	1	0,1	0,1	-	-	0,1	0,2	0,2	1	1	0,2
	2	0,5	2	2	-	5	1	5	0,4	0,4	-	-	0,5	1	1	5	5	1
14.099	0,4	0,1	0,4	0,4	-	1	0,2	-	0,1	0,1	-	-	0,1	0,2	0,2	1	1	0,2
	2	0,5	2	2	-	5	1	-	0,4	0,4	-	-	0,5	1	1	5	5	1

Table II.1.2 Normal and Maximum use levels (mg/kg) for the candidate substances in FGE.17Rev3 (EFFA, 2003q; EFFA, 2007a; EFFA, 2007e; EFFA, 2007f; Flavour Industry, 2010m).

FL-no	Food Categories																	
	Normal use levels (mg/kg) Maximum use levels (mg/kg)																	
	01.0	02.0	03.0	04.1	04.2	05.0	06.0	07.0	08.0	09.0	10.0	11.0	12.0	13.0	14.1	14.2	15.0	16.0
14.101	0,4 2	0,1 0,5	0,4 2	0,4 2	- -	1 5	0,2 1	- -	0,1 0,4	0,1 0,4	- -	- -	0,1 0,5	0,2 1	0,2 1	1 5	1 5	0,2 1
14.102	0,4 2	0,1 0,5	0,4 2	0,4 2	- -	1 5	0,2 1	1 5	0,1 0,4	0,1 0,4	- -	- -	0,1 0,5	0,2 1	0,2 1	1 5	1 5	0,2 1
14.108	0,4 2	0,1 0,5	0,4 2	0,4 2	- -	1 5	0,2 1	0,2 1	0,1 0,4	0,1 0,4	- -	- -	0,1 0,5	0,2 1	0,2 1	1 5	1 5	0,2 1
14.113	0,4 2	0,1 0,5	0,4 2	0,4 2	- -	1 5	0,2 1	1 5	0,1 0,4	0,1 0,4	- -	- -	0,1 0,5	0,2 1	0,2 1	1 5	1 5	0,2 1
14.122	0,4 2	0,1 0,5	0,4 2	0,4 2	- -	1 5	0,2 1	1 5	0,1 0,4	0,1 0,4	- -	- -	0,1 0,5	0,2 1	0,2 1	1 5	1 5	0,2 1
14.127	0,4 2	0,1 0,5	0,4 2	0,4 2	- -	1 5	0,2 1	1 5	0,1 0,4	0,1 0,4	- -	- -	0,1 0,5	0,2 1	0,2 1	1 5	1 5	0,2 1
14.129	0,4 2	0,1 0,5	0,4 2	0,4 2	- -	1 5	0,2 1	2 10	0,2 1	0,2 1	- -	- -	0,1 0,5	0,2 1	0,2 1	1 5	1 5	0,2 1
14.139	0,4 2	0,1 0,5	0,4 2	0,4 2	- -	1 5	0,2 1	1 5	0,1 0,4	0,1 0,4	- -	- -	0,1 0,5	0,2 1	0,2 1	1 5	1 5	0,2 1
14.147	0,4 2	0,1 0,5	0,4 2	0,4 2	- -	1 5	0,2 1	1 5	0,1 0,4	0,1 0,4	- -	- -	0,1 0,5	0,2 1	0,2 1	1 5	1 5	0,2 1
14.148	0,4 2	0,1 0,5	0,4 2	0,4 2	- -	1 5	0,2 1	1 5	0,1 0,4	0,1 0,4	- -	- -	0,1 0,5	0,2 1	0,2 1	1 5	1 5	0,2 1
14.161	0,4 2	0,1 0,5	0,4 2	0,4 2	- -	1 5	0,2 1	2 10	0,2 1	0,2 1	- -	- -	0,1 0,5	0,2 1	0,2 1	1 5	1 5	0,2 1
14.170	1,72 3,6	- -	1,1 2,33	- -	- -	2,92 6	- -	3,13 6	2 4	- -	- -	- -	- -	- -	1,93 2,5	0,3 1	- -	- -

II.2 mTAMDI CALCULATIONS

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

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

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

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

- Beverages (SCF, 1995) correspond to food category 14.1 (EC, 2000a)
- Foods (SCF, 1995) correspond to the food categories 1, 2, 3, 4.1, 4.2, 6, 7, 8, 9, 10, 13, and/or 16 (EC, 2000a)

- Exception a (SCF, 1995) corresponds to food category 5 and 11 (EC, 2000a)
- Exception b (SCF, 1995) corresponds to food category 15 (EC, 2000a)
- Exception c (SCF, 1995) corresponds to food category 14.2 (EC, 2000a)
- Exception d (SCF, 1995) corresponds to food category 12 (EC, 2000a)
- Exception e (SCF, 1995) corresponds to others, e.g. chewing gum.

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

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

The mTAMDI values (see Table II.2.3) are presented for 21 of the substances in the present flavouring group, for which Industry has provided use and use levels (EFFA, 2003q; EFFA, 2007a; EFFA, 2007e; EFFA, 2007f; Flavour Industry, 2010m). The mTAMDI values are only given for highest reported normal use levels.

TableII.2.3 Estimated intakes based on the mTAMDI approach

FL-no	EU Register name	mTAMDI (µg/person/day)	Structural class	Threshold of concern (µg/person/day)
14.057	2-Isopropyl-3-methoxypyrazine		Class II	540
14.081	5-Acetyl-2,3-dimethylpyrazine	270	Class II	540
14.083	2-Acetyl-5-ethylpyrazine	270	Class II	540
14.084	2-Acetyl-5-methylpyrazine	270	Class II	540
14.086	2-Acetyl-6-ethylpyrazine	270	Class II	540
14.087	2-Acetyl-6-methylpyrazine	270	Class II	540
14.091	2-Butyl-3-methylpyrazine	270	Class II	540
14.097	2,5-Diethylpyrazine	270	Class II	540
14.099	6,7-Dihydro-5,7-dimethyl-5H-cyclopentapyrazine	190	Class II	540
14.101	2,5-Dimethyl-3-isopropylpyrazine	190	Class II	540
14.102	5,6-Dimethyldihydrocyclopentapyrazine	270	Class II	540
14.111	3-Ethyl-2,5-dimethylpyrazine		Class II	540
14.113	5-Ethyl-6,7-dihydro-5H-cyclopentapyrazine	270	Class II	540
14.127	2-Methoxy-3-propylpyrazine	270	Class II	540
14.129	2-Methyl-3-propylpyrazine	400	Class II	540

TableII.2.3 Estimated intakes based on the mTAMDI approach

FL-no	EU Register name	mTAMDI (µg/person/day)	Structural class	Threshold of concern (µg/person/day)
14.148	5,6,7,8-Tetrahydro-5-methylquinoxaline	270	Class II	540
14.161	6,7-Dihydro-2,5-dimethyl-5H-cyclopentapyrazine	400	Class II	540
14.170	5-Ethyl-2,3-dimethyl pyrazine	1100	Class II	540
14.052	Isopropenylpyrazine	400	Class II	540
14.051	2,5 or 6-Methoxy-3-ethylpyrazine		Class II	540
14.108	2,3-Dimethylquinoxaline	190	Class III	90
14.109	2-Ethoxy-3-methylpyrazine		Class III	90
14.112	2-Ethyl-3-methoxypyrazine		Class III	90
14.126	2-Methoxy-3-methylpyrazine		Class III	90
14.122	2-Isopropyl-3-methylthiopyrazine	270	Class III	90
14.128	2-Methyl-3-methylthiopyrazine		Class III	90
14.139	2-Methylquinoxaline	270	Class III	90
14.147	Quinoxaline	270	Class III	90

ANNEX III: METABOLISM

III.1. Introduction

This group of flavouring substances consists of 28 candidate substances, all of which contain a pyrazine ring. In 20 substances [FL-no: 14.051, 14.052, 14.057, 14.081, 14.083, 14.084, 14.086, 14.087, 14.091, 14.097, 14.101, 14.109, 14.111, 14.112, 14.122, 14.126, 14.127, 14.128, 14.129 and 14.170] only one heterocyclic ring is present. In five candidate substances a pyrazine ring is fused with either cyclopentane [FL-no: 14.099, 14.102, 14.113 and 14.161] or cyclohexane [FL-no: 14.148]. All of these 25 substances have different substituents on the rings. In 16 of them, the substituents are simple alkyl chains and / or ketones. In nine, the substituents are, next to alkyl chains, either a methoxy- ([FL-no: 14.051, 14.057, 14.112, 14.126 and 14.127], an ethoxy- [FL-no: 14.109], a thiomethyl- [FL-no: 14.122 and 14.128]) or an isopropenyl residue [FL-no: 14.052].

In the remaining three candidate substances the pyrazine ring is fused with benzene giving quinoxalines. In two of these substances [FL-no: 14.139 and 14.108] the pyrazine ring also bears one or two methyl substituents and in the third no substituents are present (quinoxaline [FL-no 14.147]).

A group with 41 related supporting substances has been evaluated by the JECFA (JECFA, 2002a).

III.2. Absorption, Distribution and Elimination

No pertinent absorption, distribution or elimination studies were found in the published or available unpublished literature for the candidate substances. Some information on supporting substances has been found, but the available information is still very limited.

In rats, orally administered substituted pyrazines are absorbed from the gastrointestinal tract and excreted (Hawksworth and Scheline, 1975). Approximately 90 - 100 % of a 100 mg/kg dose of 2-methylpyrazine, 2,5-dimethylpyrazine, 2,6-dimethylpyrazine or 2-methoxypyrazine administered to male Wistar rats by stomach tube was excreted in the urine as polar metabolites within 24 hours. Also, 50 % of the orally administered dose of 100 mg 2,3-dimethylpyrazine per kg was recovered in the urine within 24 hours (Hawksworth and Scheline, 1975).

The supporting substance pyrazine [FL-no: 14.144] is a weak base with a pK_a = 0.6 (Damani and Crooks, 1982). At intestinal pH (pH = 5 - 7), absorption of weak amine bases such as pyrazine is optimal, because at these pHs such substances occur largely in the non-ionised state, which facilitates their absorption through the gastro-intestinal membranes (Hogben et al., 1959; Schranker et al., 1957). For the same reason it may be expected that the pyrazine derivatives in this group will be absorbed mainly after the passage through the stomach into the intestinal lumen.

Groups of five anaesthetised male Sprague-Dawley rats were administered a single intravenous bolus dose of 2, 5 or 10 mg tetramethylpyrazine/kg body weight (bw) through the femoral vein. Blood samples were withdrawn directly *via* heart puncture of the rat and collected from the same animals at 2.5, 5, 10, 15, 20, 30, 45, 60, 90 and 120 minutes following administration to determine pharmacokinetic profiles in plasma. Distribution of tetramethylpyrazine was studied in various parts of the brain. It was determined that a two-compartment open model best described the plasma concentration-time curve for all the dose levels. The plasma distribution half-life ($t_{1/2,alpha}$) ranged from about 2.6 to 9.2 minutes and the elimination half-life ($t_{1/2,beta}$) ranged from 20 to 28 minutes for the 2 and 10 mg tetramethylpyrazine/kg bw doses, respectively. Area

under the concentration curve (AUC) ranged from about 23 to 227 microg x min/ml and clearance (CL) ranged from 92 to 45 ml/min/kg for the 2 and 10 mg tetramethylpyrazine/kg bw doses, respectively. These results indicate that rapid distribution, elimination and clearance occur in rats within the tested concentration range of 2 - 10 mg tetramethylpyrazine/kg bw. Fifteen minutes after intravenous administration of 10 mg tetramethylpyrazine/kg bw, the cerebral cortex concentration and plasma concentration of tetramethylpyrazine were 1.45 ± 0.09 microg/g and 6.14 ± 0.38 microg/ml, respectively. There were no significant differences in tetramethylpyrazine concentration among the various regions of the brain (Liang et al., 1999). The data on plasma clearance also indicate that the kinetics of tetramethylpyrazine may not be linear with the dose level.

An experimental design allowing simultaneous and continuous monitoring of tetramethylpyrazine concentrations in rat blood and brain was employed to study the distribution of an intravenously administered dose of 10 mg tetramethylpyrazine/kg bw. Microdialysis probes were inserted into the right jugular vein and striatum of four anaesthetised male Sprague-Dawley rats. Results indicate that both blood and brain pharmacokinetics of unbound tetramethylpyrazine fit best to a two-compartment model. The elimination half-life ($t_{1/2,beta}$) of tetramethylpyrazine in rat blood and brain were about 28 and 53 minutes and the AUCs were about 82 and 185 microg x min/ml, respectively (Tsai and Liang, 2001).

In conclusion:

Very few data on absorption, distribution and elimination of the candidate or supporting substances are available. The available data indicate that the (weak) basic heterocyclic substances in this group may be well absorbed, mainly from the intestinal lumen, and may be rapidly excreted.

III.3. Metabolism

III.3.1. Alkyl-, Alicyclic- and Alkylaryl-substituted Pyrazine Derivatives

For two candidate substances [FL-no: 14.147 and 14.108], some information on metabolism has been submitted. Supportive data have also been taken from general reviews on metabolism and from submitted studies, in which biotransformation of substances with remote resemblance to the candidate substances has been described.

In general, the biotransformation of the alkyl-, alicyclic-, and alkylaryl-substituted pyrazine derivatives is expected to occur *via* oxidation of the side-chains (see Figure III.1). For example, methyl-substituted pyrazines are oxidised to yield the corresponding pyrazine-carboxylic acids, which may be excreted as glycine conjugates (Hawksworth and Scheline, 1975). An alternative pathway for substituted pyrazines and primary pathway for pyrazine involves hydroxylation of the pyrazine ring (Hawksworth and Scheline, 1975; Whitehouse et al., 1987; Yamamoto et al., 1987a; Yamamoto et al., 1987b). For example, 2,5-dimethylpyrazine and 2,6-dimethylpyrazine are oxidised in rats almost exclusively *via* their aliphatic side-chains to carboxylic acid derivatives. Conversely, 2,3-dimethylpyrazine primarily undergoes ring hydroxylation, because side-chain oxidation is retarded (only 13 % of the administered dose oxidised) by the steric hindrance between the methyl groups (Hawksworth and Scheline, 1975).

At least 89 % of a 100 mg/kg oral dose of 2-methylpyrazine, 2,5-dimethylpyrazine, or 2,6-dimethylpyrazine was metabolised in the rat by side-chain oxidation to yield the corresponding pyrazine-2-carboxylic acid derivative. The acids were mainly excreted unconjugated, although 9 and 14 % of the administered doses were excreted as the corresponding glycine conjugates for 2-methylpyrazine and 2,5-dimethylpyrazine, respectively. No glycine conjugation was observed with 2-methylpyrazine-6-carboxylic acid. 2,3-Dimethylpyrazine was metabolised to 2-methylpyrazine-3-carboxylic acid (not conjugated with glycine) and 2,3-dimethyl-5-hydroxypyrazine (in total 50 % of the dose). No N-oxygenation products could be detected in

the urine (Hawksworth and Scheline, 1975). Methyl side-chain oxidation to yield the corresponding alcohols has also been demonstrated for quinoxaline derivatives (Turesky et al., 1988; Knize et al., 1989; Sjödin et al., 1989; Wallin et al., 1989).

Alicyclic-substituted pyrazines, such as candidate substances 6,7-dihydro-5,7-dimethyl-5H-cyclopentapyrazine [FL-no: 14.099], 5-ethyl-6,7-dihydro-5H-cyclopentapyrazine [FL-no: 14.113], 6,7-dihydro-2,5-dimethyl-5H-cyclopentapyrazine [FL-no: 14.161] and 5,6,7,8-tetrahydro-5-methylquinoxaline [FL-no: 14.148], are expected to undergo side-chain oxidation similar to that previously described for alkyl substituted pyrazines (> C_1), but no experimental evidence for this has been submitted. In addition, hydroxylation at various positions on the alicyclic ring is likely, based on general knowledge of metabolic conversion of alicyclic ring systems. The products of these oxidative metabolism reactions may be excreted unchanged or may be conjugated with glycine, glucuronic acid, or sulphate prior to excretion (Parkinson, 1996a).

Ring hydroxylation may be catalysed by molybdenum hydroxylases, e.g., xanthine oxidase and aldehyde oxidase, which are present in the cytosol of humans and other mammalian species, predominantly in the liver and small intestine. These enzymes catalyse ring hydroxylation of a wide range of endogenous and exogenous N-heterocycles bearing a substituent and/or a second fused ring. The molybdenum hydroxylases facilitate oxidation reactions involving nucleophilic attack by oxygen derived from water. Oxidation occurs at the most electropositive atom, which in N-heterocycles is generally the carbon adjacent to the ring nitrogen. The role of the molybdenum hydroxylases increases as the number of ring nitrogen atoms increase since each nitrogen activates the ring system toward nucleophilic attack. The oxidation action of the molybdenum hydroxylases is opposite to the microsomal monooxygenases (such as cytochrome-P450), which catalyse electrophilic attack by an oxygen atom derived from molecular oxygen (O_2) (Beedham, 1985; Beedham, 1988; Parkinson, 1996a). Although substituted monocyclic pyrazines may be substrates for the molybdenum hydroxylases when other pathways are unfavourable, as indicated by the results of (Hawksworth and Scheline, 1975), bicyclic heterocycles are the preferred substrates of these enzymes (Stubley et al., 1979; Beedham, 1985; Beedham, 2002).

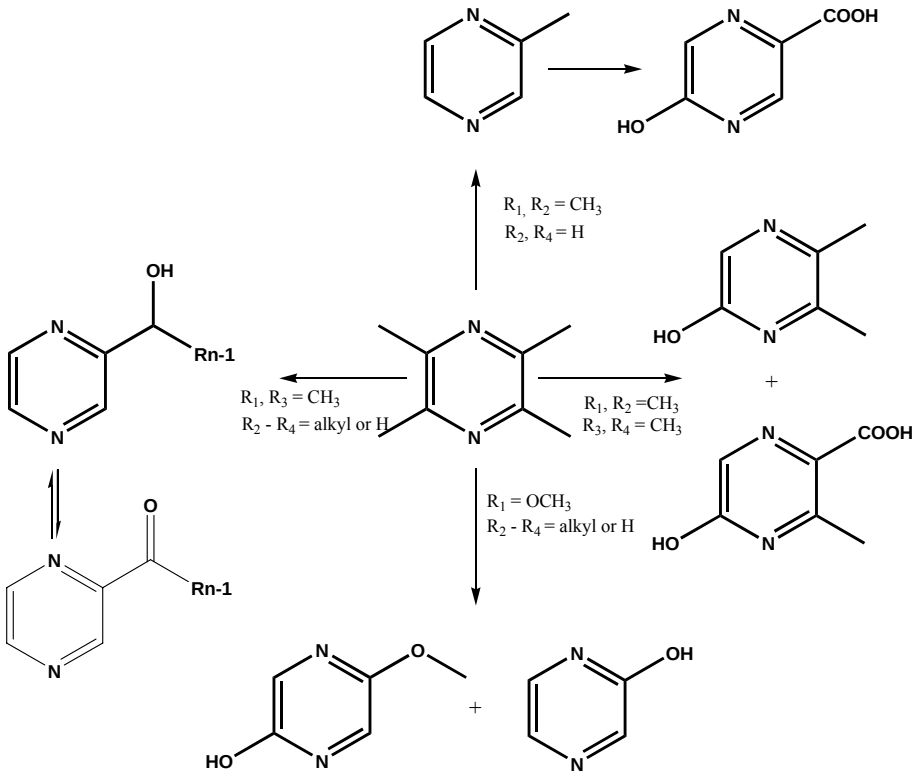


Figure III.1. *Possible metabolic pathways of alkyl- and alkoxy-substituted pyrazine derivatives. Conjugates not shown. Excretion products are displayed in bold font.*

III.3.2. N-oxidation and N-methylation

After *in vivo* administration of several methyl substituted pyrazine or pyridine derivatives to rats, no N-oxygenation products could be detected in the urine (Hawksworth and Scheline, 1975).

Generally, oxidation of ring nitrogen occurs in structures where the nitrogen atom has a nucleophilic character. N-oxidation of ring nitrogen has been reported in pyridine derivatives e.g. 3-acetyl pyridine is oxidised to give 3-acetyl pyridine N-oxide in *in vivo* and *in vitro* studies in rats and other laboratory animal species (Damani et al., 1980). The nucleophilicity of the ring nitrogen of pyrazine is much lower than in pyridine (pyrazine having a pK_a of 0.6 in comparison with pyridine having a pK_a of 5.17), suggesting that N-oxidation in pyrazine may not occur.

N-methylation of ring nitrogen in heterocyclic compounds has been reported, e.g. [2,6-^{14}C]pyridine is methylated to N-methylpyridine in various mammals *in vivo* (D`Souza et al., 1980).

However, similarly as above, methylation of ring nitrogen occurs in structures where the nitrogen atom has nuclephilic character. N-methylation of pyrazine derivatives therefore is unlikely to occur, in contrast to the situation with pyridine derivatives.

Candidate substances

Quinoxaline [FL-no: 14.147]

Quinoxaline has been incubated in *in vitro* subcellular fractions to study the contribution of aldehyde oxidase (Mo-hydroxylase) and cytochrome P-450 in its metabolism. With aldehyde oxidase, formation of 2-hydroxyquinoxaline could be determined (K_m: 1.6 x 10^{-4} M) which could be further metabolised to 2,3-dihydroxyquinoxaline. With cytochrome P-450 only a trace of a phenolic reaction product was obtained (not further characterised). As with quinoxaline, with several other mono-aza and di-aza bicyclic heterocyclic compounds (quinolines, phthalazine, quinazolines, cinnoline), similar phenolic conversions were observed. Except with cinnoline, in all cases with aldehyde oxidase, but not with cytochrome P-450, the hydroxylation occurred at the carbon adjacent to an N atom. The study indicates that the conversions by aldehyde oxidase may be more efficient than those by cytochrome P-450 (Stubley et al., 1977).

Quinoxaline incubated *in vitro* with rabbit liver aldehyde oxidase is ring hydroxylated at a carbon atom adjacent to a ring nitrogen atom to yield 2-hydroxyquinoxaline and 2,3-dihydroxyquinoxaline. The apparent Michaelis-Menten constant of rabbit liver aldehyde oxidase for quinoxaline is $K_m = 1.76 \times 10^{-4}$ (at pH = 7 and 30°C). Incubation of quinoxaline with rat liver preparation yields qualitatively the same results as those using rabbit liver, but smaller amounts of the oxidation products are detected from rat liver incubations. Comparing the conversion of quinoxaline by rabbit and rat liver (10,000 *g* supernatants) during one hour incubations at 37°C, shows that the percentage conversion for rabbit is 6 and 5 % and for rat 4.4 and 4.5 % for 2-hydroxyquinoxaline and 2,3-dihydroxyquinoxaline, respectively (Stubley et al., 1979).

2,3-Dimethylquinoxaline [FL-no: 14.108]

Repeated administration of 2,3-dimethylquinoxaline [FL-no: 14.108] at a daily oral dose of 500 mg/kg bw for four days induces total hepatic cytochrome P-450 and cytochrome P-450-mediated biotransformations (aniline ring-hydroxylation, *p*-nitroanisole O-demethylation, aminopyrine N-demethylation, N-methylaniline N-demethylation) in female rat liver (Béraud et al., 1975).

III.3.3. Pyrazine Derivatives Containing an Oxygenated Functional Group in the Side-chain

Candidate substances with an oxygenated functional group in the side chain are [FL-no: 14.051, 14.057, 14.081, 14.083, 14.084, 14.086, 14.087, 14.109, 14.112, 14.126 and 14.127].

Rats were dosed with 100 mg/kg bw of 2-methoxypyrazine via gavage. Only 20 % of the urinary metabolites were identified as 2-hydroxypyrazine (i.e. the O-demethylated product). The other 80 % was one substance which was identified as a ring-hydroxylated 2-methoxypyrazine (Hawksworth and Scheline, 1975). Similar reactions can be expected to occur with candidate substances 2-, 5- or 6-methoxy-3-ethylpyrazine [FL-no: 14.051], 2-isopropyl-3-methoxypyrazine [FL-no: 14.057], 2-ethyl-3-methoxypyrazine [FL-no: 14.112], 2-methoxy-3-methylpyrazine [FL-no: 14.126], 2-methoxy-3-propylpyrazine [FL-no: 14.127]. O-deethylation can be expected to some extent for 2-ethoxy-3-methylpyrazine [FL-no: 14.109].

Ring hydroxylation of the anti-tubercular agent, pyrazinamide, has been reported *in vitro* (Yamamoto et al., 1987b) and *in vivo* (Whitehouse et al., 1987; Yamamoto et al., 1987a) in both humans and rats. Within 12 hours after dosing, male rats with 150 mg pyrazinamide/kg bw by gavage, 60 % of the dose was excreted as hydrolysis products via the urine (25 % as 5-hydroxypyrazine-2-carboxylic acid and 35 % as pyrazine-2-carboxylic acid). Parent compound and 5-hydroxypyrazinamide accounted for 14 and 3 % of the dose, respectively (Whitehouse et al., 1987). The same 5-hydroxylation products were detected in a urine sample of a man dosed with 12.5 mg pyrazinamide/kg bw. The hydroxylation of pyrazinamide and pyrazinoic acid *in vitro* to form 5-hydroxypyrazinamide and 5-hydroxypyrazine-2-carboxylic acid, respectively, occurred in the presence of xanthine oxidase-rich human liver cytosol (Yamamoto et al., 1987b).

It has been demonstrated that *in vitro* and *in vivo* 3-acetylpyridine can be reduced at the carbonyl group to give 1-(3-pyridyl)-ethanol (Damani et al., 1980). Based on this observation, it may be expected that the structurally related candidate acetylated pyrazines, such as 2-acetyl-5-methylpyrazine [FL-no: 14.084], 2-acetyl-6-methylpyrazine [FL-no: 14.087], 2-acetyl-5-ethylpyrazine [FL-no: 14.083], 2-acetyl-6-ethylpyrazine [FL-no: 14.086], and 5-acetyl-2,3-dimethylpyrazine [FL-no: 14.081] may also be metabolised by reduction of the ketone functional group resulting in the formation of the corresponding secondary alcohol. Subsequently, the terminal methyl group in the acetyl-chain may be oxidised to yield the corresponding alpha-hydroxy-carboxylic acid. Alternatively, the terminal carbon atom of the acetyl side chain may be completely removed, which results in the formation of the corresponding pyrazine-carboxylic acid. Similar conversions have been demonstrated for acetophenone, which can be metabolised to mandelic acid or benzoic acid (Sullivan et al., 1976).

Conclusion on metabolism of monocyclic or alicyclic- or aryl-bicyclic pyrazine derivatives with alkyl- or oxygenated functional group ring substituents:

Very little information has been submitted to describe the metabolism of the pyrazines and alkyl-, aryl- or alicyclic-substituted pyrazines in this group of flavouring substances. The available information shows that pyrazine with a simple alkyl substituent may be oxidised at the side chain to give the corresponding carboxylic acid [FL-no: 14.097]. If such oxidations are not possible, e.g. due to steric hindrance, hydroxylation of the pyrazine ring may also occur [FL-no: 14.091, 14.101, 14.111 14.129 and 14.170]. The bicyclic pyrazine derivatives with an additional alicyclic or aryl ring substituent [FL-no: 14.099, 14.102, 14.108, 14.113, 14.139, 14.147, 14.148 and 14.161] may be better substrates for ring hydroxylation, which seems to be carried out preferably by molybdenum hydroxylases. The candidate substances bearing a ketone ring substituent [FL-no: 14.081, 14.083, 14.084, 14.086 and 14.087] may be reduced at the carbonyl in the side chain to give the corresponding alcohol. The five monocyclic pyrazine derivatives with a methoxy side chain [FL-no: 14.051, 14.057, 14.112, 14.126 and 14.127] may also be expected to be metabolised via both ring hydroxylation and O-demethylation of the methoxy side chain. Accordingly, 2-ethoxy-3-methylpyrazine [FL-no: 14.109] may be expected to be metabolised via both ring hydroxylation and O-deethylation of the ethoxy side chain. With the resulting products of any of these flavouring substances, conjugation with glycine, sulphate or glucuronide may occur. In addition, with some related substances N-oxidation or N-methylation have been observed, which may lead to biologically active metabolites. However, after *in vivo* administration of several methyl substituted pyrazine derivatives to rats, no N-oxidation products could be

detected in the urine. Additionally, the nucleophilicity of the pyrazine ring nitrogen is much lower than that of the pyridine ring nitrogen. It is concluded, therefore, that N-oxidation or N-methylation are unlikely to occur in the pyrazines.

III.3.4. Pyrazine Derivatives Containing a Thiol or Sulphide Functional Group in the Side-chain

The presence of sulphur in the side chain of pyrazines and alkylpyrazines provides a further metabolic option. The reactive lone pair of electrons on divalent sulphur in thiols and monosulphides permits rapid oxidation. Alkyl and aromatic sulphides, such as the candidate substances 2-isopropyl-3-methylthiopyrazine [FL-no: 14.122] and 2-methyl-3-methylthiopyrazine [FL-no: 14.128], can be oxidised to sulphoxides and then to sulphones (Hoodi and Damani, 1984; Nickson and Mitchell, 1994; Nickson et al., 1995). The oxidation to sulphoxides is catalysed by at least two enzyme systems, cytochrome P-450s and the flavin-containing monooxygenases (FMO) (Cashman and Williams, 1990; Cashman et al., 1990; Cashman et al., 1995a; Cashman et al., 1995b; Elfarra et al., 1995) and (Hoodi and Damani, 1984; Nnane and Damani, 1995; Rettie et al., 1990; Sadeque et al., 1992; Sadeque et al., 1995; Yoshihara and Tatsumi, 1990; Ziegler, 1980). The contribution of each system is highly dependent on molecular shape and nucleophilicity. For simple aliphatic, alicyclic and aromatic sulphides, oxidation is primarily catalysed by FMO and, to a lesser extent, by cytochrome P-450 (Hoodi and Damani, 1984). However, it is not clear which of these systems is of relevance for the sulphur containing candidate substances [FL-no: 14.122 and 14.128] in this group of flavouring substances. The FMO enzymes are easily saturated, already at very low sulphide concentrations (Ziegler, 1980). However, quantitative data to assess the actual role of FMOs in thioether sulphoxidation *in vivo* were not provided (Ziegler, 1980) but the observation might indicate that their role could be limited at higher levels of exposure.

The final oxidation of the sulphoxide to the sulphone is an irreversible reaction (Damani, 1987; Williams et al., 1966). Essentially, all low molecular weight aliphatic and aromatic sulphones are metabolically stable. Hence, sulphoxides and sulphones are excreted in the urine of animals exposed to sulphides.

As described above, the major reactions by which simple sulphides can be metabolised involve oxidation of the S to give a sulphoxide, which can be further converted to a sulphone. Alternatively, sulphides can undergo oxidation of the carbon alpha to the -S- resulting in the formation of an unstable hydroxyalkyl intermediate, which can be split to give an aldehyde and a free thiol. The aldehyde can be oxidised to its corresponding acid (Damani, 1987; Richardson et al., 1991). Similar reactions might occur with the candidate substances [FL-no: 14.122 and 14.128]. Thiols, such as the supporting substances 2-(mercaptomethyl) pyrazine and 2-pyrazinylethanethiol, are very reactive substances. *In vivo*, they become even more reactive, mainly because most thiols exist in the ionised form at physiologic pH. Metabolic options for thiols include: oxidation to form unstable sulphenic acids (RSOH), which may be oxidised to sulphinic acid (RSO_2H) and sulphonic acid (RSO_3H); methylation to yield methyl sulphides, which then form sulphoxides and sulphones; reaction with physiologic thiols to form mixed disulphides and conjugation with glucuronic acid; or oxidation of the alpha-carbon, which results in desulphuration and the formation of an aldehyde (McBain and Menn, 1969; Dutton and Illing, 1972; Maiorino et al., 1989; Richardson et al., 1991).

Extensive discussions on the metabolism of sulphur-containing flavouring substances have been presented in FGE.08 and FGE.13.

Candidate substances

No pertinent metabolism studies were found in the published or available unpublished literature for the candidate substances.

III.4. Summary and Conclusions

This group of flavouring substances consists of 28 candidate substances, all of which contain a pyrazine ring. In 20 substances [FL-no: 14.051, 14.052, 14.057, 14.081, 14.083, 14.084, 14.086, 14.087, 14.091, 14.097, 14.101, 14.109, 14.111, 14.112, 14.122, 14.126, 14.127, 14.128, 14.129 and 14.170] only one heterocyclic ring is present. In five candidate substances a pyrazine ring is fused with either cyclopentane [FL-no: 14.099, 14.102, 14.113 and 14.161] or cyclohexane [FL-no: 14.148]. All of these 25 substances have different substituents on the rings. In 16 of them, the substituents are simple alkyl chains and / or ketones. In nine, the substituents are, next to alkyl chains, either a methoxy- ([FL-no: 14.051, 14.057, 14.112, 14.126 and 14.127], an ethoxy- [FL-no: 14.109], a thiomethyl- [FL-no: 14.122 and 14.128]) or an isopropenyl residue [FL-no: 14.052].

In the remaining three candidate substances the pyrazine ring is fused with benzene giving quinoxalines. In two of the quinoxalines [FL-no 14.108 and 14.139] the pyrazine ring also bears one or two methyl substituents; in the third no substituents are present (quinoxaline; [FL-no 14.147]).

A group with 41 related supporting substances has been evaluated by the JECFA (JECFA, 2002a).

Very few data on absorption, distribution and elimination of the candidate or supporting flavouring substances are available. The available data indicate that the weak basic heterocyclic substances in this group may be well absorbed, mainly from the intestinal lumen, and may be rapidly excreted.

Limited information has been submitted to describe the metabolism of the pyrazines and alkyl-, aryl- or alicyclic-substituted pyrazines in this group of flavouring substances. Almost all data available come from one paper (Hawksworth and Scheline, 1975) and a few review papers (Beedham, 1985; Beedham, 1988; Parkinson, 1996a). Additional information provided in other papers is supportive of the metabolic conversions that have been described, but of little quantitative relevance as they concern substances that are widely different from the candidate substances in this group and the supporting ones evaluated by the JECFA.

Pyrazines with a simple alkyl substituent (e.g. [FL-no: 14.097]) may be expected to be oxidised at the side chain to give the corresponding carboxylic acid. If such oxidations are not possible, e.g. due to steric hindrance, hydroxylation of the pyrazine ring may also occur [FL-no: 14.091, 14.101, 14.111, 14.129 and 14.170]. The bicyclic pyrazine derivatives with an additional alicyclic or aryl ring substituent [FL-no: 14.099, 14.102, 14.108, 14.113, 14.139, 14.147, 14.148 and 14.161] may be better substrates for ring hydroxylation, which seems to be carried out preferably by molybdenum hydroxylases. The candidate substances bearing a ketone ring substituent [FL-no: 14.081, 14.083, 14.084, 14.086 and 14.087] may be reduced at the carbonyl in the side chain to give the corresponding alcohol. The five monocyclic pyrazine derivatives with a methoxy side chain [FL-no: 14.051, 14.057, 14.112, 14.126 and 14.127] may be expected to be metabolised via both ring hydroxylation and O-demethylation of the methoxy side chain. Accordingly, the monocyclic pyrazine derivatives with an ethoxy side chain, 2-ethoxy-3-methylpyrazine [FL-no: 14.109], may be expected to be metabolised via both ring hydroxylation and O-deethylation of the ethoxy side chain. With the resulting products of any of these flavouring substances, conjugation with glycine, sulphate or glucuronide may occur. In none of the studies, N-oxidation or N-methylation, which would lead to the formation of bioactive metabolites, has been observed. This is in agreement with the reactive properties of the heterocyclic nitrogen in the pyrazine moieties of the various substances studied.

Two candidate substance in this group, 2-isopropyl-3-methylthiopyrazine [FL-no: 14.122] and 2-methyl-3-methylthiopyrazine [FL-no: 14.128], are thioethers, which may be detoxified by formation of a sulphoxide and subsequently a sulphone, which are both stable and usually rapidly excreted. Alternatively, they may also be bioactivated via S-demethylation, resulting in the formation of a reactive free thiol. No data were provided to show that either route (sulphoxidation or S-demethylation) has prevalence over the other.

The limited amount of information on the metabolism of the substances in this flavouring group does not indicate that these substances will be metabolised to toxic products, except for the sulphur-containing flavouring substances [FL-no: 14.122 and 14.128], which may be converted to a reactive free thiol. For that reason, these two sulphur-containing pyrazine derivatives cannot be expected to be metabolised to innocuous products. Also [FL-no: 14.052], having a terminal double bond, which may be epoxidised giving rise to reactive metabolites cannot be expected to be metabolised to innocuous products.

Originally (FGE.17), it was considered that the results from the genotoxicity studies with quinoxaline and derivatives [FL-no: 14.108, 14.139 and 14.147] indicate that these three may be metabolised into substances that are reactive to DNA. However, new data have become available for the structurally related substance, 5-methylquinoxaline [FL-no: 14.028], considered in FGE.50Rev1. Based on these and other available data the Panel concluded that the *in vitro* genotoxicity alert could be ruled out for 5-methylquinoxaline [FL-no: 14.028] (FGE.50Rev1) as well as for the structurally related substance 2,3-dimethylquinoxaline [FL-no: 14.108] (FGE.17Rev2), but not for 2-methylquinoxaline [FL-no: 14.139] and quinoxaline itself [FL-no: 14.147].

Therefore, based on the available data, the following substances in this group [FL-no: 14.051, 14.057, 14.081, 14.083, 14.084, 14.086, 14.087, 14.091, 14.097, 14.099, 14.101, 14.102, 14.108, 14.109, 14.111, 14.112, 14.113, 14.126, 14.127, 14.129, 14.148, 14.161 and 14.170] may be expected to be metabolised to innocuous products. Three substances [FL-no: 14.122, 14.128 and 14.052] cannot be anticipated to be metabolised to innocuous products. For the remaining two quinoxalines [FL-no: 14.139 and 14.147] a concern for genotoxicity has been identified (see also Section 8.4).

ANNEX IV: TOXICITY

Oral acute toxicity data are available for none of the candidate substances of the present flavouring group evaluation but for 18 supporting substances evaluated by the JECFA at the 57th meeting.

Table IV.1: ACUTE TOXICITY

Chemical Name [FL-no]	Species	Sex	Route	LD_{50} (mg/kg bw)	Reference	Comments
(2-Methylpyrazine [14.027])	Rat	NR	Gavage	1800	(Moran et al., 1980)	
(2,3-Dimethylpyrazine [14.050])	Rat	NR	Gavage	613	(Moran et al., 1980)	
(2,5-Dimethylpyrazine [14.020])	Rat	NR	Gavage	1020	(Moran et al., 1980)	
(2,6-Dimethylpyrazine [14.121])	Rat	NR	Gavage	880	(Moran et al., 1980)	
(2-Ethyl-3-methylpyrazine [14.006])	Rat	NR	Gavage	600	(Moran et al., 1980)	
(2-Ethyl-5-methylpyrazine [14.017])	Rat	NR	Gavage	900	(Moran et al., 1980)	
(2,3,5-Trimethylpyrazine [14.019])	Rat	NR	Gavage	806	(Moran et al., 1980)	
(2-Ethyl-3, (5 or 6)-dimethylpyrazine [14.100])	Rat	NR	Gavage	456	(Moran et al., 1980)	
(2-Ethyl-3,5-dimethylpyrazine [14.024])	Rat	M, F	Gavage	504	(Posternak et al., 1975)	
(2,3,5,6-Tetramethylpyrazine [14.018])	Rat	NR	Gavage	1910	(Oser, 1969c)	
(2-Isobutyl-3-methoxypyrazine [14.043])	Mouse	NR	Gavage	2000	(Quest International, 1983a)	
	Rat	NR	Gavage	> 4000	(Roure Inc., 1974)	
(Acetylpyrazine [14.032])	Rat	M, F	Gavage	> 3000	(Posternak et al., 1975)	
(2-(sec-Butyl)-3-methoxypyrazine [14.062])	Mouse	NR	Gavage	2000	(Quest International, 1983b)	
(Cyclohexylmethylpyrazine [14.069])	Mouse	M, F	Gavage	2673	(Babish, 1978a)	
(Mercaptomethylpyrazine [14.053])	Rat	M, F	Gavage	2100	(Burdock and Ford, 1990a)	
(Pyrazinylethanethiol [14.031])	Rat	NR	Gavage	158	(Posternak et al., 1975)	
(Pyrazinyl methyl sulfide [14.034])	Rat	M, F	Gavage	2500	(Posternak et al., 1975)	
(2-Methyl-3,5 or 6-methylthiopyrazine [14.035])	Rat	NR	Gavage	1970	(Posternak et al., 1975)	

[1] Aspartic acid-fructose extracts were examined; 2,5-diethylpyrazine was one of 29 components identified in aspartic acid-fructose extract.

Subacute / Subchronic / Chronic / Carcinogenic toxicity data are available for none of the candidate substances of the present Flavouring Group Evaluation but for 17 supporting substances evaluated by the JECFA at the 57th meeting.

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

Chemical Name [FL-no]	Species; Sex No./Group	Route	Dose levels	Duration	NOAEL (mg/kg bw/day)	Reference	Comments
(2-Ethyl-3-methylpyrazine [14.006])	Rat; M, F 32	Diet		90 days	M: 5.31[1] F: 5.22[1]	(Posternak et al., 1969)	
(2-Ethyl-5-methylpyrazine [14.017])	Rat; M, F 30	Diet		90 days	M: 17[1] F: 18[1,2]	(Oser, 1969d)	
(2,3-Diethylpyrazine [14.005])	Rat; M, F 32	Diet		90 days	1.75[1]	(Posternak et al., 1969)	
(2,3,5-Trimethylpyrazine [14.019])	Rat; M, F 30	Diet		90 days	18[1]	(Oser, 1969e)	
(2-Ethyl-3, (5 or 6)-dimethylpyrazine [14.100])	Rat; M, F 30	Diet		90 days	18[1]	(Oser, 1969f)	
(2-Ethyl-3,5-dimethylpyrazine [14.024])	Rat; M, F 32	Diet		84 days	M: 12.7[1] F: 12.3[1]	(Posternak et al., 1975)	
(2,3,5,6-Tetramethylpyrazine [14.018])	Rat; M, F 30	Diet		90 days	M: 50[1] F: 55[2]	(Oser, 1969e)	
(6,7-dihydro-5-Methyl-5H-cyclopentapyrazine [14.037])	Rat; M 10	Diet		90 days	50	(Wheldon et al., 1967)	This study can not be evaluated as a complete report could not be provided.
(5,6,7,8-Tetrahydroquinoxaline [14.015])	Rat; M, F 30	Diet		90 days	M: 18.6[1] F: 19.3[1]	(Oser, 1970d)	
(Acetylpyrazine [14.032])	Rat; M, F 32	Diet		91 days	M: 8.25[1] F: 8.15[1]	(Posternak et al., 1975)	
(Methoxypyrazine [14.054])	Rat; M, F 10-16	Diet	20, 63, 200 mg/kg	91 days	20	(Osborne et al., 1981)	
((2,5 or 6)-Methoxy-3- methylpyrazine [14.025])	Rat; M, F 32	Diet		90 days	M: 45[1] F: 53[1]	(Posternak et al., 1969)	70-80 % 2-Methoxy-3-methylpyrazine, 20-30 % (5 or 6)-Methoxy-3-methylpyrazine.
(Cyclohexylmethylpyrazine [14.069])	Rat; M, F 30	Diet		90 days	M: 0.44[1] F: 0.47[1]	(Babish, 1978b)	
(Pyrazinylethanethiol [14.031])	Rat; M, F 32	Diet		91 days	M: 16.56[1] F: 16.30[1]	(Posternak et al., 1975)	
(Pyrazinyl methyl sulphide [14.034])	Rat; M, F 32	Diet		91 days	M: 1.66[1] F: 1.63[1]	(Posternak et al., 1975)	This study was not performed in accordance with modern guidelines. No treatment related effects were observed in either haematological examination, on blood urea determinations or in histilogical examination of 25 organs or tissues. A complete clinical biochemical examination was not performed.
(2-Methyl-3,5 or 6-methylthiopyrazine [14.035])	Rat; M, F 32	Diet		91 days	4[1]	(Posternak et al., 1975)	This study was not performed in accordance with modern guidelines. No treatment related effects were observed in either haematological examination, on blood urea determinations or in histilogical examination of 25 organs or tissues. A complete clinical biochemical examination was not performed.
(5-Methylquinoxaline [14.028])	Rat; M, F 32	Diet		90 days	17.1[1]	(Posternak et al., 1969)	

[1] This study was performed at a single dose level.

[2]Growth rate and food utilisation efficiency effects were observed, but not accompanied by any evidence of pathology.

Developmental and reproductive toxicity data are available for none of the candidate substances of the present flavouring group evaluation but for four supporting substance evaluated by the JECFA at the 57th meeting. Supporting substance listed in brackets.

Table IV.3: Developmental and Reproductive Toxicity Studies

Chemical Name [FL-no]	Study type Durations	Species/Sex No / group	Route	Dose levels	NOAEL (mg/kg bw/day), Including information of possible maternal toxicity	Reference	Comments
(2,3-Dimethylpyrazine [14.050])	Developmental toxicity 2 weeks	Rat; M 5 – 7	SC	0, 10, 30, 70, 100	100[1]	(Yamada et al., 1993)	This study is considered of limited relevance, since the subcutaneous route of administration was used. [7]
(2,5-Dimethylpyrazine [14.020])	Developmental toxicity : To 1st oestrus; 1, 2 or 4 days	Rat; F 6 – 10	SC	100[2,3]		(Yamada et al., 1992)	This study is considered of limited relevance, since the subcutaneous route of administration was used. [7]
	Developmental toxicity : 2 weeks	Rat; M 5 – 7	SC	0, 10, 30, 70, 100	30[1]	(Yamada et al., 1993)	This study is considered of limited relevance, since the subcutaneous route of administration was used. [7]
	Developmental toxicity : 2 weeks	Rat; M 5	SC	0, 10, 30, 70, 100, 300	30[6] 100[5]	(Yamada et al., 1994)	This study is considered of limited relevance, since the subcutaneous route of administration was used. [7]
(2,6-Dimethylpyrazine [14.021])	Developmental toxicity : 2 weeks	Rat; M 5 - 7	SC	0, 10, 30, 70, 100	70[1]	(Yamada et al., 1993)	This study is considered of limited relevance, since the subcutaneous route of administration was used. [7]
(2,3,5,6-Tetramethylpyrazine [14.018])	Reproductive/ Developmental Toxicity[4]	Rat; F 10	Gavage	25, 125, 250	Maternal: 25 Foetal: 250	(Vollmuth et al., 1997)	

[1]Five to seven four-week old (juvenile) male Wistar rats/group were dosed subcutaneously once/day for two weeks.

[2]Subcutaneous injections of female Wistar rats beginning at the age of three weeks with 100 mg/kg bw once daily until the first oestrus.

[3]2,5-Dimethylpyrazine pretreatment of seven week old females twice per day for one, two and four days prior to oestradiol injection.

[4]Virgin rats administered 0, 25, 125, or 250 mg tetramethylpyrazine/kg body weight by gavage, seven days prior to and through cohabitation, gestation, delivery, and a four-day postparturition period.

[5]Five six-week old (mature) male Wistar rats/group were dosed subcutaneously once/day for two weeks at doses of 100 or 300 mg/kg bw.

[6] Five four-week old (juvenile) male Wistar rats/group were dosed subcutanously once/day for two weeks at doses of 10, 30, 70, 100, or 300 mg/kg bw.

[7]It was concluded that the findings were of little relevance for the risk assessment of pyrazines as flavourings.

In vitro mutagenicity/genotoxicity data are available for three candidate substances of the present flavouring group evaluation and for 11 supporting substances evaluated by the JECFA at the 57th meeting. Supporting substances are listed in brackets.

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

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.
(2,5-Dimethylpyrazine [14.020])	Ames test	*S. typhimurium*	97200 µg/plate	Negative[1]	(Aeschbacher et al.,	97200 µg/plate: highest non-bactericidal dose. Well conducted study, valid

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

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
		TA98; TA100; TA102			1989)	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	135000 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-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.
	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.
	DNA Polymerase deficiency assay	*E. coli*	NR	Negative[3]	(Rosenkranz and Leifer, 1980)	Review, of limited value (concentrations tested not reported; without metabolic activation).

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

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
	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* WP2 uvrA	Up to 5000 microgram/plate	Negative[1]	(Ogura and 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).
	Reverse mutation	*E.coli* strain WP2 uvrA	Up to 5000 microgram/plate	Negative[1]	(Ogura and 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 and 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.

In vivo mutagenicity/genotoxicity data are available for one candidate substance of the present flavouring group evaluation from chemical group 24 and for two supporting substances evaluated by the JECFA at the 57th meeting. The supporting substance is listed in brackets.

Table IV.5: 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		87, 174, 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 and Kawaguchi, 2004b)	Valid. GLP-study mainly in compliance with OECD 474 (only 5 male mice per group instead of 5 males and 5 females).

REFERENCES

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

Ajimu S and Kawaguchi J, 2004a. Report of Chromosomal Aberration Test in Cultured Mammalian Cells of 5-Methylquinoxaline. Chemicals Evaluation and Research Institute, Japan (CERI), Hota Laboratory. Unpublished report submitted to IOFI by the Japan Ministry of Health Labor and Welfare.

Ajimu S and Kawaguchi J, 2004b. Report of Micronucleus Assay of 5-Methylquinoxaline. Chemicals Evaluation and Research Institute, Japan (CERI), Hota Laboratory. Unpublished report submitted to IOFI by the Japan Ministry of Health Labor and Welfare.

Anderson D and Styles JA, 1978. An evaluation of 6 short-term tests for detecting organic chemical carcinogens. Appendix 2. The bacterial mutation test. Br. J. Cancer 37, 924-930.

Babish JG, 1978a. Acute oral toxicity in albino mice. (Cyclohexylmethyl)pyrazine. Food and Drug Research Laboratories. Lab. no. 5662b. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Babish JG, 1978b. 90-Day feeding study in Sprague Dawley rats. (Cyclohexylmethyl)pyrazine. Food and Drug Research Laboratories. Lab. no. 5664a. August 8, 1978. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Beedham C, 1985. Molybdenum hydroxylases as drug metabolising enzymes. Drug Metab. Rev. 16(1&2), 119-156.

Beedham C, 1988. Molybdenum hydroxylases. In: Gorrod JW, Oelschlager H and Caldwell J, (Eds.). Metabolism of xenobiotics. Taylor and Francis, London, pp. 51-58.

Beedham C, 2002. Molybdenum hydroxylases. Chapter 5. In: Ioannides, C. (Ed.). Enzyme systems that metabolise drugs and other xenobiotics. John Wiley & Sons, Ltd, Chichester, pp. 147-187.

Béraud M, Gaillard D and Derache R, 1975. The influence of dietary regimen on the stimulation of microsomal monooxygenases in rat liver induced by 2,3-dimethylquinoxaline. Eur. J. Toxicol. Environ. Hyg. 8(4), 212-219.

Beutin L, Preller E and Kowalski B, 1981. Mutagenicity of quindoxin, its metabolites, and two substituted quinoxaline-do-N-oxides. Antimicrob. Agents Chemother. 20, 336-343.

Burdock GA and Ford RA, 1990a. Acute oral toxicity (LD50) study in the rat with 2-mercaptomethylpyrazine. J. Am. Coll. Toxicol. Part B 1(1), 4.

Cashman JR and Williams DE, 1990. Enantioselective S-oxygenation of 2-aryl-1,3-dithiolanes by rabbit lung enzyme preparations. Mol. Pharmacol. 37, 333-339.

Cashman JR, Olsen LD and Bornheim LM, 1990. Enantioselective S-oxygenation by flavin containing cytochrome P-450 mono-oxygenases. Chem. Res. Toxicol. 3, 344-349.

Cashman J R, Yang ZC, Yang L and Wrighton SA, 1995a. Stereo- and regioselective N- and S-oxygenation of tertiary amines and sulfides in adult human liver microsomes. ISSX Proceedings (ISSN 1061-3439). 8, 34.

Cashman JR, Park SB, Yang ZC, Washington CB, Gomez DY, Giacomini K and Brett CM, 1995b. Chemical, enzymatic and human enantioselective S-oxygenation of cimetidine. ISSX Proceedings (ISSN 1061-3439). 8, 133.

CoE, 1992. Flavouring substances and natural sources of flavourings. 4th Ed. vol. I. Chemically defined flavouring substances. Council of Europe, partial agreement in the social and public health field. Strasbourg.

Cramer GM, Ford RA and Hall RL, 1978. Estimation of toxic hazard - a decision tree approach. Food Cosmet. Toxicol. 16(3), 255-276.

D`Souza J, Caldwell J and Smith RL, 1980. Species variation in N-methylation and quaterization of [14C] pyridine. Xenobiotica 10(2), 151-157.

Damani LA and Crooks PA, 1982. Oxidative metabolism of heterocyclic ring systems. In: Jakoby WB, Bend JR and Caldwe J (Ed.). Metabolic Basis of Detoxication. 2nd Ed. Academic Press, New York, pp. 69-89.

Damani LA, Bryan JB, Cowan DA and Gorrod JW, 1980. The origin of l-(3-pyridyl-N-oxide)ethanol as a metabolite of 3-acetylpyridine. Xenobiotica 10(7/8), 645-653.

Damani LA, 1987. Metabolism of sulphur-containing drugs. In: Benford, D.J., Bridges, J.W., Gibson, G.G. (Eds.). Drug metabolism - from molecules to man. Taylor & Francis, London, New York, Philadelphia, pp. 581-603.

Dutton GJ and Illing HPA, 1972. Mechanism of biosynthesis of thio-beta-D-glucuronides. Biochem. J., 129, 539-550.

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

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

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

EC, 2002b. Commission Regulation No 622/2002 of 11 April 2002 establishing deadlines for the submission of information for the evaluation of chemically defined flavouring substances used in or on foodstuffs. Official Journal of the European Communities 12.4.2002, L 95, 10-11.

EC, 2009a. Commission Decision 2009/163/EC of 26 February 2009 amending Decision 1999/217/EC as regards the Register of flavouring substances used in or on foodstuffs. Official Journal of the European Union 27.2.2009, L 55, 41.

EFFA, 2002i. Letter from EFFA to Dr. Joern Gry, Danish Veterinary and Food Administration. Dated 31 October 2002. Re.: Second group of questions. FLAVIS/8.26.

EFFA, 2003q. Submission 2003-8. Flavouring Group Evaluation of 18 Flavouring Substances (Candidate Chemicals) of the Chemical Group 24 (Annex I of 1565/2000/EC) Structurally Related to Pyrazine Derivatives [FAO/WHO JECFA 48/57] used as flavouring substances. 9 October 2003. Unpublished report submitted by EFFA to FLAVIS Secretariat. FLAVIS/8.25.

EFFA, 2003r. Submission 2003-8. Flavouring Group Evaluation of 18 Flavouring Substances (Candidate Chemicals) of the Chemical Group 24 (Annex I of 1565/2000/EC) Structurally Related to to Pyrazine Derivatives [FAO/WHO JECFA 48/57] used as Flavouring Substances. 9 October 2003. FLAVIS/8.25. European inquiry on volume of use. IOFI, International Organization of the Flavor Industry, 1995. Private communication to FEMA. Unpublished report submitted by EFFA to FLAVIS Secretariat.

EFFA, 2004e. Intake - Collection and collation of usage data for flavouring substances. Letter from Dan Dils, EFFA to Torben Hallas-Møller, EFSA. May 31, 2004.

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

EFFA, 2007e. Addendum of 1 flavouring substance (candidate substance) to the flavouring group evaluation of the chemical group 24 (Annex I of 1565/2000/EC) structurally related to pyrazine derivatives used as flavouring substances. addendum to FGE.17 (EFFA submission 2003-8). 21 December 2006. Unpublished report submitted by EFFA to FLAVIS Secretariat. FLAVIS/8.85.

EFFA, 2007f. Addendum of 1 flavouring substance (candidate chemical) to the flavouring group evaluation of the chemical group 24 (Annex I of 1565/2000/EC) structurally related to pyrazine derivatives from chemical group 24 used as flavouring substances Addendum to FGE.17 (EFFA submission 2003-8). 21 December 2006. Unpublished report submitted by EFFA to FLAVIS Secretariat. FLAVIS/8.86.

EFFA, 2007m. EFFA Letters to EFSA for clarification of specifications and isomerism for which data were requested in published FGEs. FLAVIS/8.58rev5

EFFA, 2011a. EFFA Letters to EFSA for clarification of specifications and isomerism for which data were requested in published FGEs.

EFFA, 2011e. Specifications and poundage data for 42 Register substances submitted by EFFA/Industry to FLAVIS Secretariat. August 2011. FLAVIS/8.124

EFFA, 2011j. E-mail from EFFA to FLAVIS Secretariat, Danish Food Institute, Technical University of Denmark . Dated 5 October 2011. FGE.17Rev3: [FL-no: 14.111] specifications. FLAVIS/8..

EFSA, 2004a. Minutes of the 7th Plenary meeting of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food, Held in Brussels on 12-13 July 2004. Brussels, 28 September 2004. [Online]. Available: http://www.efsa.europa.eu/cs/BlobServer/Event_Meeting/afc_minutes_07_en1.pdf?ssbinary=true

Elfarra AA, Duescher RJ, Sausen PJ, Lawton MP and Philpot RM, 1995. Potential role of the flavin-containing monooxygenases in the metabolism of endogenous compounds. ISSX Proceedings (ISSN 1061-3439) 8, 9.

Epler JL, Larimer FW, Rao TK, Nix CE and Ho T, 1978. Energy-related pollutants in the environment: Use of short-term tests for mutagenicity in the isolation and identification of biohazards. Environ. Health Perspect. 27, 11-20.

Eurostat, 1998. Total population. Cited in Eurostat, 2004. The EU population, Total population. [Online]. Available: http://epp.eurostat.ec.europa.eu/portal/page?_pageid=1090,30070682,1090_33076576&_dad=portal&_schema=PORTAL, Population and social conditions, Population, Demography, Main demographic indicators, Total population. December 2008.

Flavour Industry, 2009a. Unpublished information submitted by Flavour Industry to FLAVIS Secretariat. A-50rev1 [FL-no: 14.028].

Flavour Industry, 2010m. Unpublished information submitted by Flavour Industry to the European Food Safety Authority (EFSA) and forwarded to FLAVIS Secretariat. A-17rev3 [Fl-no:14.170].

Fung VA, Cameron TP, Hughes TJ, Kirby PE and Dunkel VC, 1988. Mutagenic activity of some coffee flavor ingredients. Mutat. Res. 204(2), 219-228.

Galloway SM, 2000. Cytotoxicity and chromosome aberrations *in vitro*: experience in industry and the case for an upper limit on toxicity in the aberration assay. Environ. Mol. Mutag. 35, 191-201.

Hashimoto T, Negishi T, Namba T, Hayakawa S and Hayatsu H, 1979. Mutagenicity of quinoline derivatives and analogs: quinoxaline 1,4-dioxide is a potent mutagen. Chem. Pharm. Bull. 27(8), 1954-1956.

Hawksworth G and Scheline RR, 1975. Metabolism in the rat of some pyrazine derivatives having flavour importance in foods. Xenobiotica 5(7), 389-399.

Hogben CA, Tocco DJ, Brodie BB and Schanker LS, 1959. On the mechanism of intestinal absorption of drugs. J. Pharm. Exp. Ther. 125, 275-282.

Hoodi AA and Damani LA, 1984. Cytochrome P-450 and non-P-450 sulphoxidations. Indian J. Pharm. Pharmacol. 36, 62P.

IOFI, 1995. European inquiry on volume of use. IOFI, International Organization of the Flavor Industry, 1995.

JECFA, 1995. Evaluation of certain food additives and contaminants. Forty-fourth Meeting of the Joint FAO/WHO Expert Committee on Food Additives. 14-23 February 1995. WHO Technical Report Series, no. 859. Geneva.

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

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

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

JECFA, 2001c. Compendium of food additive specifications. Addendum 9. Joint FAO/WHO Expert Committee of Food Additives 57th session. Rome, 5-14 June 2001. FAO Food and Nutrition paper 52 Add. 9.

JECFA, 2002a. Safety evaluation of certain food additives and contaminants. Fifty-seventh meeting of the Joint FAO/WHO Expert Committee on Food Additives, WHO Food Additives Series: 48. IPCS, WHO, Geneva.

JECFA, 2002b. Evaluation of certain food additives and contaminants. Fifty-seventh report of the Joint FAO/WHO Expert Committee on Food Additives. WHO Technical Report Series, no. 909. Geneva, 5-14 June 2001.

Knize MG, Övervik E, Midtvedt T, Turteltaub KW, Happe JA, Gustafsson JA and Felton JS, 1989. The metabolism of 4,8-DiMeIQx in conventional and germ-free rats. Carcinogenesis 10, 1479-1484.

Koyama S, 2004. Primer effects by conspecific odors in house mice: a new perspective in the study of primer effects on productive activities. Hormon and Behaviour, 46, 303-310.

Lee H, Bian SS and Chen YL, 1994a. Genotoxicity of 1,3-dithiane and 1,4-dithiane in the CHO/SCE assay and the Salmonella/microsomal test. Mutat. Res. 321, 213-218.

Liang CC, Hong CY, Chen CF and Tsai TH, 1999. Measurement and pharmacokinetic study of tetramethylpyrazine in rat blood and its regional brain tissue by high-performance liquid chromatography. J. Chromatogr. B. 724(2), 303-309.

Maiorino RM, Bruce DC and Aposhian HV, 1989. Determination and metabolism of dithiol chelating agents VI. Isolation and identification of the mixed disulfides of meso-2,3-dimercaptosuccinic acid with L-cysteine in human urine. Toxicol. Appl. Pharmacol. 97, 338-349.

McBain JB and Menn JJ, 1969. S-methylation, oxidation, hydroxylation, and conjugation of thiophenol in the rat. Biochem. Pharmacol. 18(9), 2282-2285.

McMahon RE, Cline JC and Thompson CZ, 1979. Assay of 855 test chemicals in ten tester strains using a new modification of the ames test for bacterial mutagens. Cancer Res. 39, 682-693.

Moran EJ, Easterday DD and Oser BL, 1980. Acute oral toxicity of selected flavor chemicals. Drug Chem. Toxicol. 3(3), 249-258.

National Cancer Institute, 1998. L5178Y +/- mouse lymphoma mutagenesis assay. Quinoxaline. MA BioService. San, R.H.C. Study No. G95AN40.703. Date 28/2/1998. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Nickson RM and Mitchell SC, 1994. Fate of dipropyl sulphide and dipropyl sulphoxide in rat. Xenobiotica 24(2), 157-168.

Nickson RM, Mitchell SC and Zhang AQ, 1995. Fate of dipropyl sulfone in rat. Xenobiotica 25(12), 1391-1398.

Nnane P and Damani LA, 1995. The involvement of rat liver CYP2B1 and CYP2D1 in the microsomal sulphoxidation of 4-chlorophenyl methyl sulphide. ISSX Proceedings 8, 110.

Novotny M, Jemiolo B, Harvey S, Wiesler D and Marchlewski-Koj A, 1986. Adrenal mediated endogenous metabolites inhibit puberty in female mice. Science. 231, 722-725.

Ogura S and Wakamatsu S, 2004. Report of Bacterial Mutation (Ames) Assay of 5-Methylquinoxaline. Chemicals Evaluation and Research Institute, Japan (CERI), Hota Laboratory. Unpublished report submitted to IOFI by the Japan Ministry of Health Labor and Welfare

Osborne BE, Plawiuk M, Graham C, Bier C, Losos G, Broxup B and Procter BG, 1981. A 91-day multiple dose level dietary toxicity study of methoxypyrazine and dibenzyl ether in the albino rat. Bio-Research Laboratories Ltd. Project no. 9205. April 29, 1981. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Oser BL, 1969c. The acute oral toxicity to rats of nine pyrazine derivatives. Food and Drugs Research Laboratories, Inc. Lab. No. 90480-90488. May 23, 1969. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Oser BL, 1969d. 90-day feeding study with 2-ethyl, 5-methyl pyrazine in rats. Food and Drugs Research Laboratories, Inc. Lab. No. 90612. December 15, 1969. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Oser BL, 1969e. 90-day feeding study with 2,3,5-trimethyl pyrazine in rats. Food and Drugs Research Laboratories, Inc. Lab. No. 90611. December 15, 1969. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Oser BL, 1969f. 90-day feeding study with 2-ethyl,3,5(6)-dimethyl pyrazine in rats. Food and Drugs Research Laboratories, Inc. Lab. No. 90613. December 15, 1969. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Oser BL, 1969e. 90-day feeding study with 2,3,5,6-tetramethyl pyrazine in rats. Food and Drugs Research Laboratories, Inc. Lab. No. 90614. December 15, 1969. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Oser BL, 1969c. The acute oral toxicity to rats of nine pyrazine derivatives. Food and Drugs Research Laboratories, Inc. Lab. No. 90480-90488. May 23, 1969. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Oser BL, 1970d. 90-day feeding study with 5,6,7,8-tetrahydroquinoxaline (cyclohexapyrazine) in rats. Food and Drugs Research Laboratories, Inc. Lab. No. 90618. January 22, 1970. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Parkinson, A., 1996a. Biotransformation of xenobiotics. In: Klaassen, C.D. (Ed.). Casarret and Doull's Toxicology: The Basic Science of Poisons. 5th Ed. McGraw-Hill, New York, pp.113-186.

Posternak NM, Linder A and Vodoz CA, 1969. Summaries of toxicological data. Toxicological tests on flavouring matters. Food Cosmet. Toxicol. 7, 405-407.

Posternak JM, Dufour JJ, Rogg C and Vodoz CA, 1975. Summaries of toxicological data. Toxicological tests on flavouring matters. II. Pyrazines and other compounds. Food Cosmet. Toxicol. 13, 487-490.

Quest International, 1983a. Acute oral range-finding toxicity test of 2-sec-butyl-3-methoxy pyrazine in mice. Wilson, A.S. Project no. 43000. Date 25.4.1983.

Quest International, 1983b. Acute oral range-finding toxicity test of isobutyl methoxy pyrazine in mice. Wilson, A.S. Project no. 43000. Date 25.4.1983.

Rettie AE, Bogucki BD, Lim I and Meier GP, 1990. Stereoselective sulfoxidation of a series of alkyl p-tolyl sulfides by microsomal and purified flavincontaining monooxygenases. Mol. Pharmacol. 37, 643-651.

Richardson KA, Edward VT, Jones BC and Hutson DH, 1991. Metabolism in the rat of a model xenobiotic plant metabolite S-benzyl-N-malonyl-L-cysteine. Xenobiotica 21(3), 371-382.

Rosenkranz HS and Leifer Z, 1980. Determining the DNA-modifying activity of chemicals using DNApolymerase-deficient *Escherichia coli*. In: Serres, F.J., Hollaender, A. (Eds.). Chemical Mutagens. Principles and Methods for Their Detection. Vol. 6. Plenum Press, New York and London, pp. 109-147.

Roure Inc., 1974. Acute toxicity test of fragrance materials in mice and rats. Pellmont, B. Marts 11, 1974.

Sadeque AJM, Eddy AC, Meierand GP and Rettie AE, 1992. Stereoselective sulfoxidation by human flavin-containing monooxygenase. Drug Metab. Disposition 20(6), 832-839.

Sadeque AJM, Philpot RM and Rettie AE, 1995. Chiral sulfoxidation by human liver FMO3 and FMO5. ISSX Proceedings 8, 387.

San RHC, 1995. Salmonella mutagenicity assay (ames test). Test article code #08614. Quinoxaline. Microbiological Associates, Inc. MA study no. G95AN40.501017. Date 7/13/95. Unpublished data submitted by EFFA to FLAVIS Secretariat.

SCF, 1995. Scientific Committee for Food. First annual report on chemically defined flavouring substances. May 1995, 2nd draft prepared by the SCF Working Group on Flavouring Substances (Submitted by the SCF Secretariat, 17 May 1995). CS/FLAV/FL/140-Rev2. Annex 6 to Document III/5611/95, European Commission, Directorate-General III, Industry.

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

Schranker LS, Shore AP, Brodie BB and Hogben CAM, 1957. Absorption of drugs from the stomach. I. The rat. J. Pharmacol. Exp. Ther. 120, 528-539.

Sjödin P, Wallin H, Alexander J and Jägerstad M, 1989. Disposition and metabolism of the food mutagen 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline (MeIQx) in rats. Carcinogenesis 10, 1269-1275.

Stich HF, Stich W, Rosin MP and Powrie WD, 1980. Mutagenic activity of Pyrazine derivatives: A comparative study with *Salmonella Typhimurium, Saccharomyces Cerevisiae* and Chinese hamster ovary cells. Food Cosmet. Toxicol. 18, 581-584.

Stubley C, Subryan L, Stell JG, Perrett RH, Ingle PB and Mathieson DW, 1977. The metabolism of N-heterocycles. J. Pharm. Pharmacol. 29, 77P.

Stubley C, Stell JGP and Matheison DW, 1979. Oxidation of azaheterocycles with mammalian liver alcohol oxidase. Xenobiotica 9, 475-484.

Sullivan HR, Miller WM and McMahon RE, 1976. Reaction pathways of *in vivo* stereoselective conversion of ethylbenzene to (-)-mandelic acid. Xenobiotica. 6(1), 49-54.

TNO, 2000. Volatile Compounds in Food - VCF Database. TNO Nutrition and Food Research Institute. Boelens Aroma Chemical Information Service BACIS, Zeist, The Netherlands.

TNO, 2009. Volatile Compounds in Food - VCF Database. TNO Nutrition and Food Research Institute. Boelens Aroma Chemical Information Service BACIS, Zeist, The Netherlands.

TNO, 2011. Volatile Compounds in Food - VCF Database. TNO Nutrition and Food Research Institute. Boelens Aroma Chemical Information Service BACIS, Zeist, The Netherlands.

Topham JC, 1980. Do induced sperm-head abnormalities in mice specifically identify mammalian mutagens rather than cacinogens? Mutat. Res. 74, 379-387.

Tsai TH and Liang C, 2001. Pharmacokinetics of tetramethylpyrazine in rat blood and brain using microdialysis. Int. J. Pharm. 216(1-2), 61-66.

Turesky RJ, Aeschbacher HU, Würzner HP, Skipper PL and Tannenbaum SR, 1988. Major routes of metabolism of the food-borne carcinogen 2-amino-3,8-dimethyl-imidazo[4,5-f]quinoxaline in the rat. Carcinogenesis 9, 1043-1048.

Vollmuth TA, Bennett MB, Hoberman AM and Christian MS, 1997. An evaluation of food flavoring ingredients using an *in vivo* reproductive and developmental toxicity screening test. Argus Research Laboratories, Inc.

Wallin H, Holme JA, Becher G and Alexander J, 1989. Metabolism of the food carcinogen 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline in isolated rat liver cells. Carcinogenesis 10, 1277-1283.

Wheldon GH, Krajkeman AJ, Mawdesley-Thomas LE, Ginn HB and Street AE, 1967. The effects of ten food-flavoring additives administered to rats over a period of thirteen weeks. 1-Methyl-2,3-cyclohexadione, 3,5-dimethyl-1,2-cyclopentadione, 3,4-dimethyl-1,2-cyclopentadione. Huntingdon Research Center. Study no. 51a. June 30, 1967. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Whitehouse LW, Lodge BA, By AW and Thomas BH, 1987. Metabolic disposition of pyrazinamide in the rat: identification of a novel *in vivo* metabolite common to both rat and human. Biopharm. Drug Disposition 8, 307-318.

Wild D, King MT, Gocke E and Eckhard K, 1983. Study of artificial flavouring substances for mutagenicity in the Salmonella/microsome, BASC and micronucleus tests. Food Chem. Toxicol. 21(6), 707-719.

Williams KIH, Burstein SH and Layne DS, 1966. Metabolism of dimethyl sulfide, dimethyl sulfoxide, and dimethyl sulfone in the rabbit. Arch. Biochem. Biophys. 117(1), 84-87.

Yamada K, Takahashi H and Ohta A, 1992. Effects of 2,5-dimethylpyrazine on reproductive and accessory reproductive organs in female rats. Res. Commun. Chem. Pathol. Pharmacol. 75(1), 99-107.

Yamada K, Shimizu A and Ohta A, 1993. Effects of dimethylpyrazine isomers on reproductive and accessory reproductive organs in male rats. Biol. Pharm. Bull. 16(2), 203-206.

Yamada K, Shimizu A, Komatsu H, Sakata R and Ohta A, 1994. Effects of 2,5-dimethylpyrazine on plasma testosterone and polyamines and acid phosphatase-levels in the rat prostate. Biol. Pharm. Bull. 17(5), 730-731.

Yamamoto T, Moriwaki Y, Takahashi S, Hada T and Higishano K, 1987a. 5-hydroxypyrazinamide, a human metabolite of pyrazinamide. Biochem. Pharmacol. 36, 2415-2416.

Yamamoto T, Moriwaki Y, Takahashi S, Hada T and Higishano K, 1987b. *In vitro* conversion of pyrazinamide into 5-hydroxypyrazinamide and that of pyrazinoic acid into 5-hydroxypyrazinoic acid by xanthine oxidase from human liver. Biochem. Pharmacol. 36, 3317-3318.

Yoshihara S and Tatsumi K, 1990. Metabolism of diphenyl sulphoxide in perfused guinea pig liver. Drug Metab. Disposition 18, 876-881.

Zeiger E and Margolin BH, 2000. The proportions of mutagens among chemicals in commerce. Reg. Toxicol. Pharmacol. 32, 219-225.

Ziegler DM, 1980. Microsomal flavin-coenzyme monooxygenase: Oxygenation of nucleophilic nitrogen and sulfur compounds. In: Jakoby, W.B. (Ed.). Enzymatic Basis of Detoxification. vol. 1. Academic Press, New York, pp. 201-227.

ABBREVIATIONS

ADI	Acceptable Daily Intake
AUC	Area Under Curve
BW	Body weight
CAS	Chemical Abstract Service
CEF	Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids Chemical Abstract Service
CHO	Chinese hamster ovary (cells)´
CL	Clearance
CoE	Council of Europe
DNA	Deoxyribonucleic acid
EC	European Commission
EFSA	The European Food Safety Authority
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)
FMO	Flavin-containing Monooxygenase
DLP	Dood Laboratory Practice
ID	Identity
IOFI	International Organization of the Flavour Industry
IR	Infrared spectroscopy
JECFA	The Joint FAO/WHO Expert Committee on Food Additives
LD_{50}	Lethal Dose, 50 %; Median lethal dose
MNPCE	Micronucleated polychromatic erythrocytes
MS	Mass spectrometry
MSDI	Maximised Survey-derived Daily Intake
mTAMDI	Modified Theoretical Added Maximum Daily Intake
NAD	Nicotinamide Adenine Dinucleotide
NADP	Nicotinamide Adenine Dinucleotide Phosphate
NCE	Normochromatic erythrocyte
No	Number
NOAEL	No Observed Adverse Effect Level
NOEL	No Observed Effect Level
NTP	National Toxicology Program
OECD	Organisation for Economic Co-operation and Development

PCE	Polychromatic erythrocytes
SC	Subcutaneous
SCE	Sister Chromatid Exchange
SCF	Scientific Committee on Food
SMART	Somatic Mutation and Recombination Test
TAMDI	Theoretical Added Maximum Daily Intake
UDS	Unscheduled DNA Synthesis
WHO	World Health Organisation