EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF); Scientific Opinion on Flavouring Group Evaluation 301 (FGE.301): A sulphur substituted pyrimidin-derivative and its hydrochloride salt

EFSA Publication

Link to article, DOI:
10.2903/j.efsa.2011.1994

Publication date:
2011

Document Version
Publisher's PDF, also known as Version of record

SCIENTIFIC OPINION

Scientific Opinion on Flavouring Group Evaluation 301 (FGE.301):

A sulphur substituted pyrimidin-derivative and its hydrochloride salt from Chemical Group 30[1]

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

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

The Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids of the European Food Safety Authority was requested to evaluate two flavouring substances, 4-amino-5,6-dimethylthieno[2,3-d]pyrimidin-2(1H)-one [FL-no: 16.116] and 4-amino-5,6-dimethylthieno[2,3-d]pyrimidin-2(1H)-one hydrochloride [FL-no: 16.120] in the Flavouring Group Evaluation 301, using the Procedure in Commission Regulation (EC) No 1565/2000. None of the substances were considered to have genotoxic potential. The substances were evaluated through a stepwise approach (the Procedure) that integrates information on structure-activity relationships, intake from current uses, toxicological threshold of concern, and available data on metabolism and toxicity. The Panel concluded that the two substances [FL-no: 16.116 and 16.120] do not give rise to safety concerns at their levels of dietary intake, estimated on the basis of the MSDI approach.

SUMMARY

The European Food Safety Authority (EFSA) asked the Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (the Panel) to provide scientific advice to the Commission on the implications for human health of chemically defined flavouring substances used in or on foodstuffs in the Member States. In particular, the Panel was requested to evaluate two flavouring substances in the

1 On request from the Commission, Question No EFSA-Q-2009-00581, adopted on 19 May 2011.

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

Suggested citation: EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF); Scientific Opinion on Flavouring Group Evaluation 301 (FGE.301):
A sulphur substituted pyrimidin-derivative and its hydrochloride salt. EFSA Journal 2011; 9(6):1994. [28 pp.]. doi:10.2903/j.efsa.2011.1994. Available online: www.efsa.europa.eu/efsajournal.htm

Flavouring Group Evaluation 301 (FGE.301), using the Procedure as referred to in the Commission Regulation (EC) No 1565/2000. These two flavouring substances belong to chemical group 30, Annex I of the Commission Regulation (EC) No 1565/2000.

The present Flavouring Group Evaluation deals with a sulphur substituted pyrimidin-derivative and its hydrochloride salt from chemical group 30.

The two flavouring substances (candidate substances) have no possibility for geometrical or optical isomers.

Both of the flavouring substances are classified into structural class III.

Neither of the substances in the present group has been reported to occur naturally in food items.

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

In the absence of more precise information that would enable the Panel to make a more realistic estimate of the intakes of the flavouring substances, the Panel has decided also to perform an estimate of the daily intakes per person using a modified Theoretical Added Maximum Daily Intake (mTAMDI) approach based on the normal use levels reported by Industry. In those cases where the mTAMDI approach indicated that the intake of a flavouring substance might exceed its corresponding threshold of concern, the Panel decided not to carry out a formal safety assessment using the Procedure. In these cases the Panel requires more precise data on use and use levels.

The results for the available genotoxicity studies do not raise a concern for genotoxicity and hence do not preclude the evaluation of the two candidate substances in this FGE through the Procedure.

From the data available it is not possible to conclude that the two candidate substances in this group [FL-no: 16.116 and 16.120] would be metabolised to innocuous products at the reported levels of intake as flavouring substances.

According to the default MSDI approach, the two flavouring substances in this group have a total intake in Europe of 610 microgram/capita/day which is above the threshold of concern value for structural class III of 90 microgram/person/day. However, an adequate NOAEL exists of 10 microgram/kg bw/day from a 13-weeks study for the candidate substance 4-amino-5,6-dimethylthieno[2,3-d]pyrimidine-2(1H)-one hydrochloride [FL-no: 16.120] which provides a margin of safety of 5900.

When the estimated intakes were based on the mTAMDI approach they were 3600 and 4200 microgram/person/day for the two flavouring substances belonging to structural class III. These intakes are above the threshold of concern of 90 microgram/person/day for structural class III substances. Therefore, for these two substances more reliable exposure data are required. On the basis of such additional data, these flavouring substances should be reconsidered along the steps of the Procedure. Following this procedure additional toxicological data might become necessary.

In order to determine whether the conclusion for the two candidate substances can be applied to the materials of commerce, it is necessary to consider the available specifications. Adequate specifications including complete purity criteria and identity for the materials of commerce have been provided for both flavouring substances.

The Panel concluded that the two candidate substances 4-amino-5,6-dimethylthieno[2,3-d]pyrimidin-2(1H)-one [FL-no: 16.116] and 4-amino-5,6-dimethylthieno[2,3-d]pyrimidin-2(1H)-one hydrochloride [FL-no: 16.120] would present no safety concern at the estimated levels based on the MSDI approach.

KEYWORDS

Flavourings, pyrimidin-derivative, food, safety, FGE.301.

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BACKGROUND

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

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

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

TERMS OF REFERENCE

The European Food Safety Authority (EFSA) is requested to carry out a risk assessment on flavouring substances in the Register prior to their authorisation and inclusion in a Union List according to Commission Regulation (EC) No 1565/2000 (EC, 2000a). In addition, the Commission requested EFSA to evaluate newly notified flavouring substances, where possible, before finalising the evaluation programme.

In addition, in letter of 11 May 2009 the Commission requested EFSA to carry out a risk assessment on 4-amino-5,6-dimethylthieno[2,3-d]pyrimidin-2(1H)-one [FL-no: 16.116] and 4-amino-5,6-dimethylthieno[2,3-d]pyrimidin-2(1H)-one hydrochloride [FL-no: 16.120] in accordance with Commission Regulation (EC) No 1565/2000 (EC, 2000a):

"The European Commission requests the European Food Safety Authority to carry out a risk assessment on eighteen new flavouring substances in accordance with Commission Regulation (EC) No 1565/2000, if possible by the end of the authorisation programme, if not within nine months from the finalisation of that programme."

The deadline of the Terms of Reference for 4-amino-5,6-dimethylthieno[2,3-d]pyrimidin-2(1H)-one [FL-no: 16.116] and 4-amino-5,6-dimethylthieno[2,3-d]pyrimidin-2(1H)-one hydrochloride [FL-no: 16.120] was negotiated to 31 May 2011.

The remaining substances of this request were evaluated in other FGEs.

ASSESSMENT

1. Presentation of the Substances in Flavouring Group Evaluation 301

1.1. Description

The present Flavouring Group Evaluation 301 (FGE.301), using the Procedure as referred to in the Commission Regulation (EC) No 1565/2000 (EC, 2000a) (The Procedure - shown in schematic form in Annex I of this FGE), deals with a sulphur substituted pyrimidin-derivative and its hydrochloride salt from chemical group 30, Annex I of Commission Regulation (EC) No 1565/2000 (EC, 2000a).

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

The two flavouring substances (candidate substances) are 4-amino-5,6-dimethylthieno[2,3-d]pyrimidin-2(1H)-one [FL-no: 16.116] and 4-amino-5,6-dimethylthieno[2,3-d]pyrimidin-2(1H)-one hydrochloride [FL-no: 16.120]. No sufficiently structurally related substances to support the evaluation of the candidate substances were identified.

The outcome of the Safety Evaluation is summarised in Table 2a.

1.2. Stereoisomers

The two candidate substances cannot exist as geometrical or optical isomers.

1.3. Natural Occurrence in Food

The candidate substances [FL-no: 16.116 and 16.120] have not been reported to occur naturally in any food items (TNO, 2010).

2. Specifications

Purity criteria for the two substances have been provided by the Flavour Industry (Flavour Industry, 2009l) (Table 1).

Judged against the requirements in Annex II of Commission Regulation (EC) No 1565/2000 (EC, 2000a), this information is adequate for both candidate substances.

3. Intake Data

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

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

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

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

One of the alternatives is the "Theoretical Added Maximum Daily Intake" (TAMDI) approach, which is calculated on the basis of standard portions and upper use levels (SCF, 1995) for flavourable beverages and foods in general, with exceptional levels for particular foods. This method is regarded as a conservative estimate of the actual intake by most consumers because it is based on the

assumption that the consumer regularly eats and drinks several food products containing the same flavouring substance at the upper use level.

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

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

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

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

The anticipated annual volume of production of 4-amino-5,6-dimethylthieno[2,3-d]pyrimidin-2(1H)-one [FL-no: 16.116] and its hydrochloride salt [FL-no: 16.120] from use as flavouring substances in Europe has been reported to be approximately 5000 kg (Flavour Industry, 2009l).

The annual production volume is given as a total for the two flavouring substances rather than individual values. Based on this production volume the daily *per capita* intake is calculated to 610 µg in total (Table 2).

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

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

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

For the two candidate substances information on food categories and normal and maximum use levels[5,6] were submitted by the Flavour Industry (Flavour Industry, 2009l). The two candidate substances are used in flavoured food products divided into the food categories, outlined in Annex III of the Commission Regulation (EC) No 1565/2000 (EC, 2000a), as shown in Table 3.1. For the present calculation of mTAMDI, the reported normal use levels were used. In the case where different use levels were reported for different food categories the highest reported normal use level was used.

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

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

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

Table 3.1 Use of Candidate Substances

Food category	Description	Flavourings used
01.0	Dairy products, excluding products of category 2	Yes
02.0	Fats and oils, and fat emulsions (type water-in-oil)	No
03.0	Edible ices, including sherbet and sorbet	Yes
04.1	Processed fruits	No
04.2	Processed vegetables (incl. mushrooms & fungi, roots & tubers, pulses and legumes), and nuts & seeds	No
05.0	Confectionery	Yes
06.0	Cereals and cereal products, incl. flours & starches from roots & tubers, pulses & legumes, excluding bakery	Yes
07.0	Bakery wares	Yes
08.0	Meat and meat products, including poultry and game	No
09.0	Fish and fish products, including molluscs, crustaceans and echinoderms	No
10.0	Eggs and egg products	No
11.0	Sweeteners, including honey	No
12.0	Salts, spices, soups, sauces, salads, protein products etc.	No
13.0	Foodstuffs intended for particular nutritional uses	Yes
14.1	Non-alcoholic ("soft") beverages, excl. dairy products	Yes
14.2	Alcoholic beverages, incl. alcohol-free and low-alcoholic counterparts	Yes
15.0	Ready-to-eat savouries	No
16.0	Composite foods (e.g. casseroles, meat pies, mincemeat) - foods that could not be placed in categories 1 – 15	Yes

According to the Flavour Industry the normal use levels for the candidate substances are in the range of 4 - 10 mg/kg food and the maximum use levels are in the range of 8.5 - 25 mg/kg (Flavour Industry, 2009l).

The mTAMDI values for the two candidate substances are 3600 and 4200 microgram/person/day for [FL-no: 16.116 and 16.120], respectively.

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

4. Absorption, Distribution, Metabolism and Elimination

There are structural similarities between the candidate substances and the DNA bases cytosine and guanine which may indicate that oxidation of the amino group and eventually ring opening occur in this part of the molecule. However, there is no experimental evidence of the metabolism of the thiophene moiety of the molecule, and therefore the candidate substances cannot be anticipated to be metabolised to innocuous products in accordance with the decision for other thiophenes evaluated in FGE.21.

Because both candidate substances have the same pharmacokinetics after oral administration, leading to the same C_{max}, the systemic toxicity of the two substances will be similar.

For more detailed information, see Annex III.

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

The application of the Procedure is based on intakes estimated on the basis of the MSDI approach. Where the mTAMDI approach indicates that the intake of a flavouring substance might exceed its corresponding threshold of concern, a formal safety assessment is not carried out using the Procedure.

In these cases the Panel requires more precise data on use and use levels. For comparison of the intake estimations based on the MSDI approach and the mTAMDI approach, see Section 6.

For the safety evaluation of the two candidate substances from chemical group 30 the Procedure as outlined in Annex I was applied, based on the MSDI approach. The stepwise evaluations of the substances are summarised in Table 2.

Step 1

Both candidate substances are classified according to the decision tree approach by Cramer et al. (Cramer et al., 1978) into structural class III.

Step 2

Step 2 requires consideration of the metabolism of the candidate substances. The two candidate substances [FL-no: 16.116 and 16.120], cannot be anticipated to be metabolised to innocuous products and thus the evaluation proceeds via the B-side of the Procedure scheme.

Step B3

The total estimated daily per capita intake of the two candidate substances [FL-no: 16.116 and 16.120] is 610 microgram, which is above the threshold for their structural class of 90 microgram/person/day (class III).

The Panel therefore considered a 13-week oral sub-chronic toxicity study carried out using the candidate substance 4-amino-5,6-dimethylthieno[2,3-d]pyrimidin-2(1H)-one hydrochloride [FL-no: 16.120] in rats (see Section 8.2). Based on this study, a No Observed Adverse Effect Level (NOAEL) of 60 mg/kg body weight (bw)/day was identified. The MSDI value of 610 microgram/*capita*/day is equivalent to 10.2 microgram/kg bw/day, at a body weight of 60 kg. Thus, the margin of safety is 5900.

Based on results of the safety evaluation sequence of the Procedure, 4-amino-5,6-dimethylthioeno[2,3-d]pyrimidin-2(1H)-one [FL-no: 16.116] and its hydrochloride salt [FL-no: 16.120] are not anticipated to pose a safety concern when used as flavouring substances at the estimated levels of intake, based on the MSDI approach.

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

The estimated intakes of the substances [FL-no: 16.116 and 16.120] assigned to structural class III, based on the mTAMDI, are 3600 and 4200 microgram/person/day, respectively, which are above the threshold of concern for structural class III of 90 microgram/person/day.

Thus, for the two candidate substances [FL-no: 16.116 and 16.120] further information is required. This would include more reliable intake data and then, if required, additional toxicological data.

For comparison of the MSDI and mTAMDI values, see Table 6.1.

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

FL-no	EU Register name	MSDI* (µg/*capita*/day)	mTAMDI (µg/person/day)	Structural class	Threshold of concern (µg/person/day)
16.116	4-Amino-5,6-dimethylthioenol[2,3-d]pyrimidin-2(1H)-one	0.0	3600	Class III	90
16.120	4-Amino-5,6-dimethylthioenol[2,3-d]pyrimidin-2(1H)-one hydrochloride	610	4200	Class III	90

* Industry has provided a total production volume for 16.116 and 16.120 of 5000 kg/year, MSDI covers both substances, meaning that the value may range from 0 to 610 µg/capita/day for each of the two substances.

7. Considerations of Combined Intakes from Use as Flavouring Substances

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

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

On the basis of the reported annual production volumes in Europe (Flavour Industry, 2009l), the combined estimated daily *per capita* intake as flavourings of the two candidate substances belonging to structural class III is 610 microgram. This value exceeds the threshold of concern for structural class III of 90 microgram/person/day. However, as there is an appropriate NOAEL from an adequate 13-week study on the candidate substance [FL-no: 16.120], no safety concern would be anticipated for these flavouring compounds at the anticipated level of use as a flavouring substance. No structurally related supporting substances have been identified that should be taken into account for the combined intake calculation.

8. Toxicity

8.1. Acute Toxicity

A study is available for one of the candidate substances. Based on clinical observations and gross necropsy, 4-amino-5,6-dimethylthieno[2,3-d]pyrimidin-2(1H)-one [FL-no: 16.116] did not show any evidence of toxicity when administered orally to rats at dose levels up to 50 mg/kg bw (Arulnesan, 2007).

8.2. Subacute, Subchronic, Chronic and Carcinogenicity Studies

Subacute and subchronic toxicity data are available for the two candidate substances, 4-amino-5,6-dimethylthieno[2,3-d]pyrimidin-2(1H)-one [FL-no: 16.116] and 4-amino-5,6-dimethylthieno[2,3-d]pyrimidin-2(1H)-one hydrochloride [FL-no: 16.120].

A 21-day oral toxicity screen test was carried out using the candidate substance [FL-no: 16.116] in Sprague Dawley Crl:CD® (SD) rats (5/sex/group) at doses that were calculated to be 10.3, 29.4 and 101.0 mg/kg/day for males and 10.9, 31.1 and 103.0 mg/kg/day for females (Ross, 2008). There were no clinical signs that were attributable to treatment and no animals were killed prematurely. No effect of treatment upon body weight gain or food consumption was observed. Liver weights were slightly higher in males and females receiving 100 mg/kg/day. There were no treatment-related macroscopic or microscopic changes in the intact animals or in any of the tissues examined.

A 13-week oral sub-chronic toxicity study was carried out using the candidate substance 4-amino-5,6-dimethylthieno[2,3-d]pyrimidin-2(1H)-one hydrochloride [FL-no: 16.120] in Sprague Dawley Crl:CD® (SD) rats (20/sex/group) by dietary ingestion resulting in doses of 10.3, 30.8 and 61.9 mg/kg bw/day for males and 10.3, 30.6 and 60.3 mg/kg body weight (bw)/day for females (Ross, 2009). The

test material induced no treatment-related changes in mortality, appearance, behavior, body weight, food consumption or ophthalmic function.

Although some changes were reported in certain hematological parameters, they were inconsistent, and minor, either occurred in one sex only or lacked a dose relationship and therefore were not considered toxicologically significant. Clinical chemistry revealed statistically significantly increased total plasma cholesterol concentrations in low-dose females on day 14 (+29 %) and week 13 (+25 %) and in mid- (30 mg/kg bw/day) and high-dose females on day 14 (+23 % and +46 %, respectively), week 6 (+28 % and +44 %, respectively) and week 13 (+34 % and +49 %, respectively) compared to the control group. In males, a significant increase in total plasma cholesterol concentration was reported at the high-dose level, but only on day 14 (+25 %). Total triglyceride concentration were slightly but statistically significantly increased in high dose males (+59 %), but only at day 14, and in high dose females at week 13 (+50 %). The increased total plasma cholesterol and triglyceride levels were considered to be associated with increased liver weights (liver weights of females: control: 10.833 g (mean of 20 rats); low-dose: 11.099 g; mid-dose: 11.819 g; high-dose: 11.855 g. Liver weights of male: control: 20.154 g; low-dose: 20.637 g; mid-dose: 22.053 g; high-dose: 23.095 g). No other dose related changes were noted in clinical chemistry parameters.

Urinalysis revealed a dose-dependent increase in protein excretion attaining statistical significance in males and a non-dose-dependent reduction in specific gravity at all dose levels in females. The increase in urinary protein levels in males was attributed to high values in a single animal.

After 13 weeks of treatment, relative liver weights were elevated in a dose-related manner in animals given 30 or 60 mg/kg/day, relative kidney weights were slightly high in males given 60 mg/kg/day and relative thyroid weights were slightly high in females given 60 mg/kg/day. There were no treatment-related macroscopic changes after 13 weeks of treatment. Histopathological changes related to treatment with [FL-no: 16.120] were confined to minimal centrilobular hypertrophy in the liver of a few animals given 60 mg/kg/day. There was no evidence of hepatocyte vacuolation, indicative of disturbances in intra-hepatocyte fat metabolism associated with the changes in plasma total cholesterol and triglycerides found in clinical chemistry.

The Panel noted that histopathological changes were confined to minimal centrilobular hypertrophy in the liver of a few animals at the top dose. The Panel considered that these changes were adaptive in nature, and that the increases in plasma total cholesterol in female animals at all doses and in males receiving 60 mg/kg bw/day, also the increases in plasma total triglycerides in both males and females receiving 60 mg/kg bw/day, were associated with the adaptive changes. The Panel considered therefore that these clinical chemistry changes were not of toxicological concern, and that since the changes in liver weight were not accompanied by any significant histopathological change, the top dose of 60 mg/kg bw/day could be considered as a NOAEL, as proposed by the authors of the study (Ross, 2009).

Because both candidate substances have the same pharmacokinetics after oral administration, leading to the same C_{max} the systemic toxicity of the two substances will be similar. Therefore the 13-weeks study for [FL-no: 16.120] is also valid for [FL-no: 16.116].

Repeated dose toxicity data are summarised in Annex IV, Table IV.2.

8.3. Developmental / Reproductive Toxicity Studies

No data are submitted or available from literature search.

8.4. Genotoxicity Studies

A reverse mutation assay was carried out on the candidate substance 4-amino-5,6-dimethylthieno[2,3-d]pyrimidine-2(1H)-one hydrochloride [FL-no: 16.120] in *Salmonella typhimurium* strains TA98,

TA100, TA1535 and TA1537 and in *Escherichia coli* strain WP2 *uvrA* in the presence and absence of S9 and in accordance with the OECD Guideline 471[7] (Zhang, 2008a). It was concluded that 4-amino-5,6-dimethylthieno[2,3-d]pyrimidine-2(1H)-one hydrochloride [FL-no: 16.120] was not mutagenic to *S. typhimurium* strains TA98, TA100, TA1535 and TA1537 and to *E. coli* strain WP2 *uvrA* under the test conditions.

An *in vitro* chromosome aberration test was performed on 4-amino-5,6-dimethylthieno[2,3-d]pyrimidine-2(1H)-one hydrochloride [FL-no: 16.120] in the presence and absence of S9 according to OECD Guideline 473[8] (Zhang, 2008c).Chromosome aberrations were not observed in the solvent control cultures and only sporadically encountered in the cultures treated with the test material. The percentage of cells with chromosome abnormalities was at a level similar to the historical solvent control data from this laboratory (1-4 %) and did not meet the criteria for a positive response. It is concluded that 4-amino-5,6-dimethylthieno[2,3-d]pyrimidine-2(1H)-one hydrochloride [FL-no: 16.120] did not induce chromosomal aberrations in cultured WBL Chinese hamster ovary cells under the conditions of the test.

A mouse micronucleus test was performed on 4-amino-5,6-dimethylthieno[2,3-d]pyrimidine-2(1H)-one hydrochloride [FL-no: 16.120] in accordance with the OECD Guideline 474[9] (Zhang, 2008b). Five groups of 14 male mice received the test material by gavage, suspended in 0.5 % methyl cellulose in water at dose levels of 500, 1000 and 2000 mg/kg. A negative control group and a positive control group (cyclophosphamide at 70 mg/kg) were included.

Seven animals from each group were sacrificed at 24 hours and seven were sacrificed at 48 hours after dosing. 2000 polychromatic erythrocytes (PCEs) per animal were scored for presence of micronuclei. In addition, the numbers of normochromatic erythrocytes with micronuclei per 2000 polychromatic erythrocytes were scored and the polychromatic/normochromatic ratio was established. The presence/absence of micronuclei was also confirmed by applying a DNA specific stain (Feulgen stain) to slides from the positive control group, and to the high dose group at 24 hours. There was no statistically significant or dose dependent increase in the number of PCEs with micronuclei at any of the dose levels or time points compared to the negative control group.

Based on the results above, it was concluded that the test material did not induce micronuclei in this test at the dose levels up to 2000 mg/kg administered orally by gavage. There was no reduction in the ratio of normochromatic to polychromatic erythrocytes and therefore it is not possible to conclude that the test compound reaches the bone marrow. However, as the substance did not raise a concern for chromosomal damage in the *in vitro* assay, this limitation would not put a constraint on the evaluation of the substance.

The *in vitro* and *in vivo* genotoxicity studies on the candidate substance [FL-no: 16.120] have been carried out to OECD Guidelines and are of good quality and are considered to be supporting for the candidate substance [FL-no: 16.116]. Negative results were obtained in all of the genotoxicity studies carried out on the candidate substance [FL-no: 16.120].

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

9. Conclusions

The two candidate substances are a sulphur-substituted pyrimidine derivative [FL-no: 16.116] and its hydrochloride salt [FL-no: 16.120] that belong to EU chemical group 30.

[7] OECD Guideline for Testing of Chemicals – 471, Bacterial Reverse Mutation Test" (OECD, 1997).
[8] OECD Guideline for the Testing of Chemicals – 473, In Vitro Mammalian Chromosome Aberration Test (1997).
[9] OECD Guideline for the Testing of Chemicals, Section 474 (OECD, 1997).

The two candidate substances cannot exist as geometrical or optical isomers.

Both of the flavouring substances are classified into structural class III.

Neither of the substances in the present group has been reported to occur naturally in any food items.

The results for the available genotoxicity studies do not raise a concern for genotoxicity and hence do not preclude the evaluation of the two candidate substances in this FGE through the Procedure.

From the data available it is not possible to conclude that the two candidate substances in this group [FL-no: 16.116 and 16.120] would be metabolised to innocuous products at the reported levels of intake as flavouring substances.

According to the default MSDI approach, the two flavouring substances in this group have a total intake in Europe of 610 microgram/capita/day which is above the threshold of concern value for structural class III of 90 microgram/person/day. However, a NOAEL of 60 mg/kg bw/day could be derived from a 13-weeks study for the candidate substance 4-amino-5,6-dimethylthieno[2,3-d]pyrimidine-2(1H)-one hydrochloride [FL-no: 16.120] which provides a margin of safety of 5900.

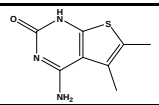
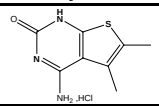
When the estimated intakes were based on the mTAMDI approach they were 3600 and 4200 microgram/person/day for the two flavouring substances belonging to structural class III. These intakes are above the threshold of concern of 90 microgram/person/day for structural class III substances. Therefore, for these two substances more reliable exposure data are required. On the basis of such additional data, these flavouring substances should be reconsidered along the steps of the Procedure. Following this procedure additional toxicological data might become necessary.

In order to determine whether the conclusion for the two candidate substances can be applied to the materials of commerce, it is necessary to consider the available specifications. Adequate specifications including complete purity criteria and identity for the materials of commerce have been provided for both flavouring substances.

The Panel concluded that the two candidate substances 4-amino-5,6-dimethylthieno[2,3-d]pyrimidin-2(1H)-one [FL-no: 16.116] and 4-amino-5,6-dimethylthieno[2,3-d]pyrimidin-2(1H)-one hydrochloride [FL-no: 16.120] would present no safety concern at the estimated levels based on the MSDI approach.

TABLE 1: SPECIFICATION SUMMARY OF THE SUBSTANCES IN THE FLAVOURING GROUP EVALUATION 301

Table 1: Specification Summary of the Substances in the Flavouring Group Evaluation 301

FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	Specification comments
16.116	4-Amino-5,6-dimethylthioenol[2,3-d]pyrimidin-2(1H)-one		4669 121746-18-7	Solid $C_8H_9N_3OS$ 195.20	Slightly soluble Soluble	 Decompose IR NMR MS 98 %	n.a. n.a.	
16.120	4-Amino-5,6-dimethylthioenol[2,3-d]pyrimidin-2(1H)-one hydrochloride		4669 1033366-59-4	Solid $C_8H_9N_3OS,HCl$ 231.70	Sparingly soluble Soluble	 Decompose IR NMR MS 99 %	n.a. n.a.	

1) Solubility in water, if not otherwise stated.

2) Solubility in 95 % ethanol, if not otherwise stated.

3) At 1013.25 hPa, if not otherwise stated.

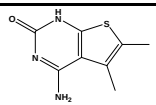
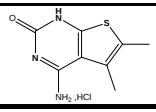
4) At 20°C, if not otherwise stated.

5) At 25°C, if not otherwise stated.

n.a.: not applicable.

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

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

FL-no	EU Register name	Structural formula	MSDI 1) (µg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
16.116	4-Amino-5,6-dimethylthioenol[2,3-d]pyrimidin-2(1H)-one		0.0	Class III B3: Intake above threshold, Data available Adequate NOAEL exists	4)	6)	
16.120	4-Amino-5,6-dimethylthioenol[2,3-d]pyrimidin-2(1H)-one hydrochloride		610	Class III B3: Intake above threshold, Data available Adequate NOAEL exists	4)	6)	

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

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

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

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

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

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

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

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

ANNEX I: PROCEDURE FOR THE SAFETY EVALUATION

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

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

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

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

- can the flavourings be predicted to be metabolised to innocuous products[10] (Step 2)?
- do their exposures exceed the threshold of concern for the structural class (Step A3 and B3)?
- are the flavourings or their metabolites endogenous[11] (Step A4)?
- does a NOAEL exist on the flavourings or on structurally related substances (Step A5 and B4)?

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

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

[10] *"Innocuous metabolic products": Products that are known or readily predicted to be harmless to humans at the estimated intakes of the flavouring agent" (JECFA, 1997a).*

[11] *"Endogenous substances": Intermediary metabolites normally present in human tissues and fluids, whether free or conjugated; hormones and other substances with biochemical or physiological regulatory functions are not included (JECFA, 1997a).*

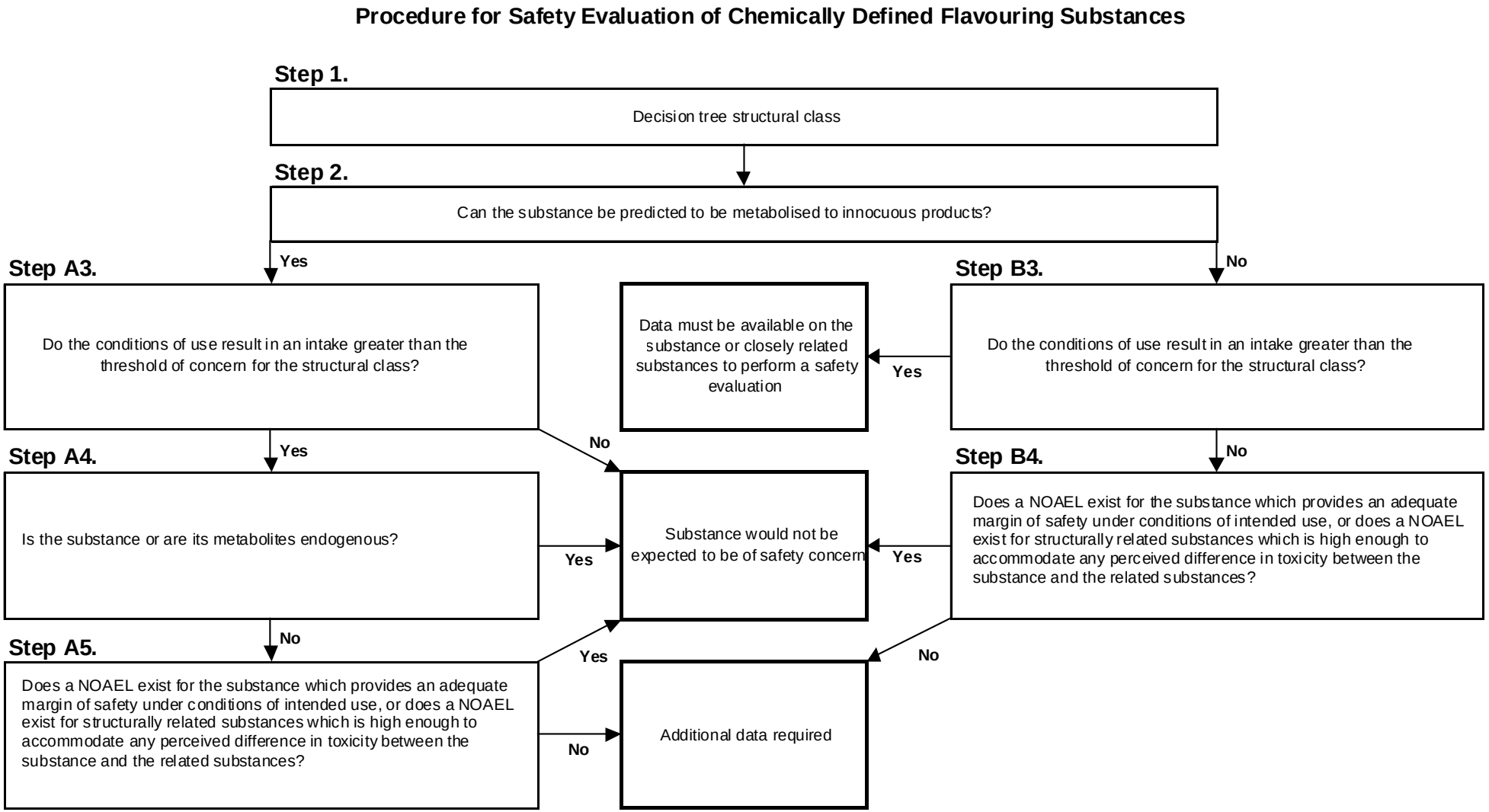


Figure I.1 *Procedure for Safety Evaluation of Chemically Defined Flavouring Substances*

ANNEX II: USE LEVELS / MTAMDI

II.1 Normal and Maximum Use Levels

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

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

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

The "normal and maximum use levels" are provided by Industry for the two candidate substances in the present flavouring group (Table II.1.2).

Table II.1.2 Normal and Maximum use levels (mg/kg) for the candidate substances in FGE.301 (Flavour Industry, 2009l)

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
16.116	6,4	-	6,4	-	-	8,5	8,5	4	-	-	-	-	-	8,5	6,4	6,4	-	6,4
	13	-	13	-	-	21	17	8,5	-	-	-	-	-	17	13	13	-	13
16.120	7,5	-	7,5	-	-	10	10	5	-	-	-	-	-	10	7,5	7,5	-	7,5
	15	-	15	-	-	25	20	10	-	-	-	-	-	20	15	15	-	15

II.2 mTAMDI Calculations

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

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

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

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

- Beverages (SCF, 1995) correspond to food category 14.1 (EC, 2000a)
- Foods (SCF, 1995) correspond to the food categories 1, 2, 3, 4.1, 4.2, 6, 7, 8, 9, 10, 13, and/or 16 (EC, 2000a)
- Exception a (SCF, 1995) corresponds to food category 5 and 11 (EC, 2000a)
- Exception b (SCF, 1995) corresponds to food category 15 (EC, 2000a)
- Exception c (SCF, 1995) corresponds to food category 14.2 (EC, 2000a)
- Exception d (SCF, 1995) corresponds to food category 12 (EC, 2000a)
- Exception e (SCF, 1995) corresponds to others, e.g. chewing gum.

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

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

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

Food categories according to Commission Regulation 1565/2000	Distribution of the seven SCF food categories
placed in categories 01.0 - 15.0	

The mTAMDI values (see Table II.2.3) are presented for each of the two flavouring substances in the present flavouring group, for which Industry has provided use and use levels (Flavour Industry, 2009l). The mTAMDI values are only given for the highest reported normal use levels.

TableII.2.3 Estimated intakes based on the mTAMDI approach

FL-no	EU Register name	mTAMDI (µg/person/day)	Structural class	Threshold of concern (µg/person/day)
16.116	4-Amino-5,6-dimethylthioenol[2,3-d]pyrimidin-2(1H)-one	3600	Class III	90
16.120	4-Amino-5,6-dimethylthioenol[2,3-d]pyrimidin-2(1H)-one hydrochloride	4200	Class III	90

ANNEX III: METABOLISM

III.1. Introduction

The present FGE consists of two candidate substances, 4-amino-5,6-dimethylthieno[2,3-d]pyrimidin-2(1H)-one [FL-no: 16.116] and its hydrochloride salt [FL-no: 16.120].

III.2. Absorption, Distribution and Elimination

One study describing the absorption and elimination of the two candidate substances from the blood was published. This study was performed largely to ensure that studies carried out on 4-amino-5,6-dimethylthieno[2,3-d]pyrimidine-2(1H)-one hydrochloride [FL-no: 16.120] would be applicable to the free base [FL-no: 16.116] and vice versa (Taylor, 2008).

The test was carried out following single gavage administration of the hydrochloride salt and of the free base to Sprague-Dawley rats (6/sex/group) at a dose level of 100 mg/kg. Blood samples were taken from the orbital sinus of the test animals at 0, 30 minutes, 1, 2, 4, 8 24 and 48 hours after dosing.

Both the hydrochloride salt and the free base were identified in the plasma as the free base. Both were readily absorbed after oral administration in Sprague-Dawley rats. The maximum concentration (C_{max}) values after dosing with both test materials were comparable (25056.3 ng/mL for the free base and 28600.3 ng/mL for the hydrochloride salt). The rate of absorption (K_a) was slightly higher for the hydrochloride salt (0.821 hr^{-1}) as compared to the free base (0.550 hr^{-1}), resulting in a shorter time to reach maximum concentration (T_{max}) and a shorter half-life of absorption phase values (i.e. 2.0 and 0.844 hours after dosing with the hydrochloride salt; 4.0 and 1.26 hours after dosing with the free base, respectively). The relative bioavailability of the free base as compared to the hydrochloride salt was 109.4 % and the mean residence time (MRT) in plasma after dosing with the free base (5.4 hours) was slightly higher than the MRT after dosing with the hydrochloride salt (4.6 hours).

The elimination phase after dosing with both test materials appeared to follow first-order kinetics. The rates of elimination were slower than the rates of absorption. A slightly higher K_a and lower $T_{1/2}$(e) were observed after administration of the free base (0.098 hr^{-1}; 7.04 hr) than after administration of the hydrochloride salt (0.065 hr^{-1}; 10.67 hr). However, the apparent clearance (CL/F) after dosing with the hydrochloride salt (479.39 mL/hr/kg) was slightly higher than that after dosing with the free base (438.29 mL/hr/kg), correlating to the small increase in relative bioavailability of the free base. The potential for accumulation in plasma with repeated dosing could not be assessed. Overall, the absorption and elimination kinetics for both test materials were comparable.

Absorption and elimination kinetics for both test articles (4-amino-5,6-dimethylthieno[2,3-d]pyrimidine-2(1H)-one hydrochloride [FL-no: 16.120] and 4-amino-5,6-dimethylthieno[2,3-d]pyrimidine-2(1H)-one [FL-no: 16.116] were comparable, with the hydrochloride salt showing slightly increased rates of both absorption and clearance producing a shorter disposition phase compared to that observed for the free base. As a result, a small increase in the extent of exposure to the free base in comparison to the hydrochloride salt was observed.

III.3. Metabolism

No studies have been undertaken specifically to examine the metabolism of the two candidate substances.

Whilst the candidate substances have structural similarities to the DNA bases cytosine and guanine, they also contain a thiophene ring.

Cytosine is degraded through the pyrimidine degradation pathway involving transformation into uracil and dihydrouracil through oxidation of the amino group and finally ring opening to form beta alanine (Blakely, 1988). Guanine presents a similar structure in which the pyrimidine ring is fused with another heterocyclic moiety. Guanine is degraded through the purine degradation pathway involving transformation into xanthine and then uric acid (Voet and Voet, 1995d).

The thiophene moiety itself may undergo S-oxidation to give a sulphoxide as primary metabolite. This can subsequently undergo spontaneous conjugation with glutathione (GSH). It may also exhibit reactivity toward protein thiols (EFSA, 2009u). Other substances containing a thiophene ring were evaluated in FGE.21 where it could not be concluded that the substances would be metabolised to innocuous products, and they were therefore taken via the B-side of the Procedure. Therefore, the two substances considered in this FGE will also be evaluated through the Procedure scheme via the B-side.

III.4. Summary and Conclusions

There are structural similarities between the candidate substances and the DNA bases cytosine and guanine which may indicate that oxidation of the amino group and eventually ring opening occur in this part of the molecule. However, there is no experimental evidence of the metabolism of the thiophene moiety of the molecule, and therefore the candidate substances cannot be anticipated to be metabolised to innocuous products in accordance with the decision for other thiophenes evaluated in FGE.21.

ANNEX IV: TOXICITY

TABLE IV.1: ACUTE TOXICITY

Oral acute toxicity data are available for one candidate substances of the present Flavouring Group Evaluation

TABLE IV.1: ACUTE TOXICITY

Chemical Name [FL-no]	Species	Sex	Route	LD_{50} (mg/kg bw)	Reference	Comments
4-Amino-5,6-dimethylthioenol[2,3-d]pyrimidin-2(1H)-one [16.116]	Rats	M	Gavage	No LD_{50} obtained	(Arulnesan, 2007)	Substance tested up to 50 mg/kg without any effect.

M = Male.

Subacute / Subchronic / Chronic / Carcinogenic toxicity data are available for the two candidate substances of the present Flavouring Group Evaluation from chemical group 30.

TABLE IV.2: SUBACUTE / SUBCHRONIC / CHRONIC / CARCINOGENICITY STUDIES

Chemical Name [FL-no]	Species; Sex No./Group	Route	Dose levels	Duration	NOAEL (mg/kg bw/day)	Reference	Comments
4-Amino-5,6-dimethylthioenol[2,3-d]pyrimidin-2(1H)-one hydrochloride [16.120]	Rats; M, F 20	Feeding	M: 10.3, 30.8, 61.9 mg/kg/day F: 10.3, 30.6, 60.3 mg/kg/day	13 weeks	60	(Ross, 2009)	Good quality, guideline study.
4-Amino-5,6-dimethylthioenol[2,3-d]pyrimidin-2(1H)-one [16.116]	Rats; M, F 5	Feeding	M: 10.3, 29.4, 101 mg/kg/day F: 10.9, 31.1, 103 mg/kg/day	21 days	>100	(Ross, 2008)	Good quality, guideline study.

TABLE IV.3: DEVELOPMENTAL AND REPRODUCTIVE TOXICITY STUDIES

No developmental and reproductive toxicity data are available for the candidate substances of the present Flavouring Group Evaluation from chemical group 30.

In vitro mutagenicity/genotoxicity data are available for one candidate substance of the present Flavouring Group Evaluation from chemical group 30.

TABLE IV.4: GENOTOXICITY (*IN VITRO*)

Chemical Name [FL-no]	Test System	Test Object	Concentration	Result	Reference	Comments
4-Amino-5,6-dimethylthioenol[2,3-d]pyrimidin-2(1H)-one hydrochloride [16.120]	Ames test	*S. typhimurium* TA98; TA100; TA1535; TA1537	0, 0.021, 0.062, 0.19, 0.56, 1.67, 5 mg/plate	Negative[1]	(Zhang, 2008a)	Valid.
	Ames test	*E.coli* WP2 *uvrA*	0, 0.021, 0.062, 0.19, 0.56,1.67, 5 mg/plate	Negative[1]	(Zhang, 2008a)	Valid.
	Chromosomal aberration	Chinese hamster ovary cells	0, 0.18, 0.55, 1.67 mg/ml	Negative[1]	(Zhang, 2008c)	Valid.

[1] *With and without metabolic activation*

In vivo mutagenicity/genotoxicity data are available for one candidate substance of the present Flavouring Group Evaluation from chemical group 30.

TABLE IV.5: GENOTOXICITY (*IN VIVO*)

Chemical Name [FL-no]	Test system	Test Object	Dose	Result	Reference	Comments
4-Amino-5,6-dimethylthioenol[2,3-d]pyrimidin-2(1H)-one hydrochloride [16.120]	Micronucleus test	Mouse bone marrow cells	500, 1000, 2000 mg/kg	Negative	(Zhang, 2008b)	Valid.

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ABBREVIATIONS

ADI	Acceptable Daily Intake
BW	Body weight
CAS	Chemical Abstract Service
CEF	Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids Chemical Abstract Service
CHO	Chinese hamster ovary (cells)
CL	Clearance
CoE	Council of Europe
DNA	Deoxyribonucleic acid
EC	European Commission
EFSA	The European Food Safety Authority
EU	European Union
FAO	Food and Agriculture Organization of the United Nations
FEMA	Flavor and Extract Manufacturers Association
FGE	Flavouring Group Evaluation
FLAVIS (FL)	Flavour Information System (database)
GSH	Glutathione
ID	Identity
IOFI	International Organization of the Flavour Industry
IR	Infrared spectroscopy
JECFA	The Joint FAO/WHO Expert Committee on Food Additives
K_a	Rate of absorption
LD_{50}	Lethal Dose, 50 %; Median lethal dose
MRT	Mean residence time
MS	Mass spectrometry
MSDI	Maximised Survey-derived Daily Intake
mTAMDI	Modified Theoretical Added Maximum Daily Intake
NAD	Nicotinamide Adenine Dinucleotide
NADP	Nicotinamide Adenine Dinucleotide Phosphate
No	Number
NOAEL	No Observed Adverse Effect Level
NOEL	No Observed Effect Level
NTP	National Toxicology Program
OECD	Organisation for Economic Co-operation and Development
PCE	Polychromatic erythrocytes
SCE	Sister Chromatid Exchange

SCF	Scientific Committee on Food
SMART	Somatic Mutation and Recombination Test
TAMDI	Theoretical Added Maximum Daily Intake
UDS	Unscheduled DNA Synthesis
WHO	World Health Organisation