October 6, 2017

Dr. Paulette Gaynor
Office of Food Additive Safety (FHS-200)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Campus Drive
College Park, MD 20740

Re: GRAS Notice for Dimethyl Ether

Dear Dr. Gaynor

In accordance with 21 CFR §170 Subpart E consisting of §170.203 through 170.285, Callaghan Innovation hereby informs the United States Food and Drug Administration of the conclusion that the intended use of dimethyl ether as an extraction solvent in the processing of various food products is Generally Recognized as Safe (GRAS) as described in the enclosed notice, and thereby the intended use of dimethyl ether is not subject to the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act. I hereby certify that the enclosed electronic files were scanned for viruses prior to submission and are thus certified as being virus-free using McAfee VirusScan 8.8.

Should you have any questions or concerns regarding this GRAS Notice, please do not hesitate to contact me at any point during the review process so that we may provide a response in a timely manner.

Yours sincerely

(b) (6)

Stephen Tallon, Ph.D.
Senior Research Engineer
Callaghan Innovation

GRAS Notice for the Use of Dimethyl Ether as an Extraction Solvent

Prepared for:

Office of Food Additive Safety (FHS-200)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Campus Drive
College Park, MD
20740

Submitted by:

Callaghan Innovation
69 Gracefield Road
Lower Hutt 5040
New Zealand

October 6, 2017

GRAS Notice for the Use of Dimethyl Ether as an Extraction Solvent

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GRAS Notice for the Use of Dimethyl Ether as an Extraction Solvent

Part 1. §170.225 Signed Statements and Certification

In accordance with 21 CFR §170 Subpart E consisting of §170.203 through 170.285, Callaghan Innovation hereby informs the U.S. (United States) Food and Drug Administration (FDA) of the view that the use of dimethyl ether is not subject to the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act, based on Callaghan Innovation's conclusion that the notified substance is Generally Recognized as Safe (GRAS) under the conditions of its intended use described in Part 1.3 below. In addition, as a responsible official of Callaghan Innovation, Stephen Tallon, hereby certifies that all data and information presented in this notice constitutes a complete, representative, and balanced submission, and which considered all unfavorable as well as favorable information known to Callaghan Innovation and pertinent to the evaluation of the safety and GRAS status of dimethyl ether as an extraction solvent in the processing of food ingredients, as described herein.

Signed,

(b) (6)

9-10-17

Stephen Tallon, Ph.D.
Senior Research Engineer
Callaghan Innovation

Date

1.1 Name and Address of Notifier

Stephen Tallon, Ph.D.
Senior Research Engineer
Callaghan Innovation
69 Gracefield Road
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E-Mail: Stephen.Tallon@callaghaninnovation.govt.nz

1.2 Common Name of Notified Substance

Dimethyl ether

1.3 Conditions of Use

Dimethyl ether is intended for use as an extraction solvent in the processing of various food products including for example, marine lipids, egg phospholipids, bacteria-derived lipids, algae-derived lipids, plant lipids, egg proteins, plant proteins, meat proteins, and fruit sugars, which can then be added to food and dietary supplements. The intended use level of dimethyl ether in the extraction process is 5 to 6 volumes of dimethyl ether per unit volume of starting material (% v/v). Dimethyl ether is recycled internally and recovered, such that the net loss of dimethyl ether is approximately 2 to 5% of the amount of the extracted food products. Residual dimethyl ether that is not recovered in the process is diluted with air so that it is below its flammability limit and vented to the atmosphere.

The ingredients produced from the dimethyl ether-extracted food products may be used in the production of final food products and supplements at levels of approximately 0.5 to 10%, with some dehydrated or defatted foods potentially containing higher levels of the dimethyl ether-extracted product.

1.4 Basis for GRAS

Pursuant to 21 CFR § 170.30 (a) and (b) of the *Code of Federal Regulations* (CFR), the dimethyl ether, as used by Callaghan Innovation, has been concluded to have GRAS status for use as an extraction solvent in the processing of various food products, as described in Part 1.3, on the basis of scientific procedures.

1.5 Availability of Information

The data and information that serve as the basis for this GRAS Notification will be made available to the FDA for review and copying upon request during business hours at the offices of:

Callaghan Innovation
69 Gracefield Road
Lower Hutt 5040
New Zealand

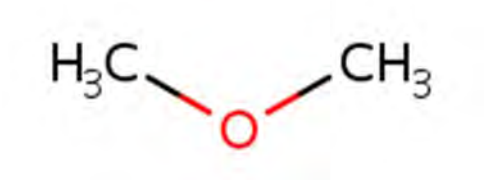
In addition, should the FDA have any questions or additional information requests regarding this notification during or after the Agency's review of the notice, Callaghan Innovation will supply these data and information as requested.

1.6 Freedom of Information Act, 5 U.S.C. Section 552

It is Callaghan Innovation's view that all data and information presented in parts 2 through 7 of this notice do not contain any trade secret, commercial, or financial information that is privileged or confidential, and therefore all data and information presented herein are not exempt from the Freedom of Information Act, 5 U.S.C. Section 552.

Part 2. §170.230 Identity, Method of Manufacture, Specifications, and Physical or Technical Effect

2.1 Identity

Chemical Name:	Methoxymethane
Other Names:	Dimethyl ether; Dimethyl oxide; oxybismethane; wood ether
Trade Names	Dymel A®, Demeon D®, Propel
Chemical Abstracts Service (CAS) Number:	115-10-6
Molecular Weight:	46.07 g/mol
Chemical Structure:	

2.2 Method of Manufacturing

2.2.1 Raw Materials and Processing Aids

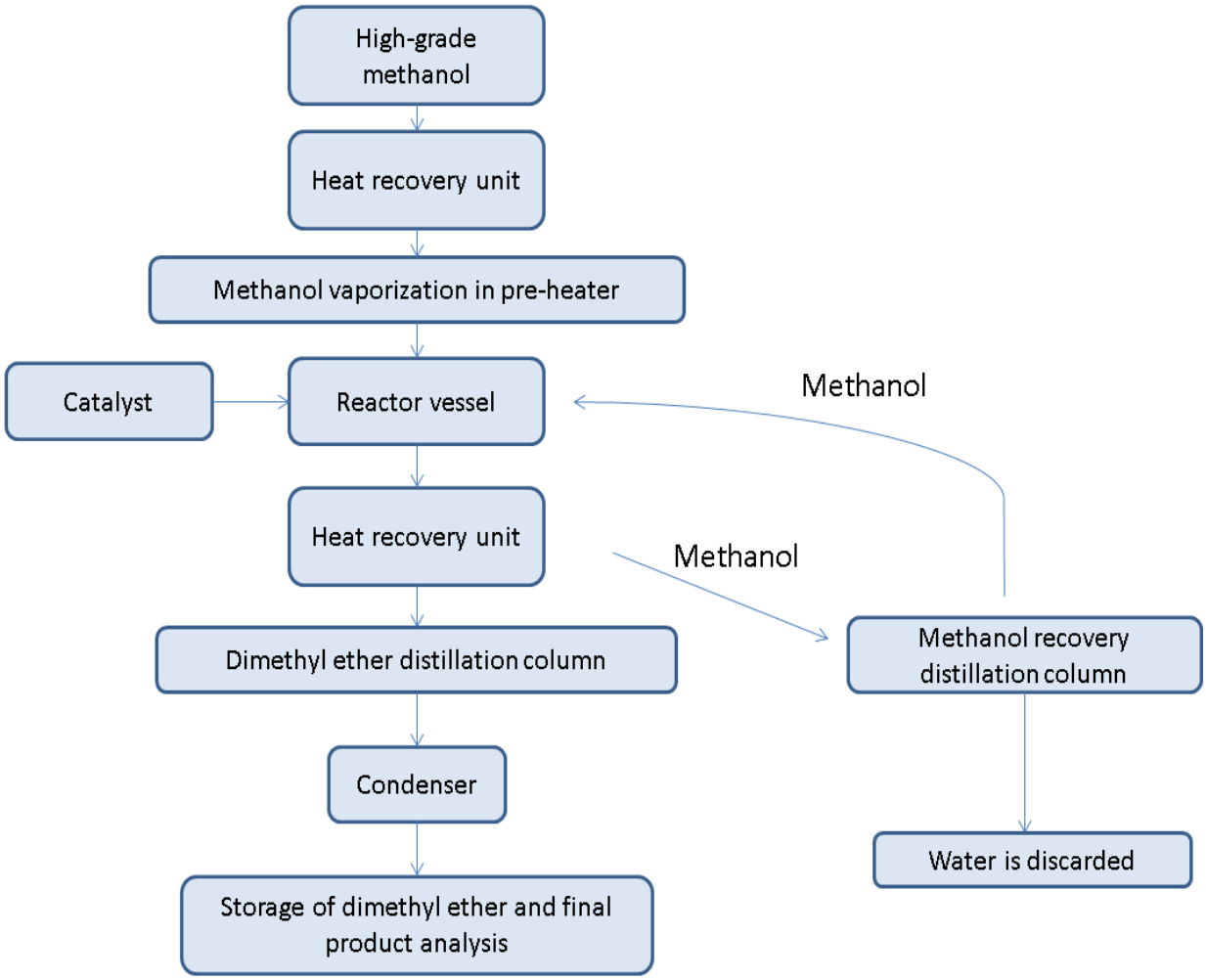
The dimethyl ether used by Callaghan is produced by catalytic dehydration of methanol using a standard catalyst, such as γ-Al_2O_3, metal alloys, transition metals, or a strong acid (sulfuric acid) (Bai *et al.,* 2013; Jamshidi *et al.,* 2013). The catalyst is removed from the dimethyl ether following production of the solvent. No other reactants or processing aids are used in the manufacture of dimethyl ether. Methanol, which is the main reactant in the manufacture of dimethyl ether, has regulatory status in the manufacture of human foods in the U.S. under 21 CFR §173.250 and §175.105.

2.2.2 Manufacturing Process

The dimethyl ether used by Callaghan Innovation is produced by catalytic dehydration of methanol under high temperature and pressure conditions. In the first step, pure, high-grade methanol is passed through a heat recovery unit and vaporized in a pre-heater. Next, 2 parts of

methanol vapor are reacted in a fixed-bed reactor vessel under pressure and at elevated temperatures in the presence of a catalyst to yield dimethyl ether and water. The reaction products (*i.e.,* dimethyl ether and water, and residual methanol) are then passed to a dimethyl ether distillation column *via* a heat recovery unit where dimethyl ether is separated from methanol and water. Dimethyl ether is passed through a condenser prior to storage, while methanol and water are separated in a methanol recovery distillation column. Methanol is recycled back into the reactor vessel and water is discarded. The dimethyl ether is analyzed to ensure compliance with product specifications. The manufacturing process consistently produces dimethyl ether with high purity (≥99.99%) (see Section 2.3.2). The final product is stored in food-grade dedicated gas cylinders or stainless steel tanks in a well-ventilated area away from heat, possible ignition sources, oxygen cylinders and other oxidizing materials. A schematic overview of the manufacturing process of the dimethyl ether is shown in Figure 2.2.2-1.

Figure 2.2.2-1 Schematic Overview of the Manufacturing Process for the Dimethyl Ether



2.3 Product Specifications and Batch Analyses

2.3.1 Product Specifications

Appropriate food-grade product specifications have been established for dimethyl ether and are presented in Table 2.3.1-1 below. The product specifications are similar to the specifications of the dimethyl ether evaluated by the European Food Safety Authority (EFSA) in their review of dimethyl ether for use as a processing aid (EFSA, 2009, 2015). Dimethyl ether is supplied and stored in a liquid state in a pressurized cylinder, and therefore a specification parameter for color is included in the product specifications.

Table 2.3.1-1 Product Specifications for Dimethyl Ether

Parameter	Specification
Purity	≥99.99%
Color (APHA)[1]	NMT 5
Methanol	<10 mg/kg
Water	<100 mg/kg
C1-C4 hydrocarbons (alkanes and CO_2)	<100 mg/kg

APHA = American Public Health Association; CO_2 = carbon dioxide; NMT = not more than

[1] Color of liquid phase when stored as a liquid under pressure.

2.3.2 Batch Analyses

Five (5) non-consecutive batches of dimethyl ether were analyzed to verify that the manufacturing process produces a consistent product that meets the product specifications. The results of the batch analyses are provided in Table 2.3.2-1.

Table 2.3.2-1 Summary of Product Analysis for 5 Non-Consecutive Batches of the Dimethyl Ether

Parameter	Specification	Manufacturing Lot No. (Date of Analysis)				
		Lot 310790 (03/04/2016)	Lot 310996 (01/05/2016)	Lot 310482 (05/06/2016)	Lot TSC0224497 (11/09/2016)	Lot TSC0224488 (14/08/2016)
Purity (%)	≥99.99	≥99.99	≥99.99	≥99.99	≥99.99	≥99.99
Color (APHA)[1]	NMT 5	NMT 5	NMT 5	NMT 5	NMT 5	NMT 5
Methanol (mg/kg)	<10	<10	<10	<10	<10	<10
Water (mg/kg)	<100	<100	<100	<100	<100	<100
C1-C4 hydrocarbons (alkanes and CO_2) (mg/kg)	<100	<100	<100	<100	<100	<100

APHA = American Public Health Association; CO_2 = carbon dioxide; NMT = not more than

[1] Color of liquid phase when stored as a liquid under pressure.

2.3.3 Additional Analytical Information

2.3.3.1 Non-volatile Impurities

Non-volatile residues in dimethyl ether (less than 0.01%) obtained from two different manufacturers were analyzed by quadrupole-time-of-flight mass spectrometry (QToF-MS). Briefly, a sample of dimethyl ether is slowly evaporated into the atmosphere. The valve assembly is removed, the cylinder rinsed with ACS-grade absolute ethanol, and the sample is analyzed by QToF-MS *via* infusion in both negative and positive modes. The major peaks observed in the ethanol phase were faintly present in the evaporated dimethyl ether extracts, indicating no contamination.

The results of the analysis showed the presence of phthalates (sodium adduct of diisooctyl phthalate, didodecyl phthalate, and dibutyl phthalate), bis(butyldiglycol)adipate, various polyethylene glycols and polypropylene glycols, and a polyfluorinated compound that was not fully identified at low concentrations. The presence of these compounds were attributed to the seals of the storage container. It should be noted that dimethyl ether is recycled during the extraction process, which would limit the introduction of additional non-volatile residues into the food ingredient.

2.4 Stability

Unsubstituted ethers, such as dimethyl ether, are stable compounds due to the presence of alpha-hydrogens that protect the compound against oxidation that cause the formation of peroxides (Naito *et al.,* 2005; Sakuth *et al.,* 2012). Dimethyl ether has been shown to be chemically stable in that no peroxide formation occurred at temperatures up to 353K (~80°C) (Naito *et al.,* 2005), while spontaneous degradation of dimethyl ether has only been observed in the presence of oxygen at temperatures higher than 500K (Rosado-Reyes *et al.,* 2005). Since the food product extraction process is performed in a closed environment with temperatures maintained at 40 to 50°C, there is limited potential for peroxide formation (see Section 3.1 for further details). The dimethyl ether used by Callaghan Innovation is manufactured in a closed environment, thereby limiting the presence of oxygen and ultimately the potential for peroxide formation. Dimethyl ether is not expected to undergo auto-oxidation. Following the manufacturing process, the dimethyl ether is stored in dedicated gas cylinders or tanks in a well-ventilated area away from sources of heat, ignition, and oxidizing compounds. Dimethyl ether does not degrade or contain any contaminants as demonstrated by analytical data showing the purity and absence of C1-C4 hydrocarbons, methanol, and water in the dimethyl ether (see Table 2.3.2-1). Furthermore, dimethyl ether is not expected to form reaction by-products with food substances due to its stable nature and is absent from various extracted food products (Table 3.2-1).

Part 3. §170.235 Dietary Exposure

3.1 Intended Conditions of Use of the Dimethyl Ether and Proposed Use Levels

Dimethyl ether is a powerful polar solvent as its density is similar to liquids and its viscosity is similar to gases. Callaghan Innovation intends to use dimethyl ether as an extraction solvent in the processing of various food products, such as preparing lipid extracts and defatted (non-lipid) products, derived from marine and animal products, plants, fruits, seeds, and micro-organisms. Examples of lipid extract products include marine lipids, egg phospholipids, bacteria-derived lipids, algae-derived lipids, and plant lipids. Examples of defatted (non-lipid) products include egg proteins, plant proteins, meat proteins, and fruit sugars. After processing, these food extracts may then be added to food and dietary supplements.

A general description of a typical extraction process is described as follows. The food or food product is loaded into stainless steel extraction vessels and liquid dimethyl ether is added (pressurized to 40 bar and heated to 40 to 50°C). The dimethyl ether phase containing the dissolved polar and non-polar extracts is passed through separation vessels where the pressure is decreased until the extracts are no longer soluble in dimethyl ether. The extract and dimethyl ether is recovered from the separation vessel; the extract is removed, whereas dimethyl ether is recycled back through the extraction vessel. The intended use level of dimethyl ether in the production process is 5 to 6 volumes of dimethyl ether per unit volume of the starting food product material. Dimethyl ether is recycled internally and recovered such that the net loss of dimethyl ether is approximately 2 to 5% of the amount of the extracted food product, which is evaporated out of the food product and recycled internally. The dimethyl ether-extracted food product may be used in the production of final food products at levels of approximately 0.5 to 10%, with some dehydrated or defatted foods containing higher levels of the dimethyl ether-extracted products.

3.2 Residue Levels of the Dimethyl Ether in Extracted Food Products

Residual levels of dimethyl ether in various extracted food products were measured by gas chromatography-mass spectroscopy (GC-MS) or flame ionization detection (GC-FID). The residue levels of dimethyl ether were below the detection limit (2 ppm). As shown in Table 3.2-1 below, dimethyl ether was not detected in a number of extracted food products. Due to the chemical nature of dimethyl ether, the solvent dissipates from both lipid extracts and residual lipid-depleted biomass at room temperature and atmospheric conditions. It should be noted that no chemical by-products are expected to form in the extracted food products.

Table 3.2-1 Residual Levels of Dimethyl Ether in Various Extracted Food Products		
DME Extracted Product	**Time of Measurement**	**Residue Level**
Lipase enzyme powder	24 hours after processing	ND
Fermented bacterial biomass	24 hours after processing	ND
DME extract from fermented biomass	Immediately after processing	ND
Defatted aqueous protein/lipid solution	After spray drying	ND
Extract from aqueous protein/lipid solution fraction	After rotary evaporation of water	ND

DME = dimethyl ether; ND = not detected

3.3 Estimated Dietary Exposure to Dimethyl Ether

Dimethyl ether is intended to be used in the extraction of food products that may then be used in the production of final food products at levels of 0.5 to 10%. As shown in Section 3.2, dimethyl ether was not detected in a number of extracted food products (*i.e.,* residue levels below the detection limit of 2 ppm). Assuming a 60-kg individual consumes 1 kg of food per day and the dimethyl ether-extracted food product is consumed as opposed to further processing into final food products (*i.e.,* the consumption level is 100%), and dimethyl ether is present at the detection limit (2 ppm), the dietary exposure to dimethyl ether is estimated to be 0.033 mg/kg body weight/day. It should be noted that this dietary exposure estimate is highly conservative in that it assumes dimethyl ether is not removed from the extracted food product and the extracted food product is consumed (without further processing in a final food product), with both of these scenarios being extremely unlikely.

EFSA estimated the dietary exposure to dimethyl ether in two worst-case scenarios based on the maximum residual levels in defatted animal protein products and gelatin (EFSA, 2009, 2015). In their evaluation, the EFSA Panel calculated an intake of 1.75 µg/kg body weight/day[1] based on the use of dimethyl ether-extracted collagen up to 3.5% of overall food intake (EFSA, 2015). In addition, the EFSA Panel calculated an intake of 0.014 µg/kg body weight/day based on the use of a dimethyl ether-extracted gelatin at levels up to 9% of overall food intake (EFSA, 2015). The EFSA Panel noted that given the conservative nature of the dietary estimate, the estimate for adults also "adequately cover the potential exposure of other age groups, including children".

As shown in Table 3.2-1, dimethyl ether was not detected in various extracted food products at various time points (immediately or 24 hours after processing, or downstream of the processing method). The results of this analysis show that dimethyl ether was below the limit of detection of 2 ppm, demonstrating that the solvent is removed or volatilizes from the extracted food product. In consideration that these food products would be further processed into final food products, it is unlikely that the consumer of such products would be exposed to any detectable

[1] The daily intake was estimated based on a proposed maximum residual level of 3 mg/kg in collagen, which is used up to 3.5% of food and a 60-kg individual consumes 1 kg food/day (EFSA, 2015).

level of dimethyl ether. Therefore, it is not expected that the consumer will be exposed to dimethyl ether at any appreciable level.

Part 4. §170.240 Self-Limiting Levels of Use

No known self-limiting levels of use are associated with the dimethyl ether.

Part 5. §170.245 Experience Based on Common Use in Food Before 1958

Not applicable.

Part 6. §170.250 Narrative

The conclusion that dimethyl ether, as described herein, is GRAS under the conditions of its intended use as an extraction solvent in the processing of various food products is based on scientific procedures using generally available data and information on dimethyl ether. A comprehensive and detailed search of the published scientific literature was conducted through October 2017 to identify toxicology studies conducted with dimethyl ether to support the safety of the solvent. Adis Clinical Trials Insight, AGRICOLA, AGRIS, Allied & Complementary Medicine™, BIOSIS® Toxicology, BIOSIS Previews®, CAB ABSTRACTS, Embase®, Foodline®: SCIENCE, FSTA®, MEDLINE®, NTIS: National Technical Information Service, and Toxfile® served as the primary sources of published literature. Toxicology studies on dimethyl ether conducted *via* the oral route were not identified in the literature, however, 2 inhalation studies were identified in the literature search (Collins *et al.,* 1978; Reuzel *et al.,* 1981). These studies are summarized in Section 6.3 below. In addition to the published inhalation toxicology studies, unpublished studies conducted *via* the inhalation route were extensively reviewed by EFSA and Food Standards Australia New Zealand (FSANZ) to support the safety of dimethyl ether when used as an extraction solvent in the processing of various food products (EFSA, 2009, 2015; FSANZ, 2011a,b). The results of the unpublished studies reviewed by EFSA and FSANZ corroborate the safety of dimethyl ether for use as an extraction solvent and are presented in Section 6.4 below.

6.1 Metabolic Fate

Given that dimethyl ether is a gas at body temperature, studies conducted from a safety standpoint have been *via* the inhalation route. Following inhalation as the route of administration, dimethyl ether has been shown to be rapidly absorbed, and distributed throughout the body prior to elimination (Kemper and Eckard, 1978; Eckard and Kemper, 1979; Daly and Kennedy, 1987). Furthermore, organ distribution of dimethyl ether has been reported to be directly proportional to the concentrations of exposure. In rats (strain not reported),

following exposure to concentrations up to 1,000 ppm, equivalent to 1,884 mg/m^3, for 60 minutes, levels of dimethyl ether were detected in muscle (14 mg/kg), lungs (15 mg/kg), liver (15 mg/kg), spleen (16 mg/kg), kidney (17 mg/kg), heart (17 mg/kg), brain (18 mg/kg), blood (19 mg/kg), and fat tissue (22 mg/kg) (Kemper and Eckard, 1978). In another inhalation study by Eckard and Kemper (1979), rats were exposed to dimethyl ether at concentrations up to 2,000 ppm, equivalent to 3,769 mg/m^3, for 180 minutes. In all tissues and organs (blood, heart, lungs, liver, spleen, kidneys, fat, muscle, and brain), levels of dimethyl ether were 16.4±1.4 mg/kg, except for muscles, where concentrations of dimethyl ether did not exceed 8 mg/kg. Daly and Kennedy (1987) reported that tissue concentrations of dimethyl ether in rats (strain not reported) rapidly increased following exposure to concentrations of 750 to 2,000 ppm, and rapidly decreased when exposure was ceased with no observed tissue storage. Steady-state levels of dimethyl ether in rats (strain not reported) were reached within 30 minutes of continuous inhalation exposure of concentrations up to 2,000 ppm (Kemper and Eckard, 1978; Eckard and Kemper, 1979; Daly and Kennedy, 1987). Unchanged dimethyl ether is rapidly excreted *via* exhaled air in a biphasic manner, with levels returning to background levels within 90 minutes. The $t_{1/2\alpha}$ and $t_{1/2\beta}$ were reported to be 10 and 90 minutes, respectively (Kemper and Eckard, 1978; Daly and Kennedy, 1987). Dimethyl ether is a stable compound and is not known to be reactive due to the absence of reactive functional groups (see Section 2.4 for further details). As a result, dimethyl ether is not metabolized and is excreted as an unchanged molecule (EFSA, 2009, 2015).

6.2 Applicability of Route-to-Route Extrapolation

As the majority of the data identified is *via* a route of administration not applicable to food ingredients, route-to-route extrapolation can be applied in the absence of toxicity studies conducted *via* the oral route (Pepelko and Withey, 1985; Rennen *et al.,* 2004). In general, the most common use of this technique is through the extrapolation from the oral to the inhalation route and vice versa (Pepelko, 1987). The general principle of route-to-route extrapolation which is an accepted scientific process as used by EFSA (2009, 2015) in their safety evaluation of dimethyl ether is to convert the external no-observed-adverse-effect level (NOAEL) (from inhalation studies) into an internal systemic dose, and to correct for the amount of the compound that does not enter the body due to incomplete absorption. This route-to-route extrapolation methodology is directly applicable for the solvent dimethyl ether in that:

1) The absorption and expression of toxicity are not influenced by local effects;
2) The absorption efficiency is the same between routes;
3) No metabolism occurs including chemical transformation by intestinal microflora or pulmonary macrophages takes place; and
4) The chemical is relatively soluble in body fluids.

As described in Section 6.1 above, dimethyl ether is rapidly absorbed and distributed following inhalation exposure, the kinetics of which is similar to oral exposure (Kemper and Eckard, 1978; Eckard and Kemper, 1979; Daly and Kennedy, 1987). Moreover, dimethyl ether is not metabolized and is eliminated unchanged in expired air. Dimethyl ether also does not cause toxicity at the site of contact (EFSA, 2009, 2015). Therefore, based on the pharmacokinetics and the available toxicity data of dimethyl ether, and the criteria described by Rennen *et al.* (2004), the available inhalation data on dimethyl ether can be bridged to support exposure through the oral route. Both EFSA (2009, 2015) and FSANZ (2011a,b) used the accepted extrapolation methodology in support of the safe use of dimethyl ether as an extraction solvent (see Section 6.4 for further details).

6.3 Published Toxicology Studies

Two published sub-chronic toxicology studies supporting the safety of dimethyl ether were identified in the literature (Collins *et al.,* 1978; Reuzel *et al.,* 1981). As they were conducted *via* the inhalation route, route-to-route extrapolation was necessary to convert the inhalation concentration to an internal dose[2].

6.3.1 13-Week Toxicity Study

Reuzel *et al.* (1981) conducted an inhalation toxicity study in CPB Wistar rats (10/sex/group) exposed to dimethyl ether (99.8% purity) in chambers containing 0, 2,000, 10,000, or 20,000 ppm for 6 hours/day, 5 days/week, for 13 weeks. Analyses showed actual concentrations of dimethyl ether to be 0, 1,943, 9,734, or 19,466 ppm, respectively, equivalent to 0, 3,661, 18,341, or 36,679 mg/m^3 (ACGIH, 2016)[3]. In terms of internal dose[2], these concentrations are equivalent to 0, 728, 3,645, or 7,289 mg/kg body weight/day for males, respectively, and 0, 748, 3,748, or 7,495 mg/kg body weight/day for females, respectively. Food and water were provided *ad libitum* during the non-exposure periods. Animals were observed for clinical signs and behavior on a daily basis. Body weight and food consumption were measured weekly. The following hematological parameters were measured on week 13: hemoglobin level, packed cell volume (PCV), red blood cell (RBC) count, and white blood cell (WBC) count and differential. In addition, the following serum biochemistry parameters were measured: aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase, total protein, and albumin. Urine samples were collected from all animals on weeks 6 and 13 and the following parameters were measured: volume, specific gravity, appearance, pH, sugar, protein, occult blood, ketones, and microscopy of the sediment. At the end of the study period, all animals were exsanguinated from the abdominal aorta under ether anesthesia and the

[2] Internal dose (mg/kg body weight/day) calculated as: (absorption efficiency x inhalation rate x duration of exposure x concentration) / body weight. Where the absorption efficiency is 75%, the exposure duration is 6 h/day, and inhalation rate and mean body weights for Wistar rats are reported by U.S EPA (1988).
[3] Concentrations in ppm were converted to mg/m^3 according to ACGIH (2016) where the molecular weight of dimethyl ether is 46.07 g/mol and the molar volume of air at NTP (25°C, 760 Torr) is 24.45 L.

following organs were removed, weighed, and examined for gross pathological changes: spleen, adrenals, kidneys, liver, heart, lungs with trachea and larynx, testes, ovaries, thymus, thyroid, brain, and pituitary. Microscopic examination of the following organs and tissues was conducted in all animals of the control and high-dose group: skin, axillary lymph nodes, preputial glands, skeletal muscle, femoral nerve, mammary glands, abdominal wall, coagulating glands, prostate, epididymides, caecum, colon, pancreas, mesenteric lymph nodes, urinary bladder, seminal vesicles, spinal cord, uterus, nasal cavity, sternum, esophagus, aorta, stomach, duodenum, jejunum, ileum, salivary glands (submaxillary, parotid, sublingual), exorbital lachrymal glands, and eyes. The lungs of animals of the low-dose and mid-dose groups were also microscopically examined.

No significant changes were reported on clinical signs, body weight, or food consumption. One female animal of the control group became ill on week 11 and was terminated. At autopsy, a tumor in the pituitary gland was observed in this animal.

A significant increase in percentage of neutrophils and a significant decrease in percentage of lymphocytes were observed in male and female animals of the high-dose group. A slight decrease in percentage of lymphocytes was observed in males of the low-dose ppm group. However, this effect was not considered treatment-related by the study authors as it was not observed in the higher concentration group (*i.e.,* mid-dose group) and occurred only in males. In females of the mid-dose group, an increase in erythrocyte count was observed. This change was within the normal range and was not considered treatment-related by the study authors.

Males of the high-dose group showed a slight but significant increase (~14%) in ALT levels compared to the control group. A slight but significant decrease in total protein was also noted in females of the high-dose group compared to the control group. No other liver effects were reported by the authors.

A significant decrease in mean specific gravity of the urine was noted in females of the low-dose and mid-dose groups at week 6, while an increase was observed in males of the mid-dose group at week 13. Additionally, mean urine volume was significantly increased in females of the high-dose group at week 6 and decreased in males of the mid-dose group at week 13. The authors noted that these changes were within the normal range and only occurred in one sex, and were therefore of no toxicological significance.

The adrenal weight relative-to-body weight was slightly increased in males of the low-dose group. This change was not considered toxicologically significant as a similar effect was not observed in animals exposed to the higher concentrations. In females of the high-dose group, a low mean ovary weight was observed. The authors noted that the low organ weight may be due to two animals with “extremely low” ovary weight, and therefore, this change was not considered to be relevant. Furthermore, the authors noted that ovary weight is “known to be highly variable under normal conditions”. A number of changes were noted upon microscopic examination,

however, they were minimal to slight, did not occur in a dose-related or sex-related manner, or occurred in a similar manner between the control and high-dose groups. Slight vacuolation of cells in the medulla and zona fasciculate of the adrenals was observed in 4 control and in 4 high-dose animals (both sexes). Based on the results of this study, the authors determined the no-observed-adverse-effects concentration (NOAEC) to be 20,000 ppm, equivalent to 7,289 and 7,495 mg/kg body weight/day in male and female animals, respectively.

6.3.2 30-Week Toxicity Study

In the study by Collins *et al.* (1978), male and female Wistar rats (25/sex/group; 150 to 220 g body weight) were exposed to dimethyl ether (purity not reported) in chambers containing concentrations of 0, 0.02, 0.2, or 2% for 6 hours/day, 5 days/week, for 30 weeks. Analysis showed the mean concentration of dimethyl ether in each group to be 0, 197, 1,964, or 18,830 ppm, respectively, equivalent to 0, 371, 3,701, or 35,481 mg/m^3 (ACGIH, 2016)[3]. In terms of internal dose[2], the concentrations were equivalent to 0, 63, 631, 6,048 mg/kg body weight/day for males, respectively, and 0, 70, 701, 6,720 mg/kg body weight/day for females, respectively. Animals were provided food and water *ad libitum* when not in exposure chambers. Animals were observed for clinical signs twice daily and body weight and food consumption were measured on a weekly basis. Ophthalmological examination was conducted before exposure and on week 26 of the study period in all control and high-dose animals. Hematology, serum biochemistry, and urinalysis were conducted in control animals prior to initiation of the study and at week 24. Examinations were performed in high-dose animals only on week 24. The following hematological parameters were measured: total RBC and WBC, hemoglobin, packed cell volume, mean corpuscular hemoglobin concentration (MCHC), mean corpuscular volume (MCV), differential WBC and platelet count, and prothrombin time. Serum biochemistry parameters evaluated include: blood urea nitrogen (BUN), alkaline phosphatase, glucose, serum proteins, and sodium and potassium concentrations. The urinalysis parameters measured include: volume, specific gravity, pH, protein, total reducing substances, glucose, ketones, bile pigments, urobilinogen, and blood pigments. Serum levels of ALT were measured in all groups on week 27 and 30 and AST levels were measured on week 30. All animals were terminated at the end of the study period and the following organs were removed and weighed: brain, pituitary glands, heart, lungs, liver, spleen, kidneys, thyroid, adrenals, uterus, and gonads. Histopathological examination was conducted on the following organs and tissues from 10 animals/sex of the control and high-dose groups: nasal passages, larynx, trachea, lungs, heart, thymus, lymph nodes (cervical and mesenteric), liver, spleen, pancreas, kidneys, urinary bladder, gonads, uterus, thyroid, adrenals, salivary gland, stomach (glandular and non-glandular), duodenum, ileum, mid-colon, bone marrow, brain (cerebral cortex, cerebellum, medulla), pituitary and eyes. It should be noted that the liver was histopathologically examined in all treatment groups.

No significant changes in clinical observations, body weight, food consumption, ophthalmologic examination, hematology, urinalysis, or histopathology were observed in any group. A significant increase in ALT was noted in males and females of the high-dose group at week 30, whereas a significant increase in AST was noted in males of the mid-dose group. When adjusted for body weight, a significant decrease in liver weight was observed in males of the high-dose group only. Non-statistically significant changes in absolute and relative-to-body liver weight were observed in males and females exposed to dimethyl ether. The authors noted that the increase in ALT level and decrease in liver weight in males of the high-dose group may be attributed to hepatic fibrosis. Based on the elevations in AST levels observed at mid-dose in this study, the authors determined the no-effect level to be the low-dose of 0.02%, equivalent to 63 and 70 mg/kg body weight/day in male and female animals, respectively.

6.4 Safety Evaluations by Authoritative Bodies

In addition to the published toxicology studies summarized in Section 6.3 above, the unpublished inhalation toxicity studies reviewed by EFSA (2009, 2015) and FSANZ (2011a,b) are presented in the sections below. No specific adverse effects were highlighted by EFSA or FSANZ upon their review, and therefore the results of these studies corroborate the safety of dimethyl ether when used as an extraction solvent.

6.4.1 European Food Safety Authority (EFSA)

In 2009, EFSA published a scientific opinion on the safety of dimethyl ether and its use as an extraction solvent in the processing of animal proteins, specifically collagen, with a proposed residual limit of 9 µg/kg (EFSA, 2009). The Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF) noted that toxicology studies conducted *via* the oral route were not available, and, as such, their review was based on inhalation exposure studies. The CEF Panel considered the findings from the inhalation studies to be applicable to the safety evaluation of orally consumed dimethyl ether as the compound is rapidly absorbed and distributed to the systemic system following inhalation exposure, similar to absorption that may occur in the gastrointestinal tract following ingestion.

A number of sub-chronic and chronic studies conducted *via* the inhalation route were reviewed by the CEF Panel. Two (2) 13-week studies were identified whereby male and female Wistar rats (10/sex/group) were exposed to dimethyl ether at concentrations up to 20,000 ppm, equivalent to 37,685 mg/m^3, in the atmosphere for 6 hours/day for 5 days/week (CIVO, 1978, 1983). No significant changes were observed in behavior, body weight, relative organ weights, urinalysis, gross pathology, and complete histopathological examinations throughout the study period. Sporadic hematological findings were observed throughout treated animals in both studies; one of these studies (CIVO, 1978) reported a significant increase in ALT in males of the high-dose group, however, this increase was not accompanied by a change in liver weight or effect on liver histopathology. The CEF Panel noted that the changes in hematology were not

considered to be biologically significant as no significant changes were observed in the histopathology in any treatment group and were not dose-related, and that the changes were within historical control ranges for this rat strain. Furthermore, the hematological findings reported by CIVO (1983) were not considered to be biologically significant as controls were reported to have lower hematology counts compared to historical controls in this strain of rat. In addition, a 13-week study was identified whereby Syrian Golden hamsters (10/sex/group) were exposed to dimethyl ether at concentrations of 0, 5,000, 10,000, or 20,000 ppm, equivalent to 0, 9,421, 18,842, or 37,685 mg/m^3 (CIVO, 1983). No significant changes were reported in behavior, growth, organ weights, body weights, urinalysis, serum biochemistry, gross pathology, and complete histopathology examinations in any treatment group with the exception of a significant increase in blood cell count in males of the 5,000 ppm and 10,000 ppm groups at day 56, and a significant decrease in blood cell count was observed in males of the 10,000 ppm group at the end of the study period. Based on these findings, the authors proposed a no-effect level of 5,000 ppm (9,421 mg/m^3) in hamsters.

A 2-year Good Laboratory Practice (GLP) inhalation study was also reviewed by the CEF Panel whereby male and female Crl:CD(SD)BR rats were exposed to concentrations of 0, 2,000, 10,000, or 25,000 ppm, equivalent to 0, 3,768, 18,842, or 47,106 mg/m^3, respectively (DuPont Co., 1986). No significant changes in 14 hematological and 10 serum biochemistry parameters, including liver enzymes, were noted between the treatment groups and control group (no further details provided). Similarly, no specific tissue damage was observed upon histopathological examination of 50 organs and tissues. A non-significant slight decrease in survival time was observed in animals of the 10,000 ppm and 25,000 ppm groups (no further details provided). Similarly, no evidence of increased cancer incidences in any tissue or organ was noted in any treatment group. Based on the slight decrease in survival time, the authors determined the no-effect level to be 2,000 ppm, equivalent to 3,768 mg/m^3, in Crl:CD(SD)BR rats.

The reproductive and developmental toxicity of dimethyl ether was considered in 2 studies conducted in rats (CIVO, 1981; Haskell, 1981). In the first study, 68 pregnant albino Wistar rats were exposed to dimethyl ether in the atmosphere at concentrations up to 2.8%, equivalent to 28,000 ppm or 52,759 mg/m^3, from gestation day (GD) 6 to GD 16 (CIVO, 1981). No significant changes in body weights, food consumption and food efficiency were observed. At necropsy, no abnormal morphological findings were observed. In addition, no teratogenic effects or adverse changes in reproductive parameters (organ weights, number of corpora lutea, implantation sites, and number of dead and alive fetuses *per* litter) were observed. Supernumerary lumbar ribs and variations in degree of ossifications were observed in the 2.0 and 2.8% group. However, the authors noted that these effects were not dose-related and changes in ossifications were considered normal variations in fetuses of this species strain. Based on the results of this study, the authors proposed a no-effects level of 2.8% or 52,759 mg/m^3.

In the second study, pregnant female Crl:CD(SD)BR rats (27/group) were exposed to dimethyl ether in the atmosphere at concentrations of 0, 0.125, 0.5, or 2.0%, equivalent to 0, 1,250, 5,000, or 20,000 ppm or 0, 2,355, 9,421, or 37,685 mg/m^3, respectively, for 6 hours/day on GD 6 to GD 15 (Haskell, 1981). No significant changes were observed in maternal body weight and organs, gross morphological examinations, and reproductive parameters (no further details provided) in any treatment group. Significantly decreased fetal body weight and increased incidence of skeletal variation were noted in the 5,000 ppm group. Based on the results of this study, the authors proposed a no-effect level of 1,250 ppm or 2,355 mg/m^3, which, upon review, was used by the CEF Panel to calculate an internal dose of 518 mg/kg body weight/day and was used to derive a margin of exposure of use of dimethyl ether as an extraction solvent (EFSA, 2015).

No mutagenic or genotoxic effects were observed in a series of studies including an *in vitro* bacterial reverse mutation assay in *Salmonella typhimurium* (*S. typhimurium*) TA98, TA100, TA1535, TA1537, and TA1538 and *Escherichia coli* (*E. coli*) WP2, *in vitro* host-mediated assay with *E. coli* K12 and *S. typhimurium* TA1538 in N:NIH mice, *in vivo* sex-linked recessive lethal test in *Drosophila melanogaster* at concentrations up to 120,000 ppm (226,110 mg/m^3), 20,000 ppm (37,685 mg/m^3), and 28,000 ppm (52,759 mg/m^3), respectively, *in vitro* unscheduled DNA synthesis (UDS) assay in primary rat liver cells at concentrations up to 75 mmol/L, and hypoxanthine-guanine phosphoribosyl transferase (HGPRT) forward mutation assay in V79 Chinese hamster cells at concentrations up to 75 mmol/L.

Based on the available toxicology dataset, the CEF Panel concluded that dimethyl ether is of low toxicity potential. Results from *in vitro* genotoxicity assays indicate that dimethyl ether does not have genotoxic potential, and results from subchronic, chronic, and carcinogenicity inhalation studies in rats and hamsters exposed to high concentrations of dimethyl ether did not produce any significant adverse effect in any evaluated study parameter. Based on the proposed residual limit of 9 µg/kg in extracted animal proteins, the exposure[4] to dimethyl ether was estimated as 0.18 µg/person/day (0.003 µg/kg body weight/day for a 60 kg individual), providing a margin of exposure in the order of 10^8 times lower than the internal dose of 630 mg/kg body weight/day calculated from the embryo-fetal inhalation toxicity study in rats conducted by Haskell (1981). As a result, the CEF Panel concluded that dimethyl ether is not of safety concern when used as a solvent at a level of up to 2% of the final food product with a maximum residual level of 9 µg/kg in extracted animal protein (EFSA, 2009).

The CEF Panel re-evaluated the safety of dimethyl ether as an extraction solvent in the processing of animal protein products such as collagen and collagen derivatives, and under a new intended condition of use, gelatin, and established maximum residual limits in each proposed use (EFSA, 2015). In their evaluation, the CEF Panel reviewed the previous safety

[4] Exposure estimate calculated as a worst-case scenario where a 60 kg individual consumes 1 kg of meat containing 2% of dimethyl ether-extracted animal protein daily.

data in addition to an *in vitro* chromosome aberration test in human peripheral lymphocytes that had become available since the CEF Panel's last review. The additional genotoxicity studies were consistent with previous negative genotoxicity and mutagenicity results (EFSA, 2009). The existing maximum residual level of 9 µg/kg in the extracted animal protein was proposed to increase to 3 mg/kg in the latest review and the use level was increased from 2.0 to 3.5%. Based on the new proposed residual limit, the exposure of dimethyl ether was estimated to be 2.0 µg/kg body weight/day[5]. In addition, a maximum residual limit of 9 µg/kg in gelatin was proposed, corresponding to an estimated exposure level of 0.014 µg/kg body weight/day[6] for a 60 kg individual. The margin of exposure was calculated to be approximately $2x10^5$, providing a large margin of exposure for the use of dimethyl ether as an extraction solvent in the preparation of animal protein materials. It should be noted that the CEF Panel used the same no-effect level reported by Haskell (1981) in deriving the margin of exposure, with the exception that a slightly lower breathing rate was used in calculating the internal dose (518 mg/kg body weight/day). As a result, the CEF Panel concluded that the proposed residual limits for defatted animal protein products (3 mg/kg) and gelatin (0.009 mg/kg) are of no safety concern.

6.4.2 Food Standards Australia New Zealand

In their review, FSANZ evaluated a similar dataset as the CEF Panel (EFSA, 2009, 2015) that was submitted as part of the applications to support the use of dimethyl ether as a processing aid in the production of dairy and non-dairy food ingredients and products (FSANZ, 2011a,b). Due to the proposed maximum permitted level and the inherent chemical properties of dimethyl ether (*e.g.*, low boiling point), exposure to dimethyl ether was considered to be negligible due to the rapid evaporation of the solvent following processing. Furthermore, FSANZ noted that the acute, subchronic, chronic, and reproductive/developmental toxicity data indicate adverse effects only at high atmospheric concentrations. Based on the available scientific information, FSANZ concluded that dimethyl ether is "of very low toxicity with regards to all toxicological aspects investigated" and the use of dimethyl ether as an extraction solvent processing aid "*does not pose any public health and safety risk*". As such, dimethyl ether is permitted for use as a processing aid for all foods, including dairy ingredients and dairy products, with maximum permitted levels of 2 mg/kg (FSANZ, 2011a,b).

[5] Exposure estimated calculated conservatively where 3.5% of the animal protein product (*e.g.,* collagen) is added to food, and a 60 kg individual consumes 1 kg of food per day.
[6] Exposure estimated calculated conservatively where 9% of gelatin is added to food, and a 60 kg individual consumes 1 kg of food per day.

6.5 Overall Conclusions Related to Safety

The available pharmacokinetic and metabolic fate data indicate that inhaled dimethyl ether can be handled very similarly to ingested dimethyl ether as it is rapidly absorbed, distributed, and eliminated (Kemper and Eckard, 1978; Eckard and Kemper, 1979; Daly and Kennedy, 1987). Moreover, dimethyl ether is not metabolized, is unreactive, and is eliminated *via* exhaled air as an unchanged compound and does not cause any site of contact toxic effects (EFSA, 2009, 2015). These dimethyl ether characteristics meet the criteria described by Rennen *et al.* (2004) for supporting a reliable route-to-route extrapolation of inhalation toxicity data to the oral route. As such, studies conducted *via* the inhalation route were considered applicable to evaluate the oral safety of dimethyl ether from its use as an extraction solvent of food products. Two published inhalation toxicology studies conducted for 13 and 30 weeks with dimethyl ether were identified and considered pivotal for safety purposes following a comprehensive search of the published literature.

In the 30-week Collins *et al.* study, a number of hematology, clinical chemistry, and urinalysis parameters were evaluated. No changes in hematology or urinalysis parameters were noted, however, several significant changes in liver enzyme levels were reported by the authors, including an increase in ALT levels in males and females of the high-dose group, an increase in AST level in males of the mid-dose group, and a decrease in relative-to-body liver weight in males of the high-dose group (Collins *et al.,* 1978). The increase in ALT level was observed in males and females exposed to concentrations of 2% dimethyl ether, equivalent to an internal dose of 6,048 or 6,720 mg/kg body weight/day for males and females, respectively. The changes in AST and ALT were accompanied by a significant decrease in liver weight when adjusted for body weight in males only at the high dose group. Non-statistically significant changes in absolute and relative-to-body liver weight were observed in males and females exposed to dimethyl ether. Collins *et al.* noted that it was “unusual” that the increase in ALT was accompanied by a decrease in liver weight, and suggested that the change may be due to hepatic fibrosis. However, based on the changes in AST observed in the mid-dose group, Collins *et al.* concluded that the no-effect level to be 0.02%, equivalent to an internal dose of 63 to 70 mg/kg body weight/day for males and females, respectively.

In the 13-week study, Reuzel *et al.* (1981) reported a minimal but significant increase in ALT levels of males of the high-dose group only compared to the control group. This increase was equivalent to approximately 14% and was observed in male animals exposed to dimethyl ether at internal doses of approximately 7,289 mg/kg body weight/day. Reuzel *et al.* reported no significant changes in organ weights or histopathological examination that may be attributable to dimethyl ether exposure in any treatment group.

On the basis of the effects noted in the Collins and Reuzel studies outlined above a close attention was paid to those unpublished studies reviewed and reported within the EFSA opinions. In 13-week studies reviewed by EFSA, a significant increase in ALT level was reported in one study conducted by CIVO (1978) in males only exposed to dimethyl ether at concentrations of 20,000 ppm. whereas no changes in ALT or AST were noted in the CIVO (1983) study. Furthermore, no corresponding changes in liver weight or liver histopathology were reported in the 13-week studies in rats and hamsters (CIVO, 1978, 1983). Furthermore, in a 2-year GLP study conducted in rats, no significant changes in hematology or serum biochemistry parameters were reported, and no specific effects were reported upon histopathological examination of 50 organs and tissues in any animal exposed to concentrations up to 25,000 ppm dimethyl ether (DuPont Co., 1986).

Taking into account the effects related to the increase in liver enzyme levels noted within the published studies by Collins *et al.* and Reuzel *et al.*studies and the associated liver weight effects, the NOAEL was determined to be the low dose reported by Collins *et al*. (1978). No significant changes in any reported study parameter was observed in animals exposed to 0.02% dimethyl ether, equivalent to an internal dose of 63 or 70 mg/kg body weight/day for males and females, respectively (Collins *et al.,* 1978). Thus, the NOAEL reported by Collins *et al.* (1978) of 0.02% dimethyl ether was used to derive a margin of exposure for the use of dimethyl ether as an extraction solvent.

When considering a worst-case exposure scenario where the residual level of dimethyl ether in the food ingredient is equivalent to the limit of detection of the method of analysis (*i.e.,* 2 mg/kg), and the dimethyl ether-extracted ingredient is used at 100% of a final food product (*i.e.,* the dimethyl ether-extracted ingredient containing 2 mg/kg of the solvent is consumed), a margin of exposure[7] of approximately 1,800 to 2,100 can be calculated using the reported NOAEL of 63 and 70 mg/kg body weight/day for males and females, respectively. These large margins of exposure in terms of 10^3 indicate no safety concerns of dimethyl ether as an extraction solvent at the proposed levels of use. It should be noted that this margin of exposure is likely greater on the basis that the extracted food ingredient will be used at lower levels in final food products (*i.e.,* less than 100%) and does not account for further volatilization of the solvent during formulation of the final food product.

In comparison, the NOAEL and margin of exposure derived from the Collins *et al.* (1978) study is less than the internal dose (~518 mg/kg body weight/day) estimated by EFSA, in which a margin of exposure of 200,000 was calculated based on the absence of embryo-fetal toxicity noted within the embryo-fetal inhalation toxicity study (EFSA, 2015). In addition, the reported no-effect level from the 2-year GLP inhalation study of 2,000 ppm, equivalent to 642 or 714 mg/kg body weight/day in male and female animals, respectively, correlated to a margin of

[7] Margin of exposure calculated as (NOAEL/exposure), where exposure is calculated with a maximum residue level of 2 mg/kg, and it is assumed that a 60 kg individual consumes 1 kg of meat per day.

exposure of approximately 20,000 (DuPont Co., 1986). Overall, the regulatory opinions established for dimethyl ether based upon the results of these unpublished toxicology studies demonstrate a large safety margin, albeit that the NOAEL was established upon the absence of embryo-fetal toxicity as opposed to effects on liver enzymes. The EFSA and FSANZ opinions therefore support the safety of dimethyl ether when used as an extraction solvent.

6.6 Conclusion

Based on the above data and information presented herein, Callaghan Innovation has concluded that the intended uses of dimethyl ether as an extraction solvent, as described in Part 1.3, are GRAS based on scientific procedures. The GRAS status of dimethyl ether is corroborated through the independent safety evaluations conducted by EFSA and FSANZ who approved the use of dimethyl ether as an extraction solvent.

Dimethyl ether, therefore, may be marketed and sold for its intended purpose in the U.S. without the promulgation of a food additive regulation under Title 21, Section 170.3 of the Code of Federal Regulations.

Part 7. §170.255 List of Supporting Data and Information

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