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Scientific opinion on the evaluation of substances as acceptable previous cargoes for edible fats and oils

EFSA Panel on Contaminants in the Food Chain (CONTAM),
Helle Katrine Knutsen, Jan Alexander, Lars Barregård, Margherita Bignami, Beat Brüschweiler, Sandra Ceccatelli, Bruce Cottrill, Michael Dinovi, Lutz Edler, Christer Hogstrand, Laurentius (Ron) Hoogenboom, Carlo Nebbia, Isabelle Oswald, Annette Petersen, Martin Rose, Alain-Claude Roudot, Tanja Schwerdtle, Günter Vollmer, Heather Wallace, Bettina Grasl-Kraupp, Konrad Grob, André Penninks, Marco Binaglia, Ruth Roldán Torres and Christiane Vleminckx

Abstract

Shipping of edible fats and oils into Europe is permitted in bulk tanks, provided that the previous cargo is included in a positive list. The European Commission requested EFSA to evaluate the acceptability as previous cargoes for fats and oils the substances calcium lignosulphonate, methyl acetate, ethyl *tert*-butyl ether (ETBE) and ammonium sulphate. The evaluation was based on the same criteria as those used for the evaluation of the substances currently on the list in the Annex to Commission Directive 96/3/EC as acceptable previous cargoes for edible fats and oils. Methyl acetate and ETBE meet the criteria for acceptability as previous cargoes. Due to uncertainties, mainly with regard to the composition and toxicity of the low molecular mass fraction, and the fact that the toxicological database is limited to the 40–65 grade and does not cover all grades of calcium lignosulphonate shipped as previous cargoes, the EFSA Panel on Contaminants in the Food Chain (CONTAM Panel) concluded that calcium lignosulphonate does not meet the criteria for acceptability as a previous cargo. Only food-grade ammonium sulphate meets the criteria for acceptability as a previous cargo due to uncertainties about impurities in other (non-food) grades.

Keywords: acceptable previous cargo, edible fats and oils, sea transport, calcium lignosulphonate, methyl acetate, ethyl *tert*-butyl ether, ammonium sulphate

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Correspondence: contam@efsa.europa.eu

Panel members: Jan Alexander, Lars Barregard, Margherita Bignami, Beat Brueschweiler (from 23 June 2016), Sandra Ceccatelli, Bruce Cottrill, Lutz Edler, Michael Dinovi, Bettina Grasl-Kraupp, Christer Hogstrand, Laurentius (Ron) Hoogenboom, Helle Katrine Knutsen, Carlo Stefano Nebbia, Isabelle Oswald, Annette Petersen, Vera Maria Rogiers (until 9 May 2016), Martin Rose, Alain-Claude Roudot, Tanja Schwerdtle, Christiane Vleminckx, Günter Vollmer and Heather Wallace.

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Summary

Shipping of edible fats and oils into Europe is permitted in bulk tanks, provided that the previous cargo is included in a positive list. The European Commission requested the European Food Safety Authority (EFSA) to evaluate the acceptability as previous cargoes for fats and oils the substances calcium lignosulphonate, methyl acetate, ethyl *tert*-butyl ether (ETBE) and ammonium sulphate. The evaluation was based on the criteria adopted by EFSA in 2009 and also used for the evaluation of the substances currently on the list of acceptable previous cargoes for edible fats and oils in the Annex to Commission Directive 96/3/EC.

Calcium lignosulphonate was re-evaluated on the basis of new information after the negative recommendations by the EFSA Panel on Contaminants in the Food Chain (CONTAM Panel) in 2011. As in 2011, the data on calcium lignosulphonate (highly purified 40–65 grade) did not provide evidence of genotoxicity, immunotoxicity, skin sensitisation and allergenicity. A no-observed-adverse-effect-level (NOAEL) of 2,000 mg/kg body weight (bw) per day (the highest dose tested) was determined in a 90-day dietary rat study. However, there are several data gaps, mainly with regard to the composition and toxicity of the low molecular mass fraction. Moreover, as in 2011, the toxicological database is limited to the 40–65 grade and does not cover all grades of calcium lignosulphonate shipped as previous cargoes for fats and oils. Therefore, the CONTAM Panel concluded that calcium lignosulphonate does not meet the criteria for acceptability as a previous cargo.

Limited data are available on the toxicity of methyl acetate. The substance is not acutely toxic by the oral route and it did not induce reverse mutations in *Salmonella* Typhimurium or *Escherichia coli*. Methyl acetate is volatile and easily removed by cleaning the tanks. It is hydrolysed to methanol and acetic acid in aqueous solution. The expected main impurities are also methanol and acetic acid. Toxicity can therefore be evaluated by the effects of these substances, and the CONTAM Panel has previously concluded that methanol and acetic acid meet the criteria for acceptability as a previous cargo. Methyl acetate easily transesterifies with triglycerides, resulting in methyl esters of fatty acids and acetylated glycerol, which are considered to be of no concern. The CONTAM Panel concluded that methyl acetate meets the criteria for acceptability as a previous cargo for fats and oils.

ETBE is metabolised by oxidation to *tert*-butyl alcohol (TBA) and acetaldehyde in the body. TBA may be further metabolised to 2-methyl-1,2-propanediol and then to 2-hydroxyisobutyrate. Since the structures of ETBE and methyl *tert*-butyl ether (MTBE) are very similar, the expected similarities in their toxicological properties have been taken into consideration where appropriate. In view of the general lack of genotoxicity of ETBE and TBA and the limited information on the carcinogenicity of ETBE, TBA and MTBE, the CONTAM Panel does not consider that ETBE represents a risk for carcinogenicity at the levels of exposure that would occur following its use as a previous cargo. ETBE is not toxic for reproduction or development in the absence of other manifestations of general toxicity. The NOAELs for developmental toxicity and fertility were 300 and 1,000 mg/kg bw per day, respectively. ETBE is not expected to be immunotoxic, skin sensitising or allergenic. The CONTAM Panel established a tolerable daily intake (TDI) of 1 mg ETBE/kg bw per day, based on the NOAEL of a 6-month study in rats and applying an uncertainty factor of 100. Expected impurities of ETBE are ethanol and isobutylene as well as impurities of isobutylene, such as 1-butylene, iso-pentanes and iso-pentenes. ETBE itself and the impurities are volatile and easily removed by cleaning the tanks. Being an ether, ETBE is expected to be chemically stable and not to react with components of fats and oils. The CONTAM Panel concluded that ETBE meets the criteria for acceptability as previous cargo.

In aqueous environments and at physiological pH, ammonium sulphate dissociates into ammonium ion and sulphate, which have been accepted as previous cargoes by the CONTAM Panel in the form of ammonium polyphosphate, ammonium hydroxide and sulfuric acid. The data available for ammonium sulphate as used in toxicity testing did not indicate concern for genotoxicity, carcinogenicity or immunotoxicity. No effects on fertility have been reported, and adverse effects on development were observed only secondarily to maternal toxicity. There was no indication that ammonium sulphate is skin sensitising or allergenic. An acceptable daily intake (ADI) for sulphate was set by the EFSA Pesticide Risk Assessment Peer Review (PRAPeR) at 12.5 mg/kg bw per day. The potential contribution of ammonium sulphate used as a previous cargo to dietary exposure is negligible and is also negligible compared to endogenous synthesis.

The CONTAM Panel concluded that the exposure to food-grade ammonium sulphate when used as a previous cargo would not give rise to toxicological concern. However, the bulk of the ammonium sulphate is likely to be of different grades, mainly used as a fertiliser or as an industrial chemical. There were insufficient data about the impurities from the various sources. The CONTAM Panel, therefore, concluded that only food-grade ammonium sulphate meets the criteria for acceptability as a previous cargo.

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1. Introduction

1.1. Background and Terms of Reference as provided by the European Commission

BACKGROUND

General hygiene requirements relating to transport of food applicable to all food business operators laid down in Regulation (EC) No 852/2004[1] (Annex II, Chapter IV) state, among others, that 'receptacles in vehicles and/or containers are not to be used for transporting anything other than foodstuffs where this may result in contamination'.

Information showed that the application of this principle to the bulk transport was not practical and imposed an unduly onerous burden on food business when applied to bulk transport in seagoing vessels of liquid oils and fats and of raw sugar. Two legal acts,[2,3] providing equivalent protection to public health are derogating from the above mentioned general hygiene requirements.

Equivalent protection to public health is guaranteed on technical (e.g. tank design) and procedural (e.g. intermediate cleaning) conditions, on record keeping (e.g. on effectiveness of cleaning and on the nature of the previous cargoes) and, in the case of bulk transport of liquid oils and fats in seagoing vessels, on a list of acceptable previous cargoes. The inclusion of a substance in the list of acceptable previous cargoes for fats and oils is based on the Scientific Opinions delivered by the European Food Safety Authority (EFSA).[4,5,6]

Four substances are proposed for evaluation: calcium lignosulphonate (CAS No 8061-52-7), methyl acetate (CAS No 79-20-9), ethyl *tert*-butyl ether (ETBE) (CAS No 637-92-3) and ammonium sulphate (inorganic salt) (CAS No 7783-20-2). For calcium lignosulphonate, the previous evaluation concluded that this substance did not meet the criteria for acceptability as a previous cargo due to a lack of information on potential impurities and absence of information on its potential reactivity with fats and oils. As additional information has been provided in relation to these aspects, this substance is proposed for re-evaluation.

The scientific opinion will be used to update the list of acceptable previous cargoes in Commission Regulation (EU) No 579/2014 and to support the European Union's position in the Codex Committee on Fats and Oils.

TERMS OF REFERENCE

In accordance with Art, 29 (1) of Regulation (EC) No 178/2002, the European Commission asks EFSA for a scientific opinion on the evaluation of the acceptability as previous cargoes for fats and oils of the substances calcium lignosulphonate (CAS No 8061-52-7), methyl acetate (CAS No 79-20-9), ethyl *tert*-butyl ether (ETBE) (CAS No 637-92-3) and ammonium sulphate (inorganic salt) (CAS No 7783-20-2).

The evaluation should be based on the criteria used for the three Scientific Opinions on the evaluation of the substances currently on the list in the Annex to Commission Directive 96/3/EC as acceptable previous cargoes for edible fats and oils.

1.2. Interpretation of the Terms of Reference

EFSA received a request from the European Commission for a scientific opinion on the evaluation of the acceptability as previous cargoes for fats and oils of the substances calcium lignosulphonate (CAS No 8061-52-7), methyl acetate (CAS No 79-20-9), ethyl *tert*-butyl ether (ETBE) (CAS No 637-92-3) and ammonium sulphate (CAS No 7783-20-2).

The EFSA Scientific Panel on Contaminants in the Food Chain (CONTAM Panel) concluded that this opinion should comprise:

[1] Regulation (EC) No 852/2004 of the European Parliament and of the Council of 29 April 2094 on the hygiene of foodstuffs (OJ L 139, 30.4.2004, p. 1–54.

[2] Commission Regulation (EU) No 579/2014 of 28 May 2014 granting derogation from certain provisions of Annex II to Regulation (EC) No 852/2004 of the European Parliament and of the Council as regards the transport of liquid oils and fats by sea (OJ L 160, 29.5.2014, p. 14–20.

[3] Commission Directive 98/28/EC of 29 April 1998 granting a derogation from certain provisions of Directive 93/43/EEC on the hygiene of foodstuffs as regards the transport by sea of bulk raw sugar. OJ L 140, 12.5.1998, p. 10–11.

[4] Scientific Opinion of the Panel on Contaminants in the Food Chain on a request from the European Commission on the review of the criteria for acceptable previous cargoes for edible fats and oils. EFSA Journal (2009), 1110, 1–21.

[5] EFSA Panel on Contaminants in the Food Chain (CONTAM); Scientific Opinion on the evaluation of substances as acceptable previous cargoes for edible fats and oils. EFSA Journal 2009; 7(11):1391.

[6] EFSA Panel on Contaminants in the Food Chain (CONTAM); Scientific Opinion on the evaluation of the substances currently on the list in the Annex to Commission Directive 96/3/EC as acceptable previous cargoes for edible fats and oils: Part I of III. EFSA Journal 2011;9(12).2482, Part II of III. EFSA Journal 2012;10(5)2703 and Part III of III. EFSA Journal 2012;10(12);2984.

a) Re-evaluation of the substance calcium lignosulphonate (CAS No 8061-52-7) following the additional information provided to EFSA (see Documentation provided to EFSA) and new data.
b) Evaluation of the substances methyl acetate (CAS No 79-20-9), ethyl *tert*-butyl ether (ETBE) (CAS No 637-92-3) and ammonium sulphate (CAS No 7783-20-2).

The evaluation is based on the criteria used for the Scientific Opinions on the evaluation of the substances currently on the list in the Annex to Commission Directive 96/3/EC as acceptable previous cargoes for edible fats and oils. (See Section 2.2).

1.3. Additional information

1.3.1. Previous risk assessments

In 1996, the Scientific Committee on Food (SCF) developed an opinion on the potential risk to human health arising from the transport of oils and fats in ship tanks from substances proposed as acceptable previous cargoes (SCF, 1997). In this opinion, the SCF set criteria and evaluated a number of substances against those. In 2003, SCF updated this opinion and considered, where available, new toxicological information (SCF, 2003).

Scientific Committee on Food (SCF)

Table 1: Criteria for the inclusion of substances in the list of acceptable previous cargoes according to the SCF (SCF, 1997, 2003)

	SCF Criteria[(a)]
1.	No toxicological concerns, particularly with regard to their genotoxic and carcinogenic potential, for which a threshold is difficult to establish
2.	Efficacy of procedures used to clean ships' tanks between cargoes
3.	Dilution factor in relation to the potential amount of residue of the previous cargo and any impurity which the previous cargo might have contained and the quantity of oil or fat transported
4.	Subsequent application of refining processes and solubility relevant to the occurrence of possible contaminating residues
5.	Availability of analytical methods to verify the presence of trace amounts of residues or the absence of contamination of oils and fats

(a): The SCF criteria have no numbering in the original reference. In the present opinion, they have been included for an easier referral throughout the document.

European Food Safety Authority (EFSA)

In 2009, EFSA received a request from the European Commission to review the criteria for acceptable previous cargoes for edible fats and oils set by the SCF (Table 1). The CONTAM Panel assessed the appropriateness of the four criteria of the Codex Committee for Fats and Oils (CCFO) (Table 2) by comparing them with those set by the SCF in 1996 (EFSA, 2009).

Table 2: Criteria proposed for immediate previous cargoes by the Codex Committee for Fats and Oils (CCFO) during their 21st meeting (CCFO, 2009) and adopted by the Codex Alimentarius Committee (CAC) (FAO/WHO, 2011)

	CCFO Criteria (adopted at Step 5)
1.	The substance is transported/stored in an appropriately designed system; with adequate cleaning routines, including the verification of the efficacy of cleaning between cargoes, followed by effective inspection and recording procedures
2.	Residues of the substance in the subsequent cargo of fat or oil should not result in adverse human health effects. The ADI (or TDI) of the substance should be greater than or equal to 0.1 mg/kg bw per day. Substances for which there is no numerical ADI (or TDI) should be evaluated on a case-by-case basis
3.	The substance should not be or contain a known food allergen, unless the identified food allergen can be adequately removed by subsequent processing of the fat or oil for its intended use
4.	Most substances do not react with edible fats and oils under normal shipping and storage conditions. However, if the substance does react with edible fats and oils, any known reaction products must comply with criteria 2 and 3

ADI: acceptable daily intake; TDI: total daily intake; bw: body weight.

'The CONTAM Panel concluded that the criteria for evaluation of acceptable previous cargoes for edible fats and oils as proposed by the CCFO are not in conflict with any of the five criteria developed by SCF. SCF criteria 1–4 are either explicitly or implicitly covered by the CCFO criteria. SCF criterion 5, dealing with the availability of analytical methods, is not explicitly addressed in the CCFO criteria. The CONTAM Panel considers that SCF criterion 5 is still important. The CCFO criteria also cover food allergens and compounds that may react with oil and fats. The CONTAM Panel considers these additions relevant. In addition, the CONTAM Panel made the following remarks:

- The CCFO criteria specifically apply to the immediate previous cargo. The CCFO criterion 1, which addresses, among other issues, documentation procedures, does not specify for how many previous cargoes records should be kept. This might be particularly important in the event that earlier previous cargoes consist of substances for which an acceptable daily intake (ADI) (or tolerable daily intake, TDI) has not been established. The CONTAM Panel was of the opinion that records of the three previous cargoes should be kept, in accordance with the Codex Recommended International Code of Practice for the Storage and Transport of Edible Oils and Fats in Bulk.
- With respect to CCFO criterion 2, the EFSA Panel on Contaminants in the Food Chain (CONTAM Panel) agreed with the proposed threshold of an ADI (or TDI) of $\geq$ 0.1 mg/kg body weight (bw). For substances for which there is no numerical ADI (or TDI) a case by case evaluation is needed. The Panel also considered the situation of second and third previous cargoes and concluded that for non-genotoxic substances their transport as second and third previous cargoes is not of concern, taking into account their very limited carry over. However, the CONTAM Panel noted that genotoxic substances would not be acceptable as previous cargoes. Also in relation to CCFO criterion 2, the CONTAM Panel noted that as consequence of the above some substances will turn out to be unacceptable as previous cargoes. This could include substances with ADI (or TDI) < 0.1 mg/kg bw or substances with genotoxic activity. The Panel was of the opinion that the exclusion of such substances as previous cargoes is appropriate.
- CCFO criterion 3 is sufficient to cover 'known food allergens'. However, the CONTAM Panel considered that the scope of the CCFO criterion is too narrow, and should apply to all known allergens, not just to known food allergens, given the fact that the same cargo may be sold for cosmetic use.
- The CONTAM Panel endorsed CCFO criterion 4 without further remarks'.

Following a request from European Commission, the CONTAM Panel of EFSA evaluated a list of substances that had been proposed at Codex level for addition to the list of Codex acceptable previous cargoes (EFSA CONTAM Panel, 2009). The evaluation was done taking into account the agreed EFSA criteria (EFSA, 2009).

In 2010, in order to ensure that all substances currently included on the list of acceptable previous cargoes were evaluated against the same criteria, EFSA received a request from European Commission to deliver a scientific opinion on the evaluation of the substances listed in the Annex to Commission Directive 96/3/EC as acceptable previous cargoes for edible fats and oils. The CONTAM Panel evaluated all the substances in the list and published three opinions (EFSA CONTAM Panel, 2011, 2012a,b).

1.3.2. Legislation

Council Directive 93/43/EEC[7] on the hygiene of foodstuffs laid down the general rules of hygiene for foodstuffs and the procedures for verification of compliance with these rules. Chapter IV of the Annex considered the hygienic transportation of foodstuffs.

Commission Directive 96/3/EC[8] derogated certain provisions of Council Directive 93/43/EEC on the hygiene of foodstuffs as regards the transport of bulk oils and fats by sea, permitting the transport by sea of bulk oils and fats in tanks which have previously been used to transport substances listed in the Annex to this Directive and subject to conditions which ensure the protection of public health and safety of foodstuffs concerned.

[7] Council Directive 93/43/EEC of 14 June 1993 on the hygiene of foodstuffs. OJ L 175, 19.7.1993, p. 1–11.
[8] Commission Directive 96/3/Euratom, ECSC, EC of 26 January 1996 granting a derogation from certain provisions of Council Directive 93/43/EEC on the hygiene of foodstuffs as regards the transport of bulk liquid oils and fats by sea. OJ L 21, 27.1.1996, p. 42–46.

In order to take account of scientific developments and based on the evaluations carried out by the SCF (SCF, 1997, 2003), the list of acceptable previous cargoes was amended by Commission Decision 2004/4/EC.

Council Directive 93/43/EEC was repealed by Regulation (EC) No 852/2004 on the hygiene on foodstuffs, which laid down general hygiene requirements relating to transport of food applicable to all food business operators. Annex II of Chapter IV states, among others, that 'receptacles in vehicles and/or containers are not to be used for transporting anything other than foodstuffs where this may result in contamination'.

The outcome of the EFSA scientific opinions on the evaluation of the substances on their acceptability as previous cargoes for fats and oils published from 2009 to 2012 was taken into account for the subsequent repeal of Directive 96/3/EC and its replacement by Commission Regulation (EU) No 579/2014. This regulation provides for derogation from point 4 of Chapter IV of Annex II to Regulation (EC) No 852/2004 as regards the transport on seagoing vessels of oils and fats intended for or likely to be used for human consumption under certain conditions and includes a revised list of acceptable previous cargoes.

As the combined entry for 'ammonium nitrate solution and calcium nitrate (CN-9) solution and their double salt' created confusion to ship charterers and competent authorities, the Commission replaced this entry in the list of acceptable previous cargoes by the separate entries of 'ammonium nitrate solution' and 'calcium ammonium nitrate'. Further, having identical hazard profiles and only differing in the amount of crystal water, 'calcium ammonium nitrate', 'calcium (II) nitrate dehydrate' and 'calcium nitrate tetrahydrate' were inserted. At its 68th plenary meeting,[9] the EFSA CONTAM Panel confirmed that these changes have no impact on the toxicological properties and chemical reactivity. Therefore, the list of acceptable previous cargoes in the Annex to Regulation (EU) No 579/2014 has been amended by Commission Regulation (EU) 2016/238.[10]

2. Data and methodologies

2.1. Data

2.1.1. Documents submitted to EFSA for the re-evaluation of calcium lignosulphonate

Borregaard AS, one of the main producers of lignosulphonates, provided the CONTAM Panel information mainly on the chemical composition of calcium lignosulphonate and the toxicity of some components. Borregaard also provided a report on determining if reaction products are formed between lignosulphonates and vegetable oil (see Documentation provided to EFSA).

2.1.2. Data retrieved by search

For the present evaluation of the substances as acceptable previous cargoes, the CONTAM Panel considered literature made publicly available until 18 April 2016.

The evaluation is based on available studies/information from literature searches carried out on public databases, e.g. PubMed, the International Uniform Chemical Information Database (IUCLID), the European Chemicals Agency (ECHA), evaluations made by the national and international bodies, e.g. the World Health Organization (WHO). A comprehensive search for literature was conducted for peer-reviewed original research published pertaining to the toxicity and allergenicity of the compounds. Reviews were also considered for the current risk assessment.

The CONTAM Panel considered the previous assessment on calcium lignosulphonate as a previous cargo (EFSA CONTAM Panel, 2011) as comprehensive, covering all relevant publications up to that date.

2.2. Methodologies

In the years 2009–2012, the CONTAM Panel evaluated the acceptability of the substances listed in the Annex to Commission Directive 96/3/EC as acceptable previous cargoes for edible fats and oils, based on its review of the criteria described in Section 1.3.1 (EFSA, 2009) and the experience gained in its subsequent evaluations, which highlighted the importance of addressing impurities that might be present (EFSA CONTAM Panel, 2009, 2011, 2012a,b).

[9] Minutes of the 68th CONTAM Plenary meeting available online: http://www.efsa.europa.eu/en/events/event/141125b-m.pdf

[10] Commission Regulation (EU) 2016/238 of 19 February 2016 amending the Annex to Regulation (EU) No 579/2014 granting derogation from certain provisions of Annex II to Regulation (EC) No 852/2004 of the European Parliament and of the Council as regards the transport of liquid oils and fats by sea. OJ L 45, 20.2.2016. p. 1–2.

The present opinion is based on the criteria established by the EFSA CONTAM Panel (EFSA, 2009):

- The substance is transported/stored in an appropriately designed system, with adequate cleaning routines, including the verification of the efficacy of cleaning between cargoes, effective inspection and recording procedures. The CONTAM Panel was of the opinion that records of the three previous cargoes should be kept, in accordance with the Codex Recommended International Code of Practice for the Storage and Transport of Edible Fats and Oils in Bulk. The CONTAM Panel noted that the choices made with respect to design of the transport system and the cleaning methods are part of the responsibility of those managing the transport of previous cargoes. It was the nature and amount of substances that might be carried over into a subsequent cargo of edible fats and oils that was taken into account by the CONTAM Panel in its evaluation of previous cargoes.
- Residues of the substance in the subsequent cargo of fat or oil should not result in adverse human health effects. The ADI (or TDI) of the substance should be greater than or equal to 0.1 mg/kg bw per day. Substances for which there is no numerical ADI (or TDI) should be evaluated on a case by case basis. For non-genotoxic substances, their transport as second and third previous cargoes is not of concern, taking into account their very limited carry-over. However, genotoxic substances would not be acceptable as second and third previous cargoes.
- The substance should not be or contain a known allergen, unless the identified allergen can be adequately removed by subsequent processing of the fat or oil for its intended use. This criterion covers all allergens, not only food allergens.
- If the substance reacts with edible fats and oils, reaction products must comply with the above two criteria. Reactions may be promoted by the acidity from free fatty acids and may occur over many months or at high temperatures during raffination after the transport; they do not need to result in high yields to be potentially relevant.
- The development of analytical methods of sufficient sensitivity to check for residues in fats and oils should be feasible, e.g. for control authorities, but such methods are seldom available, since most substances used as previous cargoes are not commonly analysed in fats and oils. The CONTAM Panel therefore evaluated the feasibility of developing such methodology as part of its assessment of each substance. In those cases where, due to the nature or composition of the substance to be evaluated as a previous cargo, the feasibility of developing suitable analytical methods was considered questionable, this was indicated when discussing the substance and was used as an argument for the rejection of a substance as a previous cargo.
- It is unrealistic to assume that chemical analysis would regularly be applied to check the purity of a substance used as a previous cargo or the efficiency of the cleaning procedure. Therefore, the substances were evaluated under worst-case assumptions with regard to cleaning efficiency and material composition (in particular the potential presence of toxic impurities or the formation of reaction products with edible fats and oils).
- Potentially relevant impurities in the previous cargo should be taken into account, since they may be toxicologically more important than the substance itself. As most products exist in different grades, a reasonable worst-case product within the specification provided was assumed, the concentration of the impurity estimated from available sources and evaluated in the same way as a listed substance. Impurities are often specified for fine chemicals and highly purified products. However, these are unlikely to be shipped in bulk. Those more commonly encountered are likely to be of intermediate to low purity grade and no specific information about impurities is publicly available (sources and methods of synthesis are usually confidential). Due to this lack of information, the source and most probable way (or ways) of synthesis of the substance was investigated to determine potentially relevant impurities, such as unreacted starting substances or products of side reactions.

3. Assessment

3.1. Calcium lignosulphonate (CAS No 8061-52-7)

3.1.1. Chemical properties and use

After purification, calcium lignosulphonate is a light brown powder. Lignosulphonates are polydisperse with a molecular mass ranging up to approximately 140,000 Da.

Calcium lignosulphonate is obtained from the sulfite pulping of wood (Toledo and Kuznesof, 2008). The three main components of wood, namely cellulose, hemicellulose and lignin, are separated with the help of heating in an acidic aqueous solution of calcium sulfite. The lignin macromolecules are solubilised through sulfonation and cleavage to smaller molecules, while the hemicellulose is hydrolysed to monomeric sugars; the cellulose remains cleaved as insoluble fibres.

Wood chips are digested with acidic calcium bisulfite solution during 6–10 h at approximately 130°C. Bisulfite ions react with the native lignin to form sulphonated lignin, i.e. lignosulphonate, which increases the water solubility of the lignin. At the same time, macromolecules are cleaved, forming smaller molecules. Further reactions may cause elimination of water or sulfur dioxide (Rydholm, 1965; Hoyt and Goheen, 1971). The water-insoluble cellulose is separated by filtration. The brownish filtrate, containing the lignosulphonates, includes not only natural components of wood, such as lipids, fatty acids, wax esters, sterols and their degradation products, resin acids and long-chain alcohols, but also sulfite, inorganic salts, sugars and reaction products.

For technical grades, the refining involves steam stripping of the spent sulfite liquor, driving off most of the excess of sulfur dioxide as well as volatile substances like formaldehyde, furfural, hydroxymethyl furfural (HMF), acetic acid and formic acid. Fermentation followed by distillation of ethanol largely removes the fermentable sugars. The subsequent water evaporation to solutions containing 50% solids (the products shipped) further reduces the volatile components. Precipitated salts are removed by filtration. Purification to obtain food- and feed-grade products involves ultrafiltration through a semipermeable membrane in order to further reduce the low molecular species (EFSA ANS Panel, 2010).

The largest use of lignosulphonates is as plasticisers in concrete to improve flow properties and slowing solidification. Lignosulphonates allow concrete to be made with less water (giving stronger concrete), while maintaining the flow properties. They are also applied during the production of cement, where they act as grinding aids. Similarly, lignosulphonates serve in the production of plasterboard to reduce the amount of water required to make the stucco flow and form the layer between two sheets of paper. The reduction in water content allows lower temperatures for drying, saving energy.

Calcium lignosulphonates are also used in petroleum drilling (blocking agent, improvement of mud fluidity), for asphalt emulsification, tanning leather, as dispersant of chemicals and pesticides, additive of slurry mixture of water and coal, and as additive for feedstuff processing (deflocculant).

Lignosulphonate products (calcium, sodium or magnesium salts) are also used in animal feeds (EFSA FEEDAP Panel, 2015).

3.1.2. Previous evaluations

Calcium lignosulphonate has been evaluated by the SCF in 1996 and was considered as a acceptable previous cargo. It was noted that calcium lignosulphonate was likely to be toxicologically inert and easily removed by tank cleaning. Moreover, it was acceptable as an animal feedstuff (SCF, 1997). In the 2003, SCF evaluation of acceptable previous cargoes, it was not further evaluated as it was already considered acceptable (SCF, 2003).

Calcium lignosulphonate (40–65, specifying an intensively purified lignosulphonate product by the average molecular weight range of 40,000-65,000 Da) was evaluated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 2008 as a food additive, intended to be used as a carrier of encapsulated food ingredients (JECFA, 2009). JECFA established an ADI of 0–20 mg/kg bw based on a no-observed-effect level (NOEL) of 2,000 mg/kg bw per day from a 90-day dietary rat study and applying a safety factor of 100. It was concluded that the histiocytosis in the mesenteric lymph nodes of rats fed calcium lignosulphonate (40–65) was of no toxicological consequence. No indication for immunotoxicological effects were reported, as supported by the results of immune function assay in which the primary immune response to sheep red blood cells was comparable to control levels. Finally, calcium lignosulphonate (40–65) did not show a skin sensitisation potential in a local lymph node assay (LLNA) in mice (JECFA, 2009).

In 2010, the Panel on Food Additives and Nutrient Sources added to Food (ANS Panel) of EFSA prepared a scientific opinion on the use of calcium lignosulphonate (40–65), the same particular calcium lignosulphonate as evaluated by JECFA, as a carrier for vitamins and carotenoids intended to be added to foods for colouring and nutrient purposes (EFSA ANS Panel, 2010). The molecular mass of > 90% of calcium lignosulphonate (40–65) was specified to range between 1,000 and 250,000 Da. The degree of sulfonation, expressed as the ratio between organically bound sulfur and methoxyl groups, was in the range of 0.3 and 0.7. This lignosulphonate contained at most 5% reducing sugars.

The concentration of the low molecular mass lignin degradation products was not addressed. This specification was in agreement with that of JECFA (2008). The ANS Panel considered that the 90-day dietary rat study was inadequate for evaluating the safety of this calcium lignosulphonate due to the high incidence of lymphoid hyperplasia and lymphoid infiltration in the mandibular and mesenteric lymph nodes, in the Peyer's patches and in the liver in all animals, including controls. It considered that the available data were insufficient to establish an ADI and that long-term toxicity studies were needed to elucidate whether the histiocytosis in the mesenteric lymph nodes of the rats observed in the 90-day study may progress into a more adverse state with time. Contact allergy to calcium lignosulphonate has been described in EFSA 2010 for one single case (EFSA ANS Panel, 2010).

In 2011, the ANS Panel considered that the new information provided by the petitioner did not address the questions raised by the Panel in 2010 and that the requested chronic toxicity study of at least 12 months was still needed (EFSA ANS Panel, 2011).

In 2011, the CONTAM Panel evaluated calcium lignosulphonate (unspecified grade) as a acceptable previous cargo. The toxicological database was considered to have several data gaps (long-term toxicity, carcinogenicity and limited data on reproductive toxicity). The limited data available did not demonstrate evidence of genotoxicity or significant concern regarding allergenicity. The CONTAM Panel considered that the available information was sufficient to conclude that the exposure to the evaluated grade of calcium lignosulphonate, when used as a previous cargo, would not only give rise to toxicological concern, but also that the grades of calcium lignosulphonate varied markedly and no information on its potential reactivity with fats and oils (EFSA CONTAM Panel, 2011) was available. It concluded that calcium lignosulphonate does not meet the criteria for acceptability as a previous cargo.

In 2015, the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) Panel of EFSA delivered an opinion on the safety of an unspecified lignosulphonate for target animals, consumers and users as well as for the environment, when used as a technological additive; functional group: binders (EFSA FEEDAP Panel, 2015). It concluded that there is no concern for consumer safety from the use of lignosulphonate in animal nutrition at up to 1% in the feed. Data on typical composition was provided and purification described as ultrafiltration or fermentation, but no requirements on the grade were specified.

3.1.3. Current evaluation

3.1.3.1. Expected impurities

In reaction to the previous evaluation of calcium lignosulphonate as a previous cargo (EFSA CONTAM Panel, 2011), Borregaard (Østfold, Norway), one of the main producers of lignosulphonate, provided additional information on the composition of the substance but the CONTAM Panel stated that this information was insufficient to re-evaluate the substance.

Based on the information about some impurities in some of the products provided by Borregaard in 2014 and 2016, these were considered of no concern. However, a main part of the components (about 10%) with a molecular mass below 1,000 Da consists of unspecified lignin degradation products of unknown genotoxic potential.

There is no analytical method of sufficient sensitivity for the analysis of lignosulphonate in edible oils, and since there is no adequate method for analysing the low molecular mass lignin degradation products in the lignosulphonate, it is considered unrealistic to analyse these in edible oils of fats.

3.1.3.2. Reactivity and reaction products

Lignosulphonates include a variety of functional groups for which it will be difficult to rule out chemical reaction with lipids. This is not considered of concern for the high molecular mass constituents, since reactions will result in compounds with a molecular mass exceeding 1,000 Da that are unlikely to be absorbed. However, there is potential concern for the low molecular components.

3.1.3.3. Toxicological profile

The data available on the toxicology of calcium lignosulphonate are limited to the 40–65 grade, which is further refined than the technical grades expected to be shipped in large amounts.

- **Absorption, distribution, metabolism and elimination**

No new data have been identified since the publication of the previous opinion of the CONTAM Panel (EFSA CONTAM Panel, 2011).

- **Acute toxicity**

No new data have been identified since the publication of the previous opinion of the CONTAM Panel (EFSA CONTAM Panel, 2011).

- **Subacute, subchronic and chronic toxicity studies**

A 90-day feeding study in Wistar rats (Thiel et al., 2007), where calcium lignosulphonate (40–65, purity: 95.5%) was administered at doses of 0, 500, 1,000 and 2,000 mg/kg bw per day, was evaluated by the EFSA ANS Panel (2010, 2011). It considered that the study to be inadequate due to the potentially impaired health status of the animals based upon a high incidence of minimal lymphoid hyperplasia in mesenteric/mandibular lymph nodes and Peyer's patches, and minimal lymphoid cell infiltration in the liver in all animals. Moreover, the ANS Panel disagreed with the conclusion that the treatment-related observation of foamy histiocytosis in mesenteric lymph nodes was non-adverse and asked whether this observation would progress in something more adverse over time. Therefore, the ANS Panel considered that this study cannot be used to the safety evaluation of calcium lignosulphonate (40–65) and consider that a chronic toxicity study of at least 12 months is needed.

A Pathology Working Group of independent consulting pathologists was convened to re-assess the sections of the lymph nodes, Peyer's patches and liver, and to re-examine all lymphoid tissues. Additional evaluation of the clinical pathology data and evaluation of the animal health certificates from the regular screening programme of the performing laboratory was also done. The conclusions were published together with other study results by Thiel et al. (2013).

In the initial evaluation, large focal/multifocal aggregates of foamy histiocytes were observed in the mesenteric lymph nodes of exposed animals. Both incidence and mean severity increased with dose (dosing: 0, 500, 1,000 or 2,000 mg/kg bw per day; incidence: M: 0/20, 4/20, 17/20 and 20/20, and F: 0/20, 3/20, 8/20, 19/20). Recovery groups (additional rats from the control and high-dose groups treated for 13 weeks and then allowed a 28-day treatment-free recovery period) showed incidence 0/10 in controls and 10/10 in exposed in (both sex). No co-existing tissue damage or reaction was observed. Minimal tubular vacuolation was observed in the kidneys of females at 1,000 mg/kg bw per day and in both sexes at 2,000 mg/kg bw per day. Due to the lack of a dose-dependent increase in severity and in the absence of tubular damage or any other sign of renal toxicity, this finding was judged by the pathologists who performed the study to be not adverse. Minimal tubular vacuolation was still observed in the animals of the high-dose groups after the 28-day recovery period.

The independent consulting pathologists determined that the rats in this study were in good health (Thiel et al., 2013). The mandibular lymph nodes were not macroscopically enlarged. Germinal centres were observed in the mandibular and mesenteric lymph nodes and Peyer's patches, but these were in the expected number and size. The spectrum of histological appearance of germinal centre and plasma cells was consistent with the appearance of these lymph nodes in the strains of laboratory rat. The findings were those commonly found in control and treated rats and were not considered to be related to compound exposure. The lymphoid cellular infiltration (lymphocytes, neutrophils and/or macrophages) in the liver were typical of those commonly found in laboratory rats and were within the limits of normal variation. Foamy macrophages were present within the sinuses of the mesenteric lymph nodes (sinus histiocytosis, incidence in control and high-dose groups were 0/20 and 20/20, respectively). This change was due to administration of calcium lignosulphonate. The histiocytes were increased in size (hypertrophy) but not in number (hyperplasia). Therefore, this was not regarded as a proliferative (hyperplastic) lesion. For the high-dose group, differences in grading were noted between the study pathologist (mean grade of 2.3) and the independent pathologists (mean grade of 1.85), who uniformly assigned lower grading and concludes that the presence of the foamy histiocytes were of minimal to slight severity. The presence of these foamy macrophages is consistent with the phagocytosis of an undigested material entering the lymphatic system from intestinal absorption and has been observed with other substances of high molecular weight such as polypentosan sulphate and mineral oils (Carlton et al., 2001; Elmore, 2006; Pohlmeyer-Esch, 2015). No clinical signs or mortality was associated with the lesions. Such lesions have not been shown to progress in severity or lead to tumour formation in rats (Shoda et al., 1997; Carlton et al., 2001; Trimmer et al., 2004). As long as there is no evidence of inflammation or necrosis, adaptation can take place (Pohlmeyer-Esch, 2015). No vacuolation was present in the proximal tubules of rats receiving calcium lignosulphonate. Therefore, it is concluded that the kidneys do not present histopathological lesions.

The only treatment-related findings were seen in the mesenteric lymph nodes. The presence of foamy macrophages (histiocytosis) was considered not to be adverse and would not be expected to

progress to an adverse alteration with time. The CONTAM Panel agrees with the established no-observed-adverse-effect level (NOAEL) of 2,000 mg/kg bw per day, the highest dose tested.

The CONTAM Panel noted that the ANS Panel has an outstanding request for a chronic study of at least 12-month duration for calcium lignosulphonate (40–65).

- **Genotoxicity**

No new data have been identified since the publication of the previous opinion of the CONTAM Panel (EFSA CONTAM Panel, 2011).

- **Carcinogenicity**

No data are available on the carcinogenicity of calcium lignosulphonate.

- **Developmental and reproductive toxicity**

No new data have been identified since the publication of the previous opinion of the CONTAM Panel (EFSA CONTAM Panel, 2011).

- **Immunotoxicity**

No new data on the potential immunotoxicity of calcium lignosulphonate have been identified. See previous opinion of the CONTAM Panel (EFSA CONTAM Panel, 2011).

3.1.3.4. Allergenicity

No new data on the on potential allergenicity of calcium lignosulphonate have been identified since the publication of the previous opinion of the CONTAM Panel (EFSA CONTAM Panel, 2011). The substance can be considered to be of no concern regarding allergenicity.

3.1.4. Uncertainties

The CONTAM Panel notes uncertainties in the composition of the calcium lignosulphonates likely to be shipped as a previous cargo. There is a lack of information on the toxicity (including genotoxicity) and characterisation of the low molecular weight fraction (< 1,000 Da).

3.1.5. Summary and conclusion

The data on calcium lignosulphonate (highly purified 40–65 grade) did not provide evidence of genotoxicity, immunotoxicity, skin sensitisation or allergenicity. A NOAEL of 2,000 mg/kg bw per day (the highest dose tested) was determined in a 90-day dietary rat study. However, there are several data gaps, mainly with regard to the composition and toxicity of the low molecular mass fraction.

Moreover, the toxicological database is limited to the 40–65 grade and does not cover all grades of calcium lignosulphonate shipped as previous cargoes.

Therefore, the CONTAM Panel concluded that calcium lignosulphonate does not meet the criteria as previous cargo.

3.2. Methyl acetate (CAS No 79-20-9)

3.2.1. Chemical properties and use

Methyl acetate is a liquid with a boiling point of 57°C and around 25% solubility in water at ambient temperature. It has a significant, rather pleasant smell.

Methyl acetate is produced not only by esterification of acetic acid with methanol in the presence of a strong acid, such as sulfuric acid, but also as a reaction by-product of the synthesis of acetic acid from carbon monoxide and methanol.

Methyl acetate is used as a solvent, e.g. in paints, adhesives and cleansers.

Methyl acetate is an ingredient in cosmetics that function as a fragrance, solvents and skin-conditioning agents.

3.2.2. Previous evaluations

In 1998, JECFA (49th meeting) evaluated methyl acetate and classified it as Cramer class I (Cramer et al., 1978), with a threshold of 1,800 μg/person per day (JECFA, 1999).

The toxicology of methyl acetate has been reviewed for risk assessment by the European Union (EU) in 2003 (ECB, 2003) without concluding on a TDI or ADI as no adequate experimental data are available to derive a NOAEL/LOAEL (lowest-observed-adverse-effect level) for administration by oral route. A no-observed-adverse-effect concentration (NOAEC) of 350 ppm (1,057 mg/m^3) for systemic effects was derived from the 28-day inhalation study (HMR, 1999 reviewed by EC ECB, 2003). In a maximisation test with 25 volunteers, no sensitisation was observed after exposure to 10% methyl acetate in petrolatum. Taking into account the long experience with human exposure to the substance, and the absence of any reports on contact allergy in exposed persons, methyl acetate is not expected to exhibit skin-sensitising properties, especially since it is hydrolysed to methanol and acetic acid, substances for which a skin sensitisation potential is either absent or restricted to a few cases (ECB, 2003).

The CIR Expert Panel concluded that methyl acetate is safe in the present practice of use and concentration based on available data on alkyl acetates, and acetic acid and the alcohol to which they could be metabolised (Heldreth et al., 2012).

After absorption, methyl acetate undergoes hydrolysis to methanol and acetic acid. Therefore, evaluation of the toxicity of the substance can refer to the toxicological evaluations of methanol and acetic acid.

In 2011, the CONTAM Panel concluded that methanol meets the criteria for acceptability as a previous cargo (EFSA CONTAM Panel, 2011). The CONTAM Panel considered that methanol does not pose toxicological concerns when used as a previous cargo. Methanol is not genotoxic or allergenic and high doses are required to produce a carcinogenic response in rats by the oral route. Therefore, it is unlikely that methanol would pose a risk of carcinogenicity at the levels of exposure that would occur following its use as a previous cargo.

The ANS Panel reviewed the toxicity of methanol in the context of the safety assessment of aspartame (EFSA ANS Panel, 2013a). The Panel concluded that the data set on genotoxicity was limited but that the available reliable *in vitro* and *in vivo* data did not indicate a genotoxic potential for methanol. The Panel concluded also that the mouse and the rat oral carcinogenicity studies were inadequate for the assessment of the carcinogenic potential of methanol. The Panel identified a NOAEC of 1,300 mg methanol/m^3 in mice that were exposed to methanol via the inhalation route. Based on this NOAEC, the Panel calculated an oral NOAEL for mice of approximately 560 mg/kg bw per day.

The ANS Panel analysed also the United States Environmental Protection Agency (US-EPA) Toxicological Review of Methanol (US-EPA, 2013). The Panel noted that the combination of the developmental endpoint used (extra cervical ribs in an inhalation developmental toxicity study in mice), a benchmark dose response (BMR) of 5% ($POD_{internal}$ = 43.1 mg/L) and the uncertainty factors applied (100), resulted in a reference dose (RfD) for exogenous methanol of 2 mg/kg bw per day that was overly conservative. This RfD was in addition to dietary intakes of methanol, which were included in the background exposure estimates used by the US-EPA (EFSA ANS Panel, 2013b).

Methanol has recently been re-evaluated under Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) Regulation (EC) No. 1907/2006[11] (ECHA, 2015). Evaluation of the data presented in the registration dossier indicated that methanol affects prenatal development of offspring in mice and rats causing fetotoxic, embryotoxic and teratogenic effects. The Risk Assessment Committee (RAC) considered that marked differences between humans and rodents, which are critical when considering that developmental toxicity in rodents is only observed at high blood methanol concentrations ($\geq$ 537 mg/L in mice and $\geq$ 1,840 mg/L in rats). The same type of reasoning that has been used in classifying methanol for acute toxicity and for specific target organ toxicity should be applied, in reverse, to consideration of the data for developmental toxicity. The clear data for methanol-induced teratogenesis in rodents at high-dose levels are not considered to be a good model for human effects. The data are not relevant for classification in humans since primate data and supporting rabbit data have not demonstrated teratogenic effects, and it is not possible to expose primates and humans to such high-dose levels as rodents. It follows that methanol should not be classified for developmental toxicity for human health.

Differences in metabolism result in very different toxicity profiles in rodents and humans, and the RAC is therefore of the opinion that the rodent toxicity data cannot be used in isolation for the

[11] Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), establishing a European Chemicals Agency, amending Directive 1999/45/EC and repealing Council Regulation (EEC) No 793/93 and Commission Regulation (EC) No 1488/94 as well as Council Directive 76/769/EEC and Commission Directives 91/155/EEC, 93/67/EEC, 93/105/EC and 2000/21/EC. OJ L 396, 30.12.2006, p. 1.

classification. The RAC is of the opinion that the high acute toxicity of methanol to humans, such as blindness, will be produced before any developmental toxicity can be expressed in humans. Methanol is classified for acute toxicity and Specific target Organ Toxicity – Single Exposure (STOT – SE) (ECHA RAC 2014).

In 2012, the CONTAM Panel concluded that acetic acid meets the criteria for acceptability as a previous cargo for edible fats and oils (EFSA CONTAM Panel, 2012a). It concluded that 'on the basis of its low toxicity and its natural occurrence in food and in the body, it is not necessary to establish an ADI. Acetic acid causes adverse effects only when it is present at sufficient concentration to change the pH H+ concentration. It will be diluted and buffered by the contents of the gastrointestinal (GI) tract so that the levels that would occur following oral ingestion of fats or oils transported subsequent to acetic acid do not give rise to toxicological concern'.

Acetic acid has been evaluated for its acceptability for use as a herbicide in the EU under Directive 91/414/EEC[12] and subsequently under Regulation (EC) No 1107/2009[13]. It was considered unnecessary to establish an ADI or an acute reference dose (ARfD) for the oral uptake of acetic acid for its intended use as a herbicide based on its widespread presence in human foods, together with the fact that it is a natural component in the metabolism of all plants and animals and is formed in many microbial processes (EFSA, 2013).

3.2.3. Current evaluation

3.2.3.1. Expected impurities

Methyl acetate is easily purified by distillation and generally of high purity. Expected main impurities are methanol and acetic acid. As the substance itself, the impurities are volatile and easily removed by cleaning the tanks.

3.2.3.2. Reactivity and reaction products

Methyl acetate easily transesterifies with triglycerides, resulting in methyl esters of fatty acids and acetylated glycerol. Methyl esters of fatty acids are also formed during interesterification to produce margarines.

3.2.3.3. Toxicological profile

- **Absorption, distribution, metabolism and elimination**

When being ingested, methyl acetate is known to be hydrolysed completely to acetic acid and methanol by non-specific esterases being present in blood and tissues. As a result, methanol but not the parent compound occurs in the serum. For example, in rabbits treated orally with 20 mL/kg bw of a 5% aqueous methyl acetate solution (1,000 mg/kg), methyl acetate was not detectable in the blood between 30 min and 5 h post-application, but methanol was found in blood and urine after 30 min, reaching a peak at 3 h (0.573 mg/mL) (ECB, 2003).

In vitro analyses showed half-lives of methyl acetate in blood of 2–3 h (rat) and approximately 4 h (human) following a reaction of first order (ECB, 2003). Another *in vitro* study reported on rapid hydrolysis of methyl acetate in human blood (27.9 μg/mL) by cellular and non-cellular fractions at 36°C, i.e. 60% of methyl acetate was degraded to methanol within 2 h and almost all of methyl acetate had disappeared after 8 h (ECB, 2003). To conclude methyl acetate is hydrolysed rapidly and completely by esterases to methanol and acetic acid in the gut and/or blood. In plasma, acetic acid has a half-life of 3–5 min (EFSA CONTAM Panel, 2012a). Methanol is a dietary constituent (e.g. pectine, aspartame) and is formed additionally by the metabolism. In humans levels usually range below 1–2 μg/mL blood (Lee et al., 1992).

For methanol and acetic acid, see previous opinions of the CONTAM Panel (EFSA CONTAM Panel, 2011, 2012a).

[12] Council Directive 91/414/EEC of 15 July 1991 concerning the placing of plant protection products on the market. OJ L 230, 19.8.1991, p. 1–32.

[13] Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC. OJ L 309, 24.11.2009, p. 1–50.

- **Acute toxicity**

Oral median lethal doses (LD_{50}s) above 5,000 mg/kg bw have been reported by Smyth et al. (1962) and Opdyke (1979, cited in ECB (2003) and MAK Value Documentation (2002)). Before being hydrolysed, local irritation of methyl acetate in the GI tract cannot be excluded (ECB, 2003). Methyl acetate is hydrolysed to the metabolites acetic acid and methanol (see Section 'Absorption, distribution, metabolism and elimination'). It appears that there is no relevant systemic availability of the parent compound itself (ECB, 2003).

- **Subacute, subchronic and chronic toxicity studies**

There are no rodent data with administration other than inhalative exposure. Information on repeated human exposures to methyl acetate is of limited value due to the lack of quantitative exposure data.

- **Genotoxicity**

Methyl acetate induced aneuploidy in the yeast strain D 61.M at a very high dosage of approximately 33.8 mg/mL (Zimmermann et al., 1985). MA was negative in reverse mutation assays in *Salmonella* Typhimurium and *Escherichia coli* with or without S9-mix (rat or hamster liver S9-mix) (ECB, 2003).

- **Carcinogenicity**

There are no experimental or epidemiological data on the carcinogenic potential of methyl acetate or the metabolites, acetic acid and formic acid. The closely related compound, potassium hydrogen diformate, was negative for carcinogenicity, when tested in mice and rats at doses of up to 2,000 mg/kg bw per day (OECD, 2008).

- **Developmental and reproductive toxicity**

There are no experimental or epidemiological data on methyl acetate with regard to fertility impairment, developmental toxicity or effects in laboratory animals or humans, respectively.

With regard to the evaluation of the critical methyl acetate metabolite, methanol, marked species differences in the metabolism of methanol have to be considered. Methanol is metabolised to formaldehyde through one of at least four pathways, i.e. aldehyde dehydrogenase, CYP2E1, catalase, or a Fenton-like system (Bradford et al., 1993; Dikalova et al., 2001). Primates and humans use only hepatic alcohol dehydrogenase while in rodents all four enzymatic systems are involved. The subsequent oxidation of formaldehyde to formic acid is a rapid process. However, the degradation of formic acid is slow, requires tetrahydrofolate, and is more quickly saturated in primates than in rodents due to lower levels of tetrahydrofolate in primate liver (Black et al., 1985). As a result, uptake of large amounts of methanol may lead to formate accumulation in the blood rather in man or monkey than in rodents.

In a recent evaluation of methanol-induced developmental toxicity by the ECHA (2015), the marked toxicokinetic differences between rodents and humans were taken into account. It was concluded that the LOAEL in mice was in the order of 1,000 mg/kg bw and that there is clear evidence for developmental toxicity in mice at blood levels of 1,650 mg/L. The lowest lethal oral methanol dose reported for humans was in the range of 450–510 mg/kg bw. Thus, in humans blood concentrations similar to those seen in mice causing developmental toxicity would be lethal. It was deduced that the rodent data are irrelevant for risk assessment.

- **Immunotoxicity**

There are no data on the potential immunotoxicity of methyl acetate.

However, considering that methyl acetate is rapidly hydrolysed to non-immunotoxic methanol and acetic acid, methyl acetate is not expected to be immunotoxic.

3.2.3.4. Allergenicity

There are no data on the potential allergenicity and skin-sensitising potential of methyl acetate.

However, based on data on methanol and acetic acid methyl acetate is not expected to be allergenic or skin sensitising.

3.2.4. Summary and conclusion

Limited data are available on the toxicity of methyl acetate. The substance is not acutely toxic by the oral route. It did not induce reverse mutations in *S.* Typhimurium or *E. coli*.

Since methyl acetate is metabolically hydrolysed to methanol and acetic acid, its toxicity can be evaluated by that of methanol and acetic acid. The CONTAM Panel has previously concluded that methanol and acetic acid meets the criteria for acceptability as a previous cargo.

Expected main impurities are methanol and acetic acid. The substance itself and its expected impurities are volatile and easily removed by cleaning the tanks. Methyl acetate easily transesterifies with triglycerides, resulting in methyl esters of fatty acids and acetylated glycerol, which are of no concern. Therefore, the CONTAM Panel concluded that methyl acetate meets the criteria for acceptability as a previous cargo.

3.3. Ethyl *tert*-butyl ether (ETBE) (CAS No 637-92-3)

3.3.1. Chemical properties and use

Ethyl *tert*-butyl ether (ETBE), also called *tert*-butyl ethyl ether, is a liquid with a boiling point of 73°C and solubility in water at ambient temperature of around 1.2%.

ETBE is produced by reaction of isobutylene with ethanol.

Together with methyl *tert*-butyl ether (MTBE), ETBE is mainly used as a fuel additive in petrol to increase the octane number (maximum 15%), to reduce carbon monoxide and hydrocarbon emissions. ETBE is more expensive than MTBE and is mainly used to introduce bioethanol to the amount required by legislation.

Since the structures of ETBE and MTBE are very similar, it is expected that there will be similarities in their toxicological properties. Therefore, data available on MTBE will be reported where appropriate.

3.3.2. Previous evaluations

The toxicology of MTBE has been reviewed by the International Agency for Research on Cancer (IARC) in 1999 and McGregor in 2006. IARC concluded: 'there is inadequate evidence in humans for the carcinogenicity of methyl *tert*-butyl ether' and 'there is limited evidence in experimental animals for the carcinogenicity of methyl *tert*-butyl ether'. According to the IARC working group evaluation, MTBE is not classifiable as to its carcinogenicity to humans (Group 3) (IARC, 1999).

It was also reviewed for risk assessment by the EU in 2002 (ECB, 2002), without concluding on a TDI or ADI. For repeated dose toxicity, a NOAEC of 800 ppm for inhalation (13-week study by Lington et al., 1997) and a NOAEL of 300 mg/kg bw per day for oral administration (90-day study in rat by Robinson et al., 1990) have been established. They concluded also that based on the available information, MTBE cannot be considered a mutagen. MTBE produces tumours in mice and rats at doses $\geq$ 3,000 ppm after exposure by inhalation. In rats, tumours have been reported at doses $\geq$ 250 mg/kg bw per day following administration by gavage. A respiratory NOAEC of 400 ppm and an oral LOAEL of 250 mg/kg bw per day have been derived. They concluded: 'The treatment relation of the occurred tumours is equivocal in some studies (mouse adenoma) and the relevance of the mode of action is questionable in others (Leydig cell). Moreover, the tumours appear mostly at very high and systemically toxic doses and MTBE is not genotoxic *in vitro* or *in vivo*. On the other hand, the human relevance of the testicular interstitial adenomas observed in rats on two separate rat strains cannot be neglected. In addition, certain uncertainty remains as to the significance of the lymphatic tumours found, in the light of the limitations of the study and inadequate reporting'. MTBE does not cause significant toxicity to reproduction in Sprague–Dawley rats and based on the available data, MTBE is not considered toxic to fetal development.

For ETBE a toxicological review has been done by McGregor in 2007.

3.3.3. Current evaluation

3.3.3.1. Expected impurities

Expected impurities are ethanol, isobutylene and impurities of isobutylene, such as 1-butylene, iso-pentanes and iso-pentenes. As the substance itself, the impurities are volatile and easily removed by cleaning the tanks.

3.3.3.2. Reactivity and reaction products

Being an ether, ETBE is expected to be chemically stable and not to react with components of fats and oils.

3.3.3.3. Toxicological profile

Similarities and differences between ETBE and MTBE in terms of basic physicochemical, pharmacokinetic and other properties are compared in a comprehensive review by McGregor (2007).

- **Absorption, distribution, metabolism and elimination**

Inhalation is the most likely route of exposure to ETBE. Therefore, all *in vivo* kinetic and metabolism studies on ETBE have been done by this route of exposure. For MTBE, studies by inhalation and oral route have been performed.

About 30% of inhaled ETBE is retained by the lungs and distributed in all the body. Following cessation of exposure, the concentration of ETBE in blood falls rapidly, largely as the result of its metabolisation (oxidation) to *tert*-butyl alcohol (TBA) and acetaldehyde. TBA may be further metabolised to 2-methyl-1,2-propanediol and then to 2-hydroxyisobutyrate; the two major metabolites found in urine of volunteers and rats. TBA and its glucuronide conjugate as well as TBA sulphate are minor urinary metabolites. Acetaldehyde is rapidly oxidised; therefore, its blood concentration is unlikely to rise above normal as a result of human exposure to ETBE (McGregor, 2007). 2-Hydroxyisobutyrate is also measurable in significant amounts in urine samples from unexposed volunteers and rats as it is formed endogeneously as a product of branch-chained amino acid degradation and ketogenesis. Elimination of ETBE from blood occurred in several phases, the two-first phases after ingestion representing redistribution of ETBE from blood into slowly perfused compartments and the final phase representing clearance by exhalation and by biotransformation to TBA and other metabolites (Dekant et al., 2001). In a study from Nihlén et al. (1998), human volunteers were exposed by inhalation to ETBE. The respiratory uptake of ETBE was in the range of 32–34% and the respiratory excretion in the range of 45–50% of the respiratory uptake. They reported that the kinetic profile of ETBE in human volunteers exposed by inhalation could be described by four phases in blood (average half-lives of 2, 18 min, 1.7 and 28 h) and two phases in urine (8 min and 8.5 h). The average half-times of TBA were 12 h in blood and 8 h in urine. The excretion rate of ETBE in urine was rather high and the cumulative excretion within 22 h post-exposure was less than 0.1% of the respiratory uptake. The excretion rate of TBA was rather slow and less than 1% of the absorbed ETBE was excreted as TBA in urine within 22 h post-exposure. After exposure, acetone was detected in elevated levels in blood and also in urine compared to controls, indicating that acetone is probably a by-product of ETBE metabolism.

Studies on the metabolism of ETBE by human liver microsomal enzymes have demonstrated its initial oxidation to TBA and acetaldehyde (McGregor, 2007). In similar studies, MTBE was oxidised to TBA and formaldehyde (McGregor, 2006). CYP2A6 was the most active enzyme in the metabolism of ETBE and MTBE.

MTBE is well and rapidly absorbed in humans following oral and inhalation exposure. The highest concentrations of MTBE in blood are found within a few minutes after oral exposure. Blood concentration of TBA increases within about 30 min and high concentrations tend to persist for up to 7 h. Elimination of MTBE from blood after acute oral exposure was rapid and triphasic with $t_{1/2}$ values of about 0.25–0.8, 1–2 and 7–8 h (Dekant et al., 2001). However, elimination of TBA from blood is slower with $t_{1/2}$ values of several hours. MTBE is eliminated by exhalation as unchanged MTBE or by urinary excretion of its less volatile metabolites. Metabolism is more rapid in humans than in rats.

Dekant et al. (2001) reviewed the biotransformation of MTBE and ETBE after inhalation or ingestion in rats and humans. After inhalation, MTBE and ETBE were rapidly absorbed by both rats and humans and clearance from blood of the ethers by exhalation and biotransformation to urinary metabolites occurred with half-times of less than 7 h in rats and humans. Biotransformation of both substances was similar in humans and rats after inhalation exposure. 2-Hydroxyisobutyrate was recovered as a major metabolite in urine. All metabolites of MTBE and ETBE excreted with urine were eliminated with half-times of less than 20 h. After oral ingestion, MTBE is rapidly absorbed from the GI tract. Hepatic first-pass metabolism was not observed and a significant part of the administered dose of MTBE was transferred into blood and cleared by exhalation. The metabolic pathway and kinetics of excretion were identical after ingestion and inhalation exposures.

- **Acute toxicity**

ETBE is of low acute oral toxicity. The LD_{50} in Wistar rats was > 5,000 mg/kg bw (MB Research Laboratories, 1988; IIT Research Institute, 1989; cited in McGregor, 2007), and LD_{50} values > 2,000 mg/kg bw were reported in OFA and Sprague–Dawley rats (Institut Pasteur de Lille, 1992a; Pharmakon Europe, 1994a, cited in McGregor, 2007). In a study where albino rats were given doses from 2,000 to 10,000 mg/kg bw, a LD_{50} of 3,800 mg/kg bw was calculated. Clinical signs of toxicity included hypoactivity, muscular weakness and hyperpnoea. Prostration was observed at the highest dose. Inflammation of the stomach and intestine were noted during the pathological examination. A LD_{50} of 3,866 mg/kg bw was calculated in another rat study (ARCO, 1980; cited in EU ECB, 2002). Ataxia and central nervous system (CNS) depression, tremors and loss of righting were reported at doses > 1,900 mg/kg bw.

- **Subacute, subchronic and chronic toxicity studies**

ETBE was administered by gavage at dose levels of 0, 5, 25, 100 and 400 mg/kg bw per day to male and female rats (specific pathogen-free Cr/CD (Sprague–Dawley), 15/sex per dose) for 180 days. No effect on mortality was noted. Treatment-related clinical findings were decreased locomotor activity in both sexes at the two highest doses and decreased respiratory rate and incomplete eyelid opening in both sexes at the highest dose, but these signs mostly disappeared by week 1. Salivation was observed transiently after dosing in males of the three highest doses and in females of the highest dose group. No effect was noted on the reflex test, grip strength, motor activity count, body weight, haematology and urinalysis. Statistically significant increase in level of total cholesterol was found in males of the high-dose group. A significant increase in relative mean liver weight was noted in both sexes of the high-dose groups. Relative kidney weights were increased in both sexes in the two highest dose groups. Significant microscopic findings were observed only in livers from males and females and in kidneys of males. Hypertrophy of hepatocytes was found in rats of the highest dose group, characterised by enlargement of hepatocytes in the centrilobular area with homogeneously eosinophilic cytoplasm. An increased incidence of hyaline droplets was observed in kidneys of males at the two highest doses. These were not observed in female rats at the same dose levels. α2u-Globulin immunoreactivity was present in hyaline droplets of the renal proximal tubule epithelium of male rat kidneys. This mechanism is not relevant for human risk assessment. The NOAEL was 100 mg/kg bw per day (Miyata et al., 2014).

Studies have been performed by Gaoua in 2004 in which rats have been exposed during two generations by gavage to ETBE for 10 weeks (Gaoua, 2004a,b). At doses of ≥ 500 mg/kg bw per day, increases in kidney weight were seen in both sexes, but protein droplet accumulation (α2u-globulin) were observed only in males at 1,000 mg/kg bw per day. Increases in liver weight were also reported (Unpublished reports, cited by McGregor, 2007).

The same types of renal effects have been observed in rats exposed by inhalation to ETBE and TBA and these findings suggest that an interaction of ETBE or TBA with α2u-globulin may be the mechanistic basis for the nephropathy (McGregor, 2006).

In conclusion, there is evidence for an effect of ETBE on the kidney of rats. Increases in kidney weight were seen in both sexes, but protein droplet accumulation (with α2u-globulin involvement) occurred only in males. The NOAEL was 100 mg/kg bw per day.

- **Genotoxicity**

In vitro

ETBE was negative in reverse mutation assays in *S.* Typhimurium TA97, TA98, TA100 and TA1535 with and without metabolic activation (S9 rat and hamster) up to 10,000 μg/plate (Zeiger et al., 1992). It was also negative in *S.* Typhimurium TA98, TA1535, TA1537 and TA1538 with or without S9 (rat) up to 500 μg/plate (Unpublished Reports Institut Pasteur de Lille, 1992a–c), and in *S.* Typhimurium TA98, TA100, TA1535, TA1537 and TA1538 with or without S9 (rat) up to 5000 μg/plate (Pharmakon Europe, 1994a–e). ETBE was negative in a gene mutation assay in Chinese Hamster ovary cells (CHO) cells at the hypoxanthine phosphorybosyl transferase (hprt) locus (Unpublished Report Bushy Run research Center, 1995) and a chromosomal aberration test in CHO cells in the presence or absence of S9 (rat) up to 5,000 μg/plate (unpublished Reports Bushy Run research Center, 1995a–c). The results of the unpublished reports are reported in McGregor (2007).

TBA was negative in reverse mutation assays in *S.* Typhimurium TA100, TA98, TA1535 and TA1537 with or without metabolic activation (rat or hamster S9) at doses up to 10,000 μg/plate (Zeiger

et al., 1987; NTP, 1997). It was also negative in one assay on TA102 with or without metabolic activation (up to 5,000 μg/plate) (McGregor et al., 2005) but was positive in another assay in TA102 in the presence of an exogenous metabolic system at (750 μg/plate) and equivoval in the absence of metabolic activation (Williams-Hill et al., 1999) TBA. It was also negative in a gene mutation assay in mouse lymphoma L5178Y cells at the tk locus up to 5,000 μg/mL (McGregor et al., 1988; NTP, 1997), a chromosomal aberration test in CHO cells up to 5,000 μg/mL (Galloway et al., 1987; NTP, 1997) and a sister chromatid exchange (SCE) test in CHO cells up to 5,000 μg/mL (Galloway et al., 1987; NTP, 1995) in the presence or absence of metabolic activation. A positive result was noted in a Comet assay in human HL-60 leukaemia cells in the absence of metabolic activation at a concentration of 1 mM (Tang et al., 1997).

In vivo

Induction of micronuclei in bone marrow cells of mice exposed orally to ETBE by gavage at dose levels up to 5,000 mg/kg bw or by inhalation at concentrations up to 5,000 ppm (21,200 mg/m^3), 6 h/day, for 5 days gave also negative results (Unpublished Reports Institut Pasteur de Lille, 1992 and Bushy Run research Center, 1995; cited in McGregor, 2007).

TBA was negative in a micronucleus test in mouse peripheral blood cells after administration of 40 mg/mL drinking water for 13 weeks (NTP, 1995).

Overall, the CONTAM Panel considered that ETBE and TBA are not genotoxic.

- **Carcinogenicity**

Sprague–Dawley rats (60/sex per group) were given 0, 250 or 1,000 mg ETBE/kg bw per day (purity ≥ 94%) by gavage in olive oil on 4 days/week for 104 weeks (Maltoni et al., 1999; also cited in McGregor, 2007). No treatment-related adverse effects were noted on water and food consumption or body weights. An early reduction in survival in female rats of the high-dose group was noted around week 48, but this difference was not maintained. At this dose, survival was reduced to 50% after dosing for 87, 80 and 72 weeks, respectively, in males and 88, 85 and 84 weeks, respectively, in females. After 96 weeks of treatment, survival was reduced to about 27, 24 and 13%, respectively, in males, and 28, 29 and 34%, respectively, in females. The incidence of all malignant tumours combined was increased with treatment. According to the authors, ETBE causes a number of neoplasms in male and female rats: tumours of the epithelium of the mouth (oral cavity, tongue, lips) specially in females and forestomach specially in males (acanthomas and squamous cell carcinomas), malignant tumours of the uterus (leiomyoma, squamous cell carcinoma, adenocarcinoma, leiomyosarcoma, malignant Schwannoma and uterus and vagina malignant Schwannoma) and haemolymphoreticular neoplasms (lymphomas and leukaemias).

However, no dose–response relationship between neoplastic effects and ETBE concentrations were found. According to the authors, this may be explained (at least partly) by the high mortality in the groups treated with 1,000 mg/kg bw per day. Haematological malignancies should be grouped according to lineage. Doing this, it is concluded that the statistically non-significant variations in the incidences of lymphoid neoplasms in this study are not related to ETBE exposure. Moreover, almost all of these neoplasms were found in the lung, a phenomenon not infrequently found in this laboratory (see Belpoggi et al., 1998) and may be associated with microbial infection. The most common tumour of the uterus and vagina and the only one with a statistically significant increased incidence was malignant Schwannoma (0/60, 6/60 ($p = 0.014$), 2/60). However, no dose–response relationship was found and normally Schwannomas are associated with nerves emerging from the CNS or with peripheral nerve tissue rather than a particular non-CNS organ. Therefore, the biological relevance of these lesions is unclear. It has also to be noted that there was no mention of preneoplastic lesions in any tissue and no mention was made of any kind of renal pathology in any group, which is highly unlikely in ageing rats.

The CONTAM Panel concluded that this report does not provide an adequate basis for a thorough evaluation of the carcinogenic potential of ETBE. There is insufficient evidence for tumour induction by ETBE in the mouth, forestomach and haematological system of rats and the biological relevance of the malignant Schwannoma tumours in the uterus is unclear.

Carcinogenicity studies on TBA may be helpful for the evaluation of the carcinogenicity of ETBE. TBA has been tested by oral, drinking water exposure in F344 rats (0, 1.25, 2.5 and 5.0 mg/mL corresponding to 0, 85, 195 and 420 mg/kg bw per day in males, and 0, 2.5, 5.0 and 10.0 mg/mL corresponding to 0, 175, 330 and 650 mg/kg bw per day in females) and B6C3F1 mice (0, 5, 10 and 20 mg/mL corresponding to 0, 535, 1,035 and 2,065 mg/kg bw per day in males, and 0, 510, 1,015 and 2,105 mg/kg bw per day in females) (NTP, 1997). In male F344 rats exposed to TBA, a weak increased incidence of renal adenoma and carcinoma was observed which was statistically significant

at ≥ 2.5 mg TBA/mL. Female rats and male and female mice showed no significant increase in the incidence of renal tumours. It is postulated that high levels of TBA result in α2u-globulin nephropathy in the male rat kidney and exacerbate the development of chronic progressive nephropathy leading to the formation of tumours. This mechanism is considered of no human relevance. Treatment of rats or mice with TBA has no effect on haematopoietic neoplasms. The incidence of thyroid follicular cell adenoma was significantly increased in female mice at 20 mg TBA/mL but not in males. This result lacks any independent supporting evidence from other studies in mice with ETBE and MTBE and in rats with ETBE, MTBE or TBA. A single forestomach papilloma was noted at 5.0 mg/mL in male rats (McGregor, 2006, 2007).

In an oral carcinogenicity study on MTBE with the same protocol as the one used for ETBE, no adverse changes were reported in the kidney proximal tubules of rats. Some increases in tumour incidence have been noted, but consistency of outcome was lacking and none had human relevance: Leydig-cell adenoma in high-dose male rats and B-cell-derived lymphoma/leukaemia of doubtful pathogenesis observed mainly in lungs of orally dosed-female rats. MTBE also cause an increase in haemolymphoreticular dysplasias (Belpoggi et al., 1995, 1998; also cited in; IARC 1999; ECB, 2002; McGregor, 2006).

In conclusion, carcinogenicity studies have been conducted with ETBE, TBA and MTBE (a structurally related compound). The study with ETBE in rats was not considered adequate for the evaluation of the carcinogenic potential of ETBE. TBA induced α2μ-globulin nephropathy-related renal tubule adenomas in male rats only. These tumours are generally considered to have no human relevance. In addition, increases in the incidence of thyroid follicular cell adenoma were observed in female mice exposed to TBA. However, such effect was not observed in other studies in mice with ETBE and MTBE and rats with ETBE, MTBE or TBA, therefore, providing no supporting evidence.

In view of the general lack of genotoxicity of ETBE and TBA and based on the limited information available from the carcinogenicity studies of ETBE, MTBE or TBA, the CONTAM Panel does not consider that ETBE represents a risk for carcinogenicity at the levels of exposure that would occur following its use as a previous cargo.

- **Developmental and reproductive toxicity**

Fujii et al. (2010) conducted a one-generation reproductive toxicity study of ETBE in rats (specific pathogen-free Crl/CD (SD), 24/sex per dose). Both male and female F0 parental animals were given 0, 100, 300 and 1,000 mg/kg bw per day by gavage for 10 weeks before the initiation of mating period. No treatment-related changes were observed in F0 parents or their F1 offspring in the 100 and 300 mg/kg bw per day groups. Some parental animals in the high-dose group exhibited transient salivation (possibly a reflex to a bitter taste of ETBE) immediately after dosing. No effect was observed on their body weights, food consumption, reproductive parameters (oestrous cycles, mean oestrous cycle length, indices of copulation, fertility, gestation and delivery) and gross pathological findings. Absolute and relative liver weights were significantly increased in the highest dose group, suggesting enhanced activities of metabolic enzymes. Significant weight increases were found for relative kidney weights in males at 100 and 300 mg/kg bw per day, and for relative pituitary weight in the 300 mg/kg bw per day group. Significant increases were also noted at 1,000 mg/kg bw per day for absolute and relative kidney and adrenal weight and relative weights of the brain, pituitary and testis in F0 males as well as absolute kidney weights in F0 females. Number of implantations increased sporadically in the high-dose group. No treatment-related clinical findings were observed in F1 pups in any of the treated groups during the lactation period. Viability index on post-natal day (PND) 4 (early lactation) decreased slightly in the high-dose group, but no treatment-related reduction in body weights was observed. This was due to a slightly increased incidence of whole litter loss (3/22 = 13.6% compared to control data in the facility of 0.0%–4.8%, mean value = 0.7%). Sperm analyses of F0 males revealed no difference between the control and treated groups in any parameter examined (number, motility in the testis and/or cauda epididymis, morphology). Sexual development of the F1 offspring was not affected by the treatment (age preputial separation or vaginal opening, body weights at completion of sexual development). Gross pathological examination of F1 pups that died before weaning or were euthanised on PNDs 4 or 21 revealed no treatment-related abnormalities. No effect was found on organ weights of weanlings. The NOAEL for parental rats and their offspring was 300 mg/kg bw per day.

ETBE was administered by gavage (in olive oil) to pregnant female Sprague–Dawley rats (21 or 22/group) at dose levels of 0, 100, 300 and 1,000 mg/kg bw per day from days 5 to 19 post-coitum. No toxicological effects attributable to EBTE were noted regarding clinical signs (with the exception of

salivation observed in some animals immediately after administration of ETBE), body weight, food intake, necropsy or examination at caesarean section (weight of the ovaries and uteri) in dams. No abnormalities were detected in the number of corpora lutea, preimplantation losses, implantations, resorptions and live fetuses, as well as sex ratios and body weight of live fetuses. No toxicological effects were observed on external or visceral examinations of embryos and fetuses. No effect on skeletal malformations was observed; however, there was a non-statistically significant increase in the number of fetuses having variations and of the number of dams with fetuses having variations at the highest dose. The frequency of rudimentary lumbar ribs was significantly increased in the group given the highest dose of ETBE; but this was considered of no toxicological significance by the authors (the frequency was within the range of historical data of the same strain of rat (1.1–21.2%) used in this study, the lumbar rib was a rudimentary type and not an extra one and this variation is temporary and vanishes after birth). The NOAEL was 1,000 mg/kg bw per day, the highest dose tested, for maternal and developmental toxicity (Aso et al., 2014).

Several other reproductive toxicity and developmental toxicity studies have been conducted in rats and rabbits exposed by gavage to doses up to 1,000 mg ETBE/kg bw per day (the highest dose tested), were reviewed by de Peyster (2010). The original Reports were not available to the CONTAM Panel. ETBE does not appear to be selectively toxic to reproduction or development in the absence of other manifestations of general toxicity. The following NOAELs have been reported: 1,000 mg/kg bw per day for reproductive endpoints and for embryofetal toxicity in a 12-week study in Sprague–Dawley rats (Unpublished Report CIT, 2003), 1,000 mg/kg bw per day for reproduction and developmental toxicity in a two-generation reproduction toxicity study in Sprague–Dawley rats (parental toxicity NOAEL was 250 mg/kg bw per day) (Unpublished Report CIT, 2004a), 1,000 mg/kg bw per day for developmental toxicity in developmental toxicity studies in Sprague–Dawley rats (Unpublished Report CIT, 2004b and METI, 2008b). In the first study, maternal toxicity was observed at 1,000 mg/kg bw per day, and in the second, the NOAEL for maternal toxicity was 1,000 mg/kg bw per day. No embryofetal effects were observed in New Zealand White rabbits up to 1,000 mg/kg bw per day, a dose at which lower body weight and food consumption were noted in the dams (Unpublished Report METI, 2008b). In a single-generation reproductive toxicity study in Sprague–Dawley rats, decrease in F1 survival rate during weaning was observed at 1,000 mg/kg bw per day, due to weakening dams at this dose level (two dams showed severe illness after delivery during the lactation period and subsequently neglected their pups and lost all of their pups). Incidence of total litter loss (13.6%) exceeded historical controls (0–4.8%, mean 0.7%). The NOAEL for maternal toxicity (based on liver weight increase) and developmental toxicity was 300 mg/kg bw per day (Unpublished Report METI, 2008a).

In conclusion, ETBE does not show selectively reproductive or developmental toxicity in the absence of other manifestations of general toxicity. The NOAELs for reproductive and developmental toxicity are 1,000 mg/kg bw per day and 300 mg/kg bw per day, respectively.

- **Immunotoxicity**

The potential for immunotoxicological effects of ETBE was studied in young adult female Sprague–Dawley rats following subchronic oral exposures. Rats were exposed by gavage once daily for 28 consecutive days to 0, 250, 500 or 1,000 mg ETBE/kg bw per day; a concurrent positive control group for the splenic antibody-forming cell (AFC) assay received four intraperitoneal injections of 50 mg cyclophosphamide monohydrate (CPS)/kg/day on study days 24–27. Potential immunotoxicity was evaluated using the splenic AFC assay to assess the T-cell-dependent antibody responses in rats sensitised with sheep red blood cells (SRBC) as antigen. All rats survived to the scheduled necropsy. There were no effects on clinical observations, body weights, feed or water consumption, or macroscopic pathology findings in the ETBE-treated rats. No ETBE-related effects were observed on absolute or relative (to final body weight) spleen or thymus weights, spleen cellularity, or on the specific (AFC/10^6 spleen cells) or total activity (AFC/spleen) of splenic IgM AFC to the T-cell-dependent antigen SRBC. CPS produced expected effects consistent with its known immunosuppressive properties and validated the appropriateness of the AFC assay. Based on the results of this study, ETBE did not suppress the humoral component of the immune system in female rats. The NOEL for immunotoxicity was the highest dosage tested at 1,000 mg/kg bw per day. (Banton et al., 2011).

Other studies have focussed on the potential immunotoxicity of ETBE via inhalation exposure.

In a study of White et al. (2014), female Sprague–Dawley rats were exposed via inhalation to vapour condensates of gasoline combined with ethyl t-butyl ether (G/ETBE) to assess the potential immunotoxicity, using AFC response to the T-dependent antigen SRBC. Target concentrations were 0, 2,000, 10,000 or 20,000 mg/m^3 administered for 6 h/day, 5 days/week for 4 weeks. These exposure

concentrations are very high, at least three orders of magnitude, relative to the actual occupational or environmental exposures that might be experienced in humans exposed to gasoline evaporative emissions. Exposure to G/ETBE resulted in a statistically significant decrease in the AFC response at the middle and high exposure concentrations. This is in contrast with the absence of any effect on the AFC response upon the oral gavage dosing of neat ETBE in the study of Banton et al. (2011), although the oral gavage doses of ETBE used were calculated to be more than 2-fold the G/ETBE exposures by inhalation. No explanation can be given concerning the discrepancy in results with the oral dosing study of Banton et al. (2011).

Li et al. (2011) examined the effects of ETBE on splenocytes in mice exposed to 0 (control), 500, 1,750 or 5,000 ppm of ETBE by inhalation for 6 h/day for 5 days/week over a 6- or 13-week period. The numbers of several T-cell subsets, the percentage of T helper cells and the T helper/T suppressor cell ratio in the ETBE-exposed groups were significantly decreased in a dose-dependent manner. The numbers of splenic NK cells, B cells, or macrophages or the total number of splenocytes were not affected. This observation might be in line with the results of the study of White et al. (2014) that showed a suppression of the AFC response, as T cells play an important role in the development of an antibody response against SRBC.

Based on the available studies focussed on the potential immunotoxicity of ETBE, it can be concluded that upon oral exposure ETBE is not expected to be immunotoxic.

3.3.3.4. Allergenicity

There are no data on the potential allergenicity of ETBE available. ETBE is not a skin sensitiser in a guinea pig maximisation test (Pharmakon Europe, 1994d. Unpublished report cited in McGregor, 2007).

3.3.4. Summary and conclusion

ETBE is metabolised by oxidation to TBA and acetaldehyde. TBA may be further metabolised to 2-methyl-1,2-propanediol and then to 2-hydroxyisobutyrate, the two major metabolites found in urine of volunteers and rats.

ETBE is of low acute oral toxicity (LD_{50} > 2,000 mg/kg bw). In a 6-month study in rats, administration of ETBE by gavage resulted in toxic effects in the liver and the kidneys. A NOAEL was set at 100 mg/kg bw per day.

Neither ETBE nor TBA is genotoxic.

In view of the general lack of genotoxicity of ETBE and TBA and the limited information on the carcinogenicity of ETBE, TBA and MTBE, the CONTAM Panel does not consider that ETBE represent a risk for carcinogenicity at the levels of exposure that would occur following its use as a previous cargo.

ETBE is not toxic for the reproduction or development in the absence of other manifestations of general toxicity. The NOAELs for developmental toxicity and fertility were 300 and 1,000 mg/kg bw per day, respectively. Oral exposure to ETBE is not expected to have immunotoxic effects. ETBE is neither a skin sensitiser nor an allergen.

The CONTAM Panel established a TDI of 1 mg ETBE/kg bw per day, based on the NOAEL of a 6-month study in rats and applying an uncertainty factor of 100.

Expected impurities of ETBE are ethanol and isobutylene as well as impurities of isobutylene, such as 1-butylene, iso-pentanes and iso-pentenes. ETBE itself and the impurities are volatile and easily removed by cleaning the tanks. Being an ether, ETBE is expected to be chemically stable and not to react with components of fats and oils.

The CONTAM Panel considers that the available information is sufficient to conclude that the exposure to ETBE, when used as a previous cargo, would not give rise to toxicological concern. The CONTAM Panel therefore concludes that ETBE meets the criteria for acceptability as a previous cargo.

3.4. Ammonium sulphate (CAS No 7783-20-2)

3.4.1. Chemical properties and use

Ammonium sulphate is a bulk chemical. It is a well water-soluble, inorganic salt (facilitating cleaning) hardly soluble in edible oils and fats. It is made from various sources and exists in various grades (Song et al., 2013). Some is produced by reacting ammonia with sulfuric acid or by addition of ammonium carbonate solution to gypsum (calcium carbonate being precipitated and leaving ammonium sulphate in solution). A major source is from coking. The coking gases contain ammonia,

which is converted to the sulphate by sulfuric acid obtained from the same source by oxidising hydrogen sulfide. Another major source is the production of caprolactam (monomer of polyamide): cyclohexanone is converted to its oxime, which is then treated with sulfuric acid. The resulting salt is neutralised with ammonia to release the caprolactam, leaving behind ammonium sulphate.

A recently introduced, rapidly expanding process yielding ammonium sulphate is the desulfurisation of flue gas from power plants running on fossil fuel. It partly replaces the classical wet scrubbing with limestone slurry. An intense beam of electrons is fired into the flue gas to promote oxidation of sulfur dioxide, while ammonia is added to form ammonium sulphate (Chou et al., 2005). As an alternative technology, for SO_2 scrubbing, a saturated solution of ammonium sulphate is used in a spray tower absorber. The reagent, ammonia, is fed into the absorber recirculation tank. The primary reaction product, ammonium sulfite/bisulfite, is converted to the sulphate by oxidation. Using the heat of the flue gas, some ammonium sulphate is crystallised from the saturated absorber liquor (Wallach, 1997; Marsulex Environmental Technologies, 2007; Ueda et al., 2012).

The main use of ammonium sulphate is as a fertiliser, but there are many other uses in chemical industry, such as for precipitating proteins and to produce flame retardants. A minor amount is used as food additive (E-517; mainly acidity regulator in flours and breads).

3.4.2. Previous evaluations

The toxicology of ammonium sulphate has been reviewed by the Organisation for Economic Co-Operation and Development (OECD) (SIDS Initial Assessment Report for SIAM 19 of 2004). Only limited data were available. As ammonium sulphate dissociates to ammonium and sulphate ions (NH_4^+, SO_4^{-2}) in aqueous media, studies with other ammonium and sulphate salts can be considered.

In 2003, the WHO estimated that the daily dietary exposure to ammonium/ammonia from food and drinking water was 18 mg per person (0.26 mg/kg bw per day for a 70 kg adult) (WHO, 2003). In 2011, the WHO reconfirmed that the exposure to ammonia from environmental sources was insignificant in comparison with endogenous synthesis (3–4 g/day, 43–57 mg/kg bw per day for a 70-kg adult) (WHO, 2011). Toxicological effects were expected only when exposure exceeds approximately 200 mg/kg bw.

In 2011, the CONTAM Panel concluded that ammonium polyphosphate meets the criteria for acceptability as a previous cargo (EFSA CONTAM Panel, 2011). JECFA had established a group maximum TDI (MTDI) for phosphates, including ammonium polyphosphate, of 70 mg/kg bw expressed as phosphorus, which the CONTAM Panel considered appropriate. In 2012, the CONTAM Panel concluded that ammonium hydroxide meets the criteria for acceptability as previous cargo for edible fats and oils (EFSA CONTAM Panel, 2012a). JECFA had established an ADI of 'not limited' and the SCF an ADI 'not specified', which the CONTAM Panel considered appropriate.

In 2011, the EFSA CEF Panel reviewed ammonia, diammonium sulfide, ammonium chloride and ammonium hydrogen sulfide in the context of flavouring substances used in foodstuffs (EFSA CEF Panel, 2011).

In 2012, EFSA produced a statement regarding the possible impact on human health of exposure to ammonium ions released from water filter cartridges. For adults, the estimated that the exposure would range from 0.014 to 0.14 mg/kg bw per day and be slightly higher for infants and children. Considering the large amounts of endogenously produced ammonium, it was concluded that additional exposure to ammonium from this source was negligible and did not pose a risk to human health, even for vulnerable groups, such as people suffering from enzyme deficiencies due to genetic disorders or severe kidney or liver failure (EFSA, 2012b).

In the assessment of calcium sulphate the Panel on food additives, flavourings, processing aids and materials in contact with food (AFC Panel) considered intakes of up to 750 mg sulphate/person per day as acceptable (EFSA, 2004). based on this assessment, the ADI for sulphate was set by the EFSA Pesticide Risk Assessment Peer Review (PRAPeR) at 12.5 mg/kg bw per day for a 60-kg adult (EFSA, 2012a).

In 2012, the CONTAM Panel accepted sulfuric acid as a previous cargo (EFSA CONTAM Panel, 2012a). Sulfuric acid was considered to be toxic only at concentration to change the pH significantly. It is diluted and buffered by the contents of the GI tract so that the levels of sulphate to be expected in fats or oils transported subsequent to sulfuric acid do not give rise to toxicological concern.

3.4.3. Current evaluation

3.4.3.1. Expected impurities

There is food-grade ammonium sulphate (> 99%) for which impurities are of no concern (selenium ≤ 30 mg/kg and lead ≤ 3 mg/kg; Regulation (EU) No 231/2012[14]). However, the bulk of the material used is lower grades, mainly as a fertiliser or as an industrial chemical. There are specifications for some impurities when used as a fertiliser (e.g. EU Regulation (EC) 2003/2003[15]), but these do not cover, e.g. organic impurities to be expected from the various sources of the substance, such as flue gases.

Using the maximum concentrations of impurities reported by the REACH registrants to the ECHA as well as the assumptions of 100 mg/kg previous cargo in the edible oil or fat transported subsequently and 50 g of such oil being regularly consumed per person and day, those impurities resulted in an exposure of no concern (by comparison with the health-based guidance values (HBGVs) or using the threshold of toxicological concern (TTC) approach for the different impurities reported). However, some registrants declared up to 20% of unknown impurities in ammonium sulphate produced by scrubbing. Impurities like polycyclic aromatic hydrocarbons or dioxins may be expected in the production of ammonium sulphate from coke-oven gas. Similar or different problems may arise when other production methods are used. The list from the ECHA on registered ammonium sulphate did not contain information on polycyclic aromatic hydrocarbons or dioxins as possible impurities.

A health concern cannot be excluded due to the lack of data on the identity and level of impurities in the aforementioned cases.

3.4.3.2. Reactivity and reaction products

Ammonium sulphate decomposes at temperatures above about 230°C, first to ammonium bisulphate, then to ammonium pyrosulphate and finally to gases including ammonia and sulfur dioxide (Song et al., 2013). In an environment of reactive components, reactions may occur at lower temperatures. However, the solubility of ammonium sulphate in edible oils is considered too low to enable the formation of reaction products in amounts to be of concern.

3.4.3.3. Toxicological profile

- **Absorption, distribution, metabolism and elimination (ADME)**

In aqueous environments and at physiological pH, ammonium sulphate dissociates completely into ammonium (NH_4^+) and sulphate (SO_4^{2-}).

ADME – Ammonia, Ammonium

At physiological pH and in aqueous solutions, more than 99% of ammonium is ionised. Ammonia (NH_3) can diffuse across the intestinal wall into the lumen of the gut and vice versa (WHO, 1986). Due to low pH in most gut segments, the equilibrium shifts towards ammonium ions, which cannot cross the intestinal wall. This results in a net flux of ammonia form the blood to the intestinal lumen. In the intestinal lumen, NH_3 is generated by bacterial deamination of unabsorbed amino acids and by bacterial urease activity at 43–57 mg/kg bw per day (EFSA, 2012b). A further endogenous source of ammonia is the deamination of glutamine in the kidneys.

The normal blood ammonium concentration is usually < 35 μmol/L and contributes to the acid–base balance (Häussinger, 2007). Ammonium is excreted via urine. Alternatively, it is converted by the hepatocellular urea cycle to urea, which is eliminated by the kidneys (WHO, 1986).

ADME – sulphate

The more sulphate is ingested the more will be absorbed. When intestinal absorption is exceeded, sulphate will be eliminated via faeces. Inorganic sulphate is formed also endogeneously from the catabolism of sulfur-containing amino acids. sulphate is a physiological blood component and the concentration is kept constant at a level of 0.33 mmol/L (95% reference interval of 0.22–0.49 mmol/L) by the kidney via renal elimination and reabsorption (Pascoe et al., 1984). After oral uptake of 5.4 g magnesium or sodium sulphate, 30–44% of sulphate was excreted in the 24-h urine (OECD, 2004).

[14] Commission Regulation (EU) No 231/2012 of 9 March 2012 laying down specifications for food additives listed in Annexes II and III to Regulation (EC) No 1333/2008 of the European Parliament and of the Council. OJ L 83, 22.3.2012, p. 1–295.

[15] Regulation (EC) No 2003/2003 of the European Parliament and of the Council of 13 October 2003 relating to fertilisers. OJ L 304, 21.11.2033, p. 1–194.

- **Acute toxicity**

Due to its osmotic activity, sulphate binds water in the intestinal lumen and may cause diarrhoea at higher doses. Accordingly, 1,200 mg sodium sulphate/L drinking water increased significantly the mean stool mass per 6-day pool from 621 to 922 g in adult humans (Heizer et al., 1997).

In humans, the normal blood ammonium concentration is < 35 μmol/L. Increases to 100 μmol/L can affect consciousness and concentrations around 200 μmol/L cause coma and convulsions (Felipo and Butterworth, 2002). The increased ammonium ion levels in the brain may interfere with energy production and the expression of some receptors being involved in neurotransmission (Kosenko et al., 1994, 2000).

Metabolic acidosis develops partly due to the formation of hydrogen ions when ammonium ions are metabolised to urea. However, the degree of acidosis also depends largely on the ability of the kidney to excrete the respective anion, e.g. when ammonium chloride is applied, hydrochloric acid is formed which induces larger decreases in plasma bicarbonate levels than the administration of equivalent quantities of hydrogen ions as nitric or sulfuric acid (De Sousa et al., 1974). As a consequence, the associated anion appears to play an important role in toxicity profile of specific ammonium salts.

The oral LD_{50} of ammonium sulphate was reported to be 4,250 mg/kg bw in rats (OECD, 2004). At doses somewhat lower than the LD_{50}, exhaustion, apathy and irregular breathing could be observed immediately after treatment. One day later, reddened and secreting eyes and secretion out of mouth and nose were evident. Later on, there were no symptoms in the surviving animals. Doses up to 2,500 mg/kg bw did not cause any symptoms (OECD, 2004).

Yamanaka et al. (1990) reported an oral LD_{50} of 2,000 mg/kg bw for rats and of 3,040 mg/kg bw for mice. Okropiridze, 1977; (cited in OECD, 2004) described the case of 18 people who drank water from a faucet in the immediate neighbourhood of a vegetable glasshouse where ammonium sulphate was used as a fertiliser. The water contained 1,500–2,000 mg/L ammonium sulphate. They developed GI dysfunction with symptoms being similar to acute dysentery. Twenty-four hours later, most had recovered.

- **Subacute, subchronic and chronic toxicity studies**

Over a period of 13 weeks, F344 rats received via diet daily doses of 0, 222, 441, 886 or 1,792 mg ammonium sulphate/kg bw (males), and of 0, 239, 484, 961 or 1,975 mg ammonium sulphate/kg bw (females). Body weights, haematology, serum parameters, and histological examinations (brain, heart, lung, liver, kidney, adrenal gland, spleen, testes and thymus) revealed no treatment-related alterations. Despite the absence of histological alterations, there were unexplainable increases in relative and absolute kidney weights in both sexes in the highest dose group and in the relative testes weight at all dose groups (Takagi et al., 1999). Based on these results, the NOAEL of ammonium sulphate was determined to be 886 mg/kg per day (equivalent to 241 mg ammonium/kg bw per day) for males and 1,975 mg/kg per day (equivalent to 539 mg ammonium/kg bw per day) for females (OECD, 2004).

Ammonium sulphate was given to male and female Fisher 344/DuCrj rats (10 animals/sex per group) at dietary concentrations of 0%, 0.1%, 0.6% and 3.0% (being equivalent to 42, 256 and 1,527 mg/kg bw per day in males, and 48, 284 and 1,490 mg/kg bw per day in females) in a 52-week toxicity study. Absolute and relative kidney weights were increased in the 3% group in both sexes. Absolute spleen weights were lowered and relative liver weights were increased at the 3% in males only. There was no treatment-related effect on survival rate, food intake, body weights, and haematological, serum biochemical or histopathological parameters. The authors concluded that the NOAEL of ammonium sulphate was at 0.6% (equivalent to 256 mg/kg bw per day in males and 284 mg/kg bw per day in females) (Ota et al., 2006).

In 2012, a statement of EFSA was published on the subacute, subchronic or chronic toxicity of ammonium chloride (EFSA, 2012b) and it was reported that no effects other than those related to metabolic acidosis were observed up to doses of approximately 3,500–4,000 mg/kg bw per day (1,200–1,400 mg ammonium/kg bw per day) in a 4- and 13-week studies, or up to approximately 1,200–1,300 mg/kg bw per day (400–440 mg ammonium/kg bw per day) in 18- and 30-month studies. No relevant studies have been published since 2012.

To conclude, in long-term studies on ammonium sulphate and ammonium chloride, there was no evidence for neoplastic alterations, indicating that ammonium and ammonium sulphate are not carcinogenic. Increases in testis or liver weights were observed in one of the three studies only. Elevated kidney weights were reported in two studies at doses of 1,490 mg/kg bw or above, which is considered to be of low toxicological relevance.

- **Genotoxicity**

BASF AG (1989, cited from OECD, 2004), tested ammonium sulphate in *S.* Typhimurium TA1535, TA100, TA1537, and TA98 with and without a metabolic activation system. Concentrations of up to 5,000 μg/plate did not show mutagenic or cytotoxic effects.

Litton Bionetics (1975, cited from OECD, 2004), studied ammonium sulphate in *S.* Typhimurium strains TA1535, TA1537 and TA1538 and in *Saccharomyces cerevisiae* D4 with and without metabolic activation. No mutagenicity or cytotoxicity became obvious up to a concentration of 50,000 ppm.

Tuschy and Obe (1988, cited from OECD, 2004), studied CHO cells treated with 3.2 M (423 mg/mL) ammonium sulphate in the absence of metabolic activation. No chromosomal aberrations were found. The same group (Obe and Kamra, 1986; cited from OECD, 2004) applied ammonium sulphate at 3.2 M to human lymphocytes without adding a metabolic activation system. Again, no chromosomal aberrations were found. However, in CHO cells and human lymphocytes, ammonium sulphate enhanced the frequency of chromosomal aberrations, which had been induced by the restriction endonuclease Alu 1. The authors speculated that the salt leads to partial dehistonisation of the chromatin which makes more recognition sites available for Alu I.

Nowak (1988) treated V79 cells with ethyl methanesulphonate (EMS) (20 mM), ammonium sulphate at concentrations up to 50 mM (corresponding to 6.6 mg/mL) or both. After single exposure to ammonium sulphate, hprt mutations were doubled and chromosomal aberrations showed a dose-dependant increase. The combined treatment with EMS led to a clear increase in the hprt mutation and chromosomal aberrations. The authors concluded that ammonium sulphate induces a hypertonic effect which increases the genotoxic potency of EMS.

There are no *in vivo* data on genotoxicity of ammonium sulphate. Based on limited *in vitro* data and on the evaluation of genotoxicity studies performed on further inorganic ammonium salts (EFSA, 2012b) the CONTAM Panel concluded that ammonium sulphate is unlikely to be genotoxic.

- **Carcinogenicity**

Ammonium sulphate was given to male and female Fisher 344/DuCrj rats (50 animals/sex per group) at dietary concentration of 650 or 1,371 mg/kg bw per day in females) in a 104-week carcinogenicity study. Ammonium sulphate did not exert any significant influence on survival and incidences of tumours in any of the organs and tissues examined. The doses of 1%, 5% and 3% increased the number of males with nephropathy at 104 weeks (Ota et al., 2006).

No further carcinogenicity studies with ammonium sulphate are available.

In mice receiving ammonium (dissolved in water) at a daily dose of 42 mg/kg bw by gavage for 4 weeks, no carcinogenic effects were observed (Uzvölgyi and Boján, 1980).

The role of ammonia as a tumour promoter has been examined in rats. After preatment with *N*-methyl-*N*'-nitro-*N*-nitrosoguanidine (83 mg/L drinking water for 24 weeks) as an initiator, for 24 weeks, rats were exposed for an additional 24 weeks to an ammonium solution (0.01% ammonia) or tap water. The administration of ammonia induced an increase in the incidence and number of malignant lesions in the glandular stomach when compared to water controls (Tsujii et al., 1992, 1995). Most of the tumours had progressed more than those of the controls and had infiltrated deeper into the surrounding tissue. Furthermore, continuous long-term application of ammonia accelerates epithelial migration, especially in the antrum, leading to mucosal atrophy and increased cell proliferation in the gastric mucosa (Tsujii et al., 1993).

The CONTAM Panel noted that ammonium or ammonia may enhance tumour formation by acting as a tumour promoter. However, the available data do not allow to assess the cancer risk of oral exposure.

There are no validated data reporting a concern for carcinogenic effects of ammonia or ammonium compounds in humans following oral exposure.

Carcinogenicity studies on other inorganic ammonium salts were evaluated previously by EFSA (EFSA CEF Panel, 2011; EFSA CONTAM Panel, 2012a,b). In 2012, the CONTAM Panel stated that 'ammonium can be considered of no carcinogenic concern' (EFSA, 2012b). No further relevant studies were identified thereafter.

- **Developmental and reproductive toxicity**

Takagi et al. (1999) reported that there were no histological changes in testes of rats treated with 1,792 mg ammonium sulphate/kg bw per day over 13 weeks (see also in the section on chronic toxicity). There are no studies available in which ammonium sulphate has been tested for its effects on fertility and development.

In a study, performed according to the OECD TG 422, diammonium phosphate was applied via gavage at daily doses of 250, 750 and 1,500 mg/kg bw to CD rats (10 females and 5 males per group) throughout the mating and gestation periods. Two weeks after start of treatment, animals were mated. There were no mortalities, including in the highest dose group of 1,500 mg diammonium phosphate/kg bw per day (equivalent to approximately 410 mg ammonium/kg bw per day). At the two highest doses, the activated partial thromboplastin time was reduced in males and body weight gain was temporarily reduced in both sexes at the highest dose. Altered enamel formation of the incisors was reported for the 750 mg/kg bw per day group most probably due to the inhibiting effect of phosphate on teeth mineralisation. No further treatment-related signs of toxicity were found. Mating performance and fertility were unaffected and macroscopic necropsy showed no effect on the pups (OECD, 2004; EFSA 2012b).

The CONTAM Panel noted that the toxicity studies were performed at very high doses compared to the potential exposure caused by previous cargo.

In 2012, EFSA stated that available information on developmental and reproductive toxicity of ammonium indicates no effect on fertility. Adverse effects on development were observed only secondarily to maternal toxicity (EFSA, 2012b). No relevant studies were identified thereafter.

- **Immunotoxicity**

F344/DuCrj rats were exposed to 0%, 0.38%, 0.75%, 1.55 and 3% ammonium sulphate (0, 222, 441, 886 and 1,792 mg/kg bw per day (males) and 0, 239, 484, 961 and 1,975 mg/kg bw per day (females)) in the feed for 90 days. Based on the results of the routine toxicology endpoints measured, there were no clear effects on cells and organs of the immune system that were measured, like haematology (e.g. WBC count and differentiation (e.g. lymphocytes, neutrophils, monocytes, eosinophils)), clinical chemistry (albumin/globulin ratio), organ weight (spleen) and pathology (thymus, spleen) (Takagi et al., 1999).

Sprague–Dawley rats were exposed to 0.5 mg/m^3 ammonium sulphate via inhalation, 5 h/day, 5 days/week, for 4 or 8 months. Among others, several immunological parameters were determined after 4 months of exposure, such as spleen weight, distribution of spleen cells and mitogenic responses of spleen cells or peripheral blood lymphocytes to concanavalin A, phytohaemagglutinin, pokeweed mitogen and lipopolysaccharide. No significant effect on the immune system parameters was observed (OECD, 2004).

Based on these limited available data, there is no indication that ammonium sulphate is immunotoxic.

3.4.3.4. Allergenicity

Available data give no indication that ammonium sulphate is a skin sensitiser.

There are no new data on the potential allergenicity of ammonium sulphate.

3.4.3.5. General remark

Exposure to ammonium and sulphate from ammonium sulphate used as previous cargoes is expected to be negligible in comparison with both uptake from food and endogenous synthesis.

3.4.4. Uncertainties

The Panel noted a lack of characterisation of the impurities in different grades of ammonium sulphate. Some of these impurities may have relevant (geno)toxic potential.

Impurities like polycyclic aromatic hydrocarbons or dioxins may be expected in the production of ammonium sulphate from coke-oven gas. Similar or different problems may arise when other production methods are used.

3.4.5. Summary and conclusion

The potential contribution of ammonium sulphate used as a previous cargo to dietary exposure is negligible and is also negligible compared to endogenous synthesis.

Ammonium polyphosphate and ammonium hydroxide as well as sulfuric acid, releasing the same ions as ammonium sulphate, are listed as acceptable previous cargoes.

The data available for ammonium sulphate used in toxicity testing did not indicate concern for genotoxicity, carcinogenicity or immunotoxicity. No effects on fertility have been reported, and adverse effects on development were observed only secondarily to maternal toxicity. There was no indication that ammonium sulphate is skin sensitising or allergenic. An ADI for sulphate was set by the PRAPeR at 12.5 mg/kg bw per day (EFSA, 2012a).

The CONTAM Panel concluded that the exposure to food-grade ammonium sulphate when used as a previous cargo would not give rise to toxicological concern. However, the bulk of the ammonium sulphate is used in lower grades, mainly as a fertiliser or as an industrial chemical. There were insufficient data on the impurities from the various sources. The CONTAM Panel, therefore, concluded that only food-grade ammonium sulphate meets the criteria for acceptability as a previous cargo.

4. Conclusions

- **Calcium lignosulphonate (CAS No 8061-52-7)**

The toxicological database on calcium lignosulphonate is limited to the highly purified 40–65 grade. It has several data gaps mainly with regard to the composition and toxicity of the low molecular mass fraction. The CONTAM Panel concluded that due to the uncertainties, calcium lignosulphonate does not meet the criteria as a previous cargo.

- **Methyl acetate (CAS No 79-20-9)**

Methyl acetate is not genotoxic in *in vitro* reverse mutations assays. As it is metabolically hydrolysed to methanol and acetic acid, its toxicity was evaluated by that of methanol and acetic acid. The CONTAM Panel has previously concluded that methanol and acetic acid meet the criteria for acceptability as a previous cargo.

The CONTAM Panel concluded that methyl acetate meets the criteria as a previous cargo.

- **Ethyl *tert*-butyl ether (ETBE) (CAS No 637-92-3)**

ETBE is metabolised by oxidation to *tert*-butyl alcohol (TBA) and acetaldehyde. TBA may be further metabolised to 2-methyl-1,2-propanediol and then to 2-hydroxyisobutyrate.

In view of the general lack of genotoxicity of ETBE and TBA and the limited information on the carcinogenicity of ETBE, TBA and MTBE, the CONTAM Panel does not consider that ETBE represents a risk for carcinogenicity at the levels of exposure that would occur following its use as a previous cargo. ETBE is not expected to be immunotoxic, skin sensitising or allergenic. The CONTAM Panel established a TDI of 1 mg ETBE/kg bw per day.

There are neither anticipated impurities nor reaction products with edible fats and oils likely to be present at levels of toxicological relevance.

The CONTAM Panel concluded that ETBE meets the criteria for acceptability as a previous cargo.

- **Ammonium sulphate (CAS No 7783-20-2)**

The CONTAM Panel considered that the available information was sufficient to conclude that the exposure to food-grade ammonium sulphate when used as a previous cargo would not give rise to toxicological concern. However, the bulk of the ammonium sulphate is likely to be of different grades, mainly used as fertiliser or as an industrial chemical. Insufficient data were available about the impurities from the various sources. The CONTAM Panel, therefore, concluded that only food grade ammonium sulphate meets the criteria for acceptability as previous cargo.

Documentation provided to EFSA

1) Borregaard_EFSA_Contam._October 2012. Submitted by Borregaard.
2) 2014-3-21_Borregaard_EFSA CONTAM. March 2014. Submitted by Borregaard.
3) 2016-9-1_Documentation to EFSA. September 2016. Submitted by Borregaard.

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Abbreviations

ADI	acceptable daily intake
ADME	absorption, distribution, metabolism and elimination
AFC	antibody-forming cells
AFC Panel	EFSA Panel on Food Additives, Flavourings, Processing Aids and materials in Contact with Food
ANS	EFSA Panel on Food Additives and Nutrient Sources Added to Food
ARfD	acute reference dose
BMR	benchmarck response
bw	body weight
CAC	Codex Alimentarius Committee
CAS	Chemical Abstracts Service
CCFO	Codex Committee for Fats and Oils
CEF	EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
CHO cells	Chinese Hamster Ovary cells
CNS	central nervous system
CONTAM	EFSA Panel on Contaminants in the Food Chain
CPS	cyclophosphamide monohydrate
Da	Dalton
ECHA	European Chemicals Agency
EMS	ethyl methanesulfonate
ETBE	ethyl *tert*-butyl ether
FAO	Food and Agriculture Organization of the United Nations
FEEDAP	EFSA Panel on Additives and Products or Substances used in Animal Feed
GC–MS	gas chromatography–mass spectrometry
G/ETBE	gasoline combined with ethyl t-butyl ether
GI	gastrointestinal
HBGV	health-based guidance value
HMF	hydroxymethyl furfural
hprt	hypoxanthine phosphorybosyl transferase
IARC	International Agency for Research on Cancer
IgM	immunoglobulin M
IUCLID	International Uniform Chemical Information Database
JECFA	The Joint FAO/WHO Expert Committee on Food Additives
LD_{50}	lethal Dose, 50%/median lethal dose
LLNA	local lymph node assay
LOAEL	lowest-observed-adverse-effect level
MSDI	maximised Survey-derived Daily Intake
MTBE	methyl *tert*-butyl ether
MTDI	maximum tolerable daily intake
NMR	nuclear magnetic resonance
NOAEC	no-observed-adverse-effect-concentration
NOAEL	no-observed-adverse-effect levels
NOEL	no-observed-effect level
NTP	US National Toxicology Program
OECD	Organisation for Economic Co-operation and Development
PND	post-natal day
PRAPeR	EFSA Pesticide Risk Assessment Peer Review
RAC	Risk Assessment Committee
REACH	Registration, Evaluation, Authorisation and Restriction of Chemicals
RfD	reference dose
SCE	sister chromatid exchange
SCF	Scientific Committee on Food
SRBC	sheep red blood cells
STOT – SE	Specific Target Organ Toxicity – Single Exposure
TBA	*tert*-Butyl alcohol
TTC	threshold of toxicological concern

TDI	tolerable daily intake
US-EPA	United States Environmental Protection Agency
WHO	World Health Organization