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Prepared by the ninetieth meeting of the
Joint FAO/WHO Expert Committee
on Food Additives (JECFA)

Safety evaluation of certain contaminants in food

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World Health Organization, Geneva, 2023

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CONTENTS

PREFACE

The monographs contained in this volume were prepared at the ninetieth meeting of the Joint Food and Agriculture Organization of the United Nations (FAO)/World Health Organization (WHO) Expert Committee on Food Additives (JECFA), which met online in November 2020. These monographs summarize the data on selected food contaminants reviewed by the Committee.

The ninetieth report of JECFA has been published by WHO as WHO Technical Report No. 1032. Reports and other documents resulting from previous meetings of JECFA are listed in Annex 1. The participants in the meeting are listed in Annex 3 of the present publication.

JECFA serves as a scientific advisory body to FAO, WHO, their Member States and the Codex Alimentarius Commission, primarily through the Codex Committee on Food Additives, the Codex Committee on Contaminants in Foods and the Codex Committee on Residues of Veterinary Drugs in Foods, regarding the safety of food additives, residues of veterinary drugs, naturally occurring toxicants and contaminants in food. Committees accomplish this task by preparing reports of their meetings and publishing specifications or residue monographs and dietary exposure and toxicological monographs, such as those contained in this volume, on substances that they have considered.

The monographs contained in this volume are based on working papers that were prepared by WHO and FAO experts. A special acknowledgement is given at the beginning of each monograph to those who prepared these working papers.

The designations employed and the presentation of the material in this publication do not imply the expression of any opinion whatsoever on the part of the organizations participating in WHO concerning the legal status of any country, territory, city or area or its authorities, or concerning the delimitation of its frontiers or boundaries. The mention of specific companies or of certain manufacturers' products does not imply that they are endorsed or recommended by the organizations in preference to others of a similar nature that are not mentioned.

Any comments or new information on the biological or toxicological properties of or dietary exposure to the compounds evaluated in this publication should be addressed to: WHO Joint Secretary of the Joint FAO/WHO Expert Committee on Food Additives, Department of Food Safety and Zoonoses, World Health Organization, 20 Avenue Appia, 1211 Geneva 27, Switzerland (jecfa@who.int).

SAFETY EVALUATIONS OF SPECIFIC CONTAMINANTS IN FOOD

PREVIOUS CARGOES

GROUP 2 – ALCOHOLS

First draft prepared by

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A. ASSESSMENT OF SUBSTANCES PROPOSED AS PREVIOUS CARGOES

1. Introduction

Fats and oils destined to be used as food are transported and stored in large volumes. Transportation in large volumes by sea is exempted from many land-based regulations as it is not practical to have fleets of ships solely dedicated to the transportation of food in large tanks, since the trade is generally unidirectional from producer to consumer. Furthermore, the construction and dependency on the availability of a limited number of single-use carriers would make the transport of fats and oils extremely expensive. To address the economic realities, certain types of ships are permitted to carry different classes of cargo in their tanks on their outbound and onward journeys. A non-food item may be carried in a tank in one direction and a single type of fat or oil on the further voyage. Since ships are constructed to have several individual tanks, each may contain a cargo destined for a different location and may be used to carry either a food or non-food item depending on the contract.

A number of organizations have been involved in the development of codes of practice, transportation contracts, ship construction, cargo segregation, environmental issues and food safety. The Codex Alimentarius Commission (CAC) adopted and published a code of practice for the storage and transport of edible fats and oils in bulk, which was developed by the Codex Committee on Fats and Oils (CCFO) in 1987 (CAC, 1987). At that time, CCFO recognized the need to assess the acceptability of previous cargoes transported in a tank subsequently used for the transportation of an edible fat or oil. Commercial trade contracts recognized the need to specify that certain chemicals should never be acceptable previous cargoes for subsequent cargoes of edible fats or oils. These substances formed the basis of the "banned lists" of previous cargoes. In 2001, a combined list of chemicals banned as previous cargoes was developed by CCFO and adopted by CAC (CAC, 2001); it was added to the Codex code of practice as Appendix 1. Other substances carried in bulk were considered to pose a low risk to public

health as a contaminant in edible fats or oils; these formed the basis of "acceptable lists" of previous cargoes. The development of a CCFO acceptable list of previous cargoes was also based on trade experience. A preliminary list was reviewed by the Scientific Committee on Food and their findings were reported to CCFO in 1999; 14 substances were identified for which there were insufficient data to make a safety determination. After further discussion at subsequent CCFO meetings, a list of 23 potentially safe previous cargoes that require evaluation was developed. CCFO asked for scientific advice from FAO/WHO on these 23 substances that lacked safety evaluations (Table 1). The present evaluation by JECFA addresses 18 of the 23 substances on the current list of chemicals acceptable as previous cargoes by CCFO.

2. Background

2.1 Global production and consumption of fats and oils

The global trade in edible fats and oils is more than 200 million metric tonnes annually and valued at approximately US$ 120 billion (USDA, 2019). By far the largest contributors are palm (36%) and soybean oil (28%), followed by rapeseed/canola (14%), sunflower seed (10%), palm kernel (4%), peanut (3%), cottonseed (3%), coconut (2%) and olive oils (2%).

Many vegetable oils are produced in regions (for example: soybean – Argentina, Brazil, USA; rapeseed – Australia, Canada; sunflower seed – Ukraine; palm – Indonesia and Malaysia; and coconut – equatorial latitudes) far from the major sites of consumption. Olive oil is produced in regions with a Mediterranean climate in both the northern and southern hemispheres. International trade in fats and oils uses the most economical method of ocean transportation since global trade in edible fats and oils is primarily unidirectional. Soybean oil from Argentina and Brazil, for example, is shipped to both Asian and European markets, but there is unlikely to be a complementary cargo of fat or oil available for transportation in the reverse direction. Similarly, oils from tropical regions are traded globally, often without reciprocal trade in fats and oils.

2.2 Regulations affecting fats and oils

Shipment of fats and oils is described in numerous national and international regulations and agreements. Land-based transportation is regulated by local and national guidelines and/or legislation, whereas international trade is subject to commercial agreements, international shipping regulations and various codes of

practice. The development of banned lists and acceptable lists of previous cargoes is founded on existing trade contracts.

About 85% of the fats and oils are traded globally using FOSFA (The Federation of Oils, Seeds and Fats Associations, London) contracts. The balance is traded under contracts issued by NIOP (National Institute of Oilseed Products) or other organizations. A contract under "banned list terms" requires that fats and oils are not shipped in tanks that have contained a substance on the banned list as the immediate previous cargo. For certain chemicals, this requirement is extended to the three previous cargoes. Alternatively, a contract may state that "the immediate previous cargo shall be a product on the FOSFA List of Acceptable Previous Cargoes". In this case, the receiver will only accept the cargo if the previous cargo is on FOSFA's acceptable list. These two lists only cover a small proportion of the chemicals transported by sea; thus many substances appear on neither list and their acceptability as a previous cargo is subject to agreement by the contracting parties.

2.3 Global transport of fats and oils

Transportation by sea is regulated by the International Maritime Organization (IMO). The International Convention for the Prevention of Pollution from Ships (MARPOL) aims to prevent operational and accidental pollution from ships. MARPOL limits the carriage of different classes of liquid cargoes to specific tanker vessels based on ship construction and the class of chemical. Under this convention, fats and oils may not be transported in vessels designated to carry cargoes of crude oil, fuel oil, heavy diesel oil or lubricating oil. The International Code for the Construction and Equipment of Ships Carrying Dangerous Chemicals in Bulk (IBC Code) lists chemicals carried as bulk liquids, their pollution category, the type of ship design and any relevant restrictions or derogations. The previous cargoes under consideration (see Table 2) are in the medium- or low-risk categories for marine pollutants. The single exception is propylene tetramer, which is considered a high-risk marine pollutant. MARPOL also deals with tank washing and material discharge. Pentane falls into an additional category of oil-like substances requiring additional attention between cargoes.

2.4 The interrelationship of national, regional and trade interests

The practice of Acceptable List trading was discussed in line with regional initiatives to protect consumer health. The adoption of the hazard analysis and critical control point (HACCP) principles and their inclusion in the Codex Alimentarius approach to the safe trade of food and food products can be applied

to the transport of oils and fats by sea. The CAC adopted the *Code of Practice for the Storage and Transport of Fats and Oils in Bulk* developed by CCFO in 1987 (CAC-RCP 36-1987). The code has been revised periodically and a banned list of substances was added in 2001. The list of acceptable previous cargoes adopted by the European Union (EU) and based on existing trade lists, was evaluated by the European Food Safety Authority (EFSA).

2.5 Development of the Codex Code of Practice for storage and transport of edible fats and oils in bulk

CCFO discussions highlighted the need for lists of banned and acceptable previous cargoes. The topic of contamination by previous cargoes led to the incorporation of the FOSFA and NIOP trade lists into the code by reference only. In 2001, CAC adopted the "banned list" and it appears in the current code of practice as Appendix 3.

The development of a List of Acceptable Previous Cargoes by CCFO began with attempts to harmonize the FOSFA and NIOP trade lists with an EU list. The Acceptable List was further refined in 1999 when CCFO considered a list of substances proposed by the EU that had been reviewed by the Scientific Committee on Food (SCF). Having developed a list of acceptable previous cargoes, it was determined that there were 14 substances on it that required further evaluation; these 14 substances formed the basis of the CCFO Proposed Draft List of Acceptable Previous Cargoes, which was adopted by CAC 34 in 2011. For consideration at this meeting a list of 23 substances was proposed to FAO/WHO (Table 2) by CCFO for scientific advice on their suitability as previous cargoes for the carriage of fats and oils by sea-going vessels upon its evaluation against the four criteria. Each substance on the list has been assigned to Groups 1–5 (1 – solvents/reactants; 2 – alcohols; 3 – oils and waxes; 4 – solutions; 5 – butyl ethers). Substances in Group 1 were not evaluated at the present meeting.

3. Development of criteria

As a result of the CCFO request to FAO/WHO for scientific advice on the development of criteria for the assessment of the safety of residues of previous cargoes in the tanks of sea-going vessels carrying edible fats and oils, a technical meeting was convened (in November 2006) at the Dutch National Institute of Public Health and the Environment (RIVM). RIVM prepared a technical background document (FAO/WHO, 2006, Appendix II) and drafted the meeting report with FAO/WHO (2007).

Table 1
List of substances submitted by CCFO for evaluation by JECFA for addition to the list of acceptable previous cargoes

Substance (synonyms)	CAS number	Assessment group[a]
Acetic anhydride (ethanoic anhydride)	108-24-7	1
1,4-Butanediol (1,4-butylene glycol)	110-63-4	2
Butyl acetate, *sec-*	105-46-4	1
Butyl acetate, *tert-*	540-88-5	1
Calcium ammonium nitrate solution	15245-12-2	4
Calcium lignosulfonate liquid (lignin liquor; sulphite lye)	8061-52-7	4
Calcium nitrate (CN-9) solution	35054-52-5	4
Cyclohexane	110-82-7	1
Fatty alcohols		
iso Decyl alcohol (isodecanol)	25339-17-7	2
Myristyl alcohol (1-tetradecanol, tetradecanol)	112-72-1	2
iso Nonyl alcohol (isononanol)	27458-94-2	2
iso Octyl alcohol (isooctanol)	26952-21-6	2
Tridecyl alcohol (1-tridecanol)	112-70-9	2
Unfractionated fatty alcohol mixture or mixtures of fatty alcohols from natural oils and fats[b]		3
Methyl tertiary butyl ether (MTBE)	1634-04-4	5
Mineral oil, medium and low viscosity, class II		3
Mineral oil, medium and low viscosity, class III		3
Montan wax	8002-53-7	3
Pentane	109-66-0	1
1,3-Propanediol (1,3-propylene glycol)	504-63-2	2
Propylene tetramer (tetrapropylene, dodecene)	6842-15-5	3
Soybean oil epoxidized	8013-07-08	3
Ethyl tertiary butyl ether (ETBE)	637-92-3	5

[a] Group 1 was not considered at this meeting.
[b] Discussed with Group 2 – alcohols.

Discussions were limited to the assessment of previous cargoes in the transport of edible fats and oils in bulk by sea and the consideration of safety implications in terms of human health. The experts accepted that the quality of the fats and oils cargo could change as a result of hydrolysis and oxidation, but they acknowledged that these changes were already taken into account in trade contracts.

The experts considered a list of parameters originating from discussions at CCFO meetings, noting that previous cargoes are generally liquid chemical substances, slurries of solid particles or aqueous solutions. To further frame the deliberations, the experts decided to consider only a generic worst-case scenario since developing criteria to cover every possible combination of previous cargo,

type of tank, cleaning regime and possible further processing of the subsequent cargo of fat or oil would not be a realistic approach.

The experts developed the following worst-case scenario: the smallest commercially viable tank size (200 m^3), coated with a polymer that absorbs the previous cargo, is filled to 60% capacity (as required by contract), and the cargo of fat or oil is not to be further processed or refined. The model also assumed that the tank and associated pipework has been cleaned according to defined standards, inspected and considered clean and dry. Under these circumstances, the maximum level of contamination in the subsequent fat or oil cargo by the previous cargo was calculated to be 100 mg/kg. This value was used to determine a single estimate of worst-case human exposure of 0.1 mg/kg bw per day. Based on this generic exposure value, the experts considered that for the evaluation of previous cargoes, the acceptable daily intake (ADI) (or tolerable daily intake (TDI)) should be greater than or equal to 0.1 mg/kg bw in order to provide sufficient protection for children and high-intake consumers. Negligent or fraudulent practices were not considered to be part of the criteria. The experts identified four criteria necessary to determine the acceptability of a previous cargo (see FAO/WHO, 2006).

The criteria as adopted by CAC 34 (2011) are listed in Table 2.

4. Basis of evaluation

4.1 Chemistry/reactivity

Edible fats and oils are normally chemically stable; however, there may be potential for reactions with residues of previous cargoes that could give rise to products that are hazardous to human health. Consideration should be given to chemical substances that can react with edible fats and oils under normal transportation conditions. Minor oxidation and hydrolysis are normally anticipated in trade contracts and are not considered a consequence of contact with a previous cargo, unless accelerated degradation occurs. Although many possible reactions require the presence of specific catalysts or temperatures well in excess of those anticipated during transportation, potential reactions of the previous cargo with triglycerides and free fatty acids or other minor components present in the fat or oil should still be considered.

Table 2
Criteria adopted by CAC 34 and included in RCP-36-1987

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.

4.2 Methods of analysis

In a few cases where contamination is considered critical there has been an international effort to develop specific analytical methods. Cases of contamination with diesel fuel (alkanes) and mineral oils (mineral oil saturated hydrocarbons, MOSH; mineral oil aromatic hydrocarbons, MOAH) led to the development of relevant international standards. Although many of the substances under review at the present meeting can be analysed by gas or liquid chromatography using appropriate detector systems, little progress has been made in the application of these technologies to their contamination of oils and fats. It is assumed that available methods with suitable modifications will be capable of determining the maximum anticipated level of 100 mg/kg of previous cargo in the subsequent cargo of fats or oils.

4.3 Dietary exposure assessment for previous cargo chemical substances

As a consequence of considering a range of previous cargo chemical substances at its ninetieth meeting, the Committee concluded that it was appropriate to review the approach to estimating dietary exposure set out in the 2006 document *Development of criteria for acceptable previous cargoes for fats and oils* (criteria document) (FAO/WHO, 2007).

The Committee noted that since the 2006 criteria document was drafted, newer and better-quality data on the consumption of fats and oils by adults, infants and young children have become available.

The Committee also noted that some of the previous cargo chemical substances assessed have additional sources of dietary exposure and expressed the view that it may be necessary to consider this in the exposure assessment.

4.3.1 Exposure estimates in the 2006 criteria document

Based on the best available data at that time, the 2006 criteria document set out the following approach to dietary exposure assessment of previous cargo chemical substances present in fats and oils:

- Estimated mean per capita consumption of 0.025 kg/day of a single type of fat or oil. The value was rounded up from the maximum per capita consumption of refined soybean oil of 22 g/person per day from the GEMS/Food cluster diets.
- A factor of 2.5 to cover children and high consumers was derived from a rounded ratio between the mean and 97.5th percentile consumption of total vegetable oil from a food consumption survey in the United Kingdom (20 and 52 g/person per day for the population aged > 18 years). The criteria document also noted that dietary exposure of children to contaminants is frequently 2.5 times that of adults.
- A worst-case concentration of 100 mg/kg for a previous cargo contaminant in fats or oils.
- A body weight of 60 kg.

These data were used to define a worst-case dietary exposure estimate:

$$\frac{\text{Consumption of oil (0.025 kg/day)} \times 2.5 \times \text{concentration (100 mg/kg fat or oil)}}{\text{60 kg body weight}}$$

= 0.1 mg/kg bw per day

Based on the **mean per capita consumption of fats and oils, and a factor of 2.5**, there would be no health concern to the general population from exposure to previous cargoes if the ADI or TDI is sufficiently protective, for example, the ADI or TDI is greater than, or equal to **0.1 mg/kg bw per day**.

4.3.2 Exposure estimates based on up-to-date consumption data for adults

Since 2006, the GEMS/Food cluster diets have been revised, and the FAO/WHO Chronic Individual Food Consumption – summary statistics database (CIFOCOss) has become available. The 2006 criteria document noted that food consumption information from dedicated surveys would be more appropriate than the food consumption estimates from the GEMS/Food cluster diets (GEMS/Food consumption database, 2012). However, it used the cluster diets, as food consumption survey data were only available from a very limited number of

countries at that time. CIFOCOss currently contains food consumption data from 37 countries.

From the current version of CIFOCOss, the maximum mean consumption for a single fat or oil type is 35 g/person per day for consumption of virgin or extra-virgin olive oil by elderly Italians. The maximum 95th percentile (p95) consumption of a single fat or oil is 138 g/person per day for edible cottonseed oil by women (age 15–49 years) from Burkina Faso. This group also has the highest 97.5th percentile consumption of 189 g/person per day.

Based on the protocols currently used by JECFA for veterinary drugs, the number of consumers of cottonseed oil in the Burkina Faso survey (n = 116) would suggest that the p95 is the highest reliable percentile (Boobis et al., 2017; Arcella et al., 2019).

These data suggest that for adults, a mean fat or oil consumption of 35 g/person per day and a high consumption of fat or oil of 140 g/person per day would be a conservative estimate consistent with available data.

The use of updated food consumption data will result in a revised estimated worst-case dietary exposure for adults:

$$\frac{\text{p95 consumption of oil (0.140 kg/day)} \times \text{concentration (100 mg/kg fat or oil)}}{\text{60 kg body weight}}$$

$$= \mathbf{0.2\ mg/kg\ bw\ per\ day}$$

4.3.3 Exposure estimates for infants and young children

Potentially vulnerable population groups, like infants and young children, were not specifically considered in the 2006 criteria document. Since then, individual consumption data for several population groups, including infants and young children, have become available through CIFOCOss and other sources. Infants and young children should be considered in the risk assessment because they could potentially experience high exposure to previous cargo chemical substances per kg body weight while they are undergoing growth and development.

Information on consumption of food oils by infants and young children was also available from the US Environmental Protection Agency's Food Commodity Intake Database (FCID) (US EPA, 2020), which in turn is based on data from the US National Health and Nutrition Survey/What We Eat In America, 2005–2010 cycles. The highest oil consumption for infants and young children based on FCID is comparable to those in the CIFOCOss database; however, oil consumption information based on FCID takes into account individual body weights.

The highest reported consumption of a specific fat or oil type was for palm oil. Estimated mean and p95 consumption by infants and young children were 7.6 and 19 g/day, respectively. Estimated mean and p95 consumption on a body weight basis were 1 g/kg bw per day and 3 g/kg bw per day, respectively.

These data were used to define a worst-case dietary exposure estimate for infants and young children:

p95 consumption of oil (0.003 kg/kg bw/day) × concentration (100 mg/kg fat or oil)

= 0.3 mg/kg bw per day

4.3.4 Exposure from other dietary sources

For some previous cargo chemical substances potentially present in food oils, there are additional sources of dietary exposure, such as contamination (e.g. contamination of drinking-water) or food additive uses (Table 3). Dietary exposures from these different sources should be considered in exposure assessment.

4.3.5 Conclusion

The Committee concluded that, based on up-to-date data on consumption of single fats and oils in the general population, which have become available since 2006, the generic human exposure value of 0.1 mg/kg bw per day used in the 2006 Criterion no. 2 to determine the acceptability of a previous cargo should be revised. Consequently, the updated, more conservative generic human exposure value of 0.3 mg/kg bw per day should be used in the evaluation of these substances.

The Committee noted that these estimates of dietary exposure were derived from a more conservative approach to using data on consumption of single fats and oils and a worst-case concentration of previous cargo chemicals in a single fat or oil of 100 mg/kg.

The Committee also concluded that additional sources of dietary exposure need to be considered in exposure assessment of previous cargo chemical substances.

4.4 Approach to toxicological evaluation

The Committee received no submitted data and, therefore, reviewed monographs from previous evaluations of individual substances conducted by JECFA, WHO, International Agency for Research on Cancer (IARC), and national and regional governmental authorities to retrieve additional relevant references for completing

Table 3
List of substances for evaluation by JECFA arising from the development of a list of acceptable previous cargoes by the Codex Committee on Fats and Oils: Other sources of exposure

Substance (synonyms)	Other sources of exposure
1,4-Butanediol (1,4-butylene glycol)	Used in food contact material
Calcium ammonium nitrate solution	Calcium, nitrate and ammonium are ubiquitous in the human diet
Calcium lignosulfonate liquid (lignin liquor; sulfite lye), molecular weight not specified	Calcium lignosulfonate (40-65) is used as a food additive, an additive in animal feed and as an ingredient in pesticides
Calcium nitrate (CN-9) solution	Calcium and nitrate are ubiquitous in the human diet
iso Decyl alcohol (isodecanol)	None
Myristyl alcohol (1-tetradecanol; tetradecanol)	Flavouring agent, formulation agent, lubricant, release agent
iso Nonyl alcohol (isononanol)	None
iso Octyl alcohol (isooctanol)	Used in food contact material
Tridecyl alcohol (1-tridecanol)	Used in food contact material
Unfractionated fatty alcohol mixture or mixtures of fatty alcohols from natural oils and fats[a]	Occurs naturally in foods
Methyl tertiary butyl ether (MTBE)	Drinking-water
Mineral oil, medium and low viscosity, class II and III	Used in food contact material, direct food additive
Montan wax	Food additive
1,3-Propylene glycol	Used in place of 1,2-propanediol as a food additive
Propylene tetramer (tetrapropylene, dodecene)	None
Soybean oil epoxidized	Used in food contact material
Ethyl tertiary butyl ether (ETBE)	Drinking-water

[a] Discussed with Group 2 – Alcohols.

the present assessment. The Committee also conducted literature searches. The details are included in the consideration of individual substances.

At its present meeting, the Committee revised the generic value for assumed worst-case human dietary exposure from 0.1 to 0.3 mg/kg bw per day and used this revised generic exposure value for the evaluation of previous cargoes. The Committee also considered data on exposure to the substances from sources other than previous cargoes. Thus, the ADI (or TDI) should be greater than or equal to the estimated dietary exposure (0.3 mg/kg bw per day plus exposure from other possible dietary sources) in order to provide sufficient protection for infants, children and high-intake consumers. In situations where no appropriate numerical ADI (or TDI) was available from JECFA, the Committee considered other previously established health-based guidance values or calculated a margin of exposure (MOE) based on a reference point characterizing the toxicological hazard (such as a no-observed-adverse-effect level (NOAEL), etc.) identified from the available data divided by the estimated dietary exposure. Interpretation

of this MOE is a matter of expert judgement that takes into account limitations in the available toxicological database.

5. Recommendations

The Committee recommended that the Codex Committee on Fats and Oils (CCFO) consider revising Criterion no. 2 in RCP-36-1987 as adopted by CAC 34 (2011).

- Based on the consumption of fats and oils by infants and young children, there is no health concern for the general population from dietary exposure to previous cargo chemical substances if the ADI or TDI is sufficiently protective, for example, the ADI or TDI is greater than, or equal to 0.3 mg/kg bw per day. Substances for which there is no numerical ADI or TDI should be evaluated on a case-by-case basis (e.g. margin of exposure (MOE) approach).
- Where there are additional sources of dietary exposure to the previous cargo chemical substances, they should be considered in the exposure assessment.

B. EVALUATION OF SUBSTANCES

I. Alcohols (Group 2)

This monograph considers the suitability of certain fatty alcohols and diols as an immediate previous cargo for a subsequent cargo of an edible fat or oil. These substances are included on the FOSFA and NIOP acceptable previous (prior) cargo lists either with or without some CAS numbers. Separate subsections of this monograph consider the linear (tridecyl, myristyl and unfractionated fatty alcohols) and branched-chain alcohols (isooctyl, isononyl and isodecyl alcohols) as well as 1,3-propanediol and 1.4-butanediol.

I.I Linear chain fatty alcohols (tridecyl alcohol, myristyl alcohol and unfractionated fatty alcohol mixtures or mixtures of fatty alcohols from natural sources)

1. Explanation

Tridecyl and myristyl alcohol were considered acceptable previous cargoes by the EU Scientific Committee on Food (SCF) (SCF, 1997, 2003).

The primary linear and saturated fatty alcohols C4 to C24 as well as oleyl alcohol have been evaluated by the SCF as substances intended for use in materials in contact with food and are listed without a specific migration limit in Commission Regulation 10/2011. Butyl, caproyl, capryl, nonyl, decyl, lauryl, tridecyl, myristyl, cetyl, stearyl and oleyl alcohols were all placed in the list (List 3) of substances for which an ADI or a TDI could not be established but where the present use could be accepted (SCF, 1995).

Tridecyl alcohol (CAS number 27458-92-0 corresponding to 11-methyldodecan-1-ol) and myristyl alcohol (CAS number 112-72-1) were included in the list of acceptable previous cargoes in the European Commission Directive 96/3/EC of 26 January 1996 (European Commission, 1996).

In 2009, the EFSA Panel on Contaminants in the Food Chain (CONTAM) concluded that unfractionated fatty alcohol mixtures, or mixtures of fatty alcohols from natural oils and fats, would not cause any health concern as previous cargoes, provided their sources are edible types of oils and fats (EFSA, 2009).

Tridecyl and myristyl alcohol were evaluated by the EFSA CONTAM Panel in 2012 and considered acceptable as previous cargoes for edible fats and oils (EFSA, 2012a,b).

For the current review, previous assessments by SCF, EFSA and the European Chemicals Agency (ECHA) were considered. A search by CAS number and name synonyms for additional toxicological studies in animals in humans was undertaken to identify any critical new data for the assessment of human health risk. Searches of PubMed, PubChem and Embase were conducted.

Searches of PubMed (all years), Embase (all years), Medline and PubChem were conducted for tridecyl alcohol (CAS number and synonyms 1-tridecanol, tridecan-1-ol), myristyl alcohol (CAS number and synonyms 1-tetradecanol, tetradecan-1-ol and tetradecyl alcohol) and unfractionated fatty alcohols versus adverse effects, toxicity, metabolism, genotoxicity, carcinogenesis, sensitization, allergy, uses and ADI, For tridecyl alcohol, 116 records were identified. All

abstracts were read and no publications of interest were identified. For myristyl alcohol, 30 records were identified. After reading all the abstracts, 10 publications were selected for full analysis. These papers were all related to skin sensitization following exposure to myristyl alcohol used in pharmaceutical formulations or as a component of water-based metal-working fluids. The cut-off date for inclusion in this monograph was 25 October 2020. Additional references with relevant toxicological information were identified from the documents reviewed.

2. Chemical and technical considerations

The physical and chemical characteristics of linear and unfractionated fatty alcohols are summarized in Table 4.

2.1 Linear fatty alcohols (tridecyl and myristyl alcohols)

Straight-chain fatty alcohols are derived both from natural sources and synthetically from petroleum by-products and are often present as mixtures in the final product.

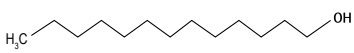
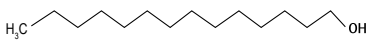
2.2 Unfractionated fatty alcohols

Commercially produced unfractionated fatty alcohols are prepared from many vegetable oils and animal fats. Fatty alcohols are present in minor amounts as free alcohols, in waxes and in the lipids of bacteria, plants, insects and animals (Dasgupta & Banerjee, 1993; Dasgupta & Macaulay, 1995a; Chance, Gerhardt & Mawhinney, 1997; Kanya, Rao & Sastry, 2007; Orozco-Solano, Ruiz-Jimenez & Luque de Castro, 2010; Rajangam et al., 2013; Giuffre, 2014; Giuffre & Capocasale, 2015; Fernandes, Martel & Cordeiro, 2020). For example, fish oil fatty alcohols range from C11 to C24 and may be saturated, unsaturated, iso-, anteiso- and diols (Chance, Gerhardt & Mawhinney, 1997).

2.3 Manufacture and uses of linear fatty alcohols

Tridecyl alcohol is produced by several different commercial processes: reaction of carbon monoxide and hydrogen with propylene tetramer (oxo process), followed by hydrogenation of the resulting aldehyde; a second type of oxo process using C15 hydrocarbons (Lewis, 2001a); or by a modified oxo process in which C11–C14 linear olefins are reacted with hydrogen and carbon monoxide over

Table 4
Some physical and chemical characteristics of linear and unfractionated fatty alcohols

Substance	CAS number[a]	Additional CAS numbers[b]
Linear fatty alcohols		
Tridecyl alcohol (1-tridecanol, tridecan-1-ol)  Molar mass: 200.37 g/mol Melting point: 32 °C Boiling point: 274 °C Insoluble in water; soluble in ether, hexanes and other solvents	112-70-9	26248-42-0 (tridecanol); 80206-82-2 (alcohols C12-14)
Myristyl alcohol (1-tetradecanol; tetradecanol; tetradecan-1-ol)  Molar mass: 214.39 g/mol Melting point: 38 °C Boiling point: 290 °C Insoluble in water; soluble in diethyl ether; slightly soluble in ethanol	**112-72-1**	67762-30-5 (alcohols C14-18); 67762-41-8 (alcohols C10-16); 68002-95-9 (alcohols C14-22 and C16-22-unsaturated); **68333-80-2** (alcohols, C14-16); 68855-56-1 (alcohols, C12-16); 71750-71-5 (alcohols C>14); **75782-87-5** (alcohols C14-15); 6338-82-8 (alcohols C12-15); **63393-82-8** (alcohols C12-15)
Unfractionated fatty alcohol mixture or mixtures of fatty alcohols from natural oils and fats (unfractionated fatty alcohols)	NA	NA

NA, not applicable.
[a] CAS number as identified in call for data.
[b] Additional CAS numbers determined from source material and process of manufacture.
Note: **Bold** indicates CAS numbers listed by the National Institute of Oilseed Products as acceptable previous cargoes.

a modified cobalt catalyst to produce a mixture of *n*- and *iso*-alcohols in which *n*-isomers predominate.

Tridecyl alcohol produced from propylene tetramer is used as a processing aid in polyvinyl chloride resin production (CFR, 2006). Tridecyl alcohol is also used as a lubricant and for the manufacture of surfactants and plasticizers.

Myristyl alcohol is manufactured by a number of different processes: sodium reduction of fatty acid esters, lithium aluminium hydride reduction of fatty acids, and from acetaldehyde plus dimethylamine (O'Neil, 2013). Myristyl alcohol may also be produced from ethylene in the presence of aluminium and hydrogen, and is coproduced with *n*-hexanol, *n*-octanol, *n*-decanol, a product called *n*-alkanol (C8–C10) and lauryl alcohol (C12). Fractionation also gives "narrow-cuts" such as *n*-alkanol (C12–C14), cetyl alcohol or stearyl alcohol, or "broad-cuts" such as *n*-alkanol (C12–C18), cetyl stearyl alcohol or *n*-alkanol (C20+) (Ashford, 1994).

Myristyl alcohol is used as a flavouring agent, release agent, lubricant, food contact material, perfume fixative for soaps and cosmetics, and in many personal care items. Myristyl alcohol is also used in specialty cleaning products, as an anti-foam agent, in some plastics and in agricultural applications.

Unfractionated fatty alcohols are produced from vegetable oils and animal fats by transesterification to form methyl esters followed by catalytic hydrogenation, or by hydrolysis into fatty acids and glycerol followed by reaction with fatty alcohols to form wax esters and final release by hydrogenolysis. Commercially, fatty alcohols are used in surfactants and detergents, as well as in cosmetic formulations (Elsner et al., 2012). Unfractionated fatty alcohols may also be directly produced by reduction of fatty acids recovered from deodorizer distillates. An alternative manufacturing process involves sodium reduction with cyclohexane.

2.4 Composition and secondary contaminants

Tridecyl alcohol usually occurs as a mixture of different isomeric compounds such as 2-tridecanol, 3-tridecanol, 4-tridecanol, 5-tridecanol, 6-tridecanol and isotridecanol.

Industrially produced **myristyl alcohol** may contain by-products of the propylene-butylene polymerization, such as isooctyl and isononyl alcohols. The commercial product can contain amounts of trimethyl-1-heptanols and dimethyl-1-octanols depending on the olefin feedstock used and this may be reflected in the assigned CAS number (Bingham, Cohrssen & Powell, 2001a).

Unfractionated fatty alcohols mostly contain even-chain-length alcohols typical of the input material (Liu et al., 2010) and may range from C4 to C20 depending on the oil source, for example: tallows, C16–C18; rapeseed/mustard seed oils, C20–C22; coconut oil, C12–C14; palm kernel oil C12–C16; palm oil C16–C18 (Kreuzer, 1984, Codex Standard 210, 2019). Commercial blends with specific ratios of major fatty alcohols are also available (Tay, 2013). Individual fatty alcohols may be saturated or retain unsaturation depending on the method of manufacture.

2.5 Reactivity and reactions of linear chain alcohols with fats and oils

Fatty alcohols react with strong oxidizing agents but are unlikely to react with fats and oils under conditions of transportation. Transesterification with glycerides

or esterification with free fatty acids present in the cargo may occur but reactions are likely to be slow at ambient temperature.

2.6 Methods of analysis

Oils can be analysed to determine the abundance of free or esterified forms of both *n*- and *iso*- fatty alcohols. Free fatty alcohols can be directly extracted with appropriate solvents and further separated by thin -layer chromatography (TLC) (Kanya et al., 2007). Esterified fatty alcohols are recovered from waxes and other esters after saponification and separation from other components of the non-saponifiable fraction by TLC (Chance, Gerhardt & Mawhinney, 1997). Owing to the presence of a hydroxyl group, fatty alcohols chromatograph poorly without first being converted into stable derivatives.

A number of different derivatization reagents have been used in both conventional heating and microwave applications. Fatty alcohols are analysed as acetyl, trifluoroacetyl and *tert*-butyldimethylsilyl derivatives (Dasgupta & Banerjee, 1993); carbethoxy-hexafluorobutyryl and heptafluorobutyryl derivatives (Dasgupta & Macaulay, 1995a); perfluorooctanoyl derivatives (Dasgupta, Thompson & Malik, 1994); and pentafluorobenzoyl derivatives (Bowden & Ford, 2011). Other common approaches include the use of silylation reagents such as N-methyl-N-*tert*-butyldimethylsilyltrifluoroace tamide (MTBSTFA) with 1%-*tert*-butyldimethylsilyl chloride (tBDMS–Cl) in combination with N-*tert*-butyldimethyl-silylimizadole (TBSIM) (Chance, Gerhardt & Mawhinney, 1997); *O*-bis(trimethylsilyl)-trifluoroacetamide (BSTFA); and N,*O*-bis(trimethylsilyl)trifluoroacetamide in combination with trimethyl-chlorosilane (Liu et al., 2010; Fernandes, Martel & Cordeiro, 2020).

Liquid chromatography was found to be less successful than gas chromatography with flame ionization in the detection and analysis of fatty alcohols (Andrisano et al., 1994; Chance, Gerhardt & Mawhinney, 1997; Kanya, Rao & Sastry, 2007; Tay, 2013; Giuffre, 2014; Giuffre & Capocasale, 2015; Chen et al., 2018). Gas chromatography coupled with mass spectrometry has been successfully applied in the determination of fatty alcohols (Dasgupta & Banerjee, 1993; Chance, Gerhardt & Mawhinney, 1997; Liu et al., 2010; Orozco-Solano, Ruiz-Jimenez & Luque de Castro, 2010; Bowden & Ford, 2011; Fernandes, Martel & Cordeiro, 2020). The use of chemical ionization mass spectrometry resulted in improved identification of individual saturated and unsaturated fatty alcohol derivatives when compared with electron ionization mass spectrometry (Dasgupta & Macaulay, 1995b). Gas chromatography coupled to electron capture negative ion chemical ionization mass spectrometry was effective in the analysis of the pentafluorobenzoyl derivatives (Bowden & Ford, 2011).

Although many research techniques have been applied to the analysis of fatty alcohols, no report of their application to the detection of a previous cargo of industrial fatty alcohols was found. It is likely that the approaches summarized above will yield acceptable results, although the presence of endogenous fatty alcohols may require cautious interpretation.

3. Biological data

3.1 Biochemical aspects

No studies of absorption, distribution, biotransformation and excretion, or studies reporting on formal pharmacokinetic analysis of tridecyl alcohol, myristyl alcohol and unfractionated fatty alcohols were identified.

The Organisation for Economic Co-operation and Development (OECD) evaluated 30 primary aliphatic alcohols, including tridecyl alcohol and myristyl alcohol, with a carbon chain length range of C6–C22 as a category (categorized as long chain alcohols – LCOH). Key attributes shared by the category members are: comparable structural features, similar metabolic pathways, a common mode of ecotoxicological action, and common levels and mode of human health-related effects. Grouping of the LCOH into a common category is possible because the group comprises a homologous series of structures that impart the predictable pattern of properties. In addition, certain branched and unsaturated structures are considered to have such similar properties that their inclusion in the category is justified (OECD, 2006a; Sanderson, 2006). Fisk et al. (2009) presented data that establish such a pattern for this homologous series of alcohols.

Aliphatic alcohols are expected to be absorbed by all common routes of exposure (dermal, oral and inhalation) (OECD, 2006a). Orally administered aliphatic alcohols are expected to show a chain-length-dependent potential for gastrointestinal absorption, with shorter chain aliphatic alcohols having a higher absorption potential than longer chain alcohols (OECD, 2006a).

According to Friedberg (1976) the oral absorption of aliphatic fatty alcohols is good. Using hexadecanol as a model compound, 23% of an orally administered dose was found as unchanged compound in the plasma of rats (Friedberg, 1976 cited in Veenstra et al., 2009).

The metabolism of aliphatic alcohols in mammals is highly efficient. The initial step in mammalian metabolism of primary alcohols is oxidation by alcohol dehydrogenase to the corresponding aldehyde. The aldehyde can be further oxidized by aldehyde dehydrogenase to the corresponding carboxylic acid. These carboxylic acids are susceptible to further degradation via acyl–

CoA intermediates by the mitochondrial β-oxidation process. This mechanism removes C2 units in a stepwise process and linear acids are more efficient in this process than the corresponding branched acids. The metabolic breakdown of mono-branched alcohol isomers is also highly efficient and involves processes that are identical to that of linear aliphatic alcohols. The presence of a side-chain does not terminate the β-oxidation process; however, in some cases, a single carbon unit is removed before the C2 elimination can proceed (OECD, 2006a).

Absorbed aliphatic alcohols could potentially be widely distributed within the body. However, as a result of the rapid and efficient metabolism, it is not anticipated that aliphatic alcohols would remain in the body for any significant length of time (OECD, 2006a). Shorter chain aliphatic alcohols can cross the blood–brain barrier more readily than longer chain alcohols (Gelman & Gilbertson, 1975 cited in Veenstra et al., 2009*)*.

Long chain alcohols are unlikely to have tissue retention or bioaccumulation potential (Bevan, 2001 cited in Veenstra et al., 2009). Longer chain aliphatic alcohols within this category may enter common lipid biosynthesis pathways and, if so, could be indistinguishable from the lipids derived from other sources (including dietary glycerides) (Kabir & Kimura, 1993, 1995; Mudge, Belenger & Nielse, 2008, cited in Veenstra et al., 2009).

Data are available from a well-conducted in vitro study using human skin in which myristyl alcohol (C14-alcohol, tetradecan-1-ol) gave a percutaneous absorption rate of 1.2% at 6 hours and 6.3% at 24 hours (P&G, 2007).[1] This study, cited in Veenstra et al., 2009, indicates that 32% of tetradecanol was absorbed after 24 hours of exposure of split-thickness cadaver skin to a 2% tetradecanol emulsion. This percentage absorption is applicable only until the residual product is removed from the skin.

3.2 Toxicological studies

3.2.1 Acute toxicity

Tridecyl alcohol administered by the oral route is reported to have very low acute toxicity, with LD_{50} values in rats of 17 200 mg/kg bw.[2]

For tridecyl alcohol, ECHA has recorded acute LD_{50} >2000 mg/kg bw for the oral and dermal routes and a lethal concentration $(LC)_{50}$ >5 mg/L air for acute inhalation.[3]

[1] Cited in https://echa.europa.eu/pt/registration-dossier/-/registered-dossier/15422/7/2/1

[2] https://comptox.epa.gov/dashboard/dsstoxdb/resultssearch=DTXSID2021947#toxicity-values

[3] https://echa.europa.eu/registration-dossier/-/registered-dossier/17532/7/3/1

Myristyl alcohol has been reported to have very low acute toxicity with an LD_{50} higher than 5000 mg/kg bw following oral (rats) and dermal (rabbits) exposure (Anonymous, 1975).

The long chain alcohols are of a low order of acute toxicity upon inhalational, oral or dermal exposure. In most cases, the acute oral LD_{50} values exceed the highest dose tested and, depending on the test protocol, range from >2000 to >10 000 mg/kg (OECD, 2006a).

3.2.2 Short-term studies of toxicity

The search of the peer-reviewed literature identified no studies on short-term exposure to tridecyl alcohol, myristyl alcohol or unfractionated fatty alcohols.

Branched tridecyl alcohol, dissolved in polyethylene glycol 300, administered by gavage for 14 days to five male Alderley Park Wistar-derived rats (single doses of 0 and 184 mg/kg bw per day) did not induce testicular atrophy, hepatomegaly, peroxisome proliferation or hypolipidaemia. No effect on body weight gain was observed in treated animals when compared to controls. Mild liver histological changes were observed, which were not clearly adverse effects: slight centrilobular hypertrophy (2/5), slight/moderate glycogen vacuolation (4/5) and slight/moderate centrilobular lipid vacuolation (2/5). No major pathological features of hepatotoxicity were observed (Rhodes et al., 1984).

A 90-day study in rats (11 males and 11 females per group), in which C14–16-branched and linear alcohol (CAS number 75782-87-5) was administered at 0%, 0.2%, 1% and 5% in the diet, was considered relevant to myristyl alcohol. These dietary concentrations were equal to mean doses of 0, 171, 759 and 3626 mg/kg bw per day in males and 0, 167, 736 and 3491 mg/kg bw per day in females. Some reduction in growth in animals in the middle and high-dose groups was associated with lower water and food consumption in both dose groups, which was reported to be probably due to reduced palatability of the diet. Feed loss due to spillage was frequently reported. Haemoglobin was significantly reduced in males given the highest dose, 15.2 g/dL (±0.5) compared to 15.9 g/dL (±0.4) for controls. Blood eosinophils in males were significantly reduced at all doses but this was not dose-related (control 1.5%, low dose 0.6%, middle dose 0.2%, high dose 0.6%). In females the only significant change (1% level) was increased white blood cells in animals given the highest dose (control 100/mm^3; 47 (±12.2) highest dose 73.2 (±16.2)). This was not accompanied by any significant changes in the differential leukocyte count. There was no increase in white blood cells in animals given the low and middle doses. An increase in liver enzymes (alkaline phosphatase (AP) and alanine amino transferase (ALT)) was observed in both males and females in the two higher dose groups. An increase of 20% and 26% in AP levels in males and 20% and 63% in females was

observed in the blood of animals receiving the two higher doses, respectively. ALT levels in blood were increased in both males (90%) and females (181%) only after receiving the higher dose. These increases were accompanied by an increase in relative liver weight. However, the authors did not consider these effects as adverse as no associated pathology was observed. Increased relative weights of a number of organs (brain, thyroid, liver, kidney, adrenal glands, testes and ovaries) were attributed to the reduction in body weight associated with reduced palatability of the feed. No treatment-related microscopic changes were observed (in the brain, hypophysis, thyroid, thymus, heart, liver, kidney, spleen, adrenal glands, testes or ovaries, stomach, pancreas, small and large intestine, lymph gland or bone marrow). Slight kidney changes such as hyaline casts, calculi and increased medullary connective tissue were observed but these were not dose-related. No histopathological changes in the liver of animals in the middle- and high-dose groups were reported. At the 0.2% dose, slight focal necrosis was observed in 1/5 females examined. No abnormalities were observed in any other organs including the gonads (Ito et al., 1978 reported in OECD, 2000).

The linear and the essentially linear alcohols are reported to be of a low order of toxicity following repeated exposure (OECD, 2006d). Typical NOAELs recorded for this category range between approximately 200 mg/kg per day to 1000 mg/kg per day in the rat upon subchronic administration via the diet.

No adverse systemic effects have been seen in reliable studies with members of the category of C6–C24 alcohols; therefore, the NOAELs represent the highest dose tested.[1]

3.2.3 Long-term studies of toxicity and carcinogenicity

No long-term toxicity or carcinogenicity studies were identified for tridecyl alcohol or myristyl alcohol.

Sicé (1966) investigated the tumour-promoting activity of a series of alkanes and alkanols including myristyl alcohol on the skin of mice. Animals were initiated with 7,12-dimethylbenz[α]anthracene and then 4 mg of myristyl alcohol was applied three times weekly for 60 days, beginning 1 week after initiation, over the area of initiated skin. Mice treated with myristyl alcohol only (non-initiated) did not develop tumours. Although some tumour-promoting activity was observed following myristyl alcohol treatment (2 of 35 surviving animals) it was also noted that skin irritation was present at the application site. Irritation at the site of application is a significant confounder in skin painting studies and the role of irritation in the tumour development following exposure to non-genotoxic chemicals has been well established (Nessel et al., 1999).

[1] https://echa.europa.eu/pt/registration-dossier/-/registered-dossier/15422/7/6/1

Myristyl alcohol was assayed for its antitumour activity against Ehrlich ascites carcinoma. Myristyl alcohol (2.5 or 10 mg/kg body weight) was administered intraperitoneally to mice for five consecutive days, 24 hours after implantation of Ehrlich ascites carcinoma. Animals were killed 30 days after implantation. Myristyl alcohol prolonged the lifespan of the treated mice but these doses were considered toxic, since the amount administered resulted in severe diarrhoea and loss of body weight (Ando et al., 1972).

3.2.4 Genotoxicity

No genotoxicity studies on tridecyl alcohol were reported.

Myristyl alcohol was tested in a *Salmonella* Typhimurium assay according to an OECD test guideline, with and without metabolic activation up to 5000 µg/plate. Myristyl alcohol was considered not to be mutagenic. The only limitation of this assay was that no cross-linking strain was used in the experiment. The original study could not be accessed but the results are sufficiently described on the ECHA site.[1]

The Screening Information Dataset (SIDS) evaluation (OECD, 2006a,b) indicates that the category members of the long chain aliphatic alcohols exhibit no structural alerts for potential mutagenic activity. The data on various linear fatty alcohols showed a consistent lack of mutagenic activity across the whole range of linear alcohols. Results of in vitro genotoxicity testing of alcohols over the carbon range (C6–C22) of category members (linear and essentially linear) and supporting substances (C5–C24–C34) provide evidence for the lack of mutagenic activity. Negative data were reported from studies on 1-decanol (*Salmonella* Typhimurium assay), C10–C16 alcohols (types B&C) (*Salmonella* Typhimurium assay, chromosome aberration, gene conversion), C12–16 (types A&B), 1-dodecanol and tetradecanol (*Salmonella* Typhimurium assay). In addition to in vitro results, 1-dodecanol, 1-octadecanol (supporting substances), 1-docosanol and C24-C34 alcohols were negative in an in vivo mouse bone marrow micronucleus test. Negative data are also available for 2-ethyl hexanol (supporting) (negative dominant lethal, micronucleus and chromosome aberration studies) and C24–34 alcohols (dominant lethal assay). Further support for the lack of mutagenicity of the category of aliphatic alcohols is provided by *n*-butanol, a substance below the minimal chain length considered in this category. *n*-Butanol showed no evidence of mutagenic activity in a bacterial assay, a test for clastogenicity in mammalian cell cultures and a mouse micronucleus assay (OECD, 2004). The conclusion of the SIDS evaluation was that the category of long chained alcohols does not have genotoxic potential.

[1] https://echa.europa.eu/pt/registration-dossier/-/registered-dossier/15422/7/7/2

3.2.5 Reproductive and developmental toxicity

A search of the peer-reviewed literature identified no studies on reproductive and developmental toxicity of tridecyl alcohol and myristyl alcohol.

Branched tridecanol (184 mg/kg bw), Alphanol C7–C9 (128 mg/kg bw), Synprol C13–15 (209 mg/kg bw), Alfol C6–C10 even (133 mg/kg bw) and Linevol C7–C9 odd 85% linear (128 mg/kg bw) administered for 14 days to male Alderley Park Wistar-derived rats (single dose by gavage) did not induce testicular atrophy (Rhodes et al., 1984). According to the SIDS dossier "the results of this study provide supportive evidence for a lack of effect of a range of alcohols on the testes following repeated oral administration as evidence of lack of effect on relative testes weights" (OECD, 2006a).

No histopathological effects in the reproductive organs were observed in the 90-day repeat-dose rat study reported above, in which C14–C16 (CAS No. 68333-80-2) was administered via the diet at doses of 0, 171, 759 and 3626 mg/kg bw per day in males and 0, 167, 736 and 3491 mg/kg bw per day in females) (Ito et al., 1978 reported in OECD, 2006b). Relative testes and ovary weights were increased in animals given the middle and high doses, but at these doses a reduction in body weight gain was observed. The effects on relative organ weights were considered to be associated with the effects on body weight rather than being a direct toxic effect.

ECHA has summarized key information on the effects of myristyl alcohol on fertility. This is based on read-across from a combined repeat-dose and reproductive and developmental toxicity screening study reporting a lack of effects on the reproductive organs of male and female rats receiving dodecan-1-ol (NOAEL > 2000 mg/kg bw) and a NOAEL for developmental effects of 2000 mg/kg bw.[1]

1-Dodecanol and 1-octadecanol were selected as supporting substances for evaluation of the effects on fertility of the long chain alcohols category by the OECD (OECD, 2006a,b). 1-Dodecanol and 1-octadecanol have been tested for potential reproductive toxicity in a combined repeat-dose reproductive and developmental toxicity screening study in rats. The materials were administered to male and female rats in the diet at 0, 100, 500 and 2000 mg/kg per day during pre-mating, mating and gestation. Pregnancy rates, uterine parameters, time to pregnancy and gestation duration indicated that fertility was not affected by exposure to 1-dodecanol or 1-octadecanol. No microscopic changes were observed in the reproductive organs. No adverse effect on the offspring was observed. The NOAEL for systemic toxicity in male and female rats and for teratogenic effects was considered by the authors to be 2000 mg/kg bw per day (highest dose tested) in the absence of toxicologically significant effects at any dose.

[1] https://echa.europa.eu/pt/registration-dossier/-/registered-dossier/15422/7/9/1

Veenstra et al. (2009) reviewed the studies on fertility of animals exposed to long chain fatty alcohols and concluded that a chain length-dependent response with maternal toxicity in rats was observed only at chain lengths of C6 and C8. No embryotoxicity or fetotoxicity was noted in any of the studies for the long chain alcohols. Veenstra et al. (2009) concluded that the studies confirm the absence of a potential for developmental toxicity for the category of the long chain fatty alcohols.

3.2.6 Irritation and allergenicity

Tridecyl alcohol and myristyl alcohols were assayed for skin irritation in CD®(SD) hrBi hairless rats (Kanikkannan & Singh, 2002). Animals were treated with 5% tridecyl alcohol or myristyl alcohol in a mixture of water:ethanol (40:60). The treatment induced slight to moderate erythema for tridecyl alcohol (median score 1.0) and myristyl alcohol (median score 1.3) after 50 hours of treatment with no increase until 100 hours (last evaluation).

Tridecyl alcohol (mainly tetramethyl-1-nonanol) was assayed for eye irritation in rabbits and considered moderately irritating (Scala & Burtis, 1973).

Myristyl alcohol applied at full strength to intact or abraded rabbit skin for 24 hours under occlusion was not irritating. Also, the occurrence of skin irritation and corneal injury in rabbits were found to be very low. Myristyl alcohol tested at 12% in petrolatum on two different panels of subjects produced no irritation after a 48-hour closed patch test on human subjects (Anonymous, 1975).

Myristyl alcohol (as Lorol 14-98 containing more than 96% of myristyl alcohol) was irritating to human skin following a 4-hour semi-occlusive exposure (OECD, 2006b).

Aliphatic alcohols in the range C6–C11 are mild irritants, but raise no concerns of tissue destruction or irreversible changes (OECD, 2006a). Aliphatic alcohols in the range C12–C16 have a low degree of skin irritation potential; alcohols with chain lengths of C18 and above are not irritating to the skin. The potential for alcohols with a chain length of C12 and above to cause eye irritation has been shown to be minimal.

Skin sensitization due to exposure to tridecyl alcohol was assessed by four different models i.e. Battery, Leadscope, SciQSAR and CASE Ultra used within the Danish QSAR database. No skin sensitization was predicted. Myristyl alcohol (as Kalcol 4098 containing more than 98% myristyl alcohol) assayed in a guinea-pig maximization test was considered not to be a sensitizer (OECD, 2006b).

3.3 Observations in humans

No studies of tridecyl alcohol in humans were identified in the literature search.

Myristyl alcohol has been reported as a contact allergen as used in medicaments and metal-working fluid (Edman & Möller, 1986; Pecegueiro et al., 1987; Tosti et al., 1996; Geier et al., 2006). Corazza et al. (2016) reported that among 310 patients who were patch tested, 11 were positive for skin sensitization reactions to myristyl alcohol. The authors also noted that patients who were allergic to emulsifiers showed concomitant allergic reactions to allergens commonly found in cosmetics.

The CONTAM Panel (EFSA, 2012a) noted that a series of fatty alcohols (myristyl alcohol, cetyl alcohol, cetylic alcohol, stearyl alcohol and oleyl alcohol) are reported to be contact allergens. The Panel concluded that the irritant and allergenic activity appears not to be very strong for any of the substances, and taking into account the high dilution factor in edible fats and oils transported as subsequent cargoes, the CONTAM Panel considered that none of the substances would represent a problem because of adjuvant, irritant or allergenic properties.

Human studies indicate that the skin irritation hazard for the aliphatic alcohols is lower than that observed in the rabbit and well below the response noted for a positive control substance. Aliphatic alcohols in the range C6–C11 are mild irritants, not anticipated to be corrosive. Those in the range C12–C16 have a low degree of skin irritation potential; alcohols with chain lengths of C18 and above are not considered irritating to the skin (OECD, 2006a).

4. Levels and patterns of contamination in food commodities

The 2006 criteria document (FAO/WHO, 2007) assumed a worst-case concentration of 100 mg/kg for previous cargo contaminants. This concentration was also supposed to be a worst-case concentration of the contaminant in oils for the current assessment. Fatty alcohols are used in surfactants and detergents, as well as in cosmetic formulations (Elsner et al., 2012). No food uses for unfractionated fatty alcohols were identified.

5. Food consumption and dietary exposure assessment

Information on consumption of food oils by infants and young children was available from the US Environmental Protection Agency's Food Commodity

Intake Database, or FCID (US EPA, 2020) which in turn is based on data from the US National Health and Nutrition Survey/What We Eat In America, 2005–2010 cycles. The highest oil consumptions for infants and young children based on information from FCID are comparable to those in the CIFOCOss database; however, oil consumption information based on FCID is available based on individual body weights.

The highest reported consumption of a specific fat or oil type was for palm oil. Estimated mean and P95 consumptions by infants and young children were 7.6 and 19 g/day, respectively. Estimated mean and P95 consumptions on a body weight basis were 1 g/kg bw per day and 3 g/kg bw per day, respectively. These data were used to define a worst-case dietary exposure estimate for infants and young children.

Estimated chronic daily dietary exposures to unfractionated fatty alcohols present in food oils are 0.1 mg/kg bw per day (mean) and 0.3 mg/kg bw per day (high consumers), assuming a worst-case concentration of 100 mg/kg, mean oil consumption of 1 g/kg bw per day, and high consumer oil consumption of 3 g/kg bw per day.

No other data on dietary exposure to unfractionated fatty alcohols were identified.

6. Comments

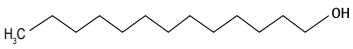
6.1 Chemical and technical considerations

Tables 5–7 summarize the chemical and technical considerations for tridecyl alcohol, myristyl alcohol and unfractionated fatty alcohols.

6.2 Biochemical aspects

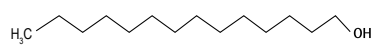
There is no specific information on absorption, metabolism, distribution and excretion of tridecyl alcohol, myristyl alcohols and unfractionated alcohols. Based on data on primary aliphatic alcohols (C6–C22), they are expected to be absorbed by all common routes of exposure. They are metabolized in mammals by alcohol dehydrogenase and the aldehyde formed is further oxidized to carboxylic acid that undergoes mitochondrial β-oxidation. Absorbed aliphatic alcohols could potentially be widely distributed within the body. However, as a result of the rapid metabolism, it is anticipated that aliphatic alcohols would be rapidly removed from the body (OECD, 2006a).

Table 5
Chemical and technical considerations for tridecyl alcohol

Name: tridecyl alcohol (1-tridecanol)	
CAS number 112-70-9	**Alternative CAS numbers** 26248-42-0 (tridecanol); 80206-82-2 (alcohols C12-14)
Chemical details	Tridecyl alcohol; 1-tridecanol White, low melting point solid  Molar mass: 200.37 g/mol Melting point: 32 °C Boiling point: 274 °C Insoluble in water; soluble in ether, hexanes and other organic solvents
Route(s) of synthesis	Manufactured by different processes: by the oxo process in which propylene tetramer is reacted with carbon monoxide and hydrogen using a catalyst, followed by hydrogenation; by a second type of oxo process using C15 hydrocarbons; or by a modified oxo process in which C11–C14 linear olefins are reacted with hydrogen and carbon monoxide over a modified cobalt catalyst.
Composition	Occurs as a mixture of mainly *n*-alcohols with minor amounts of iso-alcohols such as 2-tridecanol, 3-tridecanol, 4-tridecanol, 5-tridecanol, 6-tridecanol and isotridecanol.
Uses	Used as a processing aid in polyvinyl chloride resin production; as a lubricant and as an ingredient in the manufacture of surfactants and plasticizers.
Analytical methods	None found for previous cargoes. Possible means of analysis in fats and oils may require saponification, extraction and derivatization followed by GC-FID or GC-MS.
Potential reaction(s) with a subsequent cargo of fat or oil	Transesterification with glycerides or esterification with free fatty acids present in the cargo may occur, but are likely to be slow at ambient temperature.

GC-FID, gas chromatography with flame ionization detection; GC-MS, gas chromatography–mass spectrometry.

Table 6
Chemical and technical considerations for myristyl alcohol

Name: myristyl alcohol (1-tetradecanol)	
CAS number 112-72-1	**Alternative CAS numbers** 67762-30-5 (alcohols C14-18); 67762-41-8 (alcohols C10-16); 68002-95-9 (alcohols C14-22 and C16-22-unsaturated); 68333-80-2 (alcohols, C14-16); 68855-56-1 (alcohols C12-16); 71750-71-5 (alcohols C>14); 75782-87-5 (alcohols C14-15); 6338-82-8 (alcohols C12-15); 63393-82-8 (alcohols C12-15)
Chemical details	Myristyl alcohol; 1-tetradecanol White, low melting point solid  Molar mass: 214.39 g/mol Melting point: 38 °C Boiling point: 290 °C Insoluble in water; soluble in diethyl ether; slightly soluble in ethanol

Route(s) of synthesis	Manufactured by different processes: sodium reduction of fatty acid esters; lithium aluminium hydride reduction of fatty acids; and from acetaldehyde plus dimethylamine. May also be produced from ethylene in the presence of aluminium and hydrogen, and is coproduced with *n*-hexanol, *n*-octanol, *n*-decanol, *n*-alkanol (C8–C10) and lauryl alcohol (C12). Fractionation also gives "narrow-cuts" such as *n*-alkanol (C12–C14), cetyl alcohol or stearyl alcohol; or "broad-cuts" such as *n*-alkanol (C12–C18), cetyl stearyl alcohol or *n*-alkanol (C20+).
Composition	Occurs as a mixture and may contain by-products such as isooctyl and isononyl alcohols, trimethyl-1-heptanols, and dimethyl-1-octanols depending on the olefin feedstock.
Uses	Used as a flavouring agent, release agent, lubricant, food contact material, perfume fixative for soaps and cosmetics and in many personal care items. Also used in specialty cleaning products, as an anti-foam agent and in some plastics.
Analytical methods	None found for previous cargoes. Possible means of analysis in fats and oils may require saponification, extraction and derivatization followed by GC-FID or GC-MS.
Potential reaction(s) with a subsequent cargo of fat or oil	Transesterification with glycerides or esterification with free fatty acids present in the cargo may occur but are likely to be slow at ambient temperature.

GC-FID, gas chromatography with flame ionization detection; GC-MS, gas chromatography–mass spectrometry.

Table 7

Chemical and technical considerations for unfractionated fatty alcohol mixtures of fatty alcohols from natural oils and fats (herein referred to as unfractionated fatty alcohols)

Name: unfractionated fatty alcohols	
CAS number	**Alternative CAS numbers**
None	None
Chemical details	Mixture of fatty alcohols derived from triglycerides by saponification and reduction of the fatty acids
Route(s) of synthesis	Manufactured from natural oils and fats by either saponification and reduction of the fatty acids released or by direct reduction of fatty acids recovered from deodorizer distillates. Other manufacturing processes involve sodium reduction with cyclohexanol, hydrogenolysis or methanolysis plus hydrogenation.
Composition	Occurs as a mixture of fatty alcohols representative of the oil and/or fat used as feedstock. Chain lengths may range from C4 to C20. The fatty alcohols may be saturated or retain unsaturation depending on the method of manufacture.
Uses	Used as plasticizers (shorter chains); in surfactants, detergents and cosmetic formulations (longer chains).
Analytical methods	None found for previous cargoes. Possible means of analysis in fats and oils may require saponification, extraction and derivatization followed by GC-FID or GC-MS.
Potential reaction(s) with a subsequent cargo of fat or oil	Transesterification with glycerides or esterification with free fatty acids present in the cargo may occur but are likely to be slow at ambient temperature.

GC-FID, gas chromatography with flame ionization detection; GC-MS, gas chromatography–mass spectrometry.

6.3 Toxicological studies

There are no new data in the literature regarding the toxicological properties of tridecyl and myristyl alcohol since the 2012 EFSA evaluation. For unfractionated fatty alcohols, there are no specific toxicological data available on these mixtures of substances. Most of the toxicological evaluation was therefore based on the long chain alcohols category (C6–C22 primary aliphatic alcohols) as proposed by

the OECD in 2006 (OECD, 2006a). Some of the studies are described in detail on the ECHA website and in the previous OECD SIDS report (OECD, 2006b).

Tridecyl alcohol has very low acute oral toxicity with a reported oral LD_{50} in rats of 17 200 mg/kg bw. Myristyl alcohol is of very low acute oral toxicity with an LD_{50} exceeding 5000 mg/kg bw (Anonymous, 1975).

No repeated-dose study was available for tridecyl alcohol. Rhodes et al. (1984) administered branched tridecyl alcohol by gavage for 14 days to five male Alderley Park Wistar-derived rats (single dose level of 184 mg/kg bw per day). The Committee noted that this experiment has major limitations for the purposes of risk assessment (small group size, single species, single dose level, single sex and limited duration). No changes were reported in body, liver or testicular weight relative to controls and peroxisome proliferation and hypolipidaemia were not observed (Rhodes et al., 1984). No major pathological features of hepatotoxicity were noted. Mild liver histological changes were reported: slight centrilobular hypertrophy, slight/moderate glycogen vacuolation and slight/moderate centrilobular lipid vacuolation. The Committee did not consider these changes to be adverse.

No repeated-dose study was available for myristyl alcohol. In a 90-day study in Wistar rats (11 males and females per group), C14–16-branched and linear alcohol was administered via the diet at 0%, 0.2%, 1% and 5% (equal to 0, 171, 759 and 3626 mg/kg bw per day in males and 0, 167, 736 and 3491 mg/kg bw per day in female rats). No effects were observed at the dose of 167 mg/kg bw per day and reduced body weight gain was reported at the two higher doses. This reduction in growth was accompanied by a reduction of food and water intake, and was reported to be probably due to decreased food consumption related to unpalatability of the diet (Ito et al., 1978 described in OECD 2006b). The Committee concluded that 167 mg/kg bw per day is a NOEL for myristyl alcohol.

No data were identified on the repeated-dose toxicity of unfractionated fatty alcohols. The saturated linear alcohols C4, C6, C8, C10, C12, C14, C16 and C18, as well as oleyl alcohol are, or may be, the predominant components of these mixtures (EFSA, 2012a). NOAELs recorded for alcohols with chain length C6–C22 range from 200 mg/kg bw per day to 1000 mg/kg bw per day in the rat upon subchronic administration via the diet (OECD, 2006a).

No studies on reproductive and developmental toxicity were identified for tridecyl, myristyl and unfractionated fatty alcohols. Branched tridecanol did not induce testicular atrophy in male Alderley Park Wistar-derived rats treated with 184 mg/kg per day (single dose) for 2 weeks (Rhodes, 1984). In a 90-day repeated-dose study conducted in male and female Wistar rats, C14–16 branched and linear alcohols did not induce alterations in the gonads (weight and histology) when administered via the diet at 0.2%, which was equal to 167 mg/kg bw per day in the females (Ito et al., 1978 described in OECD, 2006b).

1-Dodecanol and 1-octadecanol, selected by the OECD as supporting substances for fertility evaluation of the linear long chain alcohols category, were tested for reproductive toxicity. Fertility was not affected; no alterations were observed in reproductive organs and no adverse effect on the offspring was observed at 2000 mg/kg bw per day (highest dose tested) (OECD, 2006a). The Committee concluded that tridecyl alcohol, myristyl alcohol and the unfractionated fatty alcohols are unlikely to induce toxic effects on reproduction and development.

Based on the data from the SIDS report (OECD, 2006a) for genotoxicity of the long chain alcohols category and taking into account that the long chain alcohols contain no structural alerts, which may be of concern for potential mutagenic activity, the Committee concluded that tridecyl alcohol and myristyl alcohol and the unfractionated fatty alcohols do not have genotoxicity potential.

No long-term exposure studies were identified for tridecyl alcohol, myristyl alcohol or unfractionated fatty alcohols.

6.4 Allergenicity

The Committee did not identify any reports of allergenicity upon oral exposure to tridecyl and myristyl alcohols or to unfractionated fatty alcohols that would indicate that they are, or they contain a known food allergen.

6.5 Food consumption and dietary exposure assessment

A worst-case concentration of 100 mg/kg has been assumed for all previous cargo chemical substances (see Part A, section 4.3).

Tridecyl alcohol has no direct food uses but is listed as an indirect additive used in food contact substances without limitations by the United States Food and Drug Administration (US FDA). No data were identified on concentrations in foods from these uses.

Myristyl alcohol is permitted as a flavouring agent (02.126) (EC, 2012). In the USA, myristyl alcohol is permitted as a formulation agent, lubricant or release agent and is generally recognized as safe (GRAS) (US FDA, 2020). No data were identified on concentrations of myristyl alcohol in foods from these uses.

No food uses for unfractionated fatty alcohols were identified.

Worst-case human dietary exposures to previous cargo chemical substances in food oils have been estimated at 0.3 mg/kg bw per day (see Part A, section 4.3).

No other data on dietary exposure to 1-tridecanol, myristyl alcohol or unfractionated fatty alcohols were identified.

7. Evaluation

The Committee noted the limitations of the current dataset of toxicological evaluations, and the need to use a read-across approach where appropriate. Based on the weight of evidence across long chain fatty alcohols, tridecyl and myristyl alcohol and unfractionated fatty alcohols can be considered not to raise concerns for genotoxicity.

For **tridecyl alcohol**, the Committee used the dose level of 184 mg/kg bw per day, at which mild histopathological changes were reported in the liver following a 14-day study of oral gavage exposure in rats (Rhodes et al., 1984), as a reference point (RP) supported by the data on other long chain alcohols, for which the NOAELs recorded in the rat upon subchronic administration via the diet range from approximately 200 to 1000 mg/kg per day (OECD, 2006a). The Committee noted limitations in the study design, but concluded that it could be used to establish a MOE in the absence of longer-term studies. Considering the estimated human dietary exposure of 0.3 mg/kg bw per day, the MOE is 610, which is adequate to address the uncertainties in the database.

For **myristyl alcohol**, the Committee identified a NOEL of 167 mg/kg bw per day as the RP from a 90-day dietary study with C14–16 branched and linear alcohols in rats (Ito et al., 1978, described in OECD, 2006b), based on decreased body weight gain at 702 mg/kg bw per day, possibly attributable to reduced palatability of the diet. Considering the estimated human dietary exposure of 0.3 mg/kg bw per day, the MOE is 560, which is adequate to address the uncertainties in the database.

For **unfractionated fatty alcohols**, the Committee adopted a read-across approach, using data on two representative fatty alcohols, tridecyl alcohol and myristyl alcohol, and long chain alcohols. NOAEL values of between 200 mg/kg bw per day and 1000 mg/kg bw per day have been reported for fatty alcohols with chain lengths in the C6–C22 range, based upon subchronic dietary studies in rats (OECD, 2006a). Based upon read-across and given that unfractionated fatty alcohols are present in natural food sources, the Committee concluded that the unfractionated fatty alcohols with components in the C6–C22 range are not of toxicological concern at the estimated dietary exposure level of 0.3 mg/kg bw per day.

There are no reports of allergenicity following oral exposure to tridecyl and myristyl alcohols and to unfractionated fatty alcohols that would indicate that they are or contain a known food allergen.

Tridecyl alcohol, myristyl alcohol and unfractionated fatty alcohols may react with a previous cargo in transesterification reactions with glycerides or esterification reactions with free fatty acids present, but the rates of reaction are

likely to be slow at ambient temperature and any products would be naturally occurring waxes.

Therefore, the Committee concluded that tridecyl alcohol, myristyl alcohol and unfractionated fatty alcohols meet the criteria for acceptability as previous cargoes.

I.II Branched-chain fatty alcohols (isodecyl, isononyl and isooctyl alcohols)

Branched-chain (iso-) fatty alcohols are produced synthetically and are often present as mixtures of analogues.

1. Explanation

The SCF evaluated isodecyl alcohol, isononyl alcohol and isooctyl alcohol as previous cargoes in 1997 and they were listed in Annex 2 of its Opinion as substances provisionally acceptable because of a lack of toxicological data and uncertainty as to their composition. It was also noted that they can be easily removed if vegetable oil is refined (SCF, 1997).

In 2003, the SCF re-evaluated a series of provisionally accepted previous cargoes, including isodecyl alcohol, isononyl alcohol and isooctyl alcohol. The information available was still considered inadequate or in need of additional clarification. On this basis, the SCF decided to maintain its previous opinion, including isodecyl alcohol, isononyl alcohol and isooctyl alcohol in the list in Annex 2 of its Opinion as substances provisionally acceptable as previous cargoes (SCF, 2003).

The CONTAM Panel of EFSA undertook an assessment of substances listed as acceptable previous cargoes for edible fats and oils in 2012 (EFSA, 2012a). The Panel noted the sparse data available to form an opinion and used a read-across approach to fill data gaps, where possible. They concluded that isodecyl alcohol, isononyl alcohol and isooctyl alcohol were of a low order of acute toxicity, and there was no evidence of genotoxicity or allergenicity. EFSA concluded that isodecyl alcohol, isononyl alcohol and isooctyl alcohol met the criteria for acceptability as previous cargoes for edible fats and oils.

For **isodecyl alcohol**, PubChem was used to identify common synonyms, namely: 25339-17-7, iso-decyl alcohol, 8-methylnonan-1-ol and 8-methyl-1-

nonanol. These terms were used as the input for a Web of Science literature search (1900–2020). The cut-off date for the search was 30 September 2020.

Common synonyms for **isononyl alcohol** identified using PubChem were: 2430-22-0, isononanol, iso-nonanol, isononyl alcohol, iso-nonyl alcohol, 7-methyl-1-octanol, 7-methyloctan-1-ol and 7-methyl-octanol. These terms were used as the input for a Web of Science literature search (1900–2020). The cut-off date for the search was 30 September 2020.

Common synonyms for **isooctyl alcohol** identified using PubChem were: 1653-40-3, isooctanol, iso-octanol, isooctyl alcohol, iso-octyl alcohol, 6-methyl-heptan-1-ol and 6-methylheptanol. The cut-off date for the search was 30 September 2020.

Other reference sources included PubChem, European Chemicals Bureau, IUCLID Dataset, ECHA and the National Institute for Occupational Safety and Health (NIOSH).

2. Chemical and technical considerations

The physical and chemical characteristics of branched-chain fatty alcohols are summarized in Table 8.

2.1 Manufacture and uses of branched-chain fatty alcohols

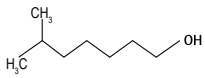
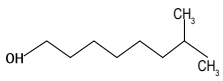
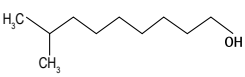
Isooctyl alcohol is manufactured by the oxo process in which heptenes are reacted with carbon monoxide and hydrogen in the presence of a catalyst, followed by hydrogenation (Bingham, Cohrssen & Powell, 2001b).

Isooctyl alcohol is used as a solvent; in the manufacture of cutting and lubricating oils and hydraulic fluids; and in the production of other chemicals. It is also an ingredient in plasticizers; an intermediate in nonionic detergent and surfactant production; in synthetic drying oils and resin solvents; emulsifiers; and antifoaming agents (Lewis, 2001b).

Isononyl alcohol is manufactured by the oxo process in which olefins are reacted with carbon monoxide and hydrogen in the presence of a catalyst, followed by hydrogenation. The commercial product typically consists of dimethyl-1-heptanols and methyl-1-octanols; the composition and CAS number depend on the olefin feedstock.

Isononyl alcohol is primarily used in further chemical reaction processes. It is not sold directly to the public for general consumer use, but may be used as an ingredient in plasticizers, stabilizers and lubricants.

Table 8
Some physical and chemical characteristics of branched-chain fatty alcohols

Substance	CAS number[a]	Additional CAS numbers[b]
Linear fatty alcohols		
Isooctyl alcohol (isooctanol; 6-methylheptan-1-ol)  Molar mass: 130.23 g/mol Melting point: −106 °C; Boiling point: 188 °C Insoluble in water; soluble in ether and ethanol	26952-21-6	1653-40-3-9 (6-methyl-1-heptanol); 68526-83-0 (alcohols C7–9-iso-, C8-rich); 68551-09-7 (alcohols C7–9-branched); 104-76-7 (2-ethylhexanol)
Isononyl alcohol (isononanol; 7-methyloctan-1-ol)  Molar mass: 144.25 g/mol Melting point: −64.5 °C Boiling point: 206 °C Insoluble in water; soluble in alcohol	**27458-94-2**	2430-22-0 (7-methyl-1-octonol); **68526-84-1** (alcohols, C8–10-iso-, C9-rich); 3452-97-9 (3,5,5-trimethylhexanol)
Isodecyl alcohol (isodecanol; 8-methylnonan-1-ol)  Molar mass: 158.28 g/mol Melting point: −2.8 °C Boiling point: 220 °C Insoluble in water, soluble in ethanol, ether, mineral oil, propylene glycol; most fixed oils	25339-17-7	68551-08-6 (alcohols C9–11-branched); 68526-85-2 (alcohols C9–11-iso-, C10-rich)

[a] CAS number as identified in call for data.
[b] Additional CAS numbers determined by source material and process of manufacture.

Isodecyl alcohol is synthesized in two different ways: by the oxo process in which nonenes are reacted with carbon monoxide and hydrogen using a cobalt catalyst followed by hydrogenation; or by polymerization of propylenes and butenes with phosphoric acid under elevated temperature and pressure to give a mixture of branched olefins, which give isodecyl alcohol upon alkaline hydrolysis (Kirk-Othmer, 1984).

Isodecyl alcohol is mainly used in the manufacture of plasticizers (70%); it is also used in lubricants, surfactants and solvents and as an antifoaming agent in textile processing. It is known to permeate the skin and has been investigated as a penetration enhancer for transdermal drug delivery.

2.2 Composition and secondary contaminants

Isooctyl alcohol is produced as a mixture of isomeric C8 alcohols and typically consists of methyl-1-heptanols and/or dimethyl-1-hexanols; the composition and assigned CAS number depend on the olefin feedstock (Kirk-Othmer, 1984).

Isononyl alcohol may be composed of a mixture of isomeric primary C9 alcohols consisting mainly of 3,5,5-trimethyl-1-hexanol, isomeric dimethyl-1-heptanols and isomeric methyl-1-octanols (e.g. 7-methyloctan-1-ol). The exact composition varies depending on the starting materials, the manufacturing process used, the reaction conditions and the purification method. It can be produced at up to 99% purity.

Isodecyl alcohol may contain by-products of the propylene-butylene polymerization such as isooctyl and isononyl alcohols. It can also contain small amounts of trimethyl-1-heptanols and dimethyl-1-octanols depending on the olefin feedstock used. This may be reflected in the assigned CAS number (Bingham, Cohrssen & Powell, 2001a).

Reactivity and reactions with fats and oils

Branched-chain fatty alcohols are anticipated to behave like linear chain fatty alcohols (see above) and react with strong oxidizing agents. They are unlikely to react with fats and oils under conditions of transportation. Transesterification with glycerides or esterification with free fatty acids present in the cargo may occur but reactions are likely to be slow at ambient temperature.

2.3 Methods of analysis

Branched-chain fatty alcohols have been identified and analysed in the general determination of the presence of fatty alcohols. No specific methodology for the separate determination of branched-chain fatty alcohols has been reported.

3. Biological data

3.1 Biochemical aspects

No studies on absorption, distribution, biotransformation and excretion, or on formal pharmacokinetic analysis of isooctyl, isononyl or isodecyl alcohol were identified.

Iso-alcohols all show similar patterns of absorption, distribution, excretion and biotransformation. They are readily absorbed from the gastrointestinal tract and rapidly cleared from the plasma due to extensive distribution around the body. Their exact fate and corresponding plasma half-lives are difficult to measure owing to the presence of endogenous iso-alcohols in animals (OECD, 2006).

Iso-alcohols are initially metabolized through NAD+/NADH-dependent enzymes to form the corresponding aldehyde. This reaction is catalysed primarily by cytosolic alcohol dehydrogenases (ADH), with a smaller contribution from CYP450s and other oxidases. These aldehyde metabolites are converted to the respective carboxylic acids by aldehyde dehydrogenases (ALDH), which are subsequently metabolized to carbon dioxide via mitochondrial β-oxidation pathways and the tricarboxylic acid cycle, in the same way as dietary fatty acids (OECD, 2006). This stepwise removal of C2 units is more efficient for linear acids than for the corresponding branched acids. The latter can also be metabolized by microsomal ω- or ω-1 oxidation followed by β-oxidation, which is relatively efficient for such compounds (Verhoeven et al., 1998). For unsaturated carboxylic acids, cleavage of C2 units continues until a double bond is reached. These double bonds will be in the *cis*-configuration and can be isomerized to the *trans*-configuration by enoyl-CoA isomerase. β-Oxidation then continues with the *trans*-isomer (Annex 1, reference *132*). Iso-alcohols, their primary metabolites and their oxidation products can be conjugated with sulfate or glucuronic acid, catalysed by sulfotransferases and UDP-glucuronosyltransferases respectively. The extent of these reactions increases with the degree and complexity of branching (Williams, 1959; Bevan, 2001; OECD, 2006).

Excretion of end-stage sulfo- and glucuronide-conjugates of iso-alcohols primarily occurs in the urine, and to a lesser extent in the faeces (OECD, 2006c).

3.2 Toxicological studies

3.2.1 Acute toxicity

For isodecyl alcohol, an acute oral LD_{50} value of 6500 µL/kg bw was reported in rats (UCDS, 1968), equivalent to 5460 mg/kg bw given a density of 0.84 for isodecyl alcohol. Nishimura et al. (1994) reported an oral LD_{50} value of 6500 mg/

kg bw. These values are within the "unclassified" category of OECD TG401 (OECD, 1981). An acute dermal LD_{50} value of 3150 mg/kg bw was reported in rabbits (Monick, 1968), a value within the "unclassified" category of OECD TG401 (OECD, 1981). No LD_{50} value has been determined for inhalation. However, Bingham, Cohrssen & Powell (2001) reported that there were no deaths in rats exposed to saturated vapours of mixed isomers of decyl iso-alcohols for 8 hours.

For isononyl alcohol, an acute oral LD_{50} value of 2980 mg/kg bw was reported in rats (Scala & Burtis, 1973), which is within the "unclassified" category of OECD TG401 (OECD, 1981). An acute dermal LD_{50} value of >3200 mg/kg bw was reported in rabbits (Scala & Burtis, 1973), a value within the "unclassified" category of OECD TG401 (OECD, 1981). No LD_{50} value has been determined for inhalation. An LC_0 value of >0.21 mg/L air (nominal) was determined in rats following a 7-hour exposure to saturated vapours (ECHA, 1982).

For isooctyl alcohol, an acute oral LD_{50} value of 1670 mg/kg bw was reported in mice (Izmerov, Sanotsky & Sidorov, 1982), and 1480 mg/kg bw in rats (Scala & Burtis, 1973). These values are within the "harmful" category of OECD TG401 (OECD, 1981). An acute dermal LD_{50} value of 2520 mg/kg bw was reported in rabbits (Monick, 1968), a value within the "unclassified" category of OECD TG401 (OECD, 1981). No LD_{50} value has been determined for inhalation. However, inhalation exposure at saturated vapour pressures generally led to no deaths (OECD, 2006).

3.2.2 Short-term studies of toxicity

Groups of five male Alderley Park Wistar-derived rats were administered isodecyl alcohol, isononyl alcohol and isooctyl alcohol at a dose of 1 mmol/kg bw per day for 14 days (Rhodes et al., 1984). This is equivalent to approximately 158 mg/kg bw per day (isodecyl alcohol), 144 mg/kg bw per day (isononyl alcohol) and 130 mg/kg bw per day (isooctyl alcohol), given a molecular mass of 158.21 g/mol, 144.25 g/mol and 130.23 g/mol, respectively.

No changes were observed in body, liver or testicular weight relative to controls. No major pathological features of hepatotoxicity were observed, but mild histological changes were noted (Table 9).

The alcohols C9–C11-iso, C9 rich, produced moderate sensory irritation of the upper airway in male mice exposed to vapours (OECD, 2006c). For isononyl alcohol, the lowest published toxic concentrations for vapour exposure are 21 700 mg/m^3 for a 6-hour exposure in mice, rats and guinea-pigs (Scala & Burtis, 1973). For isooctyl alcohol, the lowest published toxic concentrations for vapour exposure are 1500 µg/m^3 per 24 hours/96 D-continuous (Anonymous, 1972) and 600 mg/m^3/6H/2 W-intermittent for CRL CD rats (NTIS, 1992).

Table 9
Histological changes observed in the livers of male Alderley Park Wistar-derived rats

	Isodecyl alcohol	Isononyl alcohol	Isooctyl alcohol
Slight centrilobular hypertrophy	3/5	1/5	2/5
Slight/moderate glycogen vacuolation	5/5	5/5	4/5
Slight/moderate centrilobular lipid vacuolation	3/3	3/3	3/3

OECD (2006c), while noting the sparsity of robust data, reported that the available data suggest that members of the iso-alcohols C9–C13 category present a low order of subchronic toxicity.

3.2.3 Long-term and carcinogenicity studies

No carcinogenicity studies have been identified for isodecyl alcohol, isononyl alcohol or isooctyl alcohol. However, the potential for initiation, promotion or co-carcinogenicity has been investigated for several aliphatic alcohols, including the analogue linear alcohol 1-dodecanol. This provides an opportunity for read-across to fill the data gap. None of the aliphatic alcohols tested demonstrated the potential to induce local skin tumours upon repeated dermal application at or above the maximum tolerated (irritant) dose (Sicé, 1966; Bingham & Falk, 1969; Van Duuren & Goldschmidt, 1976, as cited in OECD, 2006). Furthermore, no induction of tumours was observed when members of the iso-alcohols C10–C12 category were repeatedly injected into the peritoneal cavity or implanted in the bladder of mice (Bryan & Springberg, 1966; Stoner et al., 1973, both as cited in OECD, 2006).

Based on the lack of genotoxic effects in vitro and on the absence of any structural alerts, members of the iso-alcohols C9–C13 category are unlikely to possess genotoxic carcinogenic potential.

3.2.4 Genotoxicity

Bacterial mutagenicity studies of individual chemicals and mixtures of iso-alcohols in the C9–C13 category showed a consistent lack of mutagenic activity. Negative results, with or without metabolic activation, have been reported for *Salmonella* Typhimurium strains TA98 and TA100 at 4–5000 µg/plate and *Salmonella* Typhimurium strains TA1535 and TA1537 at 0.8–2500 µg/plate (Api et al., 2014).

No specific mutagenicity studies have been identified for isodecyl alcohol, but its clastogenic potential was assessed using the in vitro mammalian chromosome aberration test in Chinese hamster ovary (CHO) cells. No significant increases in structural chromosomal aberrations were observed at concentrations up to 160 mg/mL with and without metabolic activation (Api et al., 2017).

Both isononyl alcohol and isooctyl alcohol were negative in the Ames test with and without metabolic activation (ECHA, 1986 and OECD, 2006, respectively).

3.2.5 Reproductive and developmental toxicology

In a comparative developmental toxicity study, isodecyl alcohol and two types of isononyl alcohol representing different isomer mixtures resulting from different production routes, were administered by gavage daily to Wistar rats from gestation day 6 to day 15 (Hellwig & Jäckh, 1997). Isodecyl alcohol was administered by gavage at doses of 0, 158, 790 and 1580 mg/kg bw; type 1 isononyl alcohol (isomers with a medium degree of branching including approximately 16% isodecanol) was administered at doses of 0, 144, 720 and 1440 mg/kg bw; and type 2 isononyl alcohol (isomers with a low degree of branching) at 0, 130, 650 and 975 mg/kg bw.

In rats given isodecyl alcohol, maternal toxicity was observed at 790 and 1580 mg/kg bw per day, with mortality (4/10) observed in animals in the highest dose group. Significantly reduced fetal weights, compared to controls, were observed in the highest dose group, together with a low incidence of retardations and rare malformations. Based upon these observations, NOAELs of 158 mg/kg bw and 790 mg/kg bw were identified for maternal and fetal effects, respectively.

In animals treated with type 1 isononyl alcohol, maternal toxicity was observed at 720 and 1440 mg/kg bw, with mortality (10/10) observed in animals in the highest dose group. A follow-up study reported that a dose of 1080 mg/kg bw resulted in maternal toxicity, but no mortality. An increased incidence of fetal retardations and rare malformations was reported at the 720 and 1080 mg/kg bw doses. Based upon these observations, the NOAEL for the isononyl alcohol type 1 was determined to be 144 mg/kg bw per day for both maternal and fetal toxicity.

In rats treated with type 2 isononyl alcohol, maternal toxicity was observed at 650 and 975 mg/kg bw, with mortality (3/10) observed in animals in the highest dose group. Significantly reduced fetal weights, compared to controls, were observed in the highest dose group, together with a low incidence of retardations and rare malformations. Based upon these observations, the NOAEL for the isononyl alcohol type 2 was determined to be 130 mg/kg bw and 650 mg/kg bw for maternal and fetal effects, respectively.

3.2.6 Irritation and sensitization

For isodecyl alcohol, the Union Carbide Data Sheet (UCDS, 1968) states that eye irritancy was determined as mild using the Draize test, conducted in accordance with OECD TG405 (OECD, 2020).

Dermal irritation studies in rabbits found that isononyl alcohol was irritating using the Draize test conducted in accordance with OECD TG405 (OECD, 2020). Isononyl alcohol (0.5 mL), was applied to the shaved skin of three rabbits under semi-occluded dressings for 4 hours. Dermal reactions were assessed using the Draize method 1, 24, 48 and 72 hours after removal of the dressings, and on days 6, 8, 10 and 14. The mean score for erythema at 24, 48 and 72 hours was 2.67 for each animal, and for oedema 2.67 for two animals and 3.0 for the third. The skin was dry, cracked and hardened, with scab formation, sloughing and new skin, in the three animals during days 8 to 14 (ECHA, 1986). Eye irritation studies in rabbits determined isononyl alcohol as highly irritating through the Draize test, conducted in accordance with OECD TG405 (OECD, 2020). Isononyl alcohol (0.1 mL) was instilled into the right eye of three rabbits. Responses to treatment were assessed using the Draize method 1, 24, 48 and 72 hours after instillation and again on days 6, 8, 10, 14, 17 and 21. Easily discernible areas of corneal opacity, reddening of the iris and diffuse beefy-red and slight or obvious swelling of the conjunctivae were observed in all animals. Corneal opacity and reddening of the iris persisted in one animal on day 21 (ECHA, 1986).

For isooctyl alcohol, Scala & Burtis (1973) reported that eye irritancy was severe at a dose of 100 mg as determined through the Draize test, conducted in accordance with OECD TG405 (OECD, 2020).

For isononyl alcohol, skin sensitization has been assessed through the Buehler skin sensitization assay. Twenty female guinea-pigs received three occluded dermal induction applications and one similar challenge application of isononyl alcohol. Challenge with undiluted isononyl alcohol did not cause any skin reactions 24 and 48 hours after removal of the patches (ECHA, 2008).

No reports of allergenicity upon oral exposure to isodecyl alcohol, isononyl alcohol or isooctyl alcohol were identified that would indicate that they are, or they contain, a known food allergen.

3.3 Observations in humans

No reports on the toxicity of isodecyl alcohol, isononyl alcohol or isooctyl alcohol in humans were identified. No contaminants of concern have been noted.

4. Levels and patterns of contamination in food commodities

The 2006 criteria document (FAO/WHO, 2007) assumed a worst-case concentration of 100 mg/kg for previous cargo contaminants. This concentration

was also supposed to be a worst-case concentration of the compounds of interest for the current assessment.

Isodecyl alcohol is an ingredient used in cosmetics, fragrances, shampoos and toiletries, and products such as household cleaners and detergents (McGinty et al., 2010). No data were identified on concentrations of isodecanol in foods from these uses or isodecanol exposure from non-dietary routes.

Isooctyl alcohol has no food uses but is listed as an indirect additive used in food contact substances without limitations by the FDA (US FDA, 2019). No data were identified on concentrations in food from these uses.

No data were found on isononyl alcohol uses in food or consumer goods.

5. Food consumption and dietary exposure assessment

Information on consumption of food oils by infants and young children was available from the US EPA FCID (US EPA, 2020) which, in turn, is based on data from the US National Health and Nutrition Survey/What We Eat In America, 2005–2010 cycles. The highest oil consumptions for infants and young children based on FCID are comparable to those in the CIFOCOss database; however, oil consumption information based on FCID is available based on individual body weights.

The highest reported consumption of a specific fat or oil type was for palm oil. Estimated mean and P95 consumptions by infants and young children were 7.6 and 19 g per day, respectively. Estimated mean and P95 consumption on a body weight basis were 1 g/kg bw per day and 3 g/kg bw per day, respectively. These data were used to define a worst-case dietary exposure estimate for infants and young children.

Estimated chronic daily dietary exposures to the compounds of interest present in food oils are 0.1 mg/kg bw per day (mean) and 0.3 mg/kg bw per day (high consumers), assuming a worst-case isodecanol concentration of 100 mg/kg, mean oil consumption of 1 g/kg bw per day, and high consumer oil consumption of 3 g/kg bw per day.

No other data on dietary exposure to the compounds of interest were identified.

6. Comments

6.1 Chemical and technical considerations

Chemical and technical considerations for isodecyl alcohol, isononyl alcohol and isooctyl alcohol are summarized in Tables 10, 11 and 12.

6.2 Biochemical aspects

No studies have been identified for isodecyl alcohol, isooctyl alcohol and isononyl alcohol with respect to absorption, distribution, biotransformation and excretion or formal pharmacokinetic analysis.

Iso-alcohols all show similar patterns of absorption, distribution, excretion and biotransformation. They are readily absorbed from the gastrointestinal tract and are rapidly cleared from the plasma due to extensive distribution around the body. They are initially converted to the respective carboxylic acid, which is subsequently metabolized to carbon dioxide via mitochondrial ß-oxidation pathways and the tricarboxylic acid cycle, in the same way as dietary fatty acids (OECD, 2006c).

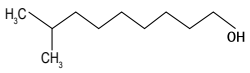
6.3 Toxicological studies

There are limited references in the literature regarding the toxicological assessment of isodecyl alcohol, isooctyl alcohol and isononyl alcohol. In many cases, experiments were undertaken with mixed isomers and/or combinations of iso-alcohols (C9–13). In addition, as might be expected for chemicals used in industrial processes, many of the citations are from unpublished reports, and access to the original data is often limited. As such, caution is needed in interpreting data to refer to a single pure chemical.

For **isodecyl alcohol**, an acute oral LD_{50} value of 6500 mg/kg bw has been reported for rat (Nishimura et al., 1994). For **isononyl alcohol**, an acute oral LD_{50} value of 2980 mg/kg bw has been reported in rat (Scala & Burtis, 1973). For **isooctyl alcohol**, acute oral LD_{50} values of 1670 mg/kg bw and 1480 mg/kg bw have been reported for mouse and rat, respectively (Scala & Burtis, 1973; Izmerov, Sanotsky & Sidorov, 1982).

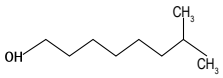
Rhodes et al. (1984) administered groups of five male Alderley Park Wistar-derived rats isodecyl alcohol, isononyl alcohol and isooctyl alcohol at a dose of 1 mmol/kg bw per day for 14 days, equivalent to approximately 158, 130 and 144 mg/kg bw per day, respectively. The Committee noted limitations in the

Table 10
Chemical and technical considerations for isodecyl alcohol

Name: isodecyl alcohol (isodecanol)	
CAS number 25339-17-7	**Alternative CAS numbers** 68551-08-6 (alcohols C9–11-branched), CAS number 68526-85-2 (alcohols C9–11-iso-, C10-rich)
Chemical details	Isodecyl alcohol, isodecanol Colourless, slightly viscous liquid  Molar mass: 158.28 g/mol Melting point: −2.8 °C Boiling point: 220 °C Insoluble in water; soluble in ethanol, ether, mineral oil, propylene glycol and most fixed oils
Route(s) of synthesis	Manufactured by two different mechanisms: by the oxo process in which nonenes are reacted with carbon monoxide and hydrogen using a cobalt catalyst, followed by hydrogenation; or by polymerization of propylenes and butenes with phosphoric acid to yield a mixture of branched olefins, which give isodecyl alcohol upon alkaline hydrolysis.
Composition	Occurs as a mixture of isomeric C10 alcohols and typically contains by-products of the propylene-butylene polymerization such as isooctyl and isononyl alcohols. May also contain small amounts of trimethyl-1-heptanols and dimethyl-1-octanols depending on the olefin feedstock used.
Uses	Used in the manufacture of plasticizers (about 70%), lubricants, surfactants and solvents; as an antifoaming agent in textile processing and as a flavouring agent.
Analytical methods	None found for previous cargoes. Possible means of analysis in fats and oils may require saponification, extraction, and derivatization followed by GC-FID or GC-MS.
Potential reaction(s) with a subsequent cargo of fat or oil	Transesterification with glycerides or esterification with free fatty acids present in the cargo may occur but are likely to be slow at ambient temperature.

GC-FID, gas chromatography with flame ionization detection; GC-MS, gas chromatography–mass spectrometry.

Table 11
Chemical and technical considerations for isononyl alcohol

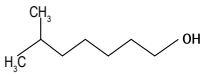
Name: isononyl alcohol (isononanol)	
	Alternative CAS numbers
CAS number 27458-94-2	2430-22-0 (7-methyl-1-octonol); 68526-84-1 (alcohols, C8–10-iso-, C9-rich); 3452-97-9 (3,5,5-trimethylhexanol)
Chemical details	Isononyl alcohol, isononanol Colourless liquid  Molar mass: 144.25 g/mol Melting point: −64.5 °C Boiling point: 206 °C Insoluble in water; soluble in alcohol

Route(s) of synthesis	Manufactured by the oxo process in which olefins are reacted with carbon monoxide and hydrogen in the presence of a catalyst, followed by hydrogenation.
Composition	Produced as a mixture of isomeric primary C9 alcohols, consisting mainly of 3,5,5-trimethyl-1-hexanol, isomeric dimethyl-1-heptanols and isomeric methyl-1-octanols (e.g. 7-methyloctan-1-ol). It can be up to 99% pure. Isononyl alcohol is characterized as a primary alcohol and is a mixture of isomers of different branched structures with both odd- and even-numbered carbon chains ranging from C8 to C10, predominantly C9. The exact composition depends on the starting materials, the manufacturing process used, the reaction conditions and the purification method.
Uses	Used as an ingredient in plasticizers, stabilizers and lubricants.
Analytical methods	None found for previous cargoes. Possible means of analysis in fats and oils may require saponification, extraction and derivatization followed by GC-FID or GC-MS.
Potential reaction(s) with a subsequent cargo of fat or oil	Transesterification with glycerides or esterification with free fatty acids present in the cargo may occur but are likely to be slow at ambient temperature.

GC-FID, gas chromatography with flame ionization detection; GC-MS, gas chromatography–mass spectrometry.

Table 12

Chemical and technical considerations for isooctyl alcohol

Name: isooctyl alcohol (isooctanol)	
	Alternative CAS numbers
CAS number 26952-21-6	1653-40-3-9 (6-methyl-1-heptanol); 68526-83-0 (alcohols C7–9-iso-, C8-rich); 68551-09-7 (alcohols C7–9-branched); 104-76-7 (2-ethylhexanol)
Chemical details	Isooctyl alcohol, isooctanol Clear colourless liquid  Molar mass: 130.23 g/mol Melting point: −106 °C Boiling point: 188 °C Insoluble in water; soluble in ether or alcohol
Route(s) of synthesis	Manufactured by the oxo process in which heptenes are reacted with carbon monoxide and hydrogen in the presence of a catalyst, followed by hydrogenation.
Composition	Occurs as a mixture of isomeric C8 alcohols and typically consists of methyl-1-heptanols and/or dimethyl-1-hexanols. The composition depends on the olefin feedstock.
Uses	Used as a solvent; in the manufacture of cutting and lubricating oils and hydraulic fluids; in the production of other chemicals; as an ingredient in plasticizers; an intermediate in nonionic detergent and surfactant production; in synthetic drying oils and resin solvents; and in emulsifiers and antifoaming agents.
Analytical methods	None found for previous cargoes. Possible means of analysis in fats and oils may require saponification, extraction, and derivatization followed by GC-FID or GC-MS.
Potential reaction(s) with a subsequent cargo of fat or oil	Transesterification with glycerides or esterification with free fatty acids present in the cargo may occur but are likely to be slow at ambient temperature.

GC-FID, gas chromatography with flame ionization detection; GC-MS, gas chromatography–mass spectrometry.

study design, including a small group size, single species, single sex, single dose level and limited duration. No changes were observed in body, liver or testicular weight relative to controls. No major pathological features of hepatotoxicity were observed. Mild histological changes in the liver were noted with all three chemicals, including slight centrilobular hypertrophy, slight/moderate glycogen vacuolation and slight/moderate centrilobular lipid vacuolation (Rhodes et al., 1984). The Committee did not consider these changes to be adverse.

In a comparative developmental toxicity study with Wistar rats, **isodecyl alcohol** was administered by gavage at doses of 0, 158, 790 and 1580 mg/kg bw per day during gestation days 6 to 15 (Hellwig & Jäckh, 1997). Maternal toxicity was observed at 790 and 1580 mg/kg bw per day, with mortality (4/10) observed at the highest dose. Significantly reduced fetal weights were observed in the highest dose group, alongside a low incidence of retardations and rare malformations (Hellwig & Jäckh, 1997). The Committee concluded that NOAELs of 158 mg/kg bw and 790 mg/kg bw could be identified for maternal and fetal effects, respectively.

In the same comparative developmental toxicity study with Wistar rats, **isononyl alcohol** was administered by gavage to rats during gestation days 6 to 15 (Hellwig & Jäckh, 1997). Type 1 isononyl alcohol (isomers with a medium degree of branching including approximately 16% isodecyl alcohol) was administered at doses of 0, 144, 720 and 1440 mg/kg bw per day, and type 2 isononyl alcohol (isomers with a low degree of branching) at 0, 130, 650 and 975 mg/kg bw per day.

For type 1 isononyl alcohol, maternal toxicity was observed at 720 and 1440 mg/kg bw per day, with mortality (10/10) observed in the highest dose group. An additional dose group, 1080 mg/kg bw per day, showed maternal toxicity, but no mortality. An increased incidence of fetal retardations and rare malformations was reported for the 720 and 1080 mg/kg bw per day dose groups (Hellwig & Jäckh, 1997). The Committee identified a NOAEL of 144 mg/kg bw per day for both maternal and fetal toxicity from isononyl alcohol type 1.

For type 2 isononyl alcohol, maternal toxicity was observed at 650 and 975 mg/kg bw per day, with mortality (3/10) observed in the highest dose group. Significantly reduced fetal weights, compared to controls, were observed in the highest dose group, alongside a low incidence of retardations and rare malformations (Hellwig & Jäckh, 1997). The Committee concluded that the NOAELs were 130 mg/kg bw per day and 650 mg/kg bw per day for maternal and fetal effects, respectively, for isononyl alcohol type 2.

No studies on reproductive or developmental toxicity have been identified for **isooctyl alcohol**.

Bacterial mutagenicity studies for individual chemicals and mixtures of iso-alcohols in the C9–C13 category showed a consistent lack of mutagenic

activity. Negative results, with or without metabolic activation, have been reported for *Salmonella* Typhimurium strains TA98, TA100, TA1535 and TA1537 (Api et al., 2014). **Isononyl alcohol** and **isooctyl alcohol** were negative in the Ames test, with and without metabolic activation, cited in OECD (2006c), and ECHA, 1986.[1] Mutagenic activity for **isodecyl alcohol** has not been reported. Clastogenic activity was not observed in the in vitro mammalian chromosome aberration test in CHO cells with and without metabolic activation (API et al., 2017).

No carcinogenicity studies have been identified for **isodecyl alcohol**, **isononyl alcohol** or **isooctyl alcohol**.

6.4 Allergenicity

The Committee did not identify any reports of allergenicity upon oral exposure to **isodecyl alcohol**, **isononyl alcohol** or **isooctyl alcohol** that would indicate that they are, or they contain, a known food allergen.

6.5 Food consumption and dietary exposure estimates

A worst-case concentration of 100 mg/kg has been assumed for all previous cargo chemical substances (see Part A, section 4.3).

Isodecyl alcohol and **isononyl alcohol** have no food uses. **Isooctyl alcohol** has no direct food uses but is listed as an indirect additive used in food contact substances without limitations by the FDA. No data were identified on concentrations in foods from these uses.

Worst-case human dietary exposures to previous cargo chemical substances in food oils have been estimated at 0.3 mg/kg bw per day (see Part A, section 4.3).

No other data on dietary exposure to isodecyl alcohol, isononyl alcohol and isooctyl alcohol were identified.

7. Evaluation

The Committee noted the limitations of the current dataset of toxicological evaluations, and the need to use a read-across approach where appropriate.

The Committee also noted the negative data for mutagenic activity for isooctyl alcohol and isononyl alcohol, lack of clastogenic activity of isodecyl

[1] https://echa.europa.eu/registration-dossier/-/registered-dossier/13404/7/3/2, accessed 14 November 2020.

alcohol, and the weight of evidence across long chain fatty alcohols for a lack of mutagenic potential. The Committee considered that isodecyl alcohol, isononyl alcohol and isooctyl alcohol can be considered non-genotoxic. The Committee noted that no carcinogenicity studies have been identified for isodecyl alcohol, isononyl alcohol and isooctyl alcohol. Based upon the weight of evidence across several aliphatic alcohols, including the linear alcohol 1-dodecanol, the Committee concluded that isodecyl alcohol, isononyl alcohol and isooctyl alcohol are unlikely to possess carcinogenic potential.

For **isodecyl alcohol,** the Committee concluded that a NOAEL of 158 mg/kg bw per day for maternal toxicity from a comparative developmental toxicity study on rats (Hellwig & Jäckh, 1997) was a suitable RP. Considering the estimated dietary exposure of 0.3 mg/kg bw per day, the MOE is approximately 520, which the Committee concluded is sufficient to address the uncertainties in the database.

For **isononyl alcohol,** the Committee considered a NOAEL of 158 mg/kg bw per day for maternal toxicity from a comparative developmental toxicity study on rats (Hellwig & Jäckh, 1997) was a suitable RP. Considering the estimated dietary exposure of 0.3 mg/kg bw per day, the MOE is approximately 520, which the Committee concluded is sufficient to address the uncertainties in the database.

For **isooctyl alcohol**, no studies on reproductive or developmental toxicity were identified. Using read-across from isodecyl alcohol and isononyl alcohol, the Committee concluded that it is highly unlikely that isooctyl alcohol possesses significant reproductive or developmental toxicity. The Committee considered that the dose of 130 mg/kg bw per day, which resulted in mild histopathological changes in the liver following a 14-day oral gavage exposure in rats, was a suitable RP (Rhodes et al., 1984). The Committee noted limitations in the study design but concluded that it could be used to establish a MOE in the absence of longer-term studies. Considering the estimated dietary exposure of 0.3 mg/kg bw per day, the MOE is approximately 430, which the Committee concluded is sufficient to address the uncertainties in the database.

There are no reports of allergenicity upon oral exposure to isodecyl alcohol, isononyl alcohol and isooctyl alcohol that would indicate that they are or contain a known food allergen.

Isodecyl alcohol, isononyl alcohol and isooctyl alcohol may react with a previous cargo in transesterification reactions with glycerides or esterification reactions with free fatty acids present, but the rates of reaction are likely to be slow at ambient temperature and any products would be naturally occurring waxes.

Therefore, the Committee concluded that isooctyl alcohol, isononyl alcohol and isodecyl alcohol meet the criteria for acceptability as previous cargoes.

I.III 1,3-Propanediol (1,3-propylene glycol)

1. Explanation

In 1996, SCF concluded that 1,3-propanediol was not acceptable as a previous cargo owing to inadequate toxicological data (SCF, 1997). In 1998, SCF considered 1,3-propanediol for use as a co-monomer in polyesters and, based on new mutagenicity and developmental toxicity studies (unpublished data), it was classified as acceptable (SCF, 1998).

Based on a subchronic toxicity study showing low oral toxicity, the SCF considered 1,3-propanediol acceptable as a previous cargo, providing that residues would be low after tank cleaning (SCF, 2003).

In 2012, the EFSA CONTAM Panel concluded that 1,3-propanediol was acceptable as a previous cargo (EFSA, 2012).

For the current review, previous assessments by SCF, EFSA and ECHA were considered. A search by CAS number and name synonyms for additional toxicological studies in animals and humans was undertaken to identify any critical new data for the assessment of human health risk. Searches were conducted on PubMed, PubChem and Embase.

A literature search was conducted of PubMed and Embase with the synonyms 1,3-propanediol, 1,3-propylene glycol and trimethylene glycol versus toxicity, NOAEL/NOEL, genotoxicity, carcinogenesis, allergenicity, sensitization, absorption, metabolism, distribution and excretion. Three hundred and forty-nine papers were identified. For all papers, the abstracts were read, and six publications were selected for full analysis. Other sources consulted included PubChem, IUCLID Dataset, EFSA documents, ECHA and the EPA High Production Volume Information System (HPVIS). The cut-off date for this search was 25 October 2020.

2. Chemical and technical considerations

The physical and chemical characteristics of 1,3-propanediol are summarized in Table 13.

Table 13
Some physical and chemical characteristics for 1,3-propanediol

Substance	CAS number	Additional CAS numbers
Diols		
1,3-Propanediol (1,3-propylene glycol; 1,3-PD; propane-1,3-diol)	504-63-2	None
		
Molar mass: 76.09 g/mol Melting point: −27 °C Boiling point: 214.4 °C Miscible with water and ethanol		

2.1 Manufacture and use

1,3-propanediol is a linear aliphatic diol produced synthetically from acrolein (Ac) or ethylene oxide (EO). In the 1990s, three production processes were developed using different feedstocks: Ac or EO using classical oleochemical processes, or from glucose/sucrose by fermentation (Haas, Wiegand & Arntz, 1994). The latter approach is currently the primary route of 1,3-propanediol production (Biddy, 2016).

1,3-Propanediol is a chemical building block in the manufacture of a variety of polymers including composites, adhesives, laminates, powder coatings, UV-cured coatings, mouldings, novel aliphatic polyesters and copolyesters, as well as solvents and lubricants (Zeng & Sabra, 2011; Vilela et al., 2014). A major driver for 1,3-propylene glycol production is as a component in polytrimethylene terephthalate (PTT) polymers used in textiles and fibres (Abraham et al., 2012). 1,3-Propanediol is also a component of cosmetics and personal care items. Increasing use of 1,3-propanediol in various applications such as PTT, polyurethane (PU), cosmetics, personal care and cleaning products is expected to drive market growth.

2.2 Composition and secondary contaminants

The Ac-based process for 1,3-propanediol production involves the hydration of Ac to form 3-hydroxypropionaldehyde (HPA) and subsequent hydrogenation to 1,3-propanediol. After the first step, the Ac-hydration product contains by-products such as 4-oxaheptanedial. The selectivity of the hydrogenation of HPA is close to 100% under optimal reaction conditions although the final product may contain up to 500 mg/kg residual carbonyl compounds. These include saturated

aliphatic carbonyl compounds such as propionic aldehyde and acetylated products such as 2-(2′-hydroxyethyl)-1,3-dioxane (Haas et al., 1994). One EO process also leads to HPA production using hydroformulation catalysts. Single-step EO processes use mixed catalyst systems of rhodium, ruthenium, phosphines and various acids as promoter (Murphy, Aguilo & Smith, 1987; Beavers, 1991) with a yield of 85–90% based on EO and the by-products including ethanol, acetaldehyde, propanol and propionaldehyde (Murphy, Smith & Aguilo, 1990).

The conversion of glucose to 1,3-propanediol by fermentation yields an aqueous fermentation broth containing residual medium components and by-products, including lactate and 2,3-butanediol. Fermentation gives rise to more product variability than chemical synthesis from petroleum feedstocks; however, raising the pH is sufficient to prevent by-product formation during water evaporation and distillation yields 1,3-propanediol of 95% purity (Ames, 2002). Impurities in 1,3-propanediol from glycerol fermentation include acetate, acetic acid, butyric acid, lactic acid, formic acid, ethanol, 2,3-butanediol, succinic acid, lactate and butanol (Lee et al., 2015).

2.3 Reactivity and reactions with fats and oils

Diols exist in a mixture of conformations with several possibilities for intramolecular and intermolecular interactions between the two hydroxyl groups depending on the temperature and the physical state. The extent of intermolecular interaction is dependent on the concentration of the compound, whereas intramolecular interaction is concentration-independent. 1,3-Propanediol is a very stable liquid at room temperature, so it is unlikely to polymerize or participate in hydrogenation or dehydrogenation reactions without the presence of a catalyst or microorganism.

2.4 Methods of analysis

Numerous chromatographic methods have been applied for the determination of 1,3-propanediol in biological specimens, particularly gas chromatography-flame ionization detection (GC-FID), GC-MS and LC-MS/MS (Cheung & Lin, 1987; Jonsson et al., 1989; Edinboro et al., 1993; Dasgupta et al., 1995; Williams et al., 2000; Maurer et al., 2001; Gembus et al., 2002; Chen, Fang & Hu, 2007; Garg et al., 2008; Imbert, Elodie & Christian, 2014). Most methods require extraction steps to both improve recovery and reduce background interference (Chatzifragkou et al., 2010; Hoa et al., 2014; Orton et al., 2016). Although no records could be found, it is likely that the determination of residual 1,3-propanediol in fats and

oils requires extraction and clean-up steps prior to analysis by GC-MS/MS or LC-MS/MS.

3. Biological data

3.1 Biochemical aspects

No studies on absorption and distribution of 1,3-propanediol in humans or animals were identified in the peer-reviewed literature searched.

One dermal absorption study (unpublished) was recorded on the ECHA information card. The experiment was performed using human abdominal skin from cadavers (epidermis peeled from dermis) mounted in an in vitro static diffusion chamber, and samples from the receptor chamber were collected during 48 hours. The permeability coefficient was calculated to be 1.50 × 10e-5 cm/hour. At the end of the 48-hour exposure period, 0.12% of the applied chemical was detected in the receptor chamber.[1]

1,3-Propanediol can be oxidized by human liver alcohol dehydrogenase (Blair & Vallee, 1966).

Homogenates of liver, but not testis, of rats that received a diet containing 500 ppm of 1,3-propanediol for 15 weeks reacted with thiobarbituric acid (TBA), suggesting that liver is able to metabolize 1,3-propanediol to malondialdehyde (MDA) (Summerfield & Tappel, 1984). MDA detection by TBA reaction has been shown not to be specific to MDA (Moselhy et al., 2013). According to Gingell, Kirkpatrick & Steup (2000), MDA, if formed from 1,3-propanediol metabolism, would be expected to be a short-lived metabolic intermediate, with further metabolism to 3-hydroxypropionic acid and malonic acid (Gingell, Kirkpatrick & Steup, 2000).

Rabbits were administered 4 g/kg bw of 1,3-propanediol by gavage and urine was collected for 3 days after dosing. Neither the unchanged compound nor malonic acid was detected in the urine, indicating that 1,3-propanediol does not undergo glucuronic acid conjugation and that it is completely oxidized in the body (Gessner, Parke & Williams, 1960).

[1] https://echa.europa.eu/pt/registration-dossier/-/registered-dossier/2099/7/2/3.

3.2 Toxicological studies

3.2.1 Acute toxicity

The lowest oral dose of 1,3-propanediol to induce death in rats was determined as 10 mL/kg bw (approximately 10 000 mg/kg bw), with depression as the systemic sign of toxicity (Van Winkle, 1941).

The acute dermal LD_{50} of 1,3-propanediol was determined to be >4 mL/kg bw, equivalent to >4200 mg/kg bw (EPA, 2007).

3.2.2 Short-term studies of toxicity

Rats (10 males and females per group) were given 1,3-propanediol by gavage at doses of 0, 100, 300 or 1000 mg/kg bw per day for 13 weeks. Clinical observation and survival, food intake and body weight, clinical pathology parameters (haematological and serum chemistry) and pathology (organ weight, macro- and microscopic examination) were recorded after 4 and 13 weeks of treatment. Spermatogenic end-points (testis weight, testicular sperm number, epididymal sperm number, sperm production rate and motile sperm) were recorded at the end of the treatment period (Gingell, Kirkpatrick & Steup, 2000). No mortality or clinical signs related to the treatment were reported. No treatment-related changes were observed in serum chemistry parameters (albumin, total protein, globulin, total bilirubin, urea nitrogen, creatinine, alkaline phosphatase, alanine transferase, aspartate transferase, glutamyl transferase and cholesterol) in any of the groups after 13 weeks of treatment. The only exception was non-dose-related decreases of 33% in total bilirubin in the females in the groups treated with 100 and 1000 mg/kg bw per day. Comparison to the control values at both 4 and 13 weeks showed a slight decrease, in females only, in aspartate aminotransferase at doses of 300 and 1000 mg/kg bw per day, in mean cholesterol at a dose of 100 mg/kg bw per day and in mean chloride at a dose of 1000 mg/kg bw per day. Glucose was increased in animals in all dose groups. Mean white blood cell counts in all treated males were decreased at week 4 (22%, 26% and 20% at doses of 100, 300 and 1000 mg/kg bw per day, respectively), but not at week 13, and no dose–response relationship was found. The authors attributed this decrease to a high control group value. The mean absolute lymphocyte value for females in the high-dose group was increased at week 13 but was similar to the week 4 value for this group (results not shown). This increase was not observed in males. The authors concluded that no relationship with treatment was evident.

Rats exposed to 0, 41, 650 or 1800 mg/m^3 of 1,3-propanediol as a vapour or vapour/aerosol mixture for 6 hours per day, 5 days per week for 2 weeks (9 exposures) did not show clinical signs of toxicity during the treatment. No compound-related alterations in clinical pathology (blood cell counts, serum chemical parameters and urinalysis) or tissue pathology (gross pathology, organ

weights and histopathology) were observed. The authors identified a NOEL of 1800 mg/m^3 (the highest concentration that could practically be generated) from this study (Scott et al., 2005).

3.2.3 Long-term studies of toxicity and carcinogenicity

No long-term or carcinogenicity studies were identified for 1,3-propanediol.

3.2.4 Genotoxicity

1,3-Propanediol was tested in the bacterial reverse mutation assay with *Salmonella* Typhimurium TA1535, TA1537, TA98, TA100 and TA102 strains at concentrations up to 5000 µg/plate with and without metabolic activation in a plate incorporation assay followed by a pre-incubation assay. 1,3-Propanediol was not mutagenic under the conditions of the assay and it did not induce forward mutations at the HPGRT locus in cultured mammalian Chinese hamster V79 cells in vitro. In an in vitro chromosomal aberration assay in Chinese hamster V79 cells in vitro, 1,3-propanediol was negative with metabolic activation but positive without metabolic activation. In a second similar study, 1,3-propanediol was negative for chromosomal aberration, both with and without metabolic activation. When tested in vivo in a mouse bone marrow micronucleus test with oral doses of 1000, 1470 and 2150 mg/kg bw, 1,3-propanediol was considered negative (SCF, 2003; EPA, 2007).

3.2.5 Reproductive and developmental toxicology

One developmental toxicity study with 1,3-propanediol was conducted by Mitterer (1992) and reported by EPA HPVIS (EPA, 2007) and ECHA.[1] Female rats (25 per group) were dosed by oral gavage from gestation day 6 until gestation day 15 with 0, 250 or 1000 mg/kg bw per day of 1,3-propanediol. No effects on the dams were observed apart from a non-significant reduction in mean maternal weight gain (32%) on days 6–9 of gestation (compared to gestation days 0–3) at the highest dose. The number of fetuses with retarded ossification was significantly increased at a dose of 1000 mg/kg bw per day (11%) compared to the concurrent control group. The incidence of incomplete ossification of the skull in fetuses was significantly increased in both treatment groups as compared to the control animals. However, according to the report, the significance observed was due to the low incidence of retarded skull ossification found in the control group in this study, which was much below the background incidence. Therefore, the increase in the incidence of this retardation was not considered treatment-related.

[1] https://echa.europa.eu/registration-dossier/-/registered-dossier/2099/7/9/3

The NOAEL for maternal and fetal toxicity was recorded on the ECHA site as 1000 mg/kg bw based on the lack of clear treatment-related effects in any parameter.[1] However, SCF identified a LOAEL of 250 mg/kg bw per day for 1,3-propanediol considering the fetal effects (retarded ossification) in this study (SCF, 2003).

3.2.6 Irritation and sensitization

1,3-Propanediol was reported as mildly irritating (Coombs & Clark, 1977) and slightly irritating to rabbits' skin (Van Beek, 1979).

1,3-Propanediol was reported as practically non-irritating (Coombs & Clark, 1977) and non-irritating (van Beek, 1979) to rabbits' eyes.

1,3-Propanediol was reported not to be a skin sensitizer (Coombs & Clark, 1977; Til & Keizer, 1979).

3.3 Observations in humans

No reports on toxicity of 1,3-propanediol in humans were identified.

4. Levels and patterns of contamination in food commodities

The 2006 criteria document (FAO/WHO, 2007) assumed a worst-case concentration of 100 mg/kg for previous cargo contaminants. This concentration was also supposed to be a worst-case concentration of 1,3-propanediol.

1,3-Propanediol has no registered food uses but has "generally recognized as safe" (GRAS) status in the USA and can be used in place of 1,2-propanediol at levels that do not exceed current good manufacturing practice (GMP) limits: maximum levels of 5% for alcoholic beverages, 24% for confections and frostings, 2.5% for frozen dairy products, 97% for seasonings and flavourings, 5% for nuts and nut products and 2% for all other food categories (excluding infant formula, fish, meat and poultry) (US FDA, 2020).

[1] https://echa.europa.eu/pt/registration-dossier/-/registered-dossier/2099/7/9/3

5. Food consumption and dietary exposure estimates

Information on consumption of food oils by infants and young children was available from the US EPA's FCID (US EPA, 2020), which in turn is based on data from the US National Health and Nutrition Survey/What We Eat In America, 2005–2010 cycles. The highest oil consumptions for infants and young children based on FCID are comparable to those in the CIFOCOss database; however, oil consumption information based on FCID is available based on individual body weights.

The highest reported consumption of a specific fat or oil type was for palm oil. Estimated mean and P95 consumptions by infants and young children were 7.6 and 19 g per day, respectively. Estimated mean and P95 consumption on a body weight basis were 1 g/kg bw per day and 3 g/kg bw per day, respectively. These data were used to define a worst-case dietary exposure estimate for infants and young children.

Estimated chronic daily dietary exposures to 1,3-propanediol present in food oils are 0.1 mg/kg bw per day (mean) and 0.3 mg/kg bw per day (high consumers), assuming a worst-case isodecanol concentration of 100 mg/kg, mean oil consumption of 1 g/kg bw per day and high consumer oil consumption of 3 g/kg bw per day.

No data were identified on actual concentrations of 1,3-propanediol in food from other sources. However, given the high levels permitted in processed foods, dietary exposure to 1,3-propanediol from this source is potentially much greater than that due to its carryover into food oils from previous cargoes.

6. Comments

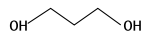
6.1 Chemical and technical considerations

Chemical and technical considerations for 1,3-propanediol are summarized in Table 14.

6.2 Biochemical aspects

No studies on absorption and distribution of 1,3-propanediol were identified. 1,3-propanediol can be metabolized by alcohol dehydrogenase. It is suggested that malondialdehyde can be formed as an intermediate metabolite that would be further metabolized to 3-hydroxypropionic and malonic acid and finally carbon dioxide (Gessner, Parke & Williams, 1960; Summerfield & Tappel, 1984; Gingell, Kirkpatrick & Steup, 2000).

Table 14
Chemical and technical considerations for 1,3-propanediol

Name: 1,3-propanediol (1,3-propylene glycol)	
CAS number	**Alternative CAS numbers**
504-63-2	None
Chemical details	1,3-propanediol, 1,3-propylene glycol, 1,3-PD Colourless to pale yellow very viscous liquid  Molar mass: 76.09 g/mol Melting point: −27 °C Boiling point: 214.4 °C Miscible with water and ethanol
Route(s) of synthesis	Manufactured by classical oleochemical processes from acrolein, ethylene oxide; or by fermentation.
Composition	The final product depends on the manufacturing process. From acrolein, the final product may contain up to 500 mg/kg residual carbonyl compounds including saturated aliphatic carbonyl compounds such as propionic aldehyde and acetylated products such as 2-(2′-hydroxyethyl)-1,3-dioxane. From ethylene oxide, by-products include ethanol, acetaldehyde, propanol and propionaldehyde. By-products in fermentation from glycerol include acetate, acetic acid, butyric acid, lactic acid, formic acid, ethanol, 2,3-butanediol, succinic acid, lactate and butanol.
Uses	Used in polymer applications such as composites, adhesives, laminates, powder and UV-cured coatings, mouldings, novel aliphatic polyesters, copolyesters, solvents and lubricants; as a component in polytrimethylene terephthalate polymers; as a component of cosmetics and personal care items
Analytical methods	None found for previous cargoes. Possible means of analysis in fats and oils may require extraction and clean-up steps prior to analysis by GC-MS/MS or LC-MS/MS.
Potential reaction(s) with a subsequent cargo of fat or oil	1,3-Propanediol is a very stable liquid at room temperature; unlikely to polymerize or participate in hydrogenation or dehydrogenation reactions without the presence of a catalyst or microorganism.

GC-MS/MS, gas chromatography with tandem mass spectrometry; LC-MS/MS, liquid chromatography with tandem mass spectrometry.

6.3 Toxicological studies

1,3-Propanediol has been reported to possess very low acute toxicity. The lowest oral dose to induce death in rats was determined by Van Winkle as 10 mL/kg bw (approximately 10 000 mg/kg bw per day) (Van Winkle, 1941).

In a 13-week study in male and female rats (10 animals of each sex per dose) 1,3-propanediol was administered by gavage at 1, 100, 300 and 1000 mg/kg bw per day. No treatment-related effects were observed (Gingell, Kirkpatrick & Steup, 2000).

In a developmental toxicity study, rats were treated with 0, 250 and 1000 mg/kg bw per day of 1,3-propanediol (Mitterer (1992) reported in EPA HPVIS (undated) and ECHA[1]). The available data indicated dose-dependent effects of

[1] https://echa.europa.eu/registration-dossier/-/registered-dossier/2099/7/9/3, accessed 14 November 2020.

1,3-propanediol on fetal skeletal retardations at the high dose and incomplete ossification of the skull at both doses. The Committee noted the limitations of the design (two dose groups only) and the incomplete data reporting (not accounting for the litter effects in the developmental study evaluation), which prohibited a robust dose–response assessment. Therefore, effects of 1,3-propanediol on the development of the rat fetus could not be discounted even at the low dose. No other alterations were observed in the mothers or fetuses. A NOAEL for maternal toxicity of 1000 mg/kg bw and a LOEL of 250 mg/kg bw per day for marginal fetal effects was determined.

1,3-Propanediol was not genotoxic in vitro or in vivo. No long-term exposure studies were identified for 1,3-propanediol.

6.4 Allergenicity

The Committee did not identify any reports of allergenicity upon oral exposure to 1,3-propanediol that would indicate that it is, or it contains a known food allergen.

6.5 Assessment of dietary exposure

A worst-case concentration of 100 mg/kg has been assumed for all previous cargo chemical substances (see Part A, section 4.3 Dietary exposure assessment for previous cargo chemical substances).

1,3-Propanediol has no registered food uses but can be used in place of 1,2-propanediol at levels not exceeding GMP (US FDA, 2020).

No data were identified on actual concentrations in food from these uses. However, given the high levels permitted for inclusion in processed foods, dietary exposure to 1,3-propanediol from this source is potentially much greater than that resulting from the carryover from previous cargoes into food oils. Worst-case human dietary exposures to previous cargo chemical substances in food oils have been estimated at 0.3 mg/kg bw per day (see Part A, section 4.3).

No other data on dietary exposure to 1,3-propanediol were identified.

7. Evaluation

1,3-Propanediol is not genotoxic.

The Committee considered that the LOEL of 250 mg/kg bw per day, based on marginal fetal effects observed in the study by Mitterer should be used

as the RP (Mitterer, 1992, cited in HPVIS, undated). Considering the estimated dietary exposure of 0.3 mg/kg bw per day, the MOE is 830, which is adequate to address the uncertainties in the databases.

There are no reports of allergenicity upon oral exposure to 1,3-propanediol that would indicate that it is or contains a known food allergen.

1,3 Propanediol is a very stable liquid at room temperature, and it is unlikely to polymerize or participate in hydrogenation or dehydrogenation reactions without the presence of a catalyst or microorganism.

Therefore, the Committee concluded that 1,3-propanediol meets the criteria for acceptability as a previous cargo.

I.IV 1,4-Butanediol (I,4-butylene glycol)

1. Explanation

SCF evaluated 1,4-butanediol (1,4-BD) in 1997 and considered it acceptable as a previous cargo, also noting that 1,4-BD is soluble in water and therefore easily cleaned from tanks (SCF, 1997). The CONTAM Panel of EFSA undertook an assessment of substances currently listed in the Annex to Commission Directive 96/3/EC as acceptable previous cargoes for edible fats and oils (SCF, 2003). They concluded that 1,4-BD is not of toxicological concern when used as a previous cargo.

SCF evaluated 1,4-BD as a substance intended for use in materials in contact with food (SCF, 1986). It concluded that 1,4-BD was a substance for which some toxicological data exist, but that an ADI or TDI could not be established. In 2001, the SCF re-evaluated 1,4-BD for this use, and again concluded that an ADI or TDI could not be established. It concluded that continued use as a substance intended for use in materials in contact with food could be accepted, and established a migration limit for 1,4-BD of 0.05 mg/kg of food. The Scientific Panel on food additives, flavourings, processing aids and materials in contact with food (AFC Panel) of EFSA re-evaluated 1,4-BD in 2004 for use in materials in contact with food. Based on additional toxicological data, a migration limit of 5 mg/kg of food was established (EFSA, 2004).

PubChem was used to identify common synonyms for 1,4-BD, namely: 110-63-4, 1,4-butanediol, 1,4-butylene glycol, tetramethylene glycol, 1,4-dihydroxybutane, 1,4-tetramethylene glycol and tetramethylene 1,4-diol.

These terms were used as the input for a Web of Science literature search (1900–2020), which was further refined with the search terms: toxic*, metaboli*, mutagen*, genotoxic*, carcinogen*, sensiti*, allerg* and ADI. The cut-off date for the search was 30 September 2020.

Other reference sources included PubChem, European Chemicals Bureau, IUCLID Dataset, ECHA and NIOSH.

2. Chemical and technical considerations

Some of the physical and chemical characteristics of 1,4-BD are summarized in Table 15.

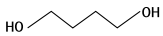
The first commercial synthesis of 1,4-BD was based on the Reppe process in which formaldehyde is reacted with acetylene, followed by hydrogenation to produce 1,4-BD. This process is still the most prevalent 1,4-BD production route worldwide (Sampat, 2011). A newer technology using butadiene and acetic acid, followed by hydrogenation and hydrolysis to form 1,4-BD was developed in the late 1970s. In the 1980s, a production process using catalytic isomerization of propylene oxide to form allyl alcohol and hydroformylation using catalysts such as rhodium was developed. During the 1990s, the maleic anhydride process was commercialized using the Davy (Kvaerner) process concept whereby maleic anhydride is converted to the ester which then undergoes hydrogenolysis to make a mixture of 1,4-BD, tetrahydrofuran and gamma-butyrolactone (GBL). GBL is further reduced to produce 1,4-BD (Sampat, 2011). Recently, additional processes have been investigated using a variety of renewable feedstocks, e.g. 1,4-BD by hydrogenation under high pressure from succinic acid from glycerol (Baidya et al., 2019).

1,4-BD is an important intermediate chemical for the manufacture of tetrahydrofuran (THF), polytetramethylene ether glycol, polybutylene terephthalate, GBL, PU and other solvents. These chemicals are widely used in fibres, engineering plastics, medicines, cosmetics, artificial leather, pesticides, plasticizers, hardeners, solvents and rust removers among others. THF production accounts for the largest proportion of the 1,4-BD market. Increasing applications of its derivatives in various industrial processes, together with the growing demand for spandex in textiles, have been responsible for driving growth in recent years.

2.1 Composition and secondary contaminants

During the production of 1,4-BD, by-products, residual reactants and various metallic catalysts are removed by purification steps including distillation or melt

Table 15
Some physical and chemical characteristics of 1,4-butanediol

Substance	CAS number	Additional CAS numbers
1,4-Butanediol (1,4-butylene glycol; 1,4-BD; butane-1,4-diol)  Molar mass: 90.12 g/mol Melting point: 20 °C Boiling point: 230 °C Soluble in water and ethanol	110-63-4	

crystallization. The purity of 1,4-BD is usually above 90%; product composition, according to the route of synthesis, is as follows:

- Reppe process: 1,4-BD > 99%, 2-methyl-1,4-butanediol < 0.4%, and 2-(4-hydroxy-butoxy)tetrahydrofuran < 0.2% (Pinkos et al., 2010);
- maleic anhydride process: 1,4-BD 90–95%, *n*-propanol and *n*-butanol 5–10% (Budge, Attig & Graham, 1991);
- propylene oxide process: 1,4-BD 86.4–91.0%, *n*-propanol and propionaldehyde 0.5–3.5%, the remainder is methyl propanediol (Dubner & Shum, 2001);
- butadiene-acetic acid process: 1,4-BD > 99%, 2-(4-hydroxybutoxy) tetrahydro-furan < 0.3% (Okuyama, 1999).

2.2 Reactivity and reactions with fats and oils

Diols exist in a mixture of conformations with several possibilities for intramolecular and intermolecular interactions between the two hydroxyl groups depending on the temperature and the physical state. The extent of intermolecular interaction is dependent on the concentration of the compound, whereas intramolecular interaction is concentration-independent. 1,4-BD was shown to have strong intramolecular hydrogen bonding in a comparative study of 1,2-ethanediol and 1,4-BD at different temperatures (Das, Das & Arunan, 2015). The possibility of intermolecular reaction between 1,4-BD molecules is very low without catalysts and a high temperature (Sato et al., 2004). Although the possibility is therefore very low at temperatures experienced during transportation and storage, residual 1,4-BD in fats and oils could react with free fatty acids to form esters.

2.3 Methods of analysis

In 2014, the WHO Expert Committee on Drug Dependence reviewed 1,4-BD in view of its possible illicit trade as a precursor of GHB (WHO, 2014).

Forensic methods of analysis of 1,4-BD in human samples have been developed (Tagliaro & Smith, 1996; von Heeren & Thormann, 1997). GC-MS and GC-FID methods have been reported (Dasgupta et al., 1995; Livesey et al., 1995; Blanchet et al., 2002; Gembus, Goulle & Lacroix, 2002). LC-UV and LC-MS/MS methods are also available (Vollmer et al., 1996; Gao et al., 2003; Johansen & Windberg, 2011; Sørensen & Hasselstrøm, 2012).

No methods for analysing 1,4-BD in fats and oils were found in the literature. However, 1,4-BD could be extracted from fats and oils using a mixture of methanol and acetonitrile, and followed by clean-up using a polymeric strong cation exchanger (Sørensen & Hasselstrøm, 2012) and analysis by LC-MS/MS or GC-MS/MS.

3. Biological data

3.1 Biochemical aspects

1,4-BD is metabolized to γ-hydroxybutyraldehyde in vivo, catalysed by alcohol dehydrogenase. This is followed by metabolism to γ-hydroxybutyric acid, catalysed by aldehyde dehydrogenase (Roth & Giarman, 1966, 1968). The rate of this two-step conversion is rapid; the plasma concentration–time curve for γ-hydroxybutyric acid following intravenous injection of 1,4-BD is indistinguishable from the plasma concentration–time curve for γ-hydroxybutyric acid following its intravenous injection (Vree et al., 1978).

γ-Hydroxybutyric acid may also be rapidly formed by the non-enzymatic hydrolysis of γ-butyrolactone. γ-Butyrolactone is rapidly and completely absorbed over a wide dose range following oral administration, with a peak plasma concentration that is proportional to the dose (Lettieri & Fung, 1978; Arena & Fung, 1980). The total plasma concentration–time curve (γ-butyrolactone plus γ-hydroxybutyric acid) following oral administration of γ-butyrolactone is indistinguishable from the plasma concentration–time curve following its intravenous injection (Lettieri & Fung, 1978). The pharmacological activity of γ-butyrolactone is essentially identical to that of 1,4-BD and γ-hydroxybutyric acid (Giarman & Roth, 1964; Sprince, Josephs & Wilpizeski, 1966; Snead, 1992); studies on γ-butyrolactone may therefore be used to inform on the effects of 1,4-BD.

The major metabolic fate of γ-hydroxybutyric acid is conversion to succinic semialdehyde and then to succinic acid, which is further processed through the tricarboxylic acid cycle (National Toxicology Programme (NTP), 1996). A secondary, minor, metabolic fate of γ-hydroxybutyric acid is through ß-oxidation (Walkenstein et al., 1964).

As a result of the metabolic processing described above, 1,4-BD is predominantly excreted as CO_2. Administration of 1-[^{14}C]-1,4-BD at 4, 40, 120 or 400 mg/kg bw to rats revealed that 50% of the administered radiolabel was eliminated as CO_2 within 2 hours. After 72 hours, 86% of the administered radiolabel was eliminated as CO_2, 4% in the urine and 0.4% in the faeces (NTP, 1996).

Because 1,4-BD is an alcohol, it can esterify free fatty acids, leading to mono- or di-esters. These products are not considered to be of any greater toxicological concern than 1,4-BD itself (EFSA, 2011).

3.2 Toxicological studies

3.2.1 Acute toxicity

Acute oral LD_{50} values have been reported for several species following administration of 1,4-BD: for mouse, 2180 mg/kg bw (Kinney et al., 1985) and 2062 mg/kg bw (Bandman et al., 1994); for rat, 1830 mg/kg bw and 2000 mg/kg bw for males and females, respectively (Jedrychowski et al., 1990a), and 1525 mg/kg bw (Bandman et al., 1994); for guinea-pig, 1200 mg/kg bw (Bandman et al., 1994); and for rabbit, 2531 mg/kg bw (Bandman et al., 1994). The values for mouse and rabbit are within the "unclassified" category of OECD TG401, whereas the values for rat and guinea-pig are within the "harmful" category (OECD 1981).

Acute LD_{50} values for mice and rats have been reported following intraperitoneal administration of 1,4-BD: for mice, 1650 mg/kg bw (Holman, Mundy & Teague, 1979); for rats, 1000 mg/kg bw (Sprince, Josephs & Wilpizeski, 1966), 1328 mg/kg bw (Zabic, Van Dam & Maickel, 1974), and 1070 mg/kg bw (Taberner & Pearce, 1974).

No LD_{50} value has been determined for inhalation of 1,4-BD. Kinney et al. (1991) exposed rats for four hours to 4.6, 9.4 and 15 gm/m^3. The lowest lethal concentration was 15 gm/m^3 for four hours: 1/10 rats died 24 hours post-exposure. Lung noise and dry red nasal discharge was reported for an unspecified number of animals in the two highest dose groups, lasting 1–9 days post-exposure.

Goodwin et al. (2009) exposed baboons to 0, 32, 56, 100, 180 and 240 mg/kg bw 1,4-BD as a single bolus infusion. The lowest published toxic dose of 56 mg/kg bw was reported for decreased food intake and alteration of classical

conditioning. Tremor and ataxia were reported at 180 mg/kg bw, and somnolence and muscle weakness at 240 mg/kg bw.

3.2.2 Short-term studies of toxicity

Jedrychowski et al. (1990a) reported a 28-day oral toxicity study in groups of eight male and female Wistar rats to which 1,4-BD was administered by gavage at doses of 0, 5, 50 and 500 mg/kg bw per day. No alterations in body or organ weight were reported. Decreased red blood cell counts were reported for both male and female rats, with the effect being significant at the lowest dose. This was mirrored by a significant increase in mean corpuscular volume and haemoglobin (both sexes) and mean corpuscular haemoglobin concentration (males only). An increase in alanine aminotransferase and sorbitol dehydrogenase activity was reported for the male rats in the highest dose group, together with a significant decrease in total protein. No effects of 1,4-BD on enzyme activity or total protein levels were reported in female rats.

No major pathological features of hepatotoxicity were reported by Jedrychowski et al. (1990a). A mild histological change of bile duct proliferation with periportal infiltration of fibroblasts and mononuclear cells was reported, which was statistically significant at the highest dose when sexes were combined. Individual response rates were: 0/5, 3/5, 1/5 and 3/5 for males and 1/5, 1/5, 3/5 and 4/5 for females at doses of 0, 5, 50 and 500 mg/kg bw per day, respectively.

Kinney et al. (1991) reported a repeat-dose experiment with inhalation of 1,4-BD. Rats were exposed, nose-only, to 0, 200, 1000 and 5000 mg/m^3 for 6 hours per day, 5 days per week for 2 weeks. A significant decrease in body weight was reported in the group exposed to 5000 mg/m^3, from 3 days exposure to 4 days post-exposure. Serum cholesterol was decreased and slight atrophy of the lymphoid cells of the thymus was reported in the highest dose group; in all cases these effects disappeared in the 14-day post-exposure recovery group.

Lowest published toxic concentration values for inhalation have been reported for both rats and mice. In mice, a value of 4 mg/m^3/30 D-intermittent was reported (Anon, 1965 as cited in NIOSH, 1998). In rats, values of 4 mg/m^3/30 D-intermittent, 1750 mg/m^3/3 W-intermittent and 400 mg/m^3/122 D-intermittent were reported (Anonymous, 1965 as cited in NIOSH, 1998), whereas Kinney et al. (1991) reported a value of 5200 mg/m^3/6H/2 W-intermittent.

The US NTP (1996) exposed rats and mice to γ-butyrolactone for 90 days, which can be considered relevant due to the rapid metabolism of both 1,4-BD and γ-butyrolactone to γ-hydroxybutyric acid in vivo. Rats were dosed by gavage with 0, 56, 112, 225, 450 or 900 mg/kg bw per day, while mice received doses of 0, 65, 131, 262, 525 or 1050 mg/kg bw per day. Increased mortality was

reported at the highest dose in male rats (10/10) and male mice (3/10), while body weight gain was significantly retarded in male rats receiving 450 mg/kg bw per day and male mice receiving 1050 mg/kg bw per day. Histopathological examination revealed an increased incidence of focal inflammation of the nasal mucosa in rats administered γ-butyrolactone. In male rats, the incidence was 1/10, 7/10, 9/9, 9/9, 9/10 and 6/10 at doses of 0, 56, 112, 225, 450 or 900 mg/kg bw per day, respectively; in female rats the incidence was 2/10, 4/9, 6/10, 9/9, 9/10 and 9/10 for the same doses. NTP (1992) reported that similar lesions have been noted with a variety of other chemicals and may be related to the reflux of gavage solution. The Committee concluded that NOAELs of 225 mg/kg bw per day in rats and 525 mg/kg bw per day in mice could be identified for γ-butyrolactone in this study, and that these could be extrapolated to 1,4-BD.

As reported in the OECD SIDS evaluation, in a combined repeat-dose and reproductive and developmental toxicity screening test, 1,4-BD was administered at doses of 0, 200, 400 or 800 mg/kg bw per day by oral gavage for 45 days in males and from 14 days before mating to day 3 of lactation in females (OECD, 2000). Acute and transient dose-related central nervous system (CNS) effects were observed in both sexes, consisting of hyperactivity in animals that received 200 mg/kg bw per day and CNS depression at higher doses. Body weight gains were reduced at doses of 400 and 800 mg/kg bw per day during the early period of administration, and food consumption was reduced in parallel. Histopathological examination revealed epithelial hyperplasia and fibrosis of the lamina propria in the bladder of animals receiving 400 or 800 mg/kg bw per day.

3.2.3 Long-term studies of toxicity and carcinogenicity

No carcinogenicity studies of 1,4-BD were identified. However, the US NTP (NTP, 1992) evaluated γ-butyrolactone in a 2-year bioassay in both B6C3F1 mice and F344 rats. As both 1,4-BD and γ-butyrolactone are rapidly converted to γ-hydroxybutyric acid in vivo, data from studies on γ-butyrolactone can be used to inform on the effects of 1,4-BD in vivo.

Male and female B6C3F1 mice received 0, 262 or 525 mg/kg bw γ-butyrolactone by oral gavage on 5 days per week for 102 weeks. The mean body weights of male mice treated with γ-butyrolactone were significantly decreased compared to the control group from 3 weeks of exposure until the end of the bioassay. In female mice, mean body weights were also significantly lower for animals receiving γ-butyrolactone than the control group, but this effect only became evident following 28 weeks of exposure. No measurement of food consumption was reported. The survival of male mice given 525 mg/kg bw γ-butyrolactone was significantly lower than that of control animals (12/50 versus 35/50, respectively). No other dose groups were reported to have survival rates

different to the control group. The report noted that the reduced survival of male animals in the high-dose group may have been due to fighting in the first year of the study, with increased aggression in the high-dose group being due to the sedative or anaesthetic properties of γ-butyrolactone. This hypothesis was based upon the observation that mice in the high-dose group were noted to be partially lethargic or sedated and inactive after dosing. The first male mice to recover were observed to attack those mice that were still sedated. Bite wounds and scratches, as well as the number of non-neoplastic lesions believed to be related to debilitation, stress or ascending infections of the urogenital tract as a result of fighting were observed in more animals in the low- and high-dose groups than in the control group. Hepatocellular adenomas or carcinomas (combined) were reported to show a statistically significant negative trend, with numbers in both low- and high-dose groups significantly lower than in the controls: 8/50 and 9/50 versus 24/50 for 262, 525 mg/kg bw γ-butyrolactone and controls, respectively. A positive correlation between body weight and the incidence of hepatocellular neoplasms in control mice in NTP studies has previously been reported (Rao et al., 1987). Based upon this finding, NTP (1992) concluded that although the lower incidence of hepatocellular neoplasms is associated with the administration of γ-butyrolactone, it may also be related to the lower body weights of dosed mice and as such it is not a chemical-specific effect. A significantly increased incidence of focal hyperplasia of the adrenal medulla was reported in male mice receiving the low dose (9/50 versus 2/48 for 262 mg/kg bw γ-butyrolactone and controls, respectively). NTP (1992) noted that the sensitivity of the study in male mice to detect a carcinogenic effect was reduced by the low survival of animals in the high-dose group associated with fighting and concluded that evidence of carcinogenic activity of γ-butyrolactone in male mice was equivocal.

Male F344 rats received 0, 112 or 225 mg/kg bw and female F344 rats received 0, 225 or 450 mg/kg bw γ-butyrolactone by oral gavage for 5 days per week for 102 weeks. The mean body weights of male rats given γ-butyrolactone were not significantly different to those of the control group throughout the 2-year study. For female rats, mean body weight of the group receiving 450 mg/kg bw γ-butyrolactone was significantly lower than that of controls from 59 weeks of exposure until the end of the bioassay. No measurement of food consumption was reported. No difference in overall survival was reported for either sex at any dose compared to controls. In female rats, a statistically significant decrease in mammary gland fibroadenomas was reported: 22/50, 14/50 and 6/50 for animals treated with 0, 225 or 450 mg/kg bw γ-butyrolactone, respectively. The incidence of mammary gland cysts also showed a statistically significant negative trend: 42/50, 35/50 and 23/50 for animals treated with 0, 225 or 450 mg/kg bw γ-butyrolactone, respectively. A statistically significant decrease in the incidence of pituitary gland cysts was reported in female rats receiving the high dose: 25/49, 13/37 and 11/48

for animals treated with 0, 225 or 450 mg/kg bw γ-butyrolactone, respectively. The incidence of mononuclear cell leukaemia in male rats was reported to show a statistically significant negative trend, with the incidence in the high-dose group being significantly lower than that in controls: 16/50, 15/50 and 9/50 for male rats treated with 0, 112 or 225 mg/kg bw γ-butyrolactone, respectively. NTP (1992) noted that tumour incidence in controls was lower than in historical controls, which could underlie the negative trends reported. NTP (1992) concluded that there was no evidence of carcinogenic activity of γ-butyrolactone in male or female rats in this study.

3.2.4 Genotoxicity

Zeiger et al. (1992) reported that 1,4-BD was negative in the Ames test, and in chromosome aberration tests in V79 and CHO cells. Studies on γ-butyrolactone can be used as an equivalent due to the rapid metabolism of 1,4-BD to γ-butyrolactone in vivo (EFSA, 2004). The US NTP (NTP, 1992) reported that γ-butyrolactone was negative in the Ames test using *Salmonella* Typhimurium strains TA100, TA1535, TA1537 and TA98, with or without metabolic activation. In the in vitro mammalian chromosome aberration test in CHO cells, significant increases in chromosomal aberrations were reported with 2580 and 3990 mg/mL γ-butyrolactone.

3.2.5 Reproductive and developmental toxicology

In an NTP-sponsored comparative developmental toxicity study with mice, 1,4-BD was administered by gavage at doses of 0, 1, 100, 300 and 600 mg/kg bw from gestation day 6 to gestation day 15 (Price et al., 1993, cited in NTP, 1996). No maternal deaths were reported, but maternal toxicity was observed in the groups receiving the 300 and 600 mg/kg bw doses. Acute CNS intoxication was reported in these two dose groups, including hypoactivity, immobility and loss of righting reflex; all symptoms were reported usually to resolve within 4 hours of dosing. In addition, body and liver weight and food consumption were statistically lower for the 300 and 600 mg/kg bw dose groups than in the control group, while kidney weights were lower for animals in the 600 mg/kg bw dose group than in the control group. Significant reductions in fetal weights were reported in mice in the 300 and 600 mg/kg bw dose groups.

As reported in the OECD SIDS evaluation, in a combined repeat-dose and reproductive and developmental toxicity screening test, 1,4-BD was administered to mice at doses of 0, 200, 400 or 800 mg/kg bw per day by oral gavage for 45 days in males and from 14 days before mating to day 3 of lactation in females (OECD, 2000). No effects on reproductive parameters for the parental animals was reported. The study also found no effect on fetal survival, or on the

incidence of morphological abnormalities. Significantly decreased fetal body weight, plus an increase in skeletal defects was reported in the females receiving 800 mg/kg bw per day. OECD (2000) considered that this effect was secondary to maternal toxicity (reduced food consumption and body weight gain).

3.2.6 Irritation and sensitization

Jedrychowski et al. (1990b) reported that 1,4-BD did not cause irritation of the skin or eyes. Using the Draize method, rabbits were treated with the undiluted test substance for 24 hours under occlusive conditions. No skin reactions were observed (mean 72-hour Draize score: 0). In a separate study using the Draize method for eye irritation, 1,4-BD was applied to rabbits' eyes using a syringe and allowed to remain for 24 hours. Slight reddening of the conjunctivae and small amounts of discharge were observed in all rabbits 1 hour after application. These changes resolved after 24 and 48 hours, respectively, and no further abnormalities were observed (mean 72-hour Draize score: 0).

In an acute inhalation toxicity study, Kinney et al. (2001) exposed rats to 4.6, 9.4 and 15 gm/m^3 1,4-BD for 4 hours per day. Lung noise and dry red nasal discharge consistent with irritation was reported for an unspecified number of animals in the two highest dose groups, lasting 1–9 days post-exposure.

The US NTP (1996) exposed rats to 0, 56, 112, 225, 450 or 900 mg/kg bw per day γ-butyrolactone for 90 days, which can be considered relevant due to the rapid metabolism of both 1,4-BD and γ-butyrolactone to γ-hydroxybutyric acid in vivo. Histopathological examination revealed an increased incidence of focal inflammation of the nasal mucosa in animals in all exposure groups.

3.3 Observations in humans

No reports of toxicity of 1,4-BD in humans were identified and no contaminants of concern have been noted.

4. Levels and patterns of contamination in food commodities

The 2006 criteria document (FAO/WHO, 2007) assumed a worst-case concentration of 100 mg/kg for previous cargo contaminants. This concentration was also supposed to be a worst-case concentration of 1,4-BD.

1,4-BD has no food uses but is listed as an indirect additive used in food contact substances without limitations by the FDA (US FDA, 2019). No data were identified on concentrations in foods from these uses.

5. Food consumption and dietary exposure estimates

Information on consumption of food oils by infants and young children was available from the US EPA's Food Commodity Intake Database, or FCID (US EPA, 2020), which in turn is based on data from the US National Health and Nutrition Survey/What We Eat In America, 2005–2010 cycles. The highest oil consumptions for infants and young children based on FCID are comparable to those in the CIFOCOss database; however, oil consumption information based on FCID is available based on individual body weights.

The highest reported consumption of a specific fat or oil type was for palm oil. Estimated mean and p95 consumptions by infants and young children were 7.6 and 19 g per day, respectively. Estimated mean and p95 consumption on a body weight basis were 1 g/kg bw per day and 3 g/kg bw per day, respectively. These data were used to define a worst-case dietary exposure estimate for infants and young children.

Estimated chronic daily dietary exposures to 1,4-BD present in food oils are 0.1 mg/kg bw per day (mean) and 0.3 mg/kg bw per day (high consumers), assuming a worst-case 1,4-BD concentration of 100 mg/kg, mean oil consumption of 1 g/kg bw per day, and high consumer oil consumption of 3 g/kg bw per day.

No data were identified on actual concentrations of 1,4-BD in food from other sources.

6. Comments

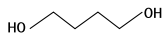
6.1 Chemical and technical considerations

Chemical and technical considerations for 1,4-BD are summarized in Table 16.

6.2 Biochemical aspects

1,4-BD is rapidly metabolized to γ-hydroxybutyraldehyde and subsequently γ-hydroxybutyric acid (Roth & Giarman, 1966, 1968). Maximum plasma

Table 16
Chemical and technical considerations for 1,4-butanediol

Name: 1,4-butanediol (1,4-butylene glycol)	
CAS number	**Alternative CAS numbers**
110-63-4	None
Chemical details	1,4-butanediol, 1,4-butylene glycol, 1,4-BD Colourless viscous liquid or crystals  Molar mass: 90.12 g/mol Melting point: 20 °C Boiling point: 230 °C Soluble in water and ethanol
Route(s) of synthesis	Manufactured by different mechanisms: by the Reppe process from formaldehyde plus acetylene; from maleic anhydride; from propylene oxide; or from butadiene plus acetic acid.
Composition	• Reppe process: 1,4-BD > 99%, 2-methyl-1,4-butanediol maximum 0.4%, and 2-(4-hydroxybutoxy) tetrahydrofuran maximum, 0.2% • maleic anhydride process: 1,4-BD 90–95%; *n*-propanol and *n*-butanol 5–10% • propylene oxide process: 1,4-BD 86–91%; *n*-propanol and propionaldehyde 0.5–3.5%; methyl propanediol typically < 13.5% • butadiene-acetic acid process: 1,4-BD > 99%; 2-(4-hydroxybutoxy)tetra-hydrofuran 0.3%.
Uses	Used in the manufacture of tetrahydrofuran (THF) and polytetramethylene ether glycol, polybutylene terephthalate, gamma-butyrolactone, polyurethane and other chemical reagents. THF production represents the largest use.
Analytical methods	None found for previous cargoes. Possible means of analysis in fats and oils may require extraction of 1,4-BD using a mixture of methanol and acetonitrile, followed by clean-up using a polymeric strong cation exchanger and analysis by LC-MS/MS or GC-MS/MS.
Potential reaction(s) with a subsequent cargo of fat or oil	1,4-Butanediol is unlikely to polymerize or participate in hydrogenation or dehydrogenation reactions without the presence of a catalyst or microorganism. There is a small possibility of ester formation with free fatty acids.

GC-MS/MS, gas chromatography with tandem mass spectrometry; LC-MS/MS, liquid chromatography with tandem mass spectrometry.

concentrations of γ-hydroxybutyric acid are approximately equivalent in humans following administration of 1,4-BD or γ-hydroxybutyric acid (Vree et al., 1978).

γ-Hydroxybutyric acid can also be rapidly formed from γ-butyrolactone. Thus, data on γ-butyrolactone can be used as a read-across for γ-hydroxybutyric acid, and by extension 1,4-BD (Irwin, 1996).

Further metabolism of γ-hydroxybutyric acid yields succinic acid, which is converted to carbon dioxide via the tricarboxylic acid cycle (Irwin, 1996).

6.3 Toxicological studies

1,4-BD has been noted to possess very low oral acute toxicity. Acute oral LD_{50} values have been reported for mouse (2062 mg/kg bw), rat (1525 mg/kg bw), guinea-pig (1200 mg/kg bw) and rabbit (2531 mg/kg bw) (Bandman et al., 1994).

In a 28-day oral toxicity study in male and female Wistar Imp:DAK rats, 1,4-BD was administered by gavage at doses of 0, 5, 50 and 500 mg/kg bw per day. No alterations in body or organ weight were reported, and only mild liver histopathology with no overt hepatotoxicity at any dose (Jedrychowski et al., 1990).

In a US NTP-sponsored developmental toxicity study with CD1 mice, 1,4-BD was administered by gavage at doses of 0, 1, 100, 300 and 600 mg/kg bw per day from gestation day 6 to day 15 (Price et al., 1993 as cited in Irwin, 1996). No maternal deaths were reported, but acute CNS intoxication was reported in the maternal 300 and 600 mg/kg bw per day dose groups, with all symptoms reported usually to resolve within 4 hours of dosing. In addition, body and liver weight, and food consumption were lower than control levels for the 300 and 600 mg/kg bw dose groups, while kidney weights were lower than control weights for the 600 mg/kg bw dose group. Significant reductions in fetal weights were reported in the 300 and 600 mg/kg bw dose groups (Price et al., 1993 as cited in Irwin, 1996). Based upon these observations, the Committee identified a NOAEL of 100 mg/kg bw per day for both maternal and fetal toxicity.

In a combined repeat-dose and reproductive and developmental toxicity study, 1,4-BD was administered to rats at doses of 0, 200, 400 or 800 mg/kg bw per day by oral gavage for 45 days in males and from 14 days before mating to day 3 of lactation in females (OECD, 2000). No effects on parental reproductive parameters, fetal survival or incidence of morphological abnormalities was reported. Epithelial hyperplasia and fibrosis of the lamina propria has been reported in the bladder of animals receiving 400 or 800 mg/kg bw per day 1,4-BD (SIDS, 2000). Acute and transient dose-related CNS effects were reported in male and female rats exposed to 1,4-BD, namely hyperactivity at a dose of 200 mg/kg bw per day and CNS depression at higher doses (400 or 800 mg/kg bw per day). The authors of the study considered that the hyperactivity seen at a dose of 200 mg/kg bw per day was not an adverse effect. The Committee, however, noted that hyperactivity would generally be considered adverse, but was not able to reach a conclusion on this study without access to the original data.

Both 1,4-BD and γ-butyrolactone are negative in the Ames test using *Salmonella* Typhimurium strains TA98, TA100, TA1535 or TA1537, with or without metabolic activation (SIDS, 2000 and Irwin, 1996). The results of an in vitro mammalian chromosome aberration assay of 1,4-BD were reported as negative, while γ-butyrolactone was reported to cause significant increases in

chromosomal aberrations in one study at high concentrations (2580 and 3990 µg/mL) (Price et al., 1993).

No chronic toxicity or carcinogenicity studies were identified for 1,4-BD. However, γ-butyrolactone has been assessed in a 2-year study reported by NTP (1992). γ-Butyrolactone was administered to F133 rats at doses of 0, 112 and 225 mg/kg bw (males) or 0, 225 and 450 mg/kg bw (females). In male and female F344 rats, exposure to γ-butyrolactone was not reported to increase the incidence of tumours. In contrast, the incidence of mammary gland fibroadenomas and pituitary gland cysts was reported to show a negative trend with dose in female rats, while mononuclear cell leukaemia was reported to show a negative trend with dose in male rats. In mice, hepatocellular adenomas or carcinomas (combined) were reported to show a dose-dependent negative trend for incidence. NTP considered that these dose-dependent negative trends were due to high incidence levels in the control groups, which were significantly higher than historical values (NTP, 1992). In the same study, B6C3F1 mice were administered γ-butyrolactone at doses of 0, 262 and 450 mg/kg bw (males and females). A significantly increased incidence of focal hyperplasia of the adrenal medulla was reported in male mice given the low dose, but this was not a dose-dependent effect (NTP, 1992). The Committee concluded that 1,4-BD was unlikely to possess genotoxic carcinogenic potential.

6.4 Allergenicity

The Committee did not identify any reports of allergenicity upon oral exposure to 1,4-BD that would indicate that it is, or it contains a known food allergen.

6.5 Assessment of dietary exposure

A worst-case concentration of 100 mg/kg has been assumed for all previous cargo chemical substances (see Part A, section 4.3).

1,4-BD has no direct food uses but is listed as an indirect additive used in food contact substances without limitations by the FDA. No data were identified on concentrations in foods from this use, and the Committee concluded that this would most likely represent a minor contribution to total exposure and did not consider this route further.

Worst-case human dietary exposures to previous cargo chemical substances in food oils have been estimated at 0.3 mg/kg bw per day.

No other data on dietary exposure to 1,4-BD were identified.

7. Evaluation

The Committee noted that both 1,4-BD and γ-butyrolactone are rapidly metabolized to γ-hydroxybutyric acid, whereupon they share metabolic fates and data on γ-butyrolactone could therefore be used for read-across to fill data gaps with 1,4-BD.

The Committee concluded that 1,4-BD is not genotoxic, and that the data for γ-butyrolactone are consistent with 1,4-BD being unlikely to possess carcinogenic potential.

The Committee noted that a range of toxic end-points have been reported for 1,4-BD and γ-butyrolactone from various studies. It concluded that acute and transient CNS effects, most notably hyperactivity, provided the most relevant end-point. A NOAEL of 100 mg/kg bw was identified by the NTP (1996), as cited in Irwin (1996), and the Committee considered that this was appropriate as an RP in the current evaluation. Considering the estimated dietary exposure of 0.3 mg/kg bw per day, the MOE is approximately 330, which the Committee concluded is sufficient to address the uncertainties in the database.

There are no reports of allergenicity upon oral exposure to 1,4-BD that would indicate that it is or contains a known food allergen.

1,4-BD is unlikely to polymerize or participate in hydrogenation or dehydrogenation reactions without the presence of a catalyst or microorganism. There is a small possibility of ester formation with free fatty acids.

Therefore, the Committee concluded that 1,4-butanediol meets the criteria for acceptability as a previous cargo.

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PREVIOUS CARGOES

GROUP 5 – BUTYL ETHERS

First draft prepared by

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A. ASSESSMENT OF SUBSTANCES PROPOSED AS PREVIOUS CARGOES

1. Introduction

Fats and oils destined to be used as food are transported and stored in large volumes. Transportation in large volumes by sea is exempted from many land-based regulations as it is not practical to have fleets of ships solely dedicated to the transportation of food in large tanks, since the trade is generally unidirectional from producer to consumer. Furthermore, the construction and dependency on the availability of a limited number of single-use carriers would make the transport of fats and oils extremely expensive. To address the economic realities, certain types of ships are permitted to carry different classes of cargo in their tanks on their outbound and onward journeys. A non-food item may be carried in a tank in one direction and a single type of fat or oil on the further voyage. Since ships are constructed to have several individual tanks, each may contain a cargo destined for a different location and may be used to carry either a food or non-food item depending on the contract.

A number of organizations have been involved in the development of codes of practice, transportation contracts, ship construction, cargo segregation, environmental issues and food safety. The Codex Alimentarius Commission (CAC) adopted and published a code of practice for the storage and transport of edible fats and oils in bulk, which was developed by the Codex Committee on Fats and Oils (CCFO) in 1987 (CAC, 1987). At that time, CCFO recognized the need

to assess the acceptability of previous cargoes transported in a tank subsequently used for the transportation of an edible fat or oil. Commercial trade contracts recognized the need to specify that certain chemicals should never be acceptable previous cargoes for subsequent cargoes of edible fats or oils. These substances formed the basis of the "banned lists" of previous cargoes. In 2001, a combined list of chemicals banned as previous cargoes was developed by CCFO and adopted by CAC (CAC, 2001); it was added to the Codex code of practice as Appendix 1. Other substances carried in bulk were considered to pose a low risk to public health as a contaminant in edible fats or oils; these formed the basis of "acceptable lists" of previous cargoes. The development of a CCFO acceptable list of previous cargoes was also based on trade experience. A preliminary list was reviewed by the Scientific Committee on Food and their findings were reported to CCFO in 1999; 14 substances were identified for which there were insufficient data to make a safety determination. After further discussion at subsequent CCFO meetings, a list of 23 potentially safe previous cargoes that require evaluation was developed. CCFO asked for scientific advice from FAO/WHO on these 23 substances that lacked safety evaluations. The present evaluation by JECFA addresses 18 of the 23 substances on the current list of chemicals acceptable as previous cargoes by CCFO.

2. Background

2.1 Global production and consumption of fats and oils

The global trade in edible fats and oils is more than 200 million metric tonnes annually and valued at approximately US$ 120 billion (USDA, 2019). By far the largest contributors are palm (36%) and soybean oil (28%), followed by rapeseed/canola (14%), sunflower seed (10%), palm kernel (4%), peanut (3%), cottonseed (3%), coconut (2%) and olive oils (2%).

Many vegetable oils are produced in regions (for example: soybean – Argentina, Brazil, USA; rapeseed – Australia, Canada; sunflower seed – Ukraine; palm – Indonesia and Malaysia; and coconut – equatorial latitudes) far from the major sites of consumption. Olive oil is produced in regions with a Mediterranean climate in both the northern and southern hemispheres. International trade in fats and oils uses the most economical method of ocean transportation since global trade in edible fats and oils is primarily unidirectional. Soybean oil from Argentina and Brazil, for example, is shipped to both Asian and European markets, but there is unlikely to be a complementary cargo of fat or oil available

for transportation in the reverse direction. Similarly, oils from tropical regions are traded globally, often without reciprocal trade in fats and oils.

2.2 Regulations affecting fats and oils

Shipment of fats and oils is described in numerous national and international regulations and agreements. Land-based transportation is regulated by local and national guidelines and/or legislation, whereas international trade is subject to commercial agreements, international shipping regulations and various codes of practice. The development of banned lists and acceptable lists of previous cargoes is founded on existing trade contracts.

About 85% of the fats and oils are traded globally using FOSFA (The Federation of Oils, Seeds and Fats Associations, London) contracts. The balance is traded under contracts issued by NIOP (National Institute of Oilseed Products) or other organizations. A contract under "banned list terms" requires that fats and oils are not shipped in tanks that have contained a substance on the banned list as the immediate previous cargo. For certain chemicals, this requirement is extended to the three previous cargoes. Alternatively, a contract may state that "the immediate previous cargo shall be a product on the FOSFA List of Acceptable Previous Cargoes". In this case, the receiver will only accept the cargo if the previous cargo is on FOSFA's acceptable list. These two lists only cover a small proportion of the chemicals transported by sea; thus many substances appear on neither list and their acceptability as a previous cargo is subject to agreement by the contracting parties.

2.3 Global transport of fats and oils

Transportation by sea is regulated by the International Maritime Organization (IMO). The International Convention for the Prevention of Pollution from Ships (MARPOL) aims to prevent operational and accidental pollution from ships. MARPOL limits the carriage of different classes of liquid cargoes to specific tanker vessels based on ship construction and the class of chemical. Under this convention, fats and oils may not be transported in vessels designated to carry cargoes of crude oil, fuel oil, heavy diesel oil or lubricating oil. The International Code for the Construction and Equipment of Ships Carrying Dangerous Chemicals in Bulk (IBC Code) lists chemicals carried as bulk liquids, their pollution category, the type of ship design and any relevant restrictions or derogations. The previous cargoes under consideration (see Table 2) are in the medium- or low-risk categories for marine pollutants. The single exception is propylene tetramer, which is considered a high-risk marine pollutant. MARPOL also deals with tank

washing and material discharge. Pentane falls into an additional category of oil-like substances requiring additional attention between cargoes.

2.4 The interrelationship of national, regional and trade interests

The practice of Acceptable List trading was discussed in line with regional initiatives to protect consumer health. The adoption of the hazard analysis and critical control point (HACCP) principles and their inclusion in the Codex Alimentarius approach to the safe trade of food and food products can be applied to the transport of oils and fats by sea. The CAC adopted the *Code of Practice for the Storage and Transport of Fats and Oils in Bulk* developed by CCFO in 1987 (CAC-RCP 36-1987). The code has been revised periodically and a banned list of substances was added in 2001. The list of acceptable previous cargoes adopted by the European Union (EU) and based on existing trade lists, was evaluated by the European Food Safety Authority (EFSA).

2.5 Development of the Codex Code of Practice for storage and transport of edible fats and oils in bulk

CCFO discussions highlighted the need for lists of banned and acceptable previous cargoes. The topic of contamination by previous cargoes led to the incorporation of the FOSFA and NIOP trade lists into the code by reference only. In 2001, CAC adopted the "banned list" and it appears in the current code of practice as Appendix 3.

The development of a List of Acceptable Previous Cargoes by CCFO began with attempts to harmonize the FOSFA and NIOP trade lists with an EU list. The Acceptable List was further refined in 1999 when CCFO considered a list of substances proposed by the EU that had been reviewed by the Scientific Committee on Food (SCF). Having developed a list of acceptable previous cargoes, it was determined that there were 14 substances on it that required further evaluation; these 14 substances formed the basis of the CCFO Proposed Draft List of Acceptable Previous Cargoes, which was adopted by CAC 34 in 2011. For consideration at this meeting a list of 23 substances was proposed to FAO/WHO (Table 2) by CCFO for scientific advice on their suitability as previous cargoes for the carriage of fats and oils by sea-going vessels upon its evaluation against the four criteria. Each substance on the list has been assigned to Groups 1–5 (1 – solvents/reactants; 2 – alcohols; 3 – oils and waxes; 4 – solutions; 5 – butyl ethers). Substances in Group 1 were not evaluated at the present meeting.

Table 1
List of substances submitted by CCFO for evaluation by JECFA for addition to the list of acceptable previous cargoes

Substance (synonyms)	CAS number	Assessment group[a]
Acetic anhydride (ethanoic anhydride)	108-24-7	1
1,4-Butanediol (1,4-butylene glycol)	110-63-4	2
Butyl acetate, *sec-*	105-46-4	1
Butyl acetate, *tert-*	540-88-5	1
Calcium ammonium nitrate solution	15245-12-2	4
Calcium lignosulfonate liquid (lignin liquor; sulphite lye)	8061-52-7	4
Calcium nitrate (CN-9) solution	35054-52-5	4
Cyclohexane	110-82-7	1
Fatty alcohols		
iso Decyl alcohol (isodecanol)	25339-17-7	2
Myristyl alcohol (1-tetradecanol, tetradecanol)	112-72-1	2
iso Nonyl alcohol (isononanol)	27458-94-2	2
iso Octyl alcohol (isooctanol)	26952-21-6	2
Tridecyl alcohol (1-tridecanol)	112-70-9	2
Unfractionated fatty alcohol mixture or mixtures of fatty alcohols from natural oils and fats[b]		3
Methyl tertiary butyl ether (MTBE)	1634-04-4	5
Mineral oil, medium and low viscosity, class II		3
Mineral oil, medium and low viscosity, class III		3
Montan wax	8002-53-7	3
Pentane	109-66-0	1
1,3-Propanediol (1,3-propylene glycol)	504-63-2	2
Propylene tetramer (tetrapropylene, dodecene)	6842-15-5	3
Soybean oil epoxidized	8013-07-08	3
Ethyl tertiary butyl ether (ETBE)	637-92-3	5

[a] Group 1 was not considered at this meeting.
[b] Discussed with Group 2 – alcohols.

3. Development of criteria

As a result of the CCFO request to FAO/WHO for scientific advice on the development of criteria for the assessment of the safety of residues of previous cargoes in the tanks of sea-going vessels carrying edible fats and oils, a technical meeting was convened (in November 2006) at the Dutch National Institute of Public Health and the Environment (RIVM). RIVM prepared a technical background document (FAO/WHO, 2006, Appendix II) and drafted the meeting report with FAO/WHO (2007).

Discussions were limited to the assessment of previous cargoes in the transport of edible fats and oils in bulk by sea and the consideration of safety

implications in terms of human health. The experts accepted that the quality of the fats and oils cargo could change as a result of hydrolysis and oxidation, but they acknowledged that these changes were already taken into account in trade contracts.

The experts considered a list of parameters originating from discussions at CCFO meetings, noting that previous cargoes are generally liquid chemical substances, slurries of solid particles or aqueous solutions. To further frame the deliberations, the experts decided to consider only a generic worst-case scenario since developing criteria to cover every possible combination of previous cargo, type of tank, cleaning regime and possible further processing of the subsequent cargo of fat or oil would not be a realistic approach.

The experts developed the following worst-case scenario: the smallest commercially viable tank size (200 m^3), coated with a polymer that absorbs the previous cargo, is filled to 60% capacity (as required by contract), and the cargo of fat or oil is not to be further processed or refined. The model also assumed that the tank and associated pipework has been cleaned according to defined standards, inspected and considered clean and dry. Under these circumstances, the maximum level of contamination in the subsequent fat or oil cargo by the previous cargo was calculated to be 100 mg/kg. This value was used to determine a single estimate of worst-case human exposure of 0.1 mg/kg bw per day. Based on this generic exposure value, the experts considered that for the evaluation of previous cargoes, the acceptable daily intake (ADI) (or tolerable daily intake (TDI)) should be greater than or equal to 0.1 mg/kg bw in order to provide sufficient protection for children and high-intake consumers. Negligent or fraudulent practices were not considered to be part of the criteria. The experts identified four criteria necessary to determine the acceptability of a previous cargo (see FAO/WHO, 2006).

The criteria as adopted by CAC 34 (2011) are listed in Table 2.

4. Basis of evaluation

4.1 Chemistry/reactivity

Edible fats and oils are normally chemically stable; however, there may be potential for reactions with residues of previous cargoes that could give rise to products that are hazardous to human health. Consideration should be given to chemical substances that can react with edible fats and oils under normal transportation conditions. Minor oxidation and hydrolysis are normally anticipated in trade contracts and are not considered a consequence of contact with a previous

Table 2
Criteria adopted by CAC 34 and included in RCP-36-1987

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.

cargo, unless accelerated degradation occurs. Although many possible reactions require the presence of specific catalysts or temperatures well in excess of those anticipated during transportation, potential reactions of the previous cargo with triglycerides and free fatty acids or other minor components present in the fat or oil should still be considered.

4.2 Methods of analysis

In a few cases where contamination is considered critical there has been an international effort to develop specific analytical methods. Cases of contamination with diesel fuel (alkanes) and mineral oils (mineral oil saturated hydrocarbons, MOSH; mineral oil aromatic hydrocarbons, MOAH) led to the development of relevant international standards. Although many of the substances under review at the present meeting can be analysed by gas or liquid chromatography using appropriate detector systems, little progress has been made in the application of these technologies to their contamination of oils and fats. It is assumed that available methods with suitable modifications will be capable of determining the maximum anticipated level of 100 mg/kg of previous cargo in the subsequent cargo of fats or oils.

4.3 Dietary exposure assessment for previous cargo chemical substances

As a consequence of considering a range of previous cargo chemical substances at its ninetieth meeting, the Committee concluded that it was appropriate to review the approach to estimating dietary exposure set out in the 2006 document Development of criteria for acceptable previous cargoes for fats and oils (criteria document) (FAO/WHO, 2007).

The Committee noted that since the 2006 criteria document was drafted, newer and better-quality data on the consumption of fats and oils by adults, infants and young children have become available.

The Committee also noted that some of the previous cargo chemical substances assessed have additional sources of dietary exposure and expressed the view that it may be necessary to consider this in the exposure assessment.

4.3.1 Exposure estimates in the 2006 criteria document

Based on the best available data at that time, the 2006 criteria document set out the following approach to dietary exposure assessment of previous cargo chemical substances present in fats and oils:

- Estimated mean per capita consumption of 0.025 kg/day of a single type of fat or oil. The value was rounded up from the maximum per capita consumption of refined soybean oil of 22 g/person per day from the GEMS/Food cluster diets.
- A factor of 2.5 to cover children and high consumers was derived from a rounded ratio between the mean and 97.5th percentile consumption of total vegetable oil from a food consumption survey in the United Kingdom (20 and 52 g/person per day for the population aged > 18 years). The criteria document also noted that dietary exposure of children to contaminants is frequently 2.5 times that of adults.
- A worst-case concentration of 100 mg/kg for a previous cargo contaminant in fats or oils.
- A body weight of 60 kg.

These data were used to define a worst-case dietary exposure estimate:

$$\frac{\text{Consumption of oil (0.025 kg/day)} \times 2.5 \times \text{concentration (100 mg/kg fat or oil)}}{\text{60 kg body weight}}$$

$$= \mathbf{0.1\ mg/kg\ bw\ per\ day}$$

Based on the mean per capita consumption of fats and oils, and a factor of 2.5, there would be no health concern to the general population from exposure to previous cargoes if the acceptable daily intake (ADI) or tolerable daily intake (TDI) is sufficiently protective, for example, the ADI or TDI is greater than, or equal to 0.1 mg/kg bw per day.

4.3.2 Exposure estimates based on up-to-date consumption data for adults

Since 2006, the GEMS/Food cluster diets have been revised, and the FAO/WHO Chronic Individual Food Consumption – summary statistics database (CIFOCOss) has become available. The 2006 criteria document noted that food consumption information from dedicated surveys would be more appropriate than the food consumption estimates from the GEMS/Food cluster diets (GEMS/Food consumption database, 2012). However, it used the cluster diets, as food consumption survey data were only available from a very limited number of countries at that time. CIFOCOss currently contains food consumption data from 37 countries.

From the current version of CIFOCOss, the maximum mean consumption for a single fat or oil type is 35 g/person per day for consumption of virgin or extra-virgin olive oil by elderly Italians. The maximum 95th percentile (p95) consumption of a single fat or oil is 138 g/person per day for edible cottonseed oil by women (age 15–49 years) from Burkina Faso. This group also has the highest 97.5th percentile consumption of 189 g/person per day.

Based on the protocols currently used by JECFA for veterinary drugs, the number of consumers of cottonseed oil in the Burkina Faso survey (n = 116) would suggest that the 95th percentile is the highest reliable percentile (Boobis et al., 2017; Arcella et al., 2019).

These data suggest that for adults, a mean fat or oil consumption of 35 g/person per day and a high consumption of fat or oil of 140 g/person per day would be a conservative estimate consistent with available data.

The use of updated food consumption data will result in a revised estimated worst-case dietary exposure for adults:

$$\frac{\text{p95 consumption of oil (0.140 kg/day)} \times \text{concentration (100 mg/kg fat or oil)}}{\text{60 kg body weight}}$$

$$= \mathbf{0.2\ mg/kg\ bw\ per\ day}$$

4.3.3 Exposure estimates for infants and young children

Potentially vulnerable population groups, like infants and young children, were not specifically considered in the 2006 criteria document. Since then, individual consumption data for several population groups, including infants and young children, have become available through CIFOCOss and other sources. Infants and young children should be considered in the risk assessment because they could potentially experience high exposure to previous cargo chemical substances per kg body weight while they are undergoing growth and development.

Information on consumption of food oils by infants and young children was also available from the US Environmental Protection Agency's Food Commodity Intake Database (FCID) (US EPA, 2020), which in turn is based on data from the US National Health and Nutrition Survey/What We Eat In America, 2005–2010 cycles. The highest oil consumption for infants and young children based on FCID is comparable to those in the CIFOCOss database; however, oil consumption information based on FCID takes into account individual body weights.

The highest reported consumption of a specific fat or oil type was for palm oil. Estimated mean and p95 consumption by infants and young children were 7.6 and 19 g/day, respectively. Estimated mean and p95 consumption on a body weight basis were 1 g/kg bw per day and 3 g/kg bw per day, respectively.

These data were used to define a worst-case dietary exposure estimate for infants and young children:

p95 consumption of oil (0.003 kg/kg bw/day) × concentration (100 mg/kg fat or oil)

= 0.3 mg/kg bw per day

4.3.4 Exposure from other dietary sources

For some previous cargo chemical substances potentially present in food oils, there are additional sources of dietary exposure, such as contamination (e.g. contamination of drinking-water) or food additive uses (Table 3). Dietary exposures from these different sources should be considered in exposure assessment.

4.3.5 Conclusion

The Committee concluded that, based on up-to-date data on consumption of single fats and oils in the general population, which have become available since 2006, the generic human exposure value of 0.1 mg/kg bw per day used in the 2006 Criterion no. 2 to determine the acceptability of a previous cargo should be revised. Consequently, the updated, more conservative generic human exposure value of 0.3 mg/kg bw per day should be used in the evaluation of these substances.

The Committee noted that these estimates of dietary exposure were derived from a more conservative approach to using data on consumption of single fats and oils and a worst-case concentration of previous cargo chemicals in a single fat or oil of 100 mg/kg.

The Committee also concluded that additional sources of dietary exposure need to be considered in exposure assessment of previous cargo chemical substances.

Table 3

List of substances for evaluation by JECFA arising from the development of a list of acceptable previous cargoes by the Codex Committee on Fats and Oils: Other sources of exposure

Substance (synonyms)	Other sources of exposure
1,4-Butanediol (1,4-butylene glycol)	Used in food contact material
Calcium ammonium nitrate solution	Calcium, nitrate and ammonium are ubiquitous in the human diet
Calcium lignosulfonate liquid (lignin liquor; sulfite lye), molecular weight not specified	Calcium lignosulfonate (40-65) is used as a food additive, an additive in animal feed and as an ingredient in pesticides
Calcium nitrate (CN-9) solution	Calcium and nitrate are ubiquitous in the human diet
iso Decyl alcohol (isodecanol)	None
Myristyl alcohol (1-tetradecanol; tetradecanol)	Flavouring agent, formulation agent, lubricant, release agent
iso Nonyl alcohol (isononanol)	None
iso Octyl alcohol (isooctanol)	Used in food contact material
Tridecyl alcohol (1-tridecanol)	Used in food contact material
Unfractionated fatty alcohol mixture or mixtures of fatty alcohols from natural oils and fats[a]	Occurs naturally in foods
Methyl tertiary butyl ether (MTBE)	Drinking-water
Mineral oil, medium and low viscosity, class II and III	Used in food contact material, direct food additive
Montan wax	Food additive
1,3-Propylene glycol	Used in place of 1,2-propanediol as a food additive
Propylene tetramer (tetrapropylene, dodecene)	None
Soybean oil epoxidized	Used in food contact material
Ethyl tertiary butyl ether (ETBE)	Drinking-water

[a] Discussed with Group 2 – Alcohols.

4.4 Approach to toxicological evaluation

The Committee received no submitted data and, therefore, reviewed monographs from previous evaluations of individual substances conducted by JECFA, WHO, International Agency for Research on Cancer (IARC), and national and regional governmental authorities to retrieve additional relevant references for completing the present assessment. The Committee also conducted literature searches. The details are included in the consideration of individual substances.

At its present meeting, the Committee revised the generic value for assumed worst-case human dietary exposure from 0.1 to 0.3 mg/kg bw per day and used this revised generic exposure value for the evaluation of previous cargoes. The Committee also considered data on exposure to the substances from sources other than previous cargoes. Thus, the ADI (or TDI) should be greater than or equal to the estimated dietary exposure (0.3 mg/kg bw per day plus exposure from other possible dietary sources) in order to provide sufficient protection for infants, children and high-intake consumers. In situations where no appropriate

numerical ADI (or TDI) was available from JECFA, the Committee considered other previously established health-based guidance values or calculated a margin of exposure (MOE) based on a reference point characterizing the toxicological hazard (such as a no-observed-adverse-effect level (NOAEL), etc.) identified from the available data divided by the estimated dietary exposure. Interpretation of this MOE is a matter of expert judgement that takes into account limitations in the available toxicological database.

5. Recommendations

The Committee recommended that the Codex Committee on Fats and Oils (CCFO) consider revising Criterion no. 2 in RCP-36-1987 as adopted by CAC 34 (2011).

- Based on the consumption of fats and oils by infants and young children, there is no health concern for the general population from dietary exposure to previous cargo chemical substances if the ADI or TDI is sufficiently protective, for example, the ADI or TDI is greater than, or equal to 0.3 mg/kg bw per day. Substances for which there is no numerical ADI or TDI should be evaluated on a case-by-case basis (e.g. margin of exposure (MOE) approach).
- Where there are additional sources of dietary exposure to the previous cargo chemical substances, they should be considered in the exposure assessment.

B. EVALUATION OF SUBSTANCES

I. Butyl ethers (Group 5)

I.I Methyl tertiary butyl ether (MTBE)

1. Explanation

This section considers the suitability of a) methyl tertiary butyl ether (MTBE) and b) ethyl tertiary butyl ether (ETBE) as an immediate previous cargo for

transporting edible fats and oils as a subsequent cargo. Both MTBE and ETBE are included on the FOSFA acceptable previous cargoes list whereas the NIOP acceptable previous cargo list includes MTBE only. The present assessment represents the first evaluation by JECFA of MTBE and ETBE as acceptable previous cargoes for edible fats and oils.

MTBE was evaluated for acceptability as a previous cargo by the Scientific Committee on Food (SCF) in 1996 and 2003 (SCF, 1997, 2003). SCF concluded that MTBE could be accepted as a previous cargo based on the determination that the solubility of MTBE in water (48 g/L) would enable effective cleaning of the cargoes by water washings at ambient temperature. More recently, the EFSA Panel on Contaminants in the Food Chain (CONTAM Panel) reviewed the available data on MTBE to evaluate its acceptability as a previous cargo for edible oils and fats (EFSA, 2012). The CONTAM Panel concluded that there was no concern regarding the carcinogenicity, developmental or reproductive toxicity of MTBE at the anticipated exposure levels from its use as a previous cargo, no reactions of concern with edible fats and oils, and no impurities likely to be present at levels of toxicological relevance. Therefore, the CONTAM Panel concluded that MTBE met the criteria for acceptability as a previous cargo for edible oils and fats. No health-based guidance values were established for MTBE under previous evaluations.

For the present assessment, the Committee identified and reviewed previous evaluations (monographs) by the WHO International Programme on Chemical Safety (WHO/IPCS), EFSA, IARC, SCF, and the United States Agency for Toxic Substances and Disease Registry (US ATSDR) and located additional references from these evaluations. This was followed by comprehensive searches for toxicological data on MTBE on PubMed and PubChem. The cut-off date for inclusion was 31 August 2020 for all searches. A total of 66 relevant records were retrieved, most of which had been included in monographs drafted by WHO/IPCS, EFSA, IARC, SCF or ATSDR; however, five additional records relevant to the present assessment were also retrieved. These included articles on physiologically based toxicokinetic (PBTK) modelling of MTBE and its main metabolite, tertiary butyl alcohol (TBA). These studies examined internal dose metrics of MTBE and/or TBA in various MTBE exposure scenarios, and the contribution of their binding with alpha (α)2-globulin in the kidneys of male rats to the observed renal toxic effects. The Committee also considered some reported data on TBA to conduct the toxicological evaluation of MTBE.

Extensive toxicological datasets on MTBE administered via different exposure routes are available. The present assessment discusses relevant toxicological data primarily on oral exposure to MTBE in animals and humans, and from genetic toxicity studies conducted using in vitro and in vivo systems.

1.1 Previous evaluations

As stated above, MTBE was evaluated for acceptability as a previous cargo by the SCF in 1996 (SCF, 1997) and 2003 (SCF, 2003). The SCF concluded that MTBE could be accepted as a previous cargo based on the determination that the solubility of MTBE in water (48 g/L) would enable effective cleaning of the cargoes by water washings at ambient temperature. Furthermore, the SCF evaluation stated that the tumour incidences reported in mice and rats exposed to MTBE probably resulted from a non-genotoxic mode of action, and thresholds could be established for the toxic events triggering carcinogenesis (SCF, 1997; 2003).

The US ATSDR (1996) concluded that the minimal risk levels (MRLs) of MTBE were 0.4 and 0.3 mg/kg per day for oral acute-duration and intermediate-duration exposure scenarios, respectively; however, a chronic-duration oral MRL was not established. IARC (1999) evaluated toxicological data on MTBE and concluded that there was inadequate evidence in humans and limited evidence in experimental animals for the carcinogenicity of MTBE. Therefore, IARC considered MTBE not classifiable as to its carcinogenicity to humans (Group 3) (IARC, 1999).

MTBE was also previously evaluated as an industrial chemical by IPCS/WHO (1998), the Organisation for Economic Co-operation and Development (OECD, 2001) and the European Union Programme on Risk Assessment of Existing Substances (ECB, 2002). The IPCS/WHO (WHO/IPCS, 1998) concluded that MTBE should be considered a rodent carcinogen; however, tumours were only observed at high levels of MTBE exposure. The OECD Safety Information Data Sheet (SIDS) programme (OECD, 2001) concluded that MTBE may present a hazard for human health (occupational exposure) because of concerns about local skin effects following repeated doses. The EU risk assessment (European Chemicals Bureau; ECB, 2002) concluded that for consumers and others exposed to MTBE indirectly via the environment there was at the time no need for further information, additional testing or risk reduction measures beyond those that were being applied already. However, the assessment stated that there was a need for limiting the risks due to long-term local effects of MTBE on the skin in occupational exposure scenarios.

The CONTAM Panel evaluated additional data on MTBE (EFSA, 2012) and concluded that MTBE meets the criteria for acceptability as a previous cargo for edible oils and fats. The CONTAM Panel's conclusion was based on the determination that there was no concern regarding the carcinogenicity, developmental or reproductive toxicity of MTBE at the levels anticipated in food from its use as a previous cargo. Furthermore, the CONTAM Panel concluded that there were no reactions of concern with edible fats and oils, and no impurities

likely to be present at levels of toxicological relevance. The CONTAM Panel also agreed with the SCF's assessment (SCF, 2003) that the solubility of MTBE in water (48 g/L) would enable effective cleaning of the cargoes by water washings at ambient temperature.

No health-based guidance values were established for MTBE under previous evaluations.

2. Chemical and technical considerations

MTBE is a colourless liquid with a terpene-like odour. It is primarily used to oxygenate gasoline to increase the octane number. The physicochemical characteristics of MTBE are listed in Table 5.

2.1 Manufacture and uses of MTBE

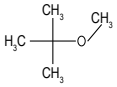
MTBE is produced by reacting methanol and isobutylene in a mixed gas–liquid phase in the presence of an acidic ion-exchange resin catalyst or in a liquid phase in the presence of sulfuric acid. The reaction mixture is distilled to produce high-purity MTBE (Jimenez et al., 2001; ICIS, 2007; Winterberg et al., 2017*)*. An additional two-step route of synthesis includes a single catalytic oxidation of isobutene with hydrogen peroxide and subsequent etherification with methanol. The process parameters are optimized to maximize purity and yield of MTBE (Van Grieken et al., 1998).

The worldwide production of MTBE was reported to be approximately 35 million metric tonnes in 2018 and is expected to be around 38 million metric tonnes by 2023 (Garside, 2019). MTBE is volatile and highly flammable and must be stored and transported in airtight containers (Diaz & Drogos, 2001). Its primary use (over 90% of total production) is as a fuel additive to oxygenate gasoline to increase the octane number and to reduce exhaust emissions. Other uses include chemical, petrochemical, pharmaceutical, paints and coatings. MTBE is used in small quantities as a laboratory solvent in place of diethyl ether.

2.2 Composition and secondary contaminants

MTBE is commercially available in two grades: gasoline grade (95–99% pure) and solvent grade (>99.8% pure). Depending on the route of synthesis, minor impurities may include isobutylene, methanol, 1-butylene, isopentanes and TBA.

Table 5
Physical and chemical characteristics of methyl tertiary butyl ether (MTBE)

Characteristic	MTBE
Chemical name; IUPAC systematic name	2-Methoxy-2-methyl-propane; tert-butyl methyl ether
Synonyms	*t*-Butyl methyl ether; *tert*-butoxymethane; 1,1-dimethylethyl methyl ether; methyl 1,1-dimethylethyl ether; 2-methyl-2-methoxypropane; methyl tertiary butyl ether; MTBE
CAS number	1634-04-4
Chemical structure	
Molecular formula; molecular mass	$C_5H_{12}O$; 88.15 g/mol
Description	Colourless liquid with terpene-like odour
Melting point; boiling point	−108.6 °C; 55.2 °C
Solubility	In water (at 25 °C): 48 g/L In ethanol and diethyl ether: very soluble

2.3 Reactivity and reactions with fats and oils

No information is available on the reactions of MTBE with edible fats and oils. Ethers are relatively inert chemically and would not be expected to react with edible fats and oils. MTBE, owing to the low formation of peroxides, is also unlikely to promote oxidation of edible fats and oils.

2.4 Methods of analysis

No analytical methods for the determination of MTBE and ETBE in edible fats and oils have been reported in the literature. Several test methods for the determination of MTBE, ETBE, tert-amyl methyl ether (TAME), di-iso propyl ether, methanol, ethanol and TBA in gasoline have been designated as official test methods by the American Society for Testing and Materials (ASTM). ASTM international test methods include: determination of gasoline oxygenates by infrared spectroscopy (ASTM D5845-01, 2016), gas chromatography (GC) with oxygen selective flame ionization detection (ASTM-D5599-18, 2018) and multidimensional GC (ASTM-D7754-19, 2019) (ASTM, 2016, 2018, 2019). The above methods determine MTBE and ETBE in the range of 0.1–20%. A gas chromatography–mass spectrometric (GC-MS) method for the determination of gasoline oxygenates, including MTBE, ETBE and TAME has also been reported (Hiromitsu et al., 1994).

Analytical methods for the determination of MTBE and ETBE and their degradation products in drinking-water, natural and groundwaters, soil and sludges are described in the literature. Two US EPA methods (EPA 502.2 and

524.2) are approved for measuring MTBE in drinking-water. EPA Method 502.2, which employs purge and trap GC (PT-GC) with a photoionization detector and electrolytic conductivity detector in series, has a method detection limit of 0.01 µg/L whereas EPA Method 524.2, which uses PT-GC with MS detection has a method detection limit in the range of 0.03–0.04 µg/L. The US EPA also recognizes the following methods as being equivalent to the EPA standard methods for measuring MTBE in drinking-water: ASTM standard method D5790-95 (ASTM, 2018), D7794 (ASTM, 2019); and American Public Health Association standard methods SM 6210D, SM 6200B and SM 6200C (APHA, AWWA & WEF, 1992, 1995, 1998). Health Canada has reported a detection limit of 0.06 µg/L for MTBE using a PT-GC technique based on EPA methods 524 and 624. Additional GC-MS methods for the determination of ETBE include EPA 524.3, EPA-OSW-4030C, USGS-NWQL O-4024-03 and USGS-NWQL O-4127-96; detection limits range from 0.01–0.06 µg/L.

In a review of the analysis of MTBE in natural waters and soils, sample preparation methodologies, enrichment and injection techniques were compared (Atienza et al., 2005). The analysis of MTBE and TBA in ground- and surface water was reviewed in 2003 (Torsten, 2003). Headspace PT-GC methods for the determination of MTBE in water were developed using a photoionization detector or MS (Rhodes & Verstuyft, 2001; O'Neill, Rochette & Ramsey, 2002). Solid phase micro-extraction GC-MS methods have been developed for the analysis of MTBE in surface water (Achten & Püttmann, 2000) and in groundwater (Dewsbury, Thornton & Lerner, 2003). Direct aqueous injection using GC-MS with a polar column gave comparable results to PT methods for the analysis in water (Church et al., 1997).

3. Biological data

3.1 Biochemical aspects

Several toxicokinetic studies in animals and humans have examined absorption, distribution, biotransformation and elimination of MTBE. After oral exposure, MTBE is rapidly absorbed such that the peak plasma concentration is observed as early as within 15 minutes after administration (Miller et al., 1997). Even though MTBE is soluble in water, it has a low molecular weight and is lipophilic enough to be extensively distributed in tissues. The metabolism of MTBE has been studied in animals as well as humans and the capacity of rats to metabolize MTBE is higher than that of humans. MTBE initially undergoes oxidation catalysed by the enzyme cytochrome P450 2A6 (CYP2A6) to form TBA and

formaldehyde (Hong et al., 1997, 1999; McGregor, 2006). The TBA generated is further metabolized to form first 2-methyl-1,2-propanediol and then 2-hydroxyisobutyrate; both metabolites are primarily eliminated in the urine (McGregor, 2006). TBA is also eliminated in the urine as TBA-glucuronide and, in trace amounts, as TBA-sulfate (Amberg, Rosner & Dekant, 1999; McGregor, 2006). Following exposure to MTBE, formaldehyde is not detected in significant amounts in the blood, possibly due to its rapid and spontaneous non-enzymatic conjugation with glutathione to form an adduct, *S*-hydroxymethylglutathione. Formaldehyde dehydrogenase (FDH) catalyses the NAD-dependent oxidation of this adduct to form *S*-formylglutathione, which is further converted into formic acid and reduced glutathione via hydrolysis catalysed by *S*-formylglutathione hydrolase (Uotila & Koivusalo, 1997).

An examination of the toxicokinetics of MTBE was conducted in human volunteers after exposure via different routes, including oral administration in the form of 2.8 mg MTBE in 250 mL of Gatorade (Prah et al., 2004). The maximum concentrations (C_{max}) of MTBE in the blood were in range of 0.2–0.7 μM after acute oral ingestion of about 0.2 mg/kg body weight. The elimination of MTBE was triphasic with half-lives ($t_{1/2}$) of 0.25 to 0.8 hours, 1–2 hours and 7–8 hours in humans. MTBE was metabolized to TBA such that the maximum concentration of TBA in the blood was reached within 30 minutes of MTBE administration. The $t_{1/2}$ of the generated TBA was estimated to be between 8 and 12 hours in humans.

The accumulation of MTBE and TBA in the male rat kidney due to specific binding to α2u-globulin has been examined using PBTK modelling (Borghoff, Short & Swenberg, 1990; Borghoff et al., 2001; Leavens & Borghoff, 2009; Borghoff, Parkinson & Leavens, 2010). This mechanism of renal effects is specific to male rats, and therefore not relevant to humans. The PBTK models investigated internal dosimetry of MTBE and TBA and their binding to α2u-globulin for different exposure scenarios, including oral bolus dose of MTBE and exposure to MTBE in drinking-water. The basic framework of the PBTK models consisted of brain, fat (adipose tissue), gastrointestinal tissues, kidney, liver, poorly perfused tissues and rapidly perfused tissues (Borghoff, Murphy & Medinsky, 1996; Leavens & Borghoff, 2009; Borghoff, Parkinson & Leavens, 2010). The PBTK models assumed that the distribution of MTBE and TBA in all tissues was blood-flow limited and the metabolism of MTBE occurred only in the liver following Michaelis-Menten kinetics of two pathways catalysed by different CYPs of varying affinities (Borghoff, Murphy & Medinsky, 1996; Hong et al., 1997). These models also assumed that TBA metabolism occurred only in the liver as a single, low-affinity pathway and was induced following repeated exposures (McComb & Goldstein, 1979; Borghoff, Murphy & Medinsky, 1996). Furthermore, the PBTK models included binding of both MTBE and TBA to α2u-globulin as a saturable and competitive process and assumed that the α2u-

globulin binding affects the rate at which it is hydrolysed in the kidney of the male rat (Leavens & Borghoff, 2009; Borghoff, Parkinson & Leavens, 2010).

A recent PBTK model by Borghoff, Parkinson & Leavens (2010) evaluated internal dosimetry of MTBE and TBA after MTBE exposure via inhalation versus orally in drinking-water. The model predicted that the concentration versus time profiles of MTBE and TBA in blood of different strains of rats (Sprague-Dawley, Fischer-344, Wistar Han) following exposure via inhalation and the oral route in drinking-water were similar. Furthermore, the model predicted that the total blood and kidney levels after continuous MTBE exposure at 7.5 mg/mL in drinking-water for 90 days were in the same range as administering an oral bolus dose of 1000 mg/kg of MTBE. However, from the animal studies, the dose metrics of MTBE and TBA in the kidneys appeared to be higher after continuous exposure to MTBE in drinking-water at 7.5 mg/mL for 90 days than following a single high-dose bolus of 1000 mg/kg of MTBE. The oral bolus dose of MTBE resulted in a greater percentage of MTBE being exhaled and a lower percentage of MTBE being metabolized to TBA as compared with a continuous dose of MTBE administered in drinking-water for 90 days.

3.2 Toxicological studies

3.2.1 Acute toxicity

MTBE has a low potential for acute toxicity with reported oral LD_{50} values of 3866 mg/kg bw in rats (ARCO, 1980) and about 4000 mg/kg bw in mice (ECETOC, 2003). The most typical symptoms are hypoactivity and decreased muscle coordination (McGregor, 2006).

3.2.2 Short-term studies of toxicity

In general, the principal organs affected in rodents following oral exposure to MTBE are the liver and the kidney. Several short-term and subchronic oral toxicity studies of MTBE have been reported. Findings common to most of these studies included effects on kidney weights and kidney morphology in the form of hyaline droplets in renal proximal tubule cells. These were potentially attributed to binding of MTBE and/or TBA to α2u-globulin in the kidneys of male rats (Robinson, Bruner & Olson, 1990; IITRI, 1992; Williams, Cattley & Borghoff, 2000). As stated above, the Committee considered this mechanism to be male-rat-specific and, therefore, such renal effects are not relevant for assessing human health risk. The short-term and subchronic oral toxicity studies of MTBE reviewed are discussed below.

Oral toxicity

I. *Fourteen-day oral toxicity study*: Robinson, Bruner & Olson (1990) studied male and female Sprague-Dawley rats (10 males and 10 females per group) treated with MTBE (with corn oil as the vehicle). MTBE was administered via oral gavage for 14 consecutive days at doses of 0, 357, 714, 1071 and 1428 mg/kg bw per day. Profound anaesthesia was reported in males and females immediately after dosing with 1428 mg/kg bw per day of MTBE. Significantly reduced body weight gain was reported in females treated with 1428 mg/kg bw per day of MTBE. Males treated with 714, 1071 and 1428 mg/kg bw per day of MTBE were reported to have increased haemoglobin values in the red blood cell compartment. Serum glucose, creatinine and blood urea nitrogen (BUN) was significantly higher in females treated with 1428 mg/kg bw per day of MTBE. Males showed statistically significant increases in aspartate transaminase (AST) and lactate dehydrogenase (LDH) after treatment with 1071 and 1428 mg/kg bw per day, and in BUN and cholesterol after treatment with 1428 mg/kg bw per day of MTBE. Females showed a significant reduction in lung weight at all doses tested. Males treated with MTBE showed significant increases in kidney weights at doses of 1071 and 1428 mg/kg bw per day, and in liver weights at the dose of 1428 mg/kg bw per day. Protein droplet nephropathy was pronounced in male rats treated with 1428 mg/kg bw per day of MTBE, and degenerative changes were characterized by hyaline droplet formation in proximal tubule epithelial cells.

II. *Twenty-eight-day oral toxicity study* (IITRI, 1992): A 28-day oral (gavage) toxicity (good laboratory practice (GLP)-compliant) study of MTBE (with reverse osmosis purified water as the vehicle) was conducted in rats. Male and female Sprague-Dawley rats (10 per sex and dose) were given 0, 90, 440 or 1750 mg/kg bw per day of MTBE. No treatment-related mortality or changes in body weights were reported. In animals in the middle and high-dose groups, salivation, hypoactivity and ataxia were observed immediately after dosing. The only statistically significant change in clinical chemistry parameters was increased cholesterol in males and females in the high-dose group. Statistically significant increases in relative kidney weight in males given the middle and high doses, and females given the low and high doses, were reported. Statistically significant relative liver weight increases in males and females in the high-dose group, and in relative adrenal weight in males given the high dose were also reported.

Microscopic kidney lesions consisting of hyaline droplet formation in the proximal convoluted tubules were observed in males in the middle and high-dose groups, probably attributable to binding to α2u-globulin. Microscopic lesions in the forestomach of both males and females given the high dose were observed; these were attributed to localized irritation.

III. *Twenty-eight-day oral toxicity study* (Williams, Cattley & Borghoff, 2000): A 28-day oral (gavage) toxicity study of MTBE (with corn oil as the vehicle) was conducted on male Sprague-Dawley rats (15 per dose) administered doses of 0, 250, 500, 1000 and 1500 mg/kg bw per day. The purpose of this study was to examine the effect of MTBE exposure on the levels of specific hormones involved in the maintenance of the hypothalamus-pituitary-testicular axis. Relative testes weights were significantly increased in animals in the high-dose group. Dose-related increases in relative liver and kidney weights were reported in animals given doses of 1000 and 1500 mg/kg bw per day, and 250, 1000 and 1500 mg/kg bw per day, respectively. No treatment-related lesions were observed in the testis or adrenal glands; however, centrilobular hypertrophy in the liver (1/15, 10/15, 11/13 and 11/11 in the 250, 500, 1000 and 1500 mg/kg bw per day groups, respectively) and protein droplet nephropathy in the kidney (12/15, 15/15, 12/13 and 10/11 in the 250, 500, 1000 and 1500 mg/kg bw per day groups, respectively) were reported at all doses tested. Significant decreases in triiodothyronine (T3) levels in the rats treated with 1000 and 1500 mg/kg bw per day of MTBE and in luteinizing hormone (LH) and dihydrotestosterone (DHT) levels in rats treated with 1500 mg/kg bw per day of MTBE were reported.

Subchronic oral toxicity

I. *Ninety-day oral toxicity study* (Robinson et al., 1990): The study by Robinson et al. (1990) referred to above (see point I) also included a subchronic (90-day) component. In this study, male and female Sprague-Dawley rats (10 males and 10 females per group) were treated daily with MTBE (with corn oil as the vehicle). MTBE was administed via oral gavage for 90 consecutive days at doses of 0, 100, 300, 900 and 1200 mg/kg bw per day. Both males and females treated with 1200 mg/kg bw per day showed profound anaesthesia immediately following dosing and all treated rats had diarrhoea throughout the duration of the study. There was no mortality in the control animals, but 11 treated rats had died by the end of the study with causation

attributed to gavage error. A statistically significant reduction in body weight was reported in females treated with 1200 mg/kg bw per day of MTBE. Statistically significant increases in red blood cell and white blood cell counts, haemoglobin and haematocrit were observed in males, and in monocyte percentage and mean corpuscular volume in females treated with 1200 mg/kg bw per day of MTBE. BUN levels were significantly decreased in both males and females in all treated groups. Males treated with 900 mg/kg bw per day of MTBE exhibited significantly elevated cholesterol, and males that received 300 and 1200 mg/kg bw per day of MTBE showed a significant increase in AST. Chronic nephropathy in the kidney was severe in rats treated with 1200 mg/kg bw per day of MTBE and tubular degenerative changes were observed. Furthermore, 5 of 10 males treated with 1200 mg/kg bw per day of MTBE also had an increased number of tubules with granular cysts and increased hyaline droplets in proximal tubular epithelial cells. Relative kidney weights were significantly higher both in males treated with 900 and 1200 mg/kg bw per day and in females treated with 300, 900 and 1200 mg/kg bw per day of MTBE. Females that received 1200 mg/kg bw per day of MTBE showed significantly higher relative adrenal weights. Relative liver weights were also significantly higher in males treated with 900 and 1200 mg/kg bw per day of MTBE. Females treated with 900 mg/kg bw per day of MTBE showed significantly higher relative liver weight; however, the relative liver weight did not differ significantly between the females treated with 1200 mg/kg bw per day of MTBE and the control group.

The Committee noted the statistically significant increases in relative kidney weight reported in females treated with MTBE at doses of 300 mg/kg bw per day and higher. However, no changes in clinical chemistry parameters or microscopic observations in the kidney were reported. The Committee also considered that no significant increases in kidney weights were observed in female Sprague-Dawley rats orally exposed to MTBE at 250 mg/kg bw per day and 1000 mg/kg bw per day in the 104-week chronic toxicity and carcinogenicity study (Belpoggi et al., 1995; 1998), discussed in section 3.2.3. In contrast, the increases in the relative liver weights reported in males in the study by Robinson et al. (1990) were accompanied by elevated cholesterol levels. Therefore, based on statistically significant increases in the relative liver weights and elevated cholesterol levels in males, the Committee identified a NOAEL of 300 mg/kg bw per day from this study (Robinson et al., 1990).

II. *Ninety-day oral toxicity study* (Zhou & Ye, 1999): A 90-day oral (gavage) toxicity study of MTBE (with soybean oil as the vehicle) was conducted on male Sprague-Dawley rats given doses of 0, 200, 600 and 1000 mg/kg bw per day, administered 5 days a week for 90 days. No treatment-related changes in body weights, food and water consumption were reported, and only rats treated with 1000 mg/kg bw per day showed signs of ataxia in the first 2 weeks of treatment. Both absolute and relative liver weights were significantly elevated at all doses tested. Furthermore, absolute and relative kidney weights were significantly increased in rats treated with 600 and 1000 mg/kg bw per day. Although increases in AST and LDH levels were reported in animals in some treatment groups, these values were within the normal range. No apparent pathological findings were noted after light-microscopic examination of samples from MTBE-treated animals and controls. However, electron microscopic examination of liver cells from all MTBE-treated groups revealed cell nuclear condensation, fat droplet and lysosome appearance in cells and smooth endoplasmic reticulum (SER) disintegration, and these observations were reported to be dose-related. No such observations in the liver cells were reported after electron microscopic examination of samples from the control group. Furthermore, no changes in liver function or hormone levels were reported that could be correlated with the hypertrophic changes in the liver at any of the MTBE doses tested. Moreover, hypertrophy and subsequent weight increase with changes in SER have been typically suggested as adaptive responses of the liver, and not necessarily indicators of functional or anatomical liver impairment or an adverse effect, particularly in the absence of data that would indicate an association with or progression to altered liver function (Hall et al., 2012). No toxic effects or carcinogenic incidences in the liver were reported in the oral chronic toxicity and carcinogenicity study of MTBE in Sprague-Dawley rats, described below (Belpoggi et al., 1995, 1998). Therefore, the Committee concluded that the microscopic changes observed in the liver upon oral subchronic exposure to MTBE in this study (Zhou & Ye, 1999) may not progress to long-term adverse effects in the liver following chronic exposure, and are therefore likely to be adaptive responses.

3.2.3 Long-term studies of toxicity and carcinogenicity

Oral carcinogenicity studies conducted on MTBE and its major metabolite, TBA, were reviewed to evaluate the risk for carcinogenicity associated with MTBE exposure.

A 104-week oral (gavage) chronic toxicity and carcinogenicity assay of MTBE (with olive oil as the vehicle) was conducted on male and female Sprague-Dawley rats (60 per sex per group) at doses of 0, 250 and 1000 mg/kg bw per day (Belpoggi et al., 1995, 1998). The MTBE dose or vehicle was administered on all days except Wednesdays and weekends. No treatment-related clinical signs, changes in body weight, or changes in water or food consumption were reported. Survival rates in males were similar in all dose groups up until week 88 after which the rate was 50%. Between weeks 104 and 136, the males in the high-dose group had survival rates 10–20% higher than those in the group treated with 250 mg/kg bw per day of MTBE and the control group. The survival rates in females at week 88 were approximately 78%, 60% and 40% for the groups treated with 0, 250 and 1000 mg/kg bw per day of MTBE, respectively. At week 120, the survival rates varied between 25% and 20%. Increased incidences of lymphoimmunoblastic dysplasia were reported in female rats (5%, 26.7% and 20% in the groups that received 0, 250 and 1000 mg/kg bw per day, respectively) and in lymphoma and leukaemia (3.3%, 11.7% and 20% in the groups treated with 0, 250 and 1000 mg/kg bw per day of MTBE, respectively). In male rats, an increased incidence of interstitial cell hyperplasia of the testes (focal + diffuse/focal) was reported at the MTBE doses tested (6.7%/25%, 13.3%/37.5% and 15%/2.2% in the groups treated with 0, 250 and 1000 mg/kg bw per day of MTBE, respectively). A significant increase in the incidence of interstitial cell adenomas in the testes or Leydig cell tumours (LCTs) was reported in males given 1000 mg/kg bw per day (18.3%) of MTBE compared to the control group (3.3%). No adverse effects were detected in the proximal tubules of the kidneys of animals in the treated groups, which is a pathological observation commonly reported in most of the short-term and subchronic oral toxicity studies on MTBE that were reviewed. Owing to deficiencies of the toxicological study design and statistical methods, previous evaluations (IPCS/WHO, 1998; IARC, 1999) reported low confidence in the data from this study. Chronic toxicity and carcinogenicity assays (Bird et al., 1997) of the effects of inhalation of MTBE on rodents also reported incidences of kidney tumours, LCTs and parathyroid gland adenoma in male Fischer-344 rats, and hepatocellular adenoma and endometrial cell (cystic) hyperplasia in female CD-1 mice. The potential underlying mechanisms of kidney tumours observed in male rats are not relevant to humans. Furthermore, the tumours in rodents were observed at very high doses of MTBE and only following exposure via the oral or inhalation route. Human exposure to such high doses of MTBE is not

expected to occur. Therefore, the tumour incidences reported in rodents after chronic exposure to MTBE were not considered relevant for assessing the human health risk of exposure to MTBE from previous cargoes.

A 2-year oral carcinogenicity assay of TBA in drinking-water was conducted in mice and rats. Male Fischer 344 rats were given doses of 0, 1.25, 2.5 and 5 mg/mL (equal to 0, 85, 195 and 420 mg/kg bw per day) and females received doses of 0, 2.5, 5 and 10 mg/mL (equal to 0, 175, 330 and 650 mg/kg bw per day). Male and female B6C3F1 mice were given doses of 0, 5, 10 and 20 mg/mL (equal to 0, 535, 1035 and 2065 mg/kg bw per day for males and 0, 510, 1015 and 2105 mg/kg bw per day for females) (NTP, 1995). Groups of male rats treated with doses ≥ 2.5 mg TBA/mL (≥ 195 mg/kg bw per day) showed increased incidence of renal adenoma and carcinoma; however, these effects are likely to be a result of α2u-globulin nephropathy; an end-point with no relevance to humans, as stated above. In contrast, renal tumours were not reported in male or female mice. An increased incidence of thyroid follicular cell adenoma was reported in male and female mice at all TBA doses; however, no clear dose-dependent relationships between these tumour incidences and TBA concentrations could be established when considering statistical significance versus controls and/or comparison with the historical control data from control mice.

Based on data indicating a lack of human or toxicological relevance of the type and severity of tumour incidences observed in rodents in the chronic toxicity and carcinogenicity studies of MTBE, and its lack of genotoxic potential, IARC (IARC, 1999) concluded that there is inadequate evidence for carcinogenicity of MTBE in humans, with limited evidence for carcinogenicity in experimental animals, and therefore classified MTBE in Group 3.

3.2.4 Genotoxicity

The genotoxic potential of MTBE has been evaluated using various in vitro as well as in vivo genetic toxicity assays some of which are summarized in Table 6. MTBE showed negative responses under the testing conditions of most of the studies reviewed. Furthermore, TBA, the major metabolite of MTBE, has been reported to be non-genotoxic (NTP, 1995).

MTBE showed negative responses in most of the in vitro studies of genetic toxicity reviewed. A positive result in an unscheduled DNA synthesis assay conducted in primary rat hepatocytes was reported at a dose of 1000 µg/mL of MTBE (Zhou et al., 2000). However, other unscheduled DNA synthesis assays (Life Science Research, 1989b; Cinelli et al., 1992) conducted in primary rat hepatocytes reported negative responses to MTBE when tested up to doses of 10 000 µg/mL. Furthermore, all genetic toxicity studies conducted using in vivo systems, including an in vivo unscheduled DNA synthesis assay conducted in

Table 6
Summary of some in vitro and in vivo genetic toxicity studies on MTBE

Test system and testing conditions	MTBE treatment	Result	Reference
Studies based on in vitro systems			
Gene mutation in mouse lymphoma cells (*L5178Y TK*$^{+/-}$; ±S9; + FDH and NAD)	1–4 µL/mL for 3 hours	Positive (but negative with FDH + NAD)	Mackerer et al., 1996
Gene mutation in hypoxanthine-guanine phosphoribosyl transferase (HPRT)-locus Chinese hamster V79 cells	625–10 000 µg/mL; 313–2500 µg/mL with S9 for 6 days	Negative	Life Science Research, 1989a
Gene mutation in HPRT-locus Chinese hamster V79 cells	1250–10 000 µg/mL	Negative	Cinelli et al., 1992
Unscheduled DNA synthesis in primary rat hepatocytes	3.16–10 000 µg/mL for 17 hours ± 30 minutes	Negative	Life Science Research, 1989b
Unscheduled DNA synthesis in primary rat hepatocytes	1000–10 000 µg/mL	Negative	Cinelli et al., 1992
Unscheduled DNA synthesis in primary rat hepatocytes	200–1000 µg/mL for 3 hours	Positive	Zhou et al., 2000
Chromosome aberration in Chinese hamster ovary cells (±S9)	0.004–5 µl/mL (–S9); 0.004–1 µl/mL (+S9)	Negative	Litton Bionetics Inc., 1980
Sister chromatid exchange in Chinese hamster ovary cells (95% MTBE)	0.004–5 µl/mL (–S9); 0.004–1 µl/mL (+S9)	Negative	Litton Bionetics Inc., 1980
Sister chromatid exchange in Chinese hamster ovary cells (99% MTBE)	0.004–5 µl/mL (–S9); 0.004–1 µl/mL (+S9)	Equivocal	Litton Bionetics Inc., 1980
Mouse micronucleus with 3T3 cells (98.8% MTBE)	5, 10, 20 µl/mL	Negative	Zhou et al., 2000
Studies based on in vivo systems			
Sex-linked recessive lethal test using *Drosophila melanogaster*	0.03–3% in sucrose	Negative	Sernau, 1989
Unscheduled DNA synthesis in CD-1 mouse hepatocytes	400, 3000 and 8000 ppm (inhalation) for 2 days, 6 hours/day	Negative	McKee et al., 1997
Gene mutation in HPRT-locus spleen lymphocytes	1, 10, 100 and 1000 mg/kg	Negative	Ward et al., 1995
Micronucleus test in CD-1 erythrocytes	400, 3000 and 8000 ppm (inhalation) for 2 days, 6 hours/day	Negative	McKee et al., 1997
Micronucleus test in Swiss Webster mice erythrocytes	250, 500, 1000, 1500 and 1750 mg/kg (intraperitoneal, single dose)	Negative	Kado et al., 1998
Bone marrow cytogenetic test in Fischer-344 rats	800, 3000 and 5000 ppm (inhalation) for 5 days, 6 hours/day	Negative	McKee et al., 1997
Chromosome aberration in bone marrow of Sprague-Dawley rats	0.014, 0.13 and 0.4 mL/kg (intraperitoneal; single or 5 doses/5 days)	Negative	Litton Bionetics Inc., 1979
Comet assay on rat lymphocytes	40, 400, 800 mg/kg (oral)	Negative	Lee, Quintana & de Peyster, 1998

hepatocytes isolated from CD-1 mice treated with MTBE at doses of 400, 3000 and 8000 ppm via inhalation (6 hours a day for 2 consecutive days) (McKee et al., 1997), reported negative responses.

3.2.5 Reproductive and developmental toxicity

The potential for reproductive toxicity of oral MTBE has been evaluated in male mice and rats following subacute exposure. A study of oral exposure to MTBE examined the effects of doses of 400 to 2000 mg/kg bw per day on the reproductive system of male CD-1 mice (de Peyster et al., 2008). MTBE was administered to the mice on days 1, 3 and 5. Animals were injected intraperitoneally with human chorionic gonadotrophin (hcG; 2.5 IU/g) on day 6 and necropsied on day 7. No effects on testis histology or testosterone levels were reported. As part of the same study, adult male BALB/c mice were exposed for 28 days and juvenile male BALB/c mice for 51 days, until postnatal day (PND) 77, to MTBE in drinking-water at concentrations of 80 to 8000 μg/mL (ppb). No significant treatment-related changes in the male reproductive system or signs of hepatic oxidative stress were reported.

A subacute (2- or 4-week) oral gavage study of the effects of exposure to MTBE on the reproductive system of male Sprague-Dawley rats was conducted at doses of 0, 400, 800 and 1600 mg/kg bw per day (Li et al., 2008). The adverse effects included a significant increase in the percentage of abnormal sperm, an irregular and disordered arrangement of the seminiferous epithelium identified by histopathological examination, changed serum levels of testosterone, LH, follicle stimulating hormone (FSH), and decreased levels of androgen-binding protein reported mainly in the high-dose groups.

The Committee did not identify any reports of studies examining the potential for reproductive and developmental toxicity as a result of exposure to MTBE in male and female animals.

3.2.6 Allergenicity

The Committee found no reports on MTBE that indicated that it elicits an allergic response upon oral exposure. There are also no data available that indicate that it contains a known food allergen.

MTBE is considered an irritant to the skin and ECHA has classified MTBE under skin irritant category 2. Acute effects such as headache, nasal and ocular irritation have been reported after inhalation exposure to gasoline containing MTBE. A study examining the effects on 10 healthy male volunteers of exposure to MTBE vapours for 2 hours at doses of 5, 25 and 50 ppm reported minimal acute effects of MTBE exposure (Nihlén et al., 1998).

3.2.7 Impurities

The Committee noted that impurities, namely methanol, isobutylene, 1-butylene, isopentanes and TBA may be expected in MTBE. As MTBE products are of high purity, the percentage contribution of these impurities to the total composition of

the substance is minor. These impurities were non-genotoxic and animal studies on oral exposure to these impurities did not report any tumour incidences of human or toxicological relevance. The Committee also identified no reports that indicated that these impurities elicit an allergic response upon oral exposure or would contain a known food allergen. Therefore, the Committee concluded that these compounds would not be expected to cause any adverse health effects at their anticipated levels of exposure as minor impurities in MTBE.

3.3 Observation in humans

MTBE has been used clinically for dissolution of gallstones by instillation through a transhepatic (Allen et al., 1985; Thistle et al., 1989; Leuschner et al., 1991; Janowitz et al., 1993) or nasobiliary catheter (Murray, LaFerla & Fullarton, 1988; Neoptolemos et al., 1990). However, the authors did not report any adverse effects of the treatment. Minimal acute effects of acute inhalation exposure to MTBE vapours at doses of up to 50 ppm were observed in humans (Nihlén et al., 1998a), as stated above (section 3.2.6).

4. Levels and patterns of contamination in food commodities

No data were found on concentrations of butyl ethers in food oils due to carryover from previous cargoes. A worst-case concentration of 100 mg/kg has been assumed for all previous cargo substances. Worst-case exposures to previous cargo substances in food oils have been estimated at 0.3 mg/kg bw per day, based on a worst-case concentration of 100 mg/kg and an oil intake of 3 g/kg bw per day by infants and young children who are high consumers (see part A, section 4.3).

MTBE has been found in drinking-water in areas where MTBE-containing gasoline has leaked into groundwater aquifers and surface waters (Johnson et al., 2000; Achten et al., 2002; Klinger et al., 2002; Morgenstern et al., 2003; Moran, Zogorski & Squillace, 2005; Tanabe et al., 2005; Kolb & Püttmann, 2006 a,b; van Wezel et al., 2009; Levchuk, Bhatnagar & Sillanpaa, 2014; Zhang et al., 2016). Most of the available data on MTBE concentrations in drinking-water were generated prior to regional bans or limitations on use of MTBE in gasoline. However, MTBE is still found in some drinking-water sources, and potential exposure to MTBE from drinking-water must therefore be considered as part of total dietary exposure to MTBE. WHO has not set a guideline value for MTBE in drinking-water, because its presence in drinking-water is associated

with objectionable odours and taste (van Wezel et al., 2009), and WHO determined that any guideline value would be well above the concentration at which MTBE could be detected by taste and odour (Fawell, 2007). The proposed advisory thresholds for MTBE in drinking-water in Europe range from 7–16 µg/L (van Wezel et al., 2009). The US EPA has not set a national standard for MTBE, but in 1997 it issued a Drinking Water Advisory stating that concentrations of MTBE in the range of 20 to 40 µg/L of water or below will probably not cause unpleasant taste and odour for most people. Various US states have set drinking-water standards of up to 60 µg MTBE/L ((California State Water Resources Control Board (2014), Minnesota Environmental Health Division (2013) and New Hampshire Department of Environmental Services (2020)).

5. Food consumption and dietary exposure assessment

Maximum mean and p95 daily drinking-water intakes by adults, based on data in the US EPA *Exposure factors handbook*, are 22.9 mL/kg bw per day and 47 mL/kg bw per day, respectively. EFSA estimated adequate intakes of drinking-water of 4 L at the p95 for adult males (EFSA, 2010); this is equivalent to 67 mL/kg bw per day based on a 60 kg body weight. The estimated chronic daily exposure to MTBE from consumption of 67 mL/kg bw per day of drinking-water containing 40 µg MTBE/L (the maximum level likely not to be associated with unpleasant odours or taste) would therefore be 0.003 mg/kg bw for adults.

For infants and toddlers, the maximum mean and p95 daily drinking-water intakes, based on data in the US EPA *Exposure factors handbook*, are 79 mL/kg bw per day and 174 mL/kg bw per day, respectively. EFSA estimated adequate intakes of drinking-water of up to 190 mL/kg bw per day for infants (EFSA, 2010). The estimated maximum daily MTBE exposure for infants and young children, assuming consumption of 190 mL/kg bw per day of drinking-water containing 40 µg/L of MTBE is 0.008 mg/kg bw per day.

Therefore, the estimated exposure to MTBE from drinking-water is considered to be a minor contributor as compared with potential exposure from MTBE in food oil commodities from previous cargoes.

No other potential sources of dietary exposure to MTBE were identified.

6. Comments

6.1 Chemical and technical considerations

The chemical and technical considerations for MTBE are summarized in Table 7.

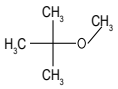
6.2 Biochemical aspects

Following oral exposure, MTBE is rapidly absorbed such that the peak plasma concentration is observed as early as within 15 minutes after administration (Miller et al., 1997). Even though MTBE is soluble in water, it has a low molecular weight and is lipophilic enough to be extensively distributed in human tissues. MTBE initially undergoes oxidation catalysed by the enzyme CYP2A6 to form TBA and formaldehyde (Hong et al., 1997, 1999; McGregor, 2006). The elimination of MTBE in humans after acute oral exposure is triphasic with elimination half-lives ($t_{1/2}$) of 0.25 to 0.8 hours, 1–2 hours and 7–8 hours (Prah et al., 2004). The $t_{1/2}$ of the generated TBA ranges from 8 to 12 hours (Prah et al., 2004). TBA is further metabolized, first to form 2-methyl-1,2-propanediol and then 2-hydroxyisobutyrate; both metabolites are primarily eliminated in the urine (McGregor, 2006). TBA is also eliminated in the urine as TBA-glucuronide and, in trace amounts, as TBA-sulfate (Amberg, Rosner & Dekant, 1999; McGregor, 2006). Formaldehyde is not detected in significant amounts in the blood upon exposure to MTBE, possibly owing to its rapid and spontaneous non-enzymatic conjugation with glutathione to form an adduct, *S*-hydroxymethylglutathione, followed by hydrolysis to formic acid and reduced glutathione catalysed by *S*-formylglutathione hydrolase (Uotila & Koivusalo, 1997). The Committee also reviewed studies that described application of PBTK modelling for investigating the accumulation of MTBE and TBA in the male rat kidney due to specific binding to α2-globulin and its association with the incidence of male rat-specific renal tumours, α2-globulin nephropathy and similar effects in the kidneys (Borghoff, Short & Swenberg, 1990; Borghoff et al., 2001; Leavens & Borghoff, 2009; Borghoff, Parkinson & Leavens, 2010). However, the Committee considered this mechanism of renal effects as male-rat-specific, and, therefore, as not relevant to humans.

6.3 Toxicological studies

There are extensive toxicological datasets on MTBE exposure via different exposure routes. The Committee evaluated relevant toxicological data on oral

Table 7
Chemical and technical considerations for methyl tertiary butyl ether (MTBE)

Name: methyl tertiary butyl ether (2-methoxy-2-methylpropane)	
CAS number	**Alternative CAS numbers**
1634-04-4	None
Chemical details	*t*-Butyl methyl ether, *tert*-butoxymethane, 1,1-dimethylethyl methyl ether, MTBE Colourless liquid with terpene-like odour  Molar mass: 88.15 g/mol Melting point: −108.6 °C Boiling point: 55.2 °C Solubility in water (25 °C): 48 g/L; very soluble in ethanol and diethyl ether
Route(s) of synthesis	Manufactured by reacting isobutylene with methanol in the presence of acidic ion-exchange resin catalysts or strong acids. An alternative two-step route of synthesis includes catalytic oxidation of isobutene with hydrogen peroxide followed by etherification with methanol.
Composition	Commercially available in two grades: gasoline grade (95–99% pure) and solvent grade (>99.8% pure); minor impurities may include methanol, isobutylene, 1-butylene, isopentanes and tertiary butyl alcohol.
Uses	Used to oxygenate gasoline to improve octane number. Minor uses include chemicals, petrochemicals, pharmaceuticals, paints and coatings.
Analytical methods	None reported for previous cargoes. Potential methods for its determination in fats and oils include head space, purge and trap or solid-phase micro-extraction techniques coupled with GC-FID/PID/ELCD or GC-MS.
Potential reaction(s) with a subsequent cargo of fat or oil	MTBE is not expected to react with edible fats and oils to form any reaction products.

GC-FID/PID/ELCD, gas chromatography with flame ionization detection/photo-ionization detection/electrolytic conductivity detection; GC-MS, gas chromatography–mass spectrometry.

exposure to MTBE in animals and humans, and from genetic toxicity studies conducted with MTBE using in vitro and in vivo systems.

The Committee concluded that the potential for acute toxicity of MTBE after oral exposure is low based on reported oral LD_{50} values of 3866 mg/kg bw in rats (ARCO, 1980) and about 4000 mg/kg in mice (ECETOC, 2003). The Committee reviewed the available short-term and subchronic oral toxicity studies of MTBE and noted that some common findings between most studies included effects on kidney weights and kidney morphology in the form of hyaline droplets in renal proximal tubule cells (Robinson, Bruner & Olson 1990; IITRI, 1992; Williams, Cattley & Borghoff, 2000). As these effects can be attributed to binding of MTBE and/or TBA to α2u-globulin in the kidneys of male rats, as stated above, the Committee considered this mechanism to be male-rat-specific and not relevant to humans.

The Committee reviewed a 90-day oral toxicity study of MTBE conducted in male and female Sprague-Dawley rats at doses of 0, 100, 300, 900 and 1200 mg/kg bw per day (Robinson, Bruner & Olson, 1990). It noted some treatment-related effects in the kidney and the liver. These included statistically significant increases in relative kidney weights in females treated with 300, 900 and 1200 mg/kg bw per day of MTBE and in the relative liver weights in males treated with 900 mg/kg bw per day and 1200 mg/kg bw per day. The Committee also noted that no changes in clinical chemistry parameters or microscopic observations in organs were reported with relative weight increases in the kidneys of females. The Committee considered that no significant increases in kidney weights were observed in female Sprague-Dawley rats following oral exposure to MTBE at 250 mg/kg bw per day and 1000 mg/kg bw per day in the 104-week chronic toxicity and carcinogenicity study discussed below (Belpoggi, Soffritti & Maltoni, 1995, 1998). In contrast, the increases in the relative liver weights reported in males in the study by Robinson et al. were accompanied by elevated cholesterol levels (Robinson, Bruner & Olson, 1990). Therefore, based on statistically significant increases in the relative liver weights and elevated cholesterol levels in males, the Committee identified a NOAEL of 300 mg/kg bw per day from this study (Robinson, Bruner & Olson, 1990).

The Committee also reviewed another 90-day oral toxicity study of MTBE, conducted in male rats only, at dose levels of 200, 600 and 1000 mg/kg bw per day, which reported significant increases in absolute and relative liver weights, and dose-related microscopic findings in the liver were reported at all tested doses (Zhou & Ye, 1999). The Committee considered the reported hypertrophic changes in the liver and subsequent weight increases observed in this study as adaptive responses and not adverse findings, particularly in the absence of data that would indicate an association with altered liver function or progression to long-term adverse effects (Zhou & Ye, 1999).

The Committee evaluated the potential for reproductive toxicity of oral MTBE exposure based on two reproductive studies, both of which looked at the effects of subacute MTBE exposure on the male reproductive system in rodents. The first study did not report any treatment-related effects of oral MTBE exposure in mice (de Peyster, 2008). In contrast, the second study reported some treatment-related effects on the male reproductive system, including a significant increase in the percentage of abnormal sperm, an irregular and disordered arrangement of the seminiferous epithelium indicated by a histopathological examination, changed serum levels of testosterone, LH, FSH and decreased levels of androgen binding protein in the high-dose groups (Li et al., 2008). However, the Committee noted that these effects were mainly reported in the high-dose groups. The Committee did not identify any studies examining the potential for

reproductive or developmental toxicity in response to oral exposure to MTBE in both male and female animals.

The Committee concluded that MTBE is not genotoxic based on negative responses reported in most of the in vivo and in vitro genetic toxicity studies reviewed, and genotoxicity data on TBA, its major metabolite, reporting that TBA is non-genotoxic (NTP, 1995).

The Committee reviewed a 104-week oral (gavage) chronic toxicity/carcinogenicity assay of MTBE conducted on male and female rats at dose levels of 0, 250 and 1000 mg/kg bw per day (Belpoggi, Soffritti & Maltoni, 1995, 1998). It also reviewed a 2-year oral carcinogenicity assay of TBA in drinking-water in male and female rats (0, 1.25, 2.5 and 5 mg/mL equal to 0, 90, 200 and 420 mg/kg bw per day in males and 0, 2.5, 5 and 10 mg/mL equal to 0, 180, 330 and 650 mg/kg bw per day in females) and mice (0, 5, 10 and 20 mg/mL equal to 0, 540, 1040 and 2070 mg/kg bw per day in males and 0, 510, 1020 and 2110 mg/kg bw per day in females) (NTP, 1995) to evaluate the potential for carcinogenicity of MTBE following oral exposure. Both reported some tumour incidences; however, the Committee noted that the observed tumour incidences in rodents lacked human or toxicological relevance, and these effects were reported at exposure levels much higher than those expected from oral exposure to MTBE as a previous cargo.

The Committee noted that MTBE has been used clinically for dissolution of gallstones by instillation through a transhepatic or nasobiliary catheter (Allen et al., 1985; Janowitz et al., 1993; Thistle et al., 1989; Leuschner et al., 1991; Murray, LaFerla & Fullarton, 1988; Neoptolemos et al., 1990). However, no adverse effects of the treatment were reported in the studies reviewed.

6.4 Allergenicity

The Committee did not identify any reports that indicated that MTBE elicits an allergenic response upon oral exposure. There are also no data available that indicate that MTBE would contain a known food allergen.

6.5 Impurities

The Committee noted that impurities, namely methanol, isobutylene, 1-butylene, isopentanes and TBA may be expected in MTBE. As MTBE products are of high purity, the percentage contribution of these impurities to the total composition of the substance is minor. The Committee noted that these impurities were non-genotoxic and studies of oral exposure to these impurities in animals did not report tumour incidences of human or toxicological relevance. The Committee also did not identify any reports that indicated that these impurities elicit an

allergenic response upon oral exposure or would contain a known food allergen. Therefore, the Committee concluded that these compounds would not be expected to cause any adverse health effects at their anticipated levels of exposure as minor impurities in MTBE.

6.6 Food consumption and dietary exposure estimates

A worst-case concentration of 100 mg/kg has been assumed for all previous cargo substances (see Part A, section 4.3). MTBE has been found in drinking-water in areas where MTBE-containing gasoline has leaked into groundwater aquifers and surface waters. The worst-case exposures to previous cargo substances in food oils have been estimated as 0.3 mg/kg bw per day (see part A, section 4.3). The maximum daily MTBE exposure from drinking-water was estimated to be 0.008 mg/kg bw per day based on drinking-water consumption of 190 mL/kg bw per day by infants and children (the maximum estimated by EFSA, 2010), and an MTBE concentration of 40 µg/L (the maximum level the US EPA determined would not have an unpleasant taste or odour (US EPA, 1997)).

7. Evaluation

Upon evaluating the available toxicity studies and examining the toxicological relevance of the effects reported, the Committee considered that the NOAEL of 300 mg/kg bw per day identified from the 90-day oral subchronic study of MTBE in rats was the most appropriate RP (Robinson, Bruner & Olson, 1990). The Committee concluded that the estimated exposure to MTBE from drinking-water is a minor contributor (0.008 mg/kg bw per day) as compared with the estimated exposure to MTBE in food oil commodities from previous cargoes (0.3 mg/kg bw per day), and that there are no other known potential sources of dietary exposure to MTBE. A comparison of the RP of 300 mg/kg bw per day with the estimated exposure of 0.3 mg/kg bw per day for MTBE as a previous cargo yields a MOE of 1000, which is sufficient to address the uncertainties in the database.

There are no data on allergenicity upon oral exposure to MTBE that indicate that it is, or it contains a known food allergen.

MTBE as a previous cargo is not expected to react with edible fats and oils to form any reaction products.

Therefore, MTBE meets the criteria for acceptability as a previous cargo for edible fats and oils.

I.II Ethyl tertiary butyl ether

1. Explanation

The acceptability of ETBE as a previous cargo was evaluated by the CONTAM Panel based on the assessment of toxicological data on both MTBE and ETBE (EFSA, 2017). The CONTAM Panel established a TDI of 1 mg/kg bw per day based on a NOAEL of 100 mg/kg bw per day from a 180-day rat study (Miyata et al., 2014) after application of an uncertainty factor of 100. The CONTAM Panel concluded that ETBE was neither genotoxic nor allergenic, and considered that ETBE would not pose a risk for carcinogenicity at levels of exposure anticipated from its use as a previous cargo. Therefore, the CONTAM Panel concluded that ETBE met the criteria for acceptability as a previous cargo for edible oils and fats. The US EPA conducted a toxicological review of ETBE and established a chronic oral reference dose of 0.5 mg/kg bw per day based on effects in the kidneys, such as urothelial hyperplasia, reported in a long-term study in rats (US EPA, 2016).

For the present assessment, the Committee reviewed previous evaluations completed by EFSA and US EPA and located additional references from these evaluations. This was followed by comprehensive searches for toxicological data on ETBE on PubMed and PubChem. The cut-off date for inclusion for all searches was 31 August 2020. A total of 34 relevant records were retrieved, most of which had been included in monographs drafted by EFSA and US EPA's Integrated Risk Information System (IRIS); however, two additional, recently published articles relevant to the present assessment were also located. These articles described application of PBTK modelling of ETBE to elucidate the effects of ETBE and/or TBA on the kidney and liver and to examine the contribution of binding of ETBE and/or TBA with α2u-globulin in the kidneys of male rats to the observed renal toxic effects.

Moreover, there are similarities between the chemical structures, toxicokinetic characteristics and toxicological properties of MTBE and ETBE. Therefore, the present assessment considered data on MTBE when evaluating the toxicological profile of ETBE. Some data reported on TBA, a major metabolite of ETBE, were also considered while evaluating the toxicological profile of ETBE.

There are extensive toxicological datasets on exposure to ETBE via different routes. The present assessment discusses relevant toxicological data on oral exposure of animals and humans to ETBE, and data from genetic toxicity studies conducted using in vitro and in vivo systems.

2. Chemical and technical considerations

ETBE is a colourless liquid with a strong objectionable odour. It is primarily used to oxygenate gasoline. The physicochemical characteristics of ETBE are summarized in Table 8.

2.1 Manufacture and uses of ETBE

ETBE is produced by the acidic etherification of isobutylene with ethanol using an acidic ion-exchange resin as a catalyst. Optimization of the ETBE production has been reported (Eliana, 2008).

ETBE is more expensive than MTBE as ethanol is produced by a more costly and complex fermentation and distillation process, whereas methanol is derived from natural gas. ETBE is volatile and highly flammable and is stable at room temperature; it must be stored and transported in airtight containers (Diaz & Drogos, 2001). It is primarily used as a fuel additive in gasoline to increase the octane number and to reduce exhaust emissions.

2.2 Composition and secondary contaminants

ETBE is generally of high purity (>95%). Minor impurities include isobutene, ethanol, isobutylene, 1-butylene, isopentanes and tertiary butanol.

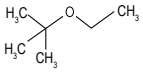
2.3 Reactivity and reactions with fats and oils

No information is available on the reactions of ETBE with edible fats and oils. Ethers are relatively inert chemically and would not be expected to react with edible fats and oils. Owing to the low formation of peroxides, ETBE is also unlikely to promote oxidation of edible fats and oils.

2.4 Methods of analysis

No analytical methods for the determination of ETBE in edible fats and oils are reported in the literature. Analytical methods for the determination of ETBE in gasoline and environmental samples are given in section 2.4 on MTBE under "Methods of analysis".

Table 8
Physical and chemical characteristics of ETBE

Characteristic	ETBE
Chemical name; IUPAC systematic name	2-ethoxy-2-methylpropane; *tert*-butylethyl ether
Synonyms	ethyl *tert*-butyl ether, ethyl *tert*-butyl oxide, methyl-2-ethoxypropan, *tert*-butyl ethyl ether, ETBE
CAS number	637-92-3
Chemical structure	
Molecular formula; molecular mass	$C_6H_{14}O$; 102.17 g/mol
Description	Colourless liquid with strong objectionable odour
Melting point; boiling point	–94 °C, 73 °C
Solubility	In water (at 25 °C): 12 g/L In ethanol and diethyl ether: very soluble

3. Biological data

3.1 Biochemical aspects

The toxicokinetic profiles of ETBE and MTBE are similar due to similarities in their chemical structures. The $t_{1/2}$ estimates of their common major metabolite, TBA, are also in the same range. Based on toxicokinetic studies of oral exposure to MTBE (Prah et al., 2004), ETBE is expected to be rapidly absorbed upon oral exposure. It undergoes oxidation catalysed by CYP2A6 to form TBA and acetaldehyde (Hong et al., 1999; McGregor, 2007). TBA is further metabolized to form first 2-methyl-1,2-propanediol and then 2-hydroxyisobutyrate; both metabolites are primarily eliminated in the urine. TBA is also eliminated in the urine as TBA-glucuronide and, in trace amounts, as TBA-sulfate (McGregor, 2007). No measurements of acetaldehyde after exposure to ETBE have been reported; however, the oxidation of acetaldehyde by aldehyde dehydrogenases (ALDH) to form acetic acid is expected (McGregor, 2007). Given its high lipophilicity and low molecular weight, ETBE is extensively distributed in human tissues. In an in vitro analysis of tissue/air partition coefficients, the blood/air partition coefficient of ETBE was estimated as 12, whereas its olive oil/blood partition coefficient was 16 (Nihlén, Löf & Johanson, 1995). Based on oral toxicokinetic data on MTBE in humans, and a comparison of toxicokinetics of MTBE and ETBE upon inhalation exposure, ETBE is expected to have a longer terminal $t_{1/2}$ in blood (about 24–33 hours) than MTBE (7–8 hours) (Nihlén, Löf & Johanson, 1998a,b).

A review of available toxicokinetic data on TBA, a major metabolite of ETBE and MTBE, indicated that the toxicokinetic profiles of the TBA generated are similar, with the $t_{1/2}$ ranging from 8 to 12 hours in humans. A comparison

of concentrations of urinary metabolites recovered after exposure to ETBE and MTBE in humans and rats indicated that the extent of metabolism of these two compounds in humans and rats is similar with no specific sex-based differences (Amberg, Rosner & Dekant, 1999, 2000).

The specific binding of ETBE and/or TBA to α2u-globulin in the kidneys of male rats after exposure to ETBE has been examined using PBTK modelling for different exposure scenarios. As stated in the assessment of MTBE, this mechanism of renal effects is male-rat-specific and, therefore, not relevant to humans.

Recently, PBTK model-based simulations for exposure to ETBE in rats were used to predict its toxicity and potential for carcinogenicity based on the internal dose metrics of ETBE and/or TBA, and/or the rate of the metabolism and exhalation of the ETBE. Salazar et al. (2015) described a PBTK model for ETBE and TBA in rats based on the framework of the MTBE PBTK models (Leavens & Borghoff, 2009; Borghoff, Parkinson & Leavens, 2010) to predict internal dose metrics of ETBE and TBA after exposure to ETBE via the oral, inhalation and intravenous routes. The model predicted that the non-cancer kidney effects, such as kidney weight changes, urothelial hyperplasia and chronic progressive nephropathy showed consistent dose–response relationships across routes of exposure, using TBA concentration in blood as the dose metric. Furthermore, relative liver weights were consistent across ETBE studies based on TBA metabolism, which was proportional to TBA concentrations in the liver. However, the incidences of kidney and liver tumours were not consistently related to any dose metric. In conclusion, this PBTK model showed that TBA mediated non-cancer kidney and liver effects following exposure to ETBE via different routes; however, the internal dose metric of TBA could not be used to explain the induction of liver and kidney tumours. An updated PBTK model was used to evaluate the contribution of ETBE and TBA kinetics, after exposure to ETBE via the oral, intravenous and inhalation routes, to tumour incidences in the kidneys and liver of male rats (Borghoff et al., 2017). This model predicted that the internal dose metrics of TBA in the kidney were similar between different ETBE exposure scenarios. It also predicted nonlinear kinetics when ETBE inhalation exposure was higher than 2000 ppm, based on the area under the curve (AUC) values of ETBE and TBA in the blood.

3.2 Toxicological studies

3.2.1 Acute toxicity

ETBE has a low potential for acute toxicity, with a reported oral LD_{50} of > 5000 mg/kg bw in Wistar rats (McGregor, 2007). The most typical clinical signs of toxicity

caused by ETBE include ataxia, hypoactivity, muscle weakness and hyperpnoea; however, rapid recovery after the cessation of exposure has been reported.

3.2.2 Short-term studies of toxicity

A 28-day oral toxicity study was conducted on female Sprague-Dawley rats (10 per group) to evaluate toxicity of ETBE at doses of 0, 250, 500 and 1000 mg/kg bw per day (Banton et al., 2011). No treatment-related effects on clinical signs, body weights, food or water consumption, or macroscopic pathological findings were reported. This study also assessed the immunotoxic potential of ETBE. Those results are discussed under MTBE in section 3.2.2.

A 180-day oral subchronic toxicity study of ETBE (with olive oil as the vehicle) was conducted on male and female Sprague-Dawley rats (15 per dose and sex) at exposure levels of 0, 5, 25, 100 and 400 mg/kg bw per day (Miyata et al., 2014). The doses were based on a 28-day repeated-dose oral toxicity study of ETBE in male and female Sprague-Dawley rats (five per dose and sex) that evaluated short-term toxicity of ETBE at levels of 0, 15, 25, 50, 100, 150, 400 and 1000 mg/kg bw per day (cited in Miyata et al., 2014). This short-term study found decreased spontaneous movement in animals given 100 mg/kg bw per day and above, and increased liver weights at 400 mg/kg bw per day of ETBE. The 180-day study was performed in accordance with the OECD guideline (OECD, 1981) for test chemicals with some modifications, and following the principles of Good Laboratory Practice (OECD no. 26). No mortality was reported. Decreased locomotor activity in both sexes following administration of 100 and 400 mg/kg bw per day, decreased respiratory rate and incomplete eyelid opening in animals of both sexes that received 400 mg/kg bw per day were reported. Salivation was observed transiently in males treated with 25, 100 and 400 mg/kg bw per day and in females treated with 400 mg/kg bw per day of ETBE. No effects on reflex test, grip strength, motor activity, body weight, haematology or urinalysis findings were reported. A significant increase in relative mean liver weights in males and females that received 400 mg/kg bw per day of ETBE was reported. An increase in relative kidney weights in males and females treated with 100 and 400 mg/kg bw per day of ETBE was also reported. An increase in total cholesterol was noted in males treated with 400 mg/kg bw per day of ETBE, but was not observed in females. Livers of males and females, and kidneys of males showed significant microscopic findings. Hepatocytes of males and females given 400 mg/kg bw per day of ETBE showed hypertrophy characterized by hepatocyte enlargement in the centrilobular area with homogeneously eosinophilic cytoplasm. An increased incidence of hyaline droplets associated with α2u-globulin immunoreactivity was reported in the kidneys of males treated with 100 and 400 mg/kg bw per day of ETBE. However, as mentioned in the description of the studies on MTBE,

the observation of hyaline droplets associated with binding to α2u-globulin in the kidneys is specific to male rats and not relevant to the assessment of human health risk. Based on the increase in relative mean liver weights and microscopic findings in the livers of male and female rats treated with 400 mg/kg bw per day and increased total cholesterol levels reported in males treated with 400 mg/kg bw per day of ETBE, the present Committee identified a NOAEL of 100 mg/kg bw per day from this study (Miyata et al., 2014).

3.2.3 Long-term studies of toxicity and carcinogenicity

The risk for chronic toxicity and carcinogenicity following exposure to ETBE was evaluated based on a review of relevant studies on ETBE and its major metabolite TBA. Data on chronic toxicity and carcinogenicity from studies of exposure to MTBE in rodents were also considered.

A 104-week assay of chronic toxicity and carcinogenicity of orally administered (gavage) ETBE (with olive oil as the vehicle) was conducted on male and female Sprague-Dawley rats (60 per sex per group) at doses of 0, 250 and 1000 mg/kg bw per day (Maltoni et al., 1999). The treatment or vehicle was administered to rats on 4 days per week throughout the study. No treatment-related effects on body weights or on food or water consumption were reported. Survival was reduced in animals that received 1000 mg/kg bw per day of ETBE at different points of the study. The authors reported that ETBE induced tumours of the epithelium of the mouth in females, tumours of the forestomach in males, malignant tumours of the uterus, and haemolymphoreticular neoplasms. No dose–response relationships between the neoplastic effects and the concentrations of ETBE were observed. A statistically significant increase in incidence of malignant schwannoma in the uterus and vagina was reported in females administered 250 mg/kg bw per day of ETBE. However, the incidence of this tumour was not statistically significant in the highest dose group. Moreover, schwannomas are mainly associated with nerves originating from the central or peripheral nervous system and are not directly linked with non-nervous system organs. Therefore, the incidence of these tumours was not toxicologically relevant.

As discussed in the assessment of MTBE, the tumour incidences reported in rodents after oral exposure to MTBE and the metabolite TBA lack human and/or toxicological relevance.

3.2.4 Genotoxicity

ETBE did not cause any genotoxic effects under the testing conditions of the studies that were reviewed (summarized in Table 9). Furthermore, TBA, the major metabolite of ETBE, has been reported to be non-genotoxic (NTP, 1995).

Table 9
Summary of some genetic toxicity studies on ETBE reviewed by the Committee

Test system and testing conditions	ETBE treatment	Result	Reference
Ames assay using *Salmonella* Typhimurium strains TA97, TA98, TA100 and TA1535	Concentrations up to 1000 µg/plate, with or without metabolic activation (rat or hamster S9)	Negative	Zeiger et al., 1992
Ames assay using *Salmonella* Typhimurium strains TA98, TA1535, TA1537 and TA1538	Concentrations up to 500 µg/plate, with or without metabolic activation (rat S9)	Negative	Institut Pasteur de Lille, 1992a
Ames assay using *Salmonella* Typhimurium strains TA98, TA100, TA1537 and TA1538	Concentrations up to 5000 µg/plate, with or without metabolic activation (rat S9)	Negative	Pharmakon Europe, 1994
Gene mutation assay using Chinese hamster V79 cells at the hypoxanthine phosphoribosyl transferase (HPRT) locus	Concentrations up to 5000 µg/mL, with or without metabolic activation	Negative	Bushy Run Research Center, 1995a
Chromosome aberration in Chinese hamster ovary cells	Concentrations up to 5000 µg/mL, with or without metabolic activation	Negative	Bushy Run Research Center, 1995b
In vivo micronucleus formation in mouse bone marrow cells	Male and female OF-1 mice treated orally with doses up to 5000 mg/kg bw	Negative	Institut Pasteur de Lille, 1992b
In vivo micronucleus formation in mouse bone marrow cells	Male and female CD1 mice exposed via inhalation to doses up to 5000 ppm (6 hours/day, 5 days)	Negative	Bushy Run Research Center, 1995c

3.2.5 Reproductive and developmental toxicity

An evaluation by the CONTAM Panel of the potential of ETBE to lead to reproductive and developmental toxicity concluded that ETBE did not show selectively reproductive or developmental toxicity in the absence of other manifestations of general parental toxicity (EFSA, 2017). A summary of the studies of reproductive and developmental toxicity following oral exposure to ETBE is provided in Table 10.

The Committee also reviewed a 10-week oral reproductive toxicity study of ETBE conducted on male and female rats administered doses of 500 and 1000 mg/kg bw per day. Renal effects in the form of increased kidney weights in male and female rats at doses ≥500 mg/kg bw per day and protein droplet accumulation in male rats treated with 1000 mg/kg bw per day of ETBE were reported (Gaoua, 2004a,b; cited by McGregor, 2007). No specific treatment-related effects on reproductive or developmental parameters were reported in this study.

3.2.6 Allergenicity

The Committee did not identify any reports indicating that ETBE elicits an allergic response upon oral exposure. There are also no data available that indicate that it contains a known food allergen.

Possible allergic symptoms were reported in eight male human volunteers with no prior history of allergies upon acute inhalation exposure to ETBE (Nihlén, Löf & Johanson, 1998c). These symptoms included discomfort

Table 10
Summary of some reproductive and developmental toxicity studies on oral exposure to ETBE reviewed for this monograph

Type of study	Species	Doses	NOAEL/LOAEL	Toxicological end-points	Reference
One-generation reproductive toxicity	Sprague-Dawley rats (24 per sex per dose)	0, 100, 300 and 1000 mg/kg bw per day, via oral gavage	NOAEL: 300 mg/kg bw per day for parental animals as well as offspring (based on no treatment-related effects in F0 parents or their F1 offspring at doses up to 300 mg/kg bw per day)	Significant increases in absolute and relative liver weights in groups treated with 1000 mg/kg bw per day. Significant increases in absolute and relative kidney and adrenal weights, and relative brain, pituitary and testes weights in F0 males and absolute kidney weights in F0 females; both sexes treated with 1000 mg/kg bw per day	Fujii et al., 2010
Prenatal developmental toxicity	Pregnant female Sprague-Dawley rats (21 or 22 per group)	0, 100, 300 and 1000 mg/kg bw per day	NOAEL: 1000 mg/kg bw per day for maternal and developmental toxicity (based on no treatment-related effects on pregnant rats, or their embryos and fetuses at all doses tested)	No treatment-related effects on clinical signs, body weight, food intake or necropsy findings. No treatment-related effects seen on external, visceral and skeletal examinations of embryos and fetuses	Aso et al., 2014
One-generation reproductive toxicity	Sprague-Dawley rats	Doses up to 1000 mg/kg bw per day	NOAEL: 300 mg/kg bw per day for maternal toxicity (based on liver weight increase) and for developmental toxicity (based on reduced F1 survival rate at 1000 mg/kg bw per day)	Decrease in F1 survival rate during weaning was noted at 1000 mg/kg bw per day due to weakening of dams at this dose and incidence of litter loss that exceeded historical controls	Cited by EFSA, 2017

in the throat and airways after exposure to ETBE at 50 ppm, and increased nasal swelling and frequency of blinking after exposure to ETBE at 5, 25 and 50 ppm; however, these effects were not further investigated.

3.2.7 Other studies

A 28-day oral toxicity study was conducted on female Sprague-Dawley rats (10 per group) to assess the immunotoxic potential of ETBE at doses of 0, 250, 500 and 1000 mg/kg bw per day (Banton et al., 2011) (see section 3.2.2). Animals in a concurrent positive control group received four intraperitoneal injections of 50 mg/kg per day of cyclophosphamide monohydrate on days 24–27. No treatment-related effects on absolute or relative spleen or thymus weights, spleen cellularity, or specific or total activity of splenic IgM antibody-forming cell (AFC) to the T-cell-dependent antigen sheep red blood cells were reported. The positive control group showed the expected immunosuppressive responses in the AFC assay.

3.2.8 Impurities

Impurities, namely ethanol, isobutylene, 1-butylene, isopentanes and TBA, may be expected in ETBE. As ETBE products are of high purity, the percentage contribution of these impurities to the total composition of the substance is minor. The Committee noted that these impurities were non-genotoxic and studies on oral exposure in animals did not report any tumour incidences of human or toxicological relevance. The Committee also did not identify any reports that indicated that these impurities elicit an allergic response upon oral exposure or would contain a known food allergen. Therefore, the Committee concluded that these compounds would not be expected to cause any adverse health effects following oral exposure at their anticipated levels as minor impurities in ETBE.

3.3 Observations in humans

There are no data on oral exposure to ETBE in humans. Some possible allergenic symptoms were reported in humans after acute inhalation exposure to ETBE vapours at doses of up to 50 ppm (Nihlén, Löf & Johanson, 1998c), as stated above (section 3.2.6).

4. Levels and patterns of contamination in food commodities

No data were found on concentrations of butyl ethers in food oils due to carryover from previous cargoes. A worst-case concentration of 100 mg/kg has been assumed for all previous cargo substances. Worst-case exposures to previous cargo substances in food oils have been estimated at 0.3 mg/kg bw per day, based on a worst-case concentration of 100 mg/kg and an oil intake of 3 g/kg bw per day by infants and young children who are high consumers (see part A, section 4.3).

ETBE, used in many areas as a substitute for MTBE in gasoline, has been found in drinking-water (van Wezel et al., 2009). Potential exposure to ETBE from drinking-water must therefore be considered as part of total dietary exposure to ETBE. Proposed advisory thresholds for ETBE in drinking-water in Europe range from 1–2 µg/L (van Wezel et al., 2009). The US State of New York has set a maximum concentration limit of 50 µg/L (New York State Department of Health, 2006).

5. Food consumption and dietary exposure estimates

The maximum, mean and p95 daily drinking-water intakes by adults, based on data from the US EPA *Exposure factors handbook*, are 22.9 mL/kg bw per day and 47 mL/kg bw per day, respectively. EFSA estimated adequate intakes of drinking-water for adult males of 4 L at the p95 (EFSA, 2010); this is equivalent to 67 mL/kg bw per day based on a body weight of 60 kg. The estimated chronic daily exposure to ETBE from consumption of 67 mL/kg bw per day of drinking-water containing 50 µg ETBE/L (the maximum identified advisory or regulatory level) is 0.003 mg/kg bw per day for adults.

For infants and toddlers, maximum mean and p95 daily drinking-water intakes, based on data in the US EPA *Exposure factors handbook*, are 79 mL/kg bw per day and 174 mL/kg bw per day, respectively. EFSA estimated adequate intakes of drinking-water of up to 190 mL/kg bw per day for infants (EFSA, 2010). The estimated maximum daily ETBE exposure for infants and young children, assuming consumption of 190 mL/kg bw per day of drinking-water containing 50 µg/L of ETBE, is 0.01 mg ETBE/kg bw per day.

The estimated ETBE exposure from drinking-water is considered to be a minor contributor as compared with the potential exposure to ETBE in food oil commodities from previous cargoes.

No other potential sources of dietary exposure to ETBE were identified.

6. Comments

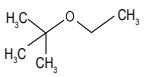
6.1 Chemical and technical considerations

The chemical and technical considerations for ETBE are summarized in Table 11.

6.2 Biochemical aspects

The toxicokinetic profiles of ETBE and MTBE are expected to be similar owing to the similarities in their chemical structures. The $t_{1/2}$ estimates of TBA generated upon metabolism of MTBE and ETBE are also expected to be in the same range. Based on oral exposure studies with MTBE (Prah et al., 2004), ETBE is expected to be rapidly absorbed following oral exposure. Given its high lipophilicity and low molecular weight, ETBE is extensively distributed in human tissues (Nihlén, Löf & Johanson, 1995). It undergoes oxidation catalysed by CYP2A6 to form TBA and acetaldehyde (Hong et al., 1997; McGregor, 2007). The terminal $t_{1/2}$ of

Table 11
Chemical and technical considerations for ethyl tertiary butyl ether (ETBE)

Name: ethyl tertiary butyl ether (2-ethoxy-2-methylpropane)	
CAS number	**Alternative CAS numbers**
637-92-3	None
Chemical details	Ethyl *tert*-butyl oxide, methyl-2-ethoxypropane, *tert*-butyl ethyl ether, ETBE Colourless liquid with strong objectionable odour  Molar mass: 102.17 g/mol Melting point: –94 °C Boiling point: 73 °C Solubility in water (25 °C): 12 g/L; very soluble in ethanol and diethyl ether
Route(s) of synthesis	Manufactured by reacting isobutylene with ethanol in the presence of acidic ion-exchange resin catalysts or strong acids.
Composition	ETBE > 95%; minor impurities may include ethanol, isobutylene, 1-butylene, isopentanes and tertiary butyl alcohol.
Uses	Used to oxygenate gasoline to improve octane number. Minor uses include chemicals, petrochemicals, pharmaceuticals, paints and coatings.
Analytical methods	None reported for previous cargoes. Potential methods for its determination in fats or oils may include head space, purge and trap or solid-phase micro-extraction techniques coupled with GC-FID/PID/ELCD or GC-MS.
Potential reaction(s) with a subsequent cargo of fat or oil	ETBE is not expected to react with edible fats and oils to form any reaction products.

GC-FID/PID/ELCD, gas chromatography with flame ionization detection/photo-ionization detection/electrolytic conductivity detection; GC-MS, gas chromatography–mass spectrometry.

ETBE in humans is predicted to be about 24–33 hours (McGregor, 2007). The $t_{1/2}$ of the generated TBA in humans is predicted to range from 8 to 12 hours. TBA is further metabolized, first to form 2-methyl-1,2-propanediol and then 2-hydroxyisobutyrate; both metabolites are primarily eliminated in the urine (McGregor, 2007). TBA is also eliminated in the urine as TBA-glucuronide and, in trace amounts, as TBA-sulfate (Amberg, Rosner & Dekant, 1999; McGregor, 2007). No measurements of acetaldehyde after exposure to ETBE have been reported; however, the oxidation of acetaldehyde by aldehyde dehydrogenases (ALDH) to form acetic acid is expected (McGregor, 2007). The Committee reviewed studies that described application of PBTK modelling to investigate specific binding of ETBE and/or TBA to α2-globulin in the male rat kidneys after ETBE exposure via different exposure scenarios, including oral administration (Salazar et al., 2015; Borghoff et al., 2017). However, the Committee considered

this mechanism of renal effects to be male-rat-specific, and, therefore, as not relevant to humans.

6.3 Toxicological studies

There are extensive toxicological datasets on ETBE exposure via different routes. The Committee evaluated relevant toxicological data on oral exposure to ETBE in animals and humans, and from genetic toxicity studies conducted with ETBE in in vitro and in vivo systems.

The Committee concluded that the potential for acute toxicity of ETBE after oral exposure is low based on the reported oral LD_{50} value of > 5000 mg/kg bw in rats (McGregor, 2007). The Committee reviewed a 180-day oral toxicity study conducted in male and female Sprague-Dawley rats at exposure levels of 0, 5, 25, 100 and 400 mg/kg bw per day and identified a NOAEL of 100 mg/kg bw per day (Miyata et al., 2014). This was based on an increase in relative mean liver weights and microscopic findings in the livers of males and females treated with 400 mg/kg bw per day, and the increase in cholesterol levels reported in males treated with 400 mg/kg bw per day of ETBE. The Committee concluded that ETBE did not show selectively reproductive or developmental toxicity in the absence of other manifestations of general parental toxicity based on an evaluation of some reproductive and developmental toxicity studies conducted in rats (Fujii et al., 2010; Aso et al., 2014; Gaoua, 2004a,b, cited by McGregor, 2007).

The Committee concluded that ETBE is not genotoxic based on negative responses reported in the in vivo and in vitro genetic toxicity studies reviewed, and genotoxicity data on TBA, its major metabolite, reporting that TBA is non-genotoxic.

The Committee evaluated a 104-week oral (gavage) chronic toxicity/carcinogenicity assay of ETBE conducted on male and female rats given doses of 0, 250 and 1000 mg/kg bw per day to assess the potential for carcinogenicity of ETBE upon oral exposure (Maltoni et al., 1999). In addition, the Committee reviewed the 2-year oral carcinogenicity assay of TBA in drinking-water conducted in male and female rats and mice at different dose levels (NTP, 1995). Both studies reported some tumour incidences; however, the Committee noted that the observed tumours in rodents lacked human or toxicological relevance, and these effects were reported at exposure levels much higher than those expected from oral exposure to ETBE as a previous cargo.

The Committee did not identify any reports of oral exposure to ETBE in humans.

6.4 Allergenicity

The Committee did not identify any reports that indicated that ETBE elicits an allergenic response upon oral exposure. There are also no data available that indicate that ETBE would contain a known food allergen.

6.5 Impurities

The Committee noted that impurities, namely ethanol, isobutylene, 1-butylene, isopentanes and TBA, may be expected in ETBE. As ETBE products are of high purity, the percentage contribution of these impurities to the total composition of the substance is minor. The Committee noted that these impurities were non-genotoxic and studies of oral exposure to these impurities in animals did not report tumour incidences of human or toxicological relevance. The Committee also did not identify any reports that indicated that these impurities elicit an allergic response upon oral exposure, or would contain a known food allergen. Therefore, the Committee concluded that these compounds would not be expected to cause any adverse health effects upon oral exposure at their anticipated levels of exposure as minor impurities in ETBE.

6.6 Assessment of dietary exposure

A worst-case concentration of 100 mg/kg has been assumed for all previous cargo substances (see part A, section 4.3). ETBE, used in many areas as a substitute for MTBE in gasoline, has been found in drinking-water (van Wezel et al., 2009). The worst-case exposures to previous cargo substances in food oils have been estimated as 0.3 mg/kg bw per day (part A, section 4.3). The estimated maximum daily ETBE exposure from drinking-water was estimated to be 0.01 mg/kg bw per day based on drinking-water consumption of 190 mL/kg bw per day by infants and children (the maximum estimated by EFSA (2010), and an ETBE concentration of 50 µg/L (New York State Department of Health, 2006).

7. Evaluation

Upon evaluating the available toxicity studies and examining the toxicological relevance of effects reported therein, the Committee concluded that the NOAEL of 100 mg/kg bw per day identified from the 180-day oral subchronic study of ETBE in rats was the most appropriate RP (Miyata et al., 2014). The Committee

concluded that the estimated exposure to ETBE from drinking-water is a minor contributor (0.01 mg/kg bw per day) compared with the estimated exposure to ETBE in food oil commodities from previous cargo (0.3 mg/kg bw per day), and that there are no other known potential sources of dietary exposure to ETBE. A comparison of the RP of 100 mg/kg bw per day with the estimated exposure of 0.3 mg/kg bw per day for ETBE as a previous cargo yields a MOE of 330, which is sufficient to address the uncertainties in the database.

There are no data on allergenicity upon oral exposure to ETBE that indicate that it is, or it contains a known food allergen.

ETBE as a previous cargo is not expected to react with edible fats and oils to form any reaction products.

Therefore, ETBE meets the criteria for acceptability as a previous cargo for edible fats and oils.

8. Conclusions

Analytical methods and data on the levels and patterns of contamination of MTBE and ETBE in fats and oils were not available. In the absence of the contaminant data, the criteria developed by FAO/WHO assuming a maximum concentration of 100 mg/kg for previous cargo contaminants (FAO/WHO, 2007), may be used in the risk assessment of MTBE and ETBE.

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PREVIOUS CARGOES

GROUP 3 – OILS AND WAXES

First draft prepared by

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A. ASSESSMENT OF SUBSTANCES PROPOSED AS PREVIOUS CARGOES

1. Introduction

Fats and oils destined to be used as food are transported and stored in large volumes. Transportation in large volumes by sea is exempted from many land-based regulations as it is not practical to have fleets of ships solely dedicated to the transportation of food in large tanks, since the trade is generally unidirectional from producer to consumer. Furthermore, the construction and dependency on the availability of a limited number of single-use carriers would make the transport of fats and oils extremely expensive. To address the economic realities, certain types of ships are permitted to carry different classes of cargo in their tanks on their outbound and onward journeys. A non-food item may be carried in a tank in one direction and a single type of fat or oil on the further voyage. Since ships are constructed to have several individual tanks, each may contain a cargo destined for a different location and may be used to carry either a food or non-food item depending on the contract.

A number of organizations have been involved in the development of codes of practice, transportation contracts, ship construction, cargo segregation, environmental issues and food safety. The Codex Alimentarius Commission (CAC) adopted and published a code of practice for the storage and transport of edible fats and oils in bulk, which was developed by CCFO in 1987 (CAC/RCP 36-1987). At that time, CCFO recognized the need to assess the acceptability of previous cargoes transported in a tank subsequently used for the transportation of an edible fat or oil. Commercial trade contracts recognized the need to specify that certain chemicals should never be acceptable previous cargoes for

subsequent cargoes of edible fats or oils. These substances formed the basis of the "banned lists" of previous cargoes. In 2001, a combined list of chemicals banned as previous cargoes was developed by CCFO and adopted by CAC (FAO/WHO 2001); it was added to the Codex Code of Practice as Appendix 1. Other substances carried in bulk were considered to pose a low risk to public health as a contaminant in edible fats or oils; these formed the basis of "acceptable lists" of previous cargoes. The development of a CCFO acceptable list of previous cargoes was also based on trade experience. A preliminary list was reviewed by the Scientific Committee on Food and their findings were reported to CCFO in 1999; 14 substances were identified for which there were insufficient data to make a safety determination. After further discussion at subsequent CCFO meetings, a list of 23 potentially safe previous cargoes that require evaluation was developed. CCFO asked for scientific advice from FAO/WHO on these 23 substances that lacked safety evaluations. The present evaluation by JECFA addresses 18 of the 23 substances on the current list of chemicals acceptable as previous cargoes by CCFO.

2. Background

2.1 Global production and consumption of fats and oils

The global trade in edible fats and oils is more than 200 million metric tonnes annually and valued at approximately US$ 120 billion (USDA, 2019). By far the largest contributors are palm (36%) and soybean oil (28%), followed by rapeseed/canola (14%), sunflower seed (10%), palm kernel (4%), peanut (3%), cottonseed (3%), coconut (2%) and olive oils (2%).

Many vegetable oils are produced in regions (for example: soybean – Argentina, Brazil, USA; rapeseed – Australia, Canada; sunflower seed – Ukraine; palm – Indonesia and Malaysia; and coconut – equatorial latitudes) far from the major sites of consumption. Olive oil is produced in regions with a Mediterranean climate in both the northern and southern hemispheres. International trade in fats and oils uses the most economical method of ocean transportation since global trade in edible fats and oils is primarily unidirectional. Soybean oil from Argentina and Brazil, for example, is shipped to both Asian and European markets, but there is unlikely to be a complementary cargo of fat or oil available for transportation in the reverse direction. Similarly, oils from tropical regions are traded globally, often without reciprocal trade in fats and oils.

2.2 Regulations affecting fats and oils

Shipment of fats and oils is described in numerous national and international regulations and agreements. Land-based transportation is regulated by local and national guidelines and/or legislation, whereas international trade is subject to commercial agreements, international shipping regulations and various codes of practice. The development of banned lists and acceptable lists of previous cargoes is founded on existing trade contracts.

About 85% of the fats and oils are traded globally using FOSFA (The Federation of Oils, Seeds and Fats Associations, London) contracts. The balance is traded under contracts issued by NIOP (National Institute of Oilseed Products) or other organizations. A contract under "banned list terms" requires that fats and oils are not shipped in tanks that have contained a substance on the banned list as the immediate previous cargo. For certain chemicals, this requirement is extended to the three previous cargoes. Alternatively, a contract may state that "the immediate previous cargo shall be a product on the FOSFA List of Acceptable Previous Cargoes". In this case, the receiver will only accept the cargo if the previous cargo is on FOSFA's acceptable list. These two lists only cover a small proportion of the chemicals transported by sea; thus many substances appear on neither list and their acceptability as a previous cargo is subject to agreement by the contracting parties.

2.3 Global transport of fats and oils

Transportation by sea is regulated by the International Maritime Organization (IMO). The International Convention for the Prevention of Pollution from Ships (MARPOL) aims to prevent operational and accidental pollution from ships. MARPOL limits the carriage of different classes of liquid cargoes to specific tanker vessels based on ship construction and the class of chemical. Under this convention, fats and oils may not be transported in vessels designated to carry cargoes of crude oil, fuel oil, heavy diesel oil or lubricating oil. The International Code for the Construction and Equipment of Ships Carrying Dangerous Chemicals in Bulk (IBC Code) lists chemicals carried as bulk liquids, their pollution category, the type of ship design and any relevant restrictions or derogations. The previous cargoes under consideration (see Table 1) are in the medium- or low-risk categories for marine pollutants. The single exception is propylene tetramer, which is considered a high-risk marine pollutant. MARPOL also deals with tank washing and material discharge. Pentane falls into an additional category of oil-like substances requiring additional attention between cargoes.

2.4 The interrelationship of national, regional and trade interests

The practice of Acceptable List trading was discussed in line with regional initiatives to protect consumer health. The adoption of the hazard analysis and critical control point (HACCP) principles and their inclusion in the Codex Alimentarius approach to the safe trade of food and food products can be applied to the transport of oils and fats by sea. The CAC adopted the *Code of Practice for the Storage and Transport of Fats and Oils in Bulk* developed by CCFO in 1987 (CAC-RCP 36-1987). The Code has been revised periodically and a banned list of substances was added in 2001. The list of acceptable previous cargoes adopted by the European Union (EU) and based on existing trade lists, was evaluated by EFSA.

2.5 Development of the Codex Code of Practice for Storage and Transport of Edible Fats and Oils in Bulk

CCFO discussions highlighted the need for lists of banned and acceptable previous cargoes. The topic of contamination by previous cargoes led to the incorporation of the FOSFA and NIOP trade lists into the Code by reference only. In 2001, CAC adopted the "Banned List" and it appears in the current code of practice as Appendix 3.

The development of a List of Acceptable Previous Cargoes by CCFO began with attempts to harmonize the FOSFA and NIOP trade lists with an EU list. The Acceptable List was further refined in 1999 when CCFO considered a list of substances proposed by the EU that had been reviewed by the Scientific Committee on Food (SCF). Having developed a list of acceptable previous cargoes, it was determined that there were 14 substances on it that required further evaluation; these 14 substances formed the basis of the CCFO Proposed Draft List of Acceptable Previous Cargoes, which was adopted by CAC 34 in 2011. For consideration at this meeting a list of 23 substances was proposed to FAO/WHO (Table 2) by CCFO for scientific advice on their suitability as previous cargoes for the carriage of fats and oils by sea-going vessels upon its evaluation against the four criteria. Each substance on the list has been assigned to Groups 1–5 (1 – solvents/reactants; 2 – alcohols; 3 – oils and waxes; 4 – solutions; 5 – butyl ethers). Substances in Group 1 were not evaluated at the present meeting.

3. Development of criteria

As a result of the CCFO request to FAO/WHO for scientific advice on the development of criteria for the assessment of the safety of residues of previous

Table 1
List of substances submitted by CCFO for evaluation by JECFA for addition to the list of acceptable previous cargoes

Substance (synonyms)	CAS number	Assessment group[a]
Acetic anhydride (ethanoic anhydride)	108-24-7	1
1,4-Butanediol (1,4-butylene glycol)	110-63-4	2
Butyl acetate, *sec*-	105-46-4	1
Butyl acetate, *tert*-	540-88-5	1
Calcium ammonium nitrate solution	15245-12-2	4
Calcium lignosulfonate liquid (lignin liquor; sulphite lye)	8061-52-7	4
Calcium nitrate (CN-9) solution	35054-52-5	4
Cyclohexane	110-82-7	1
Fatty alcohols		
iso Decyl alcohol (isodecanol)	25339-17-7	2
Myristyl alcohol (1-tetradecanol, tetradecanol)	112-72-1	2
iso Nonyl alcohol (isononanol)	27458-94-2	2
iso Octyl alcohol (isooctanol)	26952-21-6	2
Tridecyl alcohol (1-tridecanol)	112-70-9	2
Unfractionated fatty alcohol mixture or mixtures of fatty alcohols from natural oils and fats[b]		3
Methyl tertiary butyl ether (MTBE)	1634-04-4	5
Mineral oil, medium and low viscosity, class II		3
Mineral oil, medium and low viscosity, class III		3
Montan wax	8002-53-7	3
Pentane	109-66-0	1
1,3-Propanediol (1,3-propylene glycol)	504-63-2	2
Propylene tetramer (tetrapropylene, dodecene)	6842-15-5	3
Soybean oil epoxidized	8013-07-08	3
Ethyl tertiary butyl ether (ETBE)	637-92-3	5

[a] Group 1 was not considered at this meeting.
[b] Discussed with Group 2 – alcohols.

cargoes in the tanks of sea-going vessels carrying edible fats and oils, a technical meeting was convened (in November 2006) at the Dutch National Institute of Public Health and the Environment (RIVM). RIVM prepared a technical background document (FAO/WHO, 2006, Appendix II) and drafted the meeting report with FAO/WHO (2007).

Discussions were limited to the assessment of previous cargoes in the transport of edible fats and oils in bulk by sea and the consideration of safety implications in terms of human health. The experts accepted that the quality of the fats and oils cargo could change as a result of hydrolysis and oxidation, but they acknowledged that these changes were already taken into account in trade contracts.

The experts considered a list of parameters originating from discussions at CCFO meetings, noting that previous cargoes are generally liquid chemical substances, slurries of solid particles or aqueous solutions. To further frame the deliberations, the experts decided to consider only a generic worst-case scenario since developing criteria to cover every possible combination of previous cargo, type of tank, cleaning regime and possible further processing of the subsequent cargo of fat or oil would not be a realistic approach.

The experts developed the following worst-case scenario: the smallest commercially viable tank size (200 m^3), coated with a polymer that absorbs the previous cargo, is filled to 60% capacity (as required by contract), and the cargo of fat or oil is not to be further processed or refined. The model also assumed that the tank and associated pipework has been cleaned according to defined standards, inspected and considered clean and dry. Under these circumstances, the maximum level of contamination in the subsequent fat or oil cargo by the previous cargo was calculated to be 100 mg/kg. This value was used to determine a single estimate of worst-case human exposure of 0.1 mg/kg bw per day. Based on this generic exposure value, the experts considered that for the evaluation of previous cargoes, the ADI (or TDI) should be greater than or equal to 0.1 mg/kg bw in order to provide sufficient protection for children and high-intake consumers. Negligent or fraudulent practices were not considered to be part of the criteria. The experts identified four criteria necessary to determine the acceptability of a previous cargo (see FAO/WHO, 2006).

The criteria as adopted by CAC 34 (2011) are listed in Table 2.

4. Basis of evaluation

4.1 Chemistry/reactivity

Edible fats and oils are normally chemically stable; however, there may be potential for reactions with residues of previous cargoes that could give rise to products that are hazardous to human health. Consideration should be given to chemical substances that can react with edible fats and oils under normal transportation conditions. Minor oxidation and hydrolysis are normally anticipated in trade contracts and are not considered a consequence of contact with a previous cargo, unless accelerated degradation occurs. Although many possible reactions require the presence of specific catalysts or temperatures well in excess of those anticipated during transportation, potential reactions of the previous cargo with triglycerides and free fatty acids or other minor components present in the fat or oil should still be considered.

Table 2
Criteria adopted by CAC 34 and included in RCP-36-1987

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.

4.2 Methods of analysis

In a few cases where contamination is considered critical there has been an international effort to develop specific analytical methods. Cases of contamination with diesel fuel (alkanes) and mineral oils (mineral oil saturated hydrocarbons, MOSH; mineral oil aromatic hydrocarbons, MOAH) led to the development of relevant international standards. Although many of the substances under review at the present meeting can be analysed by gas or liquid chromatography using appropriate detector systems, little progress has been made in the application of these technologies to their contamination of oils and fats. It is assumed that available methods with suitable modifications will be capable of determining the maximum anticipated level of 100 mg/kg of previous cargo in the subsequent cargo of fats or oils.

4.3 Dietary exposure assessment for previous cargo chemical substances

As a consequence of considering a range of previous cargo chemical substances at its ninetieth meeting, the Committee concluded that it was appropriate to review the approach to estimating dietary exposure set out in the 2006 document *Development of criteria for acceptable previous cargoes for fats and oils* (criteria document) (FAO/WHO 2006).

The Committee noted that since the 2006 criteria document was drafted, newer and better-quality data on the consumption of fats and oils by adults, infants and young children have become available.

The Committee also noted that some of the previous cargo chemical substances assessed have additional sources of dietary exposure and expressed the view that it may be necessary to consider this in the exposure assessment.

Based on the best available data at that time, the 2006 criteria document set out the following approach to dietary exposure assessment of previous cargo chemical substances present in fats and oils:

- Estimated mean per capita consumption of 0.025 kg/day of a single type of fat or oil. The value was rounded up from the maximum per capita consumption of refined soybean oil of 22 g/person per day from the GEMS/Food cluster diets (WHO 2012).
- A factor of 2.5 to cover children and high consumers was derived from a rounded ratio between the mean and 97.5th percentile consumption of total vegetable oil from a food consumption survey in the United Kingdom (20 and 52 g/person per day for the population aged > 18 years). The criteria document also noted that dietary exposure of children to contaminants is frequently 2.5 times that of adults.
- A worst-case concentration of 100 mg/kg for a previous cargo contaminant in fats or oils.
- A body weight of 60 kg.

These data were used to define a worst-case dietary exposure estimate:

$$\frac{\text{Consumption of oil (0.025 kg/day)} \times 2.5 \times \text{concentration (100 mg/kg fat or oil)}}{\text{60 kg body weight}}$$

$$= \mathbf{0.1\ mg/kg\ bw\ per\ day}$$

Based on the **mean per capita consumption of fats and oils, and a factor of 2.5**, there would be no health concern to the general population from exposure to previous cargoes if the acceptable daily intake (ADI) or tolerable daily intake (TDI) is sufficiently protective, for example, the ADI or TDI is greater than, or equal to **0.1 mg/kg bw per day**.

4.3.1 Exposure estimates based on up-to-date consumption data for adults

Since 2006, the GEMS/Food cluster diets have been revised, and the FAO/WHO Chronic Individual Food Consumption – summary statistics database (CIFOCOss) has become available. The 2006 criteria document noted that food consumption information from dedicated surveys would be more appropriate than the food consumption estimates from the GEMS/Food cluster diets (WHO, 2012). However, it used the cluster diets, as food consumption survey data were only available from a very limited number of countries at that time. CIFOCOss currently contains food consumption data from 37 countries.

From the current version of CIFOCOss, the maximum mean consumption for a single fat or oil type is 35 g/person per day for consumption of virgin or extra-virgin olive oil by elderly Italians. The maximum 95th percentile (p95) consumption of a single fat or oil is 138 g/person per day for edible cottonseed oil by women (age 15–49 years) from Burkina Faso. This group also has the highest 97.5th percentile consumption of 189 g/person per day.

Based on the protocols currently used by JECFA for veterinary drugs, the number of consumers of cottonseed oil in the Burkina Faso survey (n = 116) would suggest that the 95th percentile is the highest reliable percentile (Boobis et al., 2017; Arcella et al., 2019).

These data suggest that for adults, a mean fat or oil consumption of 35 g/person per day and a high consumption of fat or oil of 140 g/person per day would be a conservative estimate consistent with available data.

The use of updated food consumption data will result in a revised estimated worst-case dietary exposure for adults:

$$\frac{\text{p95 consumption of oil (0.140 kg/day)} \times \text{concentration (100 mg/kg fat or oil)}}{\text{60 kg body weight}}$$

$$= \mathbf{0.2\ mg/kg\ bw\ per\ day}$$

4.3.2 Exposure estimates for infants and young children

Potentially vulnerable population groups, like infants and young children, were not specifically considered in the 2006 criteria document. Since then, individual consumption data for several population groups, including infants and young children, have become available through CIFOCOss and other sources. Infants and young children should be considered in the risk assessment because they could potentially experience high exposure to previous cargo chemical substances per kg body weight while they are undergoing growth and development.

Information on consumption of food oils by infants and young children was also available from the US Environmental Protection Agency's Food Commodity Intake Database (FCID) (US EPA, 2020a), which in turn is based on data from the US National Health and Nutrition Survey/What We Eat In America, 2005–2010 cycles. The highest oil consumption for infants and young children based on FCID is comparable to those in the CIFOCOss database; however, oil consumption information based on FCID takes into account individual body weights.

The highest reported consumption of a specific fat or oil type was for palm oil. Estimated mean and p95 consumption by infants and young children were 7.6 and 19 g/day, respectively. Estimated mean and p95 consumption on a

body weight basis were 1 g/kg bw per day and 3 g/kg bw per day, respectively.

These data were used to define a worst-case dietary exposure estimate for infants and young children:

p95 consumption of oil (0.003 kg/kg bw/day) × concentration (100 mg/kg fat or oil)

= 0.3 mg/kg bw per day

4.3.3 Exposure from other dietary sources

For some previous cargo chemical substances potentially present in food oils, there are additional sources of dietary exposure, such as contamination (for example, contaminated drinking-water) or food additive uses (Table 3). Dietary exposures from these different sources should be considered in exposure assessment.

4.3.4 Conclusion

The Committee concluded that, based on up-to-date data on consumption of single fats and oils in the general population, which have become available since 2006, the generic human exposure value of 0.1 mg/kg bw per day used in the 2006 criterion no. 2 to determine the acceptability of a previous cargo should be revised. Consequently, the updated, more conservative generic human exposure value of 0.3 mg/kg bw per day should be used in the evaluation of these substances.

The Committee noted that these estimates of dietary exposure were derived from a more conservative approach to using data on consumption of single fats and oils and a worst-case concentration of previous cargo chemicals in a single fat or oil of 100 mg/kg.

The Committee also concluded that additional sources of dietary exposure need to be considered in exposure assessment of previous cargo chemical substances.

4.4 Approach to toxicological evaluation

The Committee received no submitted data and, therefore, reviewed monographs from previous evaluations of individual substances conducted by JECFA, WHO, International Agency for Research on Cancer (IARC), and national and regional governmental authorities to retrieve additional relevant references for completing the present assessment. The Committee also conducted literature searches. The details are included in the consideration of individual substances.

At its present meeting, the Committee revised the generic value for assumed worst-case human dietary exposure from 0.1 to 0.3 mg/kg bw per day and used this revised generic exposure value for the evaluation of previous

Table 3
List of substances for evaluation by JECFA arising from the development of a list of acceptable previous cargoes by the Codex Committee on Fats and Oils: Other sources of exposure

Substance (synonyms)	Other sources of exposure
1,4-Butanediol (1,4-butylene glycol)	Used in food contact material
Calcium ammonium nitrate solution	Calcium, nitrate and ammonium are ubiquitous in the human diet
Calcium lignosulfonate liquid (lignin liquor; sulfite lye), molecular weight not specified	Calcium lignosulfonate (40-65) is used as a food additive, an additive in animal feed and as an ingredient in pesticides
Calcium nitrate (CN-9) solution	Calcium and nitrate are ubiquitous in the human diet
iso Decyl alcohol (isodecanol)	None
Myristyl alcohol (1-tetradecanol; tetradecanol)	Flavouring agent, formulation agent, lubricant, release agent
iso Nonyl alcohol (isononanol)	None
iso Octyl alcohol (isooctanol)	Used in food contact material
Tridecyl alcohol (1-tridecanol)	Used in food contact material
Unfractionated fatty alcohol mixture or mixtures of fatty alcohols from natural oils and fats[a]	Occurs naturally in foods
Methyl tertiary butyl ether (MTBE)	Drinking-water
Mineral oil, medium and low viscosity, class II and III	Used in food contact material, direct food additive
Montan wax	Food additive
1,3-Propylene glycol	Used in place of 1,2-propanediol as a food additive
Propylene tetramer (tetrapropylene, dodecene)	None
Soybean oil epoxidized	Used in food contact material
Ethyl tertiary butyl ether (ETBE)	Drinking-water

[a] Discussed with Group 2 – Alcohols.

cargoes. The Committee also considered data on exposure to the substances from sources other than previous cargoes. Thus, the ADI (or TDI) should be greater than or equal to the estimated dietary exposure (0.3 mg/kg bw per day plus exposure from other possible dietary sources) in order to provide sufficient protection for infants, children and high-intake consumers. In situations where no appropriate numerical ADI (or TDI) was available from JECFA, the Committee considered other previously established health-based guidance values or calculated a margin of exposure (MOE) based on a reference point characterizing the toxicological hazard (such as a no-observed-adverse-effect level (NOAEL), etc.) identified from the available data divided by the estimated dietary exposure. Interpretation of this MOE is a matter of expert judgement that takes into account limitations in the available toxicological database.

5. Recommendations

The Committee recommended that the Codex Committee on Fats and Oils (CCFO) consider revising Criterion no. 2 in RCP-36-1987 as adopted by CAC 34 (2011).

- Based on the consumption of fats and oils by infants and young children, there is no health concern for the general population from dietary exposure to previous cargo chemical substances if the ADI or TDI is sufficiently protective, for example, the ADI or TDI is greater than, or equal to 0.3 mg/kg bw per day. Substances for which there is no numerical ADI or TDI should be evaluated on a case-by-case basis (e.g. margin of exposure (MOE) approach).
- Where there are additional sources of dietary exposure to the previous cargo chemical substances, they should be considered in the exposure assessment.

B. EVALUATION OF SUBSTANCES

Oils and waxes (Group 3)

Montan wax, propylene tetramer, soybean oil epoxidized, and mineral oils (medium and low viscosity, class II and III)

This monograph considers the suitability of a) montan wax, b) propylene tetramer, c) soybean oil epoxidized and d) mineral oils (medium and low viscosity, class II and III), as an immediate previous cargo for transporting edible fats and oils as a subsequent cargo. Whereas propylene tetramer, epoxidized soybean oil and white mineral oil (liquid paraffin oil) – edible grade are included on the FOSFA list of acceptable previous cargoes, montan wax, propylene tetramer, epoxidized soybean oil and white mineral oil, United States Pharmacopeia (USP) grade, are on the NIOP–FOFSA joint list of acceptable previous cargoes. The present assessment represents the first evaluation of these substances by JECFA for consideration as acceptable previous cargoes for edible fats and oils. Their general characteristics are summarized in Table 4.

Table 4
Previous cargo substances under the group of oils and waxes included in the current assessment

Substance	Substance type	Components	Relative contribution (%)	Reference
Montan wax CAS number: 8002-53-7	Complex mixture (composition varies by region)	Esters Fatty acids Alcohols *n*-Alkanes Ketones Resin acids Terpenes Hydroxy acids Ash Resin + asphalt Sterols, alkenes, aldehydes and ethers	43–60 0.1–25 1–13 1–10 1–1.5 10–15 0.1–3.5 1 0.5 25 Not defined	Lawrence et al., 1982; Matthies, 2001; Noskova, 2010; Guo, 2014; Lang et al., 2020; Zhang et al., 2018
Propylene tetramer CAS number: 6842-15-5	Alkenes (C10–C15)	C12 – tetra-alkene, mixed isomers possible	C12 alkene: >71% C10/C11 alkenes: <22% C13–C15 alkenes: <15%	EFSA, 2012b
Epoxidized soybean oil CAS number: 8013-07-8	Soybean oil epoxidized at the site of double bonds	Epoxidized fatty acids	Linolenic: 7% Oleic: 25% Linoleic: 53%	Cai et al., 2008
		Saturated fatty acids	About 15%	
Mineral oils low and medium viscosity (class II and III) CAS number: 8042-47-5* *White mineral oil (liquid paraffin oil)-edible grade Liquid hydrocarbons (paraffinic and naphthenic)	Complex mixture	*n*-Alkanes Branched alkanes Cyclic alkanes	Carbon chain lengths between >17 and 23	JECFA, 2003; EFSA, 2012c; JECFA 2013 (Annex 1, reference *216*); Bevan et al., 2020

I. Montan wax

1. Explanation

The acceptability of montan wax as a previous cargo was evaluated in 1996 by the EU SCF (1997) and determined to be provisionally acceptable. The conclusion was based on the fact that montan wax itself is highly insoluble and that montan acid esters derived from montan wax were temporarily authorized as food additives for the surface treatment of certain fruits (EFSA, 2012b). Montan wax was re-evaluated in 2011 by EFSA's CONTAM Panel based on revisions to the criteria for the acceptability of previous cargoes as proposed by the Codex Committee

on Fats and Oils in 2009 (EFSA, 2012b). The CONTAM Panel noted that montan wax is an ill-defined material and that there was insufficient information on the composition and toxicological profile of the substance to determine that it does not contain constituents that would be a human health concern when used as a previous cargo. Taking into account these deficiencies, the Committee concluded that montan wax does not meet the criteria for acceptability as a previous cargo for edible fats and oils.

For the current review, previous assessments by EFSA and a recent review by Health Canada were considered (Health Canada, 2019). A search by CAS number and name for additional relevant toxicological studies in animals or humans was undertaken to identify any critical new data for the assessment of risk to human health. REACH registration data from the ECHA dissemination website were accessed, and targeted searches were conducted on the PubMed and PubChem websites as well as using the Google Scholar search engine.

The following sources and databases were also queried to obtain data on chemical specifications, route(s) of synthesis, composition and uses of montan wax, as well as information on analytical methods and potential reactions with edible fats and oils: Agricola, CAB Abstracts, Embase, FSTA, Global Health, Medline, Scopus, the grey literature and PubMed. The cut-off date for inclusion in this report was 30 September 2020.

2. Chemical and technical considerations

2.1 Methods of manufacture

Montan wax is formed along with coal and is present in lignite or brown coal (Lawrence et al., 1982; Matthies, 2001). It is extracted from dried, crushed particles of lignite through a leaching process using organic solvents such as toluene and is further refined through an oxidation process (Lawrence et al., 1982; Lolja, 2003; Hu et al., 2011; Wei et al., 2014; Wang, Herdegen & Repke, 2015, 2016; Yuan et al., 2015; Chen et al., 2020). It is available in three broad categories, crude montan wax, montan resin and refined montan wax, distinguished by the degree of refining (Chen et al., 2020).

2.2 Composition and impurities

Montan wax is a complex mixture of organic compounds belonging to many chemical classes. Montan wax, like other natural waxes, is dominated by even-

numbered carbon chain components (Lawrence et al., 1982). Its composition and characteristics are presented in Tables 5 and 6. Its composition varies depending on the geographical region, the plant material that has undergone coalification and the period in which it was formed (Ye, 1976; Tang, Hu & Chen, 1982; Li, Sun & Wu, 1987; Yu, Ma & Zhue, 1991; Matthies, 2001; Noskova, 2010; Guo, 2014; Li, 2014; Wei et al., 2014; Wang, Herdegen & Repke, 2015; Chen et al., 2020; Lang et al., 2020). Crude montan wax, deresinated montan wax and refined montan wax from lignite have long chain fatty acids (C14–C34), wax alcohols (C24–C32) and normal alkanes (C23–C33) (Wei et al., 2014). Montan resin extracts contain C18–C30 alkanes, ketones, alcohols, esters, alkenes, aldehydes and ethers (Zhang et al., 2018; Lang et al., 2020).

2.3 Uses of montan wax

Montan wax is used as a replacement for carnauba wax in shoe polish, floor wax, car wax, etc., where colour is not a concern (Wei et al., 2014). Although montan wax has been registered as a food additive in Japan, its applications are very limited (Ikeda et al., 2008; Wei et al., 2014). Montan wax is used as an insulator, road asphalt additive, in paper making, leather finishing and lubricant manufacture and also has applications in agriculture as a chemical dispersant and in waterproofing wood products (Ikeda et al., 2008; Wei et al., 2014).

2.4 Reactivity and reactions with edible fats and oils

No specific information was found on the reactions of montan wax with edible fats and oils. Montan wax is considered to be chemically stable (Wei et al., 2014). The compound classes present in montan wax are consistent with those found in plant lipids and may form esters.

2.5 Methods of analysis

No analytical methods for the detection and determination of montan wax in edible fats and oils were found in the literature. Gas chromatography (GC) with flame ionization detection (FID) has been and continues to be used for montan wax analysis, consistent with its broad application in the analysis of lipids (Lawrence et al., 1982; Matthies, 2001; Yuan et al., 2015). The use of GC coupled to mass spectrometry (MS) has also been reported in the literature (Hawthorne & Miller, 1987; Chen et al., 2020). Derivatization of polar groups is required for detection of more components as well as improved sensitivity (Lawrence

Table 5
Physical and chemical characteristics of montan wax

Characteristic	Value	Reference
Colour/form	Creamy white, light yellow, light brown, dark brown solid	Wei et al., 2014 ; Yuan et al., 2015
Melting point (°C)	80–89	Yuan et al., 2015; Chen et al., 2020
Acid value (KOH mg/g)	30–140	Wei et al., 2014; Yuan et al., 2015; Chen et al., 2020
Saponification values (KOH mg/g)	60–180	Yuan et al., 2015; Chen et al., 2020; Wei et al., 2014
Ester value (KOH mg/g)	15–105	Wei et al., 2014
Friability (%)	3.43–4.18	Chen et al., 2020

KOH, potassium hydroxide.

Table 6
Chemical and technical considerations for montan wax

Name: montan wax	
CAS number	**Alternative CAS numbers**
8002-53-7	None
Chemical details	Creamy white, light yellow, light brown or dark brown solid depending on grade Structure: complex mixture Melting point: 80–89 °C Insoluble in water; soluble in toluene and other organic solvents
Route(s) of synthesis	Montan wax is formed during coalification and is present in lignite or brown coal. It is extracted from dried, crushed particles of lignite using toluene.
Composition	Esters (43–60%) Fatty acids (0.1–25%) Alcohols (1–13%) *n*-Alkanes (1–10%) Ketones (1–1.5%) Resin acids (10–15%) Terpenes (0.1–3.5%) Hydroxy acids (1%) Ash (0.5%) Resin + asphalt (25%) Sterols, alkenes, aldehydes and ethers (not defined)
Uses	Used in shoe polish, floor wax, car wax, etc. (as a replacement for carnauba wax). Used as an insulator, road asphalt additive, in paper making, leather finishing and lubricant manufacture, as a chemical dispersant in agricultural applications, and in waterproofing of wood products; minor use as a food additive.
Analytical methods	None found for previous cargoes. Possible means of detection in fats and oils by GC-FID or GC-MS.
Potential reaction(s) with a subsequent cargo of fat or oil	No specific information is available on the reactions of montan wax with edible fats and oils.

GC-FID, gas chromatography with flame ionization detection; GC-MS, gas chromatography–mass spectrometry.

et al., 1982). Fatty acids may be detected after methylation and alcohols after acetylation. More recently, methylation using diazomethane and silylation with N,O-*Bis*(trimethylsilyl)trifluoroacetamide (BSTFA) has been reported (Yuan et al., 2015; Chen et al., 2020). Direct analysis without a prior derivatization step has been reported, following supercritical fluid extraction (Hawthorne & Miller, 1987). Thermogravimetry-Fourier-transform infrared spectroscopy (TG-FTIR) has been used to examine the structural spectra of pyrolysis products of montan waxes (Hu et al., 2011).

3. Biological data

3.1 Biochemical aspects

No data specific to the disposition of montan wax following oral exposure were identified. The composition of montan wax varies according to the geographical region, the plant material that has undergone coalification and the period in which it was formed. Crude montan wax, deresinated montan wax and refined montan wax from lignite have long chain fatty acids (C14–C34), wax alcohols (C24–C32) and normal alkanes (C23–C33). A recent compositional analysis of refined montan wax from five different sources (Chen et al., 2020) identified 40 substances, of which 29 appeared in all five samples and four were distributed in only one or two samples. The individual constituents identified tended to be large, almost invariably hydrophobic molecules that are anticipated to have low oral bioavailability, consistent with the previous conclusion of the CONTAM Panel that absorption of montan wax from the gastrointestinal tract is expected to be limited. EFSA (2012b) stated that alkane constituents of montan wax that are absorbed are expected to be metabolized to the corresponding fatty alcohols and then to fatty acids before entering normal metabolic pathways.

3.2 Toxicological studies

3.2.1 Acute toxicity

The available evidence indicates that montan wax can be expected to be of low acute toxicity. An acute oral toxicity study equivalent or similar to OECD guideline 401 is reported in the ECHA registration dossier for this substance (Anonymous, 1979; ECHA, undated). Male and female Wistar rats (10 of each sex) were administered two doses of 6000 mg/kg bw montan wax within 2 hours by oral gavage. Although one female rat died directly after administration, no

adverse effects were observed in the remaining animals over a 1-week observation period. The substance was concluded to be practically non-toxic, with an oral LD_{50} > 12 000 mg/kg bw.

3.2.2 Repeated-dose toxicity

In a subchronic oral toxicity study conducted in accordance with OECD guideline 408, montan wax was administered to male and female F344/DuCrj rats (10 per sex per group) at levels of 0, 0.56, 1.67 or 5% in the diet (equivalent to 260, 835 or 2500 mg/kg bw per day) for 90 days (Ikeda et al., 2008). It is not specifically stated whether the test article was a crude, deresinated or refined montan wax, although the following specifications were given: melting point 81.5 °C, oxidization value 11.8, saponification value 14.0. Although no deaths occurred in any group and there were no remarkable changes in general condition, haematological and serum biochemical changes were observed at all doses tested. Elevations of serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) occurred in all treated groups, while haemoglobin, haematocrit, mean corpuscular volume and mean corpuscular haemoglobin were significantly decreased at all doses. White blood cell count was significantly increased in all treated rats of both sexes, as were relative weights of the liver, spleen, lung and kidney. Lastly, multiple diffuse liver granulomas accompanied by hepatocyte effects, lymphocytic infiltration and granulomatous lesions in mesenteric lymph nodes (MLN) were also observed starting from the lowest dose tested. Most of the lesions, including the liver granulomas, exhibited no clear dose–response relationship. It was not possible to establish a NOAEL for subchronic oral toxicity on the basis of these findings.

No information on chronic studies was located for montan wax and no data on end-points such as developmental or reproductive toxicity, immunotoxicity or neurotoxicity were identified.

3.2.3 Genotoxicity and carcinogenicity

Montan wax in ethanol was non-mutagenic in bacterial reverse mutation assays (conducted according to OECD guideline 471) using *Salmonella* Typhimurium strains TA97a, TA98, TA100, TA102 and TA1535 with and without metabolic activation (Anonymous, 2010; ECHA, undated). No mutagenic effects and no cytotoxicity were observed at any of the five concentrations tested, although there is some discrepancy in the summary data. This is because the methods section details test concentrations up to 342 µg/plate while the discussion reports the same nominal concentrations in units of pg/plate, a difference of six orders of magnitude. Testing was reported to have been conducted up to precipitating conditions in line with OECD guidelines for insoluble noncytotoxic substances

although no data regarding the solubility of montan wax in ethanol were identified.

No data concerning the carcinogenicity of montan wax were located.

3.2.4 Allergenicity

The sensitization potential of montan wax was investigated with the mouse local lymph node assay according to OECD guideline 429 (Anonymous, 2009; ECHA, undated). When tested at concentrations of 2.5, 5 and 10% (w/w) in methyl ethyl ketone, montan wax was found to be nonsensitizing. Similarly, lipstick formulations containing montan wax at 1.61, 1.81, 1.93 and 2.53% were tested for irritation and sensitization properties in humans using repeated insult patch tests. The material caused no irritation or sensitization (Anonymous, 1984). Based on the available data, there is no indication that montan wax has significant sensitizing, adjuvant or irritant potential at concentrations expected from its use as a previous cargo (EFSA, 2012b).

3.3 Observations in humans

No human studies pertaining to the toxicity of montan wax were identified.

4. Food consumption and dietary exposure estimates

A worst-case concentration of 100 mg/kg has been assumed for all previous cargo substances (see part A, section 4.3). Montan wax substances are used in the protection of fruit surfaces and as food packaging additives in some countries and regions; however, no data are available on concentrations resulting from these uses.

Worst-case exposures to previous cargo substances in food oils have been estimated at 0.3 mg/kg bw per day (see part A, section 4.3). Health Canada (2019) evaluated the potential for exposure to montan wax substances from food packaging and concluded that exposure related to this use is negligible.

No other data on dietary exposure to montan wax were identified.

5. Comments

5.1 Chemical and technical considerations

Chemical and technical considerations for montan wax are summarized in Table 6.

5.2 Biochemical aspects

No data specific to the disposition of montan wax following oral exposure were identified. The composition of montan wax varies according to the geographical region, plant material that has undergone coalification and the period in which it was formed. Crude montan wax, deresinated montan wax and refined montan wax from lignite have long chain fatty acids (C14–C34), wax alcohols (C24–C32) and normal alkanes (C23–C33). Several studies have identified individual constituents of montan wax, which vary according to the source and degree of refinement. Despite this variability, most constituents identified tended to be large, hydrophobic molecules that are anticipated to have low oral bioavailability. Alkane constituents of montan wax that are absorbed are expected to be metabolized to the corresponding fatty alcohols and fatty acids.

5.3 Toxicological studies

Although assessments are based on limited information, montan wax is anticipated to be of low acute oral toxicity, with an estimated rat oral LD_{50} > 12 000 mg/kg bw (Anonymous, 1979).

In an OECD guideline-compliant subchronic oral toxicity study, male and female F344/DuCrj rats (10 per sex per group) were administered montan wax in the diet for 90 days at levels of 0, 0.56, 1.67 or 5% (equivalent to 0, 260, 835 or 2500 mg/kg bw per day) for 90 days (Ikeda et al., 2008). The authors did not specify whether the test article was a crude, deresinated or refined montan wax, nor its geographical source. No deaths occurred in any group and there were no remarkable changes in general condition. However, haematological and serum biochemical changes were observed at all doses tested, as were diffuse liver granulomas accompanied by hepatocyte effects, as well as lymphocytic infiltration and granulomatous lesions in mesenteric lymph nodes. In addition to effects occurring from the lowest dose tested, most of the lesions observed, including the liver granulomas, showed no clear dose–response relationship. A NOAEL for subchronic oral toxicity could therefore not be established on the

basis of these findings. There is some evidence to suggest that the F344 rat is particularly sensitive to certain mineral waxes and oils and that adverse effects may be more severe and consequential in this strain (Annex 1, reference *211*) (Carleton et al., 2001; Trimmer et al., 2004; Griffis et al., 2010; Adenuga, Goyak & Lewis 2017). However, these observations are based on studies of long-chain mineral oil hydrocarbons generally and are not specific to montan wax.

No chronic toxicity or carcinogenicity studies were located for montan wax and no data on end-points such as developmental or reproductive toxicity, immunotoxicity or neurotoxicity were identified. Montan wax in ethanol was non-mutagenic and non-cytotoxic in guideline-compliant bacterial reverse mutation assays using *Salmonella* Typhimurium strains TA97a, 98, 100, 102 and 1535 with and without metabolic activation (Anonymous, 2010).

5.4 Allergenicity

The Committee did not identify any reports of allergenicity upon oral exposure to montan wax that would indicate that it is or contains a food allergen.

6. Food consumption and dietary exposure estimates

A worst-case concentration of 100 mg/kg has been assumed for all previous cargo substances (see part A, section 4.3).

Montan wax substances are used for the protection of fruit surfaces and as additives for food packaging in some countries and regions; however, no data are available on concentrations resulting from these uses.

Worst-case human dietary exposures to previous cargo contaminants in food oils have been estimated at 0.3 mg/kg bw per day (see part A, section 4.3).

Health Canada (2019) evaluated the potential for exposure to montan wax substances from food packaging and concluded that exposure related to this use is negligible.

No other data on dietary exposure to montan wax were identified.

7. Evaluation

While oral bioavailability of montan wax is expected to be limited and the material appears to be of low acute toxicity, in the only repeat-dose study available (Ikeda et al., 2008), montan wax produced toxicity at all doses tested. The Committee

noted that montan wax is a highly variable and poorly defined material. Given the high degree of variability in composition, the extent to which the particular test article in the subchronic study is representative of the diversity of the various forms of crude, deresinated or refined montan wax currently in commerce is unknown. Therefore, the Committee could not characterize the hazard of montan wax shipped as a previous cargo.

No specific information was found on the reactions of montan wax with edible fats and oils.

The Committee determined that the available evidence was not sufficient to characterize the risk of montan wax; as a result, it was concluded that montan wax does not meet the criteria for acceptability as a previous cargo for edible fats and oils.

7.1 Recommendations

The Committee recommended that sufficient chemical and toxicological information that allows the evaluation of montan wax as shipped is made available prior to the next evaluation. At a minimum this information should address the following:

- degree of refinement and chemical constituents;
- repeat-dose toxicological data on representative products in a relevant animal model.

II. Propylene tetramer

1. Explanation

Propylene tetramer was evaluated by SCF in 1996, at which time it was determined to be acceptable as a previous cargo, subject to the results of ongoing genotoxicity testing (SCF, 1997) as cited by the EFSA Panel on Contaminants in the Food Chain (CONTAM) (EFSA, 2012b). At the time of the evaluation, only limited toxicity data were available, but no specific concerns based on chemical structure were identified. Moreover, it was anticipated that propylene tetramer residue levels would be low, as the substance is easily removed by cleaning as well as during refinement of oils.

In 2011, EFSA (EFSA, 2012b) evaluated the acceptability of propylene tetramer as a previous cargo, to ensure continued alignment with the revised criteria for acceptable previous cargoes proposed by the Codex Committee for Fats and Oils (EFSA, 2009). The expert panel concluded that propylene tetramer would not be of toxicological concern at the levels that would occur when used as a previous cargo for edible fats and oils, and therefore the substance met the criteria for acceptability. Although it was acknowledged that studies on carcinogenicity were lacking, the panel concluded that in the absence of genotoxicity or evidence of pathological changes in subchronic studies, which might indicate carcinogenic potential, there was no concern for carcinogenicity from the use of propylene tetramer as a previous cargo.

For the current review, previous assessments by SCF, OECD and EFSA were considered, as were recent toxicological studies conducted by the Japanese National Institute of Health Sciences. A search by CAS number and name for additional relevant toxicological studies in animals or humans was undertaken to identify any critical new data for the assessment of human health risk. Targeted searches were conducted on the PubMed and PubChem websites as well as using the Google Scholar search engine.

The following sources and databases were also queried to obtain data on chemical specifications, route(s) of synthesis, composition and uses of propylene tetramer, as well as information on analytical methods and potential reactions with edible fats and oils: Embase, FSTA, Global Health, Medline, Scopus and PubMed.

The cut-off date for inclusion in this report was 30 September 2020.

2. Chemical and technical considerations

2.1 Methods of manufacture

Propylene tetramer is formed via catalytic polymerization of propene using phosphoric acid, *ortho*-phosphoric acid or sulfuric acid as catalysts. The reaction occurs when the catalysts are present on a solid support (e.g. alumina) and propene is in the vapour phase. Fractionation of the C12 polymer is necessary to obtain optimum yield. The di- and trimers, having a lower boiling range, are separated and re-polymerized (Hinds, Mozerbolt & Reno, 1951). Owing to the complicated route of synthesis, products formed are mixtures and must be separated. Paraffin distillation with separation of the propylene tetramer from the C10–C14 fraction has been identified as another route of propylene tetramer production (China Research Institute of Daily Chemical Industry, 1971).

2.2 Composition and impurities

The composition and characteristics of propylene tetramer are presented in Tables 7 and 8. The structure is shown in Fig. 1 (Takahashi et al., 2016). It is also identified as 1-dodecene, which has a single carbon-carbon double bond (Fig. 2). The product of commerce occurs as a blend of isomers comprising C12 alkene > 71%, C10–C11 alkenes < 22% and C13–C15 alkenes < 15% (EFSA, 2012b).

Fig. 1
Structure of propylene tetramer (A and B denote expanded and condensed forms, respectively)

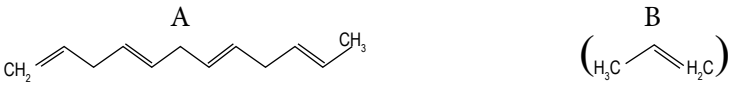
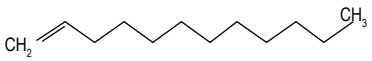


Fig. 2
Structure of propylene tetramer in 1-dodecene form



The range in molar mass seen in Table 7 is related to the inclusion of the linkage of individual propene groups forming the tetramer with the corresponding loss of hydrogen when the carbon-carbon bonds are formed. Propylene tetramer is insoluble in water, but soluble in ethanol, ether and acetone.

Propylene tetramer is a crude mixture comprising alkenes of variable chain length; alkenes with non-C12 chain lengths may be considered impurities in the product (EFSA, 2012b).

2.3 Uses of propylene tetramer

A range of products are developed using propylene tetramer including alcohols, surfactants and anti-rust agents (Janex, 2020). It is used in the production of both linear and branched alkylbenzene, dodecylphenol polyoxyethylene ether, dodecyl phenol and sodium alkyl diphenyl ether disulfonate (China Research Institute of Daily Chemical Industry, 1971; Zheng, Cui & Li, 2000). Propylene tetramer is also used in the production of detergents, plasticizers, petroleum and lubricating oil additives. In addition, the anti-rust agent (T746) can be produced by propylene tetramer and maleic anhydride (Wang & Chen, 2013).

Table 7
Physical and chemical characteristics of propylene tetramer

Characteristic	Value	Reference
Synonyms	1 propene, tetramer; dodecene; dodecene, mixture of isomers; tetrapropylene; propene tetramer; (4E,7E,10E)-dodeca-1,4,7,10-tetraene	US EPA, 2020b
Molar mass (g/mol)	162.27–168.32	US EPA, 2020b; Royal Society of Chemistry, 2020
Melting point (°C)	−33.6 to −31	EFSA, 2012b; Chemical book, 2017
Boiling point (°C)	213	Chemical book, 2017

2.4 Reactivity and reactions with edible fats and oils

No specific information was available on reactions of propylene tetramer with edible fats and oils. It is reported to be stable during transport (Chemical book, 2017). Further polymerization of propylene tetramer could occur when exposed to catalysts such as phosphoric acid under certain conditions (NOAA, 2019).

2.5 Methods of analysis

Analytical methods for the determination of propylene tetramer in fats and oils were not reported in the literature, although methods for analogous compounds (e.g. dodecenes) have been described. Extraction of dodecenes from cheese and other matrices has been reported using different solvents such as methanol or pentane:isopropyl alcohol (3:2), followed by cleanup using Florisil® or silica gel prior to GC-FID analysis (Bergaentzle et al., 1994; Gopal & Kannabiran, 2018). 1-Dodecene has been analysed following extraction from various plant materials using GC-FID and GC-MS (Gamel & Linssen, 2008; Arrabal et al., 2011; Ama et al., 2015).

3. Biological data

3.1 Biochemical aspects

No studies were identified that investigated the toxicokinetics of propylene tetramer specifically. However, propylene tetramer is a crude mixture largely of olefins (EFSA, 2012b) and some information on the disposition of these substances following oral administration is available. On the basis of their physicochemical properties (average molecular weight of 168 to 160, high lipid

solubility), EFSA (2012b) determined that the main olefins present in propylene tetramer are "likely to be absorbed from the gastrointestinal tract to a reasonable extent and distributed throughout the body". The initial step in the metabolism of olefinic compounds appears to be cytochrome P450-dependent oxidation in the liver to form unstable electrophilic epoxides. These are subsequently inactivated by the formation of protein adducts, hydrolysed to the corresponding diol by epoxide hydrolases, or form glutathione conjugates that are ultimately excreted in urine in the form of mercapturic acids (White et al., 1986; Henderson, 2001; EFSA, 2012b). Henderson (2001) has shown that there are important species-specific differences in the subcellular location and activity of these enzymes that may mediate the potential toxicity of olefins. Notably, activity of the hydrolysis pathway appears to be far more prominent in primates than in rats or mice, suggesting humans may be less sensitive to olefin toxicity, although variation in human metabolism is anticipated as a result of genetic polymorphisms (Henderson, 2001).

3.2 Toxicological studies

3.2.1 Acute toxicity

The Japanese National Institute of Health Sciences (Takahashi et al., 2016) investigated the acute oral toxicity of propylene tetramer in accordance with OECD test guideline 423 (Acute Toxic Class Method). Female Crl:CD(SD) rats (three per step) were administered propylene tetramer in olive oil by oral gavage at doses of 300 mg/kg bw (first and second steps) and 2000 mg/kg bw (third and fourth steps). At a dose of 300 mg/kg bw the substance produced diarrhoea, and, at 2000 mg/kg bw, decreased locomotor activity, diarrhoea and soiling of the perineal region were observed. No deaths were recorded at any step and the acute oral LD_{50} of propylene tetramer was estimated to be approximately 5000 mg/kg bw. Similar results were reported by Cushman et al. (1992) who administered undiluted propylene tetramer to Sprague-Dawley rats by oral gavage at a dose of 5000 mg/kg bw (five animals of each sex). No deaths or other macroscopic pathological findings were reported over the 14-day follow-up period.

The low acute oral toxicity of propylene tetramer is consistent with the findings of previous studies of the individual alkenes and mixtures of alkenes that comprise this substance; in most cases oral LD_{50} values were observed to be greater than 10 g/kg bw in rats and mice (EFSA, 2012b).

3.2.2 Repeated-dose toxicity

Studies of olefins administered via the oral route indicate that they are generally of low toxicity upon repeat administration. The most sensitive end-point is

kidney effects in male rats. However, the mechanism of action is attributed to induction of α2u-globulin and hyaline droplet accumulation in proximal tubule cells. As humans lack an analogous protein at levels sufficient to produce a similar response, α2u-globulin-mediated nephropathy is widely acknowledged as male-rat-specific and therefore not relevant to the evaluation of toxicological risk in humans.

Propylene tetramer was evaluated in a combined repeated-dose oral toxicity study with a reproduction and developmental toxicity screening test according to Japanese guidelines equivalent or similar to OCED test guideline 422 (Takahashi et al., 2016). Male and female Crl:CD(SD) rats (12 animals per sex and dose) were administered propylene tetramer daily by oral gavage at doses of 0 (vehicle control), 40, 150 or 600 mg/kg bw per day. Males were dosed for a total of 42 days, beginning from 14 days pre-mating, whereas females were dosed from 14 days pre-mating to day 4 of lactation (40–45 days). Five of 12 males in the groups that received 0 and 600 mg/kg bw per day were evaluated as a 14-day recovery group, and 10 females per dose were treated with 0 or 600 mg/kg bw per day and kept as a satellite group (without mating) to be evaluated after the administration period or following a 14-day recovery period.

Haematotoxicity (anaemia) was observed in male rats administered propylene tetramer at doses of 150 mg/kg bw per day and higher, as well as in females in the satellite group given 600 mg/kg bw per day. In all treated groups of male rats, dose-dependent α2u-globulin-mediated nephropathy was observed, including increased kidney weight at the lowest tested dose of 40 mg/kg bw per day, basophilic changes in tubular epithelium at 150 mg/kg bw per day and above, and necrosis of epithelial cells at 600 mg/kg bw per day. Hepatotoxicity was observed in both sexes; liver weights were increased in animals given doses of 150 mg/kg bw per day and higher and hypertrophy of centrilobular hepatocytes occurred at 600 mg/kg bw per day. Alterations in clinical chemistry parameters (α2-globulin fraction, γ-glutamyl transpeptidase, total cholesterol and glucose levels) were observed in both sexes as were thyroid effects (increased thyroid weight, thyroxin level and hypertrophy of follicular cells) in females at the highest dose tested. Following cessation of treatment, the liver, kidney and haematological parameters tended to resolve during the recovery period, while the thyroid effects persisted. A NOAEL for repeated-dose toxicity of 40 mg/kg bw per day was established on the basis of increased liver weight in both sexes at the next highest dose of 150 mg/kg bw per day. No developmental or reproductive toxicity effects were described, indicating a NOAEL of greater than 600 mg/kg bw per day for these end-points.

Similar results have been observed following repeated oral administration of various individual alkenes or mixtures thereof. In rats, the lowest oral NOAEL for systemic toxicity not attributed to α2u-globulin-dependent effects was for

1-octene, where a NOAEL of 50 mg/kg bw per day was established based on marginal alterations in kidney weight and serum creatinine in females at a dose of 500 mg/kg bw per day (OECD, 2001, 2004 as cited in EFSA, 2012b). In combined reproductive and developmental toxicity studies in which rats were administered 1-hexene or 1-tetradecene by oral gavage prior to mating, during mating, and throughout gestation and lactation, no effects were observed at the highest dose tested of 1000 mg/kg bw per day (OECD, 2001 as cited in EFSA, 2012b). Likewise, studies on various alkene mixtures, including C6 (internal branched/linear stream); C16/18 (internal linear and branched); and C18 (internal linear and branched) produced no evidence of developmental or reproductive toxicity with the NOAEL determined to be the highest dose tested of 1000 mg/kg bw per day in all cases (OECD, 2004 as cited in EFSA, 2012b).

3.2.3 Genotoxicity and carcinogenicity

The in vitro genotoxic potential of propylene tetramer was evaluated in bacterial reverse mutation assays using *Salmonella* Typhimurium strains TA100, TA1535, TA98 and TA1537 and *E. coli* WP2uvrA with and without metabolic activation (Takahashi et al., 2016). Studies were carried out under GLP conditions according to the Japanese *Guidelines for screening mutagenicity testing of chemicals*, which are similar or equivalent to OECD test guideline 471. Propylene tetramer in acetone with or without S9 mix did not produce revertant colonies when tested at concentrations of 156, 313, 625, 1250, 2500 or 5000 μg/plate. An in vitro cytotoxicity/chromosomal aberration test was also conducted in Chinese hamster lung (CHL/IU) cells in accordance with OECD test guideline 473. Cells (200/dose) were exposed to the test article dissolved in acetone with or without metabolic activation for short-term treatments or for continuous treatments of 24 and 48 hours. No evidence of genotoxicity was observed under any test conditions. However, propylene tetramer produced cytotoxicity (50% cell growth inhibition) at concentrations of 315.3 μg/mL (short-term), 336.7 μg/mL (24 hours, continuous) and 219.2 μg/mL (48 hours, continuous), although no cytotoxicity was observed in the presence of S9 fractions. Based on these results, it was concluded that propylene tetramer does not have genotoxic potential in vitro (Takahashi et al., 2016). Similar results have been reported for other individual olefins present in propylene tetramer or mixtures thereof in various test systems including Ames and *Salmonella* assays as well as the mouse in vivo micronucleus test. None of the olefins were found to be genotoxic (OECD, 2001, 2004 as cited in EFSA, 2012b).

No studies of the potential carcinogenicity of propylene tetramer were identified; however, the substance has no structural alerts for genotoxic or nongenotoxic carcinogenicity as set out in the Istituto Superiore di Sanita (ISS)

(Benigni/Bossa) rulebase for mutagenicity and carcinogenicity (Benigni & Bossa, 2006).

3.2.4 Allergenicity

The available data provide no indication that would suggest propylene tetramer is an allergen or an adjuvant at concentrations expected from its use as a previous cargo. The dermal sensitization potential of propylene tetramer was tested using the Buehler method in 20 female Hartley guinea-pigs (Cushman et al., 1992). Animals were induced with applications of 5% w/w propylene tetramer in ethanol under occlusion for 6 hours, three times weekly. Upon challenge 2 weeks later, no reactions were observed, and the material was determined to be non-sensitizing. No information pertaining to adjuvanticity was identified although propylene tetramer has no protein binding alerts for skin sensitization according to mechanistic and end-point-specific profiling schemes (i.e. OECD, OASIS[1]).

3.3 Observations in humans

No human studies pertaining to the toxicity of propylene tetramer were identified.

4. Food consumption and dietary exposure estimates

A worst-case concentration of 100 mg/kg has been assumed for all previous cargo substances (see part A, section 4.3).

Worst-case exposures to previous cargoes substances in food oils have been estimated at 0.3 mg/kg bw per day (see part A, section 4.3).

No other potential sources of dietary exposure to propylene tetramer were identified.

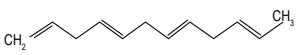
5. Comments

5.1 Chemical and technical considerations

Chemical and technical considerations for propylene tetramer are summarized in Table 8.

[1] Optimized Approach based on Structural Indices Set: available from the Laboratory of Mathematical Chemistry (https://oasis-lmc.org/) and incorporated in the OECD QSAR Toolbox.

Table 8
Chemical and technical considerations for propylene tetramer

Name: propylene tetramer	
CAS number 6842-15-5	**Alternative CAS numbers** None
Chemical details	(4*E*,7*E*,10*E*)-dodeca-1,4,7,10-tetraene Colourless liquid  Molar mass: 162.27–168.32 g/mol Melting point: −33.6 to −31 °C Boiling point: 213 °C Insoluble in water; soluble in ethanol, ether and acetone
Route(s) of synthesis	Manufactured by polymerization of propene using phosphoric acid or sulfuric acid as catalysts.
Composition	C12 alkenes (>71%) C10/C11 alkenes (<22%) C13-C15 alkenes (<15%)
Uses	Used in the production of alcohols, surfactants, detergents, plasticizers, anti-rust agents, and petroleum and lubricating oil additives.
Analytical methods	None found for previous cargoes. Possible means of analysis in fats and oils by GC-FID (methods applied to 12-carbon alkenes).
Potential reaction(s) with a subsequent cargo of fat or oil	No specific information is available on reactions of propylene tetramer with edible fats and oils.

GC-FID, gas chromatography with flame ionization detection.

5.2 Biochemical aspects

No studies were identified that investigated the toxicokinetics of propylene tetramer specifically. However, propylene tetramer is a crude mixture largely of olefins and some information on the disposition of these substances following oral administration is available (EFSA, 2012b). On the basis of physicochemical properties (average molecular weight of 168 to 160, high lipid solubility), EFSA determined that the main olefins present in propylene tetramer are "likely to be absorbed from the gastrointestinal tract to a reasonable extent and distributed throughout the body" (EFSA, 2012b). The initial step in the metabolism of olefinic compounds appears to be cytochrome P450-dependent oxidation in the liver to form unstable electrophilic epoxides, which are subsequently inactivated by the formation of protein adducts, hydrolysed to the corresponding diol by epoxide hydrolases or form glutathione conjugates that are ultimately excreted in urine in the form of mercapturic acids (White et al., 1986; Henderson, 2001; EFSA, 2012b). Henderson has shown important species-specific differences in the subcellular location and activity of these enzymes that mediate the potential toxicity of olefins

(Henderson, 2001). Notably, activity of the hydrolysis pathway appears to be far more prominent in primates than in rats or mice, suggesting humans may be less sensitive to olefin toxicity than are rodents (Henderson, 2001).

5.3 Toxicological studies

Propylene tetramer and its constituents consistently demonstrate low oral acute toxicity. A recent study conducted by the Japanese National Institute of Health Sciences in accordance with OECD test guideline 423 (Acute Toxic Class Method) estimated the acute oral LD_{50} of propylene tetramer in rats to be approximately 5000 mg/kg bw (Takahashi et al., 2016). The low acute oral toxicity of propylene tetramer is consistent with previous studies of the individual alkenes and mixtures of alkenes that comprise this substance; in most cases oral LD_{50} values were observed to be greater than 10 g/kg bw in mice and rats (EFSA, 2012b).

Similarly, studies of olefins administered via the oral route indicate that these substances are generally of low toxicity upon repeat administration. The most sensitive end-point is kidney effects in male rats; however, the mechanism of action is attributed to induction of α2u-globulin and hyaline droplet accumulation in proximal tubule cells. As humans lack an analogous protein at levels sufficient to produce a similar response, α2u-globulin-mediated nephropathy is widely acknowledged as male-rat-specific and therefore not relevant to the evaluation of toxicological risk in humans.

Propylene tetramer was evaluated in a combined repeated-dose oral toxicity study with a reproductive and developmental toxicity screening test similar to OECD test guideline 422 (Takahashi et al., 2016). Male and female Crl:CD(SD) rats (12 animals per sex and dose) were administered propylene tetramer daily by oral gavage at doses of 0 (vehicle control), 40, 150 or 600 mg/kg bw per day from 14 days pre-mating to day 4 of lactation (40–45 days). Five of 12 males in the 0 and 600 mg/kg bw per day groups were evaluated as a 14-day recovery group, and 10 females per dose were treated with 0 or 600 mg/kg bw per day and kept as a satellite group (without mating) to be evaluated after the administration period or following a 14-day recovery period.

Haematotoxicity (anaemia) was observed in male rats administered propylene tetramer at doses of 150 mg/kg bw per day and higher as well as in satellite females at 600 mg/kg bw per day. Hepatotoxicity was observed in both sexes; liver weights were increased in animals given the dose of 150 mg/kg bw per day and higher, and hypertrophy of centrilobular hepatocytes occurred at 600 mg/kg bw per day. Alterations in clinical chemistry parameters (α-2-globulin fraction, γ-glutamyl transpeptidase, total cholesterol and glucose levels) were observed in both sexes. Thyroid effects (increased thyroid weight and thyroxin level as well

as hypertrophy of follicular cells) were seen in females at the highest dose tested. Following cessation of treatment, the liver, kidney and haematological parameters resolved during the recovery period, whereas the thyroid effects, which were limited to females at the highest dose tested, persisted throughout the 14-day recovery period. No reproductive or developmental toxicity effects were described. The results of this study indicate an absence of lesions such as proliferative effects, hyperplasia or hyperplastic foci that would give rise to concerns of carcinogenicity. The Committee determined that the lowest NOAEL for repeated-dose systemic effects not attributable to α2u-globulin induction was 40 mg/kg bw per day, based on increased liver weights in rats at the next highest dose of 150 mg/kg bw per day.

The in vitro genotoxic potential of propylene tetramer was evaluated in bacterial reverse mutation assays using *Salmonella* Typhimurium strains TA100, TA1535, TA98 and TA1537 and *Escherichia coli* WP2uvrA with and without metabolic activation (Takahashi et al., 2016). Propylene tetramer in acetone with or without S9 mix did not produce revertant colonies when tested at concentrations up to 5000 µg/plate. Likewise, an in vitro cytotoxicity/chromosomal aberration test conducted in Chinese hamster lung (CHL/IU) cells produced no evidence of genotoxicity with or without metabolic activation.

5.4 Allergenicity

The Committee did not identify any reports of allergenicity upon oral exposure to propylene tetramer that would indicate this substance is or contains a known food allergen.

5.5 Food consumption and dietary exposure estimates

A worst-case concentration of 100 mg/kg has been assumed for all previous cargo substances (see part A, section 4.3).

Worst-case human dietary exposures to previous cargo substances in food oils have been estimated at 0.3 mg/kg bw per day (see part A, section 4.3).

No other potential sources of dietary exposure to propylene tetramer were identified.

6. Evaluation

Although no chronic or carcinogenic studies were identified, the Committee concluded that propylene tetramer does not have genotoxic potential in vitro

nor any structural alerts for carcinogenicity. These findings are consistent with other individual olefins present in propylene tetramer or mixtures thereof (OECD, 2001, 2004). The Committee noted the availability of a recent guideline-compliant subchronic study in rats and decided to use the NOAEL from this study of 40 mg/kg bw per day based on increased liver weights as an RP in a MOE approach to evaluate the acceptability of propylene tetramer as a previous cargo for edible fats and oils. Comparison of the generic maximum anticipated oral exposure to propylene tetramer from previous cargoes of 0.3 mg/kg bw per day with the RP of 40 mg/kg bw per day yields a MOE of approximately 130. This margin is considered adequate to address uncertainties in the health effects database. Therefore, and in consideration of the fact that this substance is not known or anticipated to be a food allergen, the Committee concluded that propylene tetramer meets the criteria for acceptability as a previous cargo for edible fats and oils.

III. Soybean oil epoxidized (ESBO)

1. Explanation

Soybean oil epoxidized (ESBO) was reviewed by the EU SCF in 1996 and assigned a provisional TDI (pTDI) of 1 mg/kg bw per day based on a chronic study in rats. The HBGV was deemed provisional pending the results of genotoxicity testing although the SCF concluded at the time that ESBO was acceptable as a previous cargo for edible fats and oils (EFSA, 2004). In 2004, having reviewed the results of genotoxicity assays and concluding that ESBO is neither carcinogenic nor genotoxic, the TDI was affirmed by EFSA and the provisional designation was removed (EFSA, 2004*)*. ESBO was also evaluated within the framework of the OECD Screening Information Dataset (SIDS) and determined to be a low priority for further assessment owing to its low hazard profile (OECD, 2006).

A review of ESBO in food contact applications was recently published in a peer-reviewed journal by scientists from the US Food and Drug Administration (US FDA) (Bandele et al., 2018). In addition, a toxicity review of ESBO was commissioned by the US Consumer Product Safety Commission (CPSC) in 2019 (CPSC, 2019).

For the current review, previous assessments by SCF, EFSA, US FDA and CPSC were considered. A search by CAS number and name for additional relevant toxicological studies in animals or humans was also undertaken to identify any

critical new data for the assessment of human health risk. Targeted searches were conducted on the PubMed and PubChem websites as well as using the Google Scholar search engine, and relevant databases from competent authorities were searched.

The following sources and databases were also queried to obtain data on chemical specifications, route(s) of synthesis, composition and uses of ESBO, as well as information on analytical methods and potential reactions with edible fats and oils: Embase, FSTA, Global Health and Medline. The cut-off date for inclusion in this report was September 2020.

2. Chemical and technical considerations

2.1 Methods of manufacture

ESBO is manufactured via epoxidation of soybean oil with: (1) carboxylic acid (e.g. acetic or formic acid) and hydrogen peroxide in the presence of sulfuric or phosphoric acid catalysts (Suman et al., 2005; Bhalerao, Kulkarni & Patwardhan, 2018; Piccolo et al., 2019); (2) enzymes (Cai et al., 2008); (3) inorganic and organic peroxides; and (4) halohydrins in the presence of hypohalous acids and oxygen in the presence of a silver catalyst (Cai et al., 2008; Bhalerao, Kulkarni & Patwardhan, 2018).

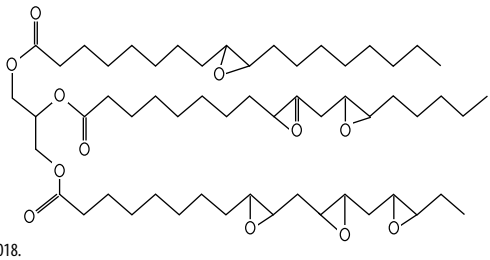
2.2 Composition and impurities

ESBO consists of epoxy compounds of triglycerides (Fig. 3 and Table 9). All double bonds in the fatty acid chains of ESBO are epoxidized (Fankhauser-Noti et al., 2006a). Given the variability of the individual fatty acid content in soybean oil, there is no single structure for ESBO; however, Fig. 3 provides a representative structure. The use of mineral acids in the reaction to form ESBO has been found to result in additional unwanted side reactions (Bhalerao, Kulkarni & Patwardhan, 2018), although no specific impurities are reported.

2.3 Uses of ESBO

ESBO is used in many applications including as a stabilizer in polyvinyl chloride (PVC) (Sreenivasan, 1990) and as a plasticizer, lubricant and cross-linking agent (Pantone et al., 2017). Owing to its use as plasticizers in food-related products such as cling films and gaskets for food containers such as baby food jars, interest

Fig. 3
Representative structure of epoxidized soybean oil



Source : Adapted from Bandele et al., 2018.

Table 9
Contributions of fatty acid to soybean oil and ESBO

Type of fatty acid	Linoleic	Linolenic	Oleic	Palmitic/stearic
Contribution to soybean oil (%)[a]	48–59	4.5–11.0	17–30	12.7–18.1
Contribution to soybean oil (%)[b]	49–57	1–2	26–36	~14
Contribution to soybean oil (%)[c]	56.5	6.5	23.1	10.5/3.0
Contribution to ESBO (%)[c]	53	7	25	Not reported
Degree of epoxidation[d]	2E	3E	1E	0E/0E

[a] Codex Standard for Named Vegetable Oils (CODEX-STAN 210 – 1999)
[b] Bandele et al., 2018
[c] Cai et al., 2008
[d] Fankhauser-Noti et al., 2006a

in the analysis of ESBO has grown (Hammarling et al., 1998; Fantoni & Simoneau, 2003; Robertson, 2011).

2.4 Reactivity and reactions with edible fats and oils

No specific information about the reaction of ESBO with edible fats and oils was identified. Migration studies have confirmed that ESBO migrates from gaskets into oily foods and oil-based food simulants (e.g. olive oil) (Fankhauser-Noti & Grob, 2006; Fankhauser-Noti, Beidermann-Brem & Grob, 2006; Fankhauser-Noti et al., 2006; Pedersen et al., 2008). ESBO is soluble in hydrocarbons, ketones, esters and higher alcohols, is slightly soluble in ethanol, and is miscible in edible fats and oils.

The epoxides or oxirane rings present in ESBO are reactive, with nucleophilic reactions resulting in the opening of the epoxide rings. Hydrolysis of ESBO in the presence of phosphoric acid and water is known to occur, as well as alcoholysis or methanolysis of ESBO in the formation of polyols (Guo et al., 2007; Heinen, Gerbase & Petzhold, 2014; Pantone et al., 2017). These reactions

are reported to proceed at temperatures of 50–90 °C (Guo et al., 2007; Holser, 2008; Heinen, Gerbase & Petzhold, 2014; Pantone et al., 2017). Owing to its use as a plasticizer, ESBO is frequently present with polymers such as PVC in gasket seals. PVC degradation can lead to the release of hydrochloric acid resulting in the production of chlorohydrins (Suman, De Dominicis & Commissati, 2010).

2.5 Methods of analysis

No reports of analysis of ESBO in fats and oils as a contaminant from previous cargoes have been published in the literature. Studies to determine ESBO migration into high-fat foods (e.g. beef and fish) (Hammarling et al., 1998) and olive oil (Coltro et al., 2014; Hanusova et al., 2015) have been performed. Food samples have been extracted using various solvent systems following addition of internal standards (Castle, Sharman & Gilbert, 1988; Castle, Mayo & Gilbert, 1990; Han & Szajer, 1994; Suman et al., 2005; Rothenbacher & Schwack, 2007). Extraction of ESBO has also been performed using dioxane (Biedermann-Brem et al., 2007) followed by methylation (Castle, Sharman & Gilbert, 1988; Castle, Mayo & Gilbert, 1990; Hammarling et al., 1998; Fantoni & Simoneau, 2003; Biedermann-Brem et al., 2007; Borcz, Franz & Stoermer, 2007; Rothenbacher & Schwack, 2007; Bueno-Ferrer, Jiminez & Garrigos, 2010; Hanusova et al., 2013; Hanusova et al., 2015).

Analyses of ESBO using numerous methods, for example, GC-MS (Castle et al., 1988; Hammarling et al., 1998; Suman et al., 2005) and GC–FID (Fankhauser-Noti et al., 2005; Biedermann-Brem et al., 2007; Borcz, Franz & Stoermer, 2007), are frequently reported. Use of LC-MS/MS (Suman et al., 2005), direct analysis in real-time mass spectrometry (Rothenbacher & Schwack, 2010) and UHPLC-HRMS (Vaclavikova et al., 2016) has also been described. Normal phase LC separation of diepoxy acids with UV detection and directly transferred to GC-FID has been reported (Fankhauser-Noti et al., 2005); reverse phase LC-MS (Suman et al., 2005) may also be used. Limits of detection for ESBO have been reported at 4–5 mg/kg (Suman et al., 2005; Rothenbacher & Schwack, 2007).

3. Biological data

3.1 Biochemical aspects

Although no studies specific to the toxicokinetics of ESBO were identified, its absorption and metabolism are assumed to be similar to that of other triglycerides

(Bassan et al., 2012; CPSC, 2019). Pancreatic lipases in the gastrointestinal tract are expected to readily hydrolyse triglycerides into mono- and diglycerides and free fatty acids, which may then be absorbed in the duodenum.

Wilson and colleagues (2002) investigated the oral absorption of epoxidized fatty acids in 13 healthy adult female volunteers. The women consumed triglycerides containing uniformly labelled [^{13}C]-monoepoxy (n = 6) or diepoxy fatty acids (n = 7). This was followed by plasma measurements of the radiolabelled fats at 0, 2, 4, 6, 8 and 24 hours. An estimated 17 ± 4% of the monoepoxy fatty acids were absorbed compared to 8 ± 1% of the diepoxy fatty acids. Statistically significant inter-individual differences were observed in the area under the curve of plasma [^{13}C]-labelled monoepoxy and diepoxy lipids, although the absorption of both was related to that of normal triglycerides.

3.2 Toxicological studies

3.2.1 Acute toxicity

ESBO has low acute oral toxicity, with a reported rat oral LD_{50} exceeding 5 g/kg bw (OECD, 2006).

3.2.2 Short-term studies of toxicity

Several repeated-dose oral toxicity studies have been reported for ESBO, although most are dated and, in many cases, only study summaries are available via secondary sources. Administration of ESBO (iodine number of 1.1 and an epoxide content of 6.8%) to Holtzman albino rats (10 animals per sex and dose) at concentrations of 0, 0.1, 0.5, 1.0, 5.0 or 10% in the diet (equivalent to 0, 50, 250, 500, 2500 or 5000 mg/kg bw per day) for 90 days suppressed growth and increased liver and kidney weights at the highest dose tested (Eagle, 1960a as cited by Bandele et al., 2018). Increases in liver weight were also observed in the group exposed to a concentration of 5% ESBO in the diet although they were determined to be equivocal.

In another subchronic study, male and female albino rats (10 animals per sex and dose; strain not reported) were fed a diet containing 0, 0.04, 0.2, 1 or 5% ESBO (iodine number of 6–14 and an epoxide content of 6.3%) (equivalent to 0, 20, 100, 500 or 2500 mg/kg bw per day) for 90 days (Mellon Institute of Industrial Research, 1960, as cited by Bandele et al., 2018). Changes in body weight and food intake were observed in animals in the higher dose groups, and liver weights were increased in females at the 1% concentration in feed and in males at the 5% concentration. ESBO also produced treatment-related kidney effects in males at concentrations in feed of 1% and higher. Based on liver and kidney effects at the

next highest dose, the authors established a NOAEL for ESBO of 0.2% in the diet, equivalent to 100 mg/kg bw per day.

Several other subchronic studies in rats reported similar results although only summary data are available. OECD (2006) reports the results of a study (cited by BIBRA, 1997) in which growth retardation and liver and kidney weight increases were observed in rats (strain and number unspecified) administered ESBO in the diet at a concentration of 2.5% (approximately 1250 mg/kg bw per day) for 15 weeks. The LOAEL based on reduced body weight and kidney and liver effects was determined to be greater than 1.5% (equivalent to 780 mg/kg bw per day based on a conversion by CPSC, 2019). Two additional studies are summarized in the CPSC review (Kieckebusch et al., 1963, as cited by BIBRA, 1997) in which rats were administered five variants of ESBO (varying iodine numbers and epoxide content) in the diet for 8 or 10 weeks at concentrations up to 20%. Critical effects observed included growth suppression, testicular degeneration, fatty infiltration of the liver, kidney effects and death, with effects being more severe in rats exposed to test articles with a higher epoxide content. Growth retardation was reported to occur at a dose of 500 mg/kg bw per day (1% in the diet, epoxide content not stated) although no NOAELs were reported, and the test articles were described by BIBRA (1997) as ESBO variants that did not correspond to the specification of those in commerce at that time.

Similar outcomes have been reported in subchronic studies in which ESBO was administered to dogs (Eagle, 1960b, as cited by Bandele et al., 2018). Beagles (two per sex and dose) fed a diet containing ESBO at a concentration of 0%, 1%, 2.5% or 5% for 14 weeks (reported to correspond to doses of 0, 250, 625 or 1250 mg/kg bw per day) exhibited reduced food intake and weight loss at the highest dose tested, although no other gross or histopathological changes were reported. The NOAEL for this study was concluded to be 2.5% (625 mg/kg bw per day) based on growth suppression at the highest dose.

Mongrel dogs (three males per dose) were fed diets containing 0, 0.1, 1.0 or 5.0% ESBO for 1 year, corresponding to doses of 0, 25, 250 or 1250 mg/kg bw per day (Larson et al., 1960). The dogs were administered two different grades of ESBO, one containing an iodine number of 6–14 and an epoxide content of 6.3%, while the other had an iodine number of 1.1 and an epoxide content of 6.8% (Bandele et al., 2018). All animals survived treatment and no changes were observed in haematological, clinical, macroscopic or histopathological parameters other than minimal fatty liver infiltration in one animal in the high epoxide content group at the highest dose tested. Dogs in the highest dose groups for both grades of ESBO exhibited weight loss and reduced food intake, which was attributed to food aversion. The NOAEL for this study appears to be 1% ESBO in the diet (250 mg/kg bw per day) although Bandele et al. (2018) noted that the study is of limited utility in establishing the safety of ESBO due to the

small group sizes and large variation in initial body weights prior to dosing in the groups that received 0.1 and 1.0% ESBO.

3.2.3 Long-term studies of toxicity and carcinogenicity

Larson and colleagues (1960) conducted chronic feeding studies of ESBO in albino rats (15 animals per sex and dose; strain not specified). Two ESBO products (identified as Paraplex® G-60 and Paraplex® G-62), with differing epoxide content and iodine numbers, were administered via the diet at concentrations of 0, 0.1, 0.5, 1.0, 2.5 or 5.0% (corresponding to doses of 0, 50, 250, 500, 1250 and 2500 mg/kg bw per day according to the conversion in BIBRA, 1997) for 2 years. In animals exposed to the test article with the lower epoxide content, growth suppression was observed in both sexes in the 5% group at 2 and 4 weeks. Similar results were observed with the higher epoxide test article where suppressed growth was observed in females at concentrations ≥ 1% and males at concentrations ≥ 2.5%. However, body weights of both sexes in all treatment groups returned to normal following 8 weeks of continued feeding. Rats exposed to the first test article (iodine number of 6–14 and an epoxide content of 6.3%) were assessed at the 1-year interim time point, when 5 of 15 rats per sex and dose were sacrificed to evaluate haematological, organ weight and histopathological parameters. The only effect observed was elevated relative liver weight in males in the 2500 mg/kg bw per day group. Two-year survival was unaffected by treatment and no significant effects on haematological or histopathological end-points were detected at the 2-year time point, although data on organ weights at terminal sacrifice were not reported. In animals exposed to the second test article (iodine number of 1.1 and an epoxide content of 6.8%), five rats per sex and dose were also assessed in an interim sacrifice group but at 6 months rather than 1 year. Decreased growth was observed in both sexes (dose groups not specified), which resolved after 8 weeks in females but not in males. Elevated relative liver weights were observed in both sexes as well as elevated relative kidney weights in females, although the organ weight effects were not accompanied by histopathological changes. While the apparent NOAEL derived from this study appears to be 0.1% (50 mg/kg bw per day), the authors estimated the lowest threshold dose was 0.5% ESBO in the diet (250 mg/kg bw per day) based on elevations in relative liver weights in females. The magnitude of change that the threshold calculation was based upon was not reported, however, and several other deficiencies limit confidence in this result (Bandele et al., 2018). Moreover, in the absence of underlying histopathological changes, such as hepatocellular hypertrophy, it is unclear if the liver weight changes would be considered adverse in accordance with current risk assessment conventions.

In what appears to be the largest chronic toxicity study of ESBO to date, Wistar rats (48 animals per sex and dose) were administered a diet containing ESBO at 0%, 0.025%, 0.25% or 2.5% (equivalent to doses of 0, 12.5, 125 or 1250 mg/kg bw per day) for 2 years (BIBRA, 1986 as cited in Bandele et al., 2018; dose conversion as per WHO, 2009). According to Bandele et al. (2018), the test article had an iodine number of 7–8 and an epoxide content of 6.3–6.4%. No treatment-related effects on survival were noted. Although the complete study report was not available for examination, the CPSC (2019) indicated that a "comprehensive" range of tissues were examined. While no adverse haematological effects were observed, no data on clinical chemistry parameters were available. In the highest dose group, slightly increased body weights in males and slightly decreased body weights in females were reported, accompanied by increased uterus weights in females and increased liver and kidney weights in males. Based on the observed changes in organ weights, a NOAEL for ESBO of 125 mg/kg bw per day was established.

The results of two chronic rodent bioassays provide no evidence of increased tumour incidence in rats administered ESBO in the diet for up to 2 years. Observed changes in organ weights were not accompanied by histopathological changes that could give rise to concerns for carcinogenicity. Overall, there is no evidence of a carcinogenic response following oral exposure to ESBO.

3.2.4 Genotoxicity

ESBO has been evaluated in a range of in vitro tests for mutagenicity and consistently produced negative results with or without metabolic activation. ESBO was not mutagenic in Ames bacterial reverse mutation assays using various strains of *Salmonella* Typhimurium nor in mammalian gene mutation assays using Chinese hamster ovary (CHO) cells. ESBO was also evaluated for clastogenicity in human and mouse lymphoma cells, and no evidence of chromosomal alterations was observed with or without metabolic activation. Although most of the information available for review is in the form of study summaries, ESBO is not considered to have genotoxic potential (Table 10).

3.2.5 Reproductive and developmental toxicity

The reproductive and developmental toxicity of ESBO was evaluated in male and female Sprague-Dawley rats in a one-generation study (CIT, 1993 as cited by Bandele et al., 2018). ESBO was administered via oral gavage (28 animals per sex and dose) at 0, 100, 300 and 1000 mg/kg bw per day. Males and females were dosed from prior to mating and throughout mating, gestation and lactation until postnatal day 21. No adverse effects were observed on any reproductive or

Table 10
Summary of in vitro genetic toxicity studies on ESBO

Test system	Conditions and doses	Result	Reference
Bacterial reverse mutation (Ames assay) with *Salmonella* Typhimurium strains TA98, TA100, TA1535, TA1538	0, 9, 18, 45, 90 or 453 µg/plate; −S9	Negative	Heath & Reilly (1982)
Bacterial reverse mutation (Ames assay) with *S.* Typhimurium strains TA98, TA100	0, 30, 90, 300, 900, or 3000 µg/plate; ± S9	Negative	Monsanto (1986) as cited in Bandele et al. (2018)
Gene mutation in hypoxanthine-guanine phosphoribosyl transferase (HPRT)-Chinese hamster ovary (CHO) cells	200–2000 µg/mL; ± S9	Negative	Monsanto (1987a) as cited in Bandele et al., (2018)
Ames tests (strain(s) not specified)	Doses not reported; ± S9	Negative	Ciba-Geigy (1981); Hazleton (1992), both as cited by CPSC (2019)
In vivo/in vitro modification of the Ames assay. Male Long-Evans rats were dosed by gavage with ESBO, and stomach and small intestine homogenates were tested in *S.* Typhimurium strains TA98 and TA100	Rats were dosed by oral gavage at 1000 mg/kg bw	Negative	Monsanto (1987b), as cited in CPSC (2019)
In vitro chromosomal aberration test in human and mouse lymphoma cells	Doses not reported; ± S9	Negative	Hazleton (1992), as cited by CPSC 2019

CPSC, Consumer Product Safety Commission

developmental end-points and the NOAEL was reported to be the highest dose tested of 1000 mg/kg bw per day.

3.2.6 Allergenicity

Two studies in guinea-pigs have investigated the dermal sensitization potential of ESBO (Ciba-Geigy, 1981; Weil et al., 1963 as cited by BIBRA, 1997). No evidence of sensitization was observed when serial intracutaneous injections of ESBO were followed by challenge injection 3 weeks later.

There is anecdotal evidence of respiratory sensitization following occupational exposure to vapours from PVC films containing ESBO and other known sensitizers such as phthalic anhydride (Pauli et al., 1980). However, the lack of subsequent reports beyond the three case studies described by Pauli and colleagues (1980) would suggest that ESBO itself is not of concern with respect to allergenicity (EFSA, 2012a).

3.3 Observations in humans

No human studies pertaining to the toxicity of ESBO were identified.

4. Levels and patterns of contamination in food commodities

A worst-case concentration of 100 mg/kg has been assumed for all previous cargo contaminants (see part A, section 4.3).

ESBO is approved in Europe, the USA, and in other parts of the world, as a stabilizer in PVC-based food contact materials, such as gaskets for glass jar lids and film wraps. It may also be used in adhesives. Surveys conducted to characterize the presence of ESBO in jarred baby foods from 1998–2001 (EFSA, 2004) found maximum concentrations of 135 mg/kg in these foods. However, the migration limit for ESBO in baby foods in Europe was lowered from 60 mg/kg to 30 mg/kg in 2007 (EC, 2007), potentially resulting in reduced concentrations in baby foods. Various market surveys have been conducted to determine ESBO migration from food packaging; ESBO concentrations in the foods tested were as high as 5511 mg/kg food (EFSA, 2006; Bandele et al., 2018). ESBO concentrations were measured in a variety of packaged foods collected by the 24th Australian Total Diet Study (ATDS) (FSANZ, 2016). The foods analysed for ESBO in the study included baked beans; beer; canned tomatoes; coffee; infant foods and formula; jam; juice; olives; peanut butter; packaged vegetables (jarred and frozen); powdered energy drinks; soup base; tea and tomato sauce. ESBO was detected only in samples of chargrilled vegetables in oil, infant dinner, infant soy formula and olives in oil, with a maximum sample concentration of 14 mg/kg in the olives.

5. Food consumption and dietary exposure estimates

Worst-case exposures to previous cargo contaminants in food oils have been estimated at 0.3 mg/kg bw per day (see part A, section 4.3).

Exposure to ESBO from food contact materials may contribute significantly to total exposures. EFSA (2006) estimated the potential high dietary exposure of adults to be 0.25 mg/kg bw per day based on 95th percentile consumption and average concentration levels of ESBO in foods packaged in glass jars with PVC-lined lids, and 0.64 mg/kg bw per day based on the 90th percentile ESBO concentrations. Potential dietary exposure of adults to ESBO from foods packaged in cling wrap was estimated by EFSA (2006) not to exceed 0.2 mg/kg bw per day.

More recent estimates of exposures to ESBO from food contact materials range from 0.13 mg/kg bw per day to 0.37 mg/kg bw per day. Food Standards Australia New Zealand (FSANZ, 2016) used a modification of the

budget method, a method for screening chemicals to determine the need for more comprehensive exposure assessments, to estimate a theoretical maximum daily exposure (TMDE) to ESBO. Using this method, it is assumed that 50% of the assumed combined weight of all foods and beverages consumed contain the substance in question at a maximum estimated level. FSANZ (2016) estimated a TMDE of 0.37 mg/kg bw per day based on the assumption that 50% of foods and beverages consumed contain the maximum level of ESBO detected in olives (14 mg/kg). Bandele et al. (2018), using international data from market surveys on ESBO migration, calculated the cumulative estimated daily intake of ESBO from its use in PVC-based food-contact articles at 0.13 mg/kg bw per day for the general US population. The figure of 0.13 mg/kg bw per day is a deterministic estimate of mean exposure based on relatively recent ESBO migration data and is likely to best represent exposure to ESBO from food packaging sources.

6. Comments

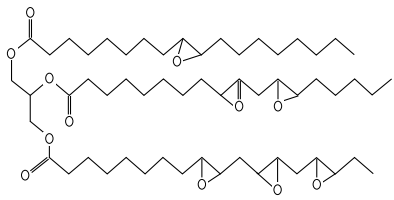
6.1 Chemical and technical considerations

Chemical and technical considerations for ESBO are summarized in Table 11.

6.2 Biochemical aspects

Although no studies specific to the toxicokinetics of ESBO were identified, ESBO is a mixture of triglycerides and therefore its absorption and metabolism are anticipated to be similar to that of other vegetable oils (OECD, 2006; Bassan et al., 2012; CPSC, 2019). Following emulsification by bile salts, pancreatic lipases in the gastrointestinal tract are expected to readily hydrolyse triglycerides into mono- and diglycerides, which may then be absorbed in the duodenum. Further metabolism by esterases is expected to yield glycerol and the corresponding free fatty acids. The oral bioavailability of epoxidized fatty acids was evaluated by Wilson et al. in healthy adult female volunteers (Wilson et al., 2002). In this study, in which the women consumed triglycerides containing uniformly labelled [^{13}C]-monoepoxy or diepoxy fatty acids, oral bioavailability decreased as the degree of epoxidation increased.

Table 11
Chemical and technical considerations for soybean oil epoxidized (ESBO)

Name: Soybean oil epoxidized	
CAS number	**Alternative CAS numbers**
8013-07-8	None
Chemical details	ESBO Oil, mixture of epoxy compounds of triglycerides Pale yellow, viscous liquid Approximate contribution of fatty acids: linolenic (7%) oleic (25%) linoleic (53%) saturated fatty acids (15%) Representative structure for ESBO:  Insoluble in water; soluble in hydrocarbons, ketones, esters and higher alcohols; slightly soluble in ethanol; miscible in edible fats and oils.
Route(s) of synthesis	Manufactured via epoxidation of soybean oil with carboxylic acid and hydrogen peroxide in the presence of sulfuric or phosphoric acid catalysts; using enzymes; using inorganic and organic peroxides; and using halohydrins in the presence of hypohalous acids and oxygen with a silver catalyst.
Composition	Occurs as a mixture with no single composition depending on the soybean oil used as feedstock. All double bonds in the fatty acid chains of ESBO are epoxidized.
Uses	Used as a plasticizer, lubricant, cross-linking agent and stabilizer in polyvinyl chloride, etc.
Analytical methods	None found for previous cargoes. From studies performed to determine ESBO migration into foods including olive oil, recommended approaches are GC-FID, LC-GC-FID, GC-MS, LC-MS/MS and LC-HRMS.
Potential reaction(s) with a subsequent cargo of fat or oil	No specific information was found on the reaction of ESBO with edible fats and oils, although migration studies have confirmed that ESBO migrates into oily foods and oil-based food simulants (e.g. olive oil).

GC-FID, gas chromatography with flame ionization detection; LC-GC-FID, on-line coupled liquid chromatography-gas chromatography-flame ionization detection; GC-MS, gas chromatography–mass spectrometry; LC-MS/MS, liquid chromatography with tandem mass spectrometry; LC-HRMS, liquid chromatography-high resolution mass spectrometry.

6.3 Toxicological studies

Although the underlying mode of action of ESBO toxicity is unknown, the substance is likely to enter normal metabolic pools and the effects observed at high doses are consistent with the general effects of high dietary lipid intake (EFSA, 2012a; CPSC, 2019). The toxicity of ESBO is relatively well characterized, although most of the studies are dated and only available through summaries

published in secondary sources. In general, studies in experimental animals indicate that ESBO produces growth suppression as well as increased liver and kidney weights when administered at relatively high doses, with increased toxicity associated with higher epoxide numbers. ESBO has low acute oral toxicity, with a reported rat oral LD_{50} exceeding 5 g/kg bw (OECD, 2006).

Several subchronic oral toxicity studies of ESBO in both rats and dogs have been reported. Administration of ESBO to Holtzman albino rats (10 animals per sex and dose) at concentrations of 0, 0.1, 0.5, 1.0, 5.0 or 10% in the diet (equivalent to 0, 50, 250, 500, 2500 or 5000 mg/kg bw per day) for 90 days suppressed growth and increased liver and kidney weights at the highest dose tested (Eagle, 1960a as cited in Bandele et al., 2018). In another subchronic study, albino rats (10 animals per sex and dose; strain not reported) were fed diets containing 0, 0.04, 0.2, 1 or 5% ESBO (equivalent to 0, 20, 100, 500 or 2500 mg/kg bw per day) for 90 days (Mellon Institute of Industrial Research, 1960, as cited in Bandele et al., 2018). Changes in body weight and food intake were observed in higher dose groups, with liver weight increases at 500 mg/kg bw per day in females and 2500 mg/kg bw per day in males. ESBO also produced treatment-related kidney effects in males at doses of 500 mg/kg bw per day and higher. In studies in which rats were administered five variants of ESBO with varying epoxide content in the diet for 8 or 10 weeks, effects were reported to be more severe in rats exposed to test articles with higher epoxide content (Kieckebusch, 1963, as cited by BIBRA, 1997).

Two subchronic studies in which ESBO was administered to dogs (Eagle, 1960b as cited in Bandele et al., 2018; Larson et al., 1960) for 14 weeks or 1 year, respectively, demonstrated reduced food intake, weight loss and growth suppression at the highest dose tested (5% in diet, equivalent to 1250 mg/kg bw per day), which was attributed to food palatability. No other adverse effects were reported other than minimal fatty liver infiltration in one animal in the highest dose group in one study (Larson et al., 1960). However, the applicability of these findings in the evaluation of the safety of ESBO are limited by the small group sizes, high variances and reporting deficiencies.

The results of two chronic rodent bioassays are available in which ESBO was administered to rats in the diet for 2 years. In the first study, Larson and colleagues conducted chronic feeding studies of two ESBO products with differing epoxide content in albino rats (15 animals per sex and dose; strain not specified) at doses up to equivalent to 2500 mg/kg bw per day (Larson et al., 1960). Two-year survival was unaffected and no significant treatment-related effects on haematological or histopathological end-points were detected at terminal sacrifice, although reporting is limited. In animals exposed to the test article with a higher epoxide content, elevated relative liver weights were observed in both sexes as well as elevated relative kidney weights in females, although the organ

weight effects were not accompanied by histopathological changes (Larson et al., 1960).

In a second chronic study, Wistar rats (48 animals per sex and dose) were administered a diet containing ESBO at 0%, 0.025%, 0.25% or 2.5% (equivalent to dose levels of 0, 12.5, 125 or 1250 mg/kg bw per day) for 2 years (BIBRA, 1986 as cited in Bandele et al., 2018). No treatment-related effects on survival were noted. In the highest dose group, slightly increased body weights in males and slightly decreased body weights in females were observed, accompanied by increased uterus weights in females and increased liver and kidney weights in males. It is not clear, however, whether the organ weight changes observed were absolute or relative. Although the complete study report was not available, a "comprehensive" range of tissues were said to have been examined and the observed changes in organ weights were not accompanied by histopathological changes (CPSC, 2019). The Committee identified a NOAEL of 125 mg/kg bw per day on the basis of organ weight changes in this chronic study.

The results of the two chronic rodent bioassays provide no evidence of increased tumour incidence in rats administered ESBO in the diet for up to 2 years. ESBO has also been evaluated in a range of in vitro tests for mutagenicity and consistently produced negative results with or without metabolic activation. ESBO was not mutagenic in Ames bacterial reverse mutation assays using various strains of *Salmonella* Typhimurium nor in mammalian gene mutation assays using CHO cells. ESBO was also evaluated for clastogenicity in human and mouse lymphoma cells, and no evidence of chromosomal alterations was observed with or without metabolic activation. Based on this information, the Committee did not consider ESBO to have genotoxic potential.

The reproductive and developmental toxicity of ESBO has also been evaluated in male and female Sprague-Dawley rats in a one-generation study (Centre International de Toxicologie, 1993, as cited in Bandele et al., 2018). No adverse effects were observed on any reproductive or developmental end-points and the NOAEL was reported to be the highest dose tested, which was 1000 mg/kg bw per day.

6.4 Allergenicity

Refined soya bean oil (non-epoxidized) has very low levels of allergenic proteins and is not regarded as a food allergen even though soya bean allergy is relatively common (Bush et al., 1985). Similarly, the Committee did not identify any reports of allergenicity upon oral exposure to ESBO that would indicate that this substance is or contains a food allergen.

6.5 Food consumption and dietary exposure assessments

A worst-case concentration of 100 mg/kg has been assumed for all previous cargo substances (see part A, section 4.3).

ESBO is used in Europe, the USA, and in other parts of the world as a stabilizer in PVC-based food contact materials, such as gaskets for glass jar lids and film wraps. It also may be used in adhesives.

Worst-case human dietary exposures to previous cargo substances in food oils have been estimated at 0.3 mg/kg bw per day (see part A, section 4.3).

Estimates of exposure to ESBO from food packaging sources have varied from 0.13 mg/kg bw per day to 0.64 mg/kg bw per day (EFSA, 2006; OECD, 2006; FSANZ, 2016). The estimate of 0.13 mg/kg bw per day (Bandele et al., 2018) is a deterministic estimate of mean exposure from food packaging sources based on the most recent available ESBO migration data, and was considered by the Committee to best represent likely exposure to ESBO from sources other than previous cargoes.

7. Evaluation

The overall toxicity database for ESBO is relatively complete, including acute, subchronic and chronic toxicity studies. ESBO is not genotoxic or carcinogenic and is not a reproductive or developmental toxicant. The overall systemic toxicity of ESBO is considered to be low and no toxicologically relevant impurities or reaction products with edible fats or oils are anticipated. The Committee decided to use the NOAEL of 125 mg/kg bw per day based on organ weight changes at the next highest dose in a 2-year rat oral bioassay as a RP to evaluate the acceptability of ESBO as a previous cargo for edible fats and oils. It should be noted that ESBO is also used in a variety of food packaging applications, which may contribute significantly to exposure. Recently, Bandele et al. estimated the cumulative daily intake of ESBO from its use in PVC-based food contact articles to be 0.13 mg/kg bw per day for the general US population (Bandele et al., 2018). A worst-case exposure estimate of 0.43 mg/kg bw per day can therefore be derived by combining the maximum estimated exposure from ESBO as a previous cargo (0.3 mg/kg bw per day) with other sources associated with food packaging. Comparison of the RP with this estimate yields a MOE of approximately 290. The Committee considered this margin adequate to account for uncertainties in the health effects and exposure databases.

ESBO is not known or anticipated to be a food allergen.

No specific information has been identified on the reaction of ESBO with edible fats and oils, although migration studies have confirmed that ESBO migrates into oily foods and oil-based food simulants (e.g. olive oil).

Therefore, ESBO meets the criteria for acceptability as a previous cargo for edible fats and oils.

IV. Mineral oils (medium and low viscosity, class II and III)

1. Explanation

White mineral oil, edible grade, contains complex mixtures of paraffinic and naphthenic liquid hydrocarbons. Medium and low viscosity mineral oil class II and III contains mineral oil saturated hydrocarbons (MOSH). These include paraffins (straight chain or *n*-alkanes and branched alkanes) and naphthenes (cyclic alkanes), with a minimal content of mineral oil aromatic hydrocarbons (MOAH). For class II, the average relative molar mass is 400–480 g/mol, with a viscosity of 7.0–8.5 mm^2/s at 100 °C. For class III, the average relative molar mass is 300–400 g/mol, with a viscosity of 3.0–7.0 mm^2/s at 100 °C. Commercial mineral oil products range from being free of MOAH (food-grade mineral oil) to containing 30% MOAH (crude mineral oil). The Committee noted that crude mineral oil is banned as a previous cargo and MOAH, which contains mutagenic and carcinogenic substances, would be unacceptable as a previous cargo. The current evaluation was conducted under the assumption that mineral oil products shipped as previous cargoes are highly refined food-grade products free of MOAH.

The previous Committee established a temporary group ADI of 0.01 mg/kg bw per day, based on an increase of histiocytosis in the mesenteric lymph nodes, for mineral oil (medium and low viscosity) classes II and III in 1995, which was extended on a number of occasions. As data supporting establishment of a full ADI had not been made available, the previously established temporary group ADI was withdrawn in 2012 (Annex 1, reference *211*).

In 2012, the CONTAM Panel (EFSA, 2012b) evaluated substances for their acceptability as previous cargoes for edible fats and oils (Part III of III) and concluded that white mineral oils met the criteria for acceptability as a previous cargo. White mineral oils include class II and class III mineral oils.

In 2012, the CONTAM Panel also evaluated mineral oil hydrocarbons (MOH) in food (EFSA, 2012c) and considered it inappropriate to establish a common health-based guidance value (HBGV) for MOSH owing to the absence of toxicological studies on MOSH mixtures typical of those humans are exposed to. The Panel used an MOE approach to the risk assessment and selected a NOAEL of 19 mg/kg bw per day for granuloma formation as a RP for background exposure.

For the current review, previous assessments by the Committee and EFSA were used to identify relevant information, and a search for additional relevant toxicological data in animals or humans was undertaken on the PubMed, PubChem and Medline websites.

A search was performed to identify previous assessments and documents published by the WHO International Programme on Chemical Safety (WHO/IPCS), JECFA, WHO technical report series, European Food Safety Authority (EFSA), the International Agency for Research on Cancer (IARC), the Scientific Committee on Food (SCF), the United States Agency for Toxic Substances and Disease Registry (US ATSDR), United States Environmental Protection Agency (US EPA), United States National Cancer Institute (US NCI), and the US Food and Drug Administration (US FDA) on mineral oil on their websites. A search was also performed on mineral oil toxicity on the PubMed, PubChem and Medline websites. Additional references with relevant toxicological information were identified from the documents reviewed. A total of 60 relevant records were retrieved.

The cut-off date for inclusion in this report was 30 September 2020.

2. Chemical and technical considerations

2.1 Methods of manufacture

Mineral oil is primarily produced from crude oil, but can also be produced from biomass, coal or natural gas (Spack et al., 2017). The manufacture of mineral oils from crude oil includes distillation (atmospheric and vacuum) and refining (extraction, crystallization, dewaxing, acid treatments and clay treatments among others) (Barp et al., 2013; Wrona, Pezo & Nerin, 2013; Pirow et al., 2019; Zoccali, Tranchida & Mondello, 2019). Hydrocarbon patterns differ among crude oils from different regions (Al-Dahhan & Mahmood, 2019) which affects patterns in mineral oil products (Spack et al., 2017). The manufacturing processes used also influence the chemical composition, purity and viscosity of mineral oil (Spack et al., 2017).

2.2 Composition and impurities

Mineral oils are complex mixtures of hydrocarbons; however, most of the compounds present in mineral oils are either MOSH or MOAH (Liu et al., 2017). The MOSH fraction also contains straight chain or *n*-alkanes and branched alkanes (paraffins) and cyclic alkanes (naphthenes) (Biedermann, Fiselier & Grob, 2009; Weber et al., 2018; Chuberre et al., 2019). Given the number of compounds present in mineral oil and their variability, they are generally identified by physicochemical properties such as viscosity (EFSA, 2012a). Mineral oils are categorized by relative viscosity at specified temperatures and grouped using more traditional naming (i.e. paraffinic, naphthenic hydrocarbons) (Table 12). Carbon chain length and molar mass are major factors affecting the viscosity of mineral oils (Barp et al., 2015b; Bevan et al., 2020).

The viscosity of mineral oil increases with greater numbers of cyclic alkanes relative to the straight and branched chain compounds present (Spack et al., 2017). Where additional components or additives are present in mineral oil (e.g. lithium salts of fatty acids), viscosity may be affected (Grob et al., 1991). Unlike plant-derived alkanes, which are dominated by odd-length carbon chains primarily in the range of C21–C35 (Fiselier & Grob, 2009; Biedermann & Grob, 2012a), mineral oil alkanes have a balanced number of even and odd carbon chain compounds (Populin et al., 2004). Impurities including polycyclic aromatic hydrocarbons and lead may be present at low concentrations. Mineral oils that are considered to be of food-grade are treated to minimize MOAH content (EFSA, 2012c). Commercial products range from being free of MOAH (food-grade mineral oil) to containing 30% MOAH (crude mineral oil) (Biedermann & Grob, 2015; Bruehl, 2016).

2.3 Uses of mineral oils (medium and low viscosity, class II and III)

Mineral oils are used for a wide variety of applications including: lubricants, food packaging, release or anti-caking agents, anti-dust agents, as diluents in printing inks, and in cosmetic products (Grob et al., 1991; Populin et al., 2004; Chuberre et al., 2019; Pirow et al., 2019; Koster et al., 2020). Mineral oils are also used as softening agents (i.e. for jute/sisal bags), although the mineral oils used in this application are not considered to be of food-grade. Certain mineral oil classes are used as food additives with functional uses identified as releasing agents and glazing agents.

Table 12
Physical and chemical properties of the class II and III medium and low viscosity mineral oils[a]

Classification	Viscosity at 40 °C (mm^2/s)	Viscosity at 100 °C (mm^2/s)	Average relative molar mass (g/mol)	Carbon number at 5% distillation point
Mineral oil (medium and low viscosity) class II		7.0–8.5	400–480	≥22
Naphthenic,[b] oil, crude	70	7.7	420	23
Mineral oil (medium and low viscosity) class III		3.0–7.0	300–400	≥17
Paraffinic,[b] oil, crude	15	3.5	350	17
Naphthenic,[b] oil, crude	15	3.5	330	17

[a] EFSA, 2012c.
[b] Hydrotreated – catalytic hydrogenation.

2.4 Reactivity and reactions with edible fats and oils

No specific information concerning the reaction of mineral oil with edible fats and oils was identified. Alkanes are very stable compounds and are not considered to be highly reactive; however, mineral oil is known to migrate into fats and oils. Despite their stability, mineral oils in the gas phase migrate into food from paperboard; migration occurs over time until equilibrium is reached (Biedermann & Grob, 2012b).

2.5 Methods of analysis

An official method for the determination of mineral oils in all types of crude and refined edible oils and fats in the concentration range of 50–1000 mg/kg has been developed. For the analysis of mineral oil in vegetable oil, the sample is first diluted with hexane (Wrona, Pezo & Nerin, 2013; Li et al., 2017). The extraction of mineral oils from food, paper packaging, etc., utilizes hexane (Biedermann & Grob, 2009a,b; Biedermann, Fiselier & Grob, 2009; Fiselier et al., 2013; Purcaro et al., 2013a; Barp et al., 2015b; Van Heyst et al., 2018). Addition of ethanol and water has been shown to improve extraction efficiency for wet foods (e.g. fish and meat) (Biedermann-Brem et al., 2012; Van Heyst et al., 2018).

MOSH and MOAH fractions can be separated by column chromatography or solid phase extraction (SPE) (Moret et al., 2012; Fiselier et al., 2013; Zoccali et al., 2016; Liu et al., 2017). Additional chromatographic methods have been used to isolate the naturally-occurring long chain alkanes and alkenes present in vegetable oil extracts (Fiselier & Grob, 2009; Wrona, Pezo & Nerin, 2013). Approaches incorporating epoxidation, bromination and saponification (Biedermann, Fiselier & Grob, 2009; Nestola & Schmidt, 2017; Spack et al., 2017)

have been described. On-line LC has also been used to remove the hydrocarbon fraction from other co-extractives (Grob et al., 1991).

Use of blank samples has been found to improve accuracy in mineral oil detection (Biedermann & Grob, 2012a). A wide variety of internal standards has been used in these techniques; 1,3,5-tri-*tert*-butyl benzene facilitates the identification of the separation of the MOSH from MOAH fractions (Biedermann, Fiselier & Grob, 2009; Barp et al., 2013; Fiselier et al., 2013; Li et al., 2017). Although LC-GC-FID has been widely applied to the analysis of mineral oil, different interfaces have been used to introduce the sample to the GC (Biedermann & Grob, 2012a; Purcaro et al., 2013b; Barp et al., 2015a; Zoccali et al., 2016; Liu et al., 2017).

Two-dimensional GC separation of the same extract using two columns of different polarity simultaneously (Beens & Brinkman, 2005) has been used to distinguish classes of compounds belonging to the MOSH group from other compounds (e.g. polyolefin oligomeric saturated hydrocarbons) (Zoccali et al., 2019). This approach coupled with MS provides qualitative information about samples and FID also allows quantitative determination (Biedermann & Grob, 2015).

On-line LC-UV/Vis – GC-FID analysis allows analysts to ensure that one fraction elutes from the LC column before entering the GC (Biedermann, Munoz & Grob, 2017). Other detection techniques have been described (Biedermann & Grob, 2009b) and additional techniques utilizing MS have been applied, including LC-GC-triple quadrupole MS-FID (Zoccali et al., 2016; Spack et al., 2017) and LC coupled to GC-time of flight mass spectrometry (LC-GC-ToF MS) (de Koning, Janssen & Brinkman, 2004).

The absence of certified reference materials and the limited number of validated methods for the analysis of mineral oil contribute to variability between laboratories (Bruehl, 2016; Koster et al., 2020). A collaboratively studied method for the analysis of aliphatic hydrocarbons (C10–C56) in animal and vegetable fats and oils has been developed using silica gel treated with silver nitrate, followed by GC-FID analysis (ISO, 2015).

Analytical methods for the determination of MOSH and MOAH have recently been reviewed (Weber et al., 2018). A method for the analysis of MOSH and MOAH (C10–C50) in vegetable fats and oils at concentrations >10 mg/kg has been validated through interlaboratory testing (CEN, 2017). In 2009, a limit of quantification (LOQ) of 1 mg/kg for MOAH in edible oils was achieved (Biedermann, Fiselier & Grob, 2009). In 2012, LOQs for MOAH and MOSH in low-fat samples were reported at approximately 0.15 mg/kg and 0.1 mg/kg, respectively (Biedermann & Grob, 2012a,b). For high-fat samples, reported LOQs ranged from 0.5 mg/kg to 2.5 mg/kg, depending on the matrix (Biedermann & Grob, 2012b).

3. Biological data

The Committee's assessment focused on animal studies tested with chemically-defined whole mixtures and subfractions rather than on single compounds or indicator substances. It also included observations in and applications to humans.

Following its evaluation (EFSA, 2012c), EFSA co-funded studies on MOSH in F344 rats to investigate bioaccumulation and toxicity (Cravedi et al., 2017). These included studies on tissue accumulation of a broad MOSH mixture (Barp et al., 2017a), tissue accumulation of subfractions and wax components (Barp et al., 2017b), toxicity in relation to liver accumulation (Nygaard et al., 2019) and studies related to immunotoxicity and autoimmunity (Andreassen et al., 2017).

Other studies investigated mineral oil in human tissues to assess the concentrations; molecular mass distributions (Barp et al., 2014); comparative toxicokinetics (low viscosity oil) in F344 rats, Sprague-Dawley rats and humans (Boogaard et al., 2012); and to characterize the accumulated hydrocarbons (Biederman et al., 2015).

3.1 Biochemical aspects

Absorption of alkanes, which decreases with increasing carbon number, may occur through the liver portal and the lymphatic system, (EFSA, 2012b). Alkanes are oxidized to the corresponding fatty alcohols through cytochrome P450s and then generally biotransformed to fatty acids. In experimental animals, MOSH with carbon numbers C16 to C35 may accumulate in different tissues including adipose tissue, lymph nodes, spleen and liver.

The repeated-dose toxicity studies of highly refined paraffinic and naphthenic mineral oils and waxes (except microcrystalline wax) showed accumulation in a dose-related fashion in the liver MLN of F344 rats (females were more sensitive than males) following subchronic exposure via gavage. For class II and III low and medium viscosity mineral oils, accumulation led to histopathological changes, mainly in the liver and MLN. MOSH concentrations observed in human tissues (mainly lymph nodes, liver, spleen and adipose tissue) showed accumulation of these compounds, mostly branched- and cycloalkanes.

Boogaard et al. (2012) compared the toxicokinetics in rodents and humans following a single oral dose of paraffinic hydrotreated white oil (P15H; low viscosity, meeting the JECFA criteria for class III) in female Sprague-Dawley and F344 rats, and in female volunteers. Sprague-Dawley rats were administered doses of 0, 200 and 1500 mg/kg bw (doses previously shown to have no adverse effects in CD rats), while F-344 rats were given doses of 0, 20, 200 and 1500 mg/kg

bw (previously shown in F344 rats to be a NOAEL, LOAEL and clear effect dose, respectively). Female human volunteers were administered one dose of 1 mg/kg bw (capsule, representing a conservative overestimate of daily human dietary consumption). Blood levels were reported as a functional surrogate measure for hepatic concentrations, as the authors showed that hepatic MOH concentration tracked the blood concentration in both rat strains. Concentrations of C19–C24 alkanes were determined as a representative measure of P15H. Analysis showed that the bioavailability of P15H oil was significantly higher in the F-344 rats, with a fourfold higher total area under the curve (AUC0-∞), and consistently higher blood and liver concentrations than Sprague-Dawley rats (24, 48 and 96 hours). Both strains showed similar elimination kinetics with a fairly short initial half-life (which may be due to redistribution) and a longer secondary half-life of around 47 hours. In human volunteers, all blood concentrations of MOH were below the detection limit of 0.16 µg/mL (at 1 and 168 hours).

In humans, accumulation of MOSH has been reported in various tissues, including liver, spleen, lung, adipose tissue, brain, heart, kidney and MLN. MOSH observed were mostly branched and cycloalkanes with a molecular mass range from C20 to C40. The fractions accumulated in human livers showed low levels of *n*-alkanes, suggesting that they are not well absorbed and/or efficiently metabolized and eliminated (Noti et al., 2003; Concin et al., 2008; Biedermann et al., 2015; Grob, 2018a,b). The carbon numbers of the MOSH to which humans are exposed via food range from C12 to C40 with centres ranging from C18 to C34. The MOSH composition in human fat tissue and lymph nodes is similar, with maximum concentrations for the C23–C24 hydrocarbons, but different from that in liver and spleen, which had maximum concentrations for the C25–C28 hydrocarbons (Barp et al., 2014; Biedermann et al., 2015). In the chromatogram, MOSH in human liver are analysed as a cloud of unresolved highly isomerized hydrocarbons, mainly naphthenes. An average of 131 mg/kg and a maximum of 901 mg/kg MOSH have been measured in livers (n = 37) (Barp et al., 2014). MOSH concentrations in tissues reached levels similar to (or higher in extreme cases than) those in F344 rats exposed to the highest experimental dose (4000 mg/kg feed) (Barp et al., 2017a,b). In extrapolating from experimental data in F344 rats to humans at an assumed mean human exposure to MOSH of 0.1 mg/kg bw per day, the values in human liver measured were 12 and 82 times higher than expected (11 mg/kg MOSH) for the mean and maximum concentrations when compared with rat intake of 2 mg MOSH/kg bw per day (40 mg/kg feed, 220 mg/kg MOSH in liver), and 47 and 322 times higher than expected (2.8 mg/kg MOSH) for the mean and maximum concentrations when compared with rat intake of 200 mg MOSH/kg bw per day (4000 mg/kg feed, 5511 mg/kg MOSH in liver) (Barp et al., 2017a). Therefore, extrapolation from high doses in experimental animals to low doses in humans can result in underestimation

of accumulated tissue (liver) concentrations (Barp 2017a,b; Cravedi et al., 2017; Grob, 2018b). Such extrapolation grossly underestimates the real values in tissues, even if F344 rats – which are reported to accumulate MOSH most strongly – are used (Barp et al., 2014). The authors noted that both the non-linear uptake and the accumulation over far longer times may explain the discrepancy between the animal data and the MOSH concentrations measured in human tissues related to the estimated exposure.

Cravedi and co-workers conducted two series of experiments in female F344 rats (reported in Barp et al. (2017a,b), Cravedi, Grob & Nygaard (2017), and Nygaard et al. (2019)). The aim was to determine the most strongly accumulated MOSH in terms of molecular mass and structure, with a focus on types of hydrocarbons in a broad mixture.

The first series was based on a broad MOSH mixture (C14–C50) representative of the whole MOSH range to which humans are exposed via the diet. Rats were given dietary concentrations of 40, 400 and 4000 mg/kg feed, corresponding to daily doses of about 2, 22 and 222 mg/kg bw, for up to 120 days. Liver, spleen, adipose tissue and the carcass were analysed after exposure for 30, 60, 90 and 120 days as well as after exposure for 90 days followed by 30 days depuration.

After 30 days of exposure to a concentration of 40 mg/kg in the feed, 10.9% of the ingested MOSH were recovered from the animal's body; after 90 days plus 30 days depuration the percentage recovered was 3.9%. In liver and spleen, the maximum retention in terms of molecular mass was at n-C29 and hydrocarbons below n-C19 and above n-C40 were virtually absent; in adipose tissue and carcass it was at n-C15/16. The data did not support a differentiation between MOSH below and above n-C25 for class I versus class II and III oils, and showed that structural characteristics seem more pertinent than molecular mass. Concentrations in the tissues increased far less than proportionally with the dose calling into question the value of linear extrapolation to low doses. No steady state was reached after 120 days. The authors noted that when compared with the concentrations in human tissues at the estimated exposure, extrapolation from animal experiments appeared to grossly underestimate human internal exposure.

Accumulation of MOSH occurred predominantly in the liver and to a lesser extent in adipose tissue and spleen. Most (roughly 50%) of the retained MOSH were located in the liver. The broad MOSH mixture contained significant amounts of *n*-alkanes, up to about n-C21, and these were largely absent in liver and spleen. After the 30-day exposure, their proportion in the adipose tissue had increased from 11% to 32% (for n-C14–21). The *n*-alkanes, *n*-alkyl monocyclic naphthenes and constituents with mainly unbranched structure were most efficiently eliminated in the liver and spleen, leaving behind a largely

unstructured cloud (chromatogram) of unresolved hydrocarbons, but this cloud of constituents gained intensity in the adipose tissue.

In a follow-up study, Barp et al. (2017b) exposed F344 rats for 120 days to three MOSH mixtures in the diet at 0, 400, 1000 and 4000 mg/kg. The oils were largely below and above C25 (S-C25 and L-C25), made using the oils that made up the central part of the broad mixture used in experiment 1 (Barp et al., 2017a).

The second series aimed to test whether maximum relative accumulation is around the hydrocarbons C29, and whether *n*-alkanes have an impact on granuloma formation. In addition, two products that were part of the broad mixture for distinguishing MOSH below and above C25, were used to clarify whether the distinction between MOSH below and above n-C25 for class I versus class II and III oils (ADI for class II and III is 1000 times lower than that of class I) is justified in terms of accumulation and granuloma formation. A third mixture contained a wax because EFSA's (2012b) toxicological evaluation when selecting a NOAEL was based on a low melting-point wax, and waxes mainly consist of *n*-alkanes, widely thought to be easily metabolized. In human tissues, *n*-alkanes were only present at low concentrations. Results showed that for all mixtures, accumulation occurred predominantly in the liver. In liver and spleen, accumulation of the C26–C30 MOSH was higher than that of the C20–C25 fraction.

n-Alkanes up to C25 were not detected in liver and spleen. Compared to the total MOSH, *n*-alkanes and *n*-alkyl monocyclic naphthenes were generally enriched in adipose tissue. In liver and spleen, *n*-alkanes up to C25 were eliminated, but strongly accumulated at around C30. Based on this profile, poor solubility and the melting points, the authors hypothesized that crystallization might prevent these wax components from being metabolized and eliminated. In the liver, relative retention of *n*-alkanes decreased again beyond C30, and was accentuated at high exposure, suggesting reduced absorption.

3.2 Toxicological studies

3.2.1 Acute toxicity

Aliphatic hydrocarbons generally show low to moderate acute oral toxicity in laboratory animals with LD_{50} values reported for some representative hydrocarbons at >5000 to >64 000 mg/kg in rats for MOSH (C9–C50) (EFSA, 2012c).

3.2.2 Short-term studies of toxicity

Overall, exposure of rats to MOSH resulted in significant increases in organ weights, especially absolute and relative liver weights. The effect was dose-related,

but was also dependent on the mixture tested. Repeated exposure to most of the food-grade MOSH led to formation of liver microgranulomas in female F344 rats (Baldwin et al., 1992; Firriolo et al., 1995; Smith et al., 1996; Griffis et al., 2010; EFSA, 2012b; Annex 1, reference *211*), more recently confirmed by Cravedi et al. (2017) and Nygaard et al. (2019).

The study by Baldwin et al. (1992) was the first to report granulomas in F344 rats following exposure to MOH (C15–C30). Rats (0, 10 or 20 males and females per group) were given oleum-treated white oil (OTWO) and hydrotreated white oil (HTWO) for 90 days at doses of 10, 100, 500, 5000, 10 000 and 20 000 mg/kg feed. Dietary intakes were given in ranges for each dose group. These dietary doses were reported as 0.93, 9.0, 45, 450, 940 and 1800 mg/kg bw per day in the evaluation of the EFSA CONTAM Panel (EFSA, 2012b). Rats fed 5000 ppm or more of either white oil showed dose-related alterations in several haematological and clinical chemistry parameters associated mainly with hepatic damage or functional alteration. At necropsy, MLNs were enlarged, and increases in weight of liver, kidney and spleen were significant. Microscopic changes were characterized by multifocal lipogranulomas in MLN and liver. The NOAEL of 500 ppm concentration in feed was 33.5–58.2 mg/kg bw per day for OTWO and 32.5–57.6 mg/kg bw per day for HTWO, reported as 45 mg/kg bw per day by EFSA (2012c).

In the study by Smith et al. (1996), F344 rats were given diets containing 2, 19, 190 or 1951 mg/kg bw per day of seven white oils (ranging from C15 to C30) and five waxes (including low melting-point wax, microcrystalline wax and high melting-point wax, among others) for 90 days. This was followed by a reversal period of 28 days or 85 days to clarify the mixed results found in other toxicity studies with laboratory animals. Feed concentrations of 20 000, 2000, 200, 20 and 0 ppm were equivalent to 1815, 173, 17 and 1.7 mg/kg bw per day in males and 1951, 190, 19 and 2.0 mg/kg bw per day in females, respectively. Animals were assigned 5, 10 or 20 per sex and group, and 60 per sex in the control group. Toxicological responses were similar in both studies and included deposition of hydrocarbons in tissues and organs, particularly the liver, spleen and MLN. Deposition of MOH in the tissues was associated with increases in the weight of liver, MLN and spleen, focal histiocytosis, liver granulomas, haematological changes indicative of underlying mild tissue inflammation and liver enzyme changes indicative of liver damage. These two studies were used by EFSA (2012c) to select a NOAEL of 19 mg/kg bw per day for background exposure (Smith et al., 1996), and a NOAEL of 45 mg/kg bw per day for high exposure scenarios related to regular consumption of bread and grains containing high levels of MOSH due to their use as release agents and use of MOSH mixtures for spraying of grains (Baldwin et al., 1992), both based on liver granuloma formation. The granuloma formation was found to be unique to Fischer 344 rats (females were

more sensitive) as they were not observed in Sprague-Dawley rats, Long-Evans rats, mice, guinea-pigs or dogs (Shubik et al., 1962; Baldwin et al., 1992; Firriolo et al., 1995; Smith et al., 1996; Miller et al., 1996; Griffis et al., 2010; Adenuga, Goyak & Lewis, 2017). These liver granulomas induced in Fischer 344 rats are associated with T and B lymphocyte influx and sometimes with central necrosis and fibrosis. In MLN, exposure to mineral oils increased microgranulomas, but these have not been associated with inflammation or necrosis (Carleton et al., 2001; Griffis et al., 2010).

In a recent subchronic study, Cravedi and co-workers (Barp et al., 2017a,b; Cravedi et al., 2017; Nygaard et al., 2019) used a MOSH mixture representative of human exposures to MOSH from food (C14–C50) and MOSH subfractions. The aim was to investigate the effects on the liver and the relationship between accumulation of MOSH in the liver and the formation of hepatic granulomas. The concentrations of MOSH in food were 0, 40, 400 and 4000 mg/kg, equivalent to dietary doses of 0, 2, 22 and 222 mg/kg bw per day for a study duration of 120 days. Groups of five female F344 rats were assigned to each experimental treatment and for each sampling time period of 30, 60, 90 and 120 days (20 groups in total). Three additional groups were assigned for a 90-day treatment with a recovery period of 30 days. There were significant changes in organ weights, in particular, increased absolute and relative liver weights, and granuloma formation in animals that received 222 mg/kg bw per day. Increased organ weights seemed to be associated with accumulation of iso-alkanes, substituted cycloalkanes and wax *n*-alkanes, whereas granuloma formation mainly appeared to be related to *n*-alkanes >C25 and not to total accumulated MOSH (Nygaard et al., 2019). Granuloma formation and increase in liver weight were not found in animals given 22 mg/kg bw per day. The effects were observed after 90 or 120 days of treatment, but not after 30 or 60 days, and the hepatic granulomas formed were not reversible within a 30-day recovery period. Granuloma density was significantly higher in the group of rats exposed to the highest dose than in the control group after 90 and 120 days. Inflammatory cell aggregates increased in parallel with strong granuloma formation. The increase in granuloma formation at the highest dose appeared to be accompanied by increased numbers of lymphoid clusters in the liver parenchyma. This increase reached statistical significance after 90 days of exposure, and a similar trend was observed after treatment for 120 days or treatment for 90 days followed by 30 days on control feed.

In the second series of experiments testing three MOSH mixtures, no clinical signs or symptoms were observed. Exposure to MOSH resulted in a significant increase in absolute and relative weights of both liver and spleen in rats exposed to the mixture L-C25, consisting of branched and cyclic MOSH ranging from n-C25 to n-C45. This was also seen in animals exposed to the mixture L-C25W, consisting of L-C25 mixed in a 1:1 ratio with a wax of similar mass range

(*n*-alkanes ranging from C23 to C45). Little or no effect was observed in animals treated with the broad MOSH mixture or the mixture that had only 27% of the hydrocarbons exceeding n-C25 (S-C25). The effects on organ weights varied considerably between the various MOSH fractions and chemical compositions. The increased liver and spleen weights were mainly related to accumulated iso-alkanes and substituted cycloalkanes, and also wax *n*-alkanes. Induction of liver granuloma appeared to be related to *n*-alkanes > C25 and not to the amount of MOSH accumulated in the liver. MOSH levels and compositions appeared to be similar to those observed in humans. The difference in slope for the L-C25 fraction with and without wax, together with the lack of increase for the S-C25 fraction, illustrates that the increased liver weights were not a direct result of the amount of MOSH accumulated, but were also dependent on the chemical composition of the MOSH.

No increase in granuloma formation was observed in animals in any of the three dose groups given the L-C25 mixture (virtually free of *n*-alkanes). However, in the groups fed 4000 mg/kg of the S-C25 mixture, granuloma formation was significantly increased compared with the control feed group. For the groups fed the L-C25W fraction, the granuloma density was significantly higher than in the control group at all doses tested. The presence of *n*-alkanes above a certain threshold concentration in the tissues seemed to be a prerequisite for granuloma formation.

The numbers of lymphoid cell clusters in the liver parenchyma and in the liver portal tract were significantly increased in animals given the highest dose of the S-C25 mixture. In animals given the low and medium doses of LC-25 W, there were significant increases in the parenchyma, but increases in the portal tract were significant only at the highest dose

3.2.3 Long-term studies of toxicity and carcinogenicity

In the study by Shubik et al. (1962), five petroleum waxes were tested by feeding them to male and female Sprague-Dawley rats at a level of 1% in the diet for 2 years; no carcinogenic or toxic effect was detected. These five petroleum waxes in benzene solution were also tested by repeated skin application to mice and rabbits and by subcutaneous implantation in mice; no carcinogenic effects were found.

Two-year dietary studies were conducted to determine the chronic toxicity and its reversibility, and the carcinogenicity of high viscosity P70(H) and P100(H) white mineral oils in F344 rats (Trimmer et al., 2004). The extent of mineral hydrocarbon deposition in liver, kidneys, MLN and spleen of female rats and reversibility of effects following cessation of exposure were evaluated. Dietary intakes were 60, 120, 240 and 1200 mg/kg per day. Mineral hydrocarbons were detected in the liver following exposure to either oil, and the maximal

concentrations were similar with both oils but were reached more rapidly with the P70(H) oil. Liver mineral hydrocarbon content returned to near-background levels during the reversibility phase. No treatment-related mortality, neoplastic lesions or changes in clinical health, haematology, serum chemistry or urine chemistry were seen in any treated group. A statistically significant increase in food consumption was noted in both males and females treated with the highest dose of either oil, and statistically significantly higher body weights were noted in the males in the highest dose group from week 33 until study termination in the P100(H) study. Higher MLN weights were accompanied by increased severity of infiltrating histiocytes. This occurred to a greater extent with the P70(H) than the P100(H) oil. No other significant changes in histopathology were observed.

A mixture of eight medium-viscosity liquid paraffin oils meeting Japanese food additive and Japanese Pharmacopoeia standards was investigated in F344/DuCrj rats (Shoda et al., 1997). Groups of rats, 50 per sex per dose group, were fed 0, 2.5 or 5% of this oil in the diet for 104 weeks, equivalent to overall achieved intakes of 0, 962.2 or 1941.9 mg/kg per day for males and 0, 1135.4 or 2291.5 mg/kg per day for females. Slight increases in food consumption and body weight were observed in animals in both high exposure groups. The only notable finding was the occurrence of infiltrating histiocytes in the lymph nodes. There were no effects on mortality, clinical signs and haematology; no differences in survival between the groups; and no statistically significant differences in the incidences of any tumour type between the test groups and the control animals.

3.2.4 Genotoxicity

No data on the mutagenicity of single cycloalkanes were identified. Generally, white highly refined paraffinic and naphthenic mineral oil mixtures with a very low content of aromatics were not mutagenic in the *Salmonella* Typhimurium mutagenicity tests, with or without metabolic activation, and they did not produce DNA-adducts upon painting of mouse skin (Granella & Clonfero, 1991; Ingram et al., 2000; Mackerer et al., 2003). In tests on alkanes, nonane, decane, undecane, dodecane, tridecane and 2-methylheptane were not found to induce morphological transformation in primary Syrian hamster embryo cells in culture (Rivedal et al., 1992).

3.2.5 Reproductive and developmental toxicity

There was limited information on reproductive and developmental toxicity of MOSH (EFSA, 2012c). In a reproductive and developmental toxicity screening test, undecane (*n*-alkane) was given orally by gavage to CD rats at doses of 0, 100, 300 and 1000 mg/kg bw per day (JMHW, 1996). Females were treated from 14 days before mating to day 3 of lactation, and killed on day 4 of lactation. Males

were treated for 46 days and killed at day 47. Changes were observed in salivation, body weight gain, food consumption, haemoglobin levels, relative liver weights and clinical serum parameters at the highest or at the two highest doses. No effects were detected on reproductive ability, reproductive organ weights, gross or histopathological findings in either sex, and there was no apparent influence on delivery or maternal behaviour of dams. Body weight gain was decreased in male and female offspring of the animals that received 1000 mg/kg. No effects on viability, general condition or autopsy findings of offspring were noted.

3.2.6 Immunological effects

Several experiments were conducted according to the OECD test guideline to investigate the possible relationship between immunological effects and oral exposure to representative MOSH mixtures in humans (Andreassen et al., 2017; Cravedi et al., 2017; Nygaard et al., 2019). A study measuring keyhole limpet haemocyanin (KLH)-specific IgM antibody production, looked at whether MOSH could impair the immune response, following antigen challenge in female F344 rats exposed for 120 days to daily MOSH doses of 0, 2, 22 and 222 mg/kg bw per day. No significant differences were observed in treated groups. Effects of mineral oils on autoimmunity in animals, based mainly on high concentrations of the inducing agent (typically 500 mL of pristane administered intraperitoneally), have been reported following parenteral administration, and at presumed high levels of exposure via inhalation or the skin in humans (Kimber & Carrillo, 2016). Therefore, an evaluation of autoimmune arthritis was performed in dark Agouti (DA) rats exposed to a MOSH mixture. Groups of male and female DA rats were exposed to the broad MOSH mixture in the diet at 0, 40, 400 and 4000 mg/kg, pristane (4000 mg/kg diet) or injected pristine (200 μL) as a positive control. Results showed no significant increase in the maximum arthritis score in the rats, and no effects on serum markers of arthritis induction that included levels of IL-17, TNFα, IL-1β and serum IgG rheumatoid factor (IgG-RF), plus expressions of TLR2 and TLR3 on splenocytes, whereas injected pristane induced arthritis and serum markers of arthritis (Andreassen et al., 2017; Cravedi et al., 2017).

3.2.7 Allergenicity

There are no data on allergenicity following oral exposure to mineral oil, medium and low viscosity, class II and class III, or MOSH that would indicate that they are or contain a known food allergen.

3.3 Observations in humans

In humans, hepatic granulomas linked to MOH by chemical analysis have been reported in the literature published between 1970 and 1985 (EFSA, 2012b). These lipogranulomas are characterized by the presence of histiocytic clusters around oil droplets, which were markedly different from the epithelioid granulomas often seen in F-344 rats, characterized by the presence of activated, cytokine-secreting giant cells. Human lipogranulomas in livers are largely asymptomatic, do not progress over the years and are not associated with abnormalities of clinical relevance (Carleton et al., 2001; Zhang et al., 2013). The incidence of hepatic granulomas related to mineral oil seems to have declined in more recent years (Lagana, Moreira & Lefkowitch, 2010). An autopsy study on mineral oil accumulation and hepatic histopathology in human tissues collected in 2013 at the Medical University of Vienna did not identify presence of granulomas (Barp et al., 2014).

Studies on MOSH accumulation in humans are discussed in more detail above (section 3.1 Biochemical aspects and section 3.2 Toxicological studies).

4. Levels and patterns of contamination in food commodities

A worst-case concentration of 100 mg/kg has been assumed for all previous cargo substances (see part A, section 4.3).

Mineral oils have direct food additive uses as fruit, vegetable, and confectionary coatings, processing aids, lubricants, release agents, plasticizers and adhesives. MOH may also be present in foods due to migration of printing inks from food packaging (e.g. jute bags used in Africa and Asia, wax paper or recycled cardboard), pesticide residues, or environmental pollution from motor oils and other sources (EFSA, 2012c; Van Heyst et al., 2018).

A number of studies have measured concentrations of MOSH from mineral oil in various foods (e.g. Vollmer et al., 2011; Biedermann et al., 2013; Foodwatch, 2015; Van Heyst et al., 2018; Zhu et al., 2019). However, the results reflect the presence of MOSH from all sources rather than from medium and low viscosity oils alone, and therefore should be viewed as worst-case concentrations of MOSH from medium and low viscosity oils. Moreover, the reliability of existing data on concentrations of MOSH has been called into question due to variability among laboratories in sample preparation and analysis procedures (Koster et al., 2020).

Vollmer et al. (2011) analysed 119 samples of dry cardboard-packaged food samples purchased in Germany. Mean MOSH concentrations ranged from 0.5 mg/kg in salt to 24 mg/kg in dry semolina. Concentrations were generally lower in products packaged with internal bags of polypropylene, acrylate-coated polypropylene, polyethylene terephthalate, or with an aluminium layer. The products were all far from the end of their shelf life, and it was estimated that MOH migration from the packaging could potentially triple before the end of shelf life. Biedermann et al. (2013) retested these samples 4 months later, and again at the expiration date or after 16 months and found increases in MOSH concentrations over this time.

A non-profit organization, Foodwatch, collected samples of 120 dry food items packed in cardboard from France, Germany and the Netherlands (Foodwatch, 2015). Foods sampled included those consumed frequently and in large quantities as well as other products packaged in cardboard. The products were analysed for MOSH by an accredited laboratory. MOSH was detected in 100 samples at concentrations ranging from < LOD to 133 mg/kg (in one brand of dry pasta). Packaging was found to be the main source of contamination, and lubricants, release agents and environmental pollution were only minor contributors to MOSH concentrations.

Van Heyst et al. (2018) analysed samples of foods collected in Belgium that were consumed in high quantities or suspected of containing mineral oils. MOSH was detected in 142 of 198 samples, with a maximum concentration of 84.8 mg/kg in a confectionary sample. Concentrations of MOSH were below the LOQ in samples of vegetables and meats, with the exception of one meat sample containing 16.86 mg/kg. MOSH was detected in samples of coffee grounds and tea leaves (up to 7.42 mg/kg), possibly due to transportation of the raw products in jute bags impregnated with mineral oil. MOSH was also found at relatively low levels in dry pudding powders, grains and legumes, possibly due to migration from cardboard packaging. It was noted that the MOSH concentrations measured were lower than those reported by Vollmer et al. (2011), possibly indicating changes in the use of mineral oils in packaging.

In a letter to the editor of *Biomedical and environmental sciences*, Zhu et al. (2019) described collection and analysis of samples of 230 foods consumed by infants and young children in China. MOSH was detected in approximately 18% of samples, mainly infant and follow-on formulas. However, the highest concentration was found in a sample of noodles, at 27.3 mg/kg.

No other sources of data on MOSH concentration generated since 2010 were identified.

5. Food consumption and dietary exposure estimates

Worst-case exposures to previous cargo substances in food oils have been estimated at 0.3 mg/kg bw per day (see part A, section 4.3).

No estimates of dietary exposure to medium and low viscosity mineral oils from any other specific source were found. However, limited estimates of exposure to MOSH from mineral oils in the total diet are available (EFSA, 2012c; van de Ven et al., 2018; Zhu et al., 2019; Van Heyst et al., 2020).

EFSA (2012c) estimated exposures to MOSH using concentration data collected in 2010 or earlier and European data from 28 food consumption surveys of individuals, conducted in 17 different European countries. Distributions of individual exposure estimates were derived for each country and age class. Total mean chronic exposures to MOSH ranged from a minimum of 0.028 mg/kg bw per day for adolescents to a maximum of 0.19 mg/kg for toddlers. Total 95th percentile exposures to MOSH ranged from 0.058–0.060 mg/kg bw per day (lower bound–upper bound) for the elderly to 0.31–0.32 mg/kg bw per day for children ≥ 3 years old to < 10 years old. Major sources of MOSH exposure varied by age group: for adults at mean consumption levels, fine bakery wares (8.3%), vegetable oil (8.1%) and bread and rolls (7.9%) were the main sources.

The Dutch National Institute for Public Health and the Environment (RIVM) (van de Ven et al., 2018) estimated exposure to MOSH based on concentration data reported by EFSA (2012c) and by Foodwatch (2015). Food consumption survey data used in the analysis covered the population aged from 2–69 years (Ocké et al., 2008; Van Rossum et al., 2011). Estimated MOSH exposures from the total diet ranged from 0.098 (ages 2–6 years, median exposures) to 0.12 mg/kg bw per day (7–69 years, 95th percentile exposures). Estimated MOSH exposures from food packaged in paperboard constituted a minor proportion of total exposures.

Zhu et al. (2019) estimated exposures to MOSH of infants and toddlers aged 0 to 36 months in China, based on data on concentrations in collected samples (described above) and results of the 2015 China Food Consumption Survey. Concentrations below the LOD were assumed to be equal to the LOD. Estimated exposures to MOSH ranged from 0.012 mg/kg bw per day (age 13–36 months, mean consumers) to 2.00 mg/kg bw per day (age 0–6 months, brand-loyal consumers).

Van Heyst et al. (2020) estimated exposure to MOSH based on analyses of Belgian food samples (Van Heyst et al., 2018) and results of the 2014/15 Belgian Food Consumption Survey. Samples below the LOD or LOQ were assumed to contain half the LOD or LOQ. Two sets of exposure estimates were calculated, based on mean analytical concentrations and on maximum analytical concentrations. Estimated exposures to MOSH ranged from 0.003 mg/kg bw per

day (for consumers aged 10–64 years, based on mean concentrations and 50th percentile intakes) to 0.044 mg/kg bw per day (for children aged 3–9 years, based on maximum concentrations and 99th percentile intakes).

In summary, for populations excluding infants whose caregivers were brand-loyal to infant formulae, recent estimates of median or mean exposures to MOSH from sources other than previous cargoes are 0.098 mg/kg bw per day or lower, and recent estimates of upper-level exposure to MOSH from these sources are 0.12 mg/kg bw per day or lower (van de Ven et al., 2018; Zhu et al., 2019; Van Heyst et al., 2020).

No other recent data (generated since 2010) on dietary exposure to MOSH were identified.

6. Comments

6.1 Chemical and technical considerations

Chemical and technical considerations for mineral oil, medium and low viscosity, class II and class III are summarized in Table 13.

6.2 Biochemical aspects

Absorption of alkanes, which decreases with increasing carbon number, may occur through the liver portal and the lymphatic system (EFSA, 2012c). Alkanes are oxidized to the corresponding fatty alcohols through cytochrome P450 and then generally biotransformed to fatty acids. In experimental animals, MOSH having carbon numbers C16 to C35 may accumulate in various tissues, including adipose tissue, lymph nodes, spleen and liver.

Similarly, in humans, MOH and MOSH accumulation has been reported in various tissues, including liver, spleen, lung, adipose tissue, brain, heart and kidney tissues. MOSH accumulations were mostly branched and cyclo-alkanes with a molecular mass range from C20 to C40. The accumulated fractions in human livers showed low levels of *n*-alkanes, suggesting that *n*-alkanes are not well absorbed and/or efficiently metabolized and eliminated (Noti et al., 2003; Concin et al., 2008; Biedermann et al., 2015; Grob, 2018a,b). MOSH in human liver samples appear as a cloud of unresolved (in the chromatogram) and highly isomerized hydrocarbons, mainly naphthenes.

Animal studies to determine MOSH accumulation in terms of molecular mass and structure were carried out on MOSH mixtures (C14-C50) representative

Table 13

Chemical and technical considerations for mineral oil, medium and low viscosity, class II and class III

Name: mineral oil, medium and low viscosity, class II and III	
CAS number 8042-47-5	**Alternative CAS numbers** None
Chemical details	White mineral oil (liquid paraffin oil)-edible grade Liquid hydrocarbons (paraffinic and naphthenic) Structure: complex mixture **Class II** – **Average relative molar mass (g/mol)**: **400–480** – **Viscosity at 100 °C (mm^2/s)**: **7.0–8.5** **Class III** – **Average relative molar mass (g/mol)**: **300–400** – **Viscosity at 100 °C (mm^2/s)**: **3.0–7.0** Insoluble in water; soluble in non-polar solvent (e.g. hexane)
Route(s) of synthesis	Manufactured from crude oil by distillation and refining (extraction, crystallization, dewaxing, acid treatments, clay treatments, etc.).
Composition	Complex mixtures of hydrocarbons Medium and low viscosity mineral oils class II and III contain saturated hydrocarbons (MOSH (paraffins: straight chain or *n*-alkanes and branched alkanes or iso-alkanes; and naphthenes: cyclic alkanes)) with minimal content of mineral oil aromatic hydrocarbons (MOAH). Commercial products range from being free of MOAH (food-grade mineral oil) to containing 30% MOAH (crude mineral oil).
Uses	Used as releasing agents, anticaking agents, glazing agents, lubricants, in food packaging, as anti-dust agents, and as diluents in printing inks and in cosmetic products.
Analytical methods	An official method for the determination of mineral oils in crude and refined edible oils and fats is available (ISO, 2015).[a] A method for the determination of MOSH and MOAH (C10–C50) is also available (CEN, 2017).[b] Other analytical methods include LC-GC-FID, LC-GC/MS, GCxGC-FID, GCxGC-MS, LC-GC-MS/MS-FID, LC-GC-ToF MS.
Potential reaction(s) with a subsequent cargo of fat or oil	No potential reactions were identified, although mineral oils are known to migrate into fats and oils.

LC-GC-FID, on-line coupled liquid chromatography-gas chromatography-flame ionization detection; LC-GC/MS, liquid chromatography-gas chromatography/mass spectrometry; GCxGC-FID, two-dimensional gas chromatography with flame ionization detection; GCxGC-MS, two-dimensional gas chromatography–mass spectrometry; LC-GC-MS/MS-FID, liquid chromatography-gas chromatography-tandem mass spectrometry-flame ionization detection; LC-GC-ToF MS, liquid chromatography-gas chromatography-time-of-flight mass spectrometry.

[a] ISO (2015). Determination of aliphatic hydrocarbons in vegetable oils. International Standard 17780; 2015.

[b] CEN. Foodstuffs – vegetable oils and foodstuff on basis of vegetable oils – determination of mineral oil saturated hydrocarbons (MOSH) and mineral oil aromatic hydrocarbons (MOAH) with on-line HPLC-GC-FID analysis. Brussels: European Committee for Standardization; 2017.

of the whole MOSH range to which humans are exposed via the diet (Barp et al., 2017a,b; Cravedi et al., 2017; Nygaard et al., 2019). Rats were given daily dietary doses of 0, 2, 22 and 222 mg/kg bw for up to 120 days. Accumulation of MOSH occurred predominantly in the liver and to a lesser extent in adipose tissue and spleen. In liver and spleen, the maximum relative retention was at n-C29, and hydrocarbons below n-C19 and above n-C40 were virtually absent. Testing with MOSH subfractions showed MOSH in the mass range of C26–C30 were more strongly accumulated than those between C20 and C25. In liver and spleen, *n*-alkanes up to C-25 were eliminated, but accumulated at around C30.

6.3 Toxicological studies

Aliphatic hydrocarbons generally show low to moderate acute oral toxicity in laboratory animals with LD_{50} values reported for some hydrocarbons at >5000 to >64 000 mg/kg in rats for MOH (C9-C50) (Concin, 2008).

In subchronic studies in F344 rats, the main findings following repeated exposure to MOH were organ weight changes, especially increased absolute and relative liver weights, and formation of liver granulomas/microgranulomas associated with inflammatory response (Baldwin et al., 1992; Firriolo et al., 1995; Smith et al., 1996; Griffis et al., 2010; Cravedi et al., 2017; Nygaard et al., 2019). The study by Baldwin et al. (1992) was the first to report on granulomas described as focal changes composed of macrophages, lymphocytes, epithelioid cells, fibroblasts and occasional multinucleate Langhans-type giant cells. More severe lesions showed areas of necrosis. There are strain and species differences in liver granuloma formation following MOH exposure in experimental studies. Granulomas were found in F344 rats (females were more sensitive), but not in Sprague-Dawley rats, Long-Evans rats, mice, guinea-pigs and dogs (Shubik et al., 1962; Baldwin et al., 1992; Firriolo et al., 1995; Miller et al., 1996; Smith et al., 1996; Griffis et al., 2010; Adenuga, 2017).

In a recent subchronic study, Cravedi and co-workers (Barp et al., 2017a,b; Cravedi et al., 2017; Nygaard et al., 2019) used a MOSH mixture representative of human exposures to MOSH from food (C14-C50) and MOSH subfractions to investigate the effects on the liver, and the relation between MOSH accumulation in the liver and the formation of hepatic granulomas. MOSH concentrations in food were 0, 40, 400 and 4000 mg/kg, equivalent to dietary doses of 0, 2, 22 and 222 mg/kg bw per day for a study duration of 120 days. Five female F344 rats were assigned to each experimental group and for each sampling period of 30, 60, 90 and 120 days (20 groups in total). Additionally, three groups were assigned to a 90-day treatment followed by a recovery period of 30 days. Significant changes in organ weights were noted, especially increased absolute and relative liver weights, and granuloma formation at 222 mg/kg bw per day. Increased organ weights seemed to be associated with accumulation of iso-alkanes, substituted cycloalkanes and wax *n*-alkanes, while granuloma formation mainly appeared to be related to *n*-alkanes >C25 and not total accumulated MOSH (Nygaard et al., 2019). Granuloma formation and increase in liver weight were not seen at 22 mg/kg bw per day. The effects were observed after 90 or 120 days of treatment, but not after 30 or 60 days, and the hepatic granulomas formed were not reversible within a 30-day recovery period. Granuloma density was significantly higher in the group of rats exposed to the highest dose level compared to the control group after 90 and 120 days. Inflammatory cell aggregates increased along with strong granuloma formation. The increase in granuloma formation at the highest dose

appeared to be accompanied by an increased number of lymphoid clusters in the liver parenchyma, reaching statistical significance after 90 days of exposure. A similar trend was observed after treatment for 120 days or treatment for 90 days followed by 30 days on control feed.

A study on mineral oil accumulation in human autopsy tissues collected in 2013 at the Medical University of Vienna did not find granulomas (Barp et al., 2014). The authors noted that granulomas linked to mineral oil hydrocarbons by chemical analysis had been reported in the literature between 1970 and 1985 (see also EFSA, 2012). The lipogranulomas reported in humans are characterized by the presence of histiocytic clusters around oil droplets, which were markedly different from the epithelioid granulomas often seen in F344 rats, which are characterized by the presence of activated, cytokine-secreting giant cells (Carlton et al., 2001; Zhang, Ouyang & Thung, 2013). Lipogranulomas in human livers are largely asymptomatic, do not progress over the years and are not associated with abnormalities of clinical relevance.

There was limited information on reproductive and developmental toxicity of MOSH (EFSA, 2012). In a reproductive/developmental toxicity screening test, undecane (*n*-alkane) was given orally by gavage to CD rats at doses of 0, 100, 300 and 1000 mg/kg bw per day (Japanese Ministry of Health and Welfare, 1996). Females were treated from 14 days before mating to day 3 of lactation, and killed on day 4 of lactation. Males were treated for 46 days and killed at day 47. Changes were observed in salivation, body weight gain, food consumption, haemoglobin levels, relative liver weights and clinical serum parameters at the highest or the two highest dose levels. No effects on reproductive ability, reproductive organ weights, gross or histopathological findings were observed in either sex, and there was no apparent influence on delivery or maternal behaviour of dams. Body weight gain was decreased in male and female offspring of animals in the highest dose group. No effects were noted on general condition or autopsy findings in offspring.

Generally, white highly refined paraffinic and naphthenic mineral oil mixtures with a very low content of aromatic hydrocarbons were not mutagenic in the *Salmonella* Typhimurium mutagenicity tests, with or without metabolic activation, and they did not produce DNA-adducts upon painting of mouse skin (Granella & Clonfero, 1991; Ingram et al., 2000; Mackerer et al., 2003).

In the study by Shubik et al. (1962), five petroleum waxes were tested by feeding them to male and female Sprague-Dawley rats at a level of 1% in the diet for 2 years; no carcinogenic or toxic effect was detected. These five petroleum waxes were also tested by repeated skin application, after dissolution in benzene solution, to mice and rabbits and by subcutaneous implantation in mice; no carcinogenic effects were found.

Two-year dietary studies were conducted to determine the chronic toxicity and reversibility and carcinogenicity of high-viscosity P70(H) and P100(H) white mineral oils in F344 rats (Trimmer et al., 2004). Mineral hydrocarbon deposition in liver, kidneys, mesenteric lymph nodes and spleen of female rats and reversibility of deposition following cessation of exposure were evaluated. Dietary intakes were 60, 120, 240 and 1200 mg/kg per day. Mineral oil hydrocarbons were detected in the liver following exposure to either oil, and the maximal concentrations were similar for both oils but occurred more rapidly with the P70(H) oil. Liver mineral hydrocarbon content returned to near-background levels during the reversibility phase. No treatment-related mortality, neoplastic lesions or changes in clinical health, haematology, serum chemistry or urine chemistry were seen in any treated group. A statistically significant increase in food consumption was noted in the highest dose groups of either oil for both males and females, and statistically significantly higher body weights were noted in the males in the highest dose group from week 33 until study termination in the P100(H) study. Higher mesenteric lymph node weights were accompanied by increased severity of infiltrating histiocytes. This occurred to a greater extent with the P70(H) than the P100(H) oil. No other histopathology of significance was observed.

The carcinogenicity of dietary administration of a mixture of eight medium-viscosity liquid paraffin oils meeting Japanese food additive and Japanese Pharmacopoeia standards was investigated in F344 rats (Shoda et al., 1997). Groups of 50 rats per sex per dose group were fed 0, 2.5 or 5% of this oil mixture in the diet for 104 weeks, equivalent to overall intakes of 0, 962.2 or 1941.9 mg/kg per day for males and 0, 1135.4 or 2291.5 mg/kg per day for females. Slight increases in food consumption and body weight were observed in both high-exposure groups. There were no effects on mortality, clinical signs and haematology; no differences in survival between the groups; and no statistically significant differences in the incidences of any tumour type between the test groups and the control animals.

Studies to determine whether oral exposure to a MOSH mixture in the diet at 0, 2, 22 or 222 mg/kg bw per day had an impact on the immune response, measured as KLH-specific IgM antibody production in response to antigen challenge, showed that MOSH did not have any effect (Cravedi et al., 2017; Nygaard et al., 1992). Effects of mineral oils on autoimmunity in animals, based mainly on high concentrations of the inducing agent (typically 500 mL of pristane administered intraperitoneally), have been reported following parenteral administration, and at presumed high levels of exposure via inhalation or the skin in humans (Kimber & Carrillo, 2016). Therefore, an evaluation of autoimmune arthritis was performed in DA rats exposed to a MOSH mixture in the diet at 0, 40, 400 and 4000 mg/kg, to pristane (4000 mg/kg diet), or injected pristane as

a positive control. None of the rats fed MOSH or pristane developed arthritis symptoms or showed any significant increase in serum markers of arthritis, while this occurred in the positive controls (Andreassen et al., 2017; Cravedi et al., 2017).

6.4 Allergenicity

The Committee did not identify any reports of allergenicity upon oral exposure to mineral oil, medium and low viscosity, class II and class III, or MOSH, that would indicate that they are or contain a known food allergen.

6.5 Food consumption and dietary exposure estimates

A worst-case concentration of 100 mg/kg has been assumed for all previous cargo substances (see part A, section 4.3). Mineral oils are used as coatings for fruit, vegetables and confectionary, and as processing aids, lubricants, release agents, plasticizers and adhesives. MOSH may also be present in foods due to migration of printing inks on food packaging, from the packaging itself (e.g. jute bags used in Africa and Asia, wax paper or recycled cardboard), pesticide residues, or environmental pollution from motor oils and other sources. A number of studies conducted in the past 10 years have measured concentrations of MOSH in foods (e.g. Vollmer et al., 2011; Biedermann et al., 2013; Foodwatch, 2015; Van Heyst et al., 2018; Zhang et al., 2019; Zhu et al., 2019). MOSH has been found in foods at levels as high as 133 mg/kg (Foodwatch, 2015).

Worst-case human dietary exposures to previous cargo substances in food oils have been estimated at 0.3 mg/kg bw per day (see part A, section 4.3).

Estimates of MOSH exposures from sources other than previous cargoes vary widely. These estimates cover MOSH exposures from all sources, not just low- and medium-viscosity oils. Upper-level exposures to MOSH for infants whose caregivers are brand-loyal to formulae containing high MOSH concentrations were estimated at 2 mg/kg bw per day (Zhu et al., 2019). However, for other populations, recent estimates of median or mean exposures to MOSH from sources other than previous cargoes are 0.1 mg/kg bw per day or lower, and recent estimates of upper-level exposure to MOSH from these sources are 0.12 mg/kg bw per day or lower (van de Ven et al., 2018; Zhu et al., 2019; Van Heyst et al., 2020). The Committee therefore selected 0.1 mg/kg bw per day as the best estimate of exposure to MOSH from sources other than previous cargoes.

7. Evaluation

The critical toxicological end-point for evaluation of MOSH is liver granuloma formation and increase in liver weight in F344 rats. The Committee acknowledged that F344 rats represent the only strain and species that have shown liver granulomas accompanied by an inflammatory response due to MOSH exposure. In humans, lipogranulomas in the liver associated with exposure to MOSH have been observed, but these have not been associated with inflammatory reactions or other adverse consequences of clinical relevance. Given the lack of sufficient information on the mechanism of liver granuloma formation in F344 rats, the Committee concluded that it could not dismiss the human relevance of these liver granulomas and used them and the increase in liver weight in its assessment of MOH as previous cargoes.

The Committee decided to use the NOAEL of 22 mg/kg bw per day of a MOSH mixture (C14–C50, including class II and class III mineral oil, medium and low viscosity) from the study of Cravedi et al. as a RP (Cravedi et al., 2017). The Committee applied an MOE approach to assess the acceptability of MOSH as a previous cargo. Considering the estimated dietary exposure of 0.4 mg/kg bw per day (0.3 mg/kg bw per day from previous cargoes, plus 0.1 mg/kg bw per day from other sources), the MOE is 55. In its judgement of this MOE, the Committee took into account that the end-point of granuloma formation is determined in the most sensitive species, sex and strain, that the RP used is one tenth of the dose showing the effect, and the uncertainty of the human health significance of the end-point. Furthermore, the exposure estimate is conservative. Based on these considerations the Committee concluded that the MOE of 55 was sufficient to address the uncertainties in the database.

There are no data on allergenicity upon oral exposure to mineral oil, medium and low viscosity, class II and class III, or MOSH that would indicate that they are or contain a known food allergen.

No potential information has been identified with respect to the reaction of mineral oil with edible fats and oils, although migration studies have confirmed that mineral oil migrates into fats and oils.

The Committee concluded that mineral oils, medium and low viscosity, class II and class III meet the criteria for acceptability as previous cargoes provided the MOH is food-grade.

Commercial MOH products range from being free of MOAH (food-grade mineral oil) to containing 30% MOAH (crude mineral oil). The Committee noted that crude mineral oil is banned as a previous cargo and MOAH, which contain mutagenic and carcinogenic substances, would be unacceptable as previous cargoes. The current evaluation is based on the assumption that MOH

products shipped as previous cargoes are highly refined food-grade products free of MOAH.

8. Conclusions

Although methods for the analysis of most of the substances considered in the oils and waxes group are available in the literature, only methods for analysis of ESBO and mineral oils in fats and oils have been developed. Research data on residues of ESBO and mineral oils have been reported in food-grade oils and foods; however, food survey data and quantitative data based on contamination by previous cargoes in subsequent cargoes of edible fats and oils are not available.

Analytical methods for the detection of propylene tetramer or montan wax in edible fats and oils are not described in the literature. GC-FID, two-dimensional GC with FID, MS or HRMS methods with sufficient validation may be developed for their detection in fats and oils.

9. Recommendations

The Committee recommended that sufficient chemical and toxicological information that allows the evaluation of montan wax as shipped are made available prior to the next evaluation. At a minimum this information should address the following:

- degree of refinement and chemical constituents;
- repeat-dose toxicological data on representative products in a relevant animal model.

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PREVIOUS CARGOES

GROUP 4 – SOLUTIONS

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A. ASSESSMENT OF SUBSTANCES PROPOSED AS PREVIOUS CARGOES

1. Introduction

Fats and oils destined to be used as food are transported and stored in large volumes. Transportation in large volumes by sea is exempted from many land-based regulations as it is not practical to have fleets of ships solely dedicated to the transportation of food in large tanks, since the trade is generally unidirectional from producer to consumer. Furthermore, the construction and dependency on the availability of a limited number of single-use carriers would make the transport of fats and oils extremely expensive. To address the economic realities, certain types of ships are permitted to carry different classes of cargo in their tanks on their outbound and onward journeys. A non-food item may be carried in a tank in one direction and a single type of fat or oil on the further voyage. Since ships are constructed to have several individual tanks, each may contain a cargo destined for a different location and may be used to carry either a food or non-food item depending on the contract.

A number of organizations have been involved in the development of codes of practice, transportation contracts, ship construction, cargo segregation, environmental issues and food safety. The Codex Alimentarius Commission (CAC) adopted and published a code of practice for the storage and transport of edible fats and oils in bulk, which was developed by CCFO in 1987 (CAC/RCP 36-1987). At that time, CCFO recognized the need to assess the acceptability of previous cargoes transported in a tank subsequently used for the transportation of an edible fat or oil. Commercial trade contracts recognized the need to specify that certain chemicals should never be acceptable previous cargoes for subsequent cargoes of edible fats or oils. These substances formed the basis of the "banned lists" of previous cargoes. In 2001, a combined list of chemicals banned as previous cargoes was developed by CCFO and adopted by CAC (FAO/WHO 2001); it was added to the Codex Code of Practice as Appendix 1. Other substances carried in bulk were considered to pose a low risk to public health as a contaminant in edible fats or oils; these formed the basis of "acceptable lists"

of previous cargoes. The development of a CCFO acceptable list of previous cargoes was also based on trade experience. A preliminary list was reviewed by the Scientific Committee on Food and their findings were reported to CCFO in 1999; 14 substances were identified for which there were insufficient data to make a safety determination. After further discussion at subsequent CCFO meetings, a list of 23 potentially safe previous cargoes that require evaluation was developed. CCFO asked for scientific advice from FAO/WHO on these 23 substances that lacked safety evaluations. The present evaluation by JECFA addresses 18 of the 23 substances on the current list of chemicals acceptable as previous cargoes by CCFO.

2. Background

2.1 Global production and consumption of fats and oils

The global trade in edible fats and oils is more than 200 million metric tonnes annually and valued at approximately US$ 120 billion (USDA, 2019). By far the largest contributors are palm (36%) and soybean oil (28%), followed by rapeseed/canola (14%), sunflower seed (10%), palm kernel (4%), peanut (3%), cottonseed (3%), coconut (2%) and olive oils (2%).

Many vegetable oils are produced in regions (for example: soybean – Argentina, Brazil, USA; rapeseed – Australia, Canada; sunflower seed – Ukraine; palm – Indonesia and Malaysia; and coconut – equatorial latitudes) far from the major sites of consumption. Olive oil is produced in regions with a Mediterranean climate in both the northern and southern hemispheres. International trade in fats and oils uses the most economical method of ocean transportation since global trade in edible fats and oils is primarily unidirectional. Soybean oil from Argentina and Brazil, for example, is shipped to both Asian and European markets, but there is unlikely to be a complementary cargo of fat or oil available for transportation in the reverse direction. Similarly, oils from tropical regions are traded globally, often without reciprocal trade in fats and oils.

2.2 Regulations affecting fats and oils

Shipment of fats and oils is described in numerous national and international regulations and agreements. Land-based transportation is regulated by local and national guidelines and/or legislation, whereas international trade is subject to commercial agreements, international shipping regulations and various codes of

practice. The development of banned lists and acceptable lists of previous cargoes is founded on existing trade contracts.

About 85% of the fats and oils are traded globally using FOSFA (The Federation of Oils, Seeds and Fats Associations, London) contracts. The balance is traded under contracts issued by NIOP (National Institute of Oilseed Products) or other organizations. A contract under "banned list terms" requires that fats and oils are not shipped in tanks that have contained a substance on the banned list as the immediate previous cargo. For certain chemicals, this requirement is extended to the three previous cargoes. Alternatively, a contract may state that "the immediate previous cargo shall be a product on the FOSFA List of Acceptable Previous Cargoes". In this case, the receiver will only accept the cargo if the previous cargo is on FOSFA's acceptable list. These two lists only cover a small proportion of the chemicals transported by sea; thus many substances appear on neither list and their acceptability as a previous cargo is subject to agreement by the contracting parties.

2.3 Global transport of fats and oils

Transportation by sea is regulated by the International Maritime Organization (IMO). The International Convention for the Prevention of Pollution from Ships (MARPOL) aims to prevent operational and accidental pollution from ships. MARPOL limits the carriage of different classes of liquid cargoes to specific tanker vessels based on ship construction and the class of chemical. Under this convention, fats and oils may not be transported in vessels designated to carry cargoes of crude oil, fuel oil, heavy diesel oil or lubricating oil. The International Code for the Construction and Equipment of Ships Carrying Dangerous Chemicals in Bulk (IBC Code) lists chemicals carried as bulk liquids, their pollution category, the type of ship design and any relevant restrictions or derogations. The previous cargoes under consideration (see Table 1) are in the medium- or low-risk categories for marine pollutants. The single exception is propylene tetramer, which is considered a high-risk marine pollutant. MARPOL also deals with tank washing and material discharge. Pentane falls into an additional category of oil-like substances requiring additional attention between cargoes.

2.4 The interrelationship of national, regional and trade interests

The practice of Acceptable List trading was discussed in line with regional initiatives to protect consumer health. The adoption of the hazard analysis and critical control point (HACCP) principles and their inclusion in the Codex Alimentarius approach to the safe trade of food and food products can be applied

to the transport of oils and fats by sea. The CAC adopted the *Code of Practice for the Storage and Transport of Fats and Oils in Bulk* developed by CCFO in 1987 (CAC-RCP 36-1987). The Code has been revised periodically and a banned list of substances was added in 2001. The list of acceptable previous cargoes adopted by the European Union (EU) and based on existing trade lists, was evaluated by EFSA.

2.5 Development of the Codex Code of Practice for storage and transport of edible fats and oils in bulk

CCFO discussions highlighted the need for lists of banned and acceptable previous cargoes. The topic of contamination by previous cargoes led to the incorporation of the FOSFA and NIOP trade lists into the Code by reference only. In 2001, CAC adopted the "Banned List" and it appears in the current code of practice as Appendix 3.

The development of a List of Acceptable Previous Cargoes by CCFO began with attempts to harmonize the FOSFA and NIOP trade lists with an EU list. The Acceptable List was further refined in 1999 when CCFO considered a list of substances proposed by the EU that had been reviewed by the Scientific Committee on Food (SCF). Having developed a list of acceptable previous cargoes, it was determined that there were 14 substances on it that required further evaluation; these 14 substances formed the basis of the CCFO Proposed Draft List of Acceptable Previous Cargoes, which was adopted by CAC 34 in 2011. For consideration at this meeting a list of 23 substances was proposed to FAO/WHO (Table 2) by CCFO for scientific advice on their suitability as previous cargoes for the carriage of fats and oils by sea-going vessels upon its evaluation against the four criteria. Each substance on the list has been assigned to Groups 1–5 (1 – solvents/reactants; 2 – alcohols; 3 – oils and waxes; 4 – solutions; 5 – butyl ethers). Substances in Group 1 were not evaluated at the present meeting.

3. Development of criteria

As a result of the CCFO request to FAO/WHO for scientific advice on the development of criteria for the assessment of the safety of residues of previous cargoes in the tanks of sea-going vessels carrying edible fats and oils, a technical meeting was convened (in November 2006) at the Dutch National Institute of Public Health and the Environment (RIVM). RIVM prepared a technical background document (FAO/WHO, 2006, Appendix II) and drafted the meeting report with FAO/WHO (2007).

Table 1
List of substances submitted by CCFO for evaluation by JECFA for addition to the list of acceptable previous cargoes

Substance (synonyms)	CAS number	Assessment group[a]
Acetic anhydride (ethanoic anhydride)	108-24-7	1
1,4-Butanediol (1,4-butylene glycol)	110-63-4	2
Butyl acetate, *sec*-	105-46-4	1
Butyl acetate, *tert*-	540-88-5	1
Calcium ammonium nitrate solution	15245-12-2	4
Calcium lignosulfonate liquid (lignin liquor; sulphite lye)	8061-52-7	4
Calcium nitrate (CN-9) solution	35054-52-5	4
Cyclohexane	110-82-7	1
Fatty alcohols		
iso Decyl alcohol (isodecanol)	25339-17-7	2
Myristyl alcohol (1-tetradecanol, tetradecanol)	112-72-1	2
iso Nonyl alcohol (isononanol)	27458-94-2	2
iso Octyl alcohol (isooctanol)	26952-21-6	2
Tridecyl alcohol (1-tridecanol)	112-70-9	2
Unfractionated fatty alcohol mixture or mixtures of fatty alcohols from natural oils and fats[b]		3
Methyl tertiary butyl ether (MTBE)	1634-04-4	5
Mineral oil, medium and low viscosity, class II		3
Mineral oil, medium and low viscosity, class III		3
Montan wax	8002-53-7	3
Pentane	109-66-0	1
1,3-Propanediol (1,3-propylene glycol)	504-63-2	2
Propylene tetramer (tetrapropylene, dodecene)	6842-15-5	3
Soybean oil epoxidized	8013-07-08	3
Ethyl tertiary butyl ether (ETBE)	637-92-3	5

[a] Group 1 was not considered at this meeting.
[b] Discussed with Group 2 – alcohols.

Discussions were limited to the assessment of previous cargoes in the transport of edible fats and oils in bulk by sea and the consideration of safety implications in terms of human health. The experts accepted that the quality of the fats and oils cargo could change as a result of hydrolysis and oxidation, but they acknowledged that these changes were already taken into account in trade contracts.

The experts considered a list of parameters originating from discussions at CCFO meetings, noting that previous cargoes are generally liquid chemical substances, slurries of solid particles or aqueous solutions. To further frame the deliberations, the experts decided to consider only a generic worst-case scenario

since developing criteria to cover every possible combination of previous cargo, type of tank, cleaning regime and possible further processing of the subsequent cargo of fat or oil would not be a realistic approach.

The experts developed the following worst-case scenario: the smallest commercially viable tank size (200 m^3), coated with a polymer that absorbs the previous cargo, is filled to 60% capacity (as required by contract), and the cargo of fat or oil is not to be further processed or refined. The model also assumed that the tank and associated pipework has been cleaned according to defined standards, inspected and considered clean and dry. Under these circumstances, the maximum level of contamination in the subsequent fat or oil cargo by the previous cargo was calculated to be 100 mg/kg. This value was used to determine a single estimate of worst-case human exposure of 0.1 mg/kg bw per day. Based on this generic exposure value, the experts considered that for the evaluation of previous cargoes, the ADI (or TDI) should be greater than or equal to 0.1 mg/kg bw in order to provide sufficient protection for children and high-intake consumers. Negligent or fraudulent practices were not considered to be part of the criteria. The experts identified four criteria necessary to determine the acceptability of a previous cargo (see FAO/WHO, 2006).

The criteria as adopted by CAC 34 (2011) are listed in Table 2.

4. Basis of evaluation

4.1 Chemistry/reactivity

Edible fats and oils are normally chemically stable; however, there may be potential for reactions with residues of previous cargoes that could give rise to products that are hazardous to human health. Consideration should be given to chemical substances that can react with edible fats and oils under normal transportation conditions. Minor oxidation and hydrolysis are normally anticipated in trade contracts and are not considered a consequence of contact with a previous cargo, unless accelerated degradation occurs. Although many possible reactions require the presence of specific catalysts or temperatures well in excess of those anticipated during transportation, potential reactions of the previous cargo with triglycerides and free fatty acids or other minor components present in the fat or oil should still be considered.

Table 2
Criteria adopted by CAC 34 and included in RCP-36-1987

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.

4.2 Methods of analysis

In a few cases where contamination is considered critical there has been an international effort to develop specific analytical methods. Cases of contamination with diesel fuel (alkanes) and mineral oils (mineral oil saturated hydrocarbons, MOSH; mineral oil aromatic hydrocarbons, MOAH) led to the development of relevant international standards. Although many of the substances under review at the present meeting can be analysed by gas or liquid chromatography using appropriate detector systems, little progress has been made in the application of these technologies to their contamination of oils and fats. It is assumed that available methods with suitable modifications will be capable of determining the maximum anticipated level of 100 mg/kg of previous cargo in the subsequent cargo of fats or oils.

4.3 Dietary exposure assessment for previous cargo chemical substances

As a consequence of considering a range of previous cargo chemical substances at its ninetieth meeting, the Committee concluded that it was appropriate to review the approach to estimating dietary exposure set out in the 2006 document *Development of criteria for acceptable previous cargoes for fats and oils* (criteria document) (FAO/WHO 2006).

The Committee noted that since the 2006 criteria document was drafted, newer and better-quality data on the consumption of fats and oils by adults, infants and young children have become available.

The Committee also noted that some of the previous cargo chemical substances assessed have additional sources of dietary exposure and expressed the view that it may be necessary to consider this in the exposure assessment.

Based on the best available data at that time, the 2006 criteria document set out the following approach to dietary exposure assessment of previous cargo chemical substances present in fats and oils:

- Estimated mean per capita consumption of 0.025 kg/day of a single type of fat or oil. The value was rounded up from the maximum per capita consumption of refined soybean oil of 22 g/person per day from the GEMS/Food cluster diets (WHO 2012).
- A factor of 2.5 to cover children and high consumers was derived from a rounded ratio between the mean and 97.5th percentile consumption of total vegetable oil from a food consumption survey in the United Kingdom (20 and 52 g/person per day for the population aged > 18 years). The criteria document also noted that dietary exposure of children to contaminants is frequently 2.5 times that of adults.
- A worst-case concentration of 100 mg/kg for a previous cargo contaminant in fats or oils.
- A body weight of 60 kg.

These data were used to define a worst-case dietary exposure estimate:

$$\frac{\text{Consumption of oil (0.025 kg/day)} \times 2.5 \times \text{concentration (100 mg/kg fat or oil)}}{\text{60 kg body weight}}$$

$$= \mathbf{0.1\ mg/kg\ bw\ per\ day}$$

Based on the **mean per capita consumption of fats and oils, and a factor of 2.5**, there would be no health concern to the general population from exposure to previous cargoes if the acceptable daily intake (ADI) or tolerable daily intake (TDI) is sufficiently protective, for example, the ADI or TDI is greater than, or equal to **0.1 mg/kg bw per day**.

4.3.1 Exposure estimates based on up-to-date consumption data for adults

Since 2006, the GEMS/Food cluster diets have been revised, and the FAO/WHO Chronic Individual Food Consumption – summary statistics database (CIFOCOss) has become available. The 2006 criteria document noted that food consumption information from dedicated surveys would be more appropriate than the food consumption estimates from the GEMS/Food cluster diets (WHO, 2012). However, it used the cluster diets, as food consumption survey data were only available from a very limited number of countries at that time. CIFOCOss currently contains food consumption data from 37 countries.

From the current version of CIFOCOss, the maximum mean consumption for a single fat or oil type is 35 g/person per day for consumption of virgin or extra-virgin olive oil by elderly Italians. The maximum 95th percentile (p95) consumption of a single fat or oil is 138 g/person per day for edible cottonseed oil by women (age 15–49 years) from Burkina Faso. This group also has the highest 97.5th percentile consumption of 189 g/person per day.

Based on the protocols currently used by JECFA for veterinary drugs, the number of consumers of cottonseed oil in the Burkina Faso survey (n = 116) would suggest that the 95th percentile is the highest reliable percentile (Boobis et al., 2017; Arcella et al., 2019).

These data suggest that for adults, a mean fat or oil consumption of 35 g/person per day and a high consumption of fat or oil of 140 g/person per day would be a conservative estimate consistent with available data.

The use of updated food consumption data will result in a revised estimated worst-case dietary exposure for adults:

$$\frac{\text{p95 consumption of oil (0.140 kg/day)} \times \text{concentration (100 mg/kg fat or oil)}}{\text{60 kg body weight}}$$

$$= \mathbf{0.2\ mg/kg\ bw\ per\ day}$$

4.3.2 Exposure estimates for infants and young children

Potentially vulnerable population groups, like infants and young children, were not specifically considered in the 2006 criteria document. Since then, individual consumption data for several population groups, including infants and young children, have become available through CIFOCOss and other sources. Infants and young children should be considered in the risk assessment because they could potentially experience high exposure to previous cargo chemical substances per kg body weight while they are undergoing growth and development.

Information on consumption of food oils by infants and young children was also available from the US Environmental Protection Agency's Food Commodity Intake Database (FCID) (US EPA, 2020a), which in turn is based on data from the US National Health and Nutrition Survey/What We Eat In America, 2005–2010 cycles. The highest oil consumption for infants and young children based on FCID is comparable to those in the CIFOCOss database; however, oil consumption information based on FCID takes into account individual body weights.

The highest reported consumption of a specific fat or oil type was for palm oil. Estimated mean and p95 consumption by infants and young children were 7.6 and 19 g/day, respectively. Estimated mean and p95 consumption on a

body weight basis were 1 g/kg bw per day and 3 g/kg bw per day, respectively.

These data were used to define a worst-case dietary exposure estimate for infants and young children:

p95 consumption of oil (0.003 kg/kg bw/day) × concentration (100 mg/kg fat or oil)

= 0.3 mg/kg bw per day

4.3.3 Exposure from other dietary sources

For some previous cargo chemical substances potentially present in food oils, there are additional sources of dietary exposure, such as contamination (for example, contaminated drinking-water) or food additive uses (Table 3). Dietary exposures from these different sources should be considered in exposure assessment.

4.3.4 Conclusion

The Committee concluded that, based on up-to-date data on consumption of single fats and oils in the general population, which have become available since 2006, the generic human exposure value of 0.1 mg/kg bw per day used in the 2006 criterion no. 2 to determine the acceptability of a previous cargo should be revised. Consequently, the updated, more conservative generic human exposure value of 0.3 mg/kg bw per day should be used in the evaluation of these substances.

The Committee noted that these estimates of dietary exposure were derived from a more conservative approach to using data on consumption of single fats and oils and a worst-case concentration of previous cargo chemicals in a single fat or oil of 100 mg/kg.

The Committee also concluded that additional sources of dietary exposure need to be considered in exposure assessment of previous cargo chemical substances.

4.4 Approach to toxicological evaluation

The Committee received no submitted data and, therefore, reviewed monographs from previous evaluations of individual substances conducted by JECFA, WHO, the International Agency for Research on Cancer (IARC), and national and regional governmental authorities to retrieve additional relevant references for completing the present assessment. The Committee also conducted literature searches. The details are included in the consideration of individual substances.

At its present meeting, the Committee revised the generic value for assumed worst-case human dietary exposure from 0.1 to 0.3 mg/kg bw per day and used this revised generic exposure value for the evaluation of previous

Table 3

List of substances for evaluation by JECFA arising from the development of a list of previous cargoes by the Codex Committee on Fats and Oils: Other sources of exposure

Substance (synonyms)	Other sources of exposure
1,4-Butanediol (1,4-butylene glycol)	Used in food contact material
Calcium ammonium nitrate solution	Calcium, nitrate and ammonium are ubiquitous in the human diet
Calcium lignosulfonate liquid (lignin liquor; sulfite lye), molecular weight not specified	Calcium lignosulfonate (40-65) is used as a food additive, an additive in animal feed and as an ingredient in pesticides
Calcium nitrate (CN-9) solution	Calcium and nitrate are ubiquitous in the human diet
iso Decyl alcohol (isodecanol)	None
Myristyl alcohol (1-tetradecanol; tetradecanol)	Flavouring agent, formulation agent, lubricant, release agent
iso Nonyl alcohol (isononanol)	None
iso Octyl alcohol (isooctanol)	Used in food contact material
Tridecyl alcohol (1-tridecanol)	Used in food contact material
Unfractionated fatty alcohol mixture or mixtures of fatty alcohols from natural oils and fats[a]	Occurs naturally in foods
Methyl tertiary butyl ether (MTBE)	Drinking-water
Mineral oil, medium and low viscosity, class II and III	Used in food contact material, direct food additive
Montan wax	Food additive
1,3-Propylene glycol	Used in place of 1,2-propanediol as a food additive
Propylene tetramer (tetrapropylene, dodecene)	None
Soybean oil epoxidized	Used in food contact material
Ethyl tertiary butyl ether (ETBE)	Drinking-water

[a] Discussed with Group 2 – Alcohols.

cargoes. The Committee also considered data on exposure to the substances from sources other than previous cargoes. Thus, the ADI (or TDI) should be greater than or equal to the estimated dietary exposure (0.3 mg/kg bw per day plus exposure from other possible dietary sources) in order to provide sufficient protection for infants, children and high-intake consumers. In situations where no appropriate numerical ADI (or TDI) was available from JECFA, the Committee considered other previously established health-based guidance values or calculated a margin of exposure (MOE) based on a reference point characterizing the toxicological hazard (such as a no observed adverse effect level or NOAEL, etc.) identified from the available data divided by the estimated dietary exposure. Interpretation of this MOE is a matter of expert judgement that takes into account limitations in the available toxicological database.

5. Recommendations

The Committee recommended that the Codex Committee on Fats and Oils (CCFO) consider revising Criterion no. 2 in RCP-36-1987 as adopted by CAC 34 (2011).

- Based on the consumption of fats and oils by infants and young children, there is no health concern for the general population from dietary exposure to previous cargo chemical substances if the ADI or TDI is sufficiently protective, for example, the ADI or TDI is greater than, or equal to 0.3 mg/kg bw per day. Substances for which there is no numerical ADI or TDI should be evaluated on a case-by-case basis (e.g. margin of exposure (MOE) approach).
- Where there are additional sources of dietary exposure to the previous cargo chemical substances, they should be considered in the exposure assessment.

B. EVALUATION OF SUBSTANCES

Group 4 – Solutions

First, previous assessments (monographs) produced by JECFA or the European Food Safety Authority (EFSA) were identified by searching the chemical name of the substance, i.e. calcium nitrate, calcium ammonium nitrate or calcium lignosulfonate, and calcium, ammonium and nitrate on the JECFA and EFSA websites, respectively. Since dolomite (calcium magnesium carbonate) and phosphate rock could be used in the manufacture of calcium ammonium nitrate and calcium nitrates, respectively, previous assessments by JECFA on magnesium salts and phosphates were located to evaluate toxicological data on magnesium and phosphate. Additional references with relevant toxicological information were identified from the JECFA and EFSA evaluations. This was followed by comprehensive searches for data on calcium nitrate, calcium ammonium nitrate and calcium lignosulfonate on PubMed and PubChem as well as on calcium, ammonium, nitrate, magnesium and phosphate on PubMed only. The cut-off date for inclusion for all searches was 20 August 2020. For calcium nitrate, six records not already included in the earlier assessments were retrieved, but only one was considered relevant to the present evaluation. For calcium ammonium nitrate, nine

records not already included in the previous assessments were retrieved, but again only one was considered relevant to the present evaluation. This reference reported a human case of contact dermatitis. Numerous records resulted from a search for data on calcium, ammonium, nitrate, magnesium and phosphate; however, only seven recently published articles relevant to the present evaluation were retrieved. These included four articles on nitrate, one article on physiologically based toxicokinetic (PBTK) modelling to assess internal exposure to nitrate from drinking-water and food sources, and three articles on the potential of nitrate to be converted to carcinogenic nitrosamines and its possible implications for human health. The remaining three recently published articles were on magnesium: one article reported on the lack of in vitro and in vivo genotoxic potential of magnesium stearate and two articles described the use of intravenous magnesium sulfate to treat exacerbations of asthma in clinical paediatric cases. A total of 11 records were retrieved on calcium lignosulfonate, but only two were considered relevant to the present evaluation; these reported a 90-day subchronic toxicity study on calcium lignosulfonate (40-65) administered by the oral route in rats.

I. Calcium nitrate (CN) and calcium ammonium nitrate (CAN) solutions

1. Explanation

The toxicological datasets on oral exposure to calcium nitrate and calcium ammonium nitrate are sparse; therefore, available toxicological data on calcium, ammonium and nitrate were reviewed to conduct a toxicological evaluation of calcium nitrate and calcium ammonium nitrate as previous cargoes for edible fats and oils. Given that dolomite and phosphate rock could be used in the manufacture of calcium ammonium nitrate and calcium nitrate, respectively, toxicological data on magnesium and phosphate were also reviewed to complete their toxicological evaluation.

The Committee has previously evaluated many calcium salts, including calcium sulfate, calcium chloride, calcium carbonate, calcium acetate and calcium gluconate for use as food additives. The Committee allocated an ADI "not specified"[1] to these salts based on their low toxicity determined from a

[1] A term applicable to a food substance of very low toxicity that does not, in the opinion of the Committee, represent a hazard to health. For that reason, and for reasons stated in individual evaluations, the establishment of an ADI expressed in numerical form is not deemed necessary (Environmental Health Criteria No. 240).

review of data available at the time of the evaluations. SCF (2003) established a tolerable upper intake level (UL) for calcium of 2500 mg/day for adults, including pregnant and lactating women. This UL was confirmed by EFSA (EFSA, 2009, 2012).

The toxicological data on nitrate were reviewed by the Committee at its sixth, eighth, seventeenth, forty-fourth (Annex 1, reference *116*) and fifty-ninth meetings (Annex 1, reference *160*). At its sixth meeting, the Committee established an ADI of 0–5 mg/kg bw for sodium nitrate, based on a NOAEL[1] of 500 mg/kg bw per day for body weight gain at a higher dose in a long-term study in rats and a short-term study of toxicity in dogs and by applying an uncertainty factor of 100. This ADI was retained at the eighth and seventeenth meetings. At the forty-fourth (Annex 1, reference *116)* and fifty-ninth meetings (Annex 1, reference *160*), the Committee concluded that the rat was an unsuitable model for evaluating toxicity of nitrate owing to quantitative differences in conversion of nitrate to nitrite between rats and humans. However, owing to limited toxicity data on nitrate and nitrite in other animal species, the Committee used toxicokinetic modelling of the rat data to estimate a conversion rate of nitrate to nitrite in humans of about 5–7% in average individuals and 20% in individuals with a high rate of conversion. Based on a re-evaluation of a long-term study in rats, and consideration of the available epidemiological data, the Committee concluded that a NOAEL of 370 mg/kg bw per day was most appropriate for the safety evaluation of nitrate and established an ADI of 0–3.7 mg/kg bw for nitrate, expressed as nitrate ion, by applying an uncertainty factor of 100 to the NOAEL (Annex 1, reference *160*).

The Committee has previously evaluated many ammonium salts, such as ammonium carbonate, ammonium bicarbonate, ammonium chloride and ammonium acetate, and allocated an ADI "not specified" to these salts based on their low toxicity determined upon review of the data available at the time of the evaluations.

The Committee has also previously evaluated many magnesium salts, including magnesium carbonate, magnesium hydrogen carbonate, magnesium sulfate, magnesium chloride, magnesium DL-lactate, magnesium gluconate, magnesium acetate, magnesium citrate, magnesium adipate, magnesium succinate, monomagnesium phosphate and magnesium stearate. Given their widespread occurrence in food from natural sources and their low toxicity assessed based on a review of available toxicological data, the Committee allocated an ADI "not specified" to the evaluated magnesium salts. The Institute of Medicine in the USA established recommended dietary allowances of magnesium at 80–420 mg/day for different age groups, which are considered to meet the nutrient

[1] At the time called NOEL.

needs of 97–98% of the individuals in a population (Institute of Medicine, 1997). SCF established an upper level of 250 mg/day for readily dissociable magnesium salts, such as magnesium chloride, magnesium sulfate, magnesium carbonate, magnesium lactate, and compounds in nutritional supplements, in water or added to food and beverages (SCF, 2006).

At its twenty-sixth meeting, the Committee established a maximum tolerable daily intake (MTDI) for phosphates, diphosphates and polyphosphates of 70 mg/kg bw, expressed as phosphorus (Annex 1, reference *59*). More recently, at its seventy-sixth meeting, the Committee stated that the approach taken to derive the MTDI of phosphates from the toxicological data was unclear. This was because the end-point considered (nephrocalcinosis in rats) for deriving this value may not be relevant to humans, leading to an overly conservative value of the MTDI and the Committee, therefore, acknowledged the need to review the toxicological basis of the MTDI for phosphate salts (Annex 1, reference *211*).

The EFSA CONTAM Panel concluded that calcium nitrate and calcium ammonium nitrate met the criteria for acceptability as previous cargoes (EFSA, 2009a), as the criterion for an ADI or TDI at that time, i.e. 0.1 mg/kg bw per day, was below the existing UL for calcium and the ADI for nitrate, and no numerical ADI was considered necessary for most ammonium salts.

For the present assessment, the Committee identified and reviewed its previous evaluations (monographs) as well as those of SCF and EFSA on calcium nitrate and calcium ammonium nitrate, or calcium, nitrate, ammonium, magnesium and phosphates, and located additional references from these evaluations. This was followed by comprehensive searches for data on calcium nitrate and calcium ammonium nitrate, or calcium, nitrate, ammonium, magnesium and phosphates on PubMed and PubChem. The cut-off date for the inclusion criteria on all searches was 20 August 2020. Some retrieved references contained relevant toxicological information not detailed under previous evaluations. These included a report of contact dermatitis upon dermal exposure to calcium ammonium nitrate and recently published articles on PBTK modelling of nitrate from dietary sources, and the potential of nitrate to be converted into carcinogenic nitrosamines and its possible implications for human health.

2. Chemical and technical considerations

CN is a water-soluble, colourless or white crystalline material. CAN is a water-soluble, white to pale yellow granule or prilled product. The physicochemical characteristics of CN and CAN solutions are summarized in Tables 4 and 5.

Table 4
Physical and chemical characteristics of calcium nitrate (CN) solution

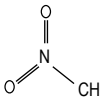
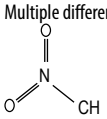
Characteristic	Calcium nitrate solution
Chemical name	Calcium nitrate
Synonyms	Nitric acid, calcium salt, CN
CAS number	35054-52-5 (hydrate) 10124-37-5 (anhydrous) 13477-34-4 (tetrahydrate)
Chemical structure	 • 1/2 Ca • × H_2O
Molecular formula; molecular mass	Anhydrous – $Ca(NO_3)_2$, 164.09 g/mol Tetrahydrate – $Ca(NO_3)_2 \cdot 4H^2O$, 236.15 g/mol
Description	Colourless or white to grey crystals or granules
Melting point	Anhydrous: 560 °C Tetrahydrate: ~ 44 °C
Solubility	Readily soluble in water

Table 5
Physical and chemical characteristics of calcium ammonium nitrate (CAN) solution

Characteristic	Calcium ammonium nitrate solution
Chemical name	Nitric acid, ammonium calcium salt
Synonyms	Nitric acid, calcium ammonium salt, CAN
CAS number	15245-12-2
Chemical structure	Multiple different but closely related products exist.  • × Ca • × NH_3
Molecular formula; molecular mass	One formulation: $H_4CaN_2O_3$, 120.12 g/mol
Description	White to pale yellow granule or prilled solid
Melting point	170 °C
Solubility	Readily soluble in water

2.1 Manufacture and uses of CN and CAN solutions

CN is manufactured by dissolving limestone ($CaCO_3$) in nitric acid (HNO_3); additional reaction products are carbon dioxide and water. CN is also created as a by-product from the manufacture of nitrophosphate fertilizers. In this process, phosphate rock (commonly fluorapatite, $Ca_5(PO_4)_3F$) is dissolved in nitric acid; the solution is cooled, and CN is precipitated out of solution (Hussain, 2012). CN is very soluble in water. It commonly occurs in the tetrahydrate form but also in the anhydrous form. CAN occurs in multiple, closely related formulations with a nominal formula of $CaH_4N_4O_3$. It is a water-soluble, white to pale yellow granule or prilled product manufactured by addition of ground limestone, dolomite ($CaMg(CO_3)_2$) or CN to molten ammonium nitrate (NH_4NO_3) (UNIDO & IFDC, 1998).

Both CN and CAN are primarily used as fertilizers. CN is also used in wastewater treatment and in the production of concrete; manufacture of disposable cold packs represents an additional, although minor, use of CAN.

2.2 Composition and secondary contaminants

Many formulations of CN and CAN fertilizers exist in commerce. Varying amounts of calcium and nitrogen (as ammoniacal nitrogen and nitrate nitrogen) are present depending on the intended use of the fertilizer. Impurities in CN and CAN fertilizer products have not been reported. Magnesium nitrate may be expected to occur in products made using dolomite. Additionally, unreacted carbonates of calcium or magnesium may be present as impurities arising from incorrect processing conditions or the use of insufficient or weak nitric acid. Phosphate impurities may arise in products manufactured using the nitrophosphate process. Limestone, dolomite and phosphate rock used in the manufacture of CN and CAN are mined materials that may contain metallic impurities including arsenic and lead. The concentrations of these impurities vary depending on the geographical origin.

2.3 Reactivity and reactions with fats and oils

In (aqueous) solution, CN and CAN will dissociate to form calcium, ammonium and nitrate ions. Magnesium ions may also be present in solutions of products manufactured using dolomite. Cargoes of edible fats or oils potentially contain free fatty acids; ions from CN and CAN solutions may react to form calcium, ammonium or magnesium salts; however, this reaction requires the presence of metal oxides or metal hydroxides, neither of which is expected.

2.4 Methods of analysis

No analytical methods for determination of residual contamination of edible fats or oils by previous cargoes of CN and CAN solutions are reported in the literature. Detection of calcium in the fats or oils could serve as an indicator of contamination from previous cargoes. Given the very low concentration of calcium expected, sensitive analytical techniques are required. Inductively coupled plasma (ICP) techniques are commonly used for analysis of elemental impurities in food matrices and are likely to be the best means of detecting and quantifying (depending on the level of contamination) calcium in cargoes of edible fats or oils.

3. Biological data

3.1 Biochemical aspects

Calcium: Calcium is an essential nutrient. In its soluble ionized form, calcium is absorbed in the human intestine via active transport across cells mainly in the duodenum and the upper jejunum, and through passive diffusion mainly in the ileum and to a lesser extent in the large intestine (Pansu, Bellaton & Bronner, 1981; Pansu & Bronner, 1999; Gueguen & Pointillart, 2000). Most of the absorbed calcium is stored in the skeleton (about 99% of the body's calcium), depending on the physiological needs related to growth and health conditions, including pregnancy and lactation (Gueguen & Pointillart, 2000). Absorbed calcium that is not retained by the body is excreted in urine, faeces and sweat (Gueguen & Pointillart, 2000; SCF, 2003).

Nitrate: The Committee evaluated available toxicological data on nitrate at its fifty-ninth meeting (Annex 1, reference *160, 161*) and concluded that nitrate is rapidly absorbed after oral administration such that its concentration in the plasma increases within 10 minutes. The elimination half-life ($t_{1/2}$) of nitrate in the plasma is 6.5 hours and 70% of the dose is excreted in the urine within 10 hours of oral administration. Some of the dietary nitrate is converted to nitrite through non-enzymatic processes and to nitric oxide by symbiotic bacteria in the oral cavity and stomach. The nitric oxide generated plays a protective role in the cardiovascular system and the gastric mucosa as well as against metabolic diseases (Lundberg et al., 2011; Ma et al., 2018). The Committee also concluded that the rat was an unsuitable model for examining the toxicokinetics of nitrate as the rat does not convert nitrate into nitrite in a quantitatively similar way to

humans. Toxicokinetic modelling based on a simple one-compartment approach and on a multi-compartment PBTK framework was used to examine the kinetics of nitrate (Wagner et al., 1983; Zeilmaker et al., 1996). These models simulated absorption of nitrate from drinking-water and vegetables, secretion of nitrate from blood into saliva, conversion of nitrate to nitrite, absorption of nitrite and interaction of nitrite with haemoglobin, yielding methaemoglobin and nitrate. The Committee determined that the range of nitrate to nitrite conversion in humans is about 5–7% in average individuals and 20% in individuals with a high rate of conversion (Annex 1, reference *160*, *161*). The model proposed by Zeilmaker et al. (1996) also predicted that a single dose of nitrate from vegetables as well as repeated intake of nitrate in drinking-water up to 44 mg/kg bw would not induce clinical methaemoglobinaemia and that lethal toxicity would occur at doses > 440 mg/kg bw. More recently, a report on the potential of nitrate to be converted to carcinogenic nitrosamines in the body under certain conditions, such as an acidic gastric environment, was published (Zeilmaker et al., 2010; Ma et al., 2018).

Ammonium: Upon oral ingestion in foods, ammonia is readily absorbed by the gastrointestinal tract. It enters into the portal circulation and undergoes transformation to urea in the liver via the urea cycle. Urea is then excreted by the kidneys. Ammonia is also produced in the gut of all mammalian species by bacterial degradation of nucleic and amino acids from ingested foods. The estimated production of ammonia in the human intestine ranges from 10 mg per day in the duodenum to 3 g per day in the colon (EFSA, 2009b).

Magnesium: Upon ingestion, magnesium salts, such as magnesium carbonate, magnesium sulfate, magnesium chloride and magnesium stearate, are dissolved under acidic gastric conditions and separate into the magnesium ion (cation) and the anion (carbonate, sulfate, chloride, stearate, etc., respectively). Magnesium is commonly found in foods, such as lettuce, spinach, turnip greens and collard, and the net absorption of dietary magnesium in a typical diet is between 40 and 60% (Schwartz, Spencer & Welsh, 1984). Magnesium is absorbed all along the intestinal tract, but the sites of maximal magnesium absorption are the ileum and jejunum (Kayne & Lee, 1993). It is an essential nutrient which serves as a cofactor for more than 300 enzyme systems (IOM, 1997). It contributes to energy metabolism, protein and nucleotide synthesis, and metabolism and activation of vitamin D and parathyroid hormone (Annex 1, reference *224*). Magnesium is mainly excreted in the urine and the kidney is the principal organ involved in its homeostasis (Institute of Medicine, 1997).

Phosphates: Phosphates and polyphosphates were previously evaluated by JECFA for their use as food additives (Annex 1, reference *59*). Phosphorus is an essential nutrient and a constituent of bones, teeth and several enzyme systems and they play an important role in carbohydrate, fat and protein metabolism. Phosphates (or phosphorus) are mainly absorbed from the diet as free orthophosphate after enzymatic hydrolysis. The intestinal absorption of phosphates depends on the requirements of the body and their levels are regulated by physiological mechanisms. The amounts of inorganic phosphates in the blood are stabilized by exchange with the mineral deposit in the skeleton by parathyroid hormone. Parathyroid hormone inhibits renal tubular reabsorption of phosphates causing demineralization of the bone tissue, and the levels of circulating parathyroid hormone are regulated by blood calcium levels. Phosphates are mainly excreted in the faeces.

3.2 Toxicological studies

Given that the toxicological datasets on CN and CAN are sparse, the present Committee considered the health-based guidance values for calcium, nitrate, ammonium, magnesium and phosphates, established under previous evaluations and briefly summarized below, to conduct a toxicological evaluation of CN and CAN at the anticipated exposure levels from their presence as previous cargoes for edible oils and fats.

Calcium: The Committee has previously evaluated many calcium salts, including calcium sulfate, calcium chloride, calcium carbonate, calcium acetate and calcium gluconate, for use as food additives. It allocated an ADI "not specified" to these salts based on their low toxicity profiles determined from a review of data available at the time of the evaluations. The UL of 2500 mg/day for adults (equivalent to about 40 mg/kg bw), was established by SCF in 2003 (SCF, 2003) and confirmed by EFSA (2009a; 2012). This was based on different human intervention studies of long duration in which total daily calcium intakes of 2500 mg from both diet and supplements were tolerated without adverse effects.

Nitrates: At its forty-fourth (Annex 1, reference *116*) and fifty-ninth meetings (Annex 1, reference *160*), the Committee concluded that nitrate was not genotoxic and the carcinogenicity studies on nitrates were negative, except when extremely high doses of both nitrate and nitrosable precursors were tested. The Committee also concluded that the rat was an unsuitable model for evaluating toxicity of nitrate due to quantitative differences in conversion of nitrate to nitrite between rats and humans. However, owing to the limited availability of toxicity

data on nitrate and nitrite in other animal species, JECFA used toxicokinetic modelling of the rat data to estimate a conversion rate of nitrate to nitrite in humans of about 5–7% in average individuals and 20% in individuals with a high rate of conversion. A review of the epidemiological data available at the time provided no evidence of an association between exposure to nitrite and risk of cancer in humans. Also, epidemiological cohort studies concluded that there was no clear evidence of an association of dietary nitrates with increased incidence of cancers, such as stomach cancer (Bryan et al., 2012). Based on a reevaluation of a long-term study in rats and consideration of the available epidemiological data, JECFA concluded that a NOAEL of 370 mg/kg bw per day was most appropriate for the safety evaluation of nitrate and established an ADI of 0–3.7 mg/kg bw for nitrate, expressed as nitrate ion, by applying an uncertainty factor of 100 to the NOAEL of 370 mg/kg bw per day (Annex 1, reference *160*).

Ammonium: The available toxicological data on ammonia and several ammonium salts, including ammonium carbonate, ammonium bicarbonate, ammonium chloride and ammonium acetate were previously evaluated by JECFA. The previous Committees decided not to establish a numerical ADI and allocated an ADI "not specified" for most ammonium salts, based on their low toxicity profiles determined upon review of the data available at the time of the evaluations. Limited genetic toxicity data are available on ammonium salts; however, there is information on an oral carcinogenicity assay conducted in Wistar rats (n = 50 per sex and group) treated with 0, 1 and 2.1% ammonium chloride in the diet over a period of 30 months. The study concluded that that ammonium chloride did not induce tumours under the testing conditions (Lina & Kuijpers, 2004).

Magnesium: The available toxicological data on several magnesium salts, including magnesium carbonate, magnesium hydrogen carbonate, magnesium sulfate, magnesium chloride, magnesium DL-lactate, magnesium gluconate, magnesium acetate, magnesium citrate, magnesium adipate, magnesium succinate, monomagnesium phosphate and magnesium stearate, for their use as food additives, were previously assessed by JECFA. The Committee noted the possibility of diarrhoea and similar gastrointestinal effects resulting from excessive intake of magnesium salts; however, no other adverse effects have been reported after long-term exposure (Annex 1, reference *224*). Given the widespread occurrence of the magnesium salts evaluated in foods from natural sources and the lack of any indication of significant toxic effects from human exposure to most of these salts, the previous Committees did not establish a numerical ADI and allocated an ADI "not specified" to the magnesium salts evaluated. More recently, JECFA evaluated data from long-term studies conducted by Kurata et al. (1989) on male and female B6C3F1 mice (50 per sex) to assess the potential

for carcinogenicity of magnesium chloride hexahydrate at doses of 0, 0.5 and 2% administered in the diet for 96 weeks (Annex 1, reference *224*). Based on the decreased body weight gain in females given the high dose, a NOAEL of 0.5% was identified for magnesium chloride hexahydrate in the diet, which was equivalent to 90 mg/kg bw per day, expressed as magnesium (Annex 1, reference *224*). Magnesium is an essential nutrient. The Institute of Medicine in the USA established recommended dietary allowances for magnesium of 80–420 mg/day for different age groups, which are considered as able to meet the nutrient needs of 97–98% of individuals in a population (Institute of Medicine, 1997). The SCF established an upper level of 250 mg/day for readily dissociable magnesium salts, such as magnesium chloride, magnesium sulfate, magnesium carbonate and magnesium lactate, and compounds in nutritional supplements, in water, or added to food and beverages (SCF, 2006).

Phosphates: At its twenty-sixth meeting, the Committee concluded that the phosphates are not genotoxic and the only consequence of excessive intake of phosphates in animals is an effect on calcium and magnesium homeostasis, which could potentially lead to bone loss, calcification of soft tissues and nephrocalcinosis (Annex 1, reference *59*). Upon evaluating the available toxicological data, the previous Committee established an MTDI for phosphates, diphosphates and polyphosphates of 70 mg/kg bw (Annex 1, reference *59*). More recently, at the seventy-sixth meeting, while evaluating magnesium dihydrogen diphosphate for use as an alternative to sodium-based acidifiers and raising agents, JECFA stated that the approach taken to derive the MTDI of phosphates from the toxicological data was unclear. This is because the end-point considered for deriving this value (nephrocalcinosis in rats) may not be relevant to humans, leading to an overly conservative value (Annex 1, reference *211*). Although there was no indication from the available toxicological data that the MTDI of 70 mg/kg bw for phosphates was insufficiently health protective, the previous Committee acknowledged the need to review the toxicological basis of the MTDI for phosphate salts expressed as phosphorus.

3.2.1 Allergenicity

There are no reports of allergenicity upon oral exposure to CN and CAN that indicate that these substances are or that they contain known food allergens.

3.3 Observations in humans

No data on oral exposure to CN and CAN in humans have been reported. However, a case of contact dermatitis in a farmer in India who had been using

urea and CAN as fertilizers was reported (Pasricha & Gupta, 1983). The recurrent episodes of dermatitis that followed visits to the field, and positive patch tests with CAN on two occasions, suggested that the allergic response was associated with the use of the fertilizer. However, these effects were not further evaluated to confirm whether they were specifically caused by exposure to CAN.

4. Levels and patterns of contamination in food commodities

Calcium, magnesium, nitrate, phosphates and ammonium are ubiquitous in the human diet:

- Calcium is an essential nutrient present in many foods, including dairy products, seeds, legumes, nuts and leafy green vegetables (Miller, 1989). Worldwide, calcium intake ranges from 171 to 314 mg/person per day (WHO, 2004).
- Nitrates occur in foods, including vegetables, fruit and processed meats (Hord, Tang & Bryan, 2009). Nitrates from a variety of sources, including pollution, are also present in water (Hill, 1996; Coss et al., 2004). They are also found in food additives, such as sodium nitrate and potassium nitrate (EFSA Panel on Food Additives, 2017). In 2002, the Committee reviewed exposure to nitrate from all food sources, based on studies from the Netherlands and the United Kingdom (Annex 1, reference *161*). The exposure assessment concluded that mean exposure to nitrate from all dietary sources was below the ADI of 0–3.7 mg/kg bw established by JECFA, but that high consumers may exceed this HBGV.
- Ammonium is present in many food additives, such as ammonium alginate, ammonium bicarbonate, ammonium phosphate and ammonium sulfate and usually permitted in accordance with the GMP (US FDA, 2019). No data on concentrations in food from these uses were identified.
- Magnesium is an essential nutrient needed for many physiological functions and commonly found in nuts, peanuts, green leafy vegetables, cereals and dairy foods. Magnesium intake varies from 21–369 mg/day (Vormann, 2003; WHO, 2004).
- Phosphates are widely distributed in tissues and commonly found in food and food ingredients (Ellinger, 2018).

5. Food consumption and dietary exposure estimates

The 2006 criteria document (FAO/WHO, 2007) assumed a worst-case concentration of 100 mg/kg for previous cargo substances. For the current assessment, this concentration was also supposed to be a worst-case concentration of the substance that could be present in oils.

Information on consumption of food oils by infants and young children is available on the US Environmental Protection Agency's Food Commodity Intake Database, or FCID (US EPA, 2020), which in turn is based on data from the US National Health and Nutrition Survey/What We Eat In America, 2005–2010 cycles. The highest oil consumptions by infants and young children based on FCID are comparable to those in the CIFOCOss database; however, oil consumption information based on FCID is available based on individual body weights. The highest reported consumption of a specific fat or oil type was for palm oil. Estimated mean and 95th percentile consumption by infants and young children were 7.6 and 19 g/day, respectively. The estimated mean and 95th percentile consumption on a body weight basis were 1 g/kg bw per day and 3 g/kg bw per day, respectively. These data were used to define a worst-case dietary exposure estimate for infants and young children. Estimated chronic daily dietary exposures to calcium, magnesium, nitrate, phosphates and ammonium present in food oils are 0.1 mg/kg bw per day (mean) and 0.3 mg/kg bw per day (high consumers), assuming a worst-case concentration of calcium, magnesium, nitrate, phosphates and ammonium of 100 mg/kg, mean oil consumption of 1 g/kg bw per day, and high-consumer oil consumption of 3 g/kg bw per day.

The worst-case exposure to CN or CAN as a previous cargo substance in oil is 0.3 mg/kg bw per day. Therefore, any potential dietary exposure to calcium, magnesium, nitrate, phosphates or ammonium from CN or CAN as previous cargo substances in food oils would be a minor contributor to overall dietary exposure.

6. Comments

6.1 Chemical and technical considerations

Chemical and technical considerations for calcium nitrate and calcium ammonium nitrate solutions are summarized in Tables 6 and 7.

Table 6
Chemical and technical considerations for calcium nitrate solution

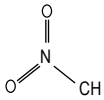
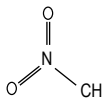
Name: Calcium nitrate solution	
CAS number	**Alternative CAS numbers**
35054-52-5 (hydrate)	10124-37-5 (anhydrous), 13477-34-4 (tetrahydrate)
Chemical details	Nitric acid, calcium salt, CN CN is a colourless or white to grey crystalline or granular material. Structure: multiple different but closely related formulations exist.  • 1/2 Ca • × H_2O Molar mass, anhydrous: 164.09 g/mol Molar mass, tetrahydrate: 236.15 g/mol Melting point, anhydrous: 560 °C Melting point, tetrahydrate: ~ 44 °C Readily soluble in water
Route(s) of synthesis	Manufactured by dissolution of limestone in nitric acid or as a by-product of the nitrophosphate fertilizer manufacturing process.
Composition	Variable formulations exist in commerce – amounts of Ca and N vary. Specific composition depends on the final use of the product.
Uses	Used as fertilizer – source of nitrogen. Additionally, used in wastewater treatment and in production of concrete.
Analytical methods	None found for previous cargoes: possible means of detection in fats and oils by inductively coupled plasma (ICP) analysis for Ca residues.
Potential reaction(s) with a subsequent cargo of fat or oil	Formation of calcium salts of free fatty acids is possible; reaction conditions are not expected to be present in a cargo of edible fats or oils.

Table 7
Chemical and technical considerations for calcium ammonium nitrate solution

Name: Calcium ammonium nitrate solution	
CAS number	**Alternative CAS numbers**
15245-12-2	None
Chemical details	Nitric acid, calcium ammonium salt, CAN CAN is a white to pale yellow granule or prilled solid.

Table 7 (continued)

	Structure: multiple different but closely related products exist.  • × Ca • × NH_3 Molar mass (as $H_4CaN_2O_3$): 120.12 g/mol Melting point: 170 °C Readily soluble in water
Route(s) of synthesis	Manufactured by addition of ground limestone, dolomite or calcium nitrate to molten ammonium nitrate.
Composition	Variable formulations exist in commerce – amounts of Ca and N vary, as do the forms of N (as ammoniacal nitrogen or nitrate nitrogen). Specific composition depends on the final use of the product.
Uses	Used as a fertilizer – source of ammoniacal nitrogen and nitrate nitrogen. Minor use in disposable cold packs is also reported.
Analytical methods	None found for previous cargoes. Possible means of detection in fats and oils by inductively coupled plasma (ICP) analysis for Ca residues.
Potential reaction(s) with a subsequent cargo of fat or oil	Formation of calcium, magnesium or ammonium salts of free fatty acids is possible; reaction conditions are not expected to be present in a cargo of edible fats or oils.

6.2 Biochemical aspects

Calcium: The Committee noted that calcium is an essential nutrient. In the soluble ionized form, calcium is absorbed by the intestine in humans via active transport across cells mainly in the duodenum and the upper jejunum, and through passive diffusion mainly in the ileum and to a lesser extent in the large intestine (Pansu, Bellaton & Bronner, 1981; Pansu & Bronner, 1999; Gueguen & Pointillart, 2000). Most of the absorbed calcium is stored in the skeleton (about 99% of the body's calcium), depending on the physiological needs related to growth and health conditions, including pregnancy and lactation (Gueguen & Pointillart, 2000). Absorbed calcium that is not retained by the body is excreted in urine, faeces and sweat (SCF, 2003; Gueguen & Pointillart, 2000).

Nitrate: The Committee revisited its previous evaluation of nitrates conducted at the fifty-ninth meeting (Annex 1, reference *160*) to examine its toxicokinetic profile for the present assessment. The Committee had previously concluded that nitrate is rapidly absorbed after oral administration such that its concentration increases in the plasma within 10 minutes (Annex 1, reference *160*, *161*). The elimination half-life ($t_{1/2}$) of nitrate in the plasma is 6.5 hours and 70% of the dose is excreted in the urine within 10 hours of oral administration. Some

of the dietary nitrate is converted to nitrite through non-enzymatic processes and to nitric oxide by symbiotic bacteria in the oral cavity and stomach. The nitric oxide generated plays a protective role in the cardiovascular system and the gastric mucosa as well as against metabolic diseases (Lundberg et al., 2011; Ma et al., 2018). The Committee also concluded that the rat was an unsuitable model for examining toxicokinetics of nitrate as the rat does not convert nitrate into nitrite in a quantitatively similar way to humans (Annex 1, reference *160*, *161*). Toxicokinetic modelling based on a simple one-compartment approach (Wagner et al., 1983) as well as on a multi-compartment framework (Zeilmaker et al., 1996) was used to examine the kinetics of nitrate. The Committee determined that the range of nitrate to nitrite conversion in humans is about 5–7% in average individuals and 20% in individuals with a high rate of conversion (Annex 1, reference *160*, *161*). The model developed by Zeilmaker et al. (1996) also predicted that a single dose of nitrate from vegetables as well as repeated intake of nitrate in drinking-water up to 44 mg/kg bw would not induce clinical methaemoglobinaemia and that lethal toxicity would occur at doses > 440 mg/kg bw. The Committee also reviewed recent reports on the potential of nitrate to be converted to carcinogenic nitrosamines in the body under certain conditions, such as acidic gastric environment (Zeilmaker et al., 1996; Ma et al., 2018). However, recent epidemiological cohort studies concluded that there is no clear evidence of an association of dietary nitrates with increased incidence of cancers, such as stomach cancer (Bryan et al., 2012).

Ammonium: The Committee noted that ammonia is produced in the gut of all mammalian species by bacterial degradation of nucleic and amino acids from ingested foods. The estimated production of ammonia in the human intestine ranges from 10 mg per day in the duodenum to 3 g per day in the colon (cited in EFSA, 2009b). Ammonia is readily absorbed by the gastrointestinal tract upon oral ingestion in foods, followed by its entry into the portal circulation and its transformation to urea in the liver via the urea cycle. It is then excreted by the kidneys as urea.

Magnesium: The Committee noted that magnesium is an essential nutrient. It is commonly found in foods, such as lettuce, spinach, turnip greens and collard, and it is an essential mineral that serves as a cofactor for more than 300 enzyme systems (Institute of Medicine, 1997). It contributes to energy metabolism, protein and nucleotide synthesis, and metabolism and activation of vitamin D and parathyroid hormone (Annex 1, reference *224*). The net absorption of dietary magnesium in a typical diet is between 40 and 60% (Schwartz, Spencer & Welsh, 1984). Upon ingestion, magnesium salts, such as magnesium carbonate, magnesium sulfate, magnesium chloride and magnesium stearate are dissolved

under acidic gastric conditions and separate into the magnesium ion (cation) and the anion (carbonate, sulfate, chloride, stearate, etc., respectively). Magnesium is absorbed all along the intestinal tract, but the sites of maximal absorption are the ileum and jejunum (Kayne & Lee, 1993). Magnesium is excreted mainly in the urine and the kidney is the principal organ involved in its homeostasis (Institute of Medicine, 1997).

Phosphates: The Committee considered its previous evaluation of phosphates conducted at the twenty-sixth meeting to assess their toxicokinetic profile (Annex 1, reference *59*). Phosphorus is an essential nutrient and a constituent of bones, teeth and several enzyme systems. Phosphates play an important role in carbohydrate, fat and protein metabolism. Phosphates (or phosphorus) are mainly absorbed from the diet as free orthophosphate after enzymatic hydrolysis. The intestinal absorption of phosphates depends on the requirements of the body and their levels are regulated by certain physiological mechanisms. The amounts of inorganic phosphates in the blood are stabilized by exchange with the mineral deposit in the skeleton by parathyroid hormone. The parathyroid hormone inhibits renal tubular reabsorption of phosphates causing demineralization of the bone tissue, and the concentrations of circulating parathyroid hormone are regulated by blood calcium concentrations. Phosphates are mainly excreted in the faeces.

6.3 Toxicological studies

Given the sparse availability of toxicological datasets on calcium nitrate and calcium ammonium nitrate, the Committee considered health-based guidance values for calcium, nitrate, ammonium, magnesium and phosphates, established under previous evaluations and briefly summarized below, to conduct the toxicological evaluation of calcium nitrate and calcium ammonium nitrate at the anticipated exposure level as previous cargoes for edible oils and fats.

Calcium: The Committee had previously evaluated many calcium salts, including calcium sulfate, calcium chloride, calcium carbonate, calcium acetate and calcium gluconate, among others, for use as food additives. It allocated an ADI "not specified" to these salts based on their low toxicity determined from a review of the data available at the time of the evaluations. The UL of 2500 mg per day for adults (equivalent to about 40 mg/kg bw), based on different intervention studies in humans of long duration, in which total daily calcium intakes of 2500 mg from both diet and supplements were tolerated without adverse effects, was established by SCF in 2003 (SCF, 2003) and confirmed by EFSA (2012).

Nitrates: At its forty-fourth and fifty-ninth meetings *(*Annex 1, references *116* and *160)*, the Committee concluded that nitrate was not genotoxic and the carcinogenicity studies on nitrates were negative, except when extremely high doses of both nitrate and nitrosable precursors were administered. A review of available epidemiological data provided no evidence of an association between human exposure to nitrite and risk of cancer. Based on a re-evaluation of a long-term study in rats and consideration of the available epidemiological data, the Committee concluded that a NOAEL of 370 mg/kg bw per day was most appropriate for the safety evaluation of nitrate, and established an ADI of 0–3.7 mg/kg bw for nitrate, expressed as nitrate ion, by applying an uncertainty factor of 100 to the NOAEL of 370 mg/kg bw per day (Annex 1, reference *160*).

Ammonium: The Committee had previously evaluated toxicological data on several ammonium salts, including ammonium carbonate, ammonium bicarbonate, ammonium chloride and ammonium acetate and concluded that these salts would not cause significant toxic effects, except for alteration of acid-base balance. At its previous meetings, the Committee decided not to establish a numerical ADI and allocated an ADI "not specified" for most ammonium salts, based on their low toxicity determined upon review of data available at the time of the evaluations.

Magnesium: The Committee had previously evaluated available toxicological data on several magnesium salts, including magnesium carbonate, magnesium hydrogen carbonate, magnesium sulfate, magnesium chloride, magnesium DL-lactate, magnesium gluconate, magnesium acetate, magnesium citrate, magnesium adipate, magnesium succinate, monomagnesium phosphate and magnesium stearate, for their use as food additives. The Committee noted the possibility of diarrhoea and similar gastrointestinal effects due to excessive intake of magnesium salts; however, no other adverse effects had been reported after long-term exposure to magnesium salts (Annex 1, reference *224*). Given their widespread occurrence in food from natural sources and no indication of significant toxic effects from human exposure to most magnesium salts, the previous Committees did not establish a numerical ADI and allocated an ADI "not specified" for the magnesium salts evaluated.

Phosphate: The Committee considered its previous evaluation of phosphates (Annex 1, reference *59*) conducted at the twenty-sixth meeting. At that time, it concluded that the phosphates are not genotoxic and the only consequence of excessive intake of phosphates in animals is an effect on calcium and magnesium homeostasis, which could potentially lead to bone loss, calcification of soft tissues and nephrocalcinosis. Upon evaluating the available

toxicological data, the previous Committee established an MTDI for phosphates, diphosphates and polyphosphates of 70 mg/kg bw (Annex 1, reference *59*). More recently, at the seventy-sixth meeting, while evaluating magnesium dihydrogen diphosphate for use as an alternative to sodium-based acidifiers and raising agents, the Committee stated that the approach taken to derive the MTDI of phosphates from the toxicological data was unclear, as the end-point considered (nephrocalcinosis in rats) for deriving this value may not be relevant to humans, leading to an overly conservative value of the MTDI (Annex 1, reference *211*). While there was no indication from the available toxicological data that the MTDI of 70 mg/kg bw for phosphates was insufficiently health protective, the Committee acknowledged the need to review the toxicological basis of the MTDI for phosphate salts expressed as phosphorus (Annex 1, reference *211*).

No data on oral exposure to calcium nitrate and calcium ammonium nitrate in humans have been reported. However, the Committee located a report of a case of contact dermatitis in a farmer in India after using urea and calcium ammonium nitrate as fertilizers (Pasricha & Gupta, 1983). The recurrent episodes of dermatitis that followed visits to the field, and positive patch tests with calcium ammonium nitrate on two occasions suggested that the allergic response was associated with the use of the fertilizer; however, these effects were not further evaluated to confirm whether they were specifically caused by exposure to calcium ammonium nitrate.

6.4 Allergenicity

The Committee did not locate any reports of allergenicity upon oral exposure to calcium nitrate and calcium ammonium nitrate that would indicate that these substances are, or contain known food allergens.

6.5 Assessment of dietary exposure

Calcium, magnesium, nitrate, phosphates and ammonium are ubiquitous in the human diet. The generic human dietary exposure value for previous cargoes of 0.3 mg/kg bw per day indicates that any potential dietary exposure to calcium, magnesium, nitrate, phosphates and ammonium from previous cargoes in food oils would be a minor contributor to the overall dietary exposure to these substances.

7. Evaluation

Given that toxicological datasets on calcium nitrate and calcium ammonium nitrate are sparse, the Committee evaluated available toxicological data on calcium, ammonium and nitrate to conduct their toxicological evaluation. The Committee also reviewed available toxicological data on magnesium and phosphates, as dolomite and phosphate rock could be used in the manufacture of calcium ammonium nitrate and calcium nitrate, respectively.

The Committee estimated exposure to calcium nitrate and calcium ammonium nitrate from previous cargoes for edible fats and oils as 0.3 mg/kg bw per day each, which is much less than the exposures to calcium, nitrate, ammonium, magnesium and phosphates expected from dietary sources. The Committee considered health-based guidance values for calcium, nitrate, ammonium, magnesium and phosphates, established under previous evaluations, to conduct the toxicological evaluation of calcium nitrate and calcium ammonium nitrate at the anticipated exposure level from previous cargoes for edible oils and fats. The estimated exposure value for calcium nitrate and calcium ammonium nitrate as previous cargoes for edible fats and oils is 0.3 mg/kg bw each, which does not exceed the ADI for nitrate of 0–3.7 mg/kg bw, expressed as nitrate ion (Annex 1, reference *160*), and the MTDI of 70 mg/kg bw for phosphates, diphosphates and polyphosphates (Annex 1, reference *59*). The previous Committees did not assign a numerical ADI but allocated an ADI "not specified" for most calcium, ammonium and magnesium salts based on their low oral toxicity profiles. Furthermore, the Committee considered that human exposure to these substances resulting from their use as previous cargoes would be a minor contributor to the total dietary exposure.

There are no data on allergenicity upon oral exposure to calcium nitrate and calcium ammonium nitrate that would indicate that these substances are, or contain, known food allergens.

The Committee concluded that the formation of calcium, ammonium or magnesium salts of free fatty acids is possible. However, owing to the anticipated absence of alkaline conditions and an insufficient concentration of counter ions and free fatty acids (necessary for the reactions to occur), these reaction products are not expected to be formed in detectable amounts in a cargo of edible fats and oils.

Therefore, calcium nitrate and calcium ammonium nitrate meet the criteria for acceptability as previous cargoes for edible fats and oils.

II. Calcium lignosulfonate liquid

1. Explanation

Calcium lignosulfonate is a complex mixture of polymers with variable degrees of cross-linking and a wide range of molecular weights, derived from the sulfite pulping of wood. Calcium lignosulfonate (40-65) is one fractionated product from sulfite pulping and is used in food applications. Calcium lignosulfonate (40-65) (a purified lignosulfonate product with an average molecular weight range of 40 000–65 000 Daltons) was evaluated by the Committee at its sixty-ninth meeting as a food additive, intended for use as a carrier for encapsulated food ingredients (Annex 1, reference *191*). The Committee identified a NOAEL of 2000 mg/kg bw per day based on results of a 90-day dietary study of calcium lignosulfonate (40-65) in rats (Thiel, Kohl & Braun, 2007). The study authors reported a dose-dependent increase in incidence of histiocytosis in the mesenteric lymph nodes. However, the Committee concluded that such histiocytosis would not be expected to progress to any adverse effect. Based on this NOAEL and an uncertainty factor of 100, the Committee established an ADI of 0–20 mg/kg bw for calcium lignosulfonate (40-65) (Annex 1, reference *190*). Calcium lignosulphonate (or calcium lignosulfonate) was evaluated by SCF. It was considered acceptable as a previous cargo and also as an additive to animal feedstuff, based on the conclusion that it is toxicologically inert and easily removable by tank cleaning (SCF, 2003). The Panel for Food Additives and Nutrient Sources added to Food (ANS Panel) evaluated the available data and concluded that the 90-day feeding study in rats (Thiel, Kohl & Braun, 2007) was inadequate for evaluating the safety of calcium lignosulfonate (40-65). This was due to a possible poor health status of the animals in this study (high incidence of lymphoid hyperplasia and lymphoid infiltration in the mandibular and mesenteric lymph nodes, in the Peyer's patches and in the livers of the animals, including controls) (EFSA, 2010). The CONTAM Panel evaluated calcium lignosulfonate as an acceptable previous cargo in 2016 and 2019 (EFSA, 2017, 2019). Based on a report of a re-evaluation of the 90-day dietary study in rats (Thiel et al., 2013), the CONTAM Panel agreed with the JECFA-identified NOAEL of 2000 mg/kg bw per day for calcium lignosulfonate (40-65) derived from this study (EFSA, 2017). However, owing to data gaps regarding the composition and toxicity of the low molecular weight fraction (LMWF) of technical grade calcium lignosulfonate, the CONTAM Panel concluded that calcium lignosulfonate did not meet the acceptability criteria for previous cargoes (EFSA, 2017). More recently, the CONTAM Panel evaluated additional genetic toxicity studies on technical grade calcium lignosulfonate

(LMWF < 1000 Daltons), which showed a lack of genotoxic potential of the test substance evaluated (EFSA, 2019). However, a review of the data on the molecular weight distribution of the test substance indicated that it was not sufficiently representative of the LMWF in products intended to be shipped as previous cargo. Therefore, the CONTAM Panel stated that the existing toxicological database did not account for all grades of calcium lignosulfonate shipped as previous cargoes.

For the present assessment, the Committee identified and reviewed its previous evaluations of calcium lignosulfonate, and those of EFSA, and located additional references from these evaluations. This was followed by comprehensive searches for data on calcium lignosulfonate on PubMed and PubChem. The cut-off date for the inclusion criteria on all searches was 20 August 2020. The retrieved references with relevant toxicological information were on the 90-day oral subchronic toxicity study of calcium lignosulfonate (40-65) conducted in rats that is discussed in the present assessment.

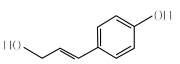
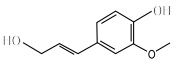
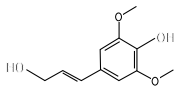
2. Chemical and technical considerations

Calcium lignosulfonate is a water-soluble anionic surfactant that is insoluble in most organic solvents. It is manufactured as an amorphous light yellowish-brown to brown powder or a brown to black liquid, depending on the level and type of processing applied and the intended use of the product. The physicochemical characteristics of calcium lignosulfonate liquid are summarized in Table 8.

2.1 Manufacture and uses of calcium lignosulfonate liquid

Lignosulfonates are manufactured from lignin, a major by-product of the sulfite wood pulping process. Lignin is the second most abundant polymer in wood, accounting for 15–40%, by weight. It is also one of the largest natural sources of aromatic compounds (Aro & Fatehi, 2017). Calcium lignosulfonate is the calcium salt of lignosulfonate obtained from the sulfite pulping of wood. Wood biomass is composed primarily of cellulose, lignin and hemicellulose. Sulfite pulping of wood chips enables the removal of the cellulose by digestion in an acidic calcium bisulfite solution for 6–10 hours at approximately 130 °C (Toledo & Kuznesof, 2008). Lignin is solubilized through cleavage of lignocellulosic bonds and formation of smaller sulfonated units. After filtration, the sulfite liquor consists of the lignosulfonate with other breakdown products and components of wood, including lipids, fatty acids, wax esters, sterols and associated degradation products, resin acids and long chain alcohols, sulfite, inorganic salts, sugars and reaction products (Li & Takkellapati, 2018; EFSA, 2019).

Table 8
Physical and chemical characteristics of calcium lignosulfonate liquid

Characteristic	Calcium lignosulfonate liquid
Chemical name; IUPAC systematic name	Lignosulfonic acid, calcium salt
Synonyms	Lignin liquor, sulfite lye
CAS number	8061-52-7
Chemical structure	Random polymer with an inconsistent degree of polymerization and cross-linking; structures of the three monomers are shown below.  A. *p*-Coumaryl alcohol  B. Coniferyl alcohol  C. Sinapyl alcohol
Molecular formula; molecular mass	No fixed molecular formula. Polydisperse, random polymer with wide molecular mass distribution ranging from approximately 1000–250 000 Da.
Description	Light brown to brown powder or a brown to black liquid.
Solubility	Readily soluble in water, insoluble in most organic solvents.

Further processing of the crude sulfite liquor is required to yield commercial calcium lignosulfonate products. Steam-stripping removes excess sulfur dioxide as well as volatile substances including formaldehyde, furfural, hydroxymethyl furfural, acetic acid and formic acid. Fermentation followed by distillation of ethanol may be used to remove fermentable sugars. Excess water and volatile components are removed by evaporation to yield commercial products, which contain approximately 50% lignosulfonate (EFSA, 2019). Food-grade calcium lignosulfonate (40-65) with a weight-average molecular weight of 40 000–65 000 Da is produced by ultrafiltration using a semi-permeable membrane to separate the high-molecular weight fraction and remove the undesirable low-molecular weight lignosulfonates and reducing-sugar monomers (Toledo & Kuznesof, 2008).

Lignosulfonates have long been valued for their colloidal properties and solubility in water. They are commercially available as calcium and sodium salts and have been used in numerous industrial applications as binders, emulsifiers and dispersants for a variety of materials (Macfarlane, Mai & Kadla, 2014). The largest single use of lignosulfonates is as plasticizers in concrete, allowing for the use of less water while increasing the strength and flow properties. This application has been reported to account for 45% of the global consumption of lignosulfonates (Holladay et al., 2007). Lignosulfonates are also used in the

following processes and products: production of cement and plasterboard; petroleum drilling; as dispersants for the application of pesticides; as emulsifiers in asphalt; and as a deflocculant in processing feedstuffs (EFSA, 2019). Products from lignosulfonates that have been further purified are used as feed additives (EFSA, 2015). One specific type of lignosulfonate, calcium lignosulfonate (40-65), is used as a food additive as a carrier for fat-soluble vitamins, carotenoids and other functional ingredients (Toledo & Kuznesof, 2008).

2.2 Composition and secondary contaminants

Calcium lignosulfonate is a random polymer with heterogeneous structures and an inconsistent degree of polymerization and cross-linking. Lignin from different plant sources contains variable amounts of three aromatic propenyl alcohol monomers: coniferyl alcohol (4-(3-hydroxy-1-propenyl)-2-methoxyphenol), *p*-coumaryl alcohol (4-[(E)-3-hydroxyprop-1-enyl]phenol) and sinapyl alcohol (4-hydroxy-3,5-dimethoxycinnamyl alcohol). The ratio of these monomers in lignin depends on the plant source (Caufield, Passaretti & Sobczynski, 1990). The variable chemical composition of lignin results in a cross-linked phenolic polymer lacking a fully defined primary structure; thus, the subsequently derived lignosulfonate similarly has no defined structure or chemical composition. The molecular masses of lignosulfonates produced from sulfite pulping generally range from 1000 to 150 000 Da, depending on a variety of factors including the type of wood and the specific sulfite pulping process used (McCarthy & Islam, 1999; Vishtal & Kraslawski, 2011). Calcium lignosulfonate products with molecular masses up to 250 000 Da and below 1000 Da have been reported (EFSA, 2011b). One report indicates that the typical weight-average molecular weight of the products shipped as previous cargoes is approximately 30 000–40 000 Da (EFSA, 2019).

Commercially available calcium lignosulfonate liquids have no single set of chemical or physical specifications. Differences in manufacturing processes, sources of wood, processing conditions and post-pulping purification steps, which may include chemical modifications to optimize products for specific industries, are responsible for the varied composition of the products shipped. Little detailed information related to chemical composition is reported for calcium lignosulfonate liquids; see Table 9 for limited specifications and typical results for some commercial products in both liquid and powder form, including calcium lignosulfonate (40-65).

Table 9
Commercial product specifications and typical analysis for calcium lignosulfonate

Product	Parameter	Minimum	Maximum	Typical analysis
BorrePlex CA LIQUID (Borregard LignoTech, 2020)	pH	3.5	5.0	4.3
	Solids (%)	50.0	56.0	53
	Insoluble matter (%)	–	0.8	–
	Total sulfur	–	–	7.0
Borresperse CA-SA POWDER (LignoTech South Africa, 2020)	pH of 10% solution	7.0	8.0	6.8
	Dry matter (%)	93.0	–	–
	Insoluble matter (%)	–	0.5	–
	Total sulfur	–	–	4.0
	Reducing sugars	–	–	2.0
	HPLC sugars	–	–	< 1.0
	Moisture (%)	–	–	6
Calcium Lignosulfonate Grades One, Two and Thee, Combined POWDERS (Green Agrochem, 2020)	pH	4	7	–
	Dry matter (%)	95%	–	–
	Insoluble matter (%)	–	2.5%	–
	Sulfate	2%	–	–
	Total calcium and magnesium sulfate	5%	8%	–
	Lignosulfonate	50%	–	–
	Sugar	9%	12%	–
	Reducing sugar	Around 7%	Around 7%	
	Ash		12–21% (depending on product)	–
Calcium Lignosulfonate 40-65 POWDER (Toledo & Kuznesof, 2008)	Weight-average molecular weight	40 000 Da	65 000 Da	–
	Molecular weight < 1000 Da	–	10%	–
	Calcium	–	5%	–
	Mannose	–	–	2.2
	Xylose	–	–	1.1
	Galactose	–	–	0.6
	Glucose	–	–	0.4
	Arabinose	–	–	0.3
	Rhamnose	–	–	0.1
	Arsenic	–	1 mg/kg	–
	Lead	–	2 mg/kg	–

HPLC, high-performance liquid chromatography.

2.3 Reactivity and reactions with fats and oils

The reactivity of commercially available calcium lignosulfonate liquids is dependent upon the types, abundance and position of reactive functional groups on the polymeric structure. Lignosulfonates exhibit numerous functional groups including sulfonic acid, carboxyl, methoxy and phenolic hydroxyl groups (Gelardi

et al., 2016). The number of sulfonic acids and carboxyl and phenolic hydroxyl functional groups has been reported to decrease with increasing molecular mass of sodium lignosulfonate (Yang et al., 2014). Higher molecular mass compounds with fewer functional groups may be less likely to react with edible fats and oils.

Free fatty acids and triglycerides present in cargoes of fats and oils are unlikely to react with lignosulfonates under the conditions of transport. Reaction products would have a molecular mass representative of the calcium lignosulfonate solution being transported. Reactions between calcium lignosulfonate components and lipid cargoes cannot be ruled out.

2.4 Methods of analysis

No analytical methods for determination of residual contamination of edible fats or oils by previous cargoes of calcium lignosulfonate liquids are reported in the literature. Detection of calcium in fats or oils could serve as an indicator of contamination. Since calcium lignosulfonate liquid is water-soluble, it is expected to be effectively removed from the tanks by required cleaning protocols, resulting in very low levels of calcium arising from the contamination. ICP techniques are capable of detecting and quantifying calcium contamination in edible fats or oils, but the limits of detection and quantification of some instruments may not be sufficiently low to assess the contamination with calcium lignosulfonate liquids. ICP-mass spectrometry (ICP-MS) instruments have greater sensitivity for the detection of calcium at low levels, which may allow detection of calcium lignosulfonate contamination in fats or oils.

3. Biological data

3.1 Biochemical aspects

The Committee evaluated the toxicokinetic profile of calcium lignosulfonate (40-65) at its sixty-ninth meeting (Annex 1, reference *191*) based on a review of two studies:

- an in vitro study based on the Caco-2 cell monolayer model performed to predict the intestinal absorption of tritiated [^{3}H]-labelled calcium lignosulfonate (40-65) (Beck, Loechleiter & Rossi, 2008); and
- an in vivo study of [^{3}H]-labelled calcium lignosulfonate (40-65) in male and female rats following single oral administration of a dose of 10 mg/kg bw (Beck & Rossi, 2005).

These studies reported that very low systemic exposure of the test substance would be expected after oral exposure. Therefore, the Committee concluded that calcium lignosulfonate (40-65) is poorly absorbed after oral exposure. No data on biotransformation of calcium lignosulfonate by the gut flora or via other mechanisms have been reported. Nonetheless, the potential of calcium lignosulfonate (40-65) to undergo biotransformation is expected to be very low considering its low systemic exposure upon oral administration.

No toxicokinetic data on molecular weight fractions different from the molecular weight fractions of the food-grade calcium lignosulfonate are available. Therefore, the Committee could not evaluate the biochemical aspects of the non-food-grade calcium lignosulfonate that is shipped as a previous cargo.

3.2 Toxicological studies

3.2.1 Acute toxicity

The oral LD_{50} values of calcium lignosulfonate (molecular weight not specified) are 31.6 g/kg bw in young albino Sprague-Dawley rats (sex not specified) and between 10 and 20 g/kg bw in male rats (strain not identified), reported in two independent studies (cited in EFSA, 2010 and EFSA, 2011).

3.2.2 Short-term studies of toxicity

Some short-term and subchronic toxicity studies of calcium lignosulfonate (40-65) previously evaluated by the Committee (Annex 1, reference *191*) are summarized below:

- *28-day repeated-dose rat feeding study* (Weber & Ramesh, 2005): A 28-day repeated-dose feeding study was conducted on male and female Wistar rats (six per sex and group) fed calcium lignosulfonate (40-65). Male rats received doses of 0, 413, 1301 and 3495 mg/kg bw per day and females 0, 453, 1345 and 3609 mg/kg bw per day. The only toxicologically relevant finding was chronic inflammation of the rectum in males in the high-dose group (minimal fibrosis with a few inflammatory cell infiltrates).
- *28-day rat feeding study* (Wolz et al., 2004): A 28-day study was conducted in male and female Wistar rats (five per sex and group) with a new additive formulated with calcium lignosulfonate (40-65) as a carrier. The tested dose levels of calcium lignosulfonate (40-65) administered in the diet were 5.4–6.4 g/kg bw per day for males and 5.8–6.9 g/kg bw per day for females. The only clinical sign observed was dark faecal discolouration from treatment week 1 onwards, which

was accompanied by soft faeces. The Committee concluded that this finding was not toxicologically relevant as it was a typical secondary effect of high dietary content of calcium lignosulfonate (40-65).

- *90-day rat feeding study* (Thiel, Kohl & Braun, 2007): A subchronic toxicity (90-day) feeding (in the diet) study on calcium lignosulfonate (40-65) was conducted in male and female Wistar rats at doses of 0, 500, 1000 or 2000 mg/kg bw per day, compliant with GLP and internationally accepted guidelines (Thiel, Kohl & Braun, 2007). The Committee (Annex 1, reference *191*) evaluated this study and noted a dose-related increase in the incidence of histiocytosis in the mesenteric lymph nodes. This observation was considered not to be an adverse finding, as histiocytosis in the mesenteric lymph nodes has been reported with other substances that have high molecular weights, such as polypentosan sulfate and copovidone (a copolymer of vinylpyrrolidone and vinyl acetate). Long-term studies of rats treated with these high-molecular weight substances did not find any progression of histiocytosis to an adverse effect or carcinogenesis (Annex 1, reference *191*). The Committee identified the highest dose tested of 2000 mg/kg bw per day as the NOAEL and established an ADI of 0–20 mg/kg bw for calcium lignosulfonate (40-65) by application of an uncertainty factor of 100 (Annex 1, reference *191*).

The EFSA ANS Panel (EFSA 2010) considered the 90-day rat feeding study (Thiel, Kohl & Braun, 2007) to be inadequate due to potentially impaired health status of the animals. This was based on 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 of the animals, including controls. Therefore, the ANS Panel disagreed with JECFA's evaluation that the treatment-related histiocytosis in mesenteric lymph nodes was not adverse and emphasized the need for a toxicity study of at least 12 months to examine the long-term effects of the observed histiocytosis in the mesenteric lymph nodes (EFSA, 2010). A reassessment of the sections of the lymph nodes, Peyer's patches, liver and all lymphoid tissue, as well as additional clinical pathological examinations of the animals from the 90-day rat feeding study of calcium lignosulfonate (40-65) (Thiel, Kohl & Braun, 2007) concluded that the rats were in good health (Thiel et al., 2013). No vacuolation was observed in the proximal tubule of the rats treated with the test substance, indicating that the kidneys did not show any histopathological lesions. Foamy macrophages were present within the sinuses of the mesenteric lymph nodes and the incidence and mean severity of the sinus histiocytosis increased with dose (incidence of histiocytosis in males: 0/20, 4/20, 17/20 and 20/20 and incidence of histiocytosis in females: 0/20, 3/20, 8/20 and

19/20, for groups treated with 0, 500, 1000 and 2000 mg/kg bw per day of the test substance). The histiocytes increased in size (hypertrophy) but not in number (hyperplasia), indicating that this was not a proliferative lesion. The presence of foamy macrophages was consistent with phagocytosis of an undigested material entering the lymphatic system from intestinal absorption. This had previously been reported for substances with high molecular weight, such as polypentosan sulfate and mineral oils (Carlton et al., 2001; Elmore, 2006), and such lesions had not been demonstrated to progress in severity or lead to tumour formation in rats (Shoda et al., 1997; Carlton et al., 2001; Trimmer et al., 2004). Based on the reassessment of the 90-day rat feeding study (Thiel et al., 2013), the EFSA CONTAM Panel concluded that histiocytosis of the mesenteric lymph nodes was not an adverse observation (EFSA, 2017). Furthermore, the CONTAM Panel agreed with the NOAEL of 2000 mg/kg bw per day for calcium lignosulfonate (40-65) identified by JECFA. However, while examining the relevance of the available data on calcium lignosulfonate (40-65) to the evaluation of calcium lignosulfonate of all grades, that is the material shipped as previous cargoes, the CONTAM Panel identified data gaps concerning the composition and toxicity of the low-molecular weight fraction (LMWF) of calcium lignosulfonate. Therefore, the Panel concluded that the existing toxicological database on calcium lignosulfonate (40-65) is inadequate to conduct a toxicological evaluation of different molecular weight fractions of calcium lignosulfonate shipped as previous cargoes.

3.2.3 Long-term studies of toxicity and carcinogenicity

There are no reports of chronic toxicity or carcinogenicity after oral exposure to calcium lignosulfonate.

3.2.4 Genotoxicity

JECFA (Annex 1, reference *191*) previously concluded that calcium lignosulfonate (40-65) did not induce reverse mutation in the bacterial strains tested (Thiel, Köhl & Sokolowski, 2005) and did not show a clastogenic response in the mammalian cells tested (Thiel et al., 2006b) under the conditions of the genetic toxicity studies reviewed.

More recently, the EFSA CONTAM Panel (EFSA, 2019) reviewed additional genetic toxicity data (not available to the present Committee) provided for a LMWF (< 1000 Daltons) isolated from a technical product that was described as a representative of grades that were intended to be shipped as previous cargoes. The two new genetic toxicity studies were:

- a reverse mutation assay in *Salmonella* Typhimurium strains TA98, TA100, TA102, TA1535 and TA1537 in the presence and absence of metabolic activation (0, 31.6, 100, 316, 1000, 3160 and 5000 µg/plate);

and

- a micronucleus assay conducted with human lymphocytes in the presence and absence of metabolic activation (250, 500 and 2000 µg/mL without metabolic activation with 4 hours of exposure and harvested 20 hours later, and 250, 500 and 2000 µg/mL with metabolic activation with 4 hours of exposure and harvested 20 hours later in the main test) (cited in EFSA, 2019).

The CONTAM Panel concluded that the test substance showed negative responses in the genetic toxicity studies (EFSA, 2019), but stated that the molecular weight distribution data on the test substance were not sufficiently representative of the LMWF in products intended to be shipped as previous cargoes; a result of an apparent loss of constituents of lower molecular masses (below 200 Daltons). As a result, the CONTAM Panel concluded that the new genotoxicity data were insufficient to assess the acceptability of calcium lignosulfonate as a previous cargo (EFSA, 2019).

3.2.5 Allergenicity

There are no reports of allergenicity upon oral exposure to calcium lignosulfonate that indicate that the substance that is shipped as a previous cargo is or contains a known food allergen. A single case of allergy attributed to dermal exposure to calcium lignosulfonate (molecular weight not specified) was reported in a 22-year-old man. This took the form of eczema of the face, hands and forearms (Andersson & Göransson, 1980). However, these effects were not further investigated.

3.2.6 Other studies

JECFA (Annex 1, reference *191*) previously evaluated a skin sensitization study on calcium lignosulfonate (40-65) in CBA mice using the local lymph node assay (van Huygevoort, 2004) and concluded that the test substance did not cause any treatment-related effects.

3.2.7 Reproductive and developmental toxicity

JECFA (Annex 1, reference *191*) evaluated the potential for reproductive and developmental toxicity of calcium lignosulfonate (40-65) based on the review of a maternal and developmental toxicity study in female Wistar rats (22 per group) (Thiel et al., 2006a). In this study, calcium lignosulfonate (40-65) was administered in the diet at doses of 0, 100, 300 or 1000 mg/kg bw per day from day 6 post-coitum to day 21 post-coitum. A NOAEL of 1000 mg/kg bw per day of

calcium lignosulfonate (40-65) was identified based on the report that there were no treatment-related or toxicologically relevant effects at any of the doses tested.

3.3 Observation in humans

No data on oral exposure to calcium lignosulfonate in humans are available. The only case of exposure to calcium lignosulfonate in humans is the report of allergy attributed to dermal exposure to calcium lignosulfonate (molecular weight not specified) discussed in section 3.2.5 (Andersson & Göransson, 1980).

No other toxicity data or data in humans on different molecular weight fractions constituting the non-food grade calcium lignosulfonate that is shipped as a previous cargo were available. Therefore, the Committee could not conduct a toxicological evaluation of the non-food grade calcium lignosulfonate that is shipped as a previous cargo.

4. Food consumption and dietary exposure estimates

The Codex Committee on Food Additives (CCFA) assigned calcium lignosulfonate (40-65) a food additive INS number of 1522 and functional class as a carrier and encapsulating agent at the forty-first session in March 2009 (CCFA, 2009). The CCFA did not take any further action relating to calcium lignosulfonate (40-65) at the forty-second session in March 2010, since no proposals for the use of the substance for inclusion in the Codex General Standard for Food Additives (GSFA) had been forwarded in response to a request for information on uses and use levels of the substance sent to the members of the CCFA.

Lignosulfonates are approved generically in European Commission Directive 70/524/EEC as additives for animal feed in the European Community. However, this application relates to calcium lignosulfonate (40-65), a more defined form of the substance than other calcium lignosulfonates. In the United States, calcium lignosulfonate (40-65) is approved as a dispersion agent and stabilizer in pesticides for preharvest or postharvest applications to bananas (US FDA, undated). In Australia and New Zealand, calcium lignosulfonate (40-65) is permitted as a food additive for the purpose of incorporating oil-soluble and dispersible nutrients (fat-soluble vitamins and carotenoids) in water-based foods (FSANZ, 2019).

As stated above, the estimated chronic daily dietary exposures to lignosulfonates present in food oils are 0.1 mg/kg bw per day (mean) and 0.3 mg/kg bw per day (high consumers), assuming a worst-case concentration of

lignosulfonates of 100 mg/kg, mean oil consumption of 1 g/kg bw per day, and high-consumer oil consumption of 3 g/kg bw per day.

A dietary exposure assessment conducted by JECFA estimated that the maximum potential dietary exposure to calcium lignosulfonate (40-65) could reach 7 mg/kg bw per day from food and supplement use (Annex 1, reference *190*). In Australia and New Zealand, the mean estimated exposure to calcium lignosulfonate (40-65) as a permitted food additive ranged from 1 mg/kg bw per day (Australian population aged 2 years and above and New Zealand population aged 15 years and above) to 4 mg/kg bw per day (Australian population aged 2 to 6 years). The estimated exposure for high consumers at the 90th percentile of exposure ranged from 2 mg/kg bw per day (New Zealand population aged 15 years and above) to 6 mg/kg bw per day (Australian population aged 2 to 6 years).

There are no data on dietary exposure to calcium lignosulfonate when added to animal feed or when used as a dispersion agent and stabilizer in pesticides for preharvest or postharvest applications.

5. Comments

5.1 Chemical and technical considerations

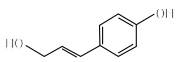
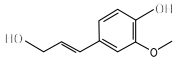
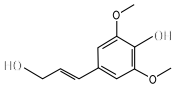
Chemical and technical considerations for calcium lignosulfonate liquid are summarized in Table 10.

5.2 Biochemical aspects

To assess the toxicokinetic profile of calcium lignosulfonate (40-65), the Committee considered its previous evaluation of in vitro (Beck, Loechleiter & Rossi, 2008) and in vivo (Beck & Rossi, 2005) studies conducted with calcium lignosulfonate (40-65), which concluded that it is poorly absorbed following oral exposure, and, therefore, has a low oral bioavailability. No data on biotransformation of calcium lignosulfonate (40-65) by the gut flora or via other mechanisms have been reported. However, the Committee expects the potential of calcium lignosulfonate (40-65) to undergo biotransformation to be very low considering its low systemic exposure following oral administration.

No toxicokinetic data on molecular weight fractions different from the molecular weight fractions of the food-grade calcium lignosulfonate are available. Therefore, the Committee could not evaluate the biochemical aspects of the non-food-grade calcium lignosulfonate that is shipped as a previous cargo.

Table 10
Chemical and technical considerations for calcium lignosulfonate liquid

Name: Calcium lignosulfonate liquid	
CAS number	**Alternative CAS numbers**
8061-52-7	None
Chemical details	Lignin liquor, sulphite lye, lignosulfonic acid, calcium salt Light brown to brown powder or a brown to black liquid Structure: Random polymer with an inconsistent degree of polymerization and cross-linking; structures of the three monomers are shown below.  A. *p*-Coumaryl alcohol  B. Coniferyl alcohol  C. Sinapyl alcohol Molar mass: polydisperse, random polymer with wide molecular mass distribution ranging from approximately 1000–250 000 Da Readily soluble in water, insoluble in most organic solvents.
Route(s) of synthesis	Manufactured as a by-product from the sulfite pulping of wood. Wood chips are digested in an acidic calcium bisulfite solution. Cellulose is filtered out of solution, leaving the spent sulfite liquor containing calcium lignosulfonate and other breakdown components of wood. The crude spent liquor is steam-stripped to remove excess sulfur dioxide and volatile substances. Sugars from hemicellulose may be removed by fermentation followed by distillation of ethanol. Evaporation removes excess water.
Composition	Variable commercial formulations exist. Composition is dependent on the wood used in the pulping process, specific processing conditions, clean-up processes and additional chemical treatments carried out to create materials with specific functionalities. Commercial products are likely to contain various sugars and sulfur (sometimes present as sulfates) as impurities. Calcium Lignosulfonate Liquid is reported to contain approximately 50% calcium lignosulfonate. One food-grade product, Calcium lignosulfonate (40-65) (a powdered product), is specified as follows: weight-average molecular weight between 40 000 and 65 000 Da; molecular weight of > 90% is between 1000 and 250 000 Da; ≤ 5.0% calcium; degree of sulfonation 0.3–0.7; ≤ 5.0% reducing sugars; ≤ 0.5% sulfite; ≤ 14.0% total ash; ≤ 8.0% moisture; pH 2.7–3.3 (10% solution).
Uses	Used as a plasticizer/dispersant in concrete (about 45% usage; weight-average molecular weight of the typical product shipped as a previous cargo is reported in the range of 30 000–40 000 Da); also used in the production of cement and plasterboard, in petroleum drilling, as a dispersant for the application of pesticides, an emulsifier in asphalt, a deflocculant in processing feedstuffs, and minor use as a food additive (as a carrier for fat-soluble vitamins, carotenoids and other functional ingredients).
Analytical methods	None found for previous cargoes. Possible means of determination in fats and oils using inductively coupled plasma (ICP) analysis for Ca residues.
Potential reaction(s) with a subsequent cargo of fat or oil	Lignosulfonates are unlikely to react with free fatty acids and triglycerides present in cargoes of fats and oils under the conditions of transport.

5.3 Toxicological studies

The Committee considered the potential for acute toxicity of calcium lignosulfonate (molecular weight not specified) to be low based on oral LD_{50} values of 31.6 g/kg bw in young albino Sprague-Dawley rats (sex not specified) and between 10 and 20 g/kg bw in male rats (strain not identified), reported in two independent studies (cited in EFSA, 2010 and EFSA, 2011b).

The Committee considered its own previous evaluation of calcium lignosulfonate (40-65) (Annex 1, reference *190*) and the data reviewed therein to conduct its toxicological evaluation. At that time, the Committee concluded that calcium lignosulfonate (40-65) was not genotoxic based on negative responses reported in a bacterial reverse mutation assay and an in vitro chromosome aberration assay (Thiel, Köhl & Sokolowski, 2005; Thiel et al., 2006a) (Annex 1, reference *190*). The Committee also concluded that calcium lignosulfonate (40-65) did not exhibit a potential for reproductive and developmental toxicity because no treatment-related or toxicologically relevant effects were observed at any dose in a maternal and developmental toxicity study in female Wistar rats (Thiel et al., 2006b) (Annex 1, reference *190*). The Committee noted a dose-related increase in the incidence of histiocytosis in the mesenteric lymph nodes in a 90-day feeding study of male and female Wistar rats administered calcium lignosulfonate (40-65) at doses of 0, 500, 1000 or 2000 mg/kg bw per day (Thiel, Kohl & Braun, 2007). However, the Committee did not consider this observation to be adverse as this finding has been reported with other substances of high molecular weights, such as polypentosan sulfate, copovidone (a copolymer of vinylpyrrolidone and vinyl acetate) and mineral oils (Carlton et al., 2001; Elmore, 2006). Furthermore, the Committee considered that long-term rat studies with such high molecular weight substances did not demonstrate any progression of histiocytosis to an adverse effect or carcinogenesis (Carlton et al., 2001; Shoda et al., 1997; Trimmer et al., 2004). The Committee identified the highest dose tested of 2000 mg/kg bw per day as the NOAEL and established an ADI of 0–20 mg/kg bw for calcium lignosulfonate (40-65) by application of an uncertainty factor of 100 (Annex 1, reference *191*).

More recently, a report detailing additional clinical pathological examinations and a reassessment of the results of the 90-day rat feeding study of calcium lignosulfonate (40-65) was published, which supported the Committee's earlier evaluation of this study (Annex 1, reference *191*) (Thiel, Kohl & Braun, 2007; Thiel et al., 2013).

The Committee determined that calcium lignosulfonate (40-65) did not cause skin sensitization based on the absence of treatment-related effects in a lymph node assay in CBA mice (Schwartz, Spencer & Welsh, 1984) (Annex 1, reference *190*).

More recently, the CONTAM Panel reviewed additional genetic toxicity data (not available to the present Committee) provided for a LMWF (< 1000 Daltons) isolated from a technical product that was described as a representative of grades that were intended to be shipped as previous cargo (EFSA, 2019). The two new genetic toxicity studies included a bacterial reverse mutation assay in *Salmonella* Typhimurium strains TA98, TA100, TA102, TA1535 and TA1537 in the presence and absence of metabolic activation, and a micronucleus assay conducted with human lymphocytes in the presence and absence of metabolic activation (cited in EFSA, 2019). The CONTAM Panel concluded that the test substance showed negative responses in the genetic toxicity studies, but stated that the molecular weight distribution data on the test substance indicated an apparent loss of constituents of lower molecular masses (below 200 Daltons) (cited in EFSA, 2019). Therefore, the CONTAM Panel concluded that the test substance was not sufficiently representative of the different molecular fractions of calcium lignosulfonate shipped as a previous cargo (EFSA, 2019).

The Committee noted that there are no other toxicity data or data in humans available on different molecular weight fractions constituting the non-food-grade calcium lignosulfonate that is shipped as a previous cargo. Therefore, the Committee could not conduct a toxicological evaluation of the non-food-grade calcium lignosulfonate that is shipped as a previous cargo.

5.4 Allergenicity

There are no reports of allergenicity upon oral exposure to calcium lignosulfonate that would indicate that the substance that is shipped as a previous cargo is, or contains, a known food allergen.

5.5 Assessment of dietary exposure

A dietary exposure assessment conducted at a previous meeting of the Committee estimated that the maximum potential dietary exposure to calcium lignosulfonate (40-65) could reach 7 mg/kg bw per day (Annex 1, reference *190*). The present Committee conducted a dietary exposure assessment based on data from Australia and New Zealand. The mean estimated exposure to calcium lignosulfonate (40-65) as a permitted food additive ranged from 1 to 4 mg/kg bw per day. The estimated exposure for high consumers at the 90th percentile of exposure ranged from 2 to 6 mg/kg bw per day. There were no data on dietary exposure to calcium lignosulfonate added to animal feed or used as a dispersion agent and stabilizer in pesticides for preharvest or postharvest applications. The generic human dietary exposure value for previous cargoes of 0.3 mg/kg bw per

day indicates that any potential dietary exposure to calcium lignosulfonate (40-65) from previous cargoes in food oils would be a minor contributor to its overall dietary exposure.

The Committee could not perform a dietary exposure assessment for the non-food-grade calcium lignosulfonate due to unavailability of the relevant data.

6. Evaluation

The Committee previously established an ADI of 0–20 mg/kg bw for the food-grade calcium lignosulfonate (40-65) (Annex 1, reference *190*) (SCF, 2003), the upper bound of which is above the estimated exposure for calcium lignosulfonate as a previous cargo for edible fats and oils of 0.3 mg/kg bw per day. There are no data on allergenicity resulting from oral exposure to calcium lignosulfonate (40-65) that would indicate that it is, or it contains, a known food allergen. Therefore, food-grade calcium lignosulfonate (40-65) meets the criteria for acceptability as a previous cargo for edible fats and oils.

Lignosulfonates are unlikely to react with free fatty acids and triglycerides present in cargoes of fats and oils under the conditions of transport.

The Committee could not determine the specific chemical composition or molecular weight distribution of the non-food-grade calcium lignosulfonate that is shipped as a previous cargo but recognized that it has a wide molecular weight distribution. The Committee acknowledged that no toxicokinetic data to determine oral bioavailability of or systemic exposure to the non-food-grade calcium lignosulfonate shipped as a previous cargo are available. Therefore, the ADI for calcium lignosulfonate (40-65) does not apply to the material that is shipped as a previous cargo unless it is food-grade calcium lignosulfonate. In the absence of adequate data on chemical specifications and toxicokinetics, the Committee concluded that the systemic effects of oral exposure to the non-food-grade calcium lignosulfonate cannot be evaluated as no oral toxicity, genotoxicity or allergenicity data are available on this substance. Therefore, in the absence of relevant toxicological data on test substances that are sufficiently representative of different molecular weight fractions constituting the non-food-grade calcium lignosulfonate that is shipped as a previous cargo, the Committee concluded that the non-food-grade calcium lignosulfonate does not meet the criteria for acceptability as a previous cargo for edible fats and oils.

6.1 Recommendations

The Committee recommended that sufficient chemical and toxicological information that allows the evaluation of non-food-grade calcium lignosulfonate liquid as shipped should be made available prior to the next evaluation. At a minimum this information should address the following:

- molecular weight range(s), chemical component identification and relative composition;
- toxicological data on representative products.

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Trichothecene toxins T-2 and HT-2 (addendum)

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1. Explanation

T-2 toxin (T-2) and HT-2 toxin (HT-2) are type A trichothecene mycotoxins, which are closely-related epoxy sesquiterpenoids. Surveys have revealed the presence of T-2 and HT-2 on a wide range of foodstuffs but they are primarily

contaminants of cereals and cereal-based products. T-2 and HT-2 have been reported to be produced by *Fusarium acuminatum*, *F. equiseti*, *F. langsethiae*, *F. poae*, *F. sibiricum* and *F. sporotrichioides*. *F. langsethiae* was first described in 2004 with a distribution largely restricted to Europe. This species has since been identified as the main producer of T-2 and HT-2 in Europe. The ecology of the species has not been elucidated and it may occur as an endophyte or a weak pathogen on cereals, with little or no disease expression.

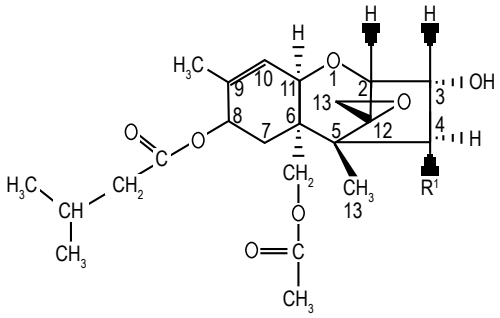
T-2 is the trivial name for 4β,15-diacetoxy-3α-hydroxy-8α-[3-methylbutyryloxy]-12,13-epoxytrichothec-9-ene (CAS no. 21259-20-1). Corresponding to the molecular formula $C_{24}H_{34}O_9$, its relative molecular mass is 466.5 g/mol. HT-2 is the trivial name for 15-acetoxy-3α,4β-dihydroxy-8α-[3-methylbutyryloxy]-12,13-epoxytrichothec-9-ene (CAS no. 21259-20-1). Its molecular formula is $C_{22}H_{32}O_8$ and the relative molecular mass is 424.5 g/mol. The structures of T-2 and HT-2 differ only in the functional group at the C4 position (Fig. 1). HT-2 is formed from the de-acetylation of T-2 which can occur as a result of metabolism of the fungus, the infected plant or animals after ingestion. These toxins co-occur with several other type A trichothecenes (for example, 4,15-diacetoxyscirpenol and neosolaniol) and modified mycotoxins – phase I and II metabolites formed in the fungus or the infected plant (for example, T-2 triol and T-2-3-glucoside).

T-2 and HT-2 were evaluated by the Committee once previously at the fifty-sixth meeting (Annex 1, references *152* and *153*). At that meeting the Committee noted that there was substantial evidence for the immunotoxicity and haematotoxicity of T-2 in several species, and that these are critical effects after short-term intake. The Committee concluded that the safety of food contaminated with T-2 could be evaluated from the lowest-observed-effect level (LOEL) of 0.029 mg/kg bw per day for changes in white and red blood cell counts identified in the 3-week dietary study in pigs. The Committee used this LOEL and a safety factor of 500 to derive a provisional maximum tolerable daily intake (PMTDI) for T-2 of 60 ng/kg bw per day. The Committee further concluded that the toxic effects of T-2 and its metabolite HT-2 could not be differentiated, and hence HT-2 was included in the PMTDI, resulting in a group PMTDI of 60 ng/kg bw per day for combined concentration of T-2 and HT-2.

T-2 and HT-2 were evaluated by the present Committee in response to a request from the Codex Committee on Contaminants in Foods (CCCF) for an updated risk assessment, including an exposure assessment. The Committee evaluated data that had become available since the last evaluation in 2000. This addendum includes the following sections:

- Transfer from feed to food
- Analytical methods

Fig. 1
Structure of type A trichothecenes HT-2 (R1=OH) and T-2 (R1=OAc)



- Sampling protocols
- Effects of processing
- Prevention and control
- Levels and patterns of contamination in food commodities
- Food consumption and dietary exposure assessment.

The toxicological evaluation and overall risk assessment were carried out at a later meeting.

2. Biological data

2.1 Transfer from feed to food

T-2 is rapidly metabolized and eliminated in different animal species (Li YS et al., 2011). In vivo studies demonstrated that the main metabolites are T-2 triol and HT-2 (Li YS et al., 2011) and the most recent analysis identified 18 metabolites of T-2 after metabolism in liver microsomes of various species (Yang SP et al., 2017). Levels of T-2 and HT-2 detected in liver and muscle of chickens given feed contaminated with T-2 and HT-2 (approximately 4 mg/kg) were less than 3 and 0.3 µg/kg respectively for HT-2 and less than 0.05 µg/kg in both tissues for T-2 (Yang LC et al., 2020). Analysis of retail pork and chicken meat samples in China identified T-2 at concentrations below 0.5 µg/kg (Zou et al., 2012b). The Committee's previous assessment of T-2 and HT-2 at its fifty-sixth meeting (Annex 1, references *152* and *153*) identified the potential for transfer via elimination into eggs and milk with conversion rates of less than 1%, but no

subsequent studies were identified in the literature search. T-2 and HT-2 have not been detected in milk or milk products in the limited studies conducted (Jolankai et al., 2008; Flores-Flores & González-Peñas, 2018a; Flores-Flores & González-Peñas, 2018b). These results are in agreement with the GEMS/food contaminants database dataset, where T-2 and HT-2 were either not detected or detected at low concentrations in meat and dairy products (section 7). Based on the known rapid metabolism and elimination of T-2 (Li YS et al., 2011) and the low levels detected in animal products, the transfer from feed to food is considered to be negligible with regard to human dietary exposure to T-2 and HT-2.

3. Analytical methods

3.1 Chemistry

T-2 (4β,15-diacetoxy-3α-hydroxy-8α-(3-methylbutyryloxy)-12,13-epoxytrichothec-9-ene) is a type A trichothecene. HT-2 has a similar chemical structure but the acetoxy group at position C4 is replaced with a hydroxyl group. Trichothecenes are characterized by the 12,13-epoxy-trichothec-9-ene (scirpene) ring system, in which the type A and B groups are characterized, respectively, by the absence or presence of a carbonyl function at C8. Type A trichothecenes do not exhibit a UV maximum above 200 nm, unlike the type B trichothecenes, which possess a conjugated keto group at C8. Similar to the type B trichothecenes, naturally occurring glucosides of these toxins have been detected (see section 3.2.4). Some information on the chemistry of T-2 and HT-2 was summarized by the Committee at its fifty-sixth meeting (Annex 1, references *152* and *153*).

3.2 Description of analytical methods

3.2.1 Introduction

The Committee summarized analytical methods for T-2 and HT-2 at its fifty-sixth meeting (Annex 1, references *152* and *153*). Details were given on thin-layer chromatography (TLC), immunochemical, gas chromatography (GC) and high-performance liquid chromatography (HPLC) methods. Extractions were generally performed with aqueous methanol or acetonitrile, followed by suitable multifunctional column or solid phase extraction (SPE) clean-up protocols. In these analyses, derivatization is required to increase volatility (GC) or to allow quantification by spectrophotometric detectors (HPLC). Since the previous review, interest in TLC and GC methods has waned, whereas HPLC coupled

to mass spectrometry (MS) has become the method of choice for all mycotoxin analyses.

The following sections highlight advances in the determination of T-2 and HT-2 since the fifty-sixth meeting of the Committee. Scopus and PubMed were searched for papers published after 2001 using the search terms "T-2 toxin" OR "T2 toxin" AND "determination", resulting in 297 and 727 hits from the two databases, respectively. Additional information has largely been drawn from the annual summaries of advances in mycotoxin analytical methods previously published as general referee reports of *AOAC International* and subsequently by the *World mycotoxin journal*. Recently published reviews have covered advances in chromatographic and immunological methods for T-2 and HT-2 (Meneely et al., 2011a; Krska et al., 2014).

3.2.2 Screening tests

Although TLC has largely been superseded by more modern methods, reports of its use for T-2 can still be found in the literature (Anaya et al., 2004). Commercial enzyme-linked immunosorbent assays (ELISAs) are available for rapid screening for T-2 or T-2+HT-2 combined. These methods generally have limits of detection (LODs) of 20 μg/kg or lower and have usually been validated for cereals and animal feeds. Generally, ELISA tests are subject to matrix effects and can only be reliably used for those matrices for which they have been validated. Lateral flow immunoassays (strip tests) are also commercially available for rapid screening purposes. A fluorescence polarization immunoassay for both toxins has been developed (Porricelli et al., 2016), as well as one including their glucosylated conjugates expressed as a total sum (Lippolis et al., 2019). Comparisons of some rapid test kits have been published (Meneely et al., 2011b; Aamot et al., 2013). Screening tests are useful for situations requiring a rapid response and in laboratories with limited instrumental capabilities.

Use of some in-house screening tests has been reported, including ELISA (Yoshizawa et al., 2004) and electrochemical immunosensors (Wang et al., 2018), but again care should be taken to validate and exclude possible matrix effects. Reviews of screening methods involving biosensors, chemosensors and electrochemical methods have been published (Krska et al., 2014; Lin & Guo, 2016).

3.2.3 Quantitative methods

Although analytical methods exclusively targeted for T-2 and HT-2 have been described, multimycotoxin methods have increasingly come to the fore since the fifty-sixth meeting of JECFA. The major advance in chemical analysis over the past three decades has been the development of HPLC coupled to mass

spectrometry (HPLC-MS) via techniques such as electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI). These advances have opened up true multi-analyte methods for mycotoxins, whereby T-2 and HT-2 can be determined as part of a full suite of mycotoxins and/or secondary metabolites. In general, recent methods achieve LODs in the low or sub-µg/kg range. As a quality control parameter for the following methods, a certified reference material has been developed in ground oat flakes (Koppen et al., 2015). More recently, a multimycotoxin maize reference material, containing incurred residues of T-2 and HT-2 together with aflatoxins, fumonisins, deoxynivalenol, ochratoxin A and zearalenone, for use with the multimycotoxin HPLC-MS/MS methods has been developed by the US National Institute of Standards and Technology and US Food and Drug Administration (Phillips et al., 2019).

3.2.3.1 GC

The frequent co-occurrence of types A and B trichothecenes in food matrices makes combined analysis of these toxins advantageous. Determination of T-2 and HT-2 together with other type A and B trichothecenes has been reported with GC-MS using silylation (Melchert & Pabel, 2004). Alternatively, GC methods with flame-ionization detection (Eke et al., 2004) or electron capture detection (ECD) (Majerus et al., 2008; Kong et al., 2012; Amelin et al., 2013) have been described. Type A trichothecenes were determined without extract clean-up by two-dimensional GC of trifluoroacetic anhydride derivatives with time-of-flight (TOF) mass spectrometric detection (Jelen & Wasowicz, 2008). A method for determination of a mixture of types A and B trichothecenes in chicken liver has been developed using GC-MS/MS of trimethylsilyl derivatives (Mahmoud et al., 2018).

3.2.3.2 HPLC with spectrophotometric detection

Co-occurrence of type A and B trichothecenes has been determined by HPLC using coumarin-3-carbonyl chloride precolumn derivatization for fluorometric detection (Dall'Asta et al., 2004a). Lippolis et al. (2008) investigated a number of fluorescence labelling reagents for HPLC analysis, including coumarin-3-carbonyl chloride, 1-naphthoyl chloride, 2-naphthoyl chloride and pyrene-1-carbonyl cyanide. The use of 1-anthroylnitrile as a derivatizing agent for fluorometric detection after immunoaffinity column (IAC) clean-up and HPLC separation has also been described (Trebstein et al., 2008), whereas Pascale et al. (2012) developed an ultra-high-performance liquid chromatography (UHPLC) method with diode array detection (DAD) at 202 nm for T-2 and HT-2 in cereals with a LOD of 8 µg/kg. Soleimany et al. (2011) have determined as many as 12 mycotoxins in cereals using HPLC with postcolumn derivatization and both

photodiode array (PDA) and fluorescence detection. Clean-up of extracts was by multifunctional IAC.

3.2.3.3 HPLC with mass spectrometric detection

As mentioned above, the major advance in the determination of mycotoxins has been the development of MS detection or tandem mass spectrometric (MS/MS) detection coupled to HPLC. Although the vast majority of the methods described below have been developed using single or triple quadrupole mass analysers, the occasional paper has described the use of ion trap mass spectrometry (Bryla et al., 2014) or high-resolution mass spectrometry (HRMS, see section 3.2.3.3.5). Analysis of the results of a proficiency test among 41 laboratories using HPLC-MS/MS gave insight into the factors to consider when using these multimycotoxin methods (De Girolamo et al., 2013). A recent collaborative study critically evaluated a range of these new techniques applied to "regulated" mycotoxins in cereals and feeds (Solfrizzo et al., 2018).

3.2.3.3.1 Extraction

HPLC-MS/MS reaches its true potential when applied to a suite of mycotoxins; however, efficient extraction requires striking a balance between the individual chemistry and solubility across the range of mycotoxins being considered. Satisfactory extraction efficiency may sometimes be achieved by multiple extraction steps tailored to the different analytes of interest (Lattanzio et al., 2014; Habler et al., 2017; Ciasca et al., 2018). When analysing cereals or cereal-based food or feed for only type A and type B trichothecenes, conventional extraction solvents of aqueous methanol or acetonitrile can be used. Additionally, Breidbach (2017) proposed use of ethyl acetate/water with sonication and addition of sodium sulfate. Modifications to the conventional extraction mixtures enable analysis of numerous fungal metabolites: these include use of acetic acid/aqueous acetonitrile (Sulyok et al., 2006; Soleimany et al., 2012a,b); ethyl acetate/formic acid (Monbaliu et al., 2009); and aqueous methanol/ethyl acetate (Ediage et al., 2011). A microwave-assisted extraction process has been evaluated as well (Du et al., 2018). There is also a report on the use of accelerated solvent extraction of wheat, barley and oats with acetonitrile/water (90:10) (Kokkonen & Jestoi, 2009).

3.2.3.3.2 Extract purification

Extract purification for the determination of T-2 and HT-2 alone or in combination with other type A and B trichothecenes continues to be performed by SPE (Sorensen & Elbaek, 2005; Rudrabhatla & Wood, 2007) or commercial multifunctional columns (Berthiller et al., 2005; Biancardi et al., 2005; Biselli et

al., 2005; Ren et al., 2007). Specific antibodies against T-2 and HT-2 have been incorporated into IACs for extract purification (Visconti et al., 2005; Trebstein et al., 2008; Pascale et al., 2012). Several methods have been developed using a multifunctional IAC (Lattanzio et al., 2007; Soleimany et al., 2011; Takino et al., 2011; Tang et al., 2013; Vaclavikova et al., 2013), whereas other authors have described multiple SPE steps such as aminopropyl, octadecyl and ion exchange (Monbaliu et al., 2009), polymeric resin (Yang et al., 2015) or both polymeric and multifunctional columns (Tamura et al., 2012).

The development of multimycotoxin analytical methods has led to two different approaches towards extract purification for analytes with differing chemistries. The first is the use of a "dilute-and-shoot" method, in which the extract is merely diluted with appropriate solvent (e.g. HPLC mobile phase) and directly injected into the HPLC (Sulyok et al., 2006; Berthiller et al., 2007; Santini et al., 2009; Soleimany et al., 2012a,b). The second is the development of QuEChERS (quick, easy, cheap, effective, rugged and safe) to remove interfering substances (Monbaliu et al., 2010; Sospedra et al., 2010; Zachariasova et al., 2010). This is a relatively fast method involving salting out with buffer salt such as magnesium sulfate or citrate buffer followed by dispersive SPE (dSPE) with magnesium sulfate, primary/secondary amine sorbent, octadecyl sorbent and neutral alumina. Depending on the matrix being analysed, factors such as the percentage of acetic or formic acid in the extractant, ratio of water to organic solvent and exact composition of the sorbent mixture used for dSPE may need to be optimized (Jettanajit & Nhujak, 2016). Besides these, polymeric resin SPE has been used for the more restricted set of "regulated" mycotoxins (Wang et al., 2013).

3.2.3.3.3 HPLC separation

Standard gradient reversed-phase HPLC is used to separate the individual mycotoxins of interest. In some cases, sodium chloride has been added to the mobile phase to optimize the detection of the type A trichothecenes as sodium adducts (Dall'Asta et al., 2004b). UHPLC has also been increasingly used; the advantage of this system lies in the improved chromatographic efficiency and decreased retention times (Beltran et al., 2009; Frenich et al., 2009; Zachariasova et al., 2010; Pascale et al., 2012; Yang et al., 2015).

De Boevre et al. (2018) have successfully applied ultra-high-performance supercritical fluid chromatography coupled to MS/MS for a multimycotoxin method including T-2, HT-2 and T-2-3-glucoside in beer.

3.2.3.3.4 Ionization techniques

ESI is the standard method for converting soluble analytes into gas phase ions entering the MS, although the use of both APCI (Santini et al., 2009) and atmospheric pressure photo-ionization (Tanaka et al., 2010) for determination of trichothecenes have been described. The determination of type A trichothecenes as sodium, protonated or ammonium adducts requires positive ESI, whereas the type B trichothecenes have also been determined by negative ESI (Sorensen & Elbaek, 2005; Berthiller et al., 2007). In the multimycotoxin analyses, both polarities are generally required, which can be achieved by separate chromatographic runs (Sulyok et al., 2006; Ren et al., 2007) or fast switching of polarity during a single chromatographic run (Berthiller et al., 2007; Beltran et al., 2009; McElhinney et al., 2015).

3.2.3.3.5 High-resolution MS

A few authors have described the application of more advanced HRMS, such as TOF (Jelen & Wasowicz, 2008) and advanced ion trap instruments (Zachariasova et al., 2010). However, these instruments are not generally available in laboratories for routine analysis.

3.2.3.3.6 Quantification

Two techniques have been used to overcome matrix effects in the analysis of mycotoxins. The first uses stable isotope-labelled internal standards (Haubl et al., 2007; Zachariasova et al., 2010; Habler & Rychlik, 2016); the second uses matrix-matched standards (Sulyok et al., 2006; Frenich et al., 2009; Kokkonen & Jestoi, 2009; Rasmussen et al., 2010). Apart from these, the standard addition method has also been used (Vaclavik et al., 2013). Single-level standard addition is a practical solution where isotope-labelled internal standards are not available (Fabregat-Cabello et al., 2016).

3.2.4 Modified forms of T-2 and HT-2

The formation of glucoside conjugates of T-2 and HT-2 by *Fusarium sporotrichioides* in both solid (oats and wheat) and liquid cultures was demonstrated by Busman et al. (2011). Subsequently, the application of HRMS in the analysis of maize powder reference material revealed that monoglucosides of both toxins and a di-glucoside of HT-2 occur naturally, based on accurate mass and mass fragmentation profiles (Nakagawa et al., 2012, 2013). Similarly, HRMS has been used to confirm the natural occurrence of mono-conjugated forms in barley, wheat and oats, and HT-2-di-glucoside in barley (Veprikova et al., 2012). A method for the determination of T-2 and HT-2 and their glucosylated and acetylated derivatives (T2-3-glucoside, 3-acetyl-T-2 and 3-acetyl-HT-2) in barley,

maize, oats, rye and wheat has also been published (Nakagawa et al., 2018). A monoclonal antibody for T-2 and its 3-glucoside, which has low cross-reactivity with HT-2, has been developed (Maragos et al., 2013), and Lippolis et al. (2019) have published a fluorescence polarization immunoassay for measuring the sum of T-2, HT-2 and their monoglucosides combined. The routine determination of the glucosides has been hampered by the absence of commercial standards.

Other modified forms of these toxins, which have been shown to occur naturally, are T-2 triol (deacetyl-HT-2) and T-2 tetraol (devaleryl-deacetyl-T-2). Apart from these hydrolysed forms and the glucosides of these toxins, malonyl, feruloyl and acetyl derivatives have been reported during studies on in planta biotransformation in wheat (Nathanail et al., 2015).

4. Sampling protocols

Sampling plays an important role in the determination of mycotoxins in food and feed. T-2 and HT-2, like other mycotoxins, are heterogeneously distributed in contaminated raw materials, especially in whole kernels (or nuts), making it difficult to carry out accurate analysis in food. Currently, sampling methods for T-2 and HT-2 detection in grains and cereals usually refer to sampling protocols for other mycotoxins. General sampling guidelines for mycotoxins, including official and non-official methods such as those of the European Commission (EC), Codex Alimentarius Commission (CAC) and International Agency for Research on Cancer (IARC), have been developed. These protocols outline the sampling process, sample size, sample handling, frequency of sampling and other relevant details.

4.1 Official methods

4.1.1 Codex Alimentarius Commission

General guidelines on sampling (CAC/GL 50-2004) are designed to ensure that fair and valid sampling procedures are used when food is being tested for compliance with a particular Codex commodity standard. CAC/GL 50-2004 enumerates the essential points that the Codex commodity committees, governments and other users should address for the selection of appropriate sampling plans when setting up specifications as follows: existence (or not) of international reference documents, nature of the control, nature of the characteristic to control, choice of the quality level, nature of the lot and composition of the sample. Appropriate sampling plans for T-2 and HT-2 should be designed according to the systematic

approach for the selection of a sampling plan for quantitative analysis of characteristics in CAC/GL 50-2004. Different sampling plans should be designed for inspection of isolated lots of bulk samples and a continuous series of lots. For example, the selection of sampling plans for the inspection by variables of bulk materials involves the following: the establishment of standard deviations, producer's risk, consumer's risk and cost of implementation.

4.1.2 European Commission

The European Commission issued EC401/2006 with recommendations outlining some specifications regarding the sampling of foodstuffs for mycotoxin determination. Sampling methods for aflatoxin B_1, total aflatoxins, aflatoxin M_1, ochratoxin A, *Fusarium* toxins and patulin are detailed for different commodities. In each method, the combined total of all the incremental samples taken from the lot or sub-lot constitutes the aggregate sample, which should be considered representative of the lots. The regulation indicates that the number of incremental samples to be taken depends on the weight of the bulk products (European Commission, 2006). EC401/2006 is also applied to the detection of T-2 and HT-2 in food (Pettersson et al., 2011).

4.1.3 International Agency for Research on Cancer

There is no sampling plan for the determination of T-2 and HT-2 in the IARC scientific publication No. 158 (IARC, 2012). IARC recommended that the sampling plans outlined for deoxynivalenol (DON) could be applied to T-2 and HT-2. Sample variance studies and sampling plans have been reported for DON, emphasizing the importance of sample selection, sample size and the number of incremental samples. It was found that the low variance associated with the sampling step is partly due to the high kernel count of wheat, which is about 10 times that for shelled maize, and 30 times that for shelled groundnuts. The sampling plans of T-2 and HT-2 proposed for surveillance purposes are based on currently available information (Table 1). The values shown are minimum sample sizes and minimum numbers of incremental samples for DON, T-2 and HT-2.

4.1.4 Sampling methods for T-2 and HT-2 detection in China

No specific sampling method has been issued for the analysis of T-2 and HT-2 in foods in China. However, GB/T 30642-2014 "General guidelines for food sampling inspection" issued by the National Standards Committee of China in 2014 could be used for T-2 and HT-2 sampling. From farm to table, the sampling parameters, including method and amount of sample, differ according to food types. The sampling protocols for aflatoxin B_1, ochratoxin, zearalenone (ZEA) and DON have been listed in the implementation rules of national food

Table 1
Summary of minimum sample sizes suggested for interpretation of surveillance data for DON, T-2 and HT-2

Commodity	Increments (n×y grams)	Minimum sample size (kg)
Maize		
Whole maize	20×100	2.0
Maize on the cob	20 cobs	3.0
Maize grits	10×50	0.5
Processed maize foods	10×50	0.5
Wheat	20×50	1.0
Barley	20×50	1.0
Oats	20×50	1.0
Rye	20×50	1.0
Flour, meal and bran of all origins	10×50	0.5
Bread	10×50	0.5

safety supervision and sampling inspection (State Administration for Market Regulation, 2020). When wheat flour is sampled during the production process, at retail and from restaurants, the rules require the corresponding quantity which is taken from different parts of the same batch product in the storage room, goods shelves and kitchen. The sample quantity should be more than two independent packages, and the total amount should be more than 3 kg. When wheat flour is sampled from unpackaged products, it should be taken from different parts of the product and the minimum total weight should be 3 kg.

4.1.5 United States Grain Standards Act (USGSA)

In the USA, sampling methods for grain inspection are clearly described in the *Grain inspection handbook – Book I sampling* (USDA, 1995), and can be used for T-2 and HT-2 determination, although this is not explicitly specified.

Three sampling methods are described: probe or trier sampling, sampling with a diverter-type sampler, and Ellis cup and pelican sampling. Details of sampling equipment, sampling procedure and sampling patterns are provided in the handbook.

4.1.6 Canadian Grain Commission (CGC)

CGC sampling directives are found in the *Sampling systems handbook and approval guide* (Canadian Grain Commission, 2015). This handbook outlines the CGC's policies and procedures for automatic mechanical sampling systems used at licensed grain handling facilities. It includes information on CGC-approved methods of sampling for the Accredited Container Sampling Program (ACSP)

and the Certified Container Sampling Program (CCSP). A licensed grain-handling facility must install CGC-approved automatic sampling equipment. A section on manual sampling is also included, which may be used at the discretion of the CGC when a lot of grain cannot be sampled by mechanical means. A guide detailing procedures for collecting a representative sample on farm and at central stores has also been published by CGC (Canadian Grain Commission, 2019).

4.2 Non-official sampling methods

In addition to the official sampling methods, some non-official ones have been designed and used. Whitaker et al. (2010) published a manual that provides sampling plans to detect mycotoxins in food. The manual also includes information on sampling protocols and the consequences of a poorly designed sampling plan for the reliability of the mycotoxin levels measured. Additional details, including sample selection, preparation for sampling, accept/reject limits, random variation, test variability reduction and procedures for mycotoxin sampling plan design were also discussed. This manual should be consulted in conjunction with the relevant legislation or guidelines on sampling for mycotoxins.

4.3 Mycotoxin sampling tool and training videos

A mycotoxin sampling tool was developed by the FAO (FAO/WHO, 2013). The sampling tool provides information required to support the analysis of the performance of sampling plans, including the sample size, the number of laboratory samples, test portion preparation, and accept/reject limits. The tool outlines sampling procedures for 26 mycotoxin–commodity combinations and determines the most appropriate mycotoxin sampling plan to minimize the risk of misclassifying lots. Two training videos have been produced to help in the implementation of appropriate sampling protocols (Brera et al., 2007; ISS, 2015).

5. Effects of processing

Various industrial processes common to food and feed (including sorting, cleaning, milling, brewing, cooking, baking and frying) can affect the concentration and fate of T-2 and HT-2 (EFSA, 2011). Owing to their stability, as with other mycotoxins, most processes do not degrade T-2 and HT-2 thoroughly, but can reduce or redistribute them.

5.1 Sorting and cleaning

Sorting and cleaning are the primary processes that can decrease the contamination of commodities with T-2 and HT-2 (Tibola et al., 2016). Cleaning and de-hulling reduce T-2 and HT-2 levels in oats by 75–99% (Scudamore et al., 2007, Pettersson et al., 2008, 2011). Schwake-Anduschus et al. (2010) also reported that de-hulling reduced the concentration of T-2 and HT-2 by over 90%, but no reduction resulted from cleaning. T-2 and HT-2 were concentrated (up to 6000 µg/kg T-2 and 20 000 µg/kg HT-2) in the residual by-product after the de-hulling process (Scudamore et al., 2007). Therefore, the use of the residual by-product should be carefully controlled.

5.2 Milling

Milling redistributes T-2 and HT-2 into different cereal fractions (Lancova et al. 2008; Pascale et al., 2011; Aprodu & Banu, 2014; Magyar et al., 2019). Following milling of 22 consignments of wheat in large commercial mills, T-2 and HT-2 were frequently detected in germ and bran samples but rarely detected in wheat and flour samples (Scudamore et al., 2009). Schollenberger et al. (2008) determined the content of 13 type A and B trichothecenes, including T-2 and HT-2, in products processed from raw maize by dry milling. Toxins were either not detected or detected at low concentrations in raw and tempered maize, grits and two types of flour, whereas markedly higher concentrations were detected in screenings, bran, germ and germ meal.

5.3 Fermentation

T-2 and HT-2 contamination can be reduced during fermentation, with reductions dependent on pH, moisture and fermentation organisms. The fates of fumonisin B1, DON, T-2 and ZEA were investigated during production of traditional Nigerian fermented bean products and sorghum beer using artificially contaminated raw materials (Chilaka et al., 2018). The reduction of mycotoxins for each process ranged from 57 to 100%. Kawtharani et al. (2020) reported that the addition of *Geotrichum candidum* reduced the proliferation of *Fusarium* spp. and the production of T-2 during the brewing process, with a 90% reduction of T-2 in 168 hours.

5.4 Cooking, roasting, frying and extruding

Cooking, baking or frying at > 150 °C, reduced T-2 and HT-2 contamination, but at lower temperatures, T-2 and HT-2 remained stable (Cazzaniga et al., 2001; Pascaria et al., 2020). Schmidt et al. (2017) systematically investigated the fate of T-2 and HT-2 during both laboratory and industrial-scale extrusion cooking of oats at 150 °C. Under laboratory conditions contamination was reduced approximately 60% and 50% for T-2 and HT-2, respectively. Moisture content, mechanical shear and temperature strongly impacted toxin reduction. In a recent study, T-2 was generally degraded at a higher rate than HT-2 by baking and roasting at temperatures above 200 °C (Kuchenbuch et al., 2018). However, Dropa and co-workers (2014) reported that the decrease of T-2 was in the range of 30–50%, while HT-2 increased by up to 60% during dough preparation and baking. It was assumed that transformation (deacetylation) of T-2 into HT-2 occurred during the baking process. Extrusion altered the distribution of toxins as they became homogenized within the matrix while high-temperature extrusion (> 200 °C) also reduced T-2 and HT-2 contamination (Schmidt et al., 2018).

5.5 Alkali treatment

Alkali treatment may be applied to cereal grains used as feeds for ruminant livestock (EFSA, 2011). Trichothecenes containing an ester group are hydrolysed to their respective parent alcohols when treated with alkali, and treatment with a dilute sodium hydroxide or ammonium hydroxide solution has been shown to hydrolyse T-2 to T-2 tetraol. The application of alkali to cereal grains may reduce the contamination of T-2 and HT-2 (Oldham et al., 1980). No studies on the impact of nixtamalization (alkali treatment of maize during tortilla production) on T-2 and HT-2 were identified.

6. Prevention and control

6.1 Preharvest control

Few studies have been published on the mycotoxigenic fungi responsible for T-2 and HT-2 contamination of food and feed. The principal species responsible are thought to be *Fusarium langsethiae* and *F. sporotrichioides* (Edwards et al., 2009, 2012; Divon et al., 2011). *F. langsethiae* was first (Langseth & Rundberget, 1999) described as a new species in 2004 (Torp & Nirenberg, 2004) and the species' known distribution is currently limited to Europe and Siberia. *F. langsethiae* has

been shown to occur on small grain cereals and levels in oats are higher than in barley and wheat (Opoku, Back & Edwards, 2013, 2018).

Field surveys have been conducted to identify the impact of agronomy on T-2 and HT-2 concentrations. A meta-analysis of 12 studies comparing organic and conventional practices determined that in most studies (7 of 12) there was less T-2 and HT-2 in organically produced than in conventionally-grown cereals (Brodal et al., 2016). The most comprehensive studies were conducted in the United Kingdom. These studies showed significantly lower T-2 and HT-2 concentrations in organic wheat and oats, but not barley, compared to conventionally-grown crops (Edwards, 2009a,b,c). The difference was greatest for oats, which had a mean concentration of combined T-2 and HT-2 that was fivefold lower in organic than in conventionally-grown samples (Edwards, 2017b). Agronomic factors that impacted T-2 and HT-2 in harvested oats in conventional production in the United Kingdom included crop rotation, interaction between previous crops and cultivation, and variety (Edwards, 2017b). For crops grown in rotation, a stepwise increase in mean T-2 and HT-2 levels was associated with an increase in the number of cereals in the rotation. Cereal intensity (the proportion of cereal crops within the rotation) and not ploughing increased T-2 and HT-2 levels in a survey of oats in Finland (Kaukoranta et al., 2019). A field survey of oats in Switzerland determined T-2 and HT-2 levels were higher when the previous crop was a cereal, after not ploughing and in winter compared to spring sown oats (Schoneberg et al., 2018). Other cereals as the previous crop were also reported to result in higher T-2 and HT-2 in both a Norwegian oat survey (Klemsdal et al., 2009; Bernhoft et al., 2012) and in a field experiment in the Czech Republic (Hasjlova, 2009). In the United Kingdom study, cultivation alone was not a significant factor but the interaction between previous crop and cultivation (ploughing versus non-ploughing) was significant and showed the lowest mean T-2 and HT-2 concentration after a non-cereal previous crop and with ploughing. This is in agreement with a study of T-2 and HT-2 in French barley (Edwards et al., 2009). Analysis of T-2 and HT-2 from national oat variety trials in the United Kingdom allowed comparison of varieties under uniform environmental conditions (Edwards, 2012, 2015). These studies showed that winter sown varieties tended to have higher T-2 and HT-2 levels and a wider range of T-2 and HT-2 concentrations than spring sown varieties. Studies using near isogenic lines of a cross between short- and long-strawed varieties found that the presence of the dwarfing gene and/or reduced height increased T-2 and HT-2 levels in oats (Stancic, 2017). Surveys have identified no significant impacts of fungicides, plant growth regulators or fertilizers on T-2 and HT-2 concentrations (Bernhoft et al., 2012; Edwards, 2017b). However, these surveys had limited statistical power due to their unbalanced structure. Further field experiments, using natural infection, have been conducted to elucidate the impact of agricultural chemicals on T-2

and HT-2 concentrations. Fungicides had no significant effect on T-2 and HT-2 concentrations in field experiments on oats (Elen et al., 2008; Pettersson, 2008; Edwards & Anderson, 2011) and, similarly, no significant effects were reported for plant growth regulators and fertilizers (Edwards & Anderson, 2011).

A survey of T-2 and HT-2 occurrence in barley in France (Edwards et al., 2009; Orlando et al., 2010) identified some similarities to oats in the United Kingdom (Edwards, 2017a). For example, wheat and barley were high-risk previous crops and the two previous years' crops being cereals significantly increased T-2 and HT-2 concentrations. There were differences between French barley varieties, with T-2 and HT-2 contamination being greater in spring varieties. The sowing date also influenced T-2 and HT-2 levels, with higher T-2 and HT-2 concentrations being associated with later sowing. This was confirmed in a field experiment with multiple varieties sown at two drilling dates (autumn and spring) (Orlando, 2009). Significant differences in T-2 (HT-2 not analysed) concentrations were also observed across a selection of Italian barley varieties, with some variation across the three years of study, indicating an interaction between genotype and environment (Beccari et al., 2017). Field experiments with barley also showed higher T-2 and HT-2 concentrations when the previous crop was cereals compared to when it was maize (Hasjlova, 2009). Hasjlova (2009) found inconsistent results for fungicides whereas Karron et al. (2020) reported no effect of fungicides on T-2 and HT-2 levels in barley.

Very few studies have looked at the impact of agronomy on T-2 and HT-2 contamination in crops other than oats and, to a lesser extent, barley. The concentration of T-2 and HT-2 was frequently low in such studies and the results were often not statistically significant and/or inconsistent. In a study on wheat, Czaban et al. (2015) showed that T-2 and HT-2 levels were correlated to *F. langsethiae* incidence and higher levels were detected in a cereal-intense rotation than after a break crop of oilseed rape. Wenda-Piesik et al. (2017) found T-2 and HT-2 levels in wheat were not affected by sowing date, previous crop or fungicide regime, although there was a difference between the two varieties tested. Suproniene et al. (2012) found no difference in the T-2 levels in wheat as a result of cultivation or fertilizer use. Plant growth regulators had no impact on the low T-2 and HT-2 concentrations present in wheat (Mankeviciene et al., 2008).

Gaurilcikiene, Mankeviciene & Suproniene (2011) reported no change in T-2 content after application of a strobilurin fungicide (azoxystrobin), except for a significant increase in T-2 in rye in one out of three field experiments (Simpson et al., 2001; Mankeviciene et al., 2008). Mikos-Szymanska & Podolska (2013) studied two triticale varieties. They reported higher T-2 and HT-2 levels in cereal-intense rotations, but no difference related to cultivation (ploughed versus non-ploughed).

Concentrations of T-2 and HT-2 varied across varieties of buckwheat showing a negative correlation with the concentration of phenolic compounds (Keriene, Mankeviciene & Cesnuleviciene, 2018). Hence, phenolics may be a useful trait for resistance in other crops.

Four maize agronomy studies were identified: Blandino et al. (2017) assessed sowing time and hybrid cultivars, De Boevre et al. (2014) compared hybrid cultivars, Baliukoniene et al. (2011) assessed cultivation, and Limay-Rios & Schaafsma (2018) assessed fungicide applications. All four studies found low levels of T-2 and HT-2 and none identified significant differences between treatments.

6.2 Postharvest control

Few studies have been published on postharvest control of T-2 and HT-2. Previously, it was believed that T-2 and HT-2 contamination occurred primarily when crops remained in the field after a delayed harvest or were stored under conditions of high moisture (fifty-sixth meeting of the Committee; Annex 1, references *152* and *153*) (Foroud et al., 2019). Recent studies have shown that production of T-2 and HT-2 by *F. langsethiae* on oat grain is largely unaffected by temperature (15–30 °C), but is restricted as water activity drops below 0.9 (Mylona & Magan, 2011), which is equivalent to approximately 19% moisture content for oat grains at 25 °C (Professor Naresh Magan, Cranfield University, personal communication, 25 August 2020). Similar temperature and moisture profiles for T-2 and HT-2 production by other *Fusarium* species were described in the review by Doohan, Brennan & Cooke (2003). It now appears that T-2 and HT-2 contamination occurs primarily preharvest (see section 6.1) and current management of stored grain minimizes any further synthesis of these contaminants.

It is also likely that cleaning grain during and after harvest will reduce T-2 and HT-2 as reported for DON, since higher mycotoxin concentrations have been found in dust, debris, and among smaller and broken grains (Cheli et al., 2013). Pascale et al. (2011) showed variable reduction of T-2 and HT-2 with cleaning during processing of durum wheat, with a mean reduction of approximately 25–79%. Postharvest cleaning will always be variable as it will depend on how clean the consignment is prior to processing, which in turn is determined by harvest conditions and the combine harvester settings (for example, fan speed). Brodal et al. (2020) found that higher mycotoxin concentrations, including of T-2 and HT-2, occurred on smaller oat grains (passing through a 2-mm screen). Screening as part of a cleaning process, either during or after harvest, will reduce T-2 and HT-2 levels.

6.3 Decontamination

Numerous studies have shown that microorganisms or their enzymes have the ability to break down mycotoxins (Li P et al., 2020). The primary detoxification method for T-2 and HT-2 is deep oxidation (Fuchs et al., 2002; He et al., 2010; Diaz, Vargas & Cortes, 2016; Gao et al., 2018; Chlebicz & Slizewska, 2020). Other studies have shown that microbes can reduce the presence of T-2 and HT-2 by adsorption (Zou et al., 2012a; Zou et al., 2015).

Various binders have been shown to absorb T-2 in vitro and in vivo (Garcia et al., 2003; Yang LC et al., 2014; Solis-Cruz et al., 2017; Olopade et al., 2019), although Garcia et al. (2003) found no correlation between binding activity in vitro and efficacy in vivo. Kutasi et al. (2012), Verbrugghe et al. (2012) and Yang LC et al. (2014) showed that a yeast preparation, modified glucomannan and montmorillonite clay, respectively, counteracted the reduced weight gain in ducks, pigs and broiler chickens due to exposure to HT-2 and/or T-2. Meissonnier et al. (2009) reported that a glucomannan binder reduced the impact of T-2 on vaccinal response in pigs.

Few studies have investigated direct chemical degradation of T-2 and HT-2. Reinholds et al. (2016) demonstrated that ozone could degrade T-2 and HT-2 in malting wheat grains. Since Wilson et al. (2005) observed that chlorine dioxide could degrade the type D trichothecenes verrucarin A and roridin A in solution, this treatment may be effective against T-2 and HT-2.

7. Levels and patterns of contamination in food commodities

7.1 Surveillance data

Data on T-2 and HT-2 or the sum of T-2 and HT-2 concentrations in foodstuffs submitted to the Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme (GEMS/Food) contaminants database by national authorities were extracted for analysis. The dataset included data from January 2000 to February 2019.

The dataset was cleaned by removing aggregated sample data (n = 218), feed samples (n = 11 417) and samples with high reported LOD values (n = 8988). In the initial dataset the LODs ranged from 0.02 to 450 µg/kg with a mean and standard deviation of 11 ± 19 µg/kg. An acceptable maximum LOD of 50 µg/kg was defined (approximately mean + 2SD). All records with a LOD greater than 50 µg/kg were from European countries and all but one result was negative. All

results were converted to the same units (μg/kg) and food names were made as consistent as possible based on the descriptions provided.

For some samples, the sum of T-2 and HT-2 concentrations was reported directly. For other samples, concentrations of T-2 and HT-2 were reported separately. For these latter samples, the individual concentrations for a sample were identified using the serial number of the record to identify analytical results relating to the same sample. Individual concentrations of T-2 and HT-2 were combined, assuming that analytical results below the LOD were true zero concentrations. For samples where neither T-2 nor HT-2 was detected, their contribution to the upper bound (UB) mean was taken as the lower of the LODs for the two toxins.

Two scenarios were considered when calculating mean mycotoxin concentrations: samples in which the concentration was below the LOQ or LOD were assumed to have a value of either zero (lower bound scenario; LB) or the limit itself (upper bound scenario; UB). Records in the GEMS/Food contaminants database sometimes report a numerical value for results between the LOD and LOQ. Such values were included in the calculation of mean values.

There were 55 399 samples for which a combined T-2 and HT-2 concentration could be calculated in the GEMS/Food contaminants database. Summary statistics are presented in tables and include number of samples, percentage positive (percentage of samples above the LOQ), lower bound (LB) mean and upper bound (UB) mean and the maximum value. The sensitivity of the assays used varied widely (LOD = 0.03–50 μg/kg) and this has an impact on the percentage occurrence and mean values. When assessing the summary statistics presented in the tables in this section, it can be observed that the concentration of T-2 and HT-2 is not normally distributed. A large proportion of samples were identified as "undetected" (left tail censorship) and there was a highly skewed right-hand tail with a maximum value three orders of magnitude above the mean value. This is typical of the distribution of mycotoxin concentrations. The distribution of samples across regions and countries is presented in Table 2. From this table it is evident that most of the results were generated within the European Union (79%) followed by Canada (16%), and the remainder of the countries and regions provided 5% of the results. In some countries, only a narrow range of commodities was tested: in the USA samples were limited to cassava and in Africa, to sorghum.

Due to the paucity of data from regions other than Europe, a literature search was conducted using Web of Science for the years 2000–2020 with the search term "TS=((mycotoxin)+(HT-2 or T-2 or HT-2 or T-2) and (surve* or occurr* or contam*) NOT (Europe*)". This search recovered 619 publications (up to August 2020), which were screened for relevance by title, abstract and then the full paper. Publications reporting HT-2 and/or T-2 for multiple samples ($n > 10$)

Table 2
Summary of T-2 and HT-2 combined concentration by region and country from the GEMS/Food contaminants database

Region and country	No. of samples	Percentage positive	Lower bound mean (µg/kg)	Upper bound mean (µg/kg)	Max. (µg/kg)
African	**1 533**	**0.07**	**0.0**	**3.8**	**11.9**
Burkina Faso	367	0.00	0.0	3.8	0.0
Ethiopia	380	0.00	0.0	3.8	0.0
Mali	336	0.00	0.0	3.8	0.0
Sudan	450	0.22	0.0	3.8	11.9
Americas	**8 899**	**1.45**	**0.6**	**10.6**	**335**
Canada	8839	1.46	0.6	10.6	335
USA	60	0.00	0.0	9.0	0.0
European	**44 259**	**24.68**	**17.0**	**23.4**	**14 452**
European Union	43 794	24.75	17.1	23.5	14 452
Norway	465	18.49	6.3	16.1	124
Western Pacific	**577**	**0.52**	**0.3**	**2.3**	**196**
Hong Kong Special Administrative Region	240	0.42	0.0	2.5	2.6
New Zealand	200	0.50	0.0	0.1	0.4
Singapore	137	0.73	1.4	6.4	196
Overall	**55 268**	**20.01**	**13.6**	**20.6**	**14 452**

of food commodities or products with a reported LOD of less than 50 µg/kg were judged relevant. The results from the GEMS/Food contaminants database and the literature search are summarized below by WHO region.

7.1.1 African region

A total of 1533 combined T-2 and HT-2 concentrations were calculated from the GEMS/Food contaminants database for food samples from African countries. The samples were fairly evenly distributed across four countries (Burkina Faso, Ethiopia, Mali and Sudan); all samples were of sorghum analysed in 2013. Concentrations in all samples were below the LOD (4 and 5 µg/kg for T-2 and HT-2, respectively) except for a single sample from Sudan that had a concentration of 12 µg/kg HT-2.

Several studies published in the scientific literature have reported T-2 and HT-2 levels to be low or below the LOD in foods from Africa. The Southern African Grain Laboratory has tested 350 maize and 40 wheat samples annually for many years and the results are published online (https://sagl.co.za/mycotoxin/). These results indicate that detection of T-2 and HT-2 (LOD 20 µg/kg for both) is very rare and only one maize sample in the 2012/2013 season

contained detectable toxin levels (combined T-2 and HT-2, 304 µg/kg). A recent (2016) study with more sensitive detection (LOD = 0.9 and 1.9 µg/kg for T-2 and HT-2, respectively) reported T-2 and HT-2 in one of 123 (0.8%) South African maize samples, with concentrations of 148 and 40 µg/kg for T-2 and HT-2, respectively (Ekwomadu et al., 2020). Other surveys of African maize have also found contamination levels predominantly below or close to the LOD. For example, in a survey of Nigerian maize (n = 32), T-2 and HT-2 were not detected (LOD = 2 and 3 µg/kg, respectively), although two samples contained detectable levels of a metabolite (T-2 tetraol; 73 and 280 µg/kg) (Bankole, Schollenberger & Drochner, 2010). A survey of maize in the United Republic of Tanzania (n = 60 composite samples) did not detect T-2 (LOD = 2 µg/kg) but detected HT-2 in 25% of samples (LOD = 0.6 µg/kg), with a maximum concentration of 25 µg/kg (Kamala et al., 2015).

In a larger survey (n = 363) of cereals in Nigeria (maize, sorghum and millet) and a fermented cereal-based product, *ogi*, T-2 was not detected (LOD = 3.6–8 µg/kg), while the highest levels of HT-2 were seen in sorghum (n = 110) with 8% of samples above the LOD (6.5 µg/kg) and a maximum concentration of 36 µg/kg (Chilaka et al., 2016). In an Algerian survey of cereals (Mahdjoubi et al., 2020), T-2 and HT-2 were found in 50% and 6% of samples (LOD = 0.7–7 and 1.3–1.5 µg/kg, respectively), with maximum concentrations of 47 and 37 µg/kg, respectively. T-2 was not detected in barley and rice samples (LOD = 0.7–1.2 µg/kg), but was detected in 100% of maize and wheat samples (LOD = 3–7 µg/kg). However, these results should be interpreted with caution, as the concentrations detected were within a narrow range for all samples (for example, range 25–26 µg/kg T-2 for wheat). These samples were either known to be imported or of unknown origin.

A Nigerian survey of melon (n = 16) and bush mango seeds (n = 40) found no detectable T-2 and HT-2 in melon seeds (LOD = 0.8 and 3 µg/kg, respectively), but the toxins were detected in 18% and 7% of bush mango seed samples, with maximum concentrations of 113 and 104 µg/kg for T-2 and HT-2, respectively (Ezekiel et al., 2016). A survey of Nigerian fermented food products (n = 191) detected T-2 and HT-2 in less than 15% of samples of each product (LOD range = 3.6–15 µg/kg). The maximum T-2 and HT-2 concentrations were in *iru* (fermented locust bean); 31 and 51 µg/kg, respectively (Adekoya et al., 2017).

A recent total diet study in sub-Saharan Africa analysed composite food samples (n = 194) representing food intake at eight locations in four countries (Benin, Cameroon, Mali and Nigeria) for numerous mycotoxins (Ingenbleek et al., 2019b). No samples contained detectable T-2 or HT-2 (LOD = 0.4 and 0.8 µg/kg, respectively).

7.1.2 Region of the Americas

Of the 8839 samples from Canada in the GEMS/Food contaminants database with calculated combined concentrations of T-2 and HT-2, results were below the LOD (range 5–20 µg/kg) for samples from all food categories except cereal and cereal products, and nuts and oilseeds (Table 3). Of the nuts and oilseeds (n = 462) a single sample of rape seed contained detectable HT-2 (70 µg/kg). In the samples of cereals and cereal products, T-2 and HT-2 were detected infrequently across all subcategories, with 1.6% of results above the LOD, and LB mean, UB mean and maximum concentrations of 0.7, 10.3 and 335 µg/kg, respectively. The highest LB mean concentration was for maize and maize products (4.2 µg/kg) and the highest concentration was found on a sample of rice (335 µg/kg).

Comparing data for commodities to intermediate and finished products or comparing imported to domestic produce highlighted no obvious differences in the concentrations of T-2 and HT-2.

There were 2660 results submitted to the GEMS/Food contaminants database from Brazil for T-2 or HT-2 that could not be combined due to lack of serial numbers. Altogether, 1770 results were reported for HT-2 and 890 for T-2. The samples were products of primary processing (for example, flour) of wheat, maize and rice and all results were below the LOD (5 µg/kg for both T-2 and HT-2).

Sixty results from the USA, for T-2 in cassava analysed in 2018, were all below the LOD (9 µg/kg).

As with the GEMS/Food contaminants database, most literature on T-2 and HT-2 in the Region of the Americas is from Canadian studies. Several surveys of Canadian cereal grains have included analysis of T-2 and/or HT-2. These studies mostly analysed composite samples from a defined region and either did not detect T-2 or HT-2 or detected them only occasionally (Campbell et al., 2002; Clear et al., 2005; Grafenhan et al., 2013; Tittlemier, Gaba & Chan, 2013; Tittlemier et al., 2019). A survey of oats sampled directly from harvest (n = 287), reported detection of T-2 and HT-2 in 6% (LOD = 60 µg/kg) and 7% (LOD = 30 µg/kg) of samples, with maximum concentrations of 108 and 256 µg/kg, respectively.

Surveys of Canadian finished cereal products, which included analysis of HT-2 but not T-2, found no HT-2 (LOQ = 40–50 µg/kg) in cereal-based infant foods (n = 363) (Lombaert et al., 2003) and detected HT-2 in a single oat product with a concentration at the LOD (20 µg/kg) in a survey of breakfast cereals (n = 156) (Roscoe et al., 2008).

A US survey of cereal-based infant foods (n = 64) detected HT-2 (LOQ = 0.5–1.0 µg/kg) in 9% of samples, with a maximum concentration of 9.6 µg/kg and T-2 (LOQ = 0.05–0.1 µg/kg) in 28% of samples, with a maximum concentration

Table 3

Summary of T-2 and HT-2 combined occurrence in foods from Canada, from the GEMS/Food contaminants database

Food category and name	No. of samples	Percentage positive	Lower bound mean (µg/kg)	Upper bound mean (µg/kg)	Max. (µg/kg)
Cereals and cereal-based products	**7070**	**1.6**	**0.7**	**10.3**	**335**
Barley	413	0.7	0.2	11.5	46
Buckwheat	140	2.9	1.0	8.1	66
Cereals and cereal-based products NES	603	0.5	0.1	7.3	17
Maize and maize products	627	6.4	4.2	12.1	271
Millet	127	3.1	2.1	9.1	98
Oats and oat products	533	1.5	0.3	9.4	51
Rice and rice products	477	0.4	1.0	8.9	335
Rye	158	3.8	0.5	9.1	20
Wheat and wheat products	3992	1.1	0.3	10.8	150
Foods for infants	**450**	**3.6**	**0.7**	**8.4**	**35**
Cereal-based food for infants	450	3.6	0.7	8.4	35
Legumes and pulses	**614**	**0.0**	**0.0**	**13.7**	**0**
Beans (dry)	14	0.0	0.0	15.0	0
Lentils (dry)	85	0.0	0.0	12.5	0
Peas (dry)	243	0.0	0.0	14.0	0
Soya bean (dry)	272	0.0	0.0	13.8	0
Nuts and oilseeds	462	0.2	0.2	14.3	70
Coconut	2	0.0	0.0	8.0	0
Linseed	131	0.0	0.0	14.2	0
Nuts and oilseeds NES	**53**	**0.0**	**0.0**	**14.2**	**0**
Rape seed	276	0.4	0.3	14.5	70
Snacks and desserts	**185**	**0.0**	**0.0**	**8.0**	**0**
Maize and maize products	41	0.0	0.0	8.0	0
Rice and rice products	82	0.0	0.0	8.0	0
Wheat and wheat products	62	0.0	0.0	8.0	0
Starchy roots and tubers	**58**	**0.0**	**0.0**	**7.2**	**0**
Arrowroot	58	0.0	0.0	7.2	0
Overall	**8839**	**1.5**	**0.6**	**10.6**	**335**

NES, not elsewhere specified.

of 3.6 µg/kg (Al-Taher et al., 2017). Another US survey of cereal-based products (n = 217 infant foods and breakfast cereals) detected no HT-2 (LOD = 2.8 µg/kg), whereas 2% of samples contained detectable but not quantifiable levels of T-2 (LOD and LOQ = 0.5 and 1.6 µg/kg respectively) (Zhang et al., 2018).

In South America, T-2 and HT-2 have been analysed in three studies of barley, maize and soya bean. A survey of barley grain in Brazil (n = 60) did not detect T-2 or HT-2 (LOD = 0.9 and 1.9 µg/kg respectively) (Iwase et al., 2020). T-2 and HT-2 were not detected (LOD = 50 µg/kg for each) in samples of maize (n = 82) in Peru (Coloma et al., 2019). A survey of 89 soya bean samples in Argentina in 2011 also did not detect T-2 or HT-2 (LOD = 10 µg/kg and 2 µg/kg, respectively) (Garrido et al., 2013).

A survey of infant food in Ecuador detected HT-2 (LOD = 20 µg/kg) in 4% of rice samples (n = 46), with a maximum concentration of 40 µg/kg, but did not detect T-2 (LOD = 2 µg/kg) (Ortiz et al., 2013). T-2 and HT-2 were not detected in oat flakes or wheat noodles.

7.1.3 Eastern Mediterranean Region

No T-2 and HT-2 analyses had been submitted to the GEMS/Food contaminants database from the Eastern Mediterranean Region.

Four studies of cereal and cereal products in North African markets identified low levels of T-2 and HT-2. A Tunisian survey of cereals and cereal products did not detect T-2 (n = 58; LOD = 1 µg/kg), whereas HT-2 was detected in 12% of samples (LOD = 1 µg/kg) with a maximum concentration of 11 µg/kg (Oueslati et al., 2012). In a second Tunisian study of barley and barley products (soup and beer), T-2 was not detected (LOD = 18 µg/kg), whereas HT-2 was detected in 6% (LOD = 18 µg/kg) of barley samples (n = 31), but not in samples of barley soups (n = 21) or beer (n = 34) (Juan et al., 2017). Two studies in Morocco analysed mycotoxins in cereal products, pasta and couscous. T-2 and HT-2 were found in 4 and 0% of samples of pasta (n = 106: LOD = 1.5 and 4.0 µg/kg, respectively) with a maximum T-2 concentration of 5.8 µg/kg (Bouafifssa et al., 2018). T-2 and HT-2 were found in 35 and 16% of samples of couscous (LOD = 2.0 and 2.5 respectively), with maximum concentrations of 50 and 419 µg/kg, respectively (Zinedine Abdellah et al., 2017b).

A study of 190 rice samples, mainly from Pakistan, did not detect HT-2 (LOD = 2.3–11 µg/kg) or T-2 (LOD = 2.4–5.0 µg/kg) (Lim et al., 2015). Similarly, in a second study (n = 180), T-2 was not detected (LOD = 22 µg/kg), but HT-2 was detected in 10% of samples (LOD = 13 µg/kg) with a maximum concentration of 32 µg/kg (Majeed et al., 2018).

7.1.4 European Region

The European Region was well represented in data from the GEMS/Food contaminants database. A total of 44 259 samples (99% from the European Union and 1% from Norway) had reported results for both T-2 and HT-2. For simplicity the combined sum concentrations of T-2 and HT-2 are reported in this section. Overall, 25% of all European samples were positive for T-2 and HT-2 (Table 4). Samples with concentrations above the LOD were reported for all food categories, but most of the positive samples were from the cereals and cereal products category and from others that do or may contain cereals.

Most samples of alcoholic beverages were from beer and beer-like beverages (n = 1049). Six per cent of these samples were positive (LOD = 0.04–25 µg/kg), with a maximum concentration of 28 µg/kg T-2 and HT-2. T-2 and HT-2 were not detected in the 175 samples of wine (LOD = 5–50 µg/kg).

For the fats and oils category, the toxins were detected in maize, olive and sunflower oils, and a single sample of walnut oil. The highest incidence and levels were reported in maize oil: 78% of samples were positive (LOD = 0.25–50 µg/kg) and LB mean, UB mean and maximum concentrations for T-2 and HT-2 were 5.9, 7.8 and 42 µg/kg, respectively.

T-2 and HT-2 were not detected in samples of fruit (n = 1074), except for two samples of cranberry (4.7% positive), which both contained approximately 300 µg/kg T-2 and HT-2.

There was a high frequency of detection of T-2 and HT-2 in samples of mushrooms (58%) although the LB mean, UB mean and maximum concentrations were all low (5.8, 6.9 and 45 µg/kg, respectively). The toxins were identified in samples of several species of fungi, both wild and cultivated.

T-2 and HT-2 were sporadically detected in samples across the category herbs, spices and condiments. The overall detection level was 18% (LOD = 0.1–50 µg/kg) and the highest concentration was detected in a sample of pepper (107 µg/kg T-2 and HT-2).

All samples in subcategories of legumes and pulses were negative for T-2 and HT-2 except for soya beans: 24% of soya bean samples were positive and LB mean, UB mean and maximum concentrations were 1.7, 4.9 and 76 µg/kg, respectively.

Data on only 14 samples of meat and meat products were available in the European dataset. Most were negative, except for samples of blood sausage (n = 4), which had a maximum concentration of 16 µg/kg, and meat paste (n = 1).

In the milk and dairy products category, all samples were negative (LOD = 0.04–15 µg/kg), except for a single sample classed as a milk imitation product, which was therefore likely to be of plant origin (0.86 µg/kg T-2 and HT-2).

Table 4
Summary of T-2 and HT-2 combined occurrence in products from the European Region, from the GEMS/Food contaminants database

Food category	No. of samples	Percentage positive	Lower bound mean (µg/kg)	Upper bound mean (µg/kg)	Max. (µg/kg)
Alcoholic beverages	1 225	5.4	0.1	3.1	28
Cereals and cereal-based products	37 400	27.2	18.7	25.2	14 452
Composite food	42	47.6	1.6	3.9	39
Fats and oils	121	26.4	1.3	2.0	42
Foods for infants	1 372	26.0	2.0	6.6	198
Fruit and fruit products	1 074	0.4	2.5	24.1	300
Fruit and vegetable juices	113	5.3	0.1	9.2	0.3
Fungi	33	57.6	5.8	6.9	45
Herbs, spices and condiments	234	17.9	2.2	10.7	107
Legumes and pulses	303	17.2	1.2	4.1	67
Meat and meat products	12	41.7	2.5	3.2	16
Milk and dairy products	60	1.7	0.0	1.2	0.9
Non-alcoholic beverages	69	24.6	2.6	4.7	35
Nuts and oilseeds	1 736	2.9	1.3	19.3	123
Products for special nutritional use	144	25.0	47.4	50.4	2341
Snacks and desserts	140	10.0	2.2	14.2	71
Starchy roots and tubers	21	19.0	0.1	1.4	0.9
Stimulant beverages	20	20.0	1.3	3.6	9.2
Sugar and confectionary	63	31.7	0.5	2.9	5.6
Vegetables and vegetable products	77	10.4	1.1	3.4	10
Overall	**44 259**	**24.7**	**17.0**	**23.4**	**14 452**

No samples of non-alcoholic beverages contained detectable T-2 and HT-2, except for malt coffee or undefined beverages. For malt coffee, 30% of samples were positive (LOD = 3–10 µg/kg) and the LB mean, UB mean and maximum concentrations were 4.0, 6.0 and 35 µg/kg, respectively.

For the nuts and oilseeds category, which comprised 1736 samples, results from many subcategories were negative (LOD = 0.25–50 µg/kg). Overall, 2.9% of the samples were positive, primarily hazelnuts, chestnuts, rape seed, sunflower seed and other oilseeds. Most positive levels were below 10 µg/kg, but two samples of sunflower seed contained approximately 120 µg/kg.

Samples from the category of products for special nutritional use had the highest mean concentrations of T-2 and HT-2 with a frequency of detection of

25% (LOD = 0.3–40 µg/kg) and LB mean, UB mean and maximum concentrations of 47, 50 and 2341 µg/kg. The highest concentrations were in samples described as dietary supplements with no further details.

For the remaining categories (snacks and desserts, starchy roots and tubers, sugar and confectionary, vegetable and vegetable products) the frequency of positive samples (LOD = 0.1–50 µg/kg) was low and the concentrations were below 10 µg/kg, unless the products contained or had the potential to contain cereals.

The category "cereals and cereal products" was well represented in data from the European Region, with 37 400 samples that included combined concentrations for T-2 and HT-2. Table 5 details the sub-categories of cereals and cereal products for the European Region, sorted into the various grains and their products and defined finished products. As can be seen from the table, the highest frequencies and concentrations occurred in the grains and products of barley, maize and oats with the highest mean concentrations found in oats (LB mean 113 µg/kg). The finished product categories tended to have lower concentrations of T-2 and HT-2. The highest levels were recorded in breakfast cereals, which is to be expected as these often have a high content of wholegrain cereal. The categories of each grain and its products were investigated further by comparing the T-2 and HT-2 concentrations in grain with the concentrations in milling products (for example, grits, flour and bran). Table 6 summarizes the occurrence data for T-2 and HT-2 for each cereal commodity and its milling products within the European Region. The data clearly show the commodities at greatest risk from T-2 and HT-2 contamination and the generally high reduction achieved during primary processing. The highest concentrations occurred predominantly in oats, followed by maize and barley grains. These commodities also showed the greatest reductions in the level of contamination during processing (approximately 70–90%). For example, the LB mean concentrations for oats and oat milling products were 241 and 15 µg/kg, respectively. The impact of processing on T-2 and HT-2 concentrations is covered in detail in section 6.

7.1.5 South-East Asia Region

No records of T-2 and HT-2 analyses had been submitted to the GEMS/Food contaminants database and no T-2 and HT-2 occurrence data were identified in the search for literature from the South-East Asia Region.

7.1.6 Western Pacific Region

Three countries supplied T-2 and HT-2 data to the GEMS/Food contaminants database from the Western Pacific Region, namely Hong Kong Special Administrative Region, New Zealand and Singapore. Of 577 samples with

Table 5

Summary of T-2 and HT-2 combined occurrence in cereals from the European Region, from the GEMS/Food contaminants database

Cereal categories	No. of samples	Percentage positive	Lower bound mean (µg/kg)	Upper bound mean (µg/kg)	Max. (µg/kg)
Barley and barley products	2 060	40.8	16.2	21.3	839
Buckwheat and buckwheat products	258	11.2	1.3	10.3	48
Cereals and cereal-based products NES	926	12.6	1.8	11.4	407
Maize and maize products	3 520	30.2	12.0	20.3	14 452
Millet and millet products	229	12.2	2.1	5.4	61
Oats and oat products	4 997	60.5	112.7	117.4	9990
Rice and rice products	661	5.3	0.1	10.7	23
Rye and rye products	2 322	11.6	0.6	7.1	85
Wheat and wheat products	20 275	20.8	2.8	8.9	1165
Breakfast cereal	1 974	25.6	3.1	14.0	293
Cereal-based dishes	38	7.9	0.2	2.1	3.1
Cereal-based food for infants	131	24.4	0.6	5.4	15
Dietetic food for diabetics	6	66.7	3.8	4.8	11.6
Milk and dairy products NES	3	0.0	0.0	3.7	0
Overall	**37 400**	**27.2**	**18.7**	**25.2**	**14 452**

NES, not elsewhere specified.

Table 6

Summary of T-2 and HT-2 combined occurrence in cereal grains and milling products[a] from the European Region, from the GEMS/Food contaminants database

Cereal category	No. of samples	Percentage positive	Lower bound mean (µg/kg)	Upper bound mean (µg/kg)	Max. (µg/kg)
Barley grain	1920	42.5	17.1	22.2	839
Barley processed	131	17.6	2.4	6.7	111
Buckwheat grain	146	11.6	1.7	9.7	40
Buckwheat milling products	112	10.7	0.9	11.0	48
Corn grain	1648	41.9	23.9	29.5	14 452
Corn milling products	1662	21.3	2.5	12.6	222
Millet grain	187	10.2	1.2	4.6	37
Millet milling products	42	21.4	5.8	9.2	61

Cereal category	No. of samples	Percentage positive	Lower bound mean (µg/kg)	Upper bound mean (µg/kg)	Max. (µg/kg)
Oat grain	2296	66.5	240.8	242.3	9990
Oat milling products	2226	56.6	15.0	21.4	1455
Rice grain	317	1.6	0.1	14.7	23
Rice milling products	300	8.0	0.1	6.7	5
Rye grain	929	12.3	0.7	6.1	61
Rye milling products	3715	11.5	0.6	7.4	85
Wheat grain[b]	8084	25.6	5.2	12.2	1165
Wheat milling products[b]	4217	13.3	1.3	8.2	143
Spelt grain	955	13.5	1.2	5.4	499
Spelt milling products	801	14.5	0.6	5.5	87
Durum grain	31	19.4	5.8	8.4	77
Durum milling products	545	11.0	0.8	3.8	131

[a] Milling products included all products from primary processing of cereals, typically this included bran, grits, flour and flakes.
[b] The categories wheat and wheat milling products may contain spelt and durum samples as well as soft wheat.

combined concentrations for T-2 and HT-2, only three had detectable (LOD = 0.04–20 µg/kg) concentrations of T-2 and HT-2 (Table 7). There were 200 samples of cereals and cereal products from New Zealand, which all had undetectable levels of T-2 and HT-2 (LOD = 0.1–0.3 and 0.04–0.11 µg/kg, respectively) except for a single snack product with a T-2 concentration of 0.4 µg/kg. Samples from Hong Kong Special Administrative Region and Singapore covered a broader range of foodstuffs, but were still dominated by samples of cereals and cereal products (38%).

There were 240 samples from Hong Kong Special Administrative Region, which all had T-2 and HT-2 concentrations below the LOD (2.5 µg/kg for both toxins), except for a single sample of breakfast cereal with a T-2 concentration of 2.6 µg/kg.

Of the 137 samples from Singapore, all had T-2 and HT-2 concentrations below the LOD (20 µg/kg for both toxins) except for a single sample of millet grain with a T-2 concentration of 196 µg/kg. There were an additional 3038 analyses reported from Singapore for T-2 and HT-2 that could not be combined to provide an HT-2+T-2 concentration due to the lack of serial numbers. These samples included approximately 50% cereals and cereal products, with the remaining 50% well distributed across other food categories. Of these samples, only two had results above the LOD (0.2–20 µg/kg) and both positive results were for HT-2, with concentrations of 12 µg/kg for a sample of buckwheat flour and 8 µg/kg for a sample of rye flour.

Very few reports of T-2 and HT-2 occurrence in samples from the Western Pacific Region were identified in the literature search. A survey of cereals

Table 7
Summary of T-2 and HT-2 combined occurrence in food from the Western Pacific Region, from the GEMS/Food contaminants database

Food categories	Number	Percentage positive
Alcoholic beverages	16	0
Cereals and cereal-based products	344	0.6
Composite food	56	0
Fats and oils	4	0
Food for infants	11	0
Fruit and fruit products	4	0
Fruit and vegetable juices	4	0
Herbs, spices and condiments	11	0
Legumes and pulses	23	0
Meat and meat products	36	0
Milk and dairy products	7	0
Non-alcoholic beverages	8	0
Nuts and oilseeds	8	0
Snacks and desserts	24	4.2
Starchy roots and tubers	13	0
Stimulant beverages	4	0
Sugar and confectionary	4	0
Overall	**577**	**0.5**

from a small geographical region in China identified a high incidence, but low concentrations of T-2 and HT-2, with 12 and 50% positive (LOD = 0.2 μg/kg) and maximum concentrations of 3.3 and 35 μg/kg, respectively (Wang XC et al., 2012). A small survey of maize (n = 24) identified very low levels of T-2 (3.3% positive (LOD = 0.02 μg/kg), maximum concentration 0.2 μg/kg) (Wang Y et al., 2013). A survey of dried fruits (n = 220) did not detect any T-2 or HT-2 (LOD = 1 and 5 μg/kg, respectively) (Wei et al., 2017). Another survey of dried fruits (n = 100) and nuts (n = 133) detected T-2 in 5% of dried fruit samples, with a maximum concentration of 7 μg/kg and did not detect any T-2 above the LOD (1 μg/kg T-2) in samples of nuts (Wang Y-J et al., 2018). A recent study of sun-dried fish (n = 12) in China detected T-2 in three samples (LOD = 0.1 μg/kg) with a maximum concentration of 1.5 μg/kg (Deng et al., 2021). Analysis for HT-2 was not included in this study. A recent review confirmed that occurrence data for type A trichothecenes were still rare in China (Shi et al., 2018).

A survey of mycotoxins in cereal grains (n = 507) in the Republic of Korea detected T-2 in two (2%) maize samples (LOD = 6.3 μg/kg), with a maximum concentration of 13.7 μg/kg, while HT-2 was detected in one (1%) mixed cereal sample (LOD = 4.0 μg/kg) with a concentration of 4.3 μg/kg (Kim et al., 2017).

No T-2 or HT-2 were detected in samples of rice, sorghum or millet (LOD = 2–15 µg/kg).

A survey of cereal grains in Malaysia (n = 80) detected T-2 and HT-2 across all cereal types tested (rice, wheat, barley, oats and maize), with an overall detection rate of 14 and 11% for T-2 and HT-2, respectively (LOD = 5 µg/kg for both). The lowest occurrence (7.5% positive), but the highest levels were found in rice, with maximum concentrations of 55 and 48 µg/kg for T-2 and HT-2, respectively (Soleimany et al., 2012).

7.2 Data on annual variation in contaminant levels

As with other *Fusarium* mycotoxins that are produced within the growing crop, T-2 and HT-2 concentrations will fluctuate between growing seasons and regions, depending on climatic conditions. Most studies reporting T-2 and HT-2 concentrations are based on single-year surveys and therefore the effect of seasonal variability cannot be assessed. A seven-year (2002–2008) investigation of *Fusarium* mycotoxins in harvested oats in the United Kingdom showed that the annual combined mean concentration of T-2 and HT-2 ranged from 121 to 727 µg/kg (Edwards, 2017).

7.3 Type A trichothecene co-occurrence and modified forms

T-2 and HT-2 usually co-occur in food commodities and finished products as they are produced by the same *Fusarium* species through the same metabolic pathway (Thrane et al., 2004). Analysis of the ratio of HT-2 to T-2 for all samples with quantifiable levels of both mycotoxins in the GEMS/Food contaminants database showed a wide range of ratios with an overall mean ratio of 3.14 (Table 8). Regression analysis identified significant differences (least significant difference, P = 0.05) between the ratios for each cereal type. Differences may be due to variation in the metabolism of the *Fusarium* species that infect different host cereals and/or in the metabolism of the mycotoxins by the host cereal. The best regression was for oat samples where a linear regression of HT-2 to T-2 accounted for 84% of the variance in HT-2 concentration and a linear equation of HT-2 = 2.8 T-2. The more extreme values at either end of the ratio range were in samples with quantifiable T-2 or HT-2 close to the LOQ. For oats with a combined T-2 and HT-2 concentration greater than 500 µg/kg the mean ratio was 3.1 with a range of 0.4–21. The mean ratios identified from the GEMS/Food contaminants database are similar to those reported in the review by van der Fels-Klerx & Stratakou (2010).

Table 8
Summary of ratio of HT-2 to T-2 for cereal samples with quantifiable concentrations of both mycotoxins from the GEMS/Food contaminants database

		Ratio of HT-2 to T-2		
Cereal	**Number**	**Mean**	**Minimum**	**Maximum**
Barley	542	4.01	0.30	32
Maize	597	1.73	0.29	13
Oats	1 746	2.87	0.04	53
Wheat	1 207	3.83	0.08	304
Overall	**4 092**	**3.14**	**0.04**	**304**

Fusarium langsethiae and *F. sporotrichioides* are both capable of producing several other derivatives of T-2 as well as HT-2 (Thrane et al., 2004). These include neosolaniol (NEO), T-2 triol and T-2 tetraol. They can also produce diacetoxyscirpenol (DAS), monoacetoxyscirpenol (MAS) and scirpentriol (SCR) as side-branch products of T-2 synthesis. These other type A trichothecenes are not commonly included in mycotoxin surveys but where they are, they are routinely detected as co-contaminants with T-2 and HT-2 toxins, particularly where analytical methods with low LODs are used. For example, Schollenberger et al. (2006) detected T-2 tetraol, T-2 triol, MAS and NEO, but not DAS, in German oat samples (n = 17). T-2 tetraol had the highest occurrence and concentration, with 94% of samples testing positive (LOD = 7 µg/kg) and mean and maximum concentrations of 150 and 577 µg/kg. HT-2 had the highest concentration of the type A trichothecenes, with mean and maximum concentrations of 181 and 494 µg/kg, respectively. Gottschalk et al. (2009) routinely detected T-2 tetraol, T-2 triol, DAS, MAS and NEO in German oat products (n = 98). T-2 tetraol had the highest occurrence and concentrations with 90% of samples testing positive (LOD < 0.5 µg/kg) and mean and maximum concentrations of 9.7 and 85 µg/kg. Combined T-2 and HT-2 had mean and maximum concentrations of 15 and 85 µg/kg, respectively. Edwards (2009b) quantified T-2 triol and neosolaniol in 41% (LOQ = 10 µg/kg) of United Kingdom oat samples (n = 458). The compounds occurred as low-concentration co-contaminants in the presence of high concentrations of the primary contaminants, T-2 and HT-2, with approximately 65% of the variance in their concentrations accounted for by the concentration of HT-2. DAS was not detected and other type A trichothecenes were not included in the analysis. Scudamore et al. (2007) studied oat processing in the United Kingdom using the same analytical methods and found similar results, with T-2 triol and NEO occurring at concentrations approximately 5% of the HT-2 concentration. More recently, isolates of *F. langsethiae* were found to

also produce glucosides of the type A trichothecenes HT-2, T-2, DAS and NEO (Lattanzio et al., 2013).

As well as the various type A trichothecenes synthesized by the *Fusarium* species, several metabolites are produced by the host plant during infection. In recent studies using host plants inoculated with isotope-labelled mycotoxins, numerous derivatives were identified or postulated in wheat, barley and oats (Meng-Reiterer et al., 2015, 2016; Nathanail et al., 2015). These metabolites were formed by typical phase I and II metabolic processes, including hydrolysis of ester groups, glycosylation and hydroxylation reactions. HT2-3-glucoside was the main metabolic intermediary of both parent mycotoxins. When the cereal plants had ripened, 15–30% of the parent mycotoxins remained following inoculation with T-2 or HT-2 (Meng-Reiterer et al., 2015, 2016; Nathanail et al., 2015). There have been several reports of glucosides of T-2 and HT-2 in naturally contaminated cereals. For maize, a single sample with a combined T-2 and HT-2 concentration of 188 µg/kg also contained 36 µg/kg HT2-3-glucoside (Ekwomadu et al., 2020). Three monoglucosides of T-2 and HT-2 were detected in naturally contaminated wheat and oats (T-2-3-glucoside, HT-2-3-glucoside and HT-2-4-glucoside), with higher relative concentrations in samples with low concentrations of the parent mycotoxin. The highest relative concentration compared to the parent mycotoxin for each glucoside was 7% in oats and 27% in wheat (Lattanzio et al., 2012). A survey of Finnish cereals (n = 95) detected HT-2-3-glucoside in approximately 50% (LOD = 3.6 µg/kg) of samples. The highest concentrations were detected in oat samples (n = 31), with mean and maximum concentrations of 41 and 300 µg/kg, compared to 159 and 1830 µg/kg for HT-2, respectively (Nathanail et al., 2015). A survey of Italian barley samples (n = 18) detected a monoglucoside of T-2 and two glucosides of HT-2. One of the HT-2 glucosides was detected more frequently and at higher concentrations. Based on other studies, this was presumably HT-2-3-glucoside. This glucoside had a maximum concentration of 163 µg/kg compared to a maximum concentration of 213 µg/kg for HT-2 (Lattanzio et al., 2015). The relative concentration of the combined glucosides compared to the combined concentration of the parent molecules ranged from 0 to 283%. Traces of DAS and NEO glucosides were also detected. A survey of oats (n = 51) in Ireland detected T-2-glucoside in 18% of samples (LOD = 6.5 µg/kg) with a maximum concentration of 270 µg/kg, compared with a maximum concentration of 1498 µg/kg for HT-2 (De Colli et al., 2020).

8. Food consumption and dietary exposure assessment

8.1 Concentrations in food used in the dietary exposure estimates

The GEMS/Food contaminants database was queried for records relating to T-2, HT-2 or the sum of T-2 and HT-2 in any food. The data extracted originated from 12 countries or country groups (European Union), representing seven of the 17 GEMS/Food cluster diets. However, it should be noted that five of the seven clusters represented (G07, G08, G10, G11 and G15) include mainly developed westernized countries. The remaining clusters (G09 and G13) include mainly African countries. However, only one analytical result from the G13 cluster was above the LOD, while all data from the G09 cluster relate to animal feed and positive results related to feed samples that were either imported or whose origin was unknown. Five clusters (G07, G08, G10, G11 and G15) cover the countries of the European Union; however, the available data on contaminant concentrations are only identified to the level of the European Union and it was not possible to examine differences in contamination profile between these clusters.

At country level, the only convincing evidence of T-2/HT-2 contamination was in domestically-produced food from the European Union, including Norway, and from Canada. Contamination detected in a small number of samples of food items from Hong Kong Special Administrative Region, New Zealand and Singapore was either identified as being associated with imported foods or highly likely (Singapore) to have been associated with imported foods.

Given these observations and the information presented in section 7, the Committee concluded that contamination of the food supply with T-2 and HT-2 is likely to be minor outside Europe and North America.

8.2 Food consumption data used in the dietary exposure estimates

8.2.1 Chronic dietary exposure

In addition to national estimates of dietary exposure published in the literature, the Committee may derive national estimates of dietary exposure using food consumption information from the FAO/WHO Chronic Individual Food Consumption Database – Summary statistics (CIFOCOss), in combination with summary concentration data from the GEMS/Food contaminants database (see section 8.1).

The Committee may also calculate international estimates of dietary exposure using the GEMS/Food cluster diets. The consumption cluster diets provide mean per capita consumption values based on FAO food balance sheet data for raw commodities and some semi-processed commodities for 17 clusters

of countries (Sy et al., 2013). Clusters G01 and G06 include primarily Middle Eastern, central Asian and north African countries; clusters G03, G13 and G16 include primarily African countries; cluster G02 includes countries in west Asia and the Balkan region of Europe; cluster G04 includes Middle Eastern and Caribbean countries; cluster G09 includes countries in Africa and Asia; clusters G07, G08, G10, G11 and G15 include European and North American countries and developed countries in Asia and the Pacific (Australia, Japan, New Zealand, the Republic of Korea); clusters G05 and G12 consist mainly of South and Central American countries; and clusters G14 and G17 include Caribbean/Asia/Pacific island states.

8.2.2 Acute dietary exposure

Techniques for assessing acute dietary exposure seek to quantify the probability of high single exposure events and require information on food consumption for a single consumption event or a single day of consumption. Food consumption data from the CIFOCOss database and the GEMS/Food cluster diets are not suitable, as these databases summarize food consumption or availability over two or more days. These data can only be used for chronic dietary exposure assessments. Therefore, the Committee accessed individual food consumption data included in the FAO/WHO Global Individual Food consumption data Tool (GIFT).[1] At the time of the assessment, this tool contained food consumption data at the individual level from 14 countries, namely Argentina, Bangladesh, Burkina Faso, the Democratic Republic of the Congo, Guatemala, India, Italy, Kenya, the Lao People's Democratic Republic, Pakistan, the Philippines, the Plurinational State of Bolivia, Uganda and Zambia. The results of two surveys carried out in Kenya were available. These data also included characteristics of the individuals, including age, sex and body weight. The data from Burkina Faso contained inconsistencies that precluded use of this dataset.

It should be noted that, of these countries, only Italy is in the geographical zone where consistent contamination of the food supply with T-2 and HT-2 is indicated.

8.3 Assessments of chronic dietary exposure

8.3.1 National estimates of chronic dietary exposure from the scientific literature

At the fifty-sixth meeting of the Committee, national estimates of dietary exposure to T-2 and HT-2 were reported for Norway and the United Kingdom. However, dietary exposures were reported separately for different cereal types (oats, rye and

[1] http://www.fao.org/gift-individual-food-consumption/overview/en/

wheat for Norway, barley, maize, oats, rye and wheat for the United Kingdom). For the Norwegian study, median and 95th percentile estimates of dietary exposure were reported for each cereal type, and for each of eight subpopulations. For T-2, the highest median exposure was 12 ng/kg bw per day from wheat consumption by 6-year-old children. The highest median dietary exposure to HT-2 was 15 ng/kg bw per day for both oat and wheat consumption by 6-year-old children. The highest 95th percentile estimates of dietary exposure were 26 and 39 ng/kg bw per day for T-2 and HT-2, respectively, from consumption of wheat and oats, respectively, by 6-year-old children.

The dietary exposure estimates for the United Kingdom were reported separately for the five cereals listed above, for two subpopulations (1.5–4.5 years and 16–64 years) and for T-2 and HT-2. Estimates were for consumers only and were not normalized to a body weight basis. Mean, median and 97.5th percentile dietary exposure estimates were reported. Aggregating the mean estimates of dietary exposure across cereal types and applying nominal body weights of 15 and 60 kg to the two subpopulations results in mean estimates of dietary exposure to T-2 of 13 and 8 ng/kg bw per day for children and adults, respectively. For HT-2, the mean estimates of dietary exposure are 18 and 12 ng/kg bw per day, respectively.

Since the evaluation of T-2 and HT-2 at the fifty-sixth meeting of the Committee in 2001, several national evaluations of chronic dietary exposure have been published. The Committee considered evaluations from Belgium, China, the Czech Republic, Ecuador, Europe, France, Ireland, Malawi, Morocco, the Netherlands, New Zealand, Nigeria, Pakistan, Romania, Serbia, Spain, Sweden, sub-Saharan Africa, Tunisia and the United Republic of Tanzania (Table 9). These reports include dietary exposure assessments for T-2 (12 studies), HT-2 (14 studies), and the sum of T-2 and HT-2 (12 studies). Summaries have also been included for studies in which these toxins were not detected or were detected so infrequently that dietary exposure could not be estimated.

(a) Belgium

A total of 174 cereal-based food items, including fibre-enriched breads, bran-enriched breads, breakfast cereals, popcorn and oatmeal were sampled from Belgian supermarkets (De Boevre et al., 2013). Samples were analysed by LC-MS/MS for a range of mycotoxins including T-2 and HT-2. LODs for individual toxins were in the range 6–11 µg/kg. HT-2 was detected in approximately 54% of samples analysed and T-2 in approximately 51%. Food consumption data were taken from the Belgian National Food Consumption Survey of 2004. The data consisted of two 24-hour dietary recalls (24HDR) for 3083 Belgians aged 15 years or older. Left-censored analytical results were substituted by either zero

Table 9

Summary of national estimates of dietary exposure to T-2, HT-2 and the sum of T-2 and HT-2 from the literature

Country	Food concentration data used	Consumption data used	Population groups (age in years)	Estimated dietary exposure, mean (high consumer)[a] in ng/kg bw per day	Major contributors	Reference
T-2						
Belgium	Survey mean	National food consumption survey (2 × 24HDR)	Adult	6–12 (15–28)	Fibre- and bran-enriched breads	De Boevre et al. (2013)
China, People's Republic	Survey mean	Agricultural production and GEMS/Food cluster diets	Adult male Adult female	0.11 0.13	Dried fruit (only dried fruit and nuts considered)	Wang Yu-Jiao et al. (2018)
Europe (Belgium, Czech Republic, France, the Netherlands, Norway)	EFSA database	2 x 24HDR	Adult (45–65)	6.4[b]	NS	De Ruyck et al. (2020)
Europe (19 countries)	EFSA database	National food consumption surveys (at least 2 survey days)	Infants Toddlers Other children Adolescents Adults Elderly Very elderly	0.7–47 (6.3–108)[c] 2.9–44 (9.1–72)[c] 2.9–42 (7.1–73)[c] 1.4–24 (3.8–44)[c] 1.0–18 2.4–40)[c] 0.8–16 (2.0–33)[c] 0.6–14 (1.6–27)[c]	Grains and grain-based products	EFSA (2017)
France	Survey mean	Individual (7-day food diary)	Adults (18–79) Children (3–17)	1.8–20 (4.8–37) 4.0–38 (9.0–73)	Pasta, bread, biscuits	Sirot, Fremy & Leblanc (2013)
France	Survey mean (Sirot et al., 2013)	Food frequency questionnaire	Pregnant women (18–45) Pre-pregnancy Third trimester	 2.0–17 (4.6–35) 1.6–13 (3.6–28)	Rice and durum wheat, pasta	Chan-Hon-Tong et al. (2013)
France	Survey mean	Individual (3-day caregiver-completed food diary)	1–4 months 5–6 months 7–12 months 13–36 months	0.4–79 (0–104)[d] 26–78 (26–100)[d] 5.9–42 (16–63)[d] 2.0–23 (5.7–42)[d]	NS	Vin et al. (2020)
Netherlands	Survey concentrations	Individual (2 consumption days)	2–6 7–69	14–43 (150–200) 2.6–10 (29–66)	Fruit juices	Sprong et al. (2016)
Nigeria	Survey mean	Survey of 10 households	Adults	4.6	Bush melon seeds only	Ezekiel et al. (2016)
Serbia	Survey mean	Serbian market basket	Adults Children	20 20	Only wheat flour included	Škrbić et al. (2012)
Serbia	Survey mean	Household survey	Adults (20–60) Adolescents (10–19) Children (6–9)	0.39–0.44 0.51–0.57 0.60–0.68	Only biscuits with fruit filling included	Skrbic, Antic & Cvejanov (2017)

Table 9 (continued)

Country	Food concentration data used	Consumption data used	Population groups (age in years)	Estimated dietary exposure, mean (high consumer)[a] in ng/kg bw per day	Major contributors	Reference
Spain	Survey mean	National database	Adults	1.7[e]	Cereal-based prepared meals	Carballo et al. (2018)
HT-2						
Belgium	Survey mean	National food consumption survey (2 × 24HDR)	Adult	10–18 (23–41)	Fibre- and bran-enriched breads	De Boevre Marthe et al. (2013)
Ecuador	Survey mean	2 × or 1 × 24HDR	Children (0–23 months)[f] Urban, mixed Urban, weaned Rural, mixed Rural, weaned	 47 (98) 54 (103) 68 (188) 90 (236)	Polished rice only	Ortiz et al. (2018)
Europe (Belgium, Czech Republic, France, the Netherlands, Norway)	EFSA database	2 × 24HDR	Adults (45–65)	13.5[b]	NS	De Ruyck et al. (2020)
Europe (28 countries)	Survey mean	Food balance sheets	General population	0.2–25[c]	Only beer included	Rodríguez-Carrasco et al. (2015)
Europe (19 countries)	EFSA database	National food consumption surveys (at least 2 survey days)	Infants Toddlers Other children Adolescents Adults Elderly Very elderly	2.3–59 (9.8–130)[c] 5.4–55 (15–96)[c] 5.1–53 (12–96)[c] 2.8–33 (6.8–57)[c] 1.2–23 (3.0–49)[c] 1.0–20 (2.9–43)[c] 1.0–18 (2.4–35)[c]	Grains and grain-based products	EFSA (2017)
France	Survey mean	Individual (7-day food diary)	Adult (18–79) Children (3–17)	7.2–32 (15–59) 11–53 (22–104)	Pasta, bread	Sirot, Fremy & Leblanc (2013)
France	Survey mean (Sirot et al., 2013)	Food frequency questionnaire	Pregnant women (18–45) Pre-pregnancy Third trimester	 4.3–22 (9.4–46) 3.7–18 (7.9–38)	Bread, pasta	Chan-Hon-Tong et al. (2013)
France	Survey mean	Individual (3-day caregiver-completed food diary)	1–4 months 5–6 months 7–12 months 13–36 months	0.4–80 (0–104)[d] 27–91 (39–158)[d] 6.8–57 (16–92)[d] 2.5–31 (6.4–55)[d]	NS	Vin et al. (2020)
Netherlands	Survey concentrations	Individual (2 consumption days)	2–6 7–69	0.0–4.1 (64–200) 0.0–2.3 (7.9–26)	Fruit juices	Sprong et al. (2016)
Nigeria	Survey mean	Survey of 10 households	Adult	8.7	Bush melon seeds only	Ezekiel et al. (2016)

Country	Food concentration data used	Consumption data used	Population groups (age in years)	Estimated dietary exposure, mean (high consumer)[a] in ng/kg bw per day	Major contributors	Reference
Pakistan	Survey mean	Food frequency questionnaire	Adult North Punjab South Punjab Children North Punjab South Punjab	 6.1–13 (11–22) 2.1–8.5 (3.4–14) 6.3–13 (10–21) 2.1–8.6 (3.6–15)	Only rice included	Majeed et al. (2018)
Spain	Survey mean	National database	Adults	8.3[e]	Cereal-based prepared meals	Carballo et al. (2018)
Spain	Survey mean	National database and nominal value	Adults Children	0.21–0.41 4.6–9.0	Only fruit juices included	Pallares et al. (2019)
United Republic of Tanzania	Distribution of survey concentrations	2 x 24HDR and food frequency	Infants (6–12 months)	40 (170)	Only maize included	Kamala et al. (2017)
Sum of T-2 and HT-2						
Belgium	Survey mean	National food consumption survey (2 x 24HDR)	Adult	Deterministic: 17–30 (38–68) Probabilistic: 26 (92)	Fibre- and bran-enriched breads	De Boevre et al. (2013)
Czech Republic	Survey mean	National dietary survey (2 x 24HDR)	Children (4–6) Male (18–59) Female (18–59)	1.8–8.2 (6.5–31) 0.5–2.7 (1.9–11) 1.0–2.9 (2.5–12)	Bread, biscuits and bakery products	Ostry et al. (2020)
Europe (Belgium, Czech Republic, France, the Netherlands, Norway)	EFSA database	2 x 24HDR	Adult (45–65)	30.6[b]	NS	De Ruyck et al. (2020)
Europe (19 countries)	EFSA database	National food consumption surveys (at least 2 survey days)	Infants Toddlers Other children Adolescents Adults Elderly Very elderly	4.4–63 (18–146)[c] 9.0–65 (24–109)[c] 8.5–62 (20–112)[c] 4.4–39 (11–72)[c] 3.5–26 (6.4–54)[c] 2.3–23 (5.4–42)[c] 1.8–21(4.2–41)[c]	Grains and grain-based products	EFSA (2017)
France	Survey mean (Sirot et al., 2013)	Web-based survey (3 non-consecutive 24HDR)	Vegetarian (18+)	9.3–27 (22–100)	Pasta, bread, rice and wheat products	Fleury et al. (2017)
France	Survey mean	Individual (3-day caregiver-completed food diary)	1–4 months 5–6 months 7–12 months 13–36 months	0.8–159 (0–208)[d] 53–169 (60–235)[d] 13–99 (33–164)[d] 4.5–54 (12–93)[d]	NS	Vin et al. (2020)
Morocco	Survey mean	Per capita	Adult	60	Only couscous included	Zinedine et al. (2017a)
Netherlands	Survey concentrations	Individual (2 consumption days)	2–6 7–69	14–47 (210–400) 2.6–13 (37–92)	Fruit juices	Sprong et al. (2016)

Table 9 (continued)

Country	Food concentration data used	Consumption data used	Population groups (age in years)	Estimated dietary exposure, mean (high consumer)[a] in ng/kg bw per day	Major contributors	Reference
Spain (Catalonia)	Survey mean	Food frequency questionnaire	Adult males (20–65) Adult females (20–65) Adolescents (10–19) Children (4–9)	12–36 (34–50)[g] 11–41 (87–89)[g] 27–55 (57–121)[g] 41–91 (86–204)[g]	NS	Cano-Sancho et al. (2012)
Spain	Survey mean	National survey	Infants (0–3) Children (5–12) Adults (18–65)	86 79 10	Wheat	Rodríguez-Carrasco et al. (2013)
Spain	Survey mean	Mean national consumption of all cereals	Adults	12	Only barley included	Ibáñez-Vea, Lizarraga & González-Peñas (2011)
Tunisia	Survey mean	National per capita consumption	Adults	0.3–12.2	Only barley and barley products included	Juan et al. (2017)

24HDR: 24-hour dietary recall, NS: not stated.
[a] 95th percentile, unless otherwise stated, LB-UB.
[b] Median.
[c] Range from minimum LB to maximum UB estimates across studies.
[d] 90th percentile.
[e] Dietary exposure was estimated separately for each of five ready-to-eat meal types. Figures presented here are the sum of the individual mean estimates for meal types in which the toxin was detected.
[f] "Mixed" refers to a diet containing breast milk and complementary foods, while "weaned" refers to a diet containing complementary foods only.
[g] The study included calculation of mean concentrations using three different treatments of left-censored data and determined dietary exposure deterministically and stochastically. The ranges given are across all method variations.

(LB), LOD/2 (MB) or LOD (UB). Deterministic estimates of dietary exposure were derived by multiplying the mean mycotoxin concentration by the mean, maximum or 95th percentile consumption level for the foods. Stochastic estimates of dietary exposure were also determined by Monte Carlo simulation, using parametric distributions fitted to mycotoxin concentration and food consumption information. For the deterministic dietary exposure assessment, the mean (LB–UB) estimates for all cereal-based foods for T-2, HT-2 and the sum of T-2 and HT-2 were 6–12, 10–18 and 17–30 ng/kg bw per day, respectively. The equivalent 95th percentile dietary exposure estimates were 15–28, 23–41 and 38–68 ng/kg bw per day, respectively. Stochastic assessment of the UB dietary exposure for the sum of T-2 and HT-2 from consumption of all cereal-based foods gave very similar results, with a mean of 26 ng/kg bw per day and a 95th percentile of 92 ng/kg bw per day. As this analysis applied the full set of analytical results to determining estimates of chronic dietary exposure, the upper percentiles of the output distribution are likely to be inflated.

(b) **China, People's Republic of**

Samples of nuts and dried fruit (n = 233) were analysed for various mycotoxins, including T-2. The samples were collected from 21 regions across China (Wang Y-J et al., 2018). Analyses were carried out by LC-MS/MS. The LOD and LOQ for T-2 were 1.0 and 5.0 µg/kg, respectively. T-2 was detected in six samples; three each of dried jujubes and dried figs, with concentrations in the range 2.8–7.0 µg/kg. Although results were reported quantitatively, all reported concentrations of T-2 in dried jujubes and at least some of the reported concentrations in dried figs were below the LOQ (5 µg/kg). Dietary exposure was estimated using mean food consumption estimates derived using agricultural production data and GEMS/Food cluster diets with adult male and female body weights of 66.2 and 56.1 kg, respectively. To calculate mean T-2 concentrations, analytical results below the LOD were assigned a value of LOD/2. Dietary exposures were calculated for each of five nut or dried fruit types. The sums of the individual dietary exposure estimates were 0.11 and 0.13 ng/kg bw per day for males and females, respectively. A probabilistic dietary exposure assessment was also carried out for adult females. Few details were given, but it appeared that the simulations were performed by replacing the mean concentration values by a statistical distribution; however, the additive exposure across the different food types was not considered.

(c) **Czech Republic**

Cereal-based food and beer samples, collected for the national total diet study, were analysed by LC-MS/MS for *Fusarium* mycotoxins, including T-2 and HT-2 (Ostry et al., 2020). LODs/LOQs for T-2 and HT-2 were 0.65/3.6 and 0.55/2.9 µg/kg, respectively. Results were reported as the sum of T-2 and HT-2, with detections reported in samples of eight food types, with the highest prevalence (79%) and mean LB–UB concentration (14.7–15.5 µg/kg) found in muesli. Food consumption data were obtained from a national dietary survey (SISP04), including repeat non-consecutive 24HDR interviews for each respondent. The respondents' body weights were recorded. Mean and 95th percentile LB–UB estimates of dietary exposure to the sum of T-2 and HT-2 were derived for three population groups; children (4–6 years), males (18–59 years) and females (18–59 years). Mean (95th percentile) estimates were 1.8–8.2 (6.5–31.0), 0.5–2.7 (1.9–11.2) and 1.0–2.9 (2.5–11.5) ng/kg bw per day, respectively, for the three population groups.

(d) **Ecuador**

Samples of cereals and cereal products (paddy rice, polished rice, oat flakes, white wheat noodles and yellow wheat noodles; n = 211) used as complementary foods or weaning foods were analysed by UHPLC-ToF/MS for a range of mycotoxins,

including T-2 and HT-2 (Ortiz et al., 2013, 2018). LODs for T-2 and HT-2 were in the range 2–7 and 5–20 μg/kg, respectively. T-2 was not detected in any samples, whereas HT-2 was detected in 2 of 46 polished rice samples at concentrations of 26.0 and 39.5 μg/kg. Information on food consumption by children aged 0–23 months was obtained through application of a 24HDR questionnaire, completed by caregivers for 998 children. For urban children (n = 650), two 24HDRs were obtained, whereas for rural children (n = 348) only one 24HDR was obtained. The mean HT-2 concentration in polished rice was determined by assigning a value of half the LOD to analytical results below the LOD. The mean estimated dietary exposures to HT-2 for children from urban areas were 47 and 54 ng/kg bw per day for children on mixed breast milk and complementary food diets and complementary food only diets, respectively. The corresponding 95th percentile estimates of dietary exposure were 98 and 103 ng/kg bw per day, respectively. For children from rural areas, the corresponding mean (95th percentile) estimates of dietary HT-2 exposure were 68 (188) and 90 (236) ng/kg bw per day.

(e) Europe

A cohort study was carried out involving 600 adults, aged 45 to 65 years, from Belgium, the Czech Republic, France, the Netherlands and Norway (De Ruyck et al., 2020). Two 1-day estimates of dietary exposure to a range of mycotoxins were derived from 24HDR records for each member of the cohort, administered 1 month apart, and concentration data for mycotoxins in foods from an EFSA database. For food types with at least one quantified result, values less than the LOD or LOQ were substituted by values of LOD/2 or LOQ/2, respectively. Dietary exposure estimates for each individual in the cohort were determined as the mean of the estimates derived from the two 24HDRs. Median dietary exposure to the sum of T-2 and HT-2 was 31 ng/kg bw per day, while median exposures to T-2 and HT-2 separately were 6 and 14 ng/kg bw per day, respectively. The maximum estimated dietary exposures were 290, 144 and 144 ng/kg bw per day for the sum of T-2 and HT-2, T-2 and HT-2, respectively. Correlations between estimates of dietary exposure for the two different days were low (Spearman's ρ approximately 0.1) but were statistically significant for the sum of T-2 and HT-2 and for HT-2. Similarly, the correlation coefficients for the relationship between estimated dietary exposure and the concentration of the mycotoxin in concurrent 24-hour urine samples was just significant for HT-2 only.

Thirty-five food consumption surveys from 19 European countries were used to assess chronic dietary exposure to T-2, HT-2 and the sum of T-2 and HT-2 (EFSA, 2017). Information from an EFSA database was used to calculate LB and UB estimates of the mean T-2, HT-2 and sum of T-2 and HT-2 concentrations for a range of food types. The concentration data for T-2 were 90% left-censored,

while those for HT-2 were 87% left-censored. Mean concentration estimates were combined with individual food consumption and body weight information and the resulting distribution of dietary exposure estimates reported in terms of the mean and high percentile (95th) of the distribution. Dietary exposure was estimated for nine population groups; infants, toddlers, other children, adolescents, adults, elderly, very elderly, pregnant women and lactating women. Across all food consumption surveys and population groups, mean estimates of dietary exposure to the sum of T-2 and HT-2 were in the range 1.8 ng/kg bw per day (minimum LB, very elderly) to 65 ng/kg bw per day (maximum UB toddlers). High-percentile exposure estimates were in the range 4.2 ng/kg bw per day (minimum LB, very elderly) to 146 ng/kg bw per day (maximum UB infants). This study also estimated acute dietary exposure.

Fusarium mycotoxins were determined by GC-MS/MS in samples of beer (n = 154) from 13 countries, collected in Valencia, Spain (Rodríguez-Carrasco et al., 2015). LODs/LOQs for T-2 and HT-2 were 4/8 and 2/4 μg/L, respectively. T-2 was not detected in any beer samples, whereas HT-2 was detected in 14 of 154 samples (9.1%), with a mean concentration (positive samples only) of 30.9 μg/L. Beer consumption for European countries was derived from FAO food balance sheets. An adult body weight of 70 kg was used. LB–UB estimates of mean dietary HT-2 exposure from beer consumption for the total European Union was reported as 7–12% of the tolerable daily intake (TDI; 100 ng/kg bw per day), equating to 7–12 ng/kg bw per day.

(f) **France**

Dietary exposures to T-2 and HT-2 were estimated as part of the second French total diet study (Sirot, Fremy & Leblanc, 2013). T-2 and HT-2 concentrations were determined by LC-MS/MS in 577 food samples collected from mainland France. LODs and LOQs for the toxins were 3 and 10 μg/kg, respectively. Individual food consumption data and body weights for 1918 adults (aged 18–79 years) and 1444 children (aged 3–17 years) were taken from the INCA2 study, a 7-day food diary study. T-2 and HT-2 were detected in most cereal-containing food types, but not in animal products or vegetables. Fruit was not analysed for these toxins. Lower- and upper-bound estimates of mean and 95th percentile dietary exposure were determined for T-2 and HT-2, separately. Mean and 95th percentile dietary exposures for adults were 1.8–19.6 and 4.8–36.5 ng/kg bw per day for T-2 and 7.2–32.3 and 14.5–58.9 ng/kg bw per day for HT-2. For children, estimated dietary exposures (mean and 95th percentile) were 4.0–38.0 and 9.0–72.8 ng/kg bw per day for T-2 and 10.5–53.1 and 22.3–104 ng/kg bw per day for HT-2. For both population groups, the major contributors to dietary exposure were bread and dried bread products, and pasta.

Using contaminant concentration data from the second French total diet study and food consumption data from the EDEN mother-child cohort study, which included pregnant women aged 18–45 years, dietary exposures to a range of contaminants, including T-2 and HT-2, were estimated (Chan-Hon-Tong et al., 2013). Food frequency questionnaires were completed covering the year before pregnancy (n = 1861) and the third trimester of pregnancy (n = 1775). Individual body weights were determined through the course of the study. Uncertainty makes comparison of estimates of dietary exposure difficult. For T-2 the mean (95th percentile) LB–UB dietary exposure before pregnancy was estimated to be 2.0–16.6 (4.6–35.4) ng/kg bw per day. The corresponding estimates of dietary exposure during the third trimester were 1.6–13.0 (3.6–27.6) ng/kg bw per day. The equivalent estimated dietary exposures to HT-2 were 4.3–22.2 (9.4–46.4) ng/kg bw per day and 3.7–18.0 (7.9–37.4) ng/kg bw per day for the year before pregnancy and the third trimester, respectively. The major contributors to dietary exposure for both toxins were cereal-based foods (bread, pasta, rice and durum wheat, pastries and cakes, pizzas, quiches and savoury pastries).

In a further study using concentration data from the second French total diet study, information on food consumption by the French vegetarian and vegan population was obtained from the NutriNet Santé study and used to derive dietary exposure estimates for a range of contaminants including T-2 and HT-2 (Fleury et al., 2017). Food consumption information was available for 1766 vegetarians, including 188 vegans, derived from three non-consecutive 24HDRs, randomly assigned over a 2-week period. The cohort had a mean age of 36.6 years (range 18–81 years). Mean and 95th percentile LB–UB estimates of dietary exposure to the sum of T-2 and HT-2 were 9.3–26.7 and 21.5–99.6 ng/kg bw per day. Dietary exposure of vegetarians to the sum of T-2 and HT-2 was greater than for the general population. The food category rice and wheat products was a major contributor to dietary exposure of vegetarians to T-2 and HT-2 and vegetarians consumed approximately twice as much of these foods as the general population.

An infant (<3 years) specific total diet study was carried out (Vin et al., 2020). Composite food samples (n = 457) were prepared and 207 of them were analysed for a range of mycotoxins including T-2 and HT-2. T-2 and HT-2 were analysed by LC-MS/MS with LODs and LOQs of 0.05–0.1 µg/kg and 1.5–3.0 µg/kg, respectively. T-2 was detected in fruit puree, infant formula, and meat or fish-based ready-to-eat meals. HT-2 was additionally detected in cereal-based infant foods, milk-based beverages, and rice and wheat products. T-2 and HT-2 were most prevalent in fruit puree, being detected in 13% of samples analysed. Food consumption data were taken from a survey of 705 infants and young children (aged 1–36 months) conducted by the Syndicat Français des Aliments de l'Enfance et de la Nutrition Clinique in 2005, using a 3-day caregiver-completed food diary. LB and UB estimates of mean and 90th percentile dietary exposure were

determined for four age groups (1–4 months, 5–6 months, 7–12 months and 13–36 months) and for T-2, HT-2 and the sum of T-2 and HT-2. For the sum of T-2 and HT-2 the mean and 90th percentile dietary exposure estimates were 0.8–159 and 0–208 ng/kg bw per day for 1–4-month-old infants, 53–169 and 60–235 ng/kg bw per day for 5–6-month-old infants, 13–99 and 33–164 for 7–12-month-old infants and 4.5–54 and 12–93 ng/kg bw per day for 13–36-month-olds.

(g) **Ireland**

As a component of a total diet study of *Fusarium* mycotoxins, the 141 food types considered to be most likely to contain the toxins were analysed for their presence (Food Safety Authority of Ireland, 2016). No *Fusarium* mycotoxins were detected in any sample; however, the LODs for the trichothecene mycotoxins were very high (50 µg/kg). No estimate of dietary exposure was possible.

(h) **Malawi**

Samples of traditional maize beer (n = 9) were collected from two districts in Malawi during 2012 and analysed by LC-MS/MS for a range of mycotoxins including T-2 and HT-2 (Matumba et al., 2014). LODs/LOQs were not reported for T-2 and HT-2. Neither of the trichothecenes were detected in any beer sample.

(i) **Morocco**

Couscous semolina samples (n = 98), based on wheat, corn or barley were collected in 15 administrative regions of Morocco during 2014–2015 and analysed by LC-MS/MS for a range of mycotoxins including T-2 and HT-2 (Zinedine et al., 2017a). The LODs/LOQs were 1.5/5 µg/kg and 4/10 µg/kg for T-2 and HT-2, respectively. T-2 was detected in three couscous samples (two wheat and one corn), with concentrations in the range 5.3–5.8 µg/kg. HT-2 was not detected in couscous from any cereal source. Mean deterministic estimates of dietary exposure were derived for the adult Moroccan population using mean mycotoxin concentrations, calculated by substituting a value of LOD/2 for analytical results below the LOD, mean consumption of the commodity and a 70-kg body weight. Food consumption data were derived from an FAO country profile. Dietary exposure to the sum of T-2 and HT-2 was estimated as 60 ng/kg bw per day.

(j) **The Netherlands**

Food composites (n = 88), representative of the food consumption patterns of the Dutch population, were analysed for a range of mycotoxins, including T-2 and HT-2 (Sprong et al., 2016). LODs were 1.0 µg/kg for both mycotoxins in cereal-based foods and 5 µg/kg for T-2 and 20 µg/kg for HT-2 in all other foods (Lopez et al., 2016). T-2 was only detected at a concentration above the LOQ in an

apple juice composite. No composite samples contained HT-2 at a concentration above the LOQ. LB–UB dietary exposures were estimated for children (aged 2–6 years) and the general population (7–69 years). For children, the median (95th percentile) estimates of dietary exposure to T-2, HT-2 and the sum of T-2 and HT-2 were 14–43 ng/kg bw per day (150–200 ng/kg bw per day), 0.0–4.1 ng/kg bw per day (64–200 ng/kg bw per day) and 14–47 ng/kg bw per day (210–400 ng/kg bw per day), respectively. The corresponding estimates of dietary exposure for the general population were 2.6–10 ng/kg bw per day (29–66 ng/kg bw per day), 0.0–2.3 ng/kg bw per day (7.9–26 ng/kg bw per day) and 2.6–13 ng/kg bw per day (37–92 ng/kg bw per day). Fruit juices were the major contributors to dietary exposure in all cases.

(k) New Zealand

A survey of trichothecene mycotoxins in cereal-based foods (n = 176) was carried out, with analysis by LC-MS/MS (Cressey, Chappell & Grounds, 2014). LODs and LOQs for T-2 were in the range 0.04–0.11 and 0.13–0.36 µg/kg, depending on the food. LODs and LOQs for HT-2 were in the range 0.1–0.3 and 0.3–1.0 µg/kg. T-2 was detected in a single sample of extruded maize-based snack food at a concentration of 0.4 µg/kg. HT-2 was not detected in any sample. These results were considered to be insufficient to carry out a dietary exposure assessment.

(l) Nigeria

A study assessed exposure to a range of mycotoxins, including T-2 and HT-2, solely from consumption of melon and bush mango seeds, materials used for the thickening of soups (Ezekiel et al., 2016). Mycotoxins were determined by LC-MS/MS, with LODs of 0.8 µg/kg (T-2) and 3 µg/kg (HT-2) for both matrices. Neither toxin was detected in melon seed samples (n = 16), whereas T-2 and HT-2 were detected in 45% and 18% of 40 bush mango seed samples, respectively. While not stated, it appears that the mean concentration of only those samples with quantifiable levels of toxin was used to estimate dietary exposure. Mean food consumption values for the two foods were estimated from the frequency of consumption in 10 households. A nominal body weight of 60 kg was used. Mean estimates of dietary exposure were 4.6 and 8.7 ng/kg bw per day, for T-2 and HT-2, respectively.

(m) Pakistan

A study in the Punjab province of Pakistan analysed rice samples (n = 180) for 23 mycotoxins, including T-2 and HT-2, by LC-MS/MS (Majeed et al., 2018). LODs/LOQs were 22/44 and 13/26 µg/kg for T-2 and HT-2, respectively. HT-2 was detected in 10% of samples, with a LB mean concentration of 2.0 µg/kg.

T-2 was not detected in any rice sample. Rice consumption data were obtained from a food frequency questionnaire administered to 548 adults and 467 children (aged 7–15 years) from the south (SP) and north (NP) Punjab regions. Deterministic estimates of dietary exposure were derived using either a fixed mean rice consumption level and percentiles of the concentration distribution or a fixed mean mycotoxin concentration and percentiles of the rice consumption distribution. Results from the latter are summarized here. For adults the mean (95th percentile) LB–UB estimates of HT-2 dietary exposure for SP and NP were 2.1–8.5 (3.4–13.7) and 6.1–12.9 (10.5–22.1) ng/kg bw per day, respectively. For children from SP and NP mean (95th percentile) LB–UB estimates of HT-2 dietary exposure were 2.1–8.6 (3.6–14.6) and 6.3–13.4 (10.0–21.1) ng/kg bw per day, respectively.

(n) Romania

Wheat-based products (n = 181) available on the Romanian market were analysed for trichothecenes and zearalenone by GC-MS/MS (Stanciu et al., 2018). LODs/LOQs for T-2 and HT-2 were 2.5/5 and 7.5/15 µg/kg, respectively. Neither trichothecenes nor zearalenone was detected in any sample and estimation of dietary exposure was not possible.

(o) Serbia

Flour samples (n = 15) were collected from supermarkets in the main wheat-growing region of Serbia and analysed by LC-MS/MS for a range of mycotoxins including T-2 and HT-2 (Škrbić et al., 2012). LODs/LOQs were 1.4/4.7 and 0.9/3.0 µg/kg for T-2 and HT-2, respectively. Total per capita consumption of wheat flour was calculated from the Serbian market basket after conversion of wheat-based foods to flour equivalents. Wheat flour consumption by children was assumed to be half that of adults (267.7 g/person per day). Body weights of 25 and 60 kg were used to determine dietary exposure estimates for children and adults. T-2 was detected in four flour samples (27%) at concentrations in the range 9.8–26.9 µg/kg. HT-2 was not detected in any flour samples. A mean T-2 concentration of 4.1 µg/kg was calculated by assigning a value of LOD/2 to analytical results below the LOD. Mean estimates of dietary exposure for children and adults were 21 and 18 ng/kg bw per day, respectively.

In a further study, the same research group analysed mycotoxins, including T-2 and HT-2, in samples of biscuits, dried fruits and fruit jams (n = 97) by LC-MS/MS (Skrbic, Antic & Cvejanov, 2017). LODs/LOQs were in the range 0.4–0.9/1.3–2.9 µg/kg for T-2 toxins and 1.1–1.2/3.7–4.0 µg/kg for HT-2. T-2, but not HT-2, was detected in fruit-filled biscuits (14 of 39 samples; 2.9–26.7 µg/kg) and fruit jams (2 of 10 samples; 3.8 and 4.9 µg/kg). Neither was

detected in cookies or dried fruit. Dietary exposure was only estimated from consumption of fruit-filled biscuits, with mean consumption amounts supplied from the Statistical Office of the Republic of Serbia, based on a survey of 4800 households per year. Body weights of 34, 52 and 68 kg were used to derive dietary exposure estimates for children (6–9 years), adolescents (10–19 years) and adults (20–60 years). LB–UB estimates of dietary exposure to T-2 from consumption of fruit-filled biscuits were 0.60–0.68, 0.51–0.57 and 0.39–0.44 ng/kg bw per day for children, adolescents and adults, respectively. Although dietary exposure estimates were derived for HT-2, the estimates were based entirely on left-censored data.

(p) **Spain**

T-2 and HT-2 were analysed by GC-CED, GC-MS or LC-DAD in 479 food composites, including corn flakes, wheat flakes, sweet corn, corn snacks, pasta, beer, sliced bread and bread, collected in 12 cities in the Catalonia region of Spain (Cano-Sancho et al., 2012). LODs were not reported, but LOQs were in the range 14–45 µg/kg (T-2) and 3–20 µg/kg (HT-2). T-2 was detected in less than 10% of samples of food composites, whereas HT-2 was detected in approximately one quarter of the samples. Mean T-2 and HT-2 concentrations were calculated by three different techniques for treatment of left-censored data: substitution by a value equal to half the LOD, a maximum likelihood estimation (MLE) method and a nonparametric Kaplan–Meier (K-M) method. Food consumption estimates for the Catalonian population were derived from a food frequency study involving 1100 respondents and frequency of consumption of 38 food types. Mean deterministic estimates of dietary exposure to the sum of T-2 and HT-2 were derived for four population groups (children, adolescents, adult males and adult females), with estimates in the range 11 ng/kg bw per day (adult females, K-M method) to 79 ng/kg bw per day (children, MLE method). Dietary exposure was also estimated by a stochastic method, representing the distribution of food consumption by gamma distributions and using mean T-2 or HT-2 concentrations derived using the same three methods for handling left-censored data. It was not stated how the covariates from consumption of various food types were included. Estimates of mean dietary exposure were in the range 18 ng/kg bw per day (adult male, K-M method) to 91 ng/kg bw per day (children, MLE method). High consumer estimates of dietary exposure (95th percentile) were approximately twice the mean estimates in all cases.

Samples of ready-to-eat meals (n = 328) purchased from a university cafeteria in Valencia, Spain, were classified according to their main ingredient (cereal, vegetable, fish, legume, meat) (Carballo et al., 2018). Both a GC-MS/MS and an LC-MS/MS method were described; however, it is unclear which

analytical results relate to which method. HT-2 was detected in 6% (vegetable) to 54% of samples (legume) and was not detected in fish-based meals, whereas T-2 was only detected in cereal-based meals (10%). LODs for all analytes were ≤1.5 µg/kg. Left-censored data were assigned a value of zero (LB) in the calculation of mean concentrations for different food types. Food consumption data were obtained from the statistical database of the Spanish Ministry of Agriculture, Food and Environment. Mean estimates of dietary exposure were calculated for each food type, but not overall. Mean dietary exposure to T-2 from cereal-based meal consumption was 1.7 ng/kg bw per day. Dietary exposure to HT-2 was in the range 0.9 ng/kg bw per day (legume-based meals) to 3.5 ng/kg bw per day (cereal-based meals) for the food types in which the toxin was detected.

Samples of cereal-based foods (n = 150), containing wheat, maize or rice were analysed by GC-MS/MS for a range of *Fusarium* mycotoxins including T-2 and HT-2 (Rodríguez-Carrasco et al., 2013). Specific LODs/LOQs for these analytes were not given but were reported to be in the range 0.6 to 5 µg/kg and 1.25 to 10 µg/kg, respectively. HT-2 was detected in 16.8% of wheat-based products and 5.9% of maize-based products but was not detected in rice-based products. T-2 was only detected in one wheat-based product. Food consumption data were derived from surveys published by the Spanish Food Safety and Nutrition Agency. Mean dietary exposures to the sum of T-2 and HT-2 for infants, children and adults were estimated to be 86, 79 and 10 ng/kg bw per day, respectively.

Type A and type B trichothecene mycotoxins, including T-2 and HT-2, were analysed by GC-MS in samples of barley (n = 44) from the 2007 harvest in the Navarra region of Spain (Ibáñez-Vea, Lizarraga & González-Peñas, 2011). LODs/LOQs were 0.4/20 and 2.0/20 µg/kg for T-2 and HT-2, respectively. The ratio of the LOQ to the LOD reported for this study seems unusually large. T-2 and HT-2 were detected in 11 and 23% of samples, with mean values in positive samples of 9.2 and 7.8 µg/kg, respectively. Overall mean concentrations were calculated by substituting a value of LOD/2 for analytical results below the LOD. No country-specific information on barley consumption was available and a figure of 239 g/day for consumption of all cereals was used, together with a body weight of 70 kg, to estimate dietary exposure to the sum of T-2 and HT-2 due to their presence in barley. Mean and maximum estimates of dietary exposure were 12 and 100 ng/kg bw per day. These estimates are likely to be inflated as barley is unlikely to be a major component of cereal intake in Spain.

Commercial fruit juices (n = 80) were collected in Valencia during 2018 and analysed by LC-MS/MS for a range of mycotoxins including T-2 and HT-2 (Pallares et al., 2019). LODs/LOQs were 2.3/7.8 µg/L and 0.6/2.0 µg/L for T-2 and HT-2, respectively. HT-2 was detected in 3% of samples, with a mean concentration in positive samples of 22.8 µg/L. Fruit juice consumption by adults was derived from per capita consumption data from a Spanish Ministry of

Agriculture, Fisheries and Food database (9.2 L/person per year = 25 mL/person per day). This estimate was considered to be too low for child consumers and a consumption volume of 200 mL/person per day was used for this population group. Mean LB–UB estimates of dietary HT-2 exposure for adults and children were 0.2–0.4 and 4.6–9.0 ng/kg bw per day, respectively.

(q) Sweden

Samples of wheat, oats and rye (n = 110) collected during 1996–1998 were analysed by GC-ECD for a range of mycotoxins including T-2 and HT-2 (Thuvander et al., 2001). LODs for T-2 and HT-2 were 10 μg/kg. Neither T-2 nor HT-2 was detected in any sample analysed.

(r) Sub-Saharan Africa (Burkina Faso, Ethiopia, Mali and Sudan)

Samples of sorghum (n = 1533) were collected from four sub-Saharan African countries and analysed for a range of mycotoxins including T-2 and HT-2 (Ssepuuya et al., 2018). The LODs and LOQs of the method used were not reported. T-2 was not detected in any sorghum sample, whereas HT-2 was detected in one sample (0.07%) at a concentration of 11.9 μg/kg. Owing to the low prevalence of T-2 and HT-2 contamination, dietary exposure to these mycotoxins was not estimated.

Composite food samples (n = 194), including dietary staples, were collected from four sub-Saharan African countries (Benin, Cameroon, Mali and Nigeria) and analysed for a wide range of mycotoxins, including T-2 and HT-2 (Ingenbleek et al., 2017, 2019a). The LODs of the method used for detecting T-2 and HT-2 ranged from 0.4–0.7 and from 0.8–1.5 μg/kg, respectively (Sulyok et al., 2020). T-2 and HT-2 were not detected in any of the composite food samples.

(s) Tunisia

Barley and barley products (soup and beer) sampled from Tunisian markets (n = 86), during 2015 and 2016, were analysed for a range of mycotoxins, including T-2 and HT-2 (Juan et al., 2017). Both GC-MS/MS and LC-MS/MS methods were described, but it is unclear which method was used to determine T-2 and HT-2. T-2 was not detected in any of the foods analysed and HT-2 was only detected in barley. Information on mean food consumption by the adult Tunisian population was used for a deterministic estimate of dietary exposure using a nominal body weight of 70 kg. Mean LB–UB dietary exposure to the sum of T-2 and HT-2 was 0.3–12.2 ng/kg bw per day for the sum of the three foods considered.

(t) United Republic of Tanzania

Samples of maize (n = 60) were analysed for a range of mycotoxins by UHPLC-ToF-MS (Kamala et al., 2017). T-2 was not detected in any samples. HT-2 was detected in 15 of 60 samples, with a mean concentration of 20 µg/kg (positive samples only). Two 24HDR questionnaires and a food frequency questionnaire were completed by mothers of 249 infants aged 6–12 months. Left-censored data were substituted by zero, half the LOD or the LOD and dietary exposure determined by Monte Carlo simulation. Mean (95th percentile) estimates of dietary exposure to HT-2 were 40 (170) ng/kg bw per day.

(u) Summary

National estimates of dietary exposure to T-2 and HT-2 described in the previous sections are summarized in Table 9. Comparability of the estimates is complicated by the different ranges of food included in the various studies. All estimates that derived LB–UB values demonstrated the considerable uncertainty in estimation of dietary exposure to T-2 and HT-2 due to the high proportion of left-censored data.

UB estimates of mean dietary exposure were <100 ng/kg bw per day for all populations studied, with the exception of dietary exposure to the sum of T-2 and HT-2 for infants in a French study (Vin et al., 2020).

Few estimates of dietary exposure to T-2 and HT-2 have been reported for non-European countries. In some cases, such as for some African countries, estimates of dietary exposure were not possible due to the absence or very low prevalence of these toxins in the foods analysed.

8.3.2 National estimates of chronic dietary exposure derived by the Committee

Additional national estimates of dietary exposure may be derived by the Committee. Previously, national estimates of chronic dietary exposure have been derived by the Committee when:

- national food consumption information was available through CIFOCOss;
- suitable concentration data have been submitted to the Committee (GEMS/Food contaminants database); and
- no existing recent dietary exposure assessment for T-2, HT-2 or the sum of T-2 and HT-2 is available for the country (section 8.3.1).

These criteria were not fully met for any country. For Burkina Faso, food consumption information and monitoring data for T-2 and/or HT-2 were available. However, only a single commodity had been monitored and no detections were reported.

Food consumption data were available for several European (including Scandinavian) countries. However, data on concentrations of T-2, HT-2, or the sum of T-2 and HT-2 were only available for the combined European Union. Recent, well-conducted estimates of chronic dietary exposure to T-2 and HT-2 are available for European countries (EFSA, 2017).

Consequently, the Committee decided not to derive additional national estimates of dietary exposure to T-2 and HT-2.

8.3.3 International estimates of dietary exposure

The available evidence suggests that contamination of the food supply with T-2 and HT-2 is unlikely or minor outside Europe and North America. The GEMS/Food cluster diets, which are used for international estimates of chronic dietary exposure, group the North American countries (Canada, the USA) with European countries (Belarus, Bulgaria, Croatia, Cyprus, Estonia, Italy, Latvia, Malta and the Russian Federation) and several other developed countries (Japan, New Zealand and the Republic of Korea) in cluster G10, whereas most other European Union countries are in clusters G07, G08, G11 and G15. The available concentration data are heavily dominated by European data and the food consumption patterns of these clusters will be aligned with European patterns. Therefore, the Committee considered that the available European estimates of dietary exposure to T-2 and HT-2 would be more informative about likely exposures in the European/North American zone than estimates based on the GEMS/Food cluster diets. Given the lack of data on concentrations in food, it was not considered appropriate to derive dietary exposure estimates for the non-European/North American clusters. Consequently, the Committee decided not to derive international estimates of dietary exposure to T-2 and HT-2.

At the fifty-sixth meeting of the Committee, international estimates of dietary exposure were only derived for the European Region. At that time, international estimates of dietary exposure were derived for five regional diets (African, European, Far Eastern, Latin American and Middle Eastern). However, the Committee considered that there were insufficient concentration data from non-European regions to allow dietary exposure estimation. For the European Region, mean estimates of dietary exposure to T-2 and HT-2 were 7.6 and 8.7 ng/kg bw per day, respectively.

8.3.4 Dietary exposures for infants

T-2 and HT-2 may potentially be transferred from mother to child through breastfeeding. Although exposure of infants to mycotoxins from maternal breast milk or infant formula only occurs for a small portion of the individual's total lifetime, infants are potentially at least as sensitive as adults to the toxicity of

contaminants. Also, their dependence on maternal breast milk or infant formula, as the sole source of nutrition, means that they are unable to avoid contamination of their food source. Therefore, infants were included in the evaluation of dietary exposure to T-2 and HT-2, where possible.

8.3.4.1 Estimated chronic dietary exposure for breastfed infants

Breast milk provides optimal nutrition and immunological benefits for infants, and the advantages of breastfeeding outweigh any possible disadvantages that may be associated with the presence of contaminants such as T-2 or HT-2 in breast milk (WHO, 2016). Very limited information is available on the T-2 and HT-2 content in breast milk.

It has been suggested that changes in dietary habits during pregnancy may result in a decrease in maternal exposure to T-2 and HT-2 (Chan-Hon-Tong et al., 2013), but it is unknown whether these changes continue into the breastfeeding period.

An Austrian study did not detect T-2 or HT-2 in 87 longitudinal breast milk samples from a single volunteer (Braun et al., 2020). However, although the LOD for T-2 was acceptably low (11 ng/L), the LOD for HT-2 was quite high (300 ng/L).

A study in Valencia, Spain, detected HT-2, but not T-2, in 10 of 35 breast milk samples (Rubert et al., 2014). Samples were collected 30 days after the mother had given birth. The mean HT-2 concentration (positive samples only) was 36.5 µg/L (range 12.2–62.5 µg/L). These results were considered to be consistent with the metabolic conversion of T-2 to HT-2.

Given that information on T-2 and HT-2 in breast milk was only available from a single study, the Committee did not prepare estimates of dietary exposure for breastfed infants.

8.3.4.2 Estimated chronic dietary exposure for fully formula-fed infants

Infant formula may be sold as a ready-to-consume liquid or as a powder that requires addition of water before consumption. Formulas with different compositions are produced for neonates from birth (starter formula) and for the period when the infant diet starts to diversify (follow-on formula). Data have been submitted to the GEMS/Food contaminants database on T-2, HT-2 or the sum of T-2 and HT-2 concentrations in a small number of starter and follow-on formulas (n = 31). No toxins were detected in any of the starter formulas or liquid follow-on formulas but were detected in two samples of powdered follow-on formula. Across all infant formula samples, the LB–UB mean concentrations were 0.005–2.1, 0.005–2.3 and 0.04–1.9 µg/kg for T-2, HT-2 and the sum of T-2 and HT-2, respectively. For powdered infant formula, results were converted to

an "as consumed" value by multiplying by 0.13. This factor was used because most common brands of infant formula are made up using about 13 g of powder to produce 100 mL of formula.

By contrast, the French infant total diet study did not detect T-2 or HT-2 in any of 28 follow-on formulas, but detected both toxins in 6 of 34 infant (starter) formulas, with LB–UB mean concentrations of 0.29–0.71 μg/kg and 0.29–0.73 μg/kg for T-2 and HT-2, respectively (Vin et al., 2020). The study by Zhang et al. (2013) did not detect T-2 in any of 18 milk-based infant formulas. However, HT-2 was not included in the analysis.

To estimate dietary exposure to mycotoxins for fully formula-fed infants, median consumption estimates for infant formula can be derived from estimated energy requirements (EERs) for fully formula-fed infants. Standard body weights and EERs for male and female infants aged 0–1, 2–3 and 5–6 months were taken from daily human energy requirements defined by FAO/WHO/United Nations University (2004). It should be noted that the EERs of formula-fed infants are greater than those of breastfed infants, although this disparity decreases with increasing age. EERs for formula-fed infants have been used here.

A further exposure scenario was considered, using high percentile daily energy intakes (95th percentile) reported for formula-fed infants (Fomon & Bell, 1993). These authors reported maximum 95th percentile energy intakes for male and female infants at 14–27 days of age of 148.7 and 146.0 kcal/kg bw per day, respectively. These energy intakes were equated to infant formula volumes using an energy density for prepared infant formula of 67 kcal/100 ml. Estimates of dietary exposure to T-2, HT-2 and the sum of T-2 and HT-2 for fully formula-fed infants are summarized in Table 10.

Dietary exposure of fully formula-fed infants to the trichothecene mycotoxins is particularly affected by uncertainty in the mean concentration of the toxins in infant formula. Consequently, while LB estimates of dietary exposure to T-2, HT-2 or the sum of T-2 and HT-2 for fully formula-fed infants are often lower than estimates for other population groups (Table 9), UB estimates are about an order of magnitude greater.

8.3.5 Chronic dietary exposure to 4,15-diacetoxyscirpenol (4,15-DAS)

At its eighty-third meeting, the Committee assessed 4,15-DAS and concluded that "4,15-DAS and T-2/HT-2 are structurally similar, and there is evidence that they cause similar effects at the biochemical and cellular levels, have similarities in toxic effects in vivo and have an additive dose effect when co-exposure occurs. Therefore, the evidence was considered sufficient by the Committee to support including 4,15-DAS in the group PMTDI for T-2 and HT-2 established at the fifty-sixth meeting of JECFA".

Table 10
Chronic dietary exposure to T-2, HT-2 and the sum of T-2 and HT-2 for fully formula-fed infants

		Estimated dietary exposure, LB–UB (ng/kg bw per day)		
Sex, age	**Summary statistic**	**T-2**	**HT-2**	**Sum of T-2 and HT-2**
Male, 0–1 month	Mean	0.9–380	0.9–420	7.2–340
Male, 2–3 months	Mean	0.8–320	0.8–350	6.1–290
Male, 5–6 months	Mean	0.6–260	0.6–290	5.0–240
Female, 0–1 month	Mean	0.9–360	0.9–390	6.8–320
Female, 2–3 months	Mean	0.8–320	0.8–350	6.0–290
Female, 5–6 months	Mean	0.6–270	0.6–290	5.1–240
Male, 14–27 days	95th percentile	1.1–470	1.1–510	8.9–420
Female, 14–27 days	95th percentile	1.1–460	1.1–500	8.7–410

LB: lower bound, UB: upper bound.

Estimates of dietary exposure to 4,15-DAS reported at that time included mean estimates from the scientific literature (≤25 ng/kg bw per day) and mean international estimates of dietary exposure (0–308 ng/kg bw per day for the lowest LB to highest UB estimates). It should be noted that the international estimates of dietary exposure were severely impacted by the high level of left-censorship in the concentration dataset.

At the eighty-third meeting of the Committee, an attempt was made to estimate combined exposure to 4,15-DAS and T-2/HT-2, by combining the LB mean dietary exposure to DAS for the European Region from the eighty-third meeting (2.8 ng/kg bw per day) with an estimate of T-2 and HT-2 dietary exposure for the European Region from the fifty-sixth meeting (16.3 ng/kg bw per day).

8.4 Assessments of acute dietary exposure

8.4.1 National estimates of acute dietary exposure from the scientific literature

Three studies have reported estimates of acute dietary exposure to T-2/HT-2 (Schothorst et al., 2005; Jekel & van Egmond, 2014; EFSA, 2017).

Acute dietary exposure was estimated for T-2, HT-2 and the sum of T-2 and HT-2 using a probabilistic technique applied to 41 dietary surveys across 23 European countries (EFSA, 2017). Available concentration data were used as empirical distributions, with concentration values for the relevant foods randomly drawn from these empirical distributions. Acute dietary exposure was assessed for each individual for each reporting day by multiplying the total

Table 11
Acute dietary exposure to T-2, HT-2 and the sum of T-2 and HT-2 for European countries

	Acute dietary exposure, median (range) across studies (ng/kg bw per day)		
Age group (number of studies)	**T-2**	**HT-2**	**Sum of T-2 and HT-2**
Mean acute dietary exposure			
Infants (6)	16.9 (10.2–40.6)	23.1 (12.9–50.9)	27.7 (15.2–54.6)
Toddlers (11)	36.2 (26.8–43.8)	47.7 (40.4–54.8)	55.7 (47.9–64.7)
Other children (20)	30.0 (20.7–41.9)	39.8 (28.5–52.6)	46.3 (35.1–62.1)
Adolescents (20)	18.2 (9.8–26.4)	24.3 (12.8–34.3)	28.1 (15.3–39.5)
Adults (22)	13.4 (9.6–18.4)	17.1 (12.5–23.4)	19.9 (14.4–26.5)
Elderly adults (16)	12.2 (9.1–16.3)	15.3 (11.9–20.3)	17.8 (13.4–23.5)
Very elderly adults (14)	13.0 (9.8–14.2)	16.7 (12.9–18.4)	18.7 (14.5–20.7)
Pregnant women (1)	(NR–15.3)	(NR–21.6)	(NR–25.7)
Lactating women (1)	(NR–11.0)	(NR–14.6)	(NR–17.3)
95th Percentile acute dietary exposure			
Infants (5)	(34.2–137)	(55.5–165)	(73.0–170)
Toddlers (11)	83.6 (59.5–96.3)	119 (97.0–133)	142 (110–154)
Other children (20)	70.9 (44.3–90.9)	96.4 (65.4–117)	116 (84.4–140)
Adolescents (20)	45.7 (21.8–60.4)	61.1 (29.3–81.1)	73.2 (36.2–100)
Adults (22)	33.6 (24.7–46.4)	44.9 (33.2–59.3)	49.8 (38.0–68.4)
Elderly adults (16)	28.9 (22.4–38.9)	38.9 (30.1–51.6)	43.1 (34.3–55.4)
Very elderly adults (14)	28.5 (24.7–36.5)	38.0 (32.8–49.9)	43.4 (37.2–55.3)
Pregnant women (1)	(NR–37.7)	(NR–57.9)	(NR–72.0)
Lactating women (1)	(NR–30.3)	(NR–41.0)	(NR–50.2)

NR: Not reported.

amount consumed for each food category by one concentration randomly drawn from the individual results available for that food category. Respective dietary exposure for each of the relevant foods consumed that day were then summed and finally divided by the individual's body weight. This process was iterated 1000 times for each reporting day. For the calculations, concentration data estimated using the UB approach were used. That is, if the value drawn from the concentration distribution was left-censored, a value equal to the analytical LOD was used in the exposure calculation. The study reported summary statistics across the various country studies for the mean and 95th percentile estimates of acute dietary exposure. These are summarized in Table 11.

Across the various surveys and countries, no more than 0.07% of exposure estimates exceeded the European acute reference dose of 300 ng/kg bw.

Complete day duplicate diet samples for young children (aged 8–12 months) from the Netherlands were collected and analysed (n = 74) for a range of trichothecene mycotoxins, including T-2 and HT-2, by GC-MS (Schothorst

et al., 2005). LODs and LOQs were 0.16 and 0.18 μg/kg for T-2 and 0.22 and 0.26 μg/kg for HT-2, respectively. HT-2 was detected in 40 of 74 duplicate diet samples (68%), whereas T-2 was detected in 14 of 74 samples (19%). Mean concentrations, assuming that analytical results below the LOD were equal to zero, were 0.054 and 0.27 μg/kg for T-2 and HT-2, respectively. The mean dietary exposure to the sum of T-2 and HT-2 was estimated to be 40 ng/kg bw per day (range 10–160 ng/kg bw per day).

A Dutch study determined T-2 and HT-2 in 128 24-hour duplicate diet portions, collected during 2011 (Jekel & van Egmond, 2014). The toxins were detectable in most of the samples, with estimated acute dietary exposures ranging from not detected to 18.6 ng/kg bw per day for the sum of T-2 and HT-2.

8.4.2 National estimates of acute dietary exposure derived by the Committee

Of the countries for which suitable information on food consumption was available for deriving national estimates of acute dietary exposure to T-2/HT-2 (FAO/WHO GIFT), only Italy is within the geographical zone where T-2/HT-2 contamination of the food supply is indicated. Assessment of acute dietary exposure for Italy would be included in the European assessment summarized above and the Committee decided not to derive additional national estimates of acute dietary exposure.

9. Comments

9.1 Analytical methods

The Committee reviewed the analytical methods for the determination of T-2 and HT-2 developed since the fifty-sixth meeting and noted considerable advances in methodology, particularly with respect to the development of multimycotoxin analytical methods based on HPLC-MS.

While TLC has largely been superseded by more modern methods, reports of its use for T-2 toxin and other trichothecenes can still be found. Screening methods, such as ELISA, lateral flow immunoassays, fluorescence polarization and various biosensors and chemosensors continue to be developed and commercialized based mainly on monoclonal antibodies. These assays can be tailored for detection of T-2 toxin alone or the sum of T-2 and HT-2 combined.

Whereas the Committee noted at its fifty-sixth meeting that GC with derivatization and detection by electron capture or MS was the primary technique for quantification, there has been a strong shift away from GC towards

the extensive use of HPLC. Depending on the extract clean-up technique, these toxins, either alone or together with other type A and B trichothecenes, can be determined by HPLC with UV or fluorescence detection. For this purpose, a number of derivatizing agents have been described.

The major advance in routine analysis since the previous Committee meeting has been the development of HPLC-MS methods, which enable simultaneous quantification and confirmation. Although capable of targeted single analyte determination, these methods can be used for multimycotoxin determination in which T-2 and HT-2 can be determined as part of a suite of toxins and/or secondary metabolites. Modern methods achieve LODs in the low or sub- μg/kg range, but require consideration of optimum conditions of extraction and extract purification to accommodate the differing chemistries of the target analytes. Two approaches for treating the extract are the "dilute-and-shoot" method in which the extract is injected into the HPLC after solvent dilution or the use of a generic clean-up (QuEChERS – quick, easy, cheap, effective, rugged and safe) to remove impurities such as lipids. A feature of MS detection, particularly with multimycotoxin determination using limited extract purification, is the occurrence of matrix effects. To overcome these problems, stable isotope-labelled internal standards or matrix-matched standards are used. Quantification can also be performed by the standard addition method. A T-2 and HT-2 certified reference material of ground oat flakes is available to aid method development and quality assurance. Modified forms of T-2 and HT-2, including numerous plant metabolites, can be identified by HPLC-MS/MS; however, validation and quantification is limited by the availability of analytical standards.

9.2 Sampling protocols

Currently, sampling methods for the analysis of T-2 and HT-2 in cereal grains use protocols for other mycotoxins. Many countries have their own sampling guidelines. For example, countries in Europe use EC 401/2006, China uses GB/T 30642-2014 and Canada and the USA have designated sampling guidelines (USDA, 1995; Canadian Grain Commission, 2015). Additional sampling guidance is available from Codex Alimentarius (CAC/GL 50-2004). In recent years, the drive towards safer food has highlighted the need to determine levels of T-2 and HT-2 contamination in different food commodities. Therefore, it is important to simplify, harmonize and validate sampling plans for T-2 and HT-2.

9.3 Effects of processing

In general, T-2 and HT-2 levels can be reduced by various processes commonly used in the food and feed industry. Cleaning and sorting are useful first steps in the reduction of T-2 and HT-2 contamination. T-2 and HT-2 are mostly located in the outer layers of cereal grains, and are recovered in higher concentrations in husk, bran and germ relative to other milling fractions. Therefore, the by-products from sorting and milling should be strictly managed. T-2 and HT-2 concentrations decrease during cooking at about 150 °C. Higher temperatures increase the extent of degradation of the toxins. Fermentation can reduce levels of contamination by T-2 and HT-2, although pH, moisture, temperature and the fermentation organisms impact concentrations.

9.4 Prevention and control

Information on the prevention and control of T-2 and HT-2 is limited to a small number of studies on a few commodities (primarily oats) and the results of these often agree with the greater volume of information available for the related trichothecene, deoxynivalenol (DON). For preharvest mitigation, decreased concentrations of T-2 and HT-2 are associated with having fewer cereals in rotation and growing resistant cultivars. Ploughing may also be beneficial, depending on the position of the host crops in the rotation. Unlike with DON, growing maize as a previous crop is not a risk factor and limited studies indicate fungicides do not reduce T-2 and HT-2 contamination. For postharvest mitigation, prevention of further T-2 and HT-2 production by *Fusarium* species is achieved by storing commodities at low moisture content. Various microbes, enzymes and chemicals have demonstrated ability to metabolize or degrade T-2 and/or HT-2, but these have been mainly tested in liquids and may not be technically feasible for most foodstuffs.

9.5 Levels and patterns of contamination in food commodities

When T-2 and HT-2 were assessed previously (Annex 1, reference 152) the percentages of analyses from 1990–2000 (n = 999) that exceeded 100 µg/kg were 0.4% and 0.9% for T-2 and HT-2, respectively. The value of 100 µg/kg was used by the Committee at the fifty-sixth meeting to allow comparison to a previous study (WHO, 1990) due to the wide range of LODs, which decreased over time. In the current assessment of data from the GEMS/Food contaminants database, there were 49 912 samples that had been analysed for T-2 and HT-2 from 2001–2020. Within this dataset, 0.8% and 1.5% of samples exceeded 100 µg/kg T-2 and HT-

2, respectively. It cannot be determined if these increases in reported frequency of high concentrations of T-2 and HT-2 are due to increases in the mycotoxin concentrations over time or to a greater focus on sampling in regions and/or commodities with higher levels of T-2 and HT-2.

Based on data from the GEMS/Food contaminants database, comparison of analyses for T-2 and HT-2 across global regions has identified stark differences in the number of tests reported, the distribution of foodstuffs analysed and the analytical results. Most of the analytical records were submitted by the European Region, with limited numbers submitted by a small number of countries within the other regions. Some of these countries only submitted results for a single foodstuff (cassava from the USA and sorghum from four African countries). Three countries in the Western Pacific Region submitted analytical results for a wide variety of foodstuffs, but they were mostly negative. Canada also submitted results for a wide variety of foodstuffs, with 1.5% positive samples, a LB mean concentration of 0.6 μg/kg, and a few samples with greater than 100 μg/kg combined T-2 and HT-2. In contrast to this, T-2 and HT-2 levels reported in Europe were much higher in cereals and any food category that does or may contain cereals. More detailed analysis of the European dataset showed that the highest levels were detected in oats, maize, barley and wheat grain (LB mean concentrations of 241, 24, 17 and 5.2 μg/kg respectively) with significantly lower concentrations occurring in milled products, excluding bran and by-products.

Although limited in quantity, the literature generally supported the conclusion that T-2 and HT-2 levels are low in all regions of the world outside of Europe. For example, a total diet study in sub-Saharan Africa analysed composite food samples (n = 194) representing food intake at eight locations across four countries (Benin, Cameroon, Mali and Nigeria) for numerous mycotoxins (Ingenbleek et al., 2019b). None of the samples had detectable T-2 or HT-2 (LOD = 0.4 and 0.8 μg/kg respectively).

As with other Fusarium mycotoxins that are produced within the growing crop, their concentrations will fluctuate between growing seasons and regions, depending on climatic conditions. Most studies reporting T-2 and HT-2 concentrations are based on single-year surveys and the effect of seasonal variability cannot be assessed. A 7-year (2002–2008) investigation of Fusarium mycotoxins in harvested oats in the United Kingdom showed that the annual combined mean concentration of T-2 and HT-2 ranged from 121 to 727 μg/kg (Edwards, 2017).

Recent studies have identified numerous modified mycotoxins which are the result of metabolism in planta; some have also been found to exist in naturally contaminated material. T-2 tetraol and HT2-3-glucoside can occur at high concentrations compared to the parent mycotoxins. There are also several other metabolites that occur individually at low concentrations compared to

the parent molecules, but may collectively contribute significantly to the overall type A trichothecene occurrence in cereals and cereal products. In recent studies using host plants inoculated with isotope-labelled mycotoxins, 70–85% of the inoculated T-2 or HT-2 was metabolized (Meng-Reiterer et al., 2015; Nathanail et al., 2015; Meng-Reiterer et al., 2016).

9.6 Food consumption and dietary exposure estimates

9.6.1 Chronic dietary exposure

Since the previous evaluation, a number of national and regional estimates of chronic dietary exposure have been published. The Committee considered evaluations from Belgium, China, the Czech Republic, Ecuador, Europe, France, Ireland, Malawi, Morocco, the Netherlands, New Zealand, Nigeria, Pakistan, Romania, Serbia, Spain, Sweden, sub-Saharan Africa, Tunisia and the United Republic of Tanzania. These reports include dietary exposure assessments for T-2 (12 studies), HT-2 (14 studies), and the sum of T-2 and HT-2 (12 studies). In several studies, these toxins were not detected or were detected so infrequently that dietary exposure could not be estimated. The estimates of dietary exposure reviewed mainly related to European and North African countries. Table 12 provides a summary of the range of exposure estimates derived from the scientific literature. Exposure estimates have been further separated into those pertaining to children, including infants and toddlers and those pertaining to adults or the general population. Dietary exposure estimates have mostly been presented as ranges from a LB to a UB. LB estimates are generally based on mean toxin concentrations calculated with results below the LOD or LOQ being assigned a value of zero. UB estimates are generally based on mean toxin concentrations calculated with results below the LOD or LOQ being assigned a value equal to the LOD or LOQ. Across studies, the foods that contributed most to chronic dietary exposure to T-2 and HT-2 are cereals and cereal-based products, particularly wheat and wheat-based products.

Based on the observed geographical distribution of T-2 and HT-2 contamination of foods (mainly Europe and North America) and available information on food consumption, the Committee, at its current meeting, decided it was unnecessary to derive additional national estimates of chronic dietary exposure to T-2 and HT-2.

At the current meeting, the Committee did not present international estimates of dietary exposure to either toxin or the sum of the toxins using the GEMS/Food cluster diets. It was concluded that dietary exposure to T-2 and HT-2 for clusters covering the known geographical distribution of T-2 and HT-2 was suitably covered by existing European estimates of chronic dietary exposure

Table 12
Summary of the range of estimates of chronic dietary exposure to T-2, HT-2 and the sum of T-2 and HT-2, derived from the literature[a]

Toxin/population group[a]	Estimated dietary exposure, range[b] (ng/kg bw per day)			
	Mean		High percentile[c]	
	LB	UB	LB	UB
T-2				
Children	0.4-26	13-79	5.7 d-150	27-200
Adults	0.1-6.4	9.1-24	1.6-29	16-66
HT-2				
Children	0.0-27	4.1-91	3.6-64	15-240
Adults	0.0-14	0.4-33	2.4-23	14-59
Sum of T-2 and HT-2				
Children	0.8-53	8.2-169	6.5-210	31-400
Adults	0.3-27	2.7-60	1.9-87	11-120

LB, lower bound; UB, upper bound.
[a] For the purpose of this summary table, "children" were taken to be any population group described as infants, toddlers or children. The maximum age for children varies from study to study, but in all cases "children" will refer to individuals aged 15 years or younger. "Adults" were taken to be any population group described as adults, adolescents, elderly or very elderly. The minimum age for adults varies from study to study, but in all cases "adults" will refer to individuals older than 10 years.
[b] Ranges are presented separately for lower and upper bound estimates of mean and high percentile estimates of dietary exposure.
[c] 95th percentile, unless otherwise indicated.
[d] 90th percentile.

and no international estimates of chronic dietary exposure were derived by the Committee.

9.6.2 Acute dietary exposure

Three studies reported in the scientific literature estimated acute dietary exposure to T-2, HT-2 or the sum of T-2 and HT-2. Two of the studies were duplicate diet studies carried out in the Netherlands, whereas the third study, by EFSA, estimated acute dietary exposure for a range of European countries. The EFSA study estimated maximum UB 95th percentile acute dietary exposures to T-2, HT-2 and the sum of T-2 and HT-2 of 137, 165 and 170 ng/kg bw, respectively (EFSA, 2017). These estimates were for infant cohorts and acute dietary exposure decreased with increasing age. The duplicate diet studies estimated mean acute dietary exposure to the sum of T-2 and HT-2 for young children (8–12 months) of 40 ng/kg bw (range 10–160 ng/kg bw). For 128 adults, acute dietary exposure to the sum of T-2 and HT-2 was in the range not detected to 18.6 ng/kg bw.

The Committee did not present additional national estimates of acute dietary exposure.

9.6.3 Combined dietary exposure

At the eighty-third meeting, JECFA assessed 4,15-diacetoxyscirpenol (DAS) and concluded that DAS was similar in structure and toxic effects to T-2 and HT-2. At that time, DAS was included in the group PMTDI for T-2 and HT-2. A combined LB mean dietary exposure estimate for the three toxins for the European Region could be derived. Depending on the cohort, the median LB dietary exposures to the sum of T-2 and HT-2 are in the range of 3.0 to 15 ng/kg bw per day. The estimated LB mean dietary exposure to DAS determined at the eighty-third meeting was 3 ng/kg bw per day, giving combined group (T-2, HT-2 and DAS) dietary exposure estimates of 6.0 to 18 ng/kg bw per day.

10. Evaluation

The Committee reviewed the information regarding analytical methods, sampling, effect of processing, prevention and control, occurrence in food commodities and dietary exposure since the last evaluation of T-2 and HT-2 at the fifty-sixth meeting in 2001 (Annex 1, reference 152). Analytical methods have been improved in the past two decades with multimycotoxin HPLC-MS methods allowing the quantification of T-2 and HT-2 at concentrations below or close to 1 µg/kg. Abundant occurrence data for T-2 and HT-2 had been submitted to the GEMS/Food contaminants database in the past two decades but these were largely from Europe and data from other regions were scarce. This may be due to the generally low incidence and low concentrations of T-2 and HT-2 found outside Europe. In Europe, T-2 and HT-2 occur frequently in cereal crops, particularly in oats. There is also evidence of co-occurrence of several other type A trichothecenes and their metabolites in cereals. It was concluded that dietary exposure to T-2 and HT-2 covering the known geographical distribution of T-2 and HT-2 was suitably covered by existing European estimates of chronic and acute dietary exposure. No additional international or national estimates of chronic or acute dietary exposure were derived by the Committee. The Committee derived dietary exposure estimates of 6.0 to 18 ng/kg bw per day for T-2, HT-2 and DAS combined. The toxicological evaluation and overall risk assessment will follow at a future meeting.

10.1 Recommendations

The Committee recommended the following:

- development of multimycotoxin methods and standards for the quantification of type A trichothecenes and their various metabolites that occur in planta;
- research to investigate the spatial distribution of T-2 and HT-2 in agricultural commodities to ensure standard sampling methods for mycotoxins are appropriate;
- that occurrence data from a wider range of countries be generated using analytical methods with suitably low LODs, to decrease the uncertainty in dietary exposure estimates and confirm the geographical distribution of these toxins.

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ANNEX 1

Reports and other documents resulting from previous meetings of the Joint FAO/WHO Expert Committee on Food Additives

1. General principles governing the use of food additives (First report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Report Series, No. 15, 1957; WHO Technical Report Series, No. 129, 1957 (out of print).
2. Procedures for the testing of intentional food additives to establish their safety for use (Second report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Report Series, No. 17, 1958; WHO Technical Report Series, No. 144, 1958 (out of print).
3. Specifications for identity and purity of food additives (antimicrobial preservatives and antioxidants) (Third report of the Joint FAO/WHO Expert Committee on Food Additives). These specifications were subsequently revised and published as Specifications for identity and purity of food additives, Vol. I. Antimicrobial preservatives and antioxidants, Rome, Food and Agriculture Organization of the United Nations, 1962 (out of print).
4. Specifications for identity and purity of food additives (food colours) (Fourth report of the Joint FAO/WHO Expert Committee on Food Additives). These specifications were subsequently revised and published as Specifications for identity and purity of food additives, Vol. II. Food colours, Rome, Food and Agriculture Organization of the United Nations, 1963 (out of print).
5. Evaluation of the carcinogenic hazards of food additives (Fifth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Report Series, No. 29, 1961; WHO Technical Report Series, No. 220, 1961 (out of print).
6. Evaluation of the toxicity of a number of antimicrobials and antioxidants (Sixth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Report Series, No. 31, 1962; WHO Technical Report Series, No. 228, 1962 (out of print).
7. Specifications for the identity and purity of food additives and their toxicological evaluation: emulsifiers, stabilizers, bleaching and maturing agents (Seventh report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 35, 1964; WHO Technical Report Series, No. 281, 1964 (out of print).
8. Specifications for the identity and purity of food additives and their toxicological evaluation: food colours and some antimicrobials and antioxidants (Eighth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 38, 1965; WHO Technical Report Series, No. 309, 1965 (out of print).
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10. Specifications for identity and purity and toxicological evaluation of food colours. FAO Nutrition Meetings Report Series, No. 38B, 1966; WHO/Food Add/66.25.

11. Specifications for the identity and purity of food additives and their toxicological evaluation: some antimicrobials, antioxidants, emulsifiers, stabilizers, flour treatment agents, acids, and bases (Ninth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 40, 1966; WHO Technical Report Series, No. 339, 1966 (out of print).

12. Toxicological evaluation of some antimicrobials, antioxidants, emulsifiers, stabilizers, flour treatment agents, acids, and bases. FAO Nutrition Meetings Report Series, No. 40A, B, C; WHO/Food Add/67.29.

13. Specifications for the identity and purity of food additives and their toxicological evaluation: some emulsifiers and stabilizers and certain other substances (Tenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 43, 1967; WHO Technical Report Series, No. 373, 1967.

14. Specifications for the identity and purity of food additives and their toxicological evaluation: some flavouring substances and non nutritive sweetening agents (Eleventh report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 44, 1968; WHO Technical Report Series, No. 383, 1968.

15. Toxicological evaluation of some flavouring substances and non nutritive sweetening agents. FAO Nutrition Meetings Report Series, No. 44A, 1968; WHO/Food Add/68.33.

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17. Specifications for the identity and purity of food additives and their toxicological evaluation: some antibiotics (Twelfth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 45, 1969; WHO Technical Report Series, No. 430, 1969.

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22. Evaluation of food additives: specifications for the identity and purity of food additives and their toxicological evaluation: some extraction solvents and certain other substances; and a review of the technological efficacy of some antimicrobial agents (Fourteenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 48, 1971; WHO Technical Report Series, No. 462, 1971.

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of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 50, 1972; WHO Technical Report Series, No. 488, 1972.

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28. Specifications for the identity and purity of some enzymes and certain other substances. FAO Nutrition Meetings Report Series, No. 50B, 1972; WHO Food Additives Series, No. 2, 1972.

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30. Evaluation of certain food additives and the contaminants mercury, lead, and cadmium (Sixteenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 51, 1972; WHO Technical Report Series, No. 505, 1972, and corrigendum.

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59. Evaluation of certain food additives and contaminants (Twenty-sixth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 683, 1982.

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62. Evaluation of certain food additives and contaminants (Twenty-seventh report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 696, 1983, and corrigenda.

63. Toxicological evaluation of certain food additives and contaminants. WHO Food Additives Series, No. 18, 1983.

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ANNEX 2

Abbreviations and acronyms used in the monographs

ADI	acceptable daily intake
bw	body weight
CAC	Codex Alimentarius Commission
CAS	Chemical Abstracts Service
CCCF	Codex Committee on Contaminants in Foods
CCFO	Codex Committee on Fats and Oils
CIFOCOss	Chronic Individual Food Consumption Database – Summary statistics
CHO	Chinese hamster ovary
CONTAM	European Food Safety Authority (EFSA) Panel on Contaminants in the Food Chain
CPSC	Consumer Product Safety Commission
ECHA	European Chemicals Agency
EFSA	European Food Safety Authority
ELSO	epoxidized linseed oil
EPA	(United States) Environmental Protection Agency
ESBO	epoxidized soybean oil
ETBE	ethyl tertiary butyl ether
EU	European Union
FAO	Food and Agriculture Organization of the United Nations
GC-FID	gas chromatography with flame ionization detection
GC-MS	gas chromatography–mass spectrometry
FCID	Food Commodity Intake Database (US Environmental Protection Agency)
GEMS/Food	Global Environment Monitoring System, Food Contamination Monitoring and Assessment Programme
HBGV	health-based guidance value
IMO	International Maritime Organization
JECFA	Joint FAO/WHO Expert Committee on Food Additives
LC-GC-FID	on-line coupled liquid chromatography-gas chromatography-flame ionization detection
LC-HRMS	liquid chromatography-high resolution mass spectrometry
LC-MS/MS	liquid chromatography with tandem mass spectrometry
LD_{50}	median lethal dose
MTBE	methyl tertiary butyl ether

MOAH	mineral oil aromatic hydrocarbons
MOE	margin of exposure
MOH	mineral oil hydrocarbons
MOSH	mineral oil saturated hydrocarbons
MTDI	maximum tolerable daily intake
NOAEL	no-observed-adverse-effect level
OECD	Organisation for Economic Co-operation and Development
p95	95th percentile
PBTK	physiologically based toxicokinetic
PCB	polychlorinated biphenyl
QSAR	quantitative structure-activity relationship
RP	reference point
SCF	EU Scientific Committee on Food
SIDS	Screening Information Dataset
TBA	tertiary butyl alcohol
TDI	tolerable daily intake
TRS	Technical Report Series
UL	upper intake level
USA	United States of America
WHO	World Health Organization

ANNEX 3

Participants in the ninetieth meeting of the Joint FAO/ WHO Expert Committee on Food Additives

Virtual meeting 26 October to 6 November 2020

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Dr R.C. Cantrill, Halifax, Nova Scotia, Canada (*Vice-chairperson*)

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This volume contains monographs prepared at the ninetieth meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), which met virtually online from 26 October to 6 November 2020.

The detailed monographs in this volume summarize the technical, analytical, dietary exposure and toxicological data on a number of groups of previous cargo contaminants: group 2 – alcohols, group 5 – butyl ethers, group 3 – oils and waxes and group 4 – solutions. It also contains an addendum on the trichothecenes, T-2 and HT-2.

This volume and others in the WHO Food Additives`series contain information that is useful to those who produce and use food additives and veterinary drugs and those involved with controlling contaminants in food, government and food regulatory officers, industrial testing laboratories, toxicological laboratories and universities.

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