

## SCIENTIFIC OPINION

### **Flavouring Group Evaluation 93 (FGE.93): Consideration of sulphur containing heterocyclic compounds evaluated by JECFA (68<sup>th</sup> meeting) structurally related to thiazoles, thiophene, thiazoline and thienyl derivatives evaluated by EFSA in FGE.21Rev1 (2009)**

#### **Scientific Opinion of the Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF)**

(Question No EFSA-Q-2009-00558)

**Adopted on 23 July 2009**

#### **PANEL MEMBERS**

Arturo Anadon, David Bell, Mona-Lise Binderup, Wilfried Bursch, Laurence Castle, Riccardo Crebelli, Karl-Heinz Engel, Roland Franz, Nathalie Gontard, Thomas Haertle, Trine Husøy, Klaus-Dieter Jany, Catherine Leclercq, Jean Claude Lhuguenot, Wim Mennes, Maria Rosaria Milana, Karla Pfaff, Kjetil Svensson, Fidel Toldra, Rosemary Waring, Detlef Wölfle.

#### **SUMMARY**

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

The present consideration concerns six sulphur containing heterocyclic substances evaluated by the JECFA (68<sup>th</sup> meeting).

The Panel concluded that the six substances in the JECFA flavouring group of sulphur containing heterocyclic substances are structurally related to the 56 substances evaluated by EFSA in the Flavouring Group Evaluation 21, Revision 1 (FGE.21Rev1), (EFSA, 2009u).

The Panel agrees with the way the application of the Procedure was applied by the JECFA for two of the six substances, 3-(methylthio)-methylthiophene [FL-no: 15.126] and 1-(3-hydroxy-5-methyl-2-thienyl)ethanone [FL-no: 15.127]. However, for 1-(3-hydroxy-5-methyl-2-thienyl)ethanone [FL-no: 15.127], the Panel concludes that no valid toxicity study from which a No Observed Adverse Effect Level (NOAEL) could be established is available. Therefore additional toxicity data are needed for this flavouring substance.

For 3-(methylthio)methylthiophene [FL-no: 15.126], the Maximised Survey-derived Daily Intake (MSDI) is below the threshold for its structural class (Cramer class III, 90 µg/person/day). The Panel agrees with the JECFA that the NOAEL of 0.29 mg/kg bw/day of the supporting substance [FL-no: 15.008] is adequate for [FL-no: 15.126] and that it provides a sufficient safety margin. It can therefore be concluded that this substance is of no safety concern when used as flavouring substance at the estimated level of intake, based on the MSDI approach.

No genotoxicity information was available on any of the six substances evaluated by the JECFA. However the Panel noted that four of the six substances, 2-acetyl-2-thiazoline [FL-no: 15.010], 2-propionyl-2-thiazoline [FL-no: 15.128], 5-ethyl-4-methyl-2-(2-methylpropyl)-thiazoline [FL-no: 15.130] and 5-ethyl-4-methyl-2-(2-butyl)-thiazoline [FL-no: 15.131] have structural similarities to 2-methylthiazolidine [FL-no: 15.090] and 2-propylthiazolidine [FL-no: 15.099] in FGE.21Rev1 (EFSA, 2009u), which are considered by the Panel to have genotoxic potential *in vitro*. The Panel therefore concluded that the Procedure should not be applied to these four flavouring substances [FL-no: 15.010, 15.128, 15.130 and 15.131] pending submission and evaluation of adequate genotoxicity data.

For one substance evaluated through the procedure [FL-no: 15.126], use levels have been provided by the Industry. The mTAMDI figures calculated are below the threshold of concern for its structural class.

For the other substance evaluated through the Procedure [FL-no: 15.127], the use levels are needed in order to calculate the mTAMDI and to identify whether more refined exposure assessment is needed to finalise the evaluation.

Although four remaining substances cannot be evaluated through the procedure [FL-no: 15.010, 15.128, 15.130 and 15.131], the corresponding available use levels were considered. It was noted that for three of these compounds [FL-no: 15.010, 15.130 and 15.131] the calculated mTAMDI are above the threshold of concern for their structural classes.

The available specifications of the six substances in this FGE were examined. Information on stereoisomeric composition is missing for two substances [FL-no: 15.130 and 15.131]; data on solubility in ethanol is missing for one substance [FL-no: 15.127]; no boiling point is given for [FL-no: 15.126]. The range for boiling point and specific gravity given is too wide for [FL-no: 15.128].

Thus, the Panel concluded that the substance [FL-no: 15.126] is of no safety concern at the estimated intake. For the substance [FL-no: 15.127], the Panel concluded that additional toxicity data are needed. For four substances [FL-no: 15.010, 15.128, 15.130 and 15.131] the Panel concluded that the Procedure could not be applied pending submission and evaluation of genotoxicity data.

## Keywords

Thiazolines, sulphur containing substances, FGE.93, JECFA 68th meeting, food safety.

## Table of Contents

|  |           |
|--|-----------|
| <b>Panel Members .....</b>   | <b>1</b>  |
| <b>Summary .....</b>   | <b>1</b>  |
| <b>Background .....</b>  | <b>4</b>  |
| <b>Terms of Reference.....</b>   | <b>4</b>  |
| <b>Acknowledgement.....</b>  | <b>4</b>  |
| <b>Assessment.....</b>   | <b>4</b>  |
| Intake.....  | 5         |
| Threshold of 1.5 Microgram/Person/Day (Step B5) Used by the JECFA.....   | 5         |
| Genotoxicity.....  | 6         |
| Specifications.....  | 6         |
| Structural Relationship.....   | 6         |
| 1. Presentation of the Substances in the JECFA Flavouring Group.....   | 6         |
| 1.1. Description.....  | 6         |
| 1.2. Isomers.....  | 7         |
| 1.3. Specifications.....   | 7         |
| 2. Intake Estimations.....   | 7         |
| 2.1. JECFA Status.....   | 7         |
| 2.2. EFSA Considerations.....  | 7         |
| 3. Genotoxicity Data.....  | 8         |
| 3.1. Genotoxicity Studies – Text Taken from the JECFA (JECFA, 2008b).....  | 8         |
| 3.2. Genotoxicity Studies - Text from FGE.21Rev1 (EFSA, 2009u).....  | 9         |
| 3.3. EFSA Considerations.....  | 10        |
| 4. Application of the Procedure.....   | 10        |
| 4.1. Application of the Procedure to Sulphur Containing Heterocyclic Compounds by the JECFA (JECFA, 2008b).....                              | 10        |
| 4.2. Application of the Procedure to Thiazoles, Thiophene, Thiazoline and Thienyl Derivatives in FGE.21Rev1 (EFSA, 2009u).....               | 11        |
| 4.3. EFSA Considerations.....  | 12        |
| 5. Conclusion.....   | 12        |
| <b>Table 1: Specification Summary for the JECFA Evaluated Substances in the Present Group.....</b>   | <b>14</b> |
| <b>Table 2: Genotoxicity Data .....</b>  | <b>15</b> |
| Table 2.1: Genotoxicity data ( <i>in vitro</i> / <i>in vivo</i> ) for Sulphur Containing Heterocyclic Substances evaluated by the JECFA..... | 15        |
| Table 2.2: Genotoxicity ( <i>in vitro</i> ) EFSA / FGE.21Rev1.....   | 15        |
| Table 2.3: Genotoxicity ( <i>in vivo</i> ) EFSA / FGE.21Rev1.....  | 17        |
| <b>Table 3: Summary of Safety Evaluation Tables.....</b>   | <b>18</b> |
| Table 3.1: Summary of Safety Evaluation of Sulphur Containing Heterocyclic Substances (JECFA, 2008b).....                                    | 18        |
| Table 3.2: Summary of Safety Evaluation Applying the Procedure (EFSA / FGE.21Rev1).....  | 20        |
| <b>References.....</b>   | <b>26</b> |
| <b>Abbreviations.....</b>  | <b>28</b> |

## BACKGROUND

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

Substances which are listed in the Register are to be evaluated according to the evaluation programme laid down in Commission Regulation (EC) No 1565/2000 (EC, 2000a), which is broadly based on the Opinion of the Scientific Committee on Food (SCF, 1999).

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

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

## TERMS OF REFERENCE

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

## ACKNOWLEDGEMENT

European Food Safety Authority wishes to thank the members of the Working Groups on Flavourings for the preparation of this Opinion: Ulla Beckman Sundh, Vibe Beltoft, Wilfried Bursch, Angelo Carere, Riccardo Crebelli, Karl-Heinz Engel, Henrik Frandsen, Jørn Gry, Rainer Gürtler, Frances Hill, Trine Husøy, John Christian Larsen, Catherine Leclercq, Pia Lund, Wim Mennes, Gerard Mulder, Karin Nørby, Gerard Pascal, Iona Pratt, Gerrit Speijers, Harriet Wallin.

## ASSESSMENT

The approach used by EFSA for safety evaluation of flavouring substances is referred to in Commission Regulation (EC) No 1565/2000 (EC, 2000a), hereafter named the “EFSA Procedure”. This Procedure is based on the Opinion of the Scientific Committee on Food (SCF, 1999), which has been derived from the evaluation procedure developed by the Joint FAO/WHO Expert Committee on Food Additives (JECFA, 1995; JECFA, 1996a; JECFA, 1997a; JECFA, 1999b), hereafter named the “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 considerations by EFSA will conclude whether the flavouring substances are of no safety concern at their estimated levels of intake, whether additional data are required or whether certain substances should not be put through the EFSA Procedure.

The following issues are of special importance.

#### Intake

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

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

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

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

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

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

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

"The Committee noted that this value was based on a risk analysis of known carcinogens which involved several conservative assumptions. The use of this value was supported by additional information on developmental toxicity, neurotoxicity and immunotoxicity. In the judgement of the Committee, flavouring substances for which insufficient data are available for them to be evaluated using earlier steps in the Procedure, but for which the intake would not exceed 1.5 microgram per person per day would not be expected to present a safety concern. The Committee recommended

that the Procedure for the Safety Evaluation of Flavouring Agents used at the forty-sixth meeting be amended to include the last step on the right-hand side of the original procedure (“Do the condition of use result in an intake greater than 1.5 microgram per day?”) (JECFA, 1999b).

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

### Genotoxicity

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

### Specifications

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

### Structural Relationship

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

## **1. Presentation of the Substances in the JECFA Flavouring Group**

### 1.1. Description

#### 1.1.1. JECFA Status

The JECFA has evaluated a group of 17 flavouring substances consisting of sulphur-containing heterocyclic substances at the 68<sup>th</sup> meeting (JECFA, 2008b).

#### 1.1.2. EFSA Considerations

The current FGE.93 deals with six [FL-no: 15.010, 15.126, 15.127, 15.128, 15.130, 15.131] of the 17 substances evaluated by JECFA.

- Seven of the substances are not in the Register [2-(4-methyl-5-thiazolyl)ethyl formate, 2-(4-methyl-5-thiazolyl)ethyl propionate, 2-(4-methyl-5-thiazolyl)ethyl butanoate, 2-(4-methyl-5-thiazolyl)ethyl isobutyrate, 2-(4-methyl-5-thiazolyl)ethyl hexanoate, 2-(4-methyl-5-thiazolyl)ethyl octanoate, 2-(4-methyl-5-thiazolyl)ethyl decanoate (JECFA-no: 1751-1757)].
- Four other substances have been evaluated by the AFC Panel of EFSA in FGE.21 [FL-no: 15.063, 15.055, 15.076 and 15.114].

This consideration therefore only deals with six substances [FL-no: 15.010, 15.126, 15.127, 15.128, 15.130, 15.131]. The Panel concluded that these six substances of the JECFA flavouring group of sulphur containing heterocyclic substances are structurally related to the group of thiazoles, thiophene, thiazoline and thienyl derivatives evaluated by EFSA in the Flavouring Group



Evaluation 21, Revision 1 (FGE.21Rev1). The substances in FGE.21Rev1 were subdivided into a number of subgroups. The six substances in the current FGE.93 are assigned to the following subgroups:

- 1-(3-Hydroxy-5-methyl-2-thienyl)ethanone [FL-no: 15.127] in subgroup A-Ib.
- 3-(methylthio)-methylthiophen [FL-no: 15.126] in subgroup A-Ic.
- 2-acetyl-2-thiazoline [FL-no: 15.010], 2-propionyl-2-thiazoline [FL-no 15.128], 5-ethyl-4-methyl-2-(2-methylpropyl)-thiazoline [FL-no: 15.130] and 5-ethyl-4-methyl-2-(2-butyl)-thiazoline [FL-no: 15.131] in subgroup B-II.

## 1.2. Isomers

### 1.2.1. *JECFA Status*

The following two substances [FL-no: 15.130 and 15.131] in the group of the JECFA evaluated sulphur containing heterocyclic substances have one or more chiral centres.

### 1.2.2. *EFSA Considerations*

Information about the stereoisomerism has not been provided for two substances [FL-no: 15.130 and 15.131].

## 1.3. Specifications

The European Flavour Industry has submitted specifications for the substances commercially used in Europe (EFFA, 2006r; EFFA, 2006s; Flavour Industry, 2004i; Flavour Industry, 2005g). Although the JECFA specifications are available, the specification used in this consideration are those submitted by the Industry. See Table 1.

Specifications including complete purity criteria and identity tests are available for one substance. Information on stereoisomerism has not been provided for two substances [FL-no: 15.130 and 15.131], data on solubility in ethanol is missing for one substance [FL-no: 15.127] and no boiling point is given for [FL-no: 15.126]. The range for boiling point and specific gravity is too wide for [FL-no: 15.128] (see Section 1.2).

## 2. **Intake Estimations**

### 2.1. JECFA Status

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

### 2.2. EFSA Considerations

For five of the six JECFA evaluated substances normal and maximum use levels have been provided by the Flavour Industry [FL-no: 15.010, 15.126, 15.128, 15.130 and 15.131] (Flavour Industry, 2005g; EFFA, 2006r; EFFA, 2006s; EFFA, 2007a) (see Table 2.2.1). Based on these normal use levels, mTAMDI figures (see Table 2.2.2) can be calculated. For definition of normal and maximum use levels and description of the method for calculation of mTAMDI consult Annex II in e.g. (EFSA, 2004d).

**Table 2.2.1 Normal and Maximum use levels (mg/kg) available for JECFA evaluated substances in FGE.93**

| 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        |  |
| 15.010 | 0,4<br>2                   | 0,2<br>1    | 0,4<br>2    | 0,3<br>1,5    | -    | 4<br>2      | 0,2<br>1     | 4<br>2      | 0,1<br>0,4    | 0,1<br>0,4 | -    | -    | 0,2<br>1      | 0,4<br>2 | 0,2<br>1     | 4<br>2      | 1<br>5      | 0,2<br>1    |  |
| 15.126 | 0,01<br>0,1                | 0,01<br>0,1 | -           | 0,005<br>0,05 | -    | -           | -            | 0,02<br>0,2 | 0,005<br>0,05 | -          | -    | -    | 0,005<br>0,05 | -        | -            | -           | 0,05<br>0,5 | 0,01<br>0,1 |  |
| 15.128 | 0,16<br>0,8                | 0,04<br>0,2 | 0,16<br>0,8 | -             | -    | 0,04<br>0,2 | 0,01<br>0,05 | 0,4<br>1    | 0,04<br>0,4   | -          | -    | -    | 0,08<br>0,8   | -        | 0,01<br>0,08 | 0,04<br>0,2 | 0,08<br>0,8 | -           |  |
| 15.130 | -                          | 0,2<br>1,1  | 0,4<br>2    | 0,3<br>1,5    | -    | 0,4<br>2    | 0,2<br>1     | 0,4<br>2    | 0,1<br>0,4    | 0,1<br>0,4 | -    | -    | 0,2<br>1      | 0,4<br>2 | 0,2<br>1     | 0,4<br>2    | 1<br>5      | 0,2<br>1    |  |
| 15.131 | 0,4<br>2                   | 0,2<br>1    | 0,4<br>2    | 0,3<br>1,5    | -    | 0,4<br>2    | 0,2<br>1     | 0,4<br>2    | 0,1<br>0,4    | 0,1<br>0,4 | -    | -    | 0,2<br>1      | 0,4<br>2 | 0,2<br>1     | 0,4<br>2    | 1<br>5      | 0,2<br>1    |  |

**Table 2.2.2 Estimated intakes based on the MSDI- and the mTAMDI approach**

| FL-no  | EU Register name                               | MSDI – EU<br>(µg/capita/day) | MSDI – USA<br>(µg/capita/day) | mTAMDI<br>(µg/person/day) | Structural class | Threshold of concern<br>(µg/person/day) |
|--------|--|------------------------------|-------------------------------|---------------------------|------------------|---|
| 15.010 | 2-Acetyl-2-thiazoline                          | 9.3                          | ND                            | 810                       | Class II         | 540                                     |
| 15.127 | 1-(3-Hydroxy-5-methyl-2-thienyl)ethanone       | 0.012                        | ND                            |                           | Class II         | 540                                     |
| 15.128 | 2-Propionyl-2-thiazoline                       | 0.012                        | ND                            | 62                        | Class II         | 540                                     |
| 15.126 | 3-(Methylthio)-methylthiophen                  | 0.012                        | ND                            | 3.8                       | Class III        | 90                                      |
| 15.130 | 5-Ethyl-4-methyl-2-(2-methylpropyl)-thiazoline | 0.012                        | ND                            | 160                       | Class III        | 90                                      |
| 15.131 | 5-Ethyl-4-methyl-2-(2-butyl)-thiazoline        | 0.012                        | ND                            | 160                       | Class III        | 90                                      |

### 3. Genotoxicity Data

#### 3.1. Genotoxicity Studies – Text Taken<sup>1</sup> from the JECFA (JECFA, 2008b)

##### *Thiophene [FL-no: 15.106] (structurally related substance)*

The results of several *in vitro* tests for genotoxicity conducted with thiophene, a structurally related substance, are described below.

No increase in mutagenic activity was observed in the reverse mutation assay (Ames test) in *Salmonella typhimurium* strains TA98, TA100, TA1535 or TA1537 at 0, 78.1, 156, 313, 625, 1250, 2500 or 5000 µg thiophene/plate with and without metabolic activation. Toxicity was noted at 1500 µg/plate in TA1537 and at 2500 µg/plate in TA98, TA100 and TA1535 (Shibuya, 2006).

Similarly, there was no increase in mutagenic activity in a mutation assay in *Escherichia coli* strain WP2uvrA at 0, 313, 625, 1250, 2500 or 5000 µg/plate with and without metabolic activation. Toxicity was noted at the 5000 µg/plate concentration (Shibuya, 2006).

There was no increase in chromosomal aberrations or polyploidy following incubation of Chinese hamster lung cells with 0, 210, 420 or 840 µg thiophene/ml (Tanaka, 2006).

<sup>1</sup> The text is taken verbatim from the indicated reference source, but text related to substances not included in the present FGE has been removed.



### *Conclusion on genotoxicity*

No genotoxicity data were available on the 17 substances evaluated by the JECFA. The JECFA evaluation refers to data on thiophene only, as summarised in Table 2.2. The JECFA concluded that in the previous evaluation of substances in this group, studies on genotoxicity, as well as studies on acute toxicity and short-term toxicity, were available and none raised safety concerns.

### 3.2. Genotoxicity Studies - Text from FGE.21Rev1 (EFSA, 2009u)

Genotoxicity data were provided for 12 of the 56 candidate substances. These 12 substances belong to subgroup A-Ia: thiophene [FL-no: 15.106]; subgroup A-Ib: 2-methylthiophene [FL-no: 15.091], 3-methylthiophene [FL-no: 15.092], 2,5-dimethylthiophene [FL-no: 15.064], 2-acetylthiophene [FL-no: 15.040], 2-acetyl-3-methylthiophene [FL-no: 15.037], thiophene-2-carbaldehyde [FL-no: 15.107], 5-ethylthiophene-2-carbaldehyde [FL-no: 15.074]; subgroup A-II: 2,4-dimethylthiazole [FL-no: 15.062]; subgroup A-III: 2-methyl-4,5-benzothiazole [FL-no: 15.088]; subgroup B-III: 2-methylthiazolidine [FL-no: 15.090] and 2-propylthiazolidine [FL-no: 15.099]. There were also mutagenicity data on four supporting substances and on four other structurally related substances. All available information on genotoxicity of the 12 candidate and the four supporting substances and of four other structurally related substances is based upon *in vitro* studies only.

In the following text from FGE.21Rev1 (EFSA, 2009u), only the information for subgroup A-I, B-I, B-II and B-III has been presented, as the information for subgroups A-II and A-III, B-IV, B-V and B-VI was not relevant to the candidate substances in the current FGE.

#### *Subgroup A-I: Thiophenes*

Thiophene [FL-no: 15.106], 2-methylthiophene [FL-no: 15.091], 3-methyl-thiophene [FL-no: 15.092] and 2,5-dimethylthiophene [FL-no: 15.064] were reported to be negative in microbial mutagenicity assays. 2-Acetylthiophene [FL-no: 15.040] was negative in microbial tests, using *S. typhimurium* strains TA 98 and TA100, with and without metabolic activation and in the SOS chromotest with metabolic activation. 2-Acetylthiophene was reported to be positive without metabolic activation in the SOS *E. coli* chromotest (Mosier et al., 2003). In the same study, 2-acetyl-3-methylthiophene [FL-no: 15.037], thiophene-2-carbaldehyde [FL-no: 15.107] and 5-ethylthiophene-2-carbaldehyde [FL-no: 15.074] gave positive results without metabolic activation in the SOS *E. coli* chromotest (Mosier et al., 2003). The concentrations tested were not reported for any of the substances subjected to the SOS *E. coli* chromotest (Mosier et al., 2003). The Panel considered the endpoint of this test inappropriate for the estimation of genotoxic potential. The supporting substance 5-methyl-2-thiophene-carboxaldehyde [FL-no: 15.004] was negative in a microbial mutagenicity assay and in the mouse lymphoma test.

#### *Subgroups B-I and B-II: Dihydrothiophenes and thiazolines*

No genotoxicity information was available for any candidate or supporting substances in these subgroups. However, considering the structural similarities between the thiazolines in subgroup B-II and the thiazolidines in subgroup B-III, the Panel also concluded that the thiazolines [FL-no: 15.060, 15.086 and 15.119] could not be evaluated through the Procedure (see Subgroup B-III below).

#### *Subgroup B-III: Thiazolidines*

The two candidate substances 2-methylthiazolidine [FL-no: 15.090] and 2-propylthiazolidine [FL-no: 15.099] as well as the structurally related ethyl, isopropyl, n-butyl and isobutyl thiazolidine have all been reported to be positive in the Ames tests (TA98 and TA100) (Mihara & Shibamoto, 1980). Owing to limited reporting, the data could not be properly evaluated. Nevertheless, these reports do raise the possibility of a genotoxic potential of these thiazolidines. Accordingly, it was concluded not to evaluate the candidate substances 2-methylthiazolidine and 2-propylthiazolidine through the Procedure.

*Overall conclusion on genotoxicity:*

It is concluded that the genotoxicity data are limited and that genotoxicity could not be assessed adequately for the flavouring substances in FGE.21Rev1. However, except for the two dihydrothiazines, 6-acetyl-2,3-dihydro-1,4-thiazine [FL-no: 15.114] (Register name: 5-acetyl-2,3-dihydro-1,4-thiazine) and 5-acetyl-2,3-dihydro-1,4-thiazine [FL-no: 15.133], the two thiazolidines 2-methylthiazolidine [FL-no: 15.090] and 2-propylthiazolidine [FL-no: 15.099] and the three structurally related thiazolines 2-methyl-2-thiazoline [FL-no: 15.086], 2,4-dimethyl-3-thiazoline [FL-no: 15.060] and 2-isobutyl-3-thiazoline [FL-no: 15.119], the genotoxicity data available do not preclude the evaluation of the remaining 49 candidate substances using the Procedure.

For a summary of *in vitro* genotoxicity data considered by EFSA, see Table 2.2.

### 3.3. EFSA Considerations

No genotoxicity information was available on the 17 substances evaluated by the JECFA, including the six substances considered in this Opinion. However, four of these six substances, 2-acetyl-2-thiazoline [FL-no: 15.010], 2-propionyl-2-thiazoline [FL-no: 15.128], 5-ethyl-4-methyl-2-(2-methylpropyl)-thiazoline [FL-no: 15.130] and 5-ethyl-4-methyl-2-(2-butyl)-thiazoline [FL-no: 15.131] have structural similarities to 2-methylthiazolidine [FL-no: 15.090] and 2-propylthiazolidine [FL-no: 15.099], evaluated by EFSA in FGE.21Rev1(subgroup B-III, thiazolidines) and reported to be positive in the Ames test (TA98 and TA100). In parallel with the EFSA conclusion on the subgroup B-II (thiazolines) in FGE.21Rev1, the Panel concluded that the Procedure could not be applied to these four thiazolines, until adequate genotoxicity data become available.

For the two substances belonging to A-Ib and A-Ic [FL-no: 15.126 and 15.127], since no genotoxicity data were available on these substances, the Panel referred to the EFSA conclusion on the substances considered in groups A-Ib and A-Ic in FGE.21Rev1 (EFSA, 2009u). For these groups, EFSA had concluded that genotoxicity data were limited, but these data did not preclude evaluating the substances through the Procedure. The Panel concluded therefore, in parallel, that the available data (from FGE.21Rev1) do not preclude taking [FL-no: 15.126 and 15.127] through the procedure.

## 4. Application of the Procedure

### 4.1. Application of the Procedure to Sulphur Containing Heterocyclic Compounds by the JECFA (JECFA, 2008b)

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

All six substances were concluded at step B4 in the JECFA Procedure – i.e. that the substances are not expected to be metabolised to innocuous products and that the estimated intakes are below the thresholds for their structural classes II and III. An adequate NOAEL was available for relevant structurally related substances for all the six substances and the JECFA concluded that the substances are therefore not expected to be of safety concern when used as flavouring substances.

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

The evaluations of the six sulphur containing heterocyclic substances are summarised in Table 3.1: Summary of Safety Evaluation of Sulphur Containing Heterocyclic Compounds (JECFA, 2008b)).

#### 4.2. Application of the Procedure to Thiazoles, Thiophene, Thiazoline and Thienyl Derivatives in FGE.21Rev1 (EFSA, 2009u)

Fifty-six candidate substances were evaluated in FGE.21Rev1. Forty-six substances were classified into structural class II and ten into structural class III using the decision tree approach presented by Cramer et al. (1978). For seven substances the Procedure could not be applied due to indication of genotoxic potential *in vitro* [FL-no: 15.060, 15.086, 15.090, 15.099, 15.114, 15.119 and 15.133].

The remaining 49 substances were allocated into 11 structural subgroups (for description and explanation, see FGE.21Rev1 (EFSA, 2009u) and were evaluated at step B4 in the Procedure, i.e. the substances are not expected to be metabolised to innocuous products and the estimated intakes are below the thresholds for their structural classes II and III.

In summary, EFSA concluded that 26 of the candidate substances evaluated through the Procedure, from the structural subgroups A-Ic (thiophenes with thiol-containing ring substituents) and A-II (thiazoles) are not of safety concern at their estimated levels of intake based on the MSDI approach, whereas for 23 candidate substances from the structural subgroups A-Ia (thiophene), A-Ib (thiophenes with non-thiol-containing ring substituents), A-III (benzothiazoles), B-I (dihydrothiophenes), B-IV (dithiazines) and B-VI (thiadiazine) additional data are required.

In the following text from EFSA (EFSA, 2009u), only the information for subgroup A-Ib, A-Ic, B-II and B-III has been presented, as the information on the application of the Procedure for the other subgroups is not relevant to the candidate substances in the current FGE.

##### *Subgroup A-Ib: Thiophenes with non-thiol-containing ring substituents*

No valid toxicity study from which a NOAEL could be established was available for the candidate or for any relevant supporting substance. Therefore, the Panel concluded that additional toxicity data are needed for the 16 substituted thiophenes in subgroup A-Ib.

##### *Subgroup A-Ic: Thiophenes with thiol-containing ring substituents*

A NOAEL of 0.29 mg/kg bw/day was reported for the supporting substance 2-thienyl disulfide [FLno: 15.008] in a single-dose level 90-day study in rats. The combined estimated daily *per capita* intake of 0.14 microgram for the three candidate substances in subgroup A-Ic corresponds to 0.0023 microgram/kg bw/day at a body weight of 60 kg. Thus a margin of safety of  $1.2 \times 10^5$  can be calculated.

On the basis of the application of the Procedure, 3-mercaptothiophene [FL-no: 15.082], 2-methyl-3-mercaptothiophene [FL-no: 15.087] and 2-thiophenemethanethiol [FL-no: 15.108] are not expected to be of safety concern at their estimated levels of intake.

#### *Subgroup B-II: Thiazolines*

The candidate substances were not evaluated through the Procedure.

#### *Subgroup B-III: Thiazolidines*

The candidate substances were not evaluated through the Procedure.

The stepwise evaluations of the 56 substances are summarised in Table 3.2: Summary of Safety Evaluation Applying the Procedure (EFSA / FGE.21Rev1).

### 4.3. EFSA Considerations

The Panel agrees with the way the application of the Procedure was applied by the JECFA for two of the six sulphur containing heterocyclic substances [FL-no: 15.126 and 15.127].

Four of the six substances evaluated by the JECFA, 2-acetyl-2-thiazoline [FL-no: 15.010], 2-propionyl-2-thiazoline [FL-no: 15.128], 5-ethyl-4-methyl-2-(2-methylpropyl)-thiazoline [FL-no: 15.130] and 5-ethyl-4-methyl-2-(2-butyl)-thiazoline [FL-no: 15.131] have structural similarities to two substances evaluated in FGE.21Rev1, 2-methylthiazolidine [FL-no: 15.090] and 2-propylthiazolidine [FL-no: 15.099], considered by the Panel to have genotoxic potential *in vitro*. The Panel therefore concludes that the Procedure should not be applied to these four flavouring substances until adequate genotoxicity data become available.

For 3-(methylthio)-methylthiophen [FL-no: 15.126], allocated to subgroup A-Ic, the intake (MSDI) of 0.12 microgram/capita/day is below the threshold for its structural class III. The Panel agrees with JECFA, and in line with the conclusion reached by EFSA for subgroup A-Ic in FGE.21Rev1, that an adequate NOAEL provides a sufficient safety margin and that this flavouring substance can be concluded at step B4 in the Procedure as of no safety concern.

The substance 1-(3-hydroxy-5-methyl-2-thienyl)ethanone [FL-no: 15.127] was allocated to the structural subgroup A-Ib (thiophenes with non-thiol-containing ring substituents) identified in FGE.21Rev1 (for description and explanation, see FGE.21Rev1). As no valid toxicity study from which a NOAEL could be established is available for the candidate or for any relevant supporting substance, the Panel concluded that additional toxicity data are needed for this flavouring substance.

## 5. Conclusion

The Panel concluded that all the six substances in the JECFA flavouring group of sulphur containing heterocyclic substances are structurally related to the 56 substances evaluated by EFSA in the Flavouring Group Evaluation 21, Revision 1 (FGE.21Rev1).

The Panel agrees with the way the application of the Procedure was applied by the JECFA for two of the six substances, 3-(methylthio)-methylthiophene [FL-no: 15.126] and 1-(3-hydroxy-5-methyl-2-thienyl)ethanone [FL-no: 15.127]. However, for 1-(3-hydroxy-5-methyl-2-thienyl)ethanone [FL-no: 15.127], the Panel concludes that no valid toxicity study from which a NOAEL could be established is available. Therefore additional toxicity data are needed for this flavouring substance.

For 3-(methylthio)methylthiophene [FL-no: 15.126], the Maximised Survey-derived Daily Intake (MSDI) is below the threshold for its structural class (Cramer class III, 90 µg/person/day). The Panel agrees with the JECFA that the NOAEL of 0.29 mg/kg bw/day of the supporting substance [FL-no: 15.008] is adequate for [FL-no: 15.126] and that it provides a sufficient safety margin. It can therefore be concluded that this substance is of no safety concern when used as flavouring substance at the estimated level of intake, based on the MSDI approach.

No genotoxicity information was available on any of the six substances evaluated by the JECFA. However the Panel noted that four of the six substances, 2-acetyl-2-thiazoline [FL-no: 15.010], 2-propionyl-2-thiazoline [FL-no: 15.128], 5-ethyl-4-methyl-2-(2-methylpropyl)-thiazoline [FL-no: 15.130] and 5-ethyl-4-methyl-2-(2-butyl)-thiazoline [FL-no: 15.131] have structural similarities to 2-methylthiazolidine [FL-no: 15.090] and 2-propylthiazolidine [FL-no: 15.099] in FGE.21Rev1 (EFSA, 2009u), which are considered by the Panel to have genotoxic potential *in vitro*. The Panel therefore concluded that the Procedure should not be applied to these four flavouring substances [FL-no: 15.010, 15.128, 15.130 and 15.131] pending submission and evaluation of adequate genotoxicity data.

For one substance evaluated through the procedure [FL-no: 15.126], use levels have been provided by the Industry. The mTAMDI figures calculated are below the threshold of concern for its structural class.

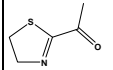
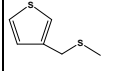
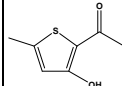
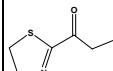
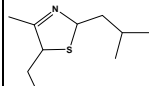
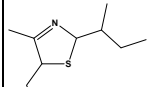
For the other substance evaluated through the Procedure [FL-no: 15.127], the use levels are needed in order to calculate the mTAMDI and to identify whether more refined exposure assessment is needed to finalise the evaluation.

Although four remaining substances cannot be evaluated through the procedure [FL-no: 15.010, 15.128, 15.130 and 15.131], the corresponding available use levels were considered. It was noted that for three of these compounds [FL-no: 15.010, 15.130 and 15.131] the calculated mTAMDI are above the threshold of concern for their structural classes.

The available specifications of the six substances in this FGE were examined. Information on stereoisomeric composition is missing for two substances [FL-no: 15.130 and 15.131]; data on solubility in ethanol is missing for one substance [FL-no: 15.127]; no boiling point is given for [FL-no: 15.126]. The range for boiling point and specific gravity given is too wide for [FL-no: 15.128].

Thus, the Panel concluded that the substance [FL-no: 15.126] is of no safety concern at the estimated intake. For the substance [FL-no: 15.127], the Panel concluded that additional toxicity data are needed. For four substances [FL-no: 15.010, 15.128, 15.130 and 15.131] the Panel concluded that the Procedure could not be applied pending submission and evaluation of genotoxicity data.

**TABLE 1: SPECIFICATION SUMMARY FOR THE JECFA EVALUATED SUBSTANCES IN THE PRESENT GROUP**

| Table 1: Specification Summary of the Substances in the the JECFA Flavouring Group of Sulphur Containing Heterocyclic Substances |   |   |                             |  |  |   |                                     |   |
|--|---|---|-----------------------------|--|--|---|-------------------------------------|---|
| FL-no<br>JECFA-no  | EU Register name                                  | Structural formula  | FEMA no<br>CoE no<br>CAS no | Phys.form<br>Mol.formula<br>Mol.weight                           | Solubility 1)<br>Solubility in ethanol 2)        | Boiling point, °C 3)<br>Melting point, °C<br>ID test<br>Assay minimum | Refrac. Index 4)<br>Spec.gravity 5) | EFSA comments /<br>References for specifications  |
| 15.010<br>1759   | 2-Acetyl-2-thiazoline                             |    | 3817<br>2335<br>29926-41-8  | Solid<br>C <sub>5</sub> H <sub>7</sub> NOS<br>129.18             | Practically insoluble or<br>insoluble<br>Soluble | 27<br>IR NMR MS<br>98 %   | n.a.<br>n.a.                        | (EFFA, 2006s.)  |
| 15.126<br>1765   | 3-(Methylthio)-methylthiophen                     |    | 61675-72-7                  | Liquid<br>C <sub>6</sub> H <sub>8</sub> S <sub>2</sub><br>144.26 | Slightly soluble<br>Slightly soluble             | n.a.<br>MS<br>97 %  | 1.580-1.586<br>1.522-1.528          | BP 7)<br>(EFFA, 2006r)  |
| 15.127<br>1750   | 1-(3-Hydroxy-5-methyl-2-thienyl)ethanone          |    | 133860-42-1                 | Solid<br>C <sub>7</sub> H <sub>8</sub> O <sub>2</sub> S<br>156.2 | Slightly soluble                                 | 74.3 (969 hPa)<br>IR NMR MS<br>98.3 %                                 | n.a.<br>n.a.                        | SE 8)<br>(Flavour Industry, 2004i)  |
| 15.128<br>1760   | 2-Propionyl-2-thiazoline                          |    | 4064<br>29926-42-9          | Liquid<br>C <sub>6</sub> H <sub>9</sub> NOS<br>143.21            | Insoluble<br>Soluble                             | 239 +/- 23<br>MS<br>99 %  | 1.510-1.525<br>1.130-1.330          | (EFFA, 2006r)   |
| 15.130<br>1761   | 5-Ethyl-4-methyl-2-(2-methylpropyl)-thiazoline 6) |   | 83418-53-5                  | Liquid<br>C <sub>10</sub> H <sub>19</sub> NS<br>185.33           | Soluble<br>Soluble                               | 253<br>MS<br>95 %   | 1.483-1.489<br>0.939-0.945          | (Flavour Industry, 2005g)<br>Industry: cis- and trans- 5-Ethyl-4-methyl-2-(2-methylpropyl)-thiazoline |
| 15.131<br>1762   | 5-Ethyl-4-methyl-2-(2-butyl)-thiazoline 6)        |  | 83418-54-6                  | Liquid<br>C <sub>10</sub> H <sub>19</sub> NS<br>185.33           | Soluble<br>Soluble                               | 253<br>MS<br>95 %   | 1.487-1.493<br>0.950-0.956          | (Flavour Industry, 2005g)   |

- 1) Solubility in water, if not otherwise stated.
- 2) Solubility in 95% ethanol, if not otherwise stated.
- 3) At 1013.25 hPa, if not otherwise stated.
- 4) At 20°C, if not otherwise stated.
- 5) At 25°C, if not otherwise stated.
- 6) Stereoisomeric composition not specified.
- 7) BP: Missing boiling point.
- 8) SE: Missing data on solubility in ethanol.



## TABLE 2: GENOTOXICITY DATA

Table 2.1: Genotoxicity data (*in vitro* / *in vivo*) for Sulphur Containing Heterocyclic Substances evaluated by the JECFA

No data are available.

Table 2.2: Genotoxicity (*in vitro*) EFSA / FGE.21Rev1

Substances listed in brackets are JECFA evaluated substances.

| Table 2.2: Summary of Genotoxicity Data ( <i>in vitro</i> ) EFSA |   |   |  |                             |                            |   |
|--|---|---|--|-----------------------------|----------------------------|---|
| Chemical Name [FL-no]  | Test System                             | Test Object   | Concentration                                | Result                      | Reference                  | Comments  |
| <b>Subgroup A-Ia</b>   |   |   |  |                             |                            |   |
| Thiophene [15.106]   | Ames assay (plate incorporation method) | <i>S. typhimurium</i><br>TA98; TA100; TA1535; TA1537      | 3 µmol/plate (all strains)<br>(252 µg/plate) | Negative (±S9)              | (Florin et al., 1980)      | Published non-GLP study. Qualitative screening in a spot-test with three strains, quantitative study (4 doses, 0.03, 0.3, 3, 30 µmol/plate) with TA 100 only. Limited report of experimental details and results. Insufficient quality, study not considered adequate for the evaluation of mutagenic activity. |
|  | Ames assay (preincubation method)       | <i>S. typhimurium</i><br>TA97;TA98; TA100; TA1535; TA1537 | Up to 10,000 µg/plate                        | Negative (±S9) <sup>1</sup> | (Zeiger et al., 1987)      | Non-GLP study roughly in accordance with OECD guideline 471. The study is considered valid.   |
|  | Ames assay (preincubation method)       | <i>S. typhimurium</i><br>TA98; TA100; TA102               | 0.01-1.2 mmol/plate<br>(100,968 µg/plate)    | Negative (±S9)              | (Aeschbacher et al., 1989) | Greatest effects are quantified by "mutation factor," no numbers are given for negative results. Limited quality (only 3 strains used), but otherwise acceptable study.   |
|  | Ames assay (plate incorporation method) | <i>S. typhimurium</i><br>TA98; TA100                      | Up to 100 µmol/plate<br>(8414 µg/plate)      | Negative (±S9)              | (Lee et al., 1994a)        | Only two strains used but otherwise acceptable study.   |
| <b>Subgroup A-Ib</b>   |   |   |  |                             |                            |   |
| 2-Methylthiophene [15.091]                                       | Ames assay (preincubation method)       | <i>S. typhimurium</i><br>TA98; TA100; TA102               | 0.00001- 1.0 mmol/plate<br>(98,170 µg/plate) | Negative (±S9)              | (Aeschbacher et al., 1989) | Greatest effects are quantified by "mutation factor," no numbers are given for negative results. Limited quality (only 3 strains used), but otherwise acceptable study.   |
|  | Ames assay (plate incorporation method) | <i>S. typhimurium</i><br>TA98; TA100                      | Up to 100 µmol/plate<br>(9817 µg/plate)      | Negative (±S9)              | (Lee et al., 1994a)        | Only two strains used but otherwise acceptable study  |
| 3-Methylthiophene [15.092]                                       | Ames assay (preincubation method)       | <i>S. typhimurium</i><br>TA98; TA100; TA102               | 0.01-1.0 mmol/plate<br>(98,170 µg/plate)     | Negative (±S9)              | (Aeschbacher et al., 1989) | Greatest effects are quantified by "mutation factor," no numbers are given for negative results. Limited quality (only 3 strains used), but otherwise acceptable study.   |
|  | Ames assay (plate incorporation method) | <i>S. typhimurium</i><br>TA98; TA100                      | Up to 100 µmol/plate<br>(9817 µg/plate)      | Negative (±S9)              | (Lee et al., 1994a)        | Only two strains used but otherwise acceptable study.   |
| 2,5-Dimethylthiophene [15.064]                                   | Ames assay (plate incorporation method) | <i>S. typhimurium</i><br>TA98; TA100                      | Up to 100 µmol/plate                         | Negative                    | (Lee et al., 1994a)        |   |

**Table 2.2: Summary of Genotoxicity Data (*in vitro*) EFSA**

| Chemical Name [FL-no]                       | Test System                             | Test Object                       | Concentration                          | Result   | Reference                  | Comments   |
|---|---|-----------------------------------|--|--|----------------------------|--|
| 2-Acetylthiophene [15.040]                  | Ames assay (plate incorporation method) | <i>S. typhimurium</i> TA98; TA100 | Up to 100 µmol/plate (12,618 µg/plate) | Negative (±S9)   | (Lee et al., 1994a)        | Only two strains used but otherwise acceptable study.  |
|   | SOS Chromotest                          | <i>E. coli</i>                    | NR                                     | Negative with rat S9, positive without rat S9  | (Mosier et al., 2003)      | Study endpoint inappropriate for the estimation of genotoxic potential.  |
| 2-Acetyl-3-Methylthiophene [15.037]         | SOS Chromotest                          | <i>E. coli</i>                    | NR                                     | Negative with rat S9, positive without rat S9  | (Mosier et al., 2003)      | Study endpoint inappropriate for the estimation of genotoxic potential.  |
| Thiophene-2-carbaldehyde [15.107]           | SOS Chromotest                          | <i>E. coli</i>                    | NR                                     | Negative with rat S9, positive without rat S9  | (Mosier et al., 2003)      | Study endpoint inappropriate for the estimation of genotoxic potential.  |
| 5-Ethylthiophene-2-carbaldehyde [15.074]    | SOS Chromotest                          | <i>E. coli</i>                    | NR                                     | Negative with rat S9, positive without rat S9  | (Mosier et al., 2003)      | Study endpoint inappropriate for the estimation of genotoxic potential.  |
| (5-Methyl-2-thiophenecarbaldehyde [15.004]) | Ames assay (plate incorporation method) | <i>S. typhimurium</i> TA98; TA100 | Up to 100 µmol/plate (12,618 µg/plate) | Negative (±S9)   | (Lee et al., 1994a)        | Only two strains used but otherwise acceptable study.  |
| <b>Subgroup B-III</b>                       |   |                                   |  |  |                            |  |
| 2-Propylthiazolidine [15.099]               | Ames assay                              | <i>S. typhimurium</i> TA98; TA100 | 1, 10, 100 µg/ml                       | 1 and 10 µg/ml: positive in TA100 (±S9);<br>100 µg/ml: positive in TA98 and TA100.(±S9)  | (Mihara & Shibamoto, 1980) | The results were stated to be positive, however, the magnitude and a positive dose effect relationship could not be assessed (no numbers are given). |
| 2-methylthiazolidine [15.090]               | Ames assay                              | <i>S. typhimurium</i> TA98; TA100 | 1, 10, 100 µg/ml                       | 1 and 10 µg/ml: positive in TA100; (±S9)<br>100 µg/ml: positive in TA98 and TA100 (±S9)  | (Mihara & Shibamoto, 1980) | The results were stated to be positive, however, the magnitude and a positive dose effect relationship could not be assessed (no numbers are given). |
| (2-ethylthiazolidine)                       | Ames assay                              | <i>S. typhimurium</i> TA98; TA100 | 1, 10, 100 µg/ml                       | 1 µg/ml: positive in TA100 (±S9) and TA98 (-S9);<br>10 µg/ml: positive in TA100 (±S9);<br>100 µg/ml: positive TA98 and TA100. (±S9)      | (Mihara & Shibamoto, 1980) | The results were stated to be positive, however, the magnitude and a positive dose effect relationship could not be assessed (no numbers are given). |
| (2-isopropylthiazolidine)                   | Ames assay                              | <i>S. typhimurium</i> TA98; TA100 | 1, 10, 100 µg/ml                       | 1 and 10 µg/ml: positive in TA100 (±S9);<br>100 µg/ml: positive in TA100 (±S9) and TA98 (-S9)  | (Mihara & Shibamoto, 1980) | The results were stated to be positive, however, the magnitude and a positive dose effect relationship could not be assessed (no numbers are given). |
| (2-butylthiazolidine)                       | Ames assay                              | <i>S. typhimurium</i> TA98; TA100 | 1, 10, 100 µg/ml                       | 1 µg/ml: positive in TA100 (+S9);<br>10 µg/ml: positive in TA100 (±S9);<br>100 µg/ml: positive in TA100 (±S9) and TA98 (-S9)             | (Mihara & Shibamoto, 1980) | The results were stated to be positive, however, the magnitude and a positive dose effect relationship could not be assessed (no numbers are given). |
| (2-isobutylthiazolidine)                    | Ames assay                              | <i>S. typhimurium</i> TA98; TA100 | 1, 10, 100 µg/ml                       | 1 µg/ml: positive in TA98 and TA100 (+S9);<br>10 µg/ml: positive in TA98 and TA100 (±S9);<br>100 µg/ml: positive in TA98 and TA100 (±S9) | (Mihara & Shibamoto, 1980) | The results were stated to be positive, however, the magnitude and a positive dose effect relationship could not be assessed (no numbers are given). |

NR: Not reported.


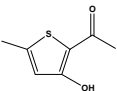
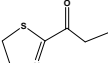

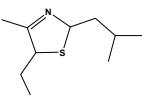
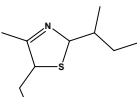
<sup>1</sup> With and without rat and hamster S9 metabolic activation.

Table 2.3: Genotoxicity (*in vivo*) EFSA / FGE.21Rev1

No data are available.

### TABLE 3: SUMMARY OF SAFETY EVALUATION TABLES

Table 3.1: Summary of Safety Evaluation of Sulphur Containing Heterocyclic Substances (JECFA, 2008b)

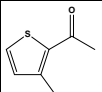
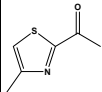
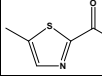
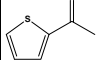
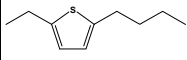
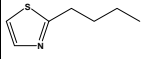
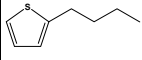
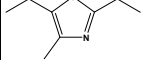
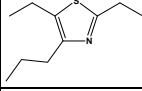
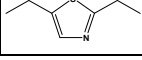
| FL-no<br>JECFA-no | EU Register name                               | Structural formula  | EU MSDI 1)<br>US MSDI ( $\mu\text{g}/\text{capita}/\text{day}$ ) | Class 2)<br>Evaluation procedure path<br>3)                           | Outcome on the<br>named compound<br>[4) or 5)] | EFSA conclusion on the<br>named compound<br>(Procedure steps, intake<br>estimates, NOAEL,<br>genotoxicity)                    | EFSA conclusion on the material<br>of commerce  |
|-------------------|--|---|--|---|--|---|---|
| 15.010<br>1759    | 2-Acetyl-2-thiazoline                          |    | 9.3<br>ND  | Class II<br>B3: Intake below threshold,<br>B4: Adequate NOAEL exists  | 4)   | The Panel concluded that the substance cannot be evaluated through the Procedure due to concern with respect to genotoxicity. | The Panel concluded that the substance cannot be evaluated through the Procedure due to concern with respect to genotoxicity.   |
| 15.127<br>1750    | 1-(3-Hydroxy-5-methyl-2-thienyl)ethanone       |    | 0.012<br>ND  | Class II<br>B3: Intake below threshold,<br>B4: Adequate NOAEL exists  | 4)   | Additional toxicity data requested.   | Additional toxicity data requested.   |
| 15.128<br>1760    | 2-Propionyl-2-thiazoline                       |    | 0.012<br>ND  | Class II<br>B3: Intake below threshold,<br>B4: Adequate NOAEL exists  | 4)   | The Panel concluded that the substance cannot be evaluated through the Procedure due to concern with respect to genotoxicity. | The Panel concluded that the substance cannot be evaluated through the Procedure due to concern with respect to genotoxicity.   |
| 15.126<br>1765    | 3-(Methylthio)-methylthiophen                  |  | 0.012<br>ND  | Class III<br>B3: Intake below threshold,<br>B4: Adequate NOAEL exists | 4)   | No safety concern at estimated level of intake as flavouring substance based on the MSDI approach.                            | Missing boiling point.  |
| 15.130<br>1761    | 5-Ethyl-4-methyl-2-(2-methylpropyl)-thiazoline |  | 0.012<br>ND  | Class III<br>B3: Intake below threshold,<br>B4: Adequate NOAEL exists | 4)   | The Panel concluded that the substance cannot be evaluated through the Procedure due to concern with respect to genotoxicity. | The Panel concluded that the substance cannot be evaluated through the Procedure due to concern with respect to genotoxicity. Stereoisomeric composition to be specified. |
| 15.131<br>1762    | 5-Ethyl-4-methyl-2-(2-butyl)-thiazoline        |  | 0.012<br>ND  | Class III<br>B3: Intake below threshold,<br>B4: Adequate NOAEL exists | 4)   | The Panel concluded that the substance cannot be evaluated through the Procedure due to concern with respect to genotoxicity. | The Panel concluded that the substance cannot be evaluated through the Procedure due to concern with respect to genotoxicity. Stereoisomeric composition to be specified. |

1) EU MSDI: Amount added to food as flavour in (kg/year) x 10E9 / (0.1 x population in Europe (= 375 x 10E6) x 0.6 x 365) =  $\mu\text{g}/\text{capita}/\text{day}$ .

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

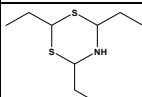
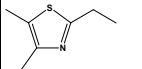
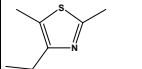
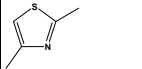
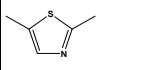
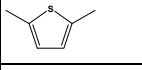
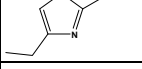
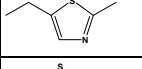
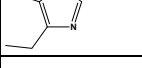
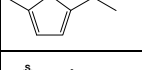
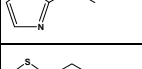
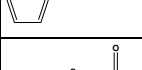
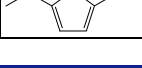
*ND: not determined.*

Table 3.2: Summary of Safety Evaluation Applying the Procedure (EFSA / FGE.21Rev1)

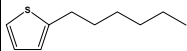
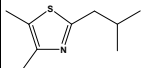
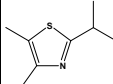
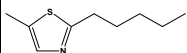
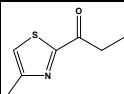



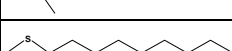
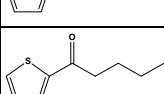
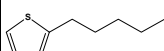
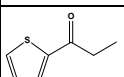
| Table 3.2: Summary of Safety Evaluation Applying the Procedure (based on intakes calculated by the MSDI approach) |                              |   |                            |  |   |   |                    |
|---|------------------------------|---|----------------------------|--|---|---|--------------------|
| FL-no   | EU Register name             | Structural formula  | MSDI 1)<br>(µg/capita/day) | Class 2)<br>Evaluation procedure path 3)                             | Outcome on the named<br>compound<br>[ 4) or 5)] | Outcome on the<br>material of commerce<br>[6), 7), or 8)] | Evaluation remarks |
| 15.037  | 2-Acetyl-3-methylthiophene   |    | 0.18                       | Class II<br>B3: Intake below threshold, B4:<br>No adequate NOAEL     | Additional data required                        |   |                    |
| 15.038  | 2-Acetyl-4-methylthiazole    |    | 0.0049                     | Class II<br>B3: Intake below threshold, B4:<br>Adequate NOAEL exists | 4)  | 6)  |                    |
| 15.039  | 2-Acetyl-5-methylthiazole    |    | 0.0024                     | Class II<br>B3: Intake below threshold, B4:<br>Adequate NOAEL exists | 4)  | 6)  |                    |
| 15.040  | 2-Acetylthiophene            |    | 2.2                        | Class II<br>B3: Intake below threshold, B4:<br>No adequate NOAEL     | Additional data required                        |   |                    |
| 15.043  | 2-Butyl-5-ethylthiophene     |    | 0.0012                     | Class II<br>B3: Intake below threshold, B4:<br>No adequate NOAEL     | Additional data required                        |   |                    |
| 15.044  | 2-Butylthiazole              |   | 0.011                      | Class II<br>B3: Intake below threshold, B4:<br>Adequate NOAEL exists | 4)  | 6)  |                    |
| 15.045  | 2-Butylthiophene             |  | 0.012                      | Class II<br>B3: Intake below threshold, B4:<br>No adequate NOAEL     | Additional data required                        |   |                    |
| 15.050  | 2,5-Diethyl-4-methylthiazole |  | 0.012                      | Class II<br>B3: Intake below threshold, B4:<br>Adequate NOAEL exists | 4)  | 6)  |                    |
| 15.051  | 2,5-Diethyl-4-propylthiazole |  | 0.0012                     | Class II<br>B3: Intake below threshold, B4:<br>Adequate NOAEL exists | 4)  | 6)  |                    |
| 15.052  | 2,5-Diethylthiazole          |  | 0.015                      | Class II<br>B3: Intake below threshold, B4:<br>Adequate NOAEL exists | 4)  | 6)  |                    |



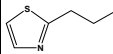
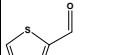
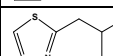
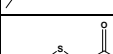
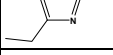
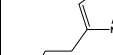
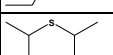
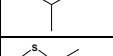
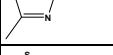
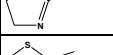
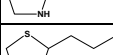
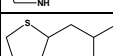
**Table 3.2: Summary of Safety Evaluation Applying the Procedure (based on intakes calculated by the MSDI approach)**

| FL-no          | EU Register name                            | Structural formula  | MSDI 1)<br>(µg/capita/day) | Class 2)<br>Evaluation procedure path 3)                             | Outcome on the named<br>compound<br>[ 4) or 5)] | Outcome on the<br>material of commerce<br>[6), 7), or 8)] | Evaluation remarks |
|----------------|---|---|----------------------------|--|---|---|--------------------|
| 15.054         | Dihydro-2,4,6-triethyl-1,3,5(4H)-dithiazine |    | 0.0012                     | Class II<br>B3: Intake below threshold, B4:<br>No adequate NOAEL     | Additional data required                        |   |                    |
| 15.058         | 4,5-Dimethyl-2-ethylthiazole                |    | 0.015                      | Class II<br>B3: Intake below threshold, B4:<br>Adequate NOAEL exists | 4)  | 6)  |                    |
| 15.061         | 2,5-Dimethyl-4-ethylthiazole                |    | 0.011                      | Class II<br>B3: Intake below threshold, B4:<br>Adequate NOAEL exists | 4)  | 6)  |                    |
| 15.062         | 2,4-Dimethylthiazole                        |    | 0.61                       | Class II<br>B3: Intake below threshold, B4:<br>Adequate NOAEL exists | 4)  | 6)  |                    |
| 15.063<br>1758 | 2,5-Dimethylthiazole                        |    | 0.0061                     | Class II<br>B3: Intake below threshold, B4:<br>Adequate NOAEL exists | 4)  | 6)  |                    |
| 15.064         | 2,5-Dimethylthiophene                       |    | 0.23                       | Class II<br>B3: Intake below threshold, B4:<br>No adequate NOAEL     | Additional data required                        |   |                    |
| 15.067         | 4-Ethyl-2-methylthiazole                    |    | 0.0037                     | Class II<br>B3: Intake below threshold, B4:<br>Adequate NOAEL exists | 4)  | 6)  |                    |
| 15.068         | 5-Ethyl-2-methylthiazole                    |   | 0.0061                     | Class II<br>B3: Intake below threshold, B4:<br>Adequate NOAEL exists | 4)  | 6)  |                    |
| 15.069         | 4-Ethyl-5-methylthiazole                    |  | 0.012                      | Class II<br>B3: Intake below threshold, B4:<br>Adequate NOAEL exists | 4)  | 6)  |                    |
| 15.070         | 2-Ethyl-5-methylthiophene                   |  | 0.061                      | Class II<br>B3: Intake below threshold, B4:<br>No adequate NOAEL     | Additional data required                        |   |                    |
| 15.071         | 2-Ethylthiazole                             |  | 0.028                      | Class II<br>B3: Intake below threshold, B4:<br>Adequate NOAEL exists | 4)  | 6)  |                    |
| 15.072         | 2-Ethylthiophene                            |  | 0.0012                     | Class II<br>B3: Intake below threshold, B4:<br>No adequate NOAEL     | Additional data required                        |   |                    |
| 15.074         | 5-Ethylthiophene-2-carbaldehyde             |  | 0.0012                     | Class II<br>B3: Intake below threshold, B4:<br>No adequate NOAEL     | Additional data required                        |   |                    |

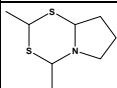



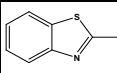




**Table 3.2: Summary of Safety Evaluation Applying the Procedure (based on intakes calculated by the MSDI approach)**

| FL-no          | EU Register name                 | Structural formula  | MSDI 1)<br>(µg/capita/day) | Class 2)<br>Evaluation procedure path 3)                             | Outcome on the named<br>compound<br>[ 4) or 5)] | Outcome on the<br>material of commerce<br>[6), 7), or 8)] | Evaluation remarks |
|----------------|----------------------------------|---|----------------------------|--|---|---|--------------------|
| 15.076<br>1764 | 2-Hexylthiophene                 |    | 0.12                       | Class II<br>B3: Intake below threshold, B4:<br>No adequate NOAEL     | Additional data required                        |   |                    |
| 15.078         | 2-Isobutyl-4,5-dimethylthiazole  |    | 0.12                       | Class II<br>B3: Intake below threshold, B4:<br>Adequate NOAEL exists | 4)  | 6)  |                    |
| 15.080         | 2-Isopropyl-4,5-dimethylthiazole |    | 0.012                      | Class II<br>B3: Intake below threshold, B4:<br>Adequate NOAEL exists | 4)  | 6)  |                    |
| 15.084         | 5-Methyl-2-pentylthiazole        |    | 0.0037                     | Class II<br>B3: Intake below threshold, B4:<br>Adequate NOAEL exists | 4)  | 6)  |                    |
| 15.085         | 4-Methyl-2-propionylthiazole     |    | 0.0037                     | Class II<br>B3: Intake below threshold, B4:<br>Adequate NOAEL exists | 4)  | 6)  |                    |
| 15.089         | 2-Methylthiazole                 |    | 0.018                      | Class II<br>B3: Intake below threshold, B4:<br>Adequate NOAEL exists | 4)  | 6)  |                    |
| 15.091         | 2-Methylthiophene                |    | 0.019                      | Class II<br>B3: Intake below threshold, B4:<br>No adequate NOAEL     | Additional data required                        |   |                    |
| 15.092         | 3-Methylthiophene                |   | 0.12                       | Class II<br>B3: Intake below threshold, B4:<br>No adequate NOAEL     | Additional data required                        |   |                    |
| 15.093         | 2-Octylthiophene                 |  | 0.012                      | Class II<br>B3: Intake below threshold, B4:<br>No adequate NOAEL     | Additional data required                        |   |                    |
| 15.094         | 2-Pentanoylthiophene             |  | 0.0012                     | Class II<br>B3: Intake below threshold, B4:<br>No adequate NOAEL     | Additional data required                        |   |                    |
| 15.096         | sec-Pentylthiophene              |  | 0.24                       | Class II<br>B3: Intake below threshold, B4:<br>No adequate NOAEL     | Additional data required                        |   |                    |
| 15.097         | 2-Propionylthiophene             |  | 0.12                       | Class II<br>B3: Intake below threshold, B4:<br>No adequate NOAEL     | Additional data required                        |   |                    |

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

| FL-no  | EU Register name                                       | Structural formula  | MSDI 1)<br>(µg/capita/day) | Class 2)<br>Evaluation procedure path 3)                             | Outcome on the named<br>compound<br>[ 4) or 5)] | Outcome on the<br>material of commerce<br>[6), 7), or 8)] | Evaluation remarks |
|--------|--|---|----------------------------|--|---|---|--------------------|
| 15.098 | 2-Propylthiazole                                       |    | 0.085                      | Class II<br>B3: Intake below threshold, B4:<br>Adequate NOAEL exists | 4)  | 6)  |                    |
| 15.107 | Thiophene-2-carbaldehyde                               |    | 0.21                       | Class II<br>B3: Intake below threshold, B4:<br>No adequate NOAEL     | Additional data required                        |   |                    |
| 15.115 | 2-Isobutyl-4-methyl thiazole                           |    | 0.011                      | Class II<br>B3: Intake below threshold, B4:<br>Adequate NOAEL exists | 4)  | 6)  |                    |
| 15.116 | 2-Acetyl-4-ethylthiazole                               |    | 0.024                      | Class II<br>B3: Intake below threshold, B4:<br>Adequate NOAEL exists | 4)  | 6)  |                    |
| 15.118 | 4-Butylthiazole  |    | 1.3                        | Class II<br>B3: Intake below threshold, B4:<br>Adequate NOAEL exists | 4)  | 6)  |                    |
| 15.129 | Tetrahydro-2,4,6-trimethyl-1,3,5(2H)-thiadiazine       |    | 0.61                       | Class II<br>B3: Intake below threshold, B4:<br>No adequate NOAEL     | Additional data required                        |   |                    |
| 15.060 | 2,4-Dimethyl-3-thiazoline                              |    | 0.012                      | Class II<br>No evaluation  |   |   | a)                 |
| 15.086 | 2-Methyl-2-thiazoline                                  |    | 0.24                       | Class II<br>No evaluation  |   |   | a)                 |
| 15.090 | 2-Methylthiazolidine                                   |   | 0.024                      | Class II<br>No evaluation  |   |   | a)                 |
| 15.099 | 2-Propylthiazolidine                                   |  | 0.012                      | Class II<br>No evaluation  |   |   | a)                 |
| 15.119 | 2-Isobutyl-3-thiazoline                                |  | 0.011                      | Class II<br>No evaluation  |   |   | a)                 |
| 15.042 | 2-Butyl-4-methyl(4H)pyrrolidino[1,2d]-1,3,5-dithiazine |  | 0.0012                     | Class III<br>B3: Intake below threshold, B4:<br>No adequate NOAEL    | Additional data required                        |   |                    |

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

| FL-no          | EU Register name                                       | Structural formula  | MSDI 1)<br>(µg/capita/day) | Class 2)<br>Evaluation procedure path 3)                              | Outcome on the named<br>compound<br>[ 4) or 5)] | Outcome on the<br>material of commerce<br>[6), 7), or 8)] | Evaluation remarks  |
|----------------|--|---|----------------------------|---|---|---|---|
| 15.055<br>1763 | 2,4-Dimethyl(4H)pyrrolidino[1,2e]-<br>1,3,5-dithiazine |    | 0.055                      | Class III<br>B3: Intake below threshold, B4:<br>No adequate NOAEL     | Additional data required                        |   |   |
| 15.077         | 4-Hydroxy-2,5-dimethylthiophen-<br>3(2H)-one           |    | 0.12                       | Class III<br>B3: Intake below threshold, B4:<br>No adequate NOAEL     | Additional data required                        |   |   |
| 15.082         | 3-Mercaptothiophene                                    |    | 0.011                      | Class III<br>B3: Intake below threshold, B4:<br>Adequate NOAEL exists | 4)  | 6)  |   |
| 15.087         | 2-Methyl-3-mercaptothiophene                           |    | 0.12                       | Class III<br>B3: Intake below threshold, B4:<br>Adequate NOAEL exists | 4)  | 6)  |   |
| 15.088         | 2-Methyl-4,5-benzothiazole                             |    | 0.0085                     | Class III<br>B3: Intake below threshold, B4:<br>No adequate NOAEL     | Additional data required                        |   |   |
| 15.106         | Thiophene  |    | 0.12                       | Class III<br>B3: Intake below threshold, B4:<br>No adequate NOAEL     | Additional data required                        |   |   |
| 15.108         | 2-Thiophenemethanethiol                                |    | 0.0073                     | Class III<br>B3: Intake below threshold, B4:<br>Adequate NOAEL exists | 4)  | 6)  |   |
| 15.114         | 6-Acetyl-2,3-dihydro-1,4-thiazine                      |   | 0.012                      | Class III<br>No evaluation  |   |   | Genotoxic alert.<br>(Register name 5-Acetyl-2,3-<br>dihydro-1,4-thiazine. |
| 15.133<br>1766 | 5-Acetyl-2,3-dihydro-1,4-thiazine                      |  | 0.61                       | Class III<br>No evaluation  |   |   | Genotoxic alert.  |

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

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

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

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

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

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

7) Tentatively regarded as presenting no safety concern (based on intake calculated by the MSDI approach) pending further information on the purity of the material of commerce and/or information on stereoisomerism.

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

a) *Genotoxic potential in vitro.*

## REFERENCES

- Aeschbacher, H.U., Wolleb, U., Loliger, J., Spadone, J.C., Liardon, R., 1989. Contribution of coffee aroma constituents to the mutagenicity of coffee. *Food Chem. Toxicol.* 27(4), 227-232.
- Cramer, G.M., Ford, R.A., Hall, R.L., 1978. Estimation of toxic hazard - a decision tree approach. *Food Cosmet. Toxicol.* 16(3), 255-276.
- EC, 1996. Regulation No 2232/96 of the European Parliament and of the Council of 28 October 1996. *Official Journal of the European Communities* 23.11.1996, L 299, 1-4.
- EC, 1999a. Commission Decision 1999/217/EC of 23 February 1999 adopting a register of flavouring substances used in or on foodstuffs. *Official Journal of the European Communities* 27.3.1999, L 84, 1-137.
- EC, 2000a. Commission Regulation No 1565/2000 of 18 July 2000 laying down the measures necessary for the adoption of an evaluation programme in application of Regulation (EC) No 2232/96. *Official Journal of the European Communities* 19.7.2000, L 180, 8-16.
- EC, 2009a. Commission Decision 2009/163/EC of 26 February 2009 amending Decision 1999/217/EC as regards the register of flavouring substances used in or on foodstuffs. *Official Journal of the European Union* 27.2.2009, L 55, 41.
- EFFA, 2006r. Addendum of 2 Flavouring Substance (Candidate Chemicals) of the Flavouring Group Evaluation of the Chemical Group 29 (Annex I of 1565/2000/EC) Structurally Related to Sulfur Containing Heterocyclic and Heteroaromatic Derivatives [FAO/WHO JECFAFAS 50/59] used as Flavouring Substances. Addendum to FGE.21 (EFFA submission 2003-10). 7 October 2006. Received 8 December 2006 Unpublished report submitted by EFFA to FLAVIS Secretariat. FLAVIS/8.109.
- EFFA, 2006s. Transfer files for FGE.93 concerning [FL-no: 15.010]. Unpublished data from EFFA to FLAVIS Secretariat.
- EFFA, 2007a. E-mail from Jan Demyttenaere, EFFA to Flavis Secretariat, National Foodinstitute, Technical University of Denmark. Dated 8 February 2007. RE: FLAVIS submissions - use levels for Category 14.2 - Alcoholic beverages FLAVIS/8.70.
- EFSA, 2004d. Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in contact with food on a request from the Commission related to Flavouring Group Evaluation 3: 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). Adopted on 7 October 2004. EFSA-Q-2003-146.
- EFSA, 2009u. Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in contact with food on a request from the Commission related to Flavouring Group Evaluation 21, Revision 1: Thiazoles, thiophene, thiazoline and thienyl derivatives from chemical group 29. Miscellaneous substances from chemical group 30 (Commission Regulation (EC) No 1565/2000 of 18 July 2000). Adopted on 26 March 2009. EFSA-Q-2009-00481.
- Flavour Industry, 2004i. Unpublished information submitted by Flavour Industry to DG SANCO and forwarded to EFSA. A-93.
- Flavour Industry, 2005g. Unpublished information submitted by Flavour Industry to DG SANCO and forwarded to EFSA. A-93.
- Florin, I., Rutberg, L., Curvall, M., Enzell, C.R., 1980. Screening of tobacco smoke constituents for mutagenicity using the Ames' test. *Toxicology.* 18, 219-232.
- JECFA, 1995. Evaluation of certain food additives and contaminants. Forty-fourth Meeting of the Joint FAO/WHO Expert Committee on Food Additives. WHO Technical Report Series, no. 859. Geneva.
- JECFA, 1996a. Toxicological evaluation of certain food additives. The forty-fourth meeting of the Joint FAO/WHO Expert Committee on Food Additives and contaminants. WHO Food Additives Series: 35. IPCS, WHO, Geneva.
- JECFA, 1997a. Evaluation of certain food additives and contaminants. Forty-sixth report of the Joint FAO/WHO Expert Committee on Food Additives. Geneva, 6-15 February 1996. WHO Technical Report Series, no. 868. Geneva.
- JECFA, 1999b. Evaluation of certain food additives and contaminants. Forty-ninth report of the Joint FAO/WHO Expert Committee on Food Additives. Rome, 17-26 June 1997. WHO Technical Report Series, no. 884. Geneva.
- JECFA, 2006c. Joint FAO/WHO Expert Committee on Food Additives. Sixty-seventh meeting Rome, 20-29 June 2006, Summary and Conclusions. Issued 7 July 2006.



- JECFA, 2008b. Safety evaluation of certain food additives and contaminants. Sixty-eight meeting of the Joint FAO/WHO Expert Committee on Food Additives, WHO Food Additives Series: 59. IPCS, WHO, Geneva 2008 [http://whqlibdoc.who.int/publications/2008/9789241660594\\_eng.pdf](http://whqlibdoc.who.int/publications/2008/9789241660594_eng.pdf) (May 2008).
- Lee, H., Bian, S.S., Chen, Y.L., 1994. Genotoxicity of 1,3-dithiane and 1,4-dithiane in the CHO/SCE assay and the Salmonella/microsomal test. *Mutat. Res.* 321, 213-218.
- Mihara, S., Shibamoto, T., 1980. Mutagenicity of products obtained from cysteamineglucose browning model systems. *J. Agric. Food Chem.* 28(1), 62-66.
- Mosier, P.D., Jurs, P.C., Custer, L.L., Durham, S.K., Pearl, G.M., 2003. Predicting the genotoxicity of thiophene derivatives from molecular structure *Chem. Res. Toxicol.* 16(6), 721-732.
- SCF, 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 the minutes of the 119th Plenary meeting. European Commission, Health & Consumer Protection Directorate-General.
- Shibuya, T., 2006. Reverse mutation test of thiophene on bacteria. Kanagawa, Japan, Hatano Research Institute, Food and Drug Safety Center.
- Tanaka, N., (2006. *In vitro* chromosomal aberration test of thiophene on cultured chinese hamster cells. Hatano Research Institute, Food and Drug Safety Center, Kanagawa, Japan. [Online] <http://wwwdb.mhlw.go.jp/ginc/dbfile1/file/file110-02-1.html> [As of January 2009].
- Zeiger, E., Anderson, B., Haworth, S., Lawlor, T., Mortelmans, K., Speck, W., 1987. Salmonella mutagenicity tests. 3. Results from the testing of 255 chemicals. *Environ. Mol. Mutag.* 9(Suppl. 9), 1-110.

**ABBREVIATIONS**

|             |   |
|-------------|---|
| 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   |
| DTU-NFI     | Danish Technical University – National Food Institute                     |
| EFSA        | The European Food Safety Authority  |
| EPA         | United States Environmental Protection Agency                             |
| EU          | European Union  |
| FAO         | Food and Agriculture Organization of the United Nations                   |
| FEMA        | Flavor and Extract Manufacturers Association                              |
| FGE         | Flavouring Group Evaluation   |
| FLAVIS (FL) | Flavour Information System (database)                                     |
| GLP         | Good laboratory practise  |
| ID          | Identity  |
| Ip          | Intraperitoneal   |
| IR          | Infrared spectroscopy   |
| ISS         | Istituto Superiore di Sanita  |
| 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   |
| PCE         | Polychromatic erythrocyte   |
| SCE         | Sister chromatic exchange   |
| SCF         | Scientific Committee on Food  |
| US EPA      | United States Environmental Protection Agency                             |
| WHO         | World Health Organisation   |