EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF); Flavouring Group Evaluation 46, Revision 1 (FGE.46Rev1): Ammonia and three ammonium salts from chemical group 30

EFSA Publication; Larsen, John Christian; Nørby, Karin Kristiane; Beltoft, Vibe Meister; Lund, Pia; Binderup, Mona-Lise

Link to article, DOI:
10.2903/j.efsa.2011.1925

Publication date:
2011

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

SCIENTIFIC OPINION

Flavouring Group Evaluation 46, Revision 1 (FGE.46Rev1):

Ammonia and three ammonium salts 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

KEYWORDS

Amminia, ammonia salts, flavourings, safety.

SUMMARY

The Scientific Panel on 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 evaluate four flavouring substances in the Flavouring Group Evaluation 46, Revision 1 (FGE.46Rev1), using the Procedure as referred to in the Commission Regulation (EC) No 1565/2000. These four flavouring substances belong to chemical group 30, Annex I of the Commission Regulation (EC) No 1565/2000.

The present Flavouring Group Evaluation deals with ammonia [FL-no: 16.009], and three ammonia salts (diammonium sulphide [FL-no: 16.002], ammonium chloride [FL-no: 16.048] and ammonium hydrogen sulphide [FL-no: 16.059]).

The flavouring substances cannot exist as geometrical or optical isomers.

---

1 On request from the Commission, Question No EFSA-Q-2010-01133 adopted on 25 November 2010.

2 Panel members Arturo Anadon, 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, Kettil Svensson, Fidel Toldra, Rosemary Waring, Detlef Wölfle. CEF-Unit@efsa.europa.eu

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 support provided to this EFSA scientific output.

Suggested citation: **EFSA Panel on Food Contact** Materials, Enzymes, Flavourings and Processing Aids (CEF); **Flavouring Group Evaluation 46, Revision 1 (**FGE.46Rev1):
Ammonia and three ammonium salts from chemical group 30. EFSA Journal 2011; 9(2):1925. [35 pp.]. doi:10.2903/j.efsa.2011.1925. Available online: www.efsa.europa.eu

Two of the flavouring substances are classified into structural class I and two are classified into structural class III according to the decision tree approach presented by Cramer et al. (1978).

The flavouring substance ammonia in the present group has been reported to occur naturally in a wide range of food items up to very high amounts. Hydrogen sulphide is also reported to occur naturally in a wide range of food items.

In its evaluation, the Panel as a default used the "Maximised Survey-derived Daily 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.

According to the default MSDI approach, the two flavouring substances [FL-no: 16.009 and 16.048] belonging to structural class I have estimated intakes in Europe of 34 and 140 microgram/*capita*/day, respectively, which are below the threshold of concern for structural class I substances (1800 microgram/person/day). The two substances belonging to structural class III have estimated intake in Europe of 62 and 5.6 microgram/*capita*/day, respectively, which is below the threshold of concern for structural class III substances (90 microgram/person/day).

Although the genotoxicity data for the flavouring substances in this group are limited, the available data on genotoxicity do not preclude an evaluation of the candidate substances through the Procedure. For the candidate substance ammonium chloride [FL-no: 16.048] there is a well-performed carcinogenicity study available, which indicates that the substance does not induce tumours.

Ammonia is a substance that is readily absorbed in the gut. It is produced endogenously in amounts that far exceed those that are to be ingested as flavourings. The three ammonium salts are expected to give rise to ammonium ion and chloride or hydrogen sulphide. Ammonia is expected to be transported by the portal circulation to the liver and metabolised to urea by the Krebs urea cycle and subsequently excreted by the kidneys. Hydrogen sulphide is a substance that is produced endogenously. The major pathway for sulphide metabolism is oxidation to sulphate and excretion by the kidney. The major oxidation product of sulphide is thiosulphate which is then converted to sulphate. The primary location for these reactions is the liver. All four substances are accordingly expected to be metabolised to innocuous substances at the anticipated levels of intake as flavouring substances.

It was noted that where toxicity data were available they were consistent with the conclusions in the present flavouring group evaluation using the Procedure.

On the basis of the default MSDI approach the Panel concluded that the flavouring substances would not give rise to safety concerns at the estimated levels of intake arising from their use as flavouring substances.

When the estimated intakes were based on the mTAMDI approach the values for the two substances from structural class I, ammonia and ammonium chloride [FL-no: 16.009 and 16.048], are 110000 microgram/person/day and 220000 microgram/person/day, respectively. These values are above the

threshold of concern for structural class I of 1800 microgram/person/day. For one of the substances from structural class III, ammonium hydrogen sulphide [FL-no: 16.059], the mTAMDI value is 220 microgram/person/day. This value is above the threshold for structural class III of 90 microgram/person/day. For the other substance from structural class III no data are available on use and use levels.

Thus, intake estimates based on the mTAMDI approach exceed the threshold of concern for the three flavouring substances in this flavouring group, and more reliable exposure data are requested for all four substances. On the basis of such additional data, these flavouring substances should be reconsidered using the Procedure. Subsequently, additional data might become necessary.

In order to determine whether this evaluation could be applied to the materials of commerce, it is necessary to consider the available specifications. Specifications including complete purity criteria for the materials of commerce have been provided for the four flavouring substances.

Identity tests is missing for one of the flavouring substances, ammoniun hydrogen sulphide [FL-no: 16.059]. Thus, the final evaluation of the materials of commerce cannot be performed for this substance, pending further information. The remaining three flavouring substances, ammonia [FL-no: 16.009], ammonium chloride [FL-no: 16.048] and diammoniun sulfide [FL-no: 16.002] would present no safety concern at the levels of intakes estimated on the basis of the MSDI approach.

TABLE OF CONTENTS

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).

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

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

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

HISTORY OF THE EVALUATION

FGE	Opinion adopted by EFSA	Link	No. of candidate substances
FGE.46	March 2008	http://www.efsa.europa.eu/en/scdocs/scdoc/955.htm	3
FGE.46Rev1	November 2010		4

The present Revision of FGE.46, FGE.46Rev1, includes the assessment of one additional candidate substance, diammonium sulfide [FL-no: 16.002]. No toxicity and/or metabolism data were provided for this substance (Flavour Industry, 2009p). A literature search for this substance did not provide any further data on toxicity or metabolism.

Furthermore, additional information on the specifications requested in FGE.46 have become available for two substances. These data have been included in the present FGE as well.

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.

ASSESSMENT

1. Presentation of the Substances in Flavouring Group Evaluation 46, Revision 1

1.1. Description

The present Flavouring Group Evaluation 46, Revision 1 (FGE.46, Rev1), 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), deals with ammonia and three ammonium salts. These four flavouring substances (candidate substances) belong to chemical group 30, Annex I of Commission Regulation (EC) No 1565/2000 (EC, 2000a). The four candidate substances, their chemical Register names, FLAVIS- (FL-), Chemical Abstract Service- (CAS-), Council of Europe- (CoE-) and Flavor and Extract Manufacturers Association- (FEMA-) numbers, structure and specifications, are listed in Table 1. This group of candidate substances includes diammonium sulphide [FL-no: 16.002], ammonia [FL-no: 16.009], ammonium chloride [FL-no: 16.048] and ammonium hydrogen sulfide [FL-no: 16.059].

The Joint FAO/WHO Expert Committee on Food Additives (the JECFA) evaluated ammonium carbonate and ammonium hydrogen carbonate in 1982 (JECFA, 1982a). Because toxicological studies were limited for these compounds, data on related ammonium salts (primarily ammonium chloride) and carbonate salts (primarily sodium and potassium carbonate and hydrogen carbonate (bicarbonate)) were also summarised and discussed. An acceptable daily intake (ADI) in mg/kg body weight (bw) was not deemed necessary: "ADI not specified".

The SCF made an recommendation on ammonium chloride in liquorice products in an Opinion expressed on 11 October 1991 (SCF, 1992), in which the SCF concluded that it is not possible to allocate an acceptable daily intake (ADI) to ammonium chloride as no data are available to establish a no adverse effect level for the ingestion of ammonium chloride.

The outcome of the safety evaluation is summarised in table 2.

1.2. Stereoisomers

The candidate substances cannot exist as geometrical or optical isomers.

1.3. Natural Occurrence in Food

Ammonia [FL-no: 16.009] and hydrogen sulphide (from ammonium hydrogen sulfide [FL-no: 16.059] and diammonium sulphide [FL-no: 16.002) have been reported to occur naturally in apple, banana, barley, beef, beer, beetroot, butter, cabbage, carrot, cauliflower, caviar, celery, cheese, chicken, coffee, sweet corn, egg, fish (fatty, lean), fruit juice, hop oil, kale, lettuce, malt, milk, mushroom, onion, pork, potato chips, radish, rhubarb, rice, rutabaga, shrimps, soybean, squid, black tea, tomato, wasabi and wine (TNO, 2000; TNO, 2010).

Quantitative data on the natural occurrence in food reported include (TNO, 2000; TNO, 2010):

- Ammonia [FL-no: 16.009]/ammonium ion: 235 mg/kg in fresh apple, 8130 mg/kg in barley, 8800 mg/kg in beetroot, up to 11060 mg/kg in cooked cabbage, 3970 mg/kg in carrot, 6376 mg/kg in cauliflower, 19600 mg/kg in celery leaves and/or stalks, 164400 mg/kg in tilsit cheese, 0.2 mg/kg in fried chicken, up to 820 mg/kg in coffee, 10030 mg/kg in sweet corn, up to 9 mg/kg in egg, up to 2928 mg/kg in fish (fatty, salted), 10660 mg/kg in hop oil, 15260 mg/kg in kale, 10260 mg/kg in lettuce, 1192 mg/kg in malt, 8450 mg/kg in radish (raw), 6340 mg/kg in rhubarb, 400 mg/kg in black tea, up to 40 mg/kg in red wine and up to 69 mg/kg in white wine.
  H_2S/HS^- ion: up to 0.004 mg/kg in beer, up to 0.002 mg/kg in butter, up to 0.8 mg/kg in cheese, up to 1.7 mg/kg in chicken, up to 0.2 mg/kg in sweet corn, up to 1.5 mg/kg in egg, trace amount in

grapefruit juice, up to 0.0005 mg/kg in milk, 0.01 mg/kg in mushroom, up to 0.03 mg/kg in orange juice, trace amount in soybean and up to 0.1 mg/kg in strawberry.

According to TNO, the diammonium sulphide [FL-no: 16.002] has not been reported in any food items (TNO, 2010).

2. Specifications

Purity criteria for the four substances have been provided by the Flavour Industry (EFFA, 2006g; Flavour Industry, 2009p) (Table 1).

Judged against the requirements in Annex II of Commission Regulation (EC) No 1565/2000 (EC, 2000), the purity criteria for one of the candidate substances are deficient in one of the parameters as the identification test is missing. Otherwise the specifications are adequate for all four candidate substances (see Section 1.2 and Table 1).

3. Intake Data

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

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

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

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

One of the alternatives is the "Theoretical Added Maximum Daily Intake" (TAMDI) approach, which is calculated on the basis of standard portions and upper use levels (SCF, 1995) for flavourable beverages and foods in general, with exceptional levels for particular foods. This method is regarded as a conservative estimate of the actual intake by most consumers because it is based on the assumption that the consumer regularly eats and drinks several food products containing the same flavouring substance at the upper use level.

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

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

The intake estimation is based on the Maximised Survey-derived Daily Intake (MSDI) approach, which involves the acquisition of data on the amounts used in food as flavourings (SCF, 1999a). These

data are derived from surveys on annual production volumes in Europe. These surveys were conducted in 1995 by the International Organization of the Flavour Industry, in which flavour manufacturers reported the total amount of each flavouring substance incorporated into food sold in the EU during the previous year (IOFI, 1995). The intake approach does not consider the possible natural occurrence in food.

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

In the present Flavouring Group Evaluation, Revision 1 (FGE.46Rev1) the total annual production volume of the four candidate substances for use as flavouring substances in Europe was reported to be approximately 2000 kg (EFFA, 2006g; Flavour Industry, 2009p). The daily *per capita* intakes for the candidate substances are 62 microgram (diammonium sulphide, [FL-no: 16.002]), 140 microgram (ammonium chloride, [FL-no: 16.048]), 34 microgram (ammonia, [FL-no: 16.009] and 5.6 microgram (ammonium hydrogen sulphide, [FL-no: 16.059]) (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 present evaluation of the four candidate substances, information on food categories and normal and maximum use levels[5,6,7] were submitted by the Flavour Industry (EFFA, 2006g; EFFA, 2007a) only for three of the four candidate substances. The four 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 case different use levels were reported for different food categories the highest reported normal use level was used.

For diammonium sulphide, [FL-no: 16.002] no use and use levels have been reported by Industry.

[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).

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

Table 3.1 Use of Candidate Substances, for which Industry has Provided Data on Food Categories and Normal and Maximum Use Levels (three of the four Candidate Substances)

Food category	Description	Flavourings used
01.0	Dairy products, excluding products of category 2	All three
02.0	Fats and oils, and fat emulsions (type water-in-oil)	Only [FL-no: 16.059]
03.0	Edible ices, including sherbet and sorbet	Only [FL-no: 16.059]
04.1	Processed fruits	Only [FL-no: 16.059]
04.2	Processed vegetables (incl. mushrooms & fungi, roots & tubers, pulses and legumes), and nuts & seeds	None
05.0	Confectionery	All three
06.0	Cereals and cereal products, incl. flours & starches from roots & tubers, pulses & legumes, excluding bakery	Only [FL-no: 16.059]
07.0	Bakery wares	Only [FL-no: 16.059]
08.0	Meat and meat products, including poultry and game	All three
09.0	Fish and fish products, including molluscs, crustaceans and echinoderms	All three
10.0	Eggs and egg products	None
11.0	Sweeteners, including honey	None
12.0	Salts, spices, soups, sauces, salads, protein products etc.	All three
13.0	Foodstuffs intended for particular nutritional uses	Only [FL-no: 16.059]
14.1	Non-alcoholic ("soft") beverages, excl. dairy products	Only [FL-no: 16.059]
14.2	Alcoholic beverages, incl. alcohol-free and low-alcoholic counterparts	All three
15.0	Ready-to-eat savouries	Only [FL-no: 16.059]
16.0	Composite foods (e.g. casseroles, meat pies, mincemeat) - foods that could not be placed in categories 1 – 15	All three

According to the Flavour Industry the normal use levels for the three candidate substances, for which use levels have been provided, are in the range of 0.1 - 1000 mg/kg food, and the maximum use levels are in the range of 1 - 3000 mg/kg (EFFA, 2002i; EFFA, 2006g; EFFA, 2007a).

The mTAMDI values for the two candidate substances, ammonia and ammonium chloride [FL-no: 16.009 and 16.048] from structural class I are 110000 and 220000 microgram/person/day, respectively. For the third candidate substance, ammonium hydrogen sulfide [FL-no: 16.059] from structural class III (see Section 5) the mTAMDI is 220 microgram/person/day. No use levels have been provided for [FL-no: 16.002].

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

Ammonia and ammonium ion are present in aqueous environment at different ratios depending on the pH.

Ammonia is produced endogenously in all mammalian species. In the gut ammonia is produced by bacterial degradation of nucleic and amino acids from ingested food. The estimated production of ammonia in the human intestine may range from 10 mg/day in the duodenum to 3 g/day in the colon. Ammonia is readily absorbed from the gastro-intestinal tract, after which it enters the portal circulation and is transformed to urea in the liver via the urea cycle, and is subsequently excreted as urea via the kidneys. Ammonium chloride is easily absorbed.

Ammonium hydrogen sulphide and diammonium sulphide are expected to give rise to ammonium ion and hydrogen sulphide in the stomach and to be rapidly absorbed.

Hydrogen sulphide is also endogenously produced. The main source is from metabolism of sulphur-containing amino acids in the intestinal tract; other sources are bacterial reduction of inorganic sulphate and sulphite in the large intestine and fermentation of sulphur-containing amino acids.

Sulphide concentrations in whole blood ranged from 10 – 100 micromol/l (corresponding to 320 – 3200 microgram/l) in six healthy adults (Richardson et al., 2000). The major pathway for sulphide metabolism is oxidation to sulphate and excretion by the kidneys. The primary location for these reactions is the liver. There is an intestinal enzymatic detoxification system for endogenously produced sulphide that may take care of the low amounts of hydrogen sulphide that may be ingested as flavouring substance.

All four candidate substances may therefore be predicted to be metabolised to innocuous products at the estimated levels of exposure as flavouring substances, according to the MSDI approach.

A more detailed discussion of the metabolism of the candidate substances in this evaluation is provided in 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 four 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 four substances are summarised in Table 2.

Step 1

The candidate substances are classified according to the decision tree approach by Cramer et al. (Cramer et al, 1978). Two substances are classified into structural class I [FL-no: 16.009 and 16.048] and two into structural class III [FL-no: 16.002 and 16.059].

Step 2

On the basis of the metabolism information the two candidate substances, ammonia and ammonium chloride [FL-no: 16.009 and 16.048] in structural class I may be predicted to be metabolised to innocuous products, and their evaluation will proceed via the A-side of the Procedure scheme.

The two substances belonging to structural class III, diammonium sulphide [FL-no: 16.002] and ammonium hydrogen sulphide [FL-no: 16.059] may also be predicted to be metabolised to innocuous products, and accordingly their evaluation proceeds via the A-side of the Procedure scheme.

Step A3

The two candidate substances [FL-no: 16.009 and 16.048] assigned to structural class I have estimated European daily *per capita* intakes (MSDI) of 34 and 140 microgram, respectively (Table 6.1). These intakes are below the threshold of concern of 1800 microgram/person/day for structural class I.

The two candidate substances [FL-no: 16.002 and 16.059] assigned to structural class III have estimated daily *per capita* intake (MSDI) of 62 and 5.6 microgram, respectively (Table 6.1). These intakes are below the threshold of concern of 90 microgram/person/day for structural class III.

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

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

The estimated intakes for the two candidate substances [FL-no: 16.009 and 16.048] in structural class I based on the mTAMDI, are 110000 and 220000 microgram/person/day, respectively. For these two substances the mTAMDI is above the threshold of concern of 1800 microgram/person/day. For comparison of the intake estimates based on the MSDI approach and the mTAMDI approach see Table 6.1.

The estimated intake of the third substance [FL-no: 16.059] assigned to structural class III, based on the mTAMDI, is 220 microgram/person/day, which is above the threshold of concern for structural class III substances of 90 microgram/person/day. For comparison of the MSDI- and mTAMDI-values, see Table 6.1.

The estimated intake of [FL-no: 16.002] assigned to structural class III, cannot be calculated as Industry has not provided information on use and use levels.

For all the candidate substances further information is required. This would include more reliable intake data and then, if required, additional toxicological data.

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)
16.009	Ammonia	34	110000	Class I	1800
16.048	Ammonium chloride	140	220000	Class I	1800
16.002	Diammonium sulfide	62		Class III	90
16.059	Ammonium hydrogen sulphide	5.6	220	Class III	90

7. Considerations of Combined Intakes from Use as Flavouring Substances

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

The combined intake of the two candidate substances [FL-no: 16.009 and 16.048] from structural class I is approximately 170 microgram/*capita*/day, which is below the threshold of concern of 1800 microgram/person/day for structural class I substances.

The combined intake of the two candidate substances [FL-no: 16.002 and 16.059] from structural class III is approximately 70 microgram/*capita*/day, which is below the threshold of concern of 90 microgram/person/day for structural class III substances.

8. Toxicity

8.1. Acute Toxicity

An oral LD_{50} of 1650 mg/kg body weight (bw) has been reported for the candidate substance ammonium chloride [FL-no: 16.048] in the rat (IUCLID Dataset, 2000d).

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

8.2. Subacute, Subchronic, Chronic and Carcinogenicity Studies

Studies on only one candidate substance, ammonium chloride [FL-no: 16.048], have been submitted. Ammonium chloride has not been tested per se, but has been used to produce metabolic acidosis, and the aim of most studies has been to study the effect of acidosis on different parameters.

In studies designed to examine the effects of feeding an acidogenic diet to rats, ammonium chloride [FL-no: 16.048] was fed to rats for periods of 4 weeks, 13 weeks (approx. 1.6-3.7 g/kg bw/day) or 18 months (approx. 0.5-1.4 g/kg bw/day). A carcinogenicity study of 30 months (approx. 0.5-1.2 g/kg bw/day) was also conducted. All animals fed NH_4Cl had reduced urinary pH. Clinical condition and death rate were not affected by treatment. Growth retardation was noted in the high treatment group, and in females of the low treatment group. Urinary calcium and phosphorus excretion was increased in treatment groups, but there was no indication that bone minerals were involved. Kidney weights were increased in all ammonium chloride-treated animals. Hypertrophy of the adrenal zona glomerulosa occurred, due to chronic stimulation of the adrenal cortex by treatment-induced acidosis. No treatment-related changes in any specific tumour type were noted among treated animals. It was not possible to derive a No Observed Adverse Effect Level (NOAEL) from these studies. The 30 month carcinogenicity study gave no indication that the feeding of ammonium chloride to rats at dose levels of approximately 0.5-1.2 g/kg bw/day would induce tumours (Lina & Kuijpers, 2004).

Dietary levels of 3 g/kg bw per day ammonium chloride [FL-no: 16.048] for 6 days gave rise to renal hypertrophy in rats (Thompson & Halliburton, 1966). The same effect occurred when ammonium chloride [FL-no: 16.048] was given *ad libitum* in drinking water for 7 days, and approximately 1 g/kg bw/day was ingested. No increase occurred in uptake of tritiated thymidine in the kidney, implying that no cell division or DNA synthesis occurred (Lotspeich, 1965).

Rats receiving 1.5 % (corresponding to approximately 1.5 g/kg bw) ammonium chloride [FL-no: 16.048]/kg bw/day in drinking water for 330 days showed significant loss of bone tissue. Changes were independent of dietary Ca, and not associated with blood Ca or P. Rats that received 2 % of the same compound in drinking water for 6 months showed significant reduction in bone density, which was accentuated by a low-Ca-diet (Barzel, 1969; Barzel & Jowsey, 1969).

Although the available data do not allow the identification of a NOAEL, high dose levels were used to study effects of ammonium chloride in several animal species (1-4 g/kg bw). The results support the conclusion of the endogenous production of ammonia.

No studies have been submitted on the candidate substances ammonium hydrogen sulphide [FL-no: 16.059] and diammonium sulphide [FL-no: 16.002] or on structurally related flavouring substances. However, it is structurally related to hydrogen sulphide, which is considered below.

The mechanism of toxicity of hydrogen sulphide is the inhibition of cytochrome oxidase and through this, the inhibition of the electron transport system.

In a U.S. Environmental Protection Agency's toxicological review on hydrogen sulphide (EPA, 2003) a 90-day study on rats is described. This study by Anderson was reported in 1987 and is not publicly available. According to the description of the study, hydrogen sulphide (presumably as solution, vehicle not known) was given by gavage to groups of 20 rats daily for 89 days in doses of 0, 1, 3.5 or 7 mg/kg bw per day. For preparation of hydrogen sulphide solution, hydrogen sulphide was purged through deoxygenated, deionised water. The solution was stored in sealed vials and fresh vials were used at each administration. Blood samples were collected at days 27-31 of treatment and at necropsy. Animals were subjected to full necropsy and extensive microscopical examination. Mortality was 50 % in high-dose males compared to 5 % in control males, cause of death in the high dose group was, however, not determined. No deaths were observed in high-dose females. Compound-related neuromuscular and behavioral signs were observed in high-dose male and female animals. There were no treatment-related effects in hematology or clinical chemistry nor in body weights. Ophthalmological findings were normal. The most common findings at necropsy were red mottled

lungs. No gross lesions could be attributed to compound administration and all microscopic findings were considered incidental to compound administration. However, the information indicates procedural problems with the dosing of the animals; a 75-100 % incidence of pneumonia was reported in vehicle control and treatment groups whereas 0 % incidence of pneumonia was reported for the non-gavaged control group. According to the EPA evaluation, the lack of quality assurance precludes the use of this study for derivation of a NOEL (EPA, 2003).

In WHO drinking water guidelines (WHO, 1996) the oral dose of sodium sulphide fatal to humans (60 kg) has been estimated at 10-15 g. For drinking water no health guideline is proposed. The organoleptic threshold for hydrogen sulphide in drinking water is < 0.05 mg/l, and it is stated that the quantity of sulphide ingested from drinking water containing sulphide at this concentration is well below the amount that the body can detoxify.

Conclusions on toxicity

From the available toxicity data it is not possible to derive NOAELs for the candidate substances. However, ammonia and hydrogen sulphide are substances that are formed endogenously in the body at levels much higher than what can be formed from use of the candidate substances at the estimated levels of exposure (6 – 140 microgram/ *capita*/ day based on the MSDI approach) and for which the body has intrinsic detoxification systems. Data on metabolism and kinetics (see Section 4 and Annex III) along with the available information on toxicity give clear indication that the amounts of the candidate substances that may be ingested from the use as flavourings, according to the MSDI approach, do not raise concern with respect to toxicity.

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

8.3. Developmental / Reproductive Toxicity Studies

No studies on developmental or reproductive toxicity have been submitted for any of the candidate substances. Developmental toxicity studies in rats exposed to hydrogen sulphide via the inhalational route throughout gestation showed no adverse effects in the embryo or foetus, nor in offspring during postnatal development, at exposures up to 80 ppm (for review, see (US ATSDR, 2006)) For ammonia (actually ammonium ion) an oral developmental toxicity study has been reported (for review, see (US ATSDR, 2004)), but the effect (reduced body weight gain in off-spring) was observed at such high dose levels (> 4000 mg/kg bw/day) that the effects in the offspring were most likely the result of maternal toxicity.

8.4. Genotoxicity Studies

Ammonia gas at concentrations of 500-25000 ppm has been reported to give a negative result in a bacterial mutagenicity assay using *Salmonella typhimurium sp.* TA98, TA100, TA1535, TA1537 and TA1538 in the presence and absence of metabolic activation (Shimoi et al., 1985). Tests using *Escherichia Coli* WRP uvrA were also negative (Shimoi et al., 1985).

There are very limited *in vivo* mammalian data on the effects of ammonia. A mouse micronucleus test (single intraperitoneal dose of ammonium at 12, 25 or 50 mg/kg bw) reported dose dependent increases in the frequencies of micronuclei when compared to controls (Yadav & Kaushik, 1997). Few details were given and no conclusions can be drawn. Slight mutagenic activity has been reported in *Drosophila* following exposure to ammonia gas, but only at very toxic levels (US ATSDR, 2004).

Ammonium chloride at concentrations up to 10 mg/plate has been reported to give negative results in a bacterial mutation assay using *S. typhimurium* strains TA92, TA94, TA98, TA100, TA1535 and TA1537 (Ishidate et al., 1984; Ishidate et al., 1988). Ammonium chloride in the absence of metabolic activation caused an increase in the incidence of cells with structural chromosomal aberrations (including gaps) in Chinese hamster fibroblasts, which the authors suggested were due to osmotic

effects rather than a direct interaction with DNA (Ishidate et al., 1984; Ishidate et al., 1988). *In vivo*, ammonium chloride did not induce an increase in the incidence of micronucleated polychromatic erythrocytes obtained from the bone marrow of male ddY mice, 24 hours after one intraperitoneal injection of 62.5-500 mg/kg bw or four intraperitoneal injections of 31.3-250 mg/kg bw (Hayashi et al., 1988).

Ammonium sulphate has also been reported to give negative results in bacterial mutagenicity tests but to enhance the frequency of chromosome type aberrations (UNEP, 2004). A similar effect has however been observed with other salts (magnesium chloride, calcium chloride and sodium chloride), and is considered to be linked to osmotic effects rather than being indicative of a clastogenic effect of ammonium sulphate or the ammonium ion.

No genotoxicity studies are available on ammonium hydrogen sulphide. No mutagenicity was observed with hydrogen sulfide gas in Ames assays using *S. typhimurium* TA97, TA98, and TA100 strains in the presence and absence of metabolic activation (US ATSDR, 2006).

Conclusion on genotoxicity

Overall, the genotoxicity data available do not preclude an evaluation of the four substances through the Procedure.

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

9. Conclusions

The present Flavouring Group Evaluation deals with ammonia [FL-no: 16.009], and three ammonia salts (diammonium sulphide [FL-no: 16.002], ammonium chloride [FL-no: 16.048] and ammonium hydrogen sulphide [FL-no: 16.059]).

The flavouring substances cannot exist as geometrical or optical isomers.

Two of the flavouring substances are classified into structural class I and two are classified into structural class III according to the decision tree approach presented by Cramer et al. (1978).

The flavouring substance ammonia in the present group has been reported to occur naturally in a wide range of food items up to very high amounts. Hydrogen sulphide is also reported to occur naturally in a wide range of food items.

In its evaluation, the Panel as a default used the Maximised Survey-derived Daily 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 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.

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

According to the default MSDI approach, the two flavouring substances [FL-no: 16.009 and 16.048] belonging to structural class I have estimated intakes in Europe of 34 and 140 microgram/*capita*/day, respectively, which are below the threshold of concern for structural class I substances (1800 microgram/person/day). The two substances belonging to structural class III have estimated intake in Europe of 62 and 5.6 microgram/*capita*/day, respectively, which is below the threshold of concern for structural class III substances (90 microgram/person/day).

Although the genotoxicity data for the flavouring substances in this group are limited, the available data on genotoxicity do not preclude an evaluation of the candidate substances through the Procedure. For the candidate substance ammonium chloride [FL-no: 16.048] there is a well-performed carcinogenicity study available, which indicates that the substance does not induce tumours.

Ammonia is a substance that is readily absorbed in the gut. It is produced endogenously in amounts that far exceed those that are to be ingested as flavourings. The three ammonium salts are expected to give rise to ammonium ion and chloride or hydrogen sulphide. Ammonia is expected to be transported by the portal circulation to the liver and metabolised to urea by the Krebs urea cycle and subsequently excreted by the kidneys. Hydrogen sulphide is a substance that is produced endogenously. The major pathway for sulphide metabolism is oxidation to sulphate and excretion by the kidney. The major oxidation product of sulphide is thiosulphate which is then converted to sulphate. The primary location for these reactions is the liver. All four substances are accordingly expected to be metabolised to innocuous substances at the anticipated levels of intake as flavouring substances.

It was noted that where toxicity data were available they were consistent with the conclusions in the present flavouring group evaluation using the Procedure.

On the basis of the default MSDI approach the Panel concluded that the flavouring substances would not give rise to safety concerns at the estimated levels of intake arising from their use as flavouring substances.

When the estimated intakes were based on the mTAMDI approach the values for the two substances from structural class I, ammonia and ammonium chloride [FL-no: 16.009 and 16.048], are 110000 microgram/person/day and 220000 microgram/person/day, respectively. These values are above the threshold of concern for structural class I of 1800 microgram/person/day. For one of the substances from structural class III, ammonium hydrogen sulphide [FL-no: 16.059], the mTAMDI value is 220 microgram/person/day. This value is above the threshold for structural class III of 90 microgram/person/day. For the other substance from structural class III no data are available on use and use levels.

Thus, intake estimates based on the mTAMDI approach exceed the threshold of concern for the three flavouring substances in this flavouring group, and more reliable exposure data are requested for all four. On the basis of such additional data, these flavouring substances should be reconsidered using the Procedure. Subsequently, additional data might become necessary.

In order to determine whether this evaluation could be applied to the materials of commerce, it is necessary to consider the available specifications. Specifications including complete purity criteria for the materials of commerce have been provided for the four flavouring substances.

Identity test is missing for one of the flavouring substances, ammonium hydrogen sulphide [FL-no: 16.059]. Thus, the final evaluation of the materials of commerce cannot be performed for this substance, pending further information. The remaining three flavouring substances, ammonia [FL-no: 16.009], ammonium chloride [FL-no: 16.048] and diammoniun sulfide [FL-no: 16.002] would present no safety concern at the levels of intakes estimated on the basis of the MSDI approach.

TABLE 1: SPECIFICATION SUMMARY OF THE SUBSTANCES IN THE FLAVOURING GROUP EVALUATION 46, REVISION 1

Table 1: Specification Summary of the Substances in the Flavouring Group Evaluation 46, Revision 1

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
16.002	Diammonium sulfide	$(NH_4)_2S$	2053 482 12135-76-1	Liquid H_8N_2S 68.15	Soluble Freely soluble	40 IR 95 %	1.378-1.398 0.995-0.999	
16.009	Ammonia	NH_3	 739 7664-41-7	Gas H_3N 17.03	Soluble Freely soluble	n.a. n.a. 95 %	n.a. n.a.	Physical form: Gas. ID test not applicable.
16.048	Ammonium chloride	NH_4^+, Cl^-	 12125-02-9	Solid H_4NCl 53.49	Very soluble Freely soluble	 decomposes 99 %	n.a. n.a.	ID test not applicable.
16.059	Ammonium hydrogen sulphide	NH_4^+, HS^-	2053 482 12124-99-1	Liquid H_5NS 51.11	Soluble Freely soluble	40 95 %	1.378-1.398 0.995-0.999	ID 6)

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) ID: Missing identification test.

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
16.009	Ammonia	NH_3	34	Class I A3: Intake below threshold	4)	7)	
16.048	Ammonium chloride	NH_4^+, Cl^-	140	Class I A3: Intake below threshold	4)	7)	
16.002	Diammonium sulfide	$(NH_4)_2S$	62	Class III A3: Intake below threshold	4)	7)	
16.059	Ammonium hydrogen sulphide	NH_4^+, HS^-	5.6	Class III A3: Intake below threshold	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, 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.

ANNEX I: PROCEDURE FOR THE SAFETY EVALUATION

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

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

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

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

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

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

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

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

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

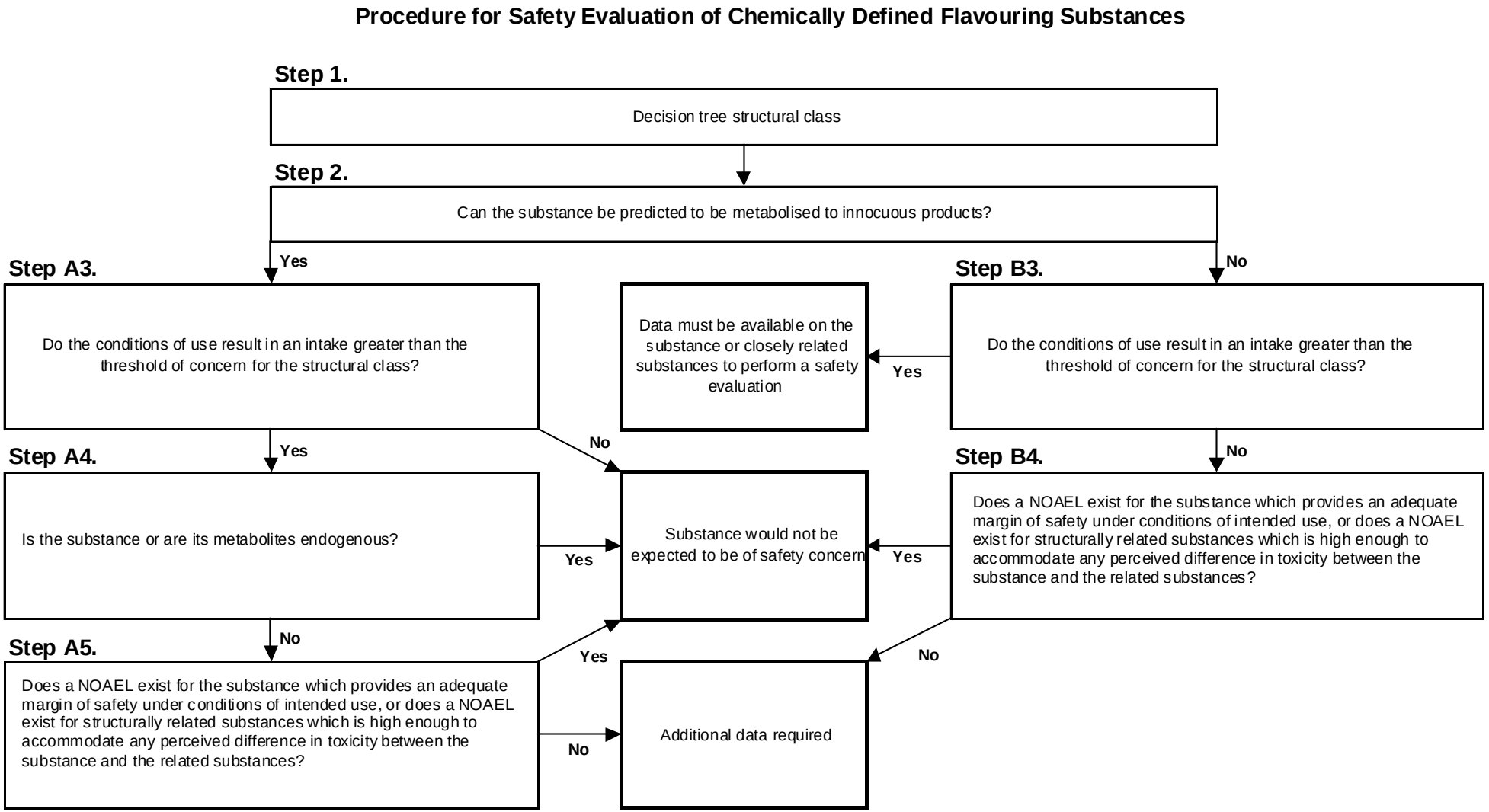

Figure I.1 *Procedure for Safety Evaluation of Chemically Defined Flavouring Substances*

ANNEX II: USE LEVELS / MTAMDI

II.1 Normal and Maximum Use Levels

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

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

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

The "normal and maximum use levels" are provided by Industry for three of the four 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.46, Revision 1 (EFFA, 2006g; EFFA, 2007a).

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
16.009	500	0	0	0	-	500	0	0	500	500	-	-	500	0	0	500	500	500
	1500	0	0	0	-	1500	0	0	1500	1500	-	-	1500	0	0	1500	1500	1500
16.048	1000	0	0	0	-	1000	0	0	1000	1000	-	-	1000	0	0	1000	1000	1000
	3000	0	0	0	-	3000	0	0	3000	3000	-	-	3000	0	0	3000	3000	3000
16.059	0,4	0,2	0,4	0,3	-	0,4	0,2	0,4	0,1	0,1	-	-	0,2	1	0,2	0,4	-	0,2
	2	1	2	1,5	-	2	1	2	0,4	0,4	-	-	1	5	1	2	-	1

II.2 mTAMDI Calculations

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

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

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

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

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

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

	Food categories according to Commission Regulation 1565/2000	Distribution of the seven SCF food categories		
Key	Food category	Food	Beverages	Exceptions
01.0	Dairy products, excluding products of category 02.0	Food		
02.0	Fats and oils, and fat emulsions (type water-in-oil)	Food		
03.0	Edible ices, including sherbet and sorbet	Food		
04.1	Processed fruit	Food		
04.2	Processed vegetables (incl. mushrooms & fungi, roots & tubers, pulses and legumes), and nuts & seeds	Food		
05.0	Confectionery			Exception a
06.0	Cereals and cereal products, incl. flours & starches from roots & tubers, pulses & legumes, excluding bakery	Food		
07.0	Bakery wares	Food		
08.0	Meat and meat products, including poultry and game	Food		
09.0	Fish and fish products, including molluscs, crustaceans and echinoderms	Food		
10.0	Eggs and egg products	Food		
11.0	Sweeteners, including honey			Exception a
12.0	Salts, spices, soups, sauces, salads, protein products, etc.			Exception d
13.0	Foodstuffs intended for particular nutritional uses	Food		
14.1	Non-alcoholic ("soft") beverages, excl. dairy products		Beverages	
14.2	Alcoholic beverages, incl. alcohol-free and low-alcoholic counterparts			Exception c
15.0	Ready-to-eat savouries			Exception b
16.0	Composite foods (e.g. casseroles, meat pies, mincemeat) - foods that could not be	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 three flavouring substances in the present Flavouring Group Evaluation 46, Revision 1, for which Industry has provided use and use levels (EFFA, 2006g; EFFA, 2007a). The mTAMDI values are only given for highest reported normal use.

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)
16.009	Ammonia	110000	Class I	1800
16.048	Ammonium chloride	220000	Class I	1800
16.002	Diammonium sulfide		Class III	90
16.059	Ammonium hydrogen sulphide	220	Class III	90

ANNEX III: METABOLISM

III.1. Absorption, Distribution and Elimination

The candidate substance ammonia [FL-no: 16.009] is produced endogenously in all mammalian species and is a metabolite from the degradation of purines and the deamination of amino acids. In the gut ammonia is formed by bacterial degradation of amino acids and nucleic acids from ingested food. The estimated production of ammonia in the human intestine may range from 10 mg/day in the duodenum to about 3 g/day in the colon. Almost all ammonia formed is absorbed. Ammonia uptake increases with higher pH of luminal contents (Castell & Moore, 1971). After absorption ammonia enters the portal circulation, and in healthy humans ammonium ions are rapidly transformed to urea in the liver, so only small amounts of ammonium reaches the systemic circulation (Summerskill & Wolpert, 1970).

For ammonium salts the anion may play an important role in determining what effects will occur. Ammonium chloride has been used in many animal studies to induce metabolic acidosis, due to the formation of hydrogen chloride. Acidosis may occur with other ammonium salts, but the degree of acidosis is determined by the ability of the kidneys to excrete the specific anion. Therefore, it is not appropriate to extrapolate the findings from one ammonium salt to another (De Sousa et al., 1974).

Ammonium chloride [FL-no: 16.048] is easily absorbed. Ammonium hydrogen sulphide [FL-no: 16.059] will produce ammonia and hydrogen sulphide after absorption. Hydrogen sulphide and soluble sulphides are rapidly absorbed following ingestion. Soluble sulphides occur as hydrogen sulphide in body fluids; in terms of their systemic effects, no distinctions are recognised between them and hydrogen sulphide (U.S. National Research Council, 1979).

Ammonia is normally present in all tissues. The distribution is pH dependent, as NH_3 diffuses more readily than NH_4^+. The lower the pH is in a compartment the higher the amount of ammonia may be (U.S. National Research Council, 1979). Although most ingested ammonia that is absorbed in the intestine is transformed to urea by the liver, some nitrogen from ammonia has been reported to be incorporated into tissue proteins (Vitti et al., 1964; Richards et al., 1968; Fürst et al., 1970).

The majority of ingested ammonia is excreted as urea in the urine (Haworth et al., 1978; Nelson & Cox, 2000c). During metabolic acidosis the concentrations of renal glutaminase, which catalyses the release of ammonia in the kidney tubular epithelium, increase proportionally to ammonium ion excretion (Davies & Yudkin, 1952; Muntwyler et al., 1956; Kamin & Handler, 1957). Ammonia may be excreted through expired air, which most likely is due to synthesis of ammonia from salivary urea by oral microflora (Biswas & Kleinberg, 1971; Hunt, 1977; Larson et al., 1977; Barrow & Steinhagen, 1980). Mammals may excrete ammonia directly into the urine; glutaminase catalyses the release of ammonia in the kidney tubular epithelium where it serves as an acceptor of H^+ and regulates the acid-base balance (White et al., 1973). Less than 1 % of the total amount of ammonia produced in the human intestinal tract is excreted in the faeces (Summerskill & Wolpert, 1970).

In humans (20 male and female volunteers orally administered 9 mg/kg bw ammonium chloride), a transient increase in arterial blood ammonia concentrations occurred in about 50 % of the subjects. At 15 minutes concentrations peaked (mean of 1.4 mg NH_3/l) and by 30 minutes concentrations returned to fasting levels (mean of 1.05 mg NH_3/l). In subjects with liver cirrhosis (50 males), fasting levels were already elevated (mean of 1.56 mg NH_3/l) and increased to much higher peak concentrations (mean of 3.7 mg NH_3/l) at 15 minutes than in volunteers without known liver disease. The return to fasting levels was slow and indicated impaired hepatic urea synthesis (Conn, 1972).

Sulphides are found in a number of foodstuffs and beverages. In cooked foods sulphides may be formed from sulphur-containing amino acids. In skimmed milk the level may be 0.8 mg/l. Hydrogen sulphide in cooked meat may range from 0.3 mg/kg in beef to 0.4 in lamb. Levels of hydrogen sulphide are higher in anaerobically packaged meat (Kraft et al., 1956).

Hydrogen sulphide is endogenously produced; the main source is from metabolism of sulphur-containing amino acids in the intestinal tract. Other sources are bacterial reduction of inorganic sulphate and sulphite in the large intestine and fermentation of sulphur-containing amino acids (Richardson et al., 2000). At pH 6 hydrogen sulphide occurs mainly as the acid, whereas at pH 7.5 a large proportion is in the anionic form, mainly as monohydrogen sulphide. At pH 10 sulphide is present in appreciable concentrations (Roediger et al., 1997). Six healthy human subjects showed blood sulphide concentrations ranging from 10 to 100 micromol/l. Whole blood sulphide did not change significantly when increasing amounts of protein from meat were fed in the diet; however, faecal sulphide showed a significant increase from 160 to 750 nmol/g in four subjects fed diets containing 60 or 420 g meat (Richardson et al., 2000). It has been shown that the proximal small intestine extracts sulphate from the circulation against the concentration gradient indicating the existence of a sulphur pump (Roediger et al., 1997).

III.2. Metabolism

In the liver the majority of excess nitrogen, either in the form of ammonia absorbed from the intestinal tract or in the form of glutamine or alanine transported from peripheral tissues, is transformed to urea in the Krebs urea cycle. The formation of urea occurs only in the liver.

Ammonia that is produced in peripheral tissues is not transported as such to the liver but is converted to glutamine or alanine. Blood levels of ammonia are kept low, since the compound is toxic and high levels may impair brain function. Most tissues convert ammonia to glutamine, using glutamine synthetase. Muscle tissue, however, uses the glucose-alanine cycle in which glycolysis produces pyruvate, which produces alanine and alpha-ketoglutarate following transamination with glutamate. In the liver alanine loses its nitrogen yelding ammonia and pyruvate. Glutamine is hydrolysed in the liver by glutaminase to release ammonium for urea synthesis.

Whatever its source, ammonium that is generated in the liver mitochondria is immediately used together with carbon dioxide (as carbonate ion) produced by mitochondrial respiration, to form carbamoyl phosphate, in an ATP-dependent reaction. The carbamoyl phosphate, which is an activated carbamoyl group donor, then enters the urea cycle. In the cycle, urea is produced from the guanidino group of arginine by the hydrolytic enzyme arginase. The other product is ornithine. The amino nitrogen of catabolised amino acids or from ammonia is used to convert ornithine back to arginine.

The cycle has four enzymatic steps:

1) Carbamoyl phosphate donates its carbamoyl group to orhithine to form citrulline, with the release of inorganic phosphor.

2) The second amino group is introduced from aspartate by a condensation reaction between the amino group of aspartate and the carbonyl group of citrulline forming arginosuccinate. Aspartate is generated in mitochondria by transamination.

3) The argininosuccinate is then reversibly cleaved to form free arginine and fumarate.

4) Arginase then cleaves arginine to yield urea and ornithine. Urea is then released into the cytosolic pool of metabolites (Fürst et al., 1969; Pitts, 1971; Haworth et al., 1978; Nelson & Cox, 2000c).

Metabolism of hydrogen sulphide may follow three different pathways. The major pathway is oxidation via thiosulphate to sulphate and subsequent excretion by the kidney. The primary location for these reactions is the liver (Bartholomew et al., 1980). The other options are methylation to methanethiol and dimethyl sulphide or reaction with disulphide- or metallo-containing proteins. In the colon, sulphate-reducing bacteria reduce sulphate to hydrogen sulphide. Protective mechanisms against injury by hydrogen sulphide may be pH regulation and enzymes of detoxification e.g. thiol methyl transferases, which methylate sulphides to methyl mercaptans. Thiol methyl transferase activity in man is high in the colon, but does not exceed the activity in the liver (Roediger et al., 1997). Although it is shown that methylation is a method of detoxification of hydrogen sulphide produced in the intestine, the extent to which the toxicity of exogenous hydrogen sulphide is detoxified by methylation is not known.

The reaction with essential proteins is the reaction that causes the toxicity of hydrogen sulphide after inhalation. The mechanism of toxicity of hydrogen sulphide is the inhibition of cytochrome oxidase and, through this, the inhibition of the electron transport system (Weisiger et al., 1979; Tabacova, 1986). However, hydrogen sulphide may also react with other metalloproteins and this may in some instances represent a detoxification pathway (Beauchamp et al., 1984).

ANNEX IV: TOXICITY

Oral acute toxicity data are available for one candidate substance of the present Flavouring Group Evaluation from chemical group 30.

TABLE IV.1: ACUTE TOXICITY

Chemical Name [FL-no]	Species	Sex	Route	LD_{50} (mg/kg bw)	Reference	Comments
Ammonium chloride [FL-no: 16.048]	Guinea pig	NR	Gavage 0.9 – 1.2 g/kg bw	NR	(Koenig & Koenig, 1949)	The dose-range gave rise to pulmonary oedema. No study details given. Study not considered relevant.
	Albino rat, guinea pig, rabbit	NR	Gavage 0.05, 0.25, 1.0 g/kg bw	NR	(Boyd & Seymour, 1946)	No untoward effects were noted. No study details given. Study not considered relevant.
	Rat	NR	Oral	1650	(IUCLID Dataset, 2000d)	No study details given.

NR: Not reported

Subacute / subchronic / chronic / carcinogenic toxicity data are available for one candidate substance 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
Ammonium chloride [FL-no: 16.048]	Rabbit (2 kg) 9 in total	Gavage in drinking water	2 %, 30-50 ml per day (approx. 300-500 mg/kg bw/day)	4 weeks	-	(Joblin & Meeker, 1936)	Study was designed to assess the effect of abnormal diets on atherosclerosis. Study not considered relevant.
	Not given	Oral in diet	3 % (approx. 3 g/kg bw/day)	6 days	-	(Thompson & Halliburton, 1966)	The study assessed mechanisms of renal hypertrophy. No details given. Study not considered relevant.
	Mongrel dogs/male 1 per group	Tablet	0, 25, 46, 91, 170, 220 mg/kg bw/day	7 days	-	(Short & Hammond, 1964)	The study assessed amount of ammonium chloride needed to acidify urine without appreciable systemic acidosis. Study not considered relevant.
	Holtzman rats/female	Drinking water	0.28 M (approx. 1 g/kg bw/day)	7 days	-	(Lotspeich, 1965)	The study assessed mechanisms of renal hypertrophy. Study not considered relevant.
	Mongrel dogs/male 1 control 5 in dosage group	Capsule	0 g, 6 g per day	7 days	-	(Pollak et al., 1965)	The study aim was to investigate enzymatic activity in the kidney in metabolic acidosis. No other parameters were studied. Study not considered relevant.
	Sprague-Dawley rats 6 per group	Drinking water Gavage Gavage	0.28 M 1.2 M; 0.1 ml/10 g bw twice daily 0.6 M; 5 ml twice daily	7 days	-	(Janicki, 1970)	The study assessed mechanisms of renal hypertrophy. No other parameters were studied. Study not considered relevant.
	Sprague-Dawley rats/male	Drinking water	0 %, 2 %, 2 % + low-Ca-diet	6 months	-	(Barzel, 1969)	The study assessed the effect of chronic ammonium chloride ingestion on bone formation. No other parameters were studied.
	Sprague-Dawley rats/male	Drinking water	0 %, 1.5 % (approx 1.5 g/kg bw) 1.5 % + low-Ca-diet	330 days	-	(Barzel & Jowsey, 1969)	The study assessed the effect of chronic ammonium chloride ingestion on bone formation. No other parameters were studied.
	Wistar rats 10 male, 10 female/group	Oral in diet	0 %, 2 %, 4 % (2 %= 40.9 mmol/bw/day=2188 mg/kg bw/day M; 41.9=2242 F; 4 % = 80.2 mol/bw/ day=4291 mg/kg bw/day M 77.9=4168 F)	4 weeks	-	(Lina & Kuijpers, 2004)	The study was designed to assess effects of acidogenic diet. Well performed study. No NOAEL may be derived.
	Wistar rat 10 male, 10 female/group	Oral in diet	0 %, 2.1 %, 4 % (2.1 %= 29.8 mmol/ bw/day=1594 mg/kg bw/day M; 33.6=1798 F; 4 %= 57.0 mmol/ bw/day=3050	13 weeks	-	(Lina & Kuijpers, 2004)	The study was designed to assess effects of acidogenic diet. Well performed study. No NOAEL may be derived.

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
			mg/kg bw/day M 69.1=3697 F), 0 % + 3 %KCl				
	Wistar rat 15 male, 15 female/group	Oral in diet	0 %, 1 %, 2.1 % (1 % = 9.0 mmol/bw/day=482 mg/kg bw/day M, 11.4=610 F; 2.1 %= 19.0 mmol/ bw/day=1017 mg/kg bw/day M, 25.6=1370 F), 0 % + 3 % KCl	18 months	-	(Lina & Kuijpers, 2004)	The study was designed to assess effects of acidogenic diet. Well performed study. No NOAEL may be derived.
	Wistar rat 50 male, 50 female/group	Oral in diet	0 %, 1 %, 2.1 % (1 %= 8.5 mmol/bw/ day=455 mg/kg bw/day M, 10.3=551 F; 2.1 %= 18.8 mmol/bw/day=1006 mg/kg bw/day M 22.5=1204 F), 0 % + 3 % KCl	30 months	-	(Lina & Kuijpers, 2004)	Carcinogenicity study. The study was designed to assess effects of acidogenic diet. Well performed study. No NOAEL may be derived. There is no indication that NH_4Cl induces tumours.

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

TABLE IV.3: DEVELOPMENTAL AND REPRODUCTIVE TOXICITY STUDIES

In vitro mutagenicity/genotoxicity data are available for two candidate substances 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
Ammonia [16.009]	Ames assay	*S. typhimurium* TA98; TA100 TA1535, TA1537, TA1538 and *E. Coli* WRP uvrA	500-25000 ppm	Negative (±S9)	(Shimizu et al., 1985)	Valid study although results for ammonia not given in paper, reported as negative. Ammonium fluoride and ammonium peroxodisulfate tested in same study with negative results.
Ammonium chloride [16.048]	Ames assay	*S. typhimurium* TA92, TA94, TA98, TA100, TA1535, and TA1537	Up to 10 mg/plate	Negative (±S9)	(Ishidate et al., 1984) (Ishidate et al., 1988)	Valid study.
	Chromosome aberration	Chinese hamster fibroblasts	-	Positive (-S9)	(Ishidate et al., 1984) (Ishidate et al., 1988)	Valid study. Positive result attributed to osmotic effects.

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

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

Chemical Name	Test System	Test Object	Route	Dose	Result	Reference	Comments
Ammonia [16.009]	Micronucleus test	Mice	intraperitoneal	12, 25 or 50 mg/kg	Positive	(Yadav & Kaushik, 1997)	Study not reliable.
Ammonium chloride [16.048]	Micronucleus test	Mice	intraperitoneal	31.3-250 mg/kg bw x 4 or single doses of 62.5-500 mg/kg bw	Negative	(Hayashi et al., 1988)	Valid study.

REFERENCES

Barrow, C.S., Steinhagen, W.H., 1980. NH3 concentrations in the expired air of the rat: Importance to inhalation toxicology. Toxicol. Appl. Pharmacol. 53, 116-121.

Bartholomew, T.C., Powell, G.M., Dodgson, K.S., Curtis C.G., 1980. Oxidation of sodium sulphide by rat liver, lungs and kidney. Biochem. Pharmacol. 29(18), 2431-7.

Barzel, U.S., Jowsey, J., 1969. The effects of chronic acid and alkali administration on bone turnover in adult rats. Clin. Sci. 36, 517-524.

Barzel, U.S., 1969. Effect of excessive acid feeding on bone. Calc. Tiss. Res. 4(2), 94-100.

Beauchamp, R.O., Jr., Bus, J.S., Popp, J.A., Boreiko, C.J., Andjekovich, D.A., 1984. A critical review of the litterature on hydrogen sulfide toxicity. Crit. JRev. Toxicol. 13(1), 25-97.

Biswas, S.D., Kleinberg, I., 1971. Effect of urea concentration on its utilisation, on the pH and the formation of ammonia and carbon dioxide in a human salivary sediment system. Arch. Oral Biol. 16, 759-780.

Boyd, E.M., Seymour, K.G.W., 1946. Ethylenediamine dihydrochloride or "chlorethamine". II. Untoward and toxic reactions. Exp. Med. Surg. 223-227.

Castell D.O., Moore E.W., 1971. Ammonia absorption from the human colon. Gastroenterology. 60(1), 33-42.

Conn, H.O., 1972. Studies of the source and significance of blood ammonia. IV. Early ammonia peaks after ingestion of ammonium salts. Yale J. Biol. Med. 45, 543-549.

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.

Davies, B.,M.,A., Yudkin, J., 1952. Studies in biochemical adaptation. The origin of urinary ammonia as indicated by the effect of chronic acidosis and alkalosis on some renal enzymes in the rat. Biochem. J. 52, 407-412.

De Sousa, R.C., Harrington, J.T., Ricanati, E.S., et al., 1974. Renal regulation of acid-base equilibrium during chronic administration of mineral acid. J Clin Invest. 53, 465.

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

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

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

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

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

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

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

EFFA 2006g. Submission 2006-30. Flavouring group evaluation of 3 flavouring substances (candidate chemicals) of chemical group 30 (annex I of 1565/2000/EC) ammonia and it's salts used as flavouring substances. Unpublished report submitted by EFFA to FLAVIS Secretariat. FLAVIS/8.XX.

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

EPA, 2003. Toxicological review of hydrogene sulfide. In Support of Summary Information on the Integrated Risk Information System (IRIS). Washington DC, June 2003.

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

Flavour Industry, 2009p. Unpublished information submitted by Flavour Industry to DG SANCO and forwarded to EFSA. A-46rev1

Fürst, P., Josephson, B., Maschio, G., and Vinnars, E., 1969. Nitrogen balance after intravenous and oral administration of ammonium salts to man. J. Appl. Physiol. 26(1), 13-22.

Fürst, P., Jonsson, A., Josephson, B., Vinnars, E., 1970. Distribution in muscle and liver vein protein of 15N administered as ammonium acidosis to man. J. Appl. Physiol. 29(3), 307-312.

Haworth, S., Koo, S., Lawlor, T., 1978. Salmonella/mammalian-microsome plate incorporation mutagenesis assay of compound #T0332.01 (MRI #78). EG_G Mason Research Institute, Rockville, MD. Unpublished report to the Flavor and Extract Manufactures Association (FEMA). Submitted to WHO by the Flavor and Extract Manufacturers Association of the United States, Washington, DC, USA.

Hayashi, M., Kishi, M., Sofuni, T., Ishidate Jr., M., 1988. Micronucleus tests in mice on 39 food additives and eight miscellaneous chemicals. Food Chem. Toxicol. 26(6), 487-500.

Hunt, R.D., 1977. Measurement of partial pressure of ammonia in breath and arterial blood. Evidence for secretion of ammonia into expired gas by metabolism of monoamines in the lung. Diss. Abst. Int. Ser. B. 37, 3279.

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

Ishidate, Jr. M., Sofuni, T., Yoshikawa, K., Hayashi, M., Nohmi, T., Sawada, M., Matsuoka, A., 1984. Primary mutagenicity screening of food additives currently used in Japan. Food Chem. Toxicol. 22(8), 623-636.

Ishidate Jr., M., Harnois MC, Sofuni T., 1988. A comparative analysis of data on the clastogenicity of 951 chemical substances tested in mammalian cell cultures. Mutat Res. 195: 151-213.

IUCLID Dataset, 2000d. European Commission - European Chemicals Bureau. Substance ID: 12125-02-9, EINECS Name ammonium chloride. Section 1 - 7.

Janicki, R.H., 1970. Renal adaptation during chronic NH4Cl acidosis in the rat: No role for hyperplasia. Am. J. Physiol 210(3), 613-618.

JECFA, 1982a. 26. Report: Twenty-sixth Meeting of the Joint FAO/WHO Expert Committee on Food Additives; Report: WHO Technical Report Series, no. 683.

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

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

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

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

Joblin, J.W., Meeker, D.R., 1936. Further investigations on experimental atherosclerosis. Arch. Pathol. 22, 293-300.

Kamin, H., Handler, P., 1957. Amino acid and protein metabolism. Annu. Rev. Biochem. 26, 419-490.

Koenig, H., Koenig, R., 1949. Production of acute pulmonary edema by ammonium salts. Proc. Soc. Exp. Biol. Med. 70, 375-380.

Kraft, A.A., Brant, A.W., Ayres, J.C., 1956. Detection of hydrogen sulfide in packaged meats and in brokenout shell eggs. Food Technol. 10, 443-444.

Larson, T.V., Covert, D.S., Frank, R., Charlson, R.J., 1977. Ammonia in the human airways: Neutralization of inspired acid sulphate aerosols. Science. 197, 161-163.

Lina, B.A.R., Kuijpers, M.H.M., 2004. Toxicity and carcinogenicity of acidogenic or alkalogenic diets in rats; effects of feeding NH4Cl, KHCO3 or KCl. Food Chem. Toxicol. 42, 135-153.

Lotspeich, W.D., 1965. Renal hypertrophy in metabolic acidosis and its relation to ammonia excretion. Am. J. Physiol. 208(6), 1135-1142.

Muntwyler, E., Iacobellis, M., Griffin, G.,E., 1956. Kidney glutaminase and carbonic anhydrase activities and renal electrolyte excretion in rats. Am. J. Physiol. 184, 83-90.

Nelson, D.L., Cox, M.M., 2000c. Lehninger Principles of Biochemistry. Chapter 18: Amino Acid Oxidation and the Production of Urea. Chapter 22: Biosynthesis of Amino Acids, Nucleotides, and Related Molecules. Worth Publishers, Inc., New York, pp. 623-658, 818-868.

Pitts, R.F., 1971. The role of ammonia production and excretion in regulation of acid-base balance. New Engl. J. Med. 284, 32-38.

Pollak, V.E., Mattenheimer, H., Debruin, H., Weinman, K.J., 1965. Experimental metabolic acidosis: The enzymic basis of ammonia production by the dog kidney. J. Clin. Invest. 44, 169-181.

Richards, P., Houghton, B.J., Metcalfe-Gibson, A., Ward, E.E., Wrong, O., 1968. Incorporation of orally administered ammonia into tissue proteins in man; the influence of diet and uraemia. In: Nutrition and renal disease, Edinburgh, E., and S. Livingstone, pp. 93-98.

Richardson, C.J., Magee, E.A.M., Cummings, J.H., 2000. A new method for the determination of sulphide in gastrointestinal contents and whole blood by microdistillation and ion chromatography. Clin. Chim. Acta. 293, 115-125.

Roediger, W.E.W., Moore, J., Babidge, W., 1997. Colonic sulfide in pathogenesis and treatment of ulcerative colitis. Dig. Dis. Sci. 42, 1571-1579.

SCF, 1992. Scientific Committee for Food. Food science and techniques. Reports of the scientific committee for food. 29th series. Second report on extration solvents. Opinion expressed on June, 21, 1991. Report no. 14482, Office for the official publications of the European Communities, Luxembourg.

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

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

Shimizu, H., Suzuki, Y., Takemura, N., Goto, S., Matsushita, H., 1985. The results of microbial mutation test for forty-three industrial chemicals. Jap. J. Ind. Health 27, 400-419.

Shimoi, K., Nakamura, Y., Noro, T., Tomita, I., Fukushima, S., Inoue, T., Kada, T., 1985. Methyl cinnamate derivatives enhance UV-induced mutagenesis due to the inhibition of DNA excision repair in *Escherichia coli* B/r. Mutat. Res. 146, 15-22.

Short, E.C., Hammond, P.B., 1964. Ammonium chloride as a urinary acidifier in the dog. J. Am. Vet. Med. Assoc . 144(8), 864-867.

Summerskill, W.H.J., Wolpert, E., 1970. Ammonia metabolism in the gut. Am. J. Clin. Nutr. 23, 633-639.

Tabacova, S., 1986. Maternal exposure to environmental chemicals. Neurotoxicology, 7(2), 421.

Thompson, R.Y., Halliburton, I.W., 1966. Effect of diet on the composition of the kidney. Biochem. J. 99(3), 44.

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

TNO, 2010. Volatile Compounds in Food - VCF Database. TNO Nutrition and Food Research Institute. Boelens Aroma Chemical Information Service BACIS, Zeist, The Netherlands.

U.S. National Research Council, 1979. Hydrogen sulphide. Committee on Medical and Biologic Effects of Environmental Pollutants, Subcommittee on Hydrogen Sulphide. University Park Press, Baltimore, MD.

UNEP, 2004. SIDS Initial Assessment Report for ammonium sulfate. UNEP Publications http://www.chem.unep.ch/irptc/sids/OECDSIDS/7783202.pdf

US ATSDR, 2004. Toxicological profile for ammonia. U.S. Department of Health and Human Services, Agency for Toxic Substances and Disease Registry.

US ATSDR, 2006. Toxicological profile for hydrogen sulfide. U.S. Department of Health and Human Services, Agency for Toxic Substances and Disease Registry.

Vitti, T.G., Vukmirovich, R., Gaebler, O.H., 1964. Utilization of ammonia nitrogen, administered by intragastric, intraperitoneal and subcutaneous routes: Effects of growth hormone. Arch. Biochem. Biophys. 106, 475-482.

Weisiger, R.A., Pinkus, L.M., Jakoby, W.B., 1979. Potential role of thilmethyltransferase in the detoxification of intestinal hydrogen sulphide and methanethiol. Gastroenterology, 76, 1269.

White, A., Handler, P., Smith E.L., 1973. Renal function and the composition of urine. In: Principles of biochemistry, 5th ed., New York, McGraw Hill Book Co., p. 604 and 928.

WHO, 1996. Guidelines for drinking-water quality. 2nd Ed. vol. 2. Health criteria and other supporting information. World Health Organization, International Programme on Chemical Safety. Geneva.

Yadav, J., S., Kaushik, V., K., 1997. Genotoxic effect of ammonia exposure on workers in a fertilizer factory. Indian J. Exp. Biol. 35, 487-92.

ABBREVIATIONS

ADI	Acceptable Daily Intake
ATP	Adenosine TriPhosphate
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)
CoE	Council of Europe
DNA	Deoxyribonucleic acid
EC	European Commission
EFFA	European Flavour and Fragrance Association
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)
ID	Identity
IOFI	International Organization of the Flavour Industry
JECFA	The Joint FAO/WHO Expert Committee on Food Additives
LD_{50}	Lethal Dose, 50 %; Median lethal dose
MS	Mass spectrometry
MSDI	Maximised Survey-derived Daily Intake
mTAMDI	Modified Theoretical Added Maximum Daily Intake
No	Number
NOAEL	No Observed Adverse Effect Level
NOEL	No Observed Effect Level
SCF	Scientific Committee on Food
TAMDI	Theoretical Added Maximum Daily Intake
UDS	Unscheduled DNA Synthesis
WHO	World Health Organisation