EFSA CEF Panel (EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids), 2014. Scientific Opinion on Flavouring Group Evaluation 77, Revision 1 (FGE.77Rev1): Consideration of Pyridine, Pyrrole and Quinoline Derivatives evaluated by JECFA (63rd meeting)

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SCIENTIFIC OPINION

Scientific Opinion on Flavouring Group Evaluation 77, Revision 1 (FGE.77Rev1): Consideration of Pyridine, Pyrrole and Quinoline Derivatives evaluated by JECFA (63rd meeting) structurally related to Pyridine, Pyrrole, Indole and Quinoline Derivatives evaluated by EFSA in FGE.24Rev2 (2013)[1]

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

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

The Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids of the European Food Safety Authority was requested to consider evaluations of flavouring substances assessed since 2000 by the Joint FAO/WHO Expert Committee on Food Additives (the JECFA), and to decide whether further evaluation is necessary, as laid down in Commission Regulation (EC) No 1565/2000. The present consideration concerns a group of 22 pyridine, pyrrole and quinoline derivatives evaluated by the JECFA (63rd meeting). The revision of this consideration is made since additional toxicity data have become available for isoquinoline [FL-no: 14.001], pyrrole [FL-no: 14.041] and 2-acetylpyrrole [FL-no: 14.047]. The toxicity data on 2-acetylpyrrole should also cover 2-propionylpyrrole [FL-no: 14.068]. Further, additional genotoxicity data on 6-methylquinoline [FL-no: 14.042] have become available. The Panel concluded that for 6-methylquinoline [FL-no: 14.042], the new genotoxicity data did not clear the concern with respect to genotoxicity *in vitro* and accordingly the substance is not evaluated through the Procedure. For 18 substances [FL-no: 14.001, 14.004, 14.007, 14.030, 14.038, 14.039, 14.041, 14.047, 14.058, 14.059, 14.060, 14.061, 14.065, 14.066, 14.068, 14.071, 14.072 and 14.164] considered in this FGE, the Panel agrees with the JECFA conclusion, "No safety concern at estimated levels of intake as flavouring substances" based on the MSDI approach. For three substances [FL-no: 13.134, 14.045 and 14.046], additional toxicological data are still required. Besides the safety assessment of these flavouring substances, the specifications for the materials of commerce have also been evaluated, and the information is considered adequate for all the substances.

[1] On request from the European Commission, Question No EFSA-Q-2012-00699, EFSA-Q-2013-00355, EFSA-Q-2013-00556, EFSA-Q-2013-00602 and EFSA-Q-2013-00816, adopted on 29 January 2014.

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KEY WORDS

pyridine, FGE.77, pyrrole, quinoline, JECFA, 63rd meeting, FGE.24Rev2

SUMMARY

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

In the previous version of Flavouring Group Evaluation 77 (FGE.77), EFSA considered 22 flavouring substances from a group of flavouring substances consisting of pyridine, pyrrole and quinoline derivatives evaluated by the JECFA at its 63rd meeting.

This revision is made due to new 90-day studies provided for isoquinoline [FL-no: 14.001], pyrrole [FL-no: 14.041] and 2-acetylpyrrole [FL-no: 14.047]. The data on 2-acetylpyrrole should also cover 2-propionylpyrrole [FL-no: 14.068]. Further, additional genotoxicity data on 6-methylquinoline [FL-no: 14.042] have also become available.

The present consideration therefore concerns these additional data and will be considered in relation to the European Food safety Authority (EFSA) evaluation of 24 pyridine, pyrrole, indole and quinoline derivatives evaluated in the Flavouring Group Evaluation 24, Revision 2 (FGE.24Rev2).

The JECFA evaluated two substances [FL-no: 13.134 and 14.030] via the B-side of the Procedure and 20 substances via the A-side.

The Panel agrees with the way the application of the Procedure has been applied by the JECFA for four of the 22 substances. Three of these four substances, methyl nicotinate [FL-no: 14.071], indole [FL-no: 14.007] and 3-methylindole [FL-no: 14.004], were evaluated by the JECFA on the A-side of the Procedure, as they were anticipated to be metabolised to innocuous products. For these three substances, EFSA agreed no safety concern at step A3 of the Procedure, as the intake is below the threshold of the structural class. For the fourth substance, 2-pyridine methanethiol [FL-no: 14.030], for which EFSA agrees with the JECFA that it should be evaluated through the B-side of the Procedure, a NOAEL was derived from a 90-day study.

The Panel concluded, contrary to the JECFA, that 6-methylquinoline [FL-no: 14.042] (evaluated via the B-side by the JECFA) should not be evaluated through the Procedure due to concern with respect to genotoxicity *in vitro*.

Also for 1-furfurylpyrrole [FL-no: 13.134], EFSA disagree with the JECFA, as the 90-day feeding study in rats was considered a poorly reported old study, the quality of which cannot be assessed.

For the remaining 16 substances the Panel, in contrast to the JECFA, did not anticipate that they will be metabolised to innocuous products and accordingly concluded that they should be evaluated along the B-side of the Procedure. However, in FGE.77, for 10 [FL-no: 14.038, 14.039, 14.058, 14.059, 14.060, 14.061, 14.065, 14.066, 14.072 and 14.164] of these 16 JECFA-evaluated pyridine derivatives evaluated via the B-side of the Procedure by EFSA, NOAELs could be derived to provide adequate margins of safety and the Panel agrees with the JECFA conclusion "no safety concern at estimated levels of intake as flavouring substances" based on the MSDI approach.

In previous version of FGE.77 it was concluded that for pyrrole and the five pyrrole derivatives as well as for isoquinoline [FL-no: 13.134, 14.001, 14.041, 14.045, 14.046, 14.047 and 14.068], No Observed Adverse Effect Levels (NOAELs) could not be derived as such or for structurally related

substances. Accordingly, additional toxicological data were required for these seven substances in FGE.77.

Since publication of FGE.77, three 90-day studies have become available for isoquinoline [FL-no: 14.001], pyrrole [FL-no: 14.041] and 2-acetylpyrrole [FL-no: 14.047] and NOAELs to provide adequate margin of safety are derived to cover these three substances as well as the structurally related 2-propionylpyrrole [FL-no: 14.068].

So, in total, for 15 substances [FL-no: 14.001, 14.030, 14.038, 14.039, 14.041, 14.047, 14.058, 14.059, 14.060, 14.061, 14.065, 14.066, 14.068, 14.072 and 14.164], evaluated via the B-side of the Procedure by EFSA, NOAELs could be derived to provide adequate margins of safety.

In order to determine whether the conclusion for the 22 JECFA evaluated substances can be applied to the materials of commerce, it is necessary to consider the available specifications. Adequate specifications including complete purity criteria and identity tests are available for the 22 JECFA-evaluated substances.

Thus, for three substances [FL-no: 13.134, 14.045 and 14.046] the Panel has reservations as additional toxicological data are still required. For one substance, 6-methylquinoline [FL-no: 14.042], the Panel concluded that the Procedure should not be applied until adequate genotoxicity data become available. For the remaining 18 JECFA evaluated pyridine, pyrrole and quinoline derivatives [FL-no: 14.001, 14.004, 14.007, 14.030, 14.038, 14.039, 14.041, 14.047, 14.058, 14.059, 14.060, 14.061, 14.065, 14.066, 14.068, 14.071, 14.072 and 14.164] the Panel agrees with the JECFA conclusion “no safety concern at estimated levels of intake as flavouring substances” based on the MSDI approach.

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BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

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

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

EFSA concluded in FGE.77 that seven substances [FL-no: 13.134, 14.001, 14.041, 14.045, 14.046, 14.047 and 14.068] should not be evaluated through the Procedure as no adequate toxicity study was available from which a no observed adverse effect level (NOAEL) could be established, neither on the substances nor on supporting substances. Further, in line with the conclusions for 2-methylquinoline, 4-methylquinoline and 4-butylquinoline [FL-no: 14.138, 14.002 and 14.094] in FGE.24Rev1, 6-methylquinoline [FL-no: 14.042] should not be evaluated through the Procedure due to concern with respect to genotoxicity *in vitro*.

Information on isoquinoline [FL-no: 14.001], pyrrole [FL-no: 14.041], 6-methylquinoline [FL-no: 14.042] and 2-acetylpyrrole [FL-no: 14.047] has now been submitted by the European Flavour Association. The information on the latter is intended to cover the re-evaluation of this substance and 2-propionylpyrrole [FL-no: 14.068].

The Commission asks EFSA to evaluate this new information and depending on the outcome proceed to the full evaluation of the flavouring substance.

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

The European Commission requests EFSA to carry out a safety assessment on the following five substances: isoquinoline [FL-no: 14.001], pyrrole [FL-no: 14.041], 6-methylquinoline [FL-no: 14.042], 2-acetylpyrrole [FL-no: 14.047] and 2-propionylpyrrole [FL-no: 14.068], in accordance with Commission Regulation (EC) N° 1565/2000.

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

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

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

ASSESSMENT

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

The following issues are of special importance.

Intake

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

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

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

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

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

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

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

"The Committee noted that this value was based on a risk analysis of known carcinogens which involved several conservative assumptions. The use of this value was supported by additional information on developmental toxicity, neurotoxicity and immunotoxicity. In the judgement of the

Committee, flavouring substances for which insufficient data are available for them to be evaluated using earlier steps in the Procedure, but for which the intake would not exceed 1.5 µg per person per day would not be expected to present a safety concern. The Committee recommended that the Procedure for the Safety Evaluation of Flavouring Agents used at the forty-sixth meeting be amended to include the last step on the right-hand side of the original procedure ("Do the condition of use result in an intake greater than 1.5 µg per day?")" (JECFA, 1999).

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

Genotoxicity

As reflected in the Opinion of SCF (SCF, 1999), the Panel has in its evaluation focussed on a possible genotoxic potential of the flavouring substances or of structurally related substances. Generally, substances for which the Panel has concluded that there is an indication of genotoxic potential *in vitro,* will not be evaluated using the EFSA Procedure until further genotoxicity data are provided. Substances for which a genotoxic potential *in vivo* has been concluded, will not be evaluated through the Procedure.

Specifications

Regarding specifications, the evaluation by the Panel could lead to a different opinion than that of the JECFA, since the Panel requests information on e.g. isomerism.

Structural Relationship

In the consideration of the JECFA evaluated substances, the Panel will examine the structural relationship and metabolism features of the substances within the flavouring group and compare this with the corresponding FGE.

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

The JECFA has evaluated a group of 22 flavouring substances consisting of pyridine, pyrrole and quinoline derivatives (JECFA, 2006a).

These 22 substances were considered by EFSA in FGE.77, in which the Panel concluded that additional toxicity data were needed for seven substances [FL-no: 13.134, 14.001, 14.041, 14.045, 14.046, 14.047 and 14.068] as no adequate toxicity studies were available from which a No Observed Adverse Effect Level (NOAEL) could be established, neither on the substances nor on supporting substances. The Panel also concluded, contrary to the JECFA, that 6-methylquinoline [FL-no: 14.042] should not be evaluated through the Procedure due to concern with respect to genotoxicity *in vitro.*

FGE	Opinion adopted by EFSA	Link	No. of candidate substances
FGE.77	31 January 2008	http://www.efsa.europa.eu/en/efsajournal/pub/936.htm	22
FGE.77Rev1	29 January 2014		22

The present Revision of FGE.77, FGE.77Rev1, includes additional toxicity data provided for isoquinoline [FL-no: 14.001], pyrrole [FL-no: 14.041] and 2-acetylpyrrole [FL-no: 14.047]; the toxicity data on 2-acetylpyrrole should also cover 2-propionylpyrrole [FL-no: 14.068]. The main studies provided are for each substance a 90-day study. Further, additional genotoxicity data for 6-methylquinoline [FL-no: 14.042] have become available.

Since the evaluation of FGE.77 in 2008, EU production volumes have been provided for four substances, [FL-no: 14.045, 14.058, 14.059 and 14.164] for which the evaluation could not be finalised, due to lack of these data. Based on the newly submitted EU production volumes, the substances have already been evaluated in FGE.96[7] (EFSA CEF Panel, 2011), but for the sake of completion, the information has also been included here as well.

Finally, information on solubility has been provided for six substances [FL-no: 13.134, 14.007, 14.030, 14.038, 14.045 and 14.046] since the previous evaluation of FGE.77.

2. Presentation of the Substances in the JECFA Flavouring Group

2.1. Description

2.1.1. JECFA Status

The JECFA has at the 63rd meeting evaluated a group of 22 flavouring substances consisting of pyridine, pyrrole and quinoline derivatives (JECFA, 2005b; JECFA, 2006a).

2.1.2. EFSA Considerations

The Panel concluded that all the substances in the JECFA flavouring group of pyridine, pyrrole and quinoline derivatives are structurally related to the group of pyridine, pyrrole, indole and quinoline derivatives from chemical group 28 evaluated by EFSA in the Flavouring Group Evaluation 24, Revision 2 (FGE.24Rev2) (EFSA CEF Panel, 2013).

2.2. Isomers

2.2.1. Status

None of the 22 flavouring substances in the group of pyridine, pyrrole and quinoline derivatives has a chiral centre.

2.2.2. EFSA Considerations

No comments.

2.3. Specifications

2.3.1. Status

The JECFA specifications are available for all 22 substances (JECFA, 2005a) (see Table 3).

2.3.2. EFSA Considerations

The specifications are considered adequate for all 22 substances.

3. Intake Estimation

3.1. Status

For all 22 substances, evaluated through the JECFA Procedure, production volumes are available for the EU (see Table 2).

3.2. EFSA Considerations

For one substance [FL-no: 14.041], the Industry has submitted use levels for normal and maximum use (EFFA, 2012) (see Table 1). Based on these normal use levels mTAMDI values can be calculated

[7] Consideration of 88 flavouring substances considered by EFSA for which EU production volumes / anticipated production volumes have been submitted on request by DG SANCO

(see Table 2), (EFSA, 2004). The mTAMDI value for [FL-no: 14.041], is below the threshold of concern of 1800 µg/person/day from structural class I.

For the remaining 21 substances, use levels are needed to calculate the mTAMDIs.

Table 1: Normal and Maximum use levels (mg/kg) available for JECFA evaluated substances in FGE.77Rev1

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**
14.041	3	-	3	-	-	3	3	3	-	-	-	-	-	-	0	0	-	-
	3	-	3	-	-	3	3	3	-	-	-	-	-	-	0	0	-	-

Table 2: Estimated intakes based on the MSDI- and the mTAMDI approach – FGE.77Rev1

FL-no	EU Register name	MSDI – EU (µg/*capita*/day)	MSDI – USA (µg/*capita*/day)	mTAMDI (µg/person/day)	Structural class	Threshold of concern (µg/person/day)
14.004	3-Methylindole	2.4	0.07		Class I	1800
14.007	Indole	26	10		Class I	1800
14.041	Pyrrole	0.11	0.01	480	Class I	1800
14.038	2-Acetylpyridine	50	68		Class II	540
14.039	3-Acetylpyridine	23	0.8		Class II	540
14.045	2-Acetyl-1-ethylpyrrole	0.12	0.009		Class II	540
14.046	2-Acetyl-1-methylpyrrole	1.2	0.02		Class II	540
14.047	2-Acetylpyrrole	3.3	0.2		Class II	540
14.059	3-Isobutylpyridine	0.049	0.07		Class II	540
14.060	2-Pentylpyridine	0.061	0.07		Class II	540
14.061	3-Ethylpyridine	9.3	3		Class II	540
14.065	2,6-Dimethylpyridine	0.26	0.007		Class II	540
14.066	5-Ethyl-2-methylpyridine	0.12	0.04		Class II	540
14.068	2-Propionylpyrrole	0.012	2		Class II	540
14.071	Methyl nicotinate	0.49	0.2		Class II	540
14.164	2-Propylpyridine	0.61	0.9		Class II	540
14.001	Isoquinoline	0.012	0.07		Class III	90
14.042	6-Methylquinoline	0.32	0.01		Class III	90
14.058	2-Isobutylpyridine	0.0061	0.9		Class III	90
14.072	2-(3-Phenylpropyl)pyridine	1.8	0.7		Class III	90
13.134	1-Furfurylpyrrole	0.12	0.07		Class III	90
14.030	2-Pyridine methanethiol	0.0012	0.007		Class III	90

SUMMARY OF SPECIFICATION DATA

Table 3: Specification Summary of the Substances in the JECFA Flavouring Group of Pyridine, Pyrrole and Quinoline Derivatives (JECFA, 2005a)

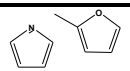
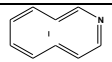
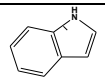
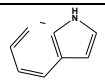
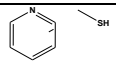
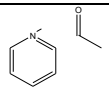
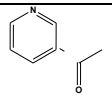
FL-no JECFA -no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility [a] Solubility in ethanol [b]	Boiling point, °C [c] Melting point, °C ID test Assay minimum	Refrac. Index [d] Spec.gravity [e]	EFSA comments
13.134 1310	1-Furfurylpyrrole		3284 2317 1438-94-4	Liquid C_9H_9ON 147.18	Insoluble Soluble	76-78 (1 hPa) NMR 98 %	1.529-1.536 1.078-1.084	
14.001 1303	Isoquinoline		2978 487 119-65-3	Solid C_9H_7N 129.16	Slightly soluble Soluble	242-243 27-29 NMR 97 %	1.621-1.627 1.097-1.103	
14.004 1304	3-Methylindole		3019 493 83-34-1	Solid C_9H_9N 131.18	Soluble Soluble	 95-97 NMR 97 %	n.a. n.a.	
14.007 1301	Indole		2593 560 120-72-9	Solid C_8H_7N 117.15	Insoluble Soluble	n.a. 51-54 NMR 97 %	n.a. n.a.	
14.030 1308	2-Pyridine methanethiol		3232 2279 2044-73-7	Liquid C_6H_7NS 125.20	Soluble Soluble	57-58 (0.8 hPa) NMR 98 %	1.573-1.580 1.150-1.157	
14.038 1309	2-Acetylpyridine		3251 2315 1122-62-9	Liquid C_7H_7ON 121.14	Insoluble Soluble	189-193 IR NMR 97 %	1.518-1.524 1.077-1.084	
14.039 1316	3-Acetylpyridine		3424 2316 350-03-8	Liquid C_7H_7ON 121.14	Soluble Soluble	230 NMR 97 %	1.530-1.540 1.103-1.112	
14.041 1314	Pyrrole		3386 2318 109-97-7	Liquid C_4H_5N 67.09	Slightly soluble Soluble	130-131 IR 98 %	1.507-1.510 0.955-0.975	

Table 3: Specification Summary of the Substances in the JECFA Flavouring Group of Pyridine, Pyrrole and Quinoline Derivatives (JECFA, 2005a)

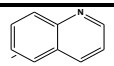
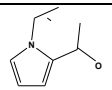
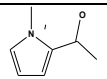
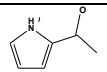
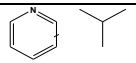
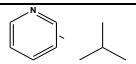
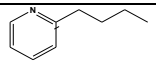
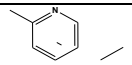
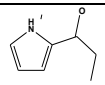
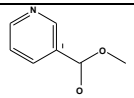
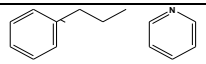
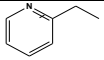
FL-no JECFA -no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility [a] Solubility in ethanol [b]	Boiling point, °C [c] Melting point, °C ID test Assay minimum	Refrac. Index [d] Spec.gravity [e]	EFSA comments
14.042 1302	6-Methylquinoline		2744 2339 91-62-3	Liquid $C_{10}H_9N$ 143.19	Slightly soluble Soluble	259 NMR 98 %	1.611-1.617 1.060-1.066	
14.045 1305	2-Acetyl-1-ethylpyrrole		3147 11371 39741-41-8	Liquid $C_8H_{11}ON$ 137.18	Slightly soluble Soluble	209-211 NMR 98 %	1.550-1.556 1.052-1.058	
14.046 1306	2-Acetyl-1-methylpyrrole		3184 11373 932-16-1	Liquid C_7H_9ON 123.16	Slightly soluble Soluble	200-202 NMR 98 %	1.539-1.545 1.037-1.043	
14.047 1307	2-Acetylpyrrole		3202 11721 1072-83-9	Solid C_6H_7ON 109.13	Soluble Soluble	n.a. 87-93 NMR 97 %	n.a. n.a.	
14.058 1311	2-Isobutylpyridine		3370 11395 6304-24-1	Liquid $C_9H_{13}N$ 135.21	Insoluble Soluble	181 NMR 97 %	1.480-1.486 0.894-0.900	
14.059 1312	3-Isobutylpyridine		3371 11396 14159-61-6	Liquid $C_9H_{13}N$ 135.21	Insoluble Soluble	68-68.5 (10hPa) NMR 97 %	1.488-1.494 0.898-0.904	
14.060 1313	2-Pentylpyridine		3383 11412 2294-76-0	Liquid $C_{10}H_{15}N$ 149.24	Insoluble Soluble	102-107 NMR 97 %	1.485-1.491 0.895-0.901	
14.061 1315	3-Ethylpyridine		3394 11386 536-78-7	Liquid C_7H_9N 107.16	Slightly soluble Soluble	166 NMR 98 %	1.499-1.505 0.951-0.957	
14.065 1317	2,6-Dimethylpyridine		3540 11381 108-48-5	Liquid C_7H_9N 107.16	Soluble Soluble	143-145 MS	1.495-1.501 0.917-0.923	

Table 3: Specification Summary of the Substances in the JECFA Flavouring Group of Pyridine, Pyrrole and Quinoline Derivatives (JECFA, 2005a)

FL-no JECFA -no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility [a] Solubility in ethanol [b]	Boiling point, °C [c] Melting point, °C ID test Assay minimum	Refrac. Index [d] Spec.gravity [e]	EFSA comments
						99 %		
14.066 1318	5-Ethyl-2-methylpyridine		3546 11385 104-90-5	Liquid $C_8H_{11}N$ 121.18	Slightly soluble Soluble	172-175 NMR 97 %	1.495-1.502 0.917-0.923	
14.068 1319	2-Propionylpyrrole		3614 11942 1073-26-3	Solid C_7H_9ON 123.16	Slightly soluble Soluble	n.a. 43-45 IR NMR 99 %	n.a. n.a.	
14.071 1320	Methyl nicotinate		3709 93-60-7	Solid $C_7H_7O_2N$ 137.14	Slightly soluble Soluble	n.a. 38-43 IR NMR MS 98 %	n.a. n.a.	
14.072 1321	2-(3-Phenylpropyl)pyridine		3751 2110-18-1	Liquid $C_{14}H_{15}N$ 197.28	Insoluble Soluble	142-143 (1 hPa) IR NMR 97 %	1.558-1.563 1.012-1.018	
14.164 1322	2-Propylpyridine		 622-39-9	Liquid $C_8H_{11}N$ 121.20	Slightly soluble Soluble	169-171 NMR 98 %	1.490-1.496 0.907-0.917	

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

4. Genotoxicity Data

4.1. Genotoxicity Studies – Text Taken[8] from the JECFA (JECFA, 2006a)

In vitro

There was no evidence of mutagenicity in the assay for reverse mutation in bacteria when various strains of *Salmonella typhimurium* (TA97, TA98, TA100, TA102, TA104, TA1535, TA1537, TA1538 and TM677) were incubated with indole [FL-no: 14.007] at a concentration of up to 30 µmol/plate (3515 µg/plate) (Anderson and Styles, 1978; Kaden et al., 1979; Florin et al., 1980; Ochiai et al., 1986; Vance et al., 1986; Sasagawa and Matsushima, 1991; Fujita et al., 1994), isoquinoline [FL-no: 14.001] at a concentration of up to 20,000 µg/ml (Sugimura et al., 1976; Nagao et al., 1977; Epler et al., 1979; Kaden et al., 1979; Sideropoulos and Specht, 1984), skatole [FL-no: 14.004] (3-methylindole) at a concentration of up to 3 µmol/plate (394 µg/plate) (Florin et al., 1980; Ochiai et al., 1986; Kim et al., 1989; Sasagawa and Matsushima, 1991), pyrrole [FL-no: 14.041] at a concentration of up to 1.4 mmol/plate (93,926 µg/plate) (Florin et al., 1980; Aeschbacher et al., 1989; Lee et al., 1994) and 3-ethylpyridine [FL-no: 14.061] at a concentration of up to 3 µmol/plate (321 µg/plate) (Florin et al., 1980) with and without metabolic activation. Methyl 2-pyrrolyl ketone [FL-no: 14.047] (2-acetylpyrrole) at concentrations of 4 to 100 µmol/plate induced a > 2-fold increase in the number of revertants/plate compared with the control when tested in *S. typhimurium* TA98 in the absence of metabolic activation (Lee et al., 1994). However, negative results were obtained with metabolic activation as well as in *S. typhimurium* TA100 (both with and without metabolic activation). Furthermore, no mutagenic activity was reported in either strain when incubated with methyl 2-pyrrolyl ketone at a concentration of up to 200 µg/plate with and without metabolic activation (Wang et al., 1994). 6-Methylquinoline [FL-no: 14.042] at a concentration of 3.3 to 3600 µg/plate gave uniformly positive results in the presence of metabolic activation (Sugimura et al., 1976; Nagao et al., 1977; Dong et al., 1978; Wild et al., 1983; Takahashi et al., 1988; Debnath et al., 1992; Zeiger et al., 1992). Methylquinolines, tested at a concentration of 400 µg/plate, showed a potent bactericidal or bacteriostatic effect, with only 6 % survival of *S. typhimurium* TA100 treated with 6-methylquinoline (Dong et al., 1978).

There was no evidence of mutagenicity when *Escherichia coli* (strains WP2 *uvr4A*/pKM101, SD-4-73, or B/r HCR+) were incubated with indole [FL-no: 14.007] at a concentration of up to 0.4 µmol/plate (47 µg/plate) (Sasagawa and Matsushima, 1991), isoquinoline [FL-no: 14.001] at a concentration of up to 50 µg/ml, skatole [FL-no: 14.004] (3-methylindole) at a concentration of up to 0.4 µmol/plate (52 µg/plate) (Szybalski, 1958; Sasagawa and Matsushima, 1991), or 3-acetylpyridine [FL-no: 14.039] at a concentration of up to 10,000 mg/plate (Pai et al., 1978).

In non-standardised assays, 2-acetylpyridine [FL-no: 14.038] at 0.50 to 0.87 % (54000 to 93960 µg/ml) and 3-acetylpyridine [FL-no: 14.039] at 0.5 to 1.11 % (55100 to 122322 µg/ml) caused a dose-dependent increase in mitotic aneuploidy in strain D61.M of *Saccharomyces ceverisiae* (Zimmermann et al., 1986). At the higher test concentrations, the growth of D61.M was strongly or completely inhibited. The authors noted that it is generally recognised that there is a threshold dose for induction of aneuploidy in yeast (Zimmermann et al., 1985a; Zimmermann et al., 1985b; Zimmermann et al., 1985c).

Assays in mammalian cell lines have been performed for isoquinoline [FL-no: 14.001] (Williams, 1984), skatole [FL-no: 14.004] (3-methylindole) (Kim et al., 1989) and pyrrole [FL-no: 14.041] (Williams, 1984). There was no evidence of increased unscheduled DNA synthesis when freshly isolated rat liver cells were incubated with pyrrole or isoquinoline (concentrations not specified) (Williams, 1984). Single-strand DNA breaks and inhibition of growth were reported when undeuterated or deuterated (at C2 or C3 positions) 3-methylindole (skatole) at 10 µmol/l to 1 mmol/l (1.31 to 131.18 µg/ml) was incubated with isolated cultured bovine kidney cells. However, there was

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

no evidence of DNA interstrand crosslinks (Kim et al., 1989). These observations are consistent with reports that, at high concentrations, indoles deplete glutathione, leading to increased formation of DNA adducts (Nichols et al., 2000; Regal et al., 2001).

In vivo

There was no evidence for mutation in a standard assay for sex-linked recessive lethal mutation when adult *Drosophila melanogaster* were fed 6-methylquinoline [FL-no: 14.042] at a concentration of 10 mmol/l (1432 µg/ml) in a 5 % sucrose solution for 3 days (Wild et al., 1983). Furthermore, 6-methylquinoline did not induce micronucleus formation in bone marrow cells obtained from male and female NMRI mice 30 hours after treatment with the test compound as a single intraperitoneal dose at 0, 286, 429, or 572 mg/kg bw (Wild et al., 1983).

Conclusion on genotoxicity

Overall, negative results were reported in assays for reverse mutation in bacteria for six representative pyridine, pyrrole and quinoline derivatives (i.e. indole [FL-no: 14.007], isoquinoline [FL-no: 14.001], skatole [FL-no: 14.004] (3-methylindole), methyl 2-pyrrolyl ketone [FL-no: 14.047], pyrrole [FL-no: 14.041] (2-acetylpyrrole) and 3-ethylpyridine [FL-no: 14.061]). Although 6-methylquinoline gave positive results with metabolic activation, it gave negative results in studies *in vivo*, indicating that there are adequate detoxication mechanisms for the rapid absorption, distribution, biotransformation and elimination of the *N*-containing heteroaromatic derivatives. 2-Acetylpyridine and 3-acetylpyridine produced positive results in yeast, but this is unlikely to occur at low doses because yeast is generally believed to have a threshold for the induction of aneuploidy. The positive results reported in bacteria for skatole (3-methylindole) are consistent with observations that, at high concentrations, indoles deplete glutathione, leading to reduced detoxification.

On the basis of the available evidence, the 22 pyridine, pyrrole and quinoline derivatives in this group do not demonstrate genotoxic potential.

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

4.2. Genotoxicity Studies – Text Taken[9] from EFSA FGE.24Rev2 (EFSA CEF Panel, 2013)

In vitro / in vivo

Genotoxicity data were provided for seven of the 24 candidate substances. In *in vitro* studies on the candidate substances 2-methylindole [FL-no: 14.131], 2-methylpyridine [FL-no: 14.134], 3-methylpyridine [FL-no: 14.135], 4-methylpyridine [FL-no: 14.136], 2,4-dimethylpyridine [FL-no: 14.104], 3,5-dimethylpyridine [FL-no: 14.106] and 4-acetylpyridine [FL-no: 14.089] in doses up to 10000 µg/plate, with and without metabolic activation, did not cause reverse mutations in various strains of *S. typhimurium* (Table 5 in present FGE.77Rev1).

Studies on induction of aneuploidy in *S. cerevisiae* D61.M available for the three candidate substances 2-methylpyridine [FL-no: 14.134], 2,4-dimethylpyridine [FL-no: 14.104] and 4-acetylpyridine [FL-no: 14.089] gave positive results. The positive results were obtained at high doses inhibiting the growth of the yeast. Furthermore, fungal systems for measuring aneuploidy have little relevance compared to the mammalian system.

No *in vivo* studies on genotoxicity of the candidate substances were available.

Genotoxicity tests are available for the eight supporting substances [FL-no: 14.004, 14.007, 14.038, 14.039, 14.041, 14.047, 14.061 and 14.065]. 2-Acetylpyrrole [FL-no: 14.047] (methyl 2-pyrrolyl

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

ketone) was positive in TA98 without metabolic activation at the two highest concentrations tested. Negative results were obtained at the lowest concentration as well as with metabolic activation. This study is considered of limited relevance. Pyrrole [FL-no: 14.041], indole [FL-no: 14.007], 3-methylindole [FL-no: 14.004] (skatole), 3-ethylpyridine [FL-no: 14.061] and 2-acetylpyridine [FL-no: 14.038] were negative in bacterial mutation assays.

Studies on induction of aneuploidy in *S. cerevisiae* D61.M are available on three supporting substances, 2,6-dimethylpyridine [FL-no: 14.065], 2-acetylpyridine [FL-no: 14.038] and 3-acetylpyridine [FL-no: 14.039], which gave positive results. However, as for the three candidate substances, the positive results were obtained at high doses inhibiting the growth of the yeast. Furthermore, fungal systems for measuring aneuploidy have little relevance compared to the mammalian system.

In vivo data are available for one supporting substance.

3-Methylindole (skatole) [FL-no: 14.004] was reported negative in the micronucleus assay in mice. The validity of this study, however, cannot be evaluated, as only an abstract is available.

Positive results were obtained for some candidate and supporting substances in the Rec, DNA breaking, CHO and DNA synthesis assays. These results are, however, not considered valid.

Conclusion on genotoxicity

The genotoxicity data available for the candidate substances do not preclude their evaluation through the Procedure.

For a summary of *in vitro / in vivo* genotoxicity data considered by EFSA, see Table 5 and Table 6.

4.3. New Genotoxicity Studies on 6-Methylquinoline [FL-no: 14.042]

6-Methylquinoline [FL-no: 14.042] was found to induce chromosome aberrations and sister chromatid exchanges (SCE) in Chinese hamster ovary (CHO) cells (NTP, 1986).

A micronucleus assay was performed by Nakajima (2005) essentially in line with the OECD Guideline 474. No significant increase of micronucleated polychromatic erythrocyte (PCE) frequency was observed in any groups of BDF1 male mice, treated by gavage at 225, 450 and 900 mg/kg body weight (bw) for two subsequent days, 24 hours apart. No significant decrease in the percentage of polychromatic erythrocytes to the analysed total erythrocytes (% PCE) was observed in any treatment group (Nakajima, 2005). The lack of cytotoxicity in the bone marrow cells does not allow a conclusion as whether the test substance or a reactive metabolite (e.g., an electrophilic epoxide) reached the bone marrow. Therefore, the results of this study have to be considered of limited relevance.

A bone marrow micronucleus assay was performed by Honarvar (2004) on a structurally related substance, 6-isopropylquinoline, which was in compliance with GLP and OECD Guideline 474. No significant increase of micronucleated PCE frequency was observed in any group of NMRI mice orally treated with 6-methylquinoline at 500, 1000 and 2000 mg/kg bw at 24 hours after treatment and for the highest dose, 2000 mg/ kg bw also 48 hours after treatment (Honarvar, 2004). Slight cytotoxic effects in the bone marrow (less than 10 % changes in PCE/NCE ratio) were observed, only at the high dose. Also at the high dose group 48 hours after treatment the percentage of micronucleated cells (0.118) was higher than the corresponding vehicle control (0.065). The value was within the historical control range (up to 0.15 %). Also in this case, due to the limited cytotoxicity, it is not clear whether the test substance/metabolite reached the target (bone marrow) in sufficient concentrations to elicit genotoxic effects.

For a summary of *in vitro / in vivo* genotoxicity data on 6-methylquinoline, see Table 7 and Table 8.

4.4. EFSA Considerations

The Panel concluded that one of the 22 substances evaluated by the JECFA, 6-methylquinoline [FL-no: 14.042], showed a genotoxic potential *in vitro*, with consistently positive results in several bacterial mutagenicity tests after metabolic activation. 6-Methylquinoline was reported negative in a test for gene mutations in *Drosophila* and in a micronucleus test in mice; however, the latter study did not meet current guidelines (PCE/NCE ratio not reported). The new genotoxicity studies submitted on 6-methylquinoline showed negative results in the micronucleus assay by Nakajima (2005), which was considered of limited relevance due to the lack of cytotoxicity in the bone marrow, which would have been indicative for target exposure. Similarly, the results of the micronucleus assay by Honarvar (2004) on the structurally related substance 6-isopropylquinoline, were considered of limited relevance due to the lack of evidence of target tissue exposure. The negative results of these two studies are not sufficient to overrule the concern on the genotoxic potential of 6-methylquinoline, which was observed *in vitro,* induction of gene mutations in bacterial cells, chromosome aberrations and sister chromatid exchanges (SCE) in cultured mammalian cells after metabolic activation (S9). Therefore, in line with the requirements in the EFSA guidance document (EFSA Scientific Committee, 2012) further *in vivo* testing is recommended with a more appropriate and sensitive assay, i.e. a Comet assay with liver as target organ to alleviate the concern for genotoxicity of 6-methylquinoline. Accordingly, the Panel concluded that 6-methylquinoline [FL-no: 14.042] could not be evaluated through the Procedure. For the remaining 21 JECFA evaluated pyridine, pyrrole and quinoline derivatives, the data available do not preclude evaluation through the Procedure.

5. New Toxicity Data

Additional toxicity data have since the publication of FGE.77 been provided for isoquinoline [FL-no: 14.001], pyrrole [FL-no: 14.041] and 2-acetylpyrrole [FL-no: 14.047], the latter also to cover the evaluation of the structurally related 2-propionylpyrrole [FL-no: 14.068]. The main studies provided are for each substance a 90-day study.

5.1. Isoquinoline [FL-no: 14.001]

A 90-day oral study in rats was performed according to the Japanese "Guidelines for designation of food additives and revision of standards for use of food additives, Notification No 29" of the Environmental Health Bureau, Ministry of Health and Welfare, Japan, March 22, 1996. The requirements of this guideline are very similar to the OECD Guideline 408. It is a GLP study. Groups (10/sex/dose) of male and female Sprague-Dawley rats were administered 0 (vehicle control), 0.03, 0.3 and 3 mg/kg bw/day of isoquinoline dissolved in corn oil by gavage daily for 90 and 91 days for males and females, respectively (Kojima, 2006). The purity of the test article was 98.5 %. Animals were weighed at the start of the study and weekly thereafter. Food consumption and efficiency were measured weekly. They rats were caged individually during the experiment. All animals were subject to ophthalmologic examination prior to the start of the study and on day 79, five animals of each sex per group were examined again. Urine was analysed on day 82 for five animals from each group. The rats were fasted for 18 - 21 hours prior to blood sampling immediately prior to necropsy. A full haematological and biochemical analysis of blood was performed. At termination of the study, animals were sacrificed and subject to full necropsy. Histopathological examination was performed on all organs (as in the OECD Guideline 408) for the control and high dose group.

No animals died through the course of the study. No clinical signs of toxicity or behavioural changes were observed. Ophthalmological examination revealed no treatment related changes. Mean body weights were comparable throughout the study between control and test groups of both sexes. Urine analysis did not reveal any treatment-related alterations when compared to controls. Haematology and blood chemistry results showed no significant differences between the test groups and controls. There were no organ weight changes or other macroscopic findings attributable to the administration of the test substance.

Histopathological examination did not show differences between controls and treated animals of either sex; some incidental findings occurred in both controls and treated animals, but there was no significant difference in their occurrence or intensity in the various organs when compared to the control groups.

Since there were no statistically significant changes due to the administration of the test material, the NOAEL of isoquinoline was determined to be 3 mg/kg bw/day in male and female rats after 90 days of administration by oral gavage (Kojima, 2006).

5.2. Pyrrole [FL-no: 14.041]

In a gavage study (Marumo, 2008), groups (10/dose/sex) of male and female Sprague-Dawley rats were administered 0 (vehicle control), 0.03, 0.30 and 3.00 mg/kg bw of aqueous pyrrole daily, by gavage for 90 days prior to necropsy. This study was performed according to "Guidelines for designation of food additives and for revision of standards for use of food additives", Notification No 29 of the Environmental Health Bureau, Ministry of Health and Welfare, Japan, March 22, 1996 which is comparable to an OECD Guideline 408 study. It is a GLP study. Clinical observations were recorded daily and body weights and food consumption were recorded weekly. On Day 79, five animals from each group were subject to ophthalmology examination. Urine samples were collected on day 82 for routine clinical chemical analysis. At termination, blood samples were taken for clinical chemistry determinations and haematological examination. At necropsy, organ weights for all organs required for an OECD Guideline 408 study were recorded. Tissues from all organs required in an OECD Guideline 408 study from both sexes of the control and 3.00 mg/kg bw/day groups were fixed and preserved for histopathological examination.

No mortality was observed throughout the course of the study and the general condition of the rats was unremarkable. Mean body weight gains and food consumption were comparable across test and control groups. Ophthalmologic examination revealed that in some animals in all male groups, controls included, and some females of the 0.03 and 3.00 mg/kg bw/day groups corneal clouding was observed.

Urine analysis revealed no toxicologically significant findings except that one male rat out of five in the 3.00 mg/kg bw/day group showed some changes, suggesting a possible kidney effect at that level; however, there were no indications of kidney pathology in the histopathological findings of this rat. In the females there were no effects observed in urinalysis except that they showed significantly higher concentrations of sodium, potassium and chloride ions, but this was not dose dependent. Males and females in the 3.00 mg/kg bw/day groups showed an increase in "urobilinogen concentrations" in blood, but this was not accompanied by associated histopathology in the liver, spleen, bone marrow or haemolysis; the effect can be attributed to the interference of pyrrole present in urine in the colorimetric assay; it gives the same reaction as urobilinogen in the detection method used.

In female rats the white blood cell count was lower for all three exposure levels than the control group, but this showed no dose-relationship; the values were 4600 ± 1500, 3600 ± 900, 3400 ± 800 and 3400 ± 1100 per µL, respectively. At the two higher dose levels, this was statistically significant ($P < 0.05$). None of the other haematological parameters was changed as compared to controls. In male rats there were no difference in white blood cell levels or any other haematological parameter. Small changes in blood biochemical findings in male rats at the highest exposure were considered incidental.

Gross pathology examination revealed some organ weight variations including decreased absolute and relative pituitary gland weights only in low dose treated male rats. All groups of male rats showed a somewhat decreased relative seminal vesicle weight due to a combination of increased body weight in the treated rats in combination with a slight decrease in absolute seminal vesicle weight. However, histopathology did not reveal abnormalities, neither in pituitary gland nor in seminal vesicles.

Histopathological examination was performed on all high dose and control animals, along with any tissues with lesions at other doses. In the lungs, alveolar accumulation of foamy cells was observed in

eight males and three females at the 3.00 mg/kg bw dose and in four male controls. Mineralisation of the pulmonary arterial wall was reported for five males and two females of the high-dose group and two male controls. Focal thickening of alveolar septum with neutrophilic infiltration was seen in two high dose male rats. Basophilic tubules were noted in the kidney cortex of eight males and five females of the high dose group and five females of the control group. Atrophy of the seminiferous tubule was observed in two male in the high dosed group but the changes were very slight. In female in the high dosed group single animals showed follicle cysts or retention of the corpus luteum with a marked decrease of eosinophils in the endometrium and myometrium or marked mucification of the vaginal mucosa. Most of these phenomena were observed in both the treated and the control groups and they are therefore considered incidental findings.

The lower white blood cell count in the females is considered an incidental finding and not considered an adverse effect since the count of all other blood cells types were normal in the female treated groups, in the males no lower blood count for any cell types was observed, the histopathological examination revealed no correlating changes in the haematopoietic tissue and there was no dose-effect relationship (raising the question whether the control value was incidentally too high; the company, unfortunately, did not give an indication of historical control values in their report). The Panel decided, based on the findings, that the NOAEL level was the highest exposure level: 3.0 mg/kg bw/day.

5.3. 2-Acetylpyrrole [FL-no: 14.047]

5.3.1. A 14-Day Range Finding Study

In a 14-day range-finding dietary study (Bauter, 2012a), groups (3/sex/dietary intake level) of male and female Sprague Dawley rats were fed a diet designed to provide 0 (dietary control), 1000, 9000 and 18000 mg/kg feed of 2-acetylpyrrole [FL-no: 14.047] daily. These estimated dietary levels correspond to the measured intake of 0, 85, 550 and 842 mg/kg bw/day for males and 0, 91, 582 and 949 mg/kg bw/day for females, respectively. Clinical observations were recorded daily and body weights were recorded on days 0, 7, 11 and 12. Individual food consumption was recorded on days 7 and 12. Due to increasing mortality in the high intake groups of both sexes, the study was terminated early at day 12. The results showed that the two higher doses were too toxic for a 90 day study. A 90 day study was started at lower exposure levels.

5.3.2. Effect on Urinary Iron and Copper Excretion

The company also studied the effect of 2-acetylpyrrole [FL-no: 14.047] on urinary excretion of iron because 2-acetylpyrrole is a strong complexing agent of metal ions. At a very high dose gavage study in rats (375 mg/kg bw orally for 10 days), the urinary excretion of total iron was increased 6-fold (Mendes, 2012); no data are provided on absorption of iron from the intestinal tract, which might be influenced by complexation of iron with 2-acetylpyrrole.

5.3.3. An 90-Day Study

In an OECD (408) compliant 90-day study, groups of rats (10/sex/dietary intake level) of male and female Sprague-Dawley CD rats were fed a diet designed to provide 0 (dietary control), 1050, 2100 and 4200 mg 2-acetylpyrrole [FL-no: 14.047]/kg feed daily (Bauter, 2012b). These dietary levels correspond to the calculated average daily intakes of 0, 68, 133 and 263 mg/kg bw for males and 0, 79, 155 and 298 mg/kg bw for females, respectively.

The test material was not stable in the diet, and in the report (Bauter, 2012b) it is suggested that part of it was probably not detected by the extraction method employed due to complexation with metal ions in the feed. It is calculated that over the course of the study the animals received concentrations of 35 - 40 % of the target intake level on average. Therefore, values for exposure levels based on the measured intake are proportionally lower. Based on this analysis of the test diets, the mean daily intakes were calculated to be 367, 754 and 1705 mg/kg feed. Assuming that the toxicity is only related

to the free 2-acetylpyrrole, these dietary concentrations correspond to average daily intakes of 24, 48 and 107 mg/kg bw for males and 28, 56 and 121 mg/kg bw for females, respectively, over 90 days.

Clinical observations of toxicity were performed on day 0 and weekly until sacrifice. Animals were weighed on day 0 at the start of the study and weekly thereafter. Food consumption and efficiency were measured and calculated weekly. Blood chemistry and haematology were performed on blood drawn via sublingual bleed at day 43 for the controls and high intake groups and at day 86 for all groups after overnight fast. Urine was collected during the 15 hours prior to the blood draw. Prior to initiation of the study and on day 91, the eyes of all rats were examined by focal illumination and indirect ophthalmoscopy. At termination of the study all survivors were sacrificed and subject to full necropsy and histopathology as required by the OECD Guideline.

There were no mortalities or ophthalmological changes associated with the presence of 2-acetylpyrrole in the diet. Most other findings, generally also noted in control animals, were not considered adverse effects of test substance administration and were regarded as incidental. Statistically significant concentration-dependent reductions in body weight, body weight gain, food consumption (males and females) and food efficiency (females) at the highest dietary level (1705 mg/kg feed measured concentration) during the study were attributed to the possible decrease in test substance palatability at high dietary levels.

Haematology parameters for both males and females were mostly unchanged during treatment. Although incidentally reaching a statistically significant difference when compared to concurrent controls, the values were in general within the range of historical controls and without associated histopathology correlate; they were therefore considered to be incidental and not related to the test material. However, statistically significantly ($p < 0.05$) decreased total white blood cell counts, erythrocyte counts, haemoglobin concentrations, haematocrit, absolute lymphocyte counts, absolute monocyte counts and absolute basophil counts and increased red cell distribution width were reported in the high intake group females on day 86. These parameters are outside of historical control levels although the variations are low in magnitude. There were no meaningful differences in coagulation parameters between test and control groups of both sexes.

Variations in clinical chemistry parameters were considered incidental and unrelated to the presence of 2-acetylpyrrole in the diet due to lack of concentration-dependence or correlated pathology.

Organ weight measurements, absolute and relative brain weight, for males were comparable to controls, with some isolated exceptions; these were without histologic correlate and were considered unrelated to test substance in the diet.

Female rats of the high intake groups displayed minimal to moderate dark bilateral thyroid glands. Microscopic changes were slight thyroid hypertrophy/hyperplasia among 4/10 and 10/10 high intake group males and females, respectively. This was characterised by enlarged subgross tall columnar appearance of the follicular epithelial cells which appeared with fine cytoplasmic vacuolation with intermittent focally piled papillary projections into the follicular lumen. The company did not provide a clear (mechanistic) explanation for this finding.

In conclusion, although some haematology and clinical parameter changes were observed in mid and high dose groups, in the mid dose were considered incidental and not of concern (not dose-related and/or very small in magnitude and/or within historic controls and without histopathology correlation). However, the thyroid effects at the exposure level are of concern, as well as the haematological changes in the high dose females. Therefore, a NOAEL for 2-acetylpyrrole is derived from the middle dose 48 mg/kg bw/day in males and 56 mg/kg bw/day in females. The NOAEL value of 48 mg/kg bw/day is used in calculating the margin of safety.

5.3.4. Metabolites of 2-Acetylpyrrole

Mendes (Mendes, 2012) analysed the urine obtained in metabolism cages from rats dosed with 2-acetylpyrrole [FL-no: 14.047] at 375 mg/kg by oral gavage as described in 5.3.2. Based on GC-MS analysis, three major components were identified in the urine of both males and females treated with 2-acetylpyrrole. Unchanged 2-acetylpyrrole and pyrrol-2,5-dione were detected; the structure of another main metabolite detected in the urine is proposed to be 1,5-dihydropyrrol-2-one, however, further experiments have yet to be performed to confirm this.

6. Application of the Procedure

6.1. Application of the Procedure to 22 Pyridine, Pyrrole and Quinoline Derivatives by the JECFA (JECFA, 2006a)

According to the JECFA, three of the substances belong to structural class I, 13 to structural class II and six to structural class III, using the decision tree approach presented by Cramer et al. (1978).

The JECFA concluded 20 pyridine, pyrrole and quinoline derivatives at step A3 in the JECFA Procedure, i.e. the substances are expected to be metabolised to innocuous products (step 2) and the intakes for all substances are below the thresholds for their structural classes I, II and III (step A3).

Two substances, 1-furfurylpyrrole [FL-no: 13.134] and 2-pyridine methanethiol [FL-no: 14.030], were evaluated via the B-side of the Procedure as the substances could not be anticipated to be metabolised to innocuous products. For these substances, the intake is below the threshold for the structural class III (step B3) and a NOAEL exists to provide an adequate margin of safety to the estimated intake as flavouring substances (step B4). For 1-furfurylpyrrole [FL-no: 13.134], a NOAEL of 12 mg/kg bw/day from a 90-day feeding study in rats (Morgareidge, 1971) is > 1,000,000 times greater than the estimated current intake of this substance as a flavouring substance. For 2-pyridine methanethiol [FL-no: 14.030], the NOAEL of 3.4 mg/kg bw/day from a 90-day feeding study in rats (Posternak et al., 1969) is > 20,000,000 times higher than the estimated current intake of this substance as a flavouring substance.

In conclusion, the JECFA evaluated all 22 substances as to be of no safety concern at the estimated levels of intake as flavouring substances based on the MSDI approach.

The evaluations of the 22 pyridine, pyrrole and quinoline derivatives are summarised in Table 9.

6.2. Application of the Procedure to 24 Pyridine, Pyrrole, Indole and Quinoline Derivatives from Chemical Group 28 evaluated by EFSA in FGE.24Rev2 (EFSA CEF Panel, 2013)

Twenty-four candidate substances were evaluated in FGE.24Rev2. Twenty-two of the 24 candidate substances are classified into structural class II and two substances into structural class III using the decision tree approach presented by Cramer et al. (1978).

Two of the substances, ethyl nicotinate [FL-no: 14.110] and isopropyl nicotinate [FL-no: 14.120], were concluded at step A3, i.e. the substances are expected to be metabolised to innocuous products (step 2) and the estimated daily intake is below the threshold for the structural class (step A3).

The remaining 22 substances were concluded at step B4, i.e. the substances could not be anticipated to be metabolised to innocuous products (step 2) and the estimated daily intake is below the threshold for the structural class (step B3). For the 22 substances, NOAELs could be derived to provide adequate margins of safety to the estimated levels of intake as flavouring substance (step B4).

For the candidate substance 2-acetyl-5-methylpyrrole [FL-no: 14.085], a NOAEL of 48 mg/kg bw/day for the supporting substance 2-acetylpyrrole [FL-no: 14.047] is derived. The estimated daily *per capita* intake of 0.0012 µg for 2-acetyl-5-methylpyrrole [FL-no: 14.085] corresponds to 0.02 ng/kg

bw/day at a body weight of 60 kg. Thus, a margin of safety of 2.4 x 10^9 can be calculated. 2-Acetyl-5-methylpyrrole is accordingly not expected to be of safety concern at the estimated level of intake.

In an oral 37 weeks feeding study in rats on indole-3-carbinole, a substance structurally related to the two indole derivatives in this FGE (FGE.24Rev2), a NOAEL of 50 mg/kg bw/day could be derived. The combined estimated daily *per capita* intake of 0.0024 µg for 1-acetylindole [FL-no: 14.088] and 2-methylindole [FL-no: 14.131] corresponds to 0.04 ng/kg bw/day at a body weight of 60 kg. Thus, a margin of safety of 1.3 x 10^9 can be calculated. 1-Acetylindole [FL-no: 14.088] and 2-methylindole [FL-no: 14.131] are accordingly not expected to be of safety concern at the estimated level of intake.

A 90 days oral feeding study in rats is available for the supporting substance 2-acetylpyridine [FL-no: 14.038]. The NOAEL derived is 37 mg/kg bw/day. The MSDI values for the 19 pyridine derivatives in this FGE (FGE.24Rev2) are between 0.012 and 0.21 µg/*capita*/day. The combined estimated daily *per capita* intake of these 19 derivatives is 1.5 µg, corresponding to 0.025 µg/kg bw/day. Thus, a margin of safety of 1.5 x 10^6 can be calculated using the NOAEL of 37 mg/kg bw/day. The 19 pyridine derivatives in this flavouring group are accordingly not expected to be of safety concern at the estimated level of intake.

In conclusion, the Panel evaluated the 24 substances as to be of no safety concern at the estimated levels of intake as flavouring substances based on the MSDI approach.

The stepwise evaluations of the 24 substances are summarised in Table 10.

6.3. EFSA Considerations

The Panel agrees with the way the application of the Procedure has been applied by the JECFA for four of the 22 substances. Methyl nicotinate [FL-no: 14.071], indole [FL-no: 14.007] and 3-methylindole [FL-no: 14.004] were evaluated via the A-side of the Procedure as they were anticipated to be metabolised to innocuous products. For these three substances, EFSA agreed no safety concern at step A3 of the Procedure, as the intake is below the threshold of the structural class (Cramer et al., 1978). 1-Furfurylpyrrole [FL-no: 13.134] and 2-pyridine methanethiol [FL-no: 14.030] were the only two substances evaluated through the B-side of the Procedure as the substances were not anticipated to be metabolised to innocuous products by the JECFA. For 1-furfurylpyrrole [FL-no: 13.134][10], EFSA disagree with the JECFA, as the 90-day feeding study in rats (Morgareidge, 1971) was considered a poorly reported old study, the quality of which cannot be assessed, as stated in FGE.24Rev1 (EFSA CEF Panel, 2013). For 2-pyridine methanethiol [FL-no: 14.030], EFSA agrees with the JECFA.

For 6-methylquinoline [FL-no: 14.042], contrary to the JECFA, the Panel concluded in FGE.77, that this substance should not be evaluated using the Procedure until adequate *in vivo* genotoxicity data become available. Additional genotoxicity data have after the publication of FGE.77 become available for 6-methylquinoline, which have been evaluated in this Revision 1 of FGE.77, however, the data are not sufficient to overrule the concern on the genotoxic potential of 6-methylquinoline. Therefore the Panel reiterated concern on the genotoxic potential of 6-methylquinoline and concluded that this substance should not be evaluated using the Procedure until adequate genotoxicity data become available.

For the remaining 16 substances the Panel, in contrast to the JECFA, did not anticipate that they will be metabolised to innocuous products due to concern with respect to *N*-oxidation of pyridines and for the pyrroles concerns about *N*-oxidation and epoxidation and accordingly concluded that they should be evaluated along the B-side. However, in FGE.77, for 10 [FL-no: 14.038, 14.039, 14.058, 14.059, 14.060, 14.061, 14.065, 14.066, 14.072 and 14.164] of these 16 substance, a NOAEL could be derived to provide adequate margins of safety to the estimated level of intakes as flavouring substance (step B4). A 90-day oral feeding study in rats is available for 2-acetylpyridine [FL-no: 14.039]. The NOAEL derived is 37 mg/kg bw/day (Til and van der Meulen, 1971). The MSDI values for the 10

[10] [FL-no: 13.134] has ben removed from FGE.24 Revision 2

pyridine derivatives in this FGE are between 0.06 and 50 µg/*capita*/day. The combined estimated daily *per capita* intake of the 10 pyridine derivatives evaluated through the B-side is 57 µg corresponding to 0.95 µg/kg bw/day. Thus, a margin of safety of approximately 39000 can be calculated using the NOAEL of 37 mg/kg bw/day. The 10 pyridine derivatives in this flavouring group evaluated through the B-side are accordingly not expected to be of safety concern at the estimated levels of intake.

For pyrrole [FL-no: 14.041] and the five pyrrole derivatives [FL-no: 13.134, 14.045, 14.046, 14.047 and 14.068] as well as for isoquinoline [FL-no: 14.001], NOAELs could not be derived as such or for structurally related substances in FGE.77. Accordingly, additional toxicological data were required for these seven substances (step B4) in FGE.77.

Additional toxicity data have after the publication of FGE.77 become available for isoquinoline [FL-no: 14.001], pyrrole [FL-no: 14.041] and 2-acetylpyrrole [FL-no: 14.047], the latter also to cover the evaluation of the structurally related 2-propionylpyrrole [FL-no: 14.068].

Based on the new data submitted (Kojima, 2006) for isoquinoline [FL-no: 14.001] a NOAEL of 3 mg/kg bw/day could be established. When comparing this NOAEL at step B4 in the Procedure to the estimated exposure based on the MSDI (0.012 µg/*capita*/day, corresponding to 0.0002 µg/kg bw/day) an adequate margin of safety of 15 x 10^6 can be calculated.

Based on the new data submitted (Marumo, 2008) for pyrrole [FL-no: 14.041] a NOAEL of 3 mg/kg bw/day could be established. When comparing this NOAEL at step B4 in the Procedure to the estimated exposure based on the MSDI (0.11 µg/*capita*/day, corresponding to 0.0018 µg/kg bw/day) an adequate margin of safety of 16 × 10^5 can be calculated.

Based on the new data submitted (Bauter, 2012b) for 2-acetylpyrrole [FL-no: 14.047] a NOAEL of 48 mg/kg bw/day could be established. When comparing the NOAEL at step B4 in the Procedure to the estimated exposure based on the MSDI (3.3 µg/*capita*/day, corresponding to 0.055 µg/kg bw/day) an adequate margin of safety of 87 × 10^4 can be calculated. For 2-propionylpyrrole [FL-no: 14.068], supported by 2-acetylpyrrole [FL-no: 14.047], the MSDI is 0.012 µg/*capita*/day, which is well below the MSDI of 2-acetylpyrrole and accordingly not expected to be of safety concern at the estimated levels of intake.

For [FL-no: 13.134, 14.045 and 14.046], data are still not available to derive NOAELs and additional toxicological data are still required for these three substances (step B4) in this revision of FGE.77 (FGE.77Rev1).

CONCLUSION

The present Revision of FGE.77, FGE.77Rev1, includes the assessment of additional toxicity data for isoquinoline [FL-no: 14.001], pyrrole [FL-no: 14.041] and 2-acetylpyrrole [FL-no: 14.047], the latter also to cover the evaluation of the structurally related 2-propionylpyrrole [FL-no: 14.068]. In addition, new submitted genotoxicity data were assessed for 6-methylquinoline [FL-no: 14.042].

The Panel concluded that the 22 substances in the JECFA flavouring group of pyridine, pyrrole and quinoline derivatives are structurally related to the group of pyridine, pyrrole, indole and quinoline derivatives from chemical group 28 evaluated by EFSA in the Flavouring Group Evaluation 24, Revision 2 (FGE.24Rev2).

The JECFA evaluated two substances [FL-no: 13.134 and 14.030] via the B-side of the Procedure and 20 substances via the A-side.

The Panel agrees with the way the application of the Procedure has been applied by the JECFA for four of the 22 substances. Three of these four substances, methyl nicotinate [FL-no: 14.071], indole

[FL-no: 14.007] and 3-methylindole [FL-no: 14.004], were evaluated by the JECFA on the A-side of the Procedure, as they were anticipated to be metabolised to innocuous products. For these three substances, EFSA agreed no safety concern at step A3 of the Procedure, as the intake is below the threshold of the structural class. For the fourth substance, 2-pyridine methanethiol [FL-no: 14.030], for which EFSA agrees with the JECFA that it should be evaluated through the B-side of the Procedure, as the substance was not anticipated to be metabolised to innocuous products. However, a NOAEL was derived from a 90-day study.

The Panel concluded, contrary to the JECFA, that 6-methylquinoline [FL-no: 14.042] (evaluated via the B-side by the JECFA) should not be evaluated through the Procedure due to concern with respect to genotoxicity *in vitro*.

Also for 1-furfurylpyrrole [FL-no: 13.134], EFSA disagree with the JECFA, as the 90-day feeding study in rats was considered a poorly reported old study, the quality of which cannot be assessed.

For the remaining 16 substances the Panel, in contrast to the JECFA, did not anticipate that they will be metabolised to innocuous products and accordingly concluded that they should be evaluated along the B-side of the Procedure. However, in FGE.77, for 10 [FL-no: 14.038, 14.039, 14.058, 14.059, 14.060, 14.061, 14.065, 14.066, 14.072 and 14.164] of these 16 JECFA-evaluated pyridine derivatives evaluated via the B-side of the Procedure by EFSA, NOAELs could be derived to provide adequate margins of safety and the Panel agrees with the JECFA conclusion "no safety concern at estimated levels of intake as flavouring substances" based on the MSDI approach.

In previous version of FGE.77 it was concluded that for pyrrole and the five pyrrole derivatives as well as for isoquinoline [FL-no: 13.134, 14.001, 14.041, 14.045, 14.046, 14.047 and 14.068], No Observed Adverse Effect Levels (NOAELs) could not be derived as such or for structurally related substances. Accordingly, additional toxicological data were required for these seven substances in FGE.77.

Since publication of FGE.77, three 90-day studies have become available for isoquinoline [FL-no: 14.001], pyrrole [FL-no: 14.041] and 2-acetylpyrrole [FL-no: 14.047] and NOAELs to provide adequate margin of safety are derived to cover these three substances as well as the structurally related 2-propionylpyrrole [FL-no: 14.068].

So, in total, for 15 substances [FL-no: 14.001, 14.030, 14.038, 14.039, 14.041, 14.047, 14.058, 14.059, 14.060, 14.061, 14.065, 14.066, 14.068, 14.072 and 14.164], evaluated via the B-side of the Procedure by EFSA, NOAELs could be derived to provide adequate margins of safety.

For one substance [FL-no: 14.041], the Industry has submitted use levels for normal and maximum use. For the remaining 21 substances, use levels are needed to calculate the mTAMDIs in order to identify those flavouring substances that need more refined exposure assessment and to finalise the evaluation.

In order to determine whether the conclusion for the 22 JECFA evaluated substances can be applied to the materials of commerce, it is necessary to consider the available specifications. Adequate specifications including complete purity criteria and identity tests are available for the 22 JECFA-evaluated substances.

Thus, for three substances [FL-no: 13.134, 14.045 and 14.046] the Panel has reservations as additional toxicological data are still required. For one substance, 6-methylquinoline [FL-no: 14.042], the Panel concluded that the Procedure should not be applied until adequate genotoxicity data become available. For the remaining 18 JECFA evaluated pyridine, pyrrole and quinoline derivatives [FL-no: 14.001, 14.004, 14.007, 14.030, 14.038, 14.039, 14.041, 14.047, 14.058, 14.059, 14.060, 14.061, 14.065, 14.066, 14.068, 14.071, 14.072 and 14.164] the Panel agrees with the JECFA conclusion "no safety concern at estimated levels of intake as flavouring substances" based on the MSDI approach.

SUMMARY OF GENOTOXICITY DATA

Table 4: Genotoxicity Data (*in vitro / in vivo*) JECFA (JECFA, 2006a)

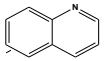
FL-no JECFA-no	EU Register name JECFA name	Structural formula	End-point	Test system	Concentration	Results	Reference
In vitro							
14.007 1301	Indole		Reverse mutation	*S. typhimurium* TA100	≤ 20 µg/plate	Negative[a]	(Ochiai et al., 1986)
			Reverse mutation	*S. typhimurium* TM677	4 mmol/l (469 µg/ml)[b]	Negative[c]	(Kaden et al., 1979)
			Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1538	4 - 2500 µg/plate	Negative[d]	(Anderson and Styles, 1978)
			Reverse mutation	*S. typhimurium* TA98, TA100	≤ 500 nmol/plate (59 µg/plate)[b]	Negative[a]	(Vance et al., 1986)
			Reverse mutation	*S. typhimurium* TA100, TA1535, TA1537	3 µmol/plate (351 µg/plate)[b]	Negative[d]	(Florin et al., 1980)
			Reverse mutation	*S. typhimurium* TA98	0.03 - 30 µmol/plate (3.5 - 3515 µg/plate)[b,e]	Negative[d]	(Florin et al., 1980)
			Reverse mutation	*S. typhimurium* TA97, TA102	10 - 1000 µg/plate	Negative[d]	(Fujita et al., 1994)
			Reverse mutation	*S. typhimurium* TA98, TA100	≤ 0.4 µmol/plate (47 µg/plate)[b]	Negative[d]	(Sasagawa and Matsushima, 1991)
			Mutation	*E. coli* WP2 uvrA/pKM101	≤ 0.4 µmol/plate (47 µg/plate)[b]	Negative[d]	(Sasagawa and Matsushima, 1991)
14.042 1302	6-Methylquinoline		Reverse mutation	*S. typhimurium* TA100	100 - 600 µg/plate	Positive[c]	(Dong et al., 1978)
			Reverse mutation	*S. typhimurium* TA98, TA100 TA1535, TA1537 and TA1538	≤ 3 600 µg/plate	Negativea , Positive[c,f]	(Wild et al., 1983)
			Reverse mutation	*S. typhimurium* TA98 and TA100	≤ 6 µmol/plate (859 µg/plate)[g]	Negative[a] Positive[c]	(Nagao et al., 1977)
			Reverse mutation	*S. typhimurium* TA98 and TA100	≤ 1000 µg/plate	Negative[a] Positive[c]	(Zeiger et al., 1992)
			Reverse mutation	*S. typhimurium* TA98 and TA100	NR	Negative[a] Positive[c]	(Sugimura et al., 1976)

Table 4: Genotoxicity Data (*in vitro / in vivo*) JECFA (JECFA, 2006a)

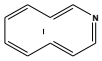
FL-no JECFA-no	EU Register name JECFA name	Structural formula	End-point	Test system	Concentration	Results	Reference
			Reverse mutation	*S. typhimurium* TA100	5 µmol/plate (716 µg/plate)[g]	Positive[c]	(Takahashi et al., 1988)
			Reverse mutation	*S. typhimurium* TA98	NR	Negative[d]	(Debnath et al., 1992)
			Reverse mutation	*S. typhimurium* TA100	3.3 - 333 µg/plate	Negative[a] Positive[c]	(Debnath et al., 1992)
14.001 1303	Isoquinoline		Reverse mutation	*S. typhimurium* TA98 and TA100	20 - 50 µg/ml	Negative[d]	(Sideropoulos and Specht, 1984)
			Reverse mutation	*S. typhimurium* TM677	≤ 8 mmol/l (1033 µg/ml)[h]	Negative[c]	(Kaden et al., 1979)
			Reverse mutation	*S. typhimurium* TA98 and TA100	NR	Negative[d]	(Sugimura et al., 1976)
			Reverse mutation	*S. typhimurium* TA98 and TA100	1 - 20 µmol/plate (129 - 2583 µg/plate)[h]	Negative[d]	(Nagao et al., 1977)
			Reverse mutation	*S. typhimurium* TA98 and TA100	10,000 - 20,000 µg/ml	Negative[d]	(Epler et al., 1979)
			Mutation	*E. coli* B/r HCR+	50 µg/ml	Negative[d]	(Sideropoulos and Specht, 1984)
			Unscheduled DNA synthesis	Rat hepatocytes	NR	Negative	(Williams, 1984)
14.004 1304	3-Methylindole		Reverse mutation	*S. typhimurium* TA100, TA1535 and TA1537	3 µmol/plate (394 µg/plate)[i]	Negative[d]	(Florin et al., 1980)
			Reverse mutation	*S. typhimurium* TA98	0.03 - 30 µmol/plate (3.9 - 3935 µg/plate)[i]	Negative[d,j]	(Florin et al., 1980)
			Reverse mutation	*S. typhimurium* TA98 and TA100	NR	Negative[c]	(Kim et al., 1989)
			Reverse mutation	*S. typhimurium* TA98 and TA100	≤ 0.4 µmol/plate (52 µg/plate)[i]	Negative[d]	(Sasagawa and Matsushima, 1991)

Table 4: Genotoxicity Data (*in vitro / in vivo*) JECFA (JECFA, 2006a)

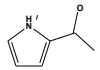
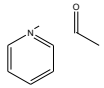
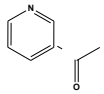
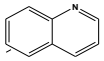
FL-no JECFA-no	EU Register name JECFA name	Structural formula	End-point	Test system	Concentration	Results	Reference
			Reverse mutation	*S. typhimurium* TA100	≤ 100 µg/plate	Negative[a]	(Ochiai et al., 1986)
			Mutation	*E. coli* WP2 *uvrA*/pKM101	≤ 0.4 µmol/plate (52 µg/plate)[i]	Negative[d]	(Sasagawa and Matsushima, 1991)
			Mutation	*E. coli* Sd-4-73	0.01 - 0.025 ml/disk	Negative	(Szybalski, 1958)
			DNA single strand break	Bovine kidney cells	10 µmol - 1 mmol/l (1.31 - 131.18 µg/ml)[i]	Positive	(Kim et al., 1989)
14.047 1307	2-Acetylpyrrole		Reverse mutation	*S. typhimurium* TA98 and TA100	12.5 - 200µg/plate	Negative[d]	(Wang et al., 1994)
			Reverse mutation	*S. typhimurium* TA98	4 - 100 µmol/plate (437 - 10,913 µg/plate)[k]	Negative[c]	(Lee et al., 1994)
			Reverse mutation	*S. typhimurium* TA100	4 - 100 µmol/plate (437 - 10,913µg/plate)[k]	Negative[d]	(Lee et al., 1994)
14.038 1309	2-Acetylpyridine		Mitotic aneuploidy	*S. cerevisiae* D61.M	0.50 - 0.87 % (54,000 - 939,600 µg/ml)[l]	Positive	(Zimmermann et al., 1986)
14.041 1314	Pyrrole		Reverse mutation	*S. typhimurium* TA98, TA100 and TA102	14 nmol/plate 1.4 mmol/plate (0.94 - 93,926 µg/plate)[m]	Negative[d]	(Aeschbacher et al., 1989)
			Reverse mutation	*S. typhimurium* TA100, TA1535 and TA1537	3 µmol/plate (201 µg/plate)[l]	Negative[d]	(Florin et al., 1980)
			Reverse mutation	*S. typhimurium* TA98	0.03 - 30 µmol/plate (2.01 - 2013 µg/plate)[m]	Negative[d]	(Florin et al., 1980)
			Reverse mutation	*S. typhimurium* TA98 and TA100	NR	Negative[d]	(Lee et al., 1994)
			Unscheduled DNA synthesis	Rat hepatocytes	NR	Negative	(Williams, 1984)
14.061 1315	3-Ethylpyridine		Reverse mutation	*S. typhimurium* TA98, TA100, TA1535 and TA1537	3 µmol/plate (321 µg/plate)[n]	Negative[d]	(Florin et al., 1980)

Table 4: Genotoxicity Data (*in vitro / in vivo*) JECFA (JECFA, 2006a)

FL-no JECFA-no	EU Register name JECFA name	Structural formula	End-point	Test system	Concentration	Results	Reference
14.039 1316	3-Acetylpyridine		Mutation	*E. coli* WP2 *uvrA*	5,000 - 10,000 µg/plate	Negative	(Pai et al., 1978)
			Mitotic aneuploidy	*S. cerevisiae* D61.M	0.5 - 1.11 % (55,100 - 122,322 µg/ml)[o]	Positive	(Zimmermann et al., 1986)
In vivo							
14.042 1302	6-Methylquinoline		Sex-linked recessive mutation	*Drosophila melanogaster*	10 mmol/l (1432 µg/ml)[g]	Negative	(Wild et al., 1983)
			Micronucleus formation	NMRI mouse	0, 286, 429, or 572 mg/kg bw	Negative	(Wild et al., 1983)

NR, not reported.
[a] Without metabolic activation.
[b] Calculated based on relative molecular mass = 117.15.
[c] With metabolic activation.
[d] With and without metabolic activation.
[e] Toxic at concentrations > 3.0 mmol/plate (351 mg/plate).
[f] TA100 and TA1535.
[g] Calculated based on relative molecular mass = 143.19.
[h] Calculated based on relative molecular mass = 129.16.
[i] Calculated based on relative molecular mass = 131.18.
[j] Toxic at concentrations of > 3.0 mmol/plate (394 mg/plate).
[k] Calculated based on relative molecular mass = 109.13.
[l] Calculated based on density = 1.08 g/ml (Sigma-Aldrich, 2003; available at http://www.sigmaaldrich.com).
[m] Calculated based on relative molecular mass = 67.09.
[n] Calculated based on relative molecular mass = 107.16.
[o] Calculated based on density = 1.102 g/ml (Sigma-Aldrich, 2003; available at http://www.sigmaaldrich.com).

Table 5: Genotoxicity Data (*in vitro*) EFSA / FGE.24Rev2 (EFSA CEF Panel, 2013)

Chemical Name [FL-no] *	Test System	Test Object	Concentration	Result	Reference	Comments
(Pyrrole [14.041])	Ames assay (modified pre-incubation method)	*S. typhimurium* TA98; TA100; TA102	1.4 mmol/plate (93926 µg/plate)	Negative[1]	(Aeschbacher et al., 1989)	
	Ames assay (pre-incubation method)	*S. typhimurium* TA100; TA1535; TA1537	3 µmol/plate (201 µg/plate)	Negative[1]	(Florin et al., 1980)	
	Ames assay (pre-incubation method)	*S. typhimurium* TA98	30 µmol/plate (2013 µg/plate)	Negative[1]		
	Ames assay (plate incorporation method)	*S. typhimurium* TA98; TA100	Not reported	Negative[3]	(Lee et al., 1994)	
	Rec assay	*B. subtilis* H17 (rec+), M45 (rec-)	4 and 20 mg/disk	Positive[3]	(Kim et al., 1987)	Poor predictive value for mutagenicity. Limited value.
	Unscheduled DNA synthesis	Rat hepatocytes	Not reported	Negative	(Williams, 1984)	
1-Methylpyrrole (former [14.007], no longer supported by Industry)	Ames assay (modified pre-incubation method)	*S. typhimurium* TA98; TA100; TA102	11 nmol – 1.1 mmol/plate	Negative[1]	(Aeschbacher et al., 1989)	6 dose levels. The study is considered valid.
	Rec assay	*B. subtilis* H17 (rec+) M45 (rec-)	2, 4, 20 and 40 mg/disk (500.5 µmol/disk)	Positive[1]	(Kim et al., 1987)	Poor predictive value for mutagenicity. Limited value.
(Indole [14.007])	Ames assay (pre-incubation method)	*S. typhimurium* TA100	20 µg/plate	Negative[2]	(Ochiai et al., 1986)	
	Ames assay	*S. typhimurium* TM677	4 mM (469 µg/ml)	Negative[3]	(Kaden et al., 1979)	
	Ames assay (plate incorporation method)	*S. typhimurium* TA98; TA100; TA1535; TA1538	2500 µg/plate	Negative[1]	(Anderson and Styles, 1978)	
	Ames assay	*S. typhimurium* TA98; TA100	500 nmol/plate (59 µg/plate)	Negative[2]	(Vance et al., 1986)	
	Ames assay (pre-incubation	*S. typhimurium* TA100; TA1535; TA1537	3 µmol/plate (351 µg/plate)	Negative[1]	(Florin et al., 1980)	

Table 5: Genotoxicity Data (*in vitro*) EFSA / FGE.24Rev2 (EFSA CEF Panel, 2013)

Chemical Name [FL-no] *	Test System	Test Object	Concentration	Result	Reference	Comments
	method)	*S. typhimurium* TA98	30 µmol/plate (3515 µg/plate)	Negative[3]		
		S. typhimurium TA97; TA102	1000 µg/plate	Negative[1]	(Fujita et al., 1994)	
		S. typhimurium TA98; TA100 *E. coli* WP2uvrA/ pKM101	0.4 µmol/plate (47 µg/plate)	Negative[1]	(Sasagawa and Matsushima, 1991)	
		S. typhimurium TA100	500 µg/plate	Negative[2]	(Hashizume et al., 1991)	
2-Methylindole [14.131]	Ames assay (pre-incubation method)	*S. typhimurium* TA98; TA100; TA1535; TA1538	4, 20, 100, 500 and 2500 µg/plate	Negative[1]	(Anderson and Styles, 1978)	The study is considered valid.
		S. typhimurium TA98; TA100; TA1535; TA1537	3 µmol/plate (394 µg/plate)	Negative[1]	(Florin et al., 1980)	Single dose study.
	Ames assay	*S. typhimurium* TA98	3 nmol - 30 µmol/plate (12 doses) (3935 µg/plate)	Negative[1]	(Curvall et al., 1982)	The study is considered valid.
		S. typhimurium TM677	2 mM (262 µg/ml)	Negative	(Kaden et al., 1979)	Single dose study.
(3-Methylindole [14.004])	Ames assay (pre-incubation method)	*S. typhimurium* TA100; TA1535; TA1537	3 µmol/plate (394 µg/plate)	Negative[1]	(Florin et al., 1980)	
		S. typhimurium TA98	30 µmol/plate (3935 µg/plate)	Negative[1]	(Florin et al., 1980)	
	Ames assay	*S. typhimurium* TA98; TA100	Not reported	Negative[3]	(Kim et al., 1989)	
	Ames assay (pre-incubation method)	*S. typhimurium* TA98; TA100 *E. coli* WP2uvrA/ pKM101	0.4 µmol/plate (52 µg/plate)	Negative[1]	(Sasagawa and Matsushima, 1991)	
		S. typhimurium TA100	100 µg/plate	Negative[2]	(Ochiai et al., 1986)	
		S. typhimurium TA100	Up to 3.33 mM (437 µg/ml)	Negative[3]	(Reddy et al., 2002)	
	Mutation assay (paper-disk method)	*E. coli* Sd-4-73	0.025 ml/disk	Negative	(Szybalski, 1958)	
	Chromosomal aberration assay	Chinese hamster ovary cells	1.3, 1.4 and 1.5 mM (+ S9) 1.4, 1.5 and 1.6 mM (- S9)	Positive[1]	(Reddy et al., 2002)	Aberrations were only detected at cytotoxic concentrations that showed marked inhibition of DNA

Table 5: Genotoxicity Data (*in vitro*) EFSA / FGE.24Rev2 (EFSA CEF Panel, 2013)

Chemical Name [FL-no] *	Test System	Test Object	Concentration	Result	Reference	Comments
						synthesis.
	Alkaline elution assay	Rat primary hepatocytes (uninduced and PB/β-NF induced)	0.5, 0.6, 0.7, 0.8, 0.9 and 1 mM	Negative	(Reddy et al., 2002)	The study is considered valid.
	DNA modification assay	Isolated human genomic DNA	25 and 500 µM (66 µg/ml)	Positive[3] Negative[2]	(Laws et al., 2001)	Assay demonstrating inhibition of PCR amplification and spots demonstrated by postlabeling. Limited predictive value.
	DNA Single strand break assay	Bovine kidney cells	10 µM - 1 mM (131.2 µg/ml)	Positive	(Kim et al., 1989)	Abstract only. Validity cannot be evaluated.
(2-Acetylpyrrole [14.047])	Ames assay (plate incorporation method)	*S. typhimurium* TA98	4, 20 and 100 µmol/plate (10913 µg/plate)	Negative[3] Positive[2]	(Lee et al., 1994)	Positive without S9 only at the two highest concentrations. High concentrations. Technically acceptable, but of limited relevance due to high concentrations.
		S. typhimurium TA100	100 µmol/plate (10913 µg/plate)	Negative[1]		
	Ames assay	*S. typhimurium* TA98; TA100	Up to 200 µg/plate	Negative[1]	(Wang et al., 1994)	
2-Methylpyridine [14.134]	Ames assay (pre-incubation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537	3 µmol/plate (279 µg/plate)	Negative[1]	(Florin et al., 1980)	Single dose study.
	Ames assay (modified pre-incubation method)	*S. typhimurium* TA98; TA100; TA102	10 nmol - 1 mmol/plate (6 doses) (93 µg/ml)	Negative[1]	(Aeschbacher et al., 1989)	The study is considered valid.
	Ames assay (plate incorporation method)	*S. typhimurium* TA97; TA98; TA100; TA102	Up to 5000 µg/plate (6 doses)	Negative[1]	(Claxton et al., 1987)	Individual dose levels not reported. The study is considered valid.
		S. typhimurium TA98; TA100; TA1535; TA1537	50, 160, 500, 1600 and 5000 nl/plate	Negative[1]	(Vleminckx et al., 1993a)	The study is considered valid.

Table 5: Genotoxicity Data (*in vitro*) EFSA / FGE.24Rev2 (EFSA CEF Panel, 2013)

Chemical Name [FL-no] *	Test System	Test Object	Concentration	Result	Reference	Comments
			(4722 µg/plate)			
	Mitotic aneuploidy assay	*S. cerevisiae* D61.M	0.5 - 0.74 % (6 doses) (6988 µg/ml)	Positive	(Zimmermann et al., 1986)	Very high doses. The effect is considered thresholded. Limited relevance.
	HGPRT Gene mutation assay	Chinese hamster V79 lung cells	4.5, 4.75, 5, 5.25 and 5.5 µl/ml (5194 µg/ml)	Negative[2]	(Vleminckx et al., 1993b)	The study is considered valid.
	Alkaline elution assay	Chinese hamster V79 lung cells	2, 3, 4, 5 and 6 µl/ml (5666 µg/ml)	Negative[2]	(Schriewer et al., 1993)	The study is considered valid.
3-Methylpyridine[14.135]	Ames assay (modified pre-incubation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537	85, 280, 840 and 8540 µg/plate	Negative	(Haworth et al., 1983)	The study is considered valid.
	Ames assay (plate incorporation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537	50, 160, 500, 1600 and 5000 nl/plate (4785 µg/plate)	Negative[1]	(Vleminckx et al., 1993a)	The study is considered valid.
	Mutagenicity assay	*E. coli* WP2 uvrA	5 - 10 mg/plate (5000 - 10,000 µg/plate)	Negative	(Pai et al., 1978)	Single dose study. Very few experimental details. The validity cannot be evaluated.
	HGPRT Gene mutation assay	Chinese hamster V79 lung cells	3, 3.25, 3.5, 3.75 and 4 µl/ml (3828 µg/ml)	Negative[2]	(Vleminckx et al., 1993b)	The study is considered valid.
	Alkaline elution assay	Chinese hamster V79 lung cells	2, 3, 4 and 5 µl/ml (4785 µg/ml)	Negative[2]	(Schriewer et al., 1993)	The study is considered valid.
4-Methylpyridine [14.136]	Ames assay (plate incorporation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537	50, 160, 500, 1600 and 5000 nl/plate (4779 µg/plate)	Negative[1]	(Vleminckx et al., 1993a)	The study is considered valid.
	HGPRT Gene mutation assay	Chinese hamster V79 lung cells	3.75, 4, 4.25 and 4.5 µl/ml (4301 µg/ml)	Negative[2]	(Vleminckx et al., 1993b)	The study is considered valid.
	Alkaline elution assay	Chinese hamster V79 lung cells	3.75, 4, 4.25 and 4.5 µl/ml (4301 µg/ml)	Negative[2]	(Schriewer et al., 1993)	The study is considered valid.
(3-Ethylpyridine [14.061])	Ames assay (pre-incubation	*S. typhimurium* TA98; TA100; TA1535; TA1537	3 µmol/plate (321µg/plate)	Negative[1]	(Florin et al., 1980)	Single dose study.

Table 5: Genotoxicity Data (*in vitro*) EFSA / FGE.24Rev2 (EFSA CEF Panel, 2013)

Chemical Name [FL-no] *	Test System	Test Object	Concentration	Result	Reference	Comments
	method)					
2,4-Dimethylpyridine [14.104]	Mitotic aneuploidy assay	*S. cerevisiae* D61.M	0.4 - 0.60 % (6 doses) (5551µg/ml)	Positive	(Zimmermann et al., 1986)	Very high doses. The effect is considered thresholded. Limited relevance.
(2,6-Dimethylpyridine [14.065])	Mitotic aneuploidy assay	*S. cerevisiae* D61.M	0.5 - 0.60 % (4 doses) (5551 µg/ml)	Positive	(Zimmermann et al., 1986)	Very high doses. The effect is considered thresholded. Limited relevance.
3,5-Dimethylpyridine [14.106]	Ames assay (pre-incubation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537	3 µmol/plate (321 µg/plate)	Negative[1]	(Florin et al., 1980)	Single dose study.
(2-Acetylpyridine [14.038])	Ames assay (plate incorporation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537; TA1538	100 - 10,000 µg/plate	Negative	(Longfellow, 1997)	Very short summary. The results cannot be validated. High doses.
	Mouse lymphoma assay	Mouse lymphocytes L5178Y tk+/–	2500 - 4500 µg/ml (-S9) 1000 - 4000 µg/ml (+S9)	Positive[1]		Very short summary. The results cannot be validated.
	Mitotic aneuploidy assay	*S. cerevisiae* D61.M	0.5 - 0.87 % (4 doses) (9396 µg/ml)	Positive	(Zimmermann et al., 1986)	Very high doses. The effect is considered thresholded. Limited relevance.
(3-Acetylpyridine [14.039])	Mutation	*E. coli* WP2uvrA	10000 µg/plate	Negative	(Pai et al., 1978)	Single dose study. Very few experimental details. The validity cannot be evaluated.
	Mitotic aneuploidy assay	*S. cerevisiae* D61.M	0.5 - 1.11 % (5 doses) (1223 µg/ml)	Positive	(Zimmermann et al., 1986)	Very high doses. The effect is considered thresholded. Limited relevance.
4-Acetylpyridine [14.089]	Ames assay (pre-incubation method)	*S. typhimurium* TA97; TA98; TA100; TA102; TA104; TA1535; TA1537; TA1538	5, 100, 300, 1000, 3000 and 10,000 µg/plate	Negative[1]	(Zeiger et al., 1992)	The study is considered valid.
	Mitotic aneuploidy	*S. cerevisiae*	0.5 - 1.19 %	Positive	(Zimmermann et al.,	Very high doses. The

Table 5: Genotoxicity Data (*in vitro*) EFSA / FGE.24Rev2 (EFSA CEF Panel, 2013)

Chemical Name [FL-no] *	Test System	Test Object	Concentration	Result	Reference	Comments
	assay	D61.M	(5 doses) (13,114 µg/ml)		1986)	effect is considered thresholded. Limited relevance.
	Mitotic aneuploidy assay	*S. cerevisiae* D61.M	Up to 11 mg/ml	Positive	(Whittaker et al., 1989)	Purity 88 %. Very high doses. The effect is considered thresholded. Limited relevance.

* Supporting substances are listed in brackets.
1 With and without metabolic activation.
2 Without metabolic activation.
3 With metabolic activation.

Table 6: Genotoxicity Data (*in vivo*) EFSA / FGE.24Rev2 (EFSA CEF Panel, 2013)

Chemical Name [FL-no]*	Test System	Test Object	Route	Dose	Result	Reference	Comments
(3-Methylindole [14.004])*	Micronucleus test	Mouse	Oral	1000 mg/kg day	Negative	(Reddy et al., 2003)	Abstract only. The validity cannot be evaluated.

* Supporting substance.

Table 7: New Genotoxicity Data (*in vitro*) on 6-Methylquinoline

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
6-Methylquinoline [14.042]	Chromosomal aberration assay	Chinese hamster ovary cells	52.7, 69.9, 174.8 and 349.5 µg/ml 50.3, 125.5, 250.9 and 374.5 µg/ml	Negative (-S9) Positive (+S9)	(NTP, 1986)	
	Sister chromatid exchanges	Chinese hamster ovary cells	16.6, 25.1, 33 and 50 µg/ml 16.7, 50.1, 166.9 and 500.7 µg/ml	Positive (-S9) Positive (+S9)	(NTP, 1986)	

Table 8: New Genotoxicity Data (*in vivo*) on 6-Methylquinoline

Chemical Name [FL-no]	Test System	Test Object	Route	Dose	Result	Reference	Comments
6-Methylquinoline [14.042]	Micronucleus test	Male mouse	Gavage	0, 225, 450 and 900 mg/kg bw	Negative	(Nakajima, 2005)	Limited relevance.
6-Isopropylquinoline	Micronucleus test	NMRI mouse	Oral	0, 500, 1000 and 2000 mg/ kg bw	Negative	(Honarvar, 2004)	Limited relevance.

SUMMARY OF SAFETY EVALUATIONS

Table 9: Summary of Safety Evaluation by the JECFA (JECFA, 2005b)

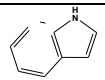
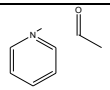
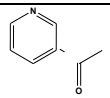
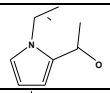
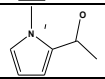
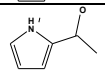
FL-no JECFA-no	EU Register name	Structural formula	EU MSDI [a] US MSDI (μg/*capita*/day)	Class [b] Evaluation procedure path [c]	Outcome on the named compound [[d] or [e]]	EFSA conclusion on the named compound (Procedure steps, intake estimates, NOAEL, genotoxicity)	EFSA conclusion on the material of commerce
14.004 1304	3-Methylindole		2.4 0.07	Class I A3: Intake below threshold	d	No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at the estimated level of intake based on the MSDI approach.
14.007 1301	Indole		26 10	Class I A3: Intake below threshold	d	No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at the estimated level of intake based on the MSDI approach.
14.041 1314	Pyrrole		0.11 0.01	Class I A3: Intake below threshold	d	No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at the estimated level of intake based on the MSDI approach.
14.038 1309	2-Acetylpyridine		50 68	Class II A3: Intake below threshold	d	No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at the estimated level of intake based on the MSDI approach.
14.039 1316	3-Acetylpyridine		23 0.8	Class II A3: Intake below threshold	d	No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at the estimated level of intake based on the MSDI approach.
14.045 1305	2-Acetyl-1-ethylpyrrole		0.12 0.009	Class II A3: Intake below threshold	d	Toxicity data required.	
14.046 1306	2-Acetyl-1-methylpyrrole		1.2 0.02	Class II A3: Intake below threshold	d	Toxicity data required.	
14.047 1307	2-Acetylpyrrole		3.3 0.2	Class II A3: Intake below threshold	d	No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at the estimated level of intake based on the MSDI approach.

Table 9: Summary of Safety Evaluation by the JECFA (JECFA, 2005b)

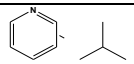
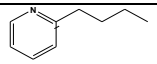
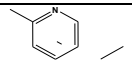
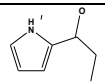
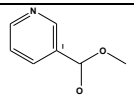
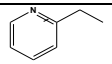
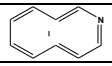
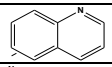
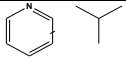
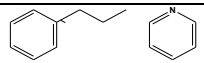
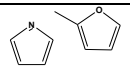
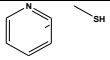
FL-no JECFA-no	EU Register name	Structural formula	EU MSDI [(a)] US MSDI (μg/*capita*/day)	Class [(b)] Evaluation procedure path [(c)]	Outcome on the named compound [[(d)] or [(e)]]	EFSA conclusion on the named compound (Procedure steps, intake estimates, NOAEL, genotoxicity)	EFSA conclusion on the material of commerce
14.059 1312	3-Isobutylpyridine		0.049 0.07	Class II A3: Intake below threshold	d	No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at the estimated level of intake based on the MSDI approach.
14.060 1313	2-Pentylpyridine		0.061 0.07	Class II A3: Intake below threshold	d	No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at the estimated level of intake based on the MSDI approach.
14.061 1315	3-Ethylpyridine		9.3 3	Class II A3: Intake below threshold	d	No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at the estimated level of intake based on the MSDI approach.
14.065 1317	2,6-Dimethylpyridine		0.26 0.007	Class II A3: Intake below threshold	d	No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at the estimated level of intake based on the MSDI approach.
14.066 1318	5-Ethyl-2-methylpyridine		0.12 0.04	Class II A3: Intake below threshold	d	No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at the estimated level of intake based on the MSDI approach.
14.068 1319	2-Propionylpyrrole		0.012 2	Class II A3: Intake below threshold	d	No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at the estimated level of intake based on the MSDI approach.
14.071 1320	Methyl nicotinate		0.49 0.2	Class II A3: Intake below threshold	d	No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at the estimated level of intake based on the MSDI approach.
14.164 1322	2-Propylpyridine		0.61 0.9	Class II A3: Intake below threshold	d	No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at the estimated level of intake based on the MSDI approach.
14.001 1303	Isoquinoline		0.012 0.07	Class III A3: Intake below threshold	d	No safety concern at the estimated level of intake	No safety concern at the estimated level of intake

Table 9: Summary of Safety Evaluation by the JECFA (JECFA, 2005b)

FL-no JECFA-no	EU Register name	Structural formula	EU MSDI [(a)] US MSDI (µg/*capita*/day)	Class [(b)] Evaluation procedure path [(c)]	Outcome on the named compound [(d) or (e)]	EFSA conclusion on the named compound (Procedure steps, intake estimates, NOAEL, genotoxicity)	EFSA conclusion on the material of commerce
						based on the MSDI approach.	based on the MSDI approach.
14.042 1302	6-Methylquinoline		0.32 0.01	Class III A3: Intake below threshold	d	Genotoxicity data required.	
14.058 1311	2-Isobutylpyridine		0.0061 0.9	Class III A3: Intake below threshold	d	No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at the estimated level of intake based on the MSDI approach.
14.072 1321	2-(3-Phenylpropyl)pyridine		1.8 0.7	Class III A3: Intake below threshold	d	No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at the estimated level of intake based on the MSDI approach.
13.134 1310	1-Furfurylpyrrole		0.12 0.07	Class III B3: Intake below threshold, B4: Adequate NOAEL exists	d	Toxicity data required.	
14.030 1308	2-Pyridine methanethiol		0.0012 0.007	Class III B3: Intake below threshold, B4: Adequate NOAEL exists	d	No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at the estimated level of intake based on the MSDI approach.

(a): EU MSDI: Amount added to food as flavour in (kg / year) x 10E9 / (0.1 x population in Europe (= 375 x 10E6) x 0.6 x 365) = µg/capita/day.
(b): Thresholds of concern: Class I = 1800 µg/person/day, Class II = 540 µg/person/day, Class III = 90 µg/person/day.
(c): Procedure path A substances can be predicted to be metabolised to innocuous products. Procedure path B substances cannot.
(d): No safety concern based on intake calculated by the MSDI approach of the named compound.
(e): Data must be available on the substance or closely related substances to perform a safety evaluation.

Table 10: Summary of Safety Evaluation by the EFSA (FGE.24Rev2) (EFSA CEF Panel, 2013)

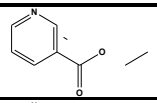
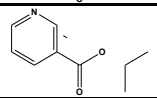
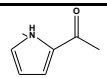
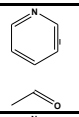
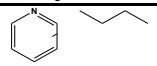
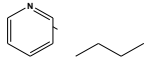
FL-no	EU Register name	Structural formula	MSDI [a] (µg/*capita*/day)	Class [b] Evaluation procedure path [c]	Outcome on the named compound [[d] or [e]]	Outcome on the material of commerce [[f], [g], or [h]]	Evaluation remarks
14.110	Ethyl nicotinate		0.013	Class II A3: Intake below threshold	d	f	
14.120	Isopropyl nicotinate		0.0012	Class II A3: Intake below threshold	d	f	
14.023	1-Methylpyrrole		0.3	Class II B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		i
14.085	2-Acetyl-5-methylpyrrole		0.0012	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	d	f	
14.089	4-Acetylpyridine		0.0073	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	d	f	
14.092	2-Butylpyridine		0.012	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	d	f	
14.093	3-Butylpyridine		0.061	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	d	f	
14.103	2,3-Dimethylpyridine		0.037	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	d	f	
14.104 2151	2,4-Dimethylpyridine		0.024	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	d	f	
14.105	3,4-Dimethylpyridine		0.13	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	d	f	

Table 10: Summary of Safety Evaluation by the EFSA (FGE.24Rev2) (EFSA CEF Panel, 2013)

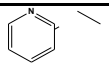
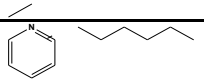
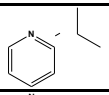
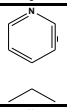
FL-no	EU Register name	Structural formula	MSDI [a] (µg/*capita*/day)	Class [b] Evaluation procedure path [c]	Outcome on the named compound [[d] or [e]]	Outcome on the material of commerce [[f], [g], or [h]]	Evaluation remarks
14.106	3,5-Dimethylpyridine		0.073	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	d	f	
14.107	2,5-Dimethylpyrrole		0.061	Class II B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		i
14.115	2-Ethylpyridine		0.027	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	d	f	
14.116	4-Ethylpyridine		0.027	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	d	f	
14.117	2-Hexylpyridine		0.012	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	d	f	
14.118	2-Hydroxypyridine		0.024	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	d	f	
14.124	2-Isopropylpyridine		0.021	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	d	f	
14.125	4-Isopropylpyridine		0.012	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	d	f	
14.134	2-Methylpyridine		0.21	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	d	f	
14.135	3-Methylpyridine		0.027	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	d	f	
14.136	4-Methylpyridine		0.73	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	d	f	

Table 10: Summary of Safety Evaluation by the EFSA (FGE.24Rev2) (EFSA CEF Panel, 2013)

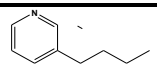
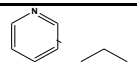
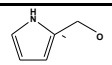
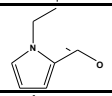
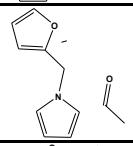
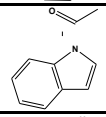
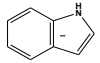
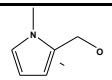
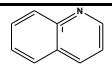
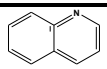
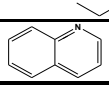
FL-no	EU Register name	Structural formula	MSDI [a] (µg/*capita*/day)	Class [b] Evaluation procedure path [c]	Outcome on the named compound [d] or [e]	Outcome on the material of commerce [f], [g], or [h]	Evaluation remarks
14.140	3-Pentylpyridine		0.0012	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	d	f	
14.143	3-Propylpyridine		0.0012	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	d	f	
14.145	Pyrrole-2-carbaldehyde		0.12	Class II B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		j
14.150	2,4,6-Trimethylpyridine		0.012	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	d	f	
14.169 2150	1-Ethyl-2-pyrrolecarboxaldehyde		0.12	Class II B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		j
13.100	2-Acetyl-1-furfurylpyrrole		0.091	Class III B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		i
14.088	1-Acetylindole		0.0012	Class III B3: Intake below threshold, B4: Adequate NOAEL exists	d	f	
14.131	2-Methylindole		0.0012	Class III B3: Intake below threshold, B4: Adequate NOAEL exists	d	f	
14.163 2152	1-Methylpyrrole-2-carboxaldehyde		0.0024	Class III B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		j
14.002	4-Methylquinoline		0.12	Class III No evaluation			i

Table 10: Summary of Safety Evaluation by the EFSA (FGE.24Rev2) (EFSA CEF Panel, 2013)

FL-no	EU Register name	Structural formula	MSDI [a] (µg/*capita*/day)	Class [b] Evaluation procedure path [c]	Outcome on the named compound [[d] or [e]]	Outcome on the material of commerce [[f], [g], or [h]]	Evaluation remarks
14.094	4-Butylquinoline		0.0012	Class III No evaluation			i
14.138	2-Methylquinoline		0.012	Class III No evaluation			i

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

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

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

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

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

(f) No safety concern at the estimated level of intake of the material of commerce meeting the specification requirement (based on intake calculated by the MSDI approach).

(g) 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.

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

(i) No longer supported by Industry (EFSA CEF Panel, 2011).

(j) No longer supported by Industry (DG SANCO, 2013).

REFERENCES

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

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

Bauter MR, 2012a. 2-Acetylpyrrole: palatability/toxicity study: a 14-day dietary study in rats. Product Safety Labs. Study no. 31097. January 9, 2012. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Bauter MR, 2012b. Draft Report. 2-Acetylpyrrole: A 90-day dietary study in rats. Product Safety Labs. Study no. 33327. Marts 2, 2012. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Claxton LD, Dearfield KL, Spanggord RJ, Riccio ES and Mortelmans K, 1987. Comparative mutagenicity of halogenated pyridines in the *Salmonella typhimurium*/mammalian microsome test. Mutation Research 176, 185-198.

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

Curvall M, Florin I and Jansson T, 1982. Mutagenicity of some indoles and related compounds in the Ames test. Toxicology 23, 1-10.

Debnath AK, Lopez de Compadre RL and Hansch C, 1992. Mutagenicity of quinolines in *Salmonella typhimurium* TA100. A QSAR study based on hydrophobicity and molecular orbital determinants. Mutation Research 280(1), 55-65.

DG SANCO (Directorate General for Health and Consumer Affairs), 2013. Information from DG SANCO 14/05 2013, concerning a list of 18 non-supported substances. FLAVIS.2.26.

Dong M, Schmeltz I, La Voie E and Hoffmann D, 1978. Aza-arenes in the respiratory environment analysis and assays for mutagenicity. In: Jones PW and Freudenthal RI (Eds.). Carcinogenesis - A comprehensive survey. Vol. 3. Raven Press, New York, pp. 97-108.

EFFA (European Flavour Association), 2012. Addendum of Additional Data Relevant to the Flavouring Group Evaluation of the Chemical Group 28 (Annex I of 1565/2000/EC) Pyridine, Pyrrole, Indole and Quinoline Derivatives [JECFA/WHO FAS 63] Used as Flavouring Substances. June 2012. FLAVIS/8.163.

EFSA CEF Panel (EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids), 2011. Scientific Opinion on Flavouring Group Evaluation 96 (FGE.96): Consideration of 88 flavouring substances considered by EFSA for which EU production volumes / anticipated production volumes have been submitted on request by DG SANCO. Addendum to FGE. 51, 52, 53, 54, 56, 58, 61, 62, 63, 64, 68, 69, 70, 71, 73, 76, 77, 79, 80, 83, 84, 85 and 87. EFSA Journal 2011;9(12):1924, 60 pp. doi:10.2903/j.efsa.2011.1924

EFSA CEF Panel (EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids), 2013. Scientific Opinion on Flavouring Group Evaluation 24, Revision 2 (FGE.24Rev2): Pyridine, pyrrole, indole and quinoline derivatives from chemical group 28. EFSA Journal 2013;11(11):3453, 64 pp. doi:10.2903/j.efsa.2013.3453

EFSA Scientific Committee, 2011. Scientific Opinion on genotoxicity testing strategies applicable to food and feed safety assessment. EFSA Journal 2011;9(9):2379, 69 pp. doi:10.2903/j.efsa.2011.2379

EFSA (European Food Safety Authority), 2004. Scientific Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in contact with food (AFC) on a request from the Commission related to Flavouring Group Evaluation 03 (FGE.03): Acetals of branched- and straight-chain aliphatic saturated primary alcohols and branched- and straight-chain saturated aldehydes, and an orthoester of formic acid, from chemical groups 1 and 2 (Commission Regulation (EC) No 1565/2000 of 18 July 2000). The EFSA Journal 2004, 107, 1-59.

EFSA (European Food Safety Authority), 2011. List of substances for which the Commission withdraw its request to EFSA for an opinion. FLAVIS/2.23Rev1.

Epler JL, Rao TK and Guerin MR, 1979. Evaluation of feasibility of mutagenic testing of shale oil products and effluents. Environmental Health Perspectives 30, 179-184.

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

Fujita H, Aoki N and Sasaki M, 1994. [Mutagenicity test of food additives with *Salmonella typhimurium* TA97 and TA102 (IX*)]. Annual Report of Tokyo Metropolitan Research Laboratory of Public Health 45, 191-199. (In Japanese)

Hashizume T, Santo H, Tsujisawa H, Kosaka K, Ozawa T, Yamashita M and Kinae N, 1991. Mutagenic activities of tryptophan metabolites before and after nitrite treatment. Food and Chemical Toxicology 29(12), 839-844.

Haworth S, Lawlor T, Mortelmans K, Speck W and Zeiger E, 1983. Salmonella mutagenicity test results for 250 chemicals. Environmental Mutagenesis 5(Suppl. 1), 3-142.

Honarvar N, 2004. Final report. Micronucleus assay in bone marrow cells of the mouse with BASE 3. RCC-Cytotest Cell Research GmbH. Study no. 809103. February 23, 2004. Unpublished report submitted by EFFA to FLAVIS Secretariat.

JECFA (Joint FAO/WHO Expert Committee on Food Additives), 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 (Joint FAO/WHO Expert Committee on Food Additives), 1996. Toxicological evaluation of certain food additives. Forty-fourth Meeting of the Joint FAO/WHO Expert Committee on Food Additives and contaminants. WHO Food Additives Series: 35. IPCS, WHO, Geneva.

JECFA (Joint FAO/WHO Expert Committee on Food Additives), 1997. 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 (Joint FAO/WHO Expert Committee on Food Additives), 1999. 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 (Joint FAO/WHO Expert Committee on Food Additives), 2005a. Compendium of food additive specifications. Addendum 12. Joint FAO/WHO Expert Committee of Food Additives 63rd session. Rome, 8-17 June 2004. FAO Food and Nutrition paper 52 Add. 12.

JECFA (Joint FAO/WHO Expert Committee on Food Additives), 2005b. Evaluation of certain food additives. Sixty-third report of the Joint FAO/WHO Expert Committee on Food Additives. WHO Technical Report Series, no. 928. Geneva, 8-17 June 2004.

JECFA (Joint FAO/WHO Expert Committee on Food Additives), 2006a. Safety evaluation of certain food additives and contaminants. Sixty-third Meeting of the Joint FAO/WHO Expert Committee on Food Additives, WHO Food Additives Series: 54. IPCS, WHO, Geneva.

JECFA (Joint FAO/WHO Expert Committee on Food Additives), 2006b. Sixty-seventh Meeting. Rome, 20-29 June 2006, Summary and Conclusions. Issued 7 July 2006.

Kaden DA, Hites RA and Thilly WG, 1979. Mutagenicity of soot and associated polycyclic aromatic hydrocarbons to *Salmonella typhimurium*. Cancer Research 39, 4152-4159.

Kim E-H, Shinohara K, Murakami H and Omura H, 1987. Pyrrole compounds as food mutagens. Journal of the Faculty of Agriculture, Kyushu University 31, 279-285.

Kim HY, Hincks JR, Huie JM, Yost GS and Coulombe Jr RA, 1989. Deuterated 3-methylindole is less genotoxic than 3-methylindole. Toxicologist 9(1), 154.

Kojima K, 2006. [Studies or tests for the standards for foods, foods additives, ect. 90-Day repeated oral dose toxicity study of isoquinoline in rats]. Hatano Research Institute, Food and Drug Safety Center, Japan. Study No. C-06-010. Unpublished report submitted by EFFA to FLAVIS Secretariat. [In Japanese - English translation included]

Laws GM, Skopek TR, Reddy MV, Storer RD and Glaab WE, 2001. Detection of DNA adducts using a quantitative long PCR technique and the fluorogenic 5' nuclease assay (TaqMan). Mutation Research 484, 3-18.

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

Longfellow D, 1997. Mutagenicity studies. Benzothiazole. Short-term test program sponsored by the Division of Cancer Etiology, National Cancer Institute.

Marumo H, 2008. 90-day repeated oral dose toxicity study of pyrrole in rats. Final report. Hatano Research Institute, Food and Drug Safety Center. Study no. C-06-009. January 25, 2008. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Mendes O, 2012. Draft Report. 2-Acetylpyrrole: A 10-day oral metabolism/toxicity study in rats. Product Safety Labs. Study no. 34496. June 11-21, 2012. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Morgareidge K, 1971. 90-Day feeding study with N-furfurylpyrrole in rats. Food and Drug Research Laboratories. February 12, 1971. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Nagao M, Yahagi T, Seino Y, Sugimura T and Ito N, 1977. Mutagenicities of quinoline and its derivatives. Mutation Research 42, 335-342.

Nakajima Y, 2005. *In vivo* micronucleus study in mice. 6-Methylquinoline. Experiment No: 8818- 079-254. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Nichols WK, Bossio JI and Yost GS, 2000. 3-Methylindole (3MI) causes both apoptosis and necrosis in cultured human lung cells. Toxicologist 54(1), 114.

NTP (National Toxicology Program), 1986. Genetic toxicology (6-Methylquinoline). Study no. 741114. http://tools.niehs.nih.gov/ntp_tox/index.cfm?fuseaction=invitro.choosestudytype&cas_no=91-62-3&endpointlist=CAB%2CSCE

Ochiai M, WakabaYashi K, Sugimura T and Nagao M, 1986. Mutagenicities of indole and 30 derivatives after nitrite treatment. Mutation Research 172, 189-197.

Pai V, Bloomfield SF, Jones J and Gorrod JW, 1978. Mutagenicity testing of nitrogenous compounds and their N-oxidised products using Trp+ reversion in *E. coli*. In: Gorrod JW (Ed.). Biological Oxidation of Nitrogen in organic compounds, Elsevier North Holland Biomedical Press, New York, pp. 375-382.

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

Reddy MV, Storer RD, Laws GM, Armstrong MJ, Barnum JE, Gara JP, McKnight CG, Skopek TR, Sina JF, DeLuca JG and Galloway SM, 2002. Genotoxicity of naturally occurring indole compounds: Correlation between covalent DNA binding and other genotoxicity tests. Environmental and Molecular Mutagenesis 40(1), 1-17.

Reddy MV, Laws GM, Armstrong MJ, Galloway SM and Storer RD, 2003. *In vivo* genotoxicity of 3-methylindole and melatonin: Formation of DNA adducts but not micronuclei. Proceedings of the American Association for Cancer Research 44, 291-292.

Regal KA, Laws GM, Yuan C, Yost GS and Skiles GL, 2001. Detection and characterization of DNA adducts of 3-methylindole. Chemical Research in Toxicology 14, 1014-1024.

Sasagawa C and Matsushima T, 1991. Mutagen formation on nitrile treatment of indole compounds derived from indole-glucosinolate. Mutation Research 250(1/2), 169-174.

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

Schriewer L, Vleminckx C, Rigaux G, Ottogali M and Lakhanisky T, 1993. Evaluation of the genotoxic potential of pyridine and methylated pyridines. Pyridine, 2-methylpyridine, 3-methylpyridine, 4-methylpyridine. C. DNA single-stranded breaks measurement in V79 cells. Reilly Industries. Institute of Hygiene and Epidemiology. Vleminckx, C. Study no. IHE-TOX-1003. EPA Doc 86-930000182, microfiche no. OTS0538163. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Sideropoulos AS and Specht SM, 1984. Evaluation of microbial testing methods for mutagenicity of quinoline and its derivatives. Current Microbiology 11, 59-65.

Sugimura T, Sato S, Nagao M, Yahagi T, Matsushima T, Seino Y, Takeuchi M and Kawachi T, 1976. Overlapping of carcinogens and mutagens. In: Magee PN, Takayama S, Sugimura T and Matsushima T (Eds.). Proceedings of the International Symposium of the Princess Takamatsu Cancer Research Fund, Tokyo, 1975. Fundamentals In Cancer Prevention. Vol. 6. University Par Press, Baltimore, pp. 191-215.

Szybalski W, 1958. Special microbiological systems. II. Observations on chemical mutagenesis in microorganisms. Annals of the New York Academy of Sciences 76, 475-489.

Takahashi K, Kamiya M, Sengoku Y, Kohda K and Kawazoe Y, 1988. Deprivation of the mutagenic property of quinoline: inhibition of mutagenic metabolism by fluorine substitution. Chemical and Pharmaceutical Bulletin 36(11), 4630-4633.

Til HP and van der Meulen HC, 1971. Subchronic (90-day) toxicity study with 2-acetylpyridine in albino rats. Centraal Instituut Voor Voedingsonderzioek, Netherlands. Report no. R3373. February, 1971. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Vance WA, Okamoto HS and Wang YY, 1986. Structure-activity relationships of nitro and methyl-nitro derivatives of indoline, indole, indazole and benzimidazole in *Salmonella typhimurium*. Mutation Research 173 (3), 169-176.

Vleminckx C, Ottogali M, Schriewer L, Rigaux G and Lakhanisky T, 1993a. Evaluation of the genotoxic potential of pyridine and methylated pyridines. Pyridine, 2-methylpyridine, 3-methylpyridine, 4-methylpyridine. A. Salmonella/microsome test. Reilly Industries with cover letter dated 03/29/93. Study no. IHE-TOX-1003. Date 3/29/93. EPA Doc. 86-930000180, microfiche no. OTS0538163. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Vleminckx C, Rigaux G, Schriewer L, Ottogali M and Lakhanisky T, 1993b. Evaluation of the genotoxic potential of pyridine and methylated pyridines. Pyridine, 2-methylpyridine, 3-methylpyridine, 4-methylpyridine. B. HGPRTgene mutation assay in V79 cells. Reilly Industries. Study no. IHE-TOX-1003. EPA Doc. 86-930000181, microfiche no. OTS0538163. Unpublished report submitted by EFFA to FLAVIS Secretariat.

Wang CJ, Lin YL and Lin JK, 1994. Mutagenicity and cytotoxicity of nitropyrrole compounds derived from the reaction of 2-acetyl pyrrole with nitrite. Food and Chemical Toxicology 32(9), 839-844.

Whittaker SG, Zimmermann FK, Dicus B, Piegorsch WW, Fogel S and Resnick MA, 1989. Detection of induced mitotic chromosome loss in *Saccharomyces cerevisiae* - an interlaboratory study. Mutation Research 224(1), 31-78.

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 and Chemical Toxicology 21(6), 707-719.

Williams GM, 1984. DNA damage and repair tests for the detection of genotoxic agents. Food Additives and Contaminants 1(2), 173-178.

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

Zimmermann FK, Mayer VW, Scheel I and Resnick MA, 1985a. Acetone, methyl ethyl ketone, ethyl acetate, acetonitrile and other polar aprotic solvents are strong inducers of aneuploidy in *Saccharomyces cerevisiae*. Mutation Research 149, 339-351.

Zimmermann FK, Groschel-Stewart U, Scheel I and Resnick MA, 1985b. Genetic change may be caused by interference with protein-protein interactions. Mutation Research 150, 203-210.

Zimmermann FK, Mayer VW, Taylor-Mayer RE, Groschel-Stewart U and Scheel I, 1985c. Induction of mitotic aneuploidy in yeast with aprotic polar solvents. In: Zimmermann PK and Taylor RE (Eds.). Mutagenicity Testing in Environmental Control. Chichester, Ellis Horwood, pp. 166-179.

Zimmermann FK, Henning JH, Schell I and Oehler M, 1986. Genetic and anti-tubulin effects induced by Pyridine derivatives. Mutation Research 163, 23-31.

ABBREVIATIONS

BW	Body Weight
CAS	Chemical Abstract Service
CEF	Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
CHO	Chinese hamster ovary (cells)
CoE	Council of Europe
DNA	Deoxyribonucleic acid
EFSA	The European Food Safety Authority
EPA	United States Environmental Protection Agency
EU	European Union
FAO	Food and Agriculture Organization of the United Nations
FEMA	Flavor and Extract Manufacturers Association
FGE	Flavouring Group Evaluation
FLAVIS (FL)	Flavour Information System (database)
GLP	Good Laboratory Practise
GC-MS	Gas chromatography-mass spectrometry
ID	Identity
I.p.	Intraperitoneal
IR	Infrared spectroscopy
JECFA	The Joint FAO/WHO Expert Committee on Food Additives
MSDI	Maximised Survey-derived Daily Intake
mTAMDI	Modified Theoretical Added Maximum Daily Intake
NCE	Normochromatic erythrocyte
No	Number
NOAEL	No observed adverse effect level
NTP	National Toxicology Program
OECD	Organisation for Economic Co-operation and Development
PCE	Polychromatic erythrocyte

PCR	Polymerase Chain Reaction
SCE	Sister chromatic exchange
SCF	Scientific Committee on Food
WHO	World Health Organisation