FOOD STANDARDS Australia New Zealand
Te Mana Kounga Kai – Ahitereiria me Aotearoa

**8 November 2024**
**315-24**

## Supporting document 1

Risk and technical assessment report – Application A1260

2-Methyloxolane as a processing aid

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# Executive summary

Food Standards Australia New Zealand (FSANZ) received an application from EcoXtract (formerly Pennakem Europa)[1] to amend the Australia New Zealand Food Standards Code (the Code) to permit the use of 2-methyloxolane (2-MeOx) as an extraction solvent processing aid. 2-MeOX would be used to extract and separate oils and proteins from plant-based products, including oilseeds. It would also be used to extract other components including flavours, fragrances and colours from plant-based sources.

2-MeOx has been assessed to be technologically justified for the production of the nominated foods and food ingredients, the latter of which may subsequently be used in the manufacture of a range of other foods. Its use as an extraction solvent has been assessed as consistent with its typical function as a processing aid. That is, 2-MeOx performs its technological purpose during the production of food and is not performing a technological purpose in the food for sale.

Some residual 2-MeOx is still present in the extracted products: refined oils, plant proteins or natural extracts (hop extract, carotenoid from algae, chlorophyll). However, toxicokinetic studies in rats and mice show that 2-MeOx is rapidly absorbed and excreted in these species and does not accumulate in any organ. In addition, its presence at residual levels can be managed through the setting of appropriate maximum permitted levels (MPL) in the Code for different categories of food.

The acute oral $LD_{50}$ of 2-MeOx in rats was in the range 300-2000 mg/kg bw. The lowest No Observed Adverse Effect Level (NOAEL) identified in animal studies was 100 mg/kg bw/day, based on a decrease in group mean live birth index in female rats dosed with ≥250 mg/kg bw/day in an extended reproductive toxicity study. No long-term or carcinogenicity studies of 2-MeOx were submitted or located by literature search. A range of *in vitro* and *in vivo* genotoxicity assays have been conducted. Collectively, these support the conclusion that 2-MeOx does not show genotoxic potential. FSANZ concludes that 2-MeOx is unlikely to be carcinogenic, because it is not genotoxic and no lesions likely to lead to neoplasia by a nongenotoxic mechanism were observed in a three-month repeat-dose study conducted in rats.

[1] EcoXtract acquired Pennakem Europa following acceptance of the application by FSANZ. The applicant is now EcoXtract.

No case reports of allergy or intolerance attributable to oral exposure to 2-MeOx were located.

The lowest NOAEL identified in animal studies, 100 mg/kg bw/day, is suitable for use for derivation of an acceptable daily intake (ADI) for 2-MeOx. FSANZ considers it appropriate to apply an Uncertainty Factor of 10 for extrapolation from animals to humans, and an Uncertainty Factor of 10 to allow for variability in sensitivity within the human population. The total Uncertainty Factor is therefore 100, from which an ADI of 1.0 mg/kg bw/day is derived.

Dietary exposure assessments (DEA) were conducted using several methods to capture all the foods/food groups requested and the different MPLs requested. The DEA covered populations in Australia and New Zealand including infants under 12 months. The dietary exposure to residual 2-MeOx from general purpose foods (including formulated meal replacements and formulated supplementary foods) at the requested MPL of 20 mg/kg of 2-MeOx was estimated to be approximately 65% of the ADI. For infants aged 3 months, the estimated mean and 90$^{th}$ percentile (P90) dietary exposures to residual 2-MeOx from only infant formula at an MPL of 3 mg/kg of 2-MeOx are 40% and 80% of the ADI respectively. It was estimated that infants aged 9 months could consume up to 1447 g of food for infants (at 5 mg/kg of 2-MeOx), or up to 2894 g of general purpose foods (at 20 mg/kg of 2-MeOx), in addition to 555 g of follow on formula (at 3 mg/kg of 2-MeOx) per day before exceeding the ADI. Infants aged 12 months could consume up to 1500 g of food for infants (at 5 mg/kg of 2-MeOx), or up to 3000 g of general purpose foods (at 20 mg/kg of 2-MeOx), in addition to 420 g of formulated supplementary food for young children per day (at 5 mg/kg of 2-MeOx) before exceeding the ADI. These amounts were well above estimated or actual food consumption amounts reported. For food for special medical purposes (FSMP) (FSMP that are not very low energy foods only), estimated dietary exposures to residual 2-MeOx at an MPL of 20 mg/kg for adults and children are 60% and 100% of the ADI respectively, based on worst-case assumptions.

Based on the safety and dietary exposure assessments, there are no safety concerns associated with use of 2-MeOx as an extraction solvent (including residual levels) at the proposed MPLs.

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# 1 Introduction

The applicant, EcoXtract, requested an amendment of section S18—8 of the Australia New Zealand Food Standards Code (the Code) to permit the use of 2-methyloxolane (2-MeOx) as a processing aid. The application also sought permission in the Code for the following maximum permitted levels (MPL) for residual 2-MeOx in foods:

- 5 mg/kg in infant formula products
- 5 mg/kg in foods for infants
- 20 mg/kg in other foods.

2-MeOx would be used as an extraction solvent, to extract and separate oils and proteins from plant-based products, including oilseeds. It would also be used to extract other components including flavours, fragrances and colours from plant-based sources before they are added to foods. 2-MeOx is produced from agricultural by-products including corn cobs, sugarcane bagasse and rice straw The applicant proposes that, as an organic compound, 2-MeOx can be used as an environmentally sustainable alternative to hexane, which is a permitted extraction solvent worldwide.

The objectives of the assessment were to:

- determine whether 2-MeOx achieves its technological purpose as a processing aid
- evaluate any potential public health and safety concerns that may arise from the use of this extraction solvent.

# 2 Food technology assessment

## 2.1 Identity

2-MeOx is a cyclic ether formed from carbohydrates derived from lignocellulosic biomass, an agricultural by-product. It is a clear liquid under standard conditions of temperature and pressure with an ether odour (Pace et al 2012). The identity and physical and chemical properties of 2-MeOx are summarised in Table 1.

***Table 1*** Identity and physical and chemical properties of 2-methyloxolane

| | |
|---|---|
| EC number | 202-507-4 |
| EC name | Tetrahydro-2-methylfuran |
| CAS number | 96-47-9 |
| IUPAC name | 2-methyltetrahydrofuran, 2-methyloxolane |
| Synonyms/other names | Tetrahydrosylvan<br>Furan, 2-methyl-tetrahydro<br>Tetrahydro-2-methylfuran<br>2-Methyltetrahydrofuran (2-Methyl-THF) |
| Abbreviation | 2-MeOx, 2-MeTHF |
| Chemical formula | $C_5H_{10}O$ |
| Formula weight (g mol-1) | 86.13 |
| Appearance and physical state | Clear colourless liquid at 20°C and 101.3 kPa |
| Relative density | 0.8552 at 20°C CRC |
| pH | 7.1 +/- 0.2 |
| Melting/freezing point | <-20°C |
| Viscosity | 0.576 mm2/s at 20°C and 0.484 mm2/s at 40°C (OECD 114) |
| Water solubility | 140 g/L organic solvents |

| | |
|---|---|
| Partition coefficient n-octanol/water (log value) | 1.85 at 25°C |
| Boiling point | 78°C at 101.3 kPa |

kPa= Kilopascals

## 2.2 Production of 2-MeOx

2-MeOx is produced from carbohydrates derived from cellulose and hemicellulose found in plant agricultural by-products such as corn cobs, oat hulls, almond husks, sugarcane bagasse and rice straw.

In producing 2-MeOx, the lignocellulosic biomass from by-products, firstly undergoes extensive acidification to produce intermediate sugars and subsequently the monomeric sugars pentose (C5) and hexose (C6). The C5- and C6-monosaccharides undergo multiple rounds of acid-catalysed reactions to produce levulinic acid (LA) and furfural (FAL) (Figure 1). Information regarding the methods of production for 2-MeOx has been published by Rapinel et al (2020) and further details on the method employed by EcoXtract were provided by the applicant in a commercial in confidence (CCI) appendix.

The applicant states that distillation of 2-MeOx achieves a product purity >99.9%. However, very low levels of impurities may be present as a result of the manufacturing process. Batch analyses of impurities were provided by the applicant in a CCI appendix. FSANZ assessed these data in determining a specification that would ensure appropriate purity criteria would be met (see section 2.4 of this report).

Unstabilised 2-MeOx reacts readily with oxygen to form hydroperoxides. The applicant indicated that the addition of butylated hydroxytoluene (BHT) or tocopherol to the final product would assist in preventing peroxide formation, particularly during periods of storage.

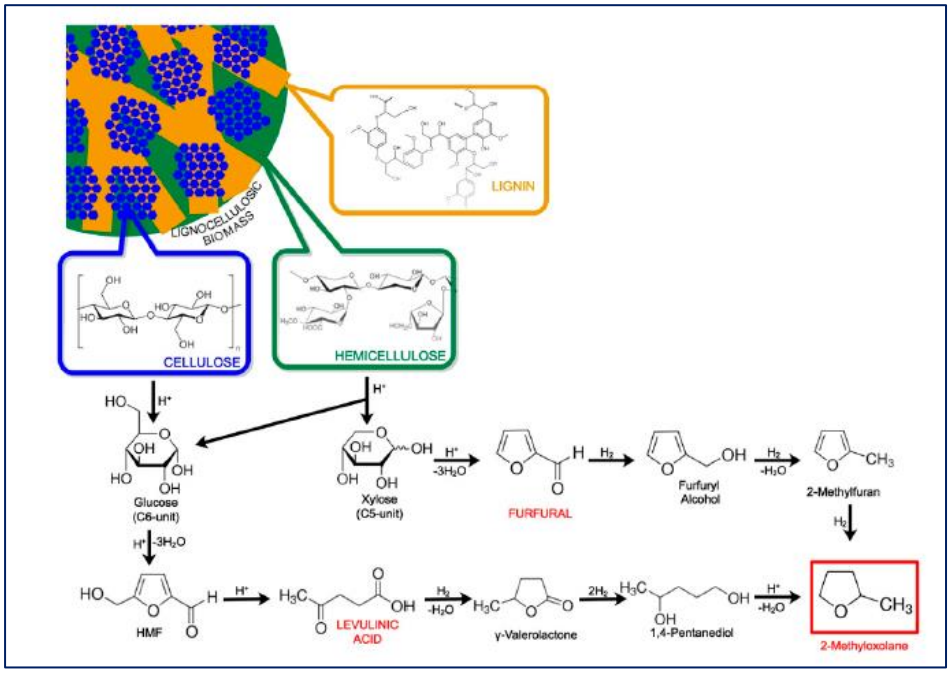


***Figure 1*** *Mechanism and pathways for 2-MeOx production (from Rapinel et al 2020)*

## 2.3 Technological purpose and justification

The technological purpose of 2-MeOx, as stated by the applicant, is as an extraction solvent processing aid. 2-MeOx would be used to separate and extract lipids from oil rich biomass

and defat protein rich biomass. It would also be used to extract natural aroma, flavours and colourants, particularly lipophilic ones (e.g. hops, annatto, carotenoids and chlorophyl).

The applicant provided information, including the results of commissioned studies, demonstrating the efficiency of oil and protein extraction using 2-MeOx and the quality of the resulting product, compared with the chemical extraction solvent hexane. A summary of this information was provided in the application, with further details provided in Appendix C of the application.

In a study examining oil extraction, 2-MeOx was found to extract slightly more oil than hexane from meals of soybean kernels and rapeseed. After refining, the oil quality and losses were determined to be more dependent on seed type rather than on the use of 2-MeOx versus hexane (OLEAD 2019).

A study examining protein extraction and characterisation from de-oiled soy meal, again comparing the efficiency of 2-Me-Ox against hexane, was also commissioned by the applicant. The study concluded that protein isolates produced using either extraction solvent had very high levels of purity. The defatted isolate produced using 2-MeOx had a higher protein content. In addition, the sample defatted with 2-MeOx retained very good gelling properties in solution, and the correct emulsifying properties (IMPROVE SAS 2019).

A 2020 review of 2-MeOx as a sustainable lipophilic solvent for the extraction of natural products investigated its extraction efficiency for lipids, natural colours and aromatic compounds from plants. Results were generally comparable to those of hexane (Rapinel et al 2020).

The technological justification for using 2-MeOx is that, if permitted, it will provide food manufacturers with an effective bio-based and environmentally sustainable alternative to other permitted extraction solvents, for example, hexane. It can be easily used at an industrial scale and, as such, existing extraction plants in Australia and New Zealand have the option of transitioning to the use of 2-MeOx as a substitute for other extraction solvents.

Although all industrial extraction processes are designed to maximise recycling of the extraction solvent, some residual 2-MeOx may still be present in the extracted products e.g. refined oils, plant proteins or natural extracts (hop extract, carotenoid from algae or chlorophyll). Based on EcoXtract's application trials, the residual 2-MeOx is expected to be less than 1 mg/kg in refined oils, liquid food and beverages, and less than 10 mg/kg in solid food.

## 2.4 Specification for 2-MeOx

There is currently no specification for 2-MeOx in the relevant monographs of Schedule 3 (subsections S3—2 and S3—3) of the Code.

The specification for 2-MeOX is established in Table 2. Limits were established for furan, 2-methylfuran and ethanol based on the potential for these substances to be present due to the manufacturing process and also in noting that in its assessment, EFSA considered that among the impurities present in 2-MeOx preparations, furan and 2-methylfuran were those with the highest hazardous potential (EFSA 2022). If 2-MeOx is permitted, the specification will be inserted into Schedule 3 of the Code.

The specification is consistent with that established in Commission Directive (EU) 2023/175.

***Table 2*** Specification for 2-MeOx

| Physical and chemical parameters | Specification |
|---|---|
| Chemical name | 2-methyloxolane |
| Chemical formula | $C_5H_{10}O$ |
| CAS Number | 96-47-9 |
| Purity (on a dry weight basis) | not less than 99.9% |
| Ethanol (on a dry weight basis) | not more than 450 mg/kg |
| Furan (on a dry weight basis) | not more than 50 mg/kg |
| 2-methylfuran (on a dry weight basis) | not more than 500 mg/kg |

## 2.5 Analytical method

The applicant provided information regarding validated analytical methods developed to measure the solvent residue in different matrices, including oil or lipophilic liquids, and powders. Further details on each method were provided as CCI. In general, the parameters (i.e. LOD and/or LOQ) would enable determination of solvent residue to check compliance with any proposed MPLs in the Code.

## 2.6 Food technology conclusion

FSANZ has assessed the technological purpose of 2-MeOx as a processing aid – in the extraction and separation of oils and proteins from plant-based products, including oilseeds; and other components from plant-based sources including flavours, fragrances and colours – as having been sufficiently justified by the applicant. The applicant has provided sufficient evidence on the levels of impurities in the product (including batch analyses of impurities) and the potential for residual amounts of 2-MeOx in extracted components.

The function of 2-MeOx is very similar to that of already permitted extraction solvents permitted in S18—8 of the Code. 2-MeOx achieves its technological purpose as an extraction solvent processing aid in the form proposed to be used whilst not exceeding the MLPs FSANZ is proposing. 2-MeOx exhibits a high level of product purity, as demonstrated by the information provided in the application.

# 3 Hazard assessment

## 3.1 Industrial use of the substance

In the European Union, 2-MeOx is registered under EC (No) 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH). The registration is for use as a solvent, in annual quantities exceeding 1000 tonnes, for the synthesis of fine chemicals, agrochemicals and pharmaceuticals. As part of the REACH process, Derived No Effect Levels (DNEL), for workers and for consumers are calculated. The DNEL for oral exposure to 2-MeOx was calculated to be 1.25 mg/kg bw/day. The DNEL applies to both acute and chronic oral exposure and was derived from a No Observed Adverse Effect Level (NOAEL) of 250 mg/kg bw/day with the application of a total of 200 in Assessment Factors (Uncertainty Factors). DNELs were also derived for dermal exposure and inhalation exposure, although these are not relevant to food consumers.

## 3.2 Use of the substance as a food processing aid in other countries

Commission Directive (EU) 2023/175[2], dated 26 January 2023, authorised the use of 2-MeOx as an extraction solvent 'in the preparation of defatted protein products, defatted flours, preparation of defatted cereal germs and flavourings from natural flavouring materials'.

In the pharmaceutical industry, 2-MeOx has been used as a process solvent to produce early intermediates of pharmaceuticals since 2007. After a worldwide call for comments in 2021, the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) has adopted a proposal to include 2-MeOx as a low toxicity (Class 3) residual solvent for use in pharmaceutical products in its document QC3(R8) 'Impurities: Guideline for residual solvents'. This proposal allows broader use of 2-MeOx in pharmaceuticals production. ICH derived a Permitted Daily Exposure level of 50 mg/day from a NOAEL of 250 mg/kg bw/day identified in a three-month repeat-dose study in rats published by Parris et al (2017). The study is summarized in section 3.4.2.

## 3.3 Toxicokinetics and metabolism

*Toxicokinetic study of radiolabelled 2-MeOx in male Fischer-344 rats and male B6C3F1 mice (Henderson et al 2007) Not GLP, no Guidelines specified.*

The test article, of unspecified purity, was radiolabelled with $^{14}C$ in the 2-methyl position. Radiochemical purity was in the range 93 to 98%. Animals, 10 to 11 weeks of age at dosing, were acclimatized to metabolism cages for 24 hours prior to dosing and remained in the cages after dosing for measurement of radioactivity in urine, faeces, expired $CO_2$ and expired volatile organic compounds. The animals, 4/group, were dosed with $^{14}C$-MeOx at 1,10, or 100 mg/kg bw by either the oral or intravenous route. The vehicle for oral dosing was water, and for intravenous dosing it was physiological saline. Stability of the dosing formulations was confirmed prior to dosing. Excreta were collected at predetermined intervals after dosing. Animals were anaesthetised for blood collection and euthanasia at 24 h for animals dosed with ≤10 mg/kg bw, and at 72 hours for animals dosed with 100 mg/kg bw. Any urine remaining in the bladder at necropsy was collected. A range of organs and tissues were collected at necropsy for measurement of radioactivity. No signs of toxicity were observed in the animals during the study. Intakes of feed and water, and urinary output, were unchanged relative to levels measured during the acclimatization period. No gross abnormalities were observed in any tissues at necropsy.

Results of the study showed that 2-MeOx was rapidly metabolised and excreted in both species. In mice, the principal route of excretion of radiolabel was urinary (60%), followed by exhalation (30%), whereas in rats, the reverse was true (25% and 50% respectively). In both species, excretion in the form of volatile organic compounds increased with dose. Faecal excretion was not a major route in either species. In mice less than 8% of radiolabel remained in the tissues at necropsy. In rats the radiolabel detected in tissues at necropsy ranged from 8 to 22%. The remaining radiolabel was principally measured in the muscle and skin, and there was no evidence of accumulation in any organ. At the high dose of 100 mg/kg bw, the exhalation of VOC increased, and the parent compound was detected in the exhaled VOC, indicating that metabolism of 2-MeOx is saturable. Analysis of urine by HPLC showed three highly polar peaks in mouse urine and two highly polar peaks in rat urine. The authors of the study considered it most likely that the peaks resulted from the radiolabelled carbon of the methyl group entering the metabolic pool and labelling intermediate dietary metabolites.

[2] https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32023L0175

After 24 hours, clearance in mice was practically complete but up to 20% of radiolabel was still present in rats, At 72 h, mean recovery of radioactivity was approximately 97% in mice dosed with 100 mg/kg bw/d, and recovery of radioactivity was approximately 91% in rats at the same dose.

## 3.4 Toxicity studies

### 3.4.1 Acute toxicity studies

*Acute oral gavage study of 2-MeOx in female Wistar rats (Harlan 2013). Regulatory status: GLP, conducted in compliance with OECD Guideline 420.*

The test article for this study had a purity of 99.93%. No vehicle was used. Female Wistar rats, aged 8 to 12 weeks, were maintained under standard laboratory conditions with *ad libitum* access to feed and water, except during pre-dosing fasting. Rats were dosed by oral gavage with a single bolus dose of 2-MeOx at a dose volume up to 10 mL/kg bw. One rat was dosed with 2-MeOx at a dose of 2000 mg/kg bw, and five rats were dosed at 300 mg/kg bw. Rats were observed at 0.5, 1, 2, 4 hours after dosing and then daily for 14 days. Bodyweight was recorded on days 0, 7, 14 or at unscheduled death. Rats that survived to Day 14 were euthanised. All rats were subject to gross necropsy.

The rat dosed at with 2000 mg/kg bw 2-MeOx was killed *in extremis* thirty minutes after dosing, showing clinical signs of coma, dyspnoea, depressed respiratory rate, hypothermia and pallor of extremities. On necropsy, haemorrhage and epithelial sloughing were observed in the non-glandular part of the stomach, and there was clear fluid in the gastric lumen.

No unscheduled deaths occurred in the rats dosed with 300 mg/kg bw, and no abnormal clinical signs were observed. The rats showed expected gains in bodyweight through to scheduled termination and necropsy on Day 14, and no gross lesions were discovered at necropsy. It was concluded that the acute oral $LD_{50}$ of 2-MeOx in the female Wistar rat is in the range 300-2000 mg/kg bw.

### 3.4.2 Short term studies

*Three-month oral gavage study of 2-MeOx in Sprague Dawley rats (Parris et al 2017). Regulatory status: GLP, no study guideline specified, based on ICH recommendations.*

The 2-MeOx used for this study had a purity of 99.95%. Formulations were prepared using water purified by reverse osmosis. Rats were 6 to 8 weeks of age when received, and were acclimatized to standard laboratory conditions of environment and husbandry for at least seven days prior to study start. Rats were assigned to five dose groups, 10 rats/sex/group, plus recovery cohorts of 5 rats/sex/group for the control and highest doses, and were gavaged with 0, 80, 250, 500 or 1000 mg/kg bw/day, at a constant dose volume of 10 mL/kg bw. Clinical observations were recorded once daily during acclimatization and the one-month recovery phase, and twice daily (prior to and 4 hours after dosing) during the dosing phase of the study. Bodyweight was recorded prior to dosing on Day 1, weekly during the dosing phase, and prior to scheduled termination. Intake of feed and water was calculated weekly. Ophthalmic examinations were performed on all rats pre-study, and during Weeks 5 and 12 on control and 1000 mg/kg bw/day rats only. Haematology and clinical chemistry were performed on selected rats mid-study and in Week 13. Urine for urinalysis was collected from all rats mid-study and prior to scheduled terminations in Week 13. Rats were killed by exsanguination and subject to detailed necropsy. Fresh organ weights were recorded for adrenal glands, brain, epididymides, heart, kidneys, liver ovaries, prostate, spleen, testes and thymus. An extensive range of tissues and organs were preserved for histopathology. The same procedures were followed for recovery cohorts after a recovery period of one

month.

Dose formulations were within 10% of target concentrations. There were three unscheduled deaths. Two rats, a 500 mg/kg bw/day female and a 1000 mg/kg/day male, were euthanized following misdosing injuries. One 80 mg/kg bw/day female did not recover from anaesthesia after mid-study blood collection. All other rats survived to scheduled termination, and there were no test article-related deaths. The only treatment-related clinical signs observed were hypersalivation immediately prior to or after dose administration. This was observed in one male and two females in the 500 mg/kg bw/day group, and six males and five females in the 1000 mg/kg bw/day group. A moderate decrease in group mean values for overall bodyweight gain was observed in rats treated with 2-MeOx when compared to sex-matched controls, reaching 15.7% and 13.4% lower than control values for male and female rats, respectively, in the 1000 mg/kg bw/day group. This effect was reversible during the recovery period. Group mean values for terminal bodyweight were slightly lower in groups treated with 2-MeOx than in sex-matched controls. No treatment-related effects were observed in ophthalmic findings, urinalysis, or gross lesions observed at necropsy. A small dose-related increase in group mean prothrombin time was observed in males, but not females, dosed with ≥500 mg/kg bw/day, but this was reversed during the recovery period. Treatment-related increases in group mean values for serum cholesterol were observed in both sexes in the 1000 mg/kg bw/day group in Weeks 6 and 13 of dosing but were reversible during the recovery phase. Group mean values for absolute and relative weights of kidneys and liver were higher than those of controls in both sexes dosed with ≥500 mg/kg bw/day. No histological correlates to these increases were found in the kidneys. In the liver, centrilobular hypertrophy was observed only at the highest dose of 1000 mg/kg bw/day. This change was minimal in 8/10 males and 6/10 females, and mild in one male. There were no associated increases in levels of any circulating liver enzymes. The hypertrophy was reversible, in that no similar changes were found in rats of either sex in the recovery cohort. No other treatment-related microscopic lesions were observed. Based on these results, the authors of the study identified a NOAEL of 250 mg/kg bw/day. In the absence of information on the possible mechanism of centrilobular hypertrophy, FSANZ considers this NOAEL is likely to be conservative, because the centrilobular hypertrophy may be an adaptive change.

An earlier three-month study by Antonucci et al (2011) tested 2-MeOx in Sprague Dawley rats at doses up to 26 mg/kg bw/day, and observed no adverse effects at this dose. Because the doses used were much lower, the study does not contradict the findings of Parris et al (2017) and does not contribute further to the assessment of the safety of 2-MeOx.

### 3.4.3 Long term and carcinogenicity studies

No long-term or carcinogenicity studies of 2-MeOx were submitted in the application or located by literature search.

### 3.4.4 Developmental and reproductive studies in animals

Developmental and reproductive studies of 2-MeOx were conducted for the applicant in Sprague-Dawley rats by contract research laboratories. Animal housing and husbandry in all the studies were under standard laboratory conditions.

*Preliminary dose range-finding developmental study of 2MeOx in pregnant Sprague Dawley rats (Envigo 2018a). Regulatory status: Not GLP, no specific guideline referenced.*

Timed-pregnant Sprague Dawley rats were assigned to groups of eight rats/group and gavaged daily from Gestational Day (GD) 3 to GD 19 at doses of 0, 250, 500 or 1000 mg/kg bw 2-MeOx (purity >99%). The control article/vehicle was Arachis oil (peanut oil). Clinical observations, bodyweights, feed consumption and water consumption were recorded during

the study. All rats were terminated on GD 20 and subjected to gross necropsy, including examination of the uterine contents and recording of number of corpora lutea; number, position and type of implantation; placental weights; and fetal weights, sex and external and internal macroscopic appearance.

All rats survived to scheduled termination and no dose-related clinical signs, or effects on bodyweights or bodyweight gains were observed. Feed and water consumption were comparable across all the groups. Two rats in the 1000 mg/kg bw/day group had compacted stomach contents at necropsy, although these findings were not associated with adverse clinical signs or gastric lesions. One rat in the 500 mg/kg bw/day group had an enlarged spleen and pale spleen, liver, kidneys, gastrointestinal tract and uterus.

There were no treatment-related effects on litter data as assessed by numbers of implantations, *in utero* fetal survival (as assessed by the mean numbers of early or late resorptions), live litter size, sex ratio or pre- and post-implantation losses. Mean fetal weight in the 1000 mg/kg bw/day group was 9.9% lower than that of the control group, although this may have been influenced by slightly larger litter sizes (i.e. number of pups/litter) in the 1000 mg/kg bw/day group compared to the control group (group mean of 14.3 pups compared to 12.4 pups for the control litters). The incidence of small fetuses was increased in the 1000 mg/kg bw/day group relative to that in the control group, but no external or internal abnormalities were observed. Mean fetal weights and sizes in the 250 and 500 mg/kg bw/day groups were similar to those of the control group, and there were no treatment-related effects on placental weights.

The highest dose tested, 1000 mg/kg bw/day, was identified as the NOAEL for maternal toxicity. A NOEL for fetal toxicity was identified at 500 mg/kg bw/day based on lower mean fetal bodyweight at a maternal dose of 1000 mg/kg/day.

FSANZ concurs with the conclusions of this study.

*Developmental study of 2-MeOx in pregnant Sprague Dawley rats (Envigo 2018b). Regulatory status: GLP, conducted in compliance with OECD Test Guideline 414, U S EPA Health Effects Test Guideline OPPTS 870.3700, Japanese Ministry of Agriculture, Forestry and Fisheries Testing guidelines for Toxicology studies 12 NouSan No 8147 and Commission Regulation (EC) No 440/2008 of 30 May 2008 test methods pursuant to Regulations (EC) No 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH).*

Based on the findings of the dose range-finding study, dose levels of 0, 100, 300 and 1000 mg/kg bw/day 2-MeOx (> 99% purity) were selected. Timed-pregnant Sprague Dawley female rats, 24/group, were administered the doses daily by oral gavage from GD 3 to 19 inclusive. The vehicle/control article was Arachis oil (peanut oil).

Parameters determined during the in-life phase included clinical signs, body weight changes, food consumption and water consumption. All rats were terminated on GD 20 and subjected to gross necropsy. The number of corpora lutea; number, position and type of implantation; placental weights; fetal weight, sex and external and internal macroscopic appearance were recorded. Half of each litter were processed for detailed examination of skeletal development and the remaining half were subjected to detailed visceral examination.

All rats survived to scheduled termination, and there were no treatment-related effects on feed consumption, water consumption or gross necropsy findings. Rats in the 1000 mg/kg bw/group showed some transient behavioural changes, such as burrowing in bedding, that suggested that the test article was unpalatable. In the same group, there was a transient (GD 14 to 17) decrease in bodyweight gain which was not statistically significant when corrected

for gravid uterus weight and was not considered sufficient to alter the previously identified maternal NOAEL of 1000 mg/kg bw/day.

There were no treatment-related effects on litter data, as assessed by pre-implantation loss, number of implantations, embryofetal survival, live litter size or sex ratio.

Fetal effects were limited to a reduction, relative to the control value, in group mean fetal bodyweight at 1000 mg/kg bw/day. Mean fetal weight in the 1000 mg/kg bw/day group was 3.777 g, 4.5% lower than the control value of 3.953 g. This was interpreted as a treatment-related effect, but not an adverse effect, because there were no associated effects on morphological development. There were no treatment-related effects observed on detailed external and internal examinations of the fetuses.

The NOAEL for maternal toxicity was identified as 1000 mg/kg bw/day, the highest dose tested. The study director identified a NOEL for fetal development of 300 mg/kg bw/day, and a NOAEL of 1000 mg/kg bw/day, because the slightly lower fetal bodyweights were not considered adverse.

FSANZ concurs with the conclusions of this study.

*Preliminary dose range-finding one-generation reproductive and fertility study of 2MeOx in Sprague Dawley rats (Charles River 2020a) Regulatory status: Not GLP, no specific guideline referenced.*

Both female and male Sprague Dawley rats of the parental generation (10/sex/group) were gavaged daily with 2-MeOx from eight days prior to mating through to termination, which for males was the end of the mating period, and for females was on Postnatal Day (PND) 21. The pups were dosed from PND 22 to 28. Dose levels used were 0, 100, 300 and 1000 mg/kg bw/day 2-MeOx (purity 99.97%). The vehicle and control article was deionised water.

Parameters recorded for the parental (P) generation included clinical signs, bodyweights, food consumption and gross necropsy findings. Litter sizes and number of pups per sex were recorded at birth. Litters were culled to 5/sex (if available) on Day 4. Clinical signs and bodyweights of pups were monitored, and food consumption was recorded after weaning. Pups were terminated on Day 29 and subject to detailed external examination.

All P generation rats survived to scheduled termination. P generation males had a lower (-5%) group mean bodyweight change in the interval Day 1 to 8, compared to the male control value. This was considered treatment-related but not adverse because of the transient nature and minor magnitude of the change. Females in the 1000 mg/kg bw/day group had lower (-18.5%) group mean feed consumption over the interval PND 4-8 (early lactation), relative to the control females. This finding was also considered to be treatment-related but not adverse because of the transient nature and minor magnitude of the change.

No treatment-related effects were observed on mating, fertility or gestation indices, or pre-coital time. Likewise, there were no observed effects on mean duration of gestation, mean number of corpora lutea, mean pre-implantation loss, mean number of pups delivered, mean post-implantation loss, or sex ratios of pups. There were no observed effects of treatment on organ weights or gross necropsy findings in the P generation rats.

In litters of rats dosed with ≥ 300 mg/kg/day, there were significant increases in numbers of dead pups between delivery and scheduled culling on PND 4. This finding was associated with a non-significant decrease in the live birth index in these groups, and in the PND 4 viability index (group mean at 1000 mg/kg/day 85.5% that of the control value). These findings were considered to be treatment-related and adverse. There was an associated

increase in number of pups with no milk visible in the stomach. There were no effects on pup survival after PND 4.

Pups of both sexes exposed to a maternal dose of 1000 mg/kg bw/day had a lower group mean bodyweight value, compared to that of sex-matched controls, in the intervals PND 4-8 and PND 8-13. Loud breathing was observed in some individuals of both sexes in the 1000 mg/kg bw/day group, and group mean terminal bodyweights on Day 29 were low in this group when compared to values for sex-matched controls (-28% for males and -20% for females). There was an associated lower group mean value for feed consumption in the interval PND 25-29 for 1000 mg/kg bw/day males, but not for females. These effects on bodyweight were considered to be adverse.

On the basis of these results, it was concluded that 1000 mg/kg bw/day was an excessive dose for the definitive study (reported below).

*Extended reproductive toxicity study of 2-MeOx in Sprague Dawley rats (Charles River 2020b). Regulatory status: GLP, compliant with OECD Test Guideline 443.*

On the basis of the findings of the dose range-finding study and because the adverse effects in that study were minor at 300 mg/kg bw/day, the dose levels of 2-MeOx (MeOx (purity 99.97%) selected for this study were 0, 100, 250 and 625 mg/kg bw/day. The vehicle and control article was deionized water. Both female and male Sprague Dawley rats of the P generation (24/sex/group) were dosed by oral gavage daily from ten weeks prior to mating through to scheduled termination, which was after pregnancy and lactation in the case of the females. For subsequent generations (F1 and F2) the dams were dosed during pregnancy and lactation, and pups dosed daily from weaning until scheduled termination. F1 litters were culled to five pups/sex/litter on PND 4. The dosing schedules of the F generations were as follows:

- Cohort 1A, dosed daily from weaning until termination at 92 to 96 days old.
- Cohort 1B, dosed daily from weaning until scheduled termination. For males, this was until PND 4 of the F2 generation. For females, it was until pups of the F2 generation were weaned, or 24 days after mating for females that did not conceive.
- Cohort 2A (20 pups/dose group from F2 generation, 10/sex, all litters represented) dosed daily from weaning until termination at PND 75 to 79, subjected to neurobehavioural assessment during life and to neurohistopathology following necropsy.
- Cohort 3 (20 pups/dose group from F2 generation, 10/sex, all litters represented) dosed daily from weaning, and injected with keyhole limpet haemagglutinin (KLH) on PND 53 to 57; terminated five days after KLH injection.

For all rats, clinical observations and mortality were subject to daily checks, and bodyweights and feed consumption were recorded at designated intervals. Mating periods of P generation and Cohort 1B generation rats were 14 days. Pregnancy and litter data were recorded. Pups were subject to detailed clinical examinations at designated intervals.

Developmental neurotoxicity and developmental immunotoxicity were tested only to 250 mg/kg bw/day in progeny because a high rate of maternal dystocia, which was not dose-related, led to insufficient numbers of pups to fully test for these toxicities. The unusually high incidence of maternal dystocia was observed in other studies conducted in the laboratory in the same time period.

Dose analysis at scheduled intervals confirmed that the dose formulations did not deviate significantly from the intended dose ranges.

One male P generation rat in the 625 mg/kg bw/day was euthanized *in extremis* on Study

Day 32. The cause of the moribund condition was not determined, but was not considered to be related to the test article because other rats were not similarly affected. One P generation male in the 250 mg/kg bw/day group was found dead after blood sampling on SD 137, and necropsy findings were consistent with anaesthetic complications. All other P generation males survived to scheduled termination.

P generation females from all dose groups were euthanized prior to scheduled termination for failure to conceive, for humane reasons in the puerperal period, or due to dystocia. Dystocia was significantly increased in the 625 mg/kg bw/day females, resulting in the unscheduled terminations of seven females, compared to three in the control group and one in each of the 100 and 250 mg/kg bw/day groups. This increase in dystocia was considered to be treatment-related and adverse.

A paternal NOAEL of 250 mg/kg bw/day was identified in P generation males, on the basis of clinical observations at 625 mg/kg bw/day that included hypoactivity, ataxic gait, abnormal startle reflex and tremors. No similar signs were observed in females.

There were no treatment-related effects on body weights, body weight changes, or feed consumption in P generation rats of either sex.

A NOAEL for reproductive and developmental toxicity of 100 mg/kg bw/day was identified on the basis of decreases in group mean live birth index in P generation females dosed with ≥250 mg/kg bw/day. Evidence of impaired maternal behaviour, including neonatal pups that were cold and/or had no milk visible in the stomach, was also present in these groups.

No treatment-related adverse effects on body weights or bodyweight changes were observed in F1 pups up to weaning. Survival of Cohort 1A and Cohort 1B rats was not affected by treatment, and there were no adverse effects on clinical observations, body weights, feed consumption, sexual development, or oestrus cycles. Group mean values for body weights and body weight changes of treated Cohort 1B females during the pregnancy period were comparable to those of control females, and there were no effects on the mean duration of gestation, number of implantation sites, percentage of post-implantation loss, mean live birth index or pups sex ratio. Survival of F2 pups to PND 4 was not affected by dose, in contrast to findings for F1 pups over the same period, and there were no treatment-related effects on clinical findings, body weights, body weight changes or sexual development in F2 pups.

No developmental neurotoxicity in Cohort 2A rats, or developmental immunotoxicity in Cohort 3 rats, was observed. The NOAEL for both these endpoints was considered to be 250 mg/kg bw/day, the highest dose tested in the F2 generation.

FSANZ concurs with the conclusions of this study.

### 3.4.5 Genotoxicity studies

A number of genotoxicity assays of 2-MeOx are available.

#### *3.4.5.1 Bacterial reverse mutation assays (Ames tests)*

*Bacterial reverse mutation assay of 2-MeOx (Antonucci et al 2011). Regulatory status: GLP, conducted according to OECD Guideline 471.*

The test strains used for this assay were *Salmonella enterocolitica* var. typhimurium TA98, TA100, TA1535, and TA1537, and *Escherichia coli* strain WP2*uvrA*. The assay was conducted using the plate incorporation method, with plates sealed in gas-tight bags during exposure to prevent evaporation of the test article. Bacterial strains were exposed to 2-MeOx

(purity unspecified) at concentrations up to 5490 µg/plate, in the presence and absence of S9 mix. No increase in revertant colonies was observed at any concentration of 2-MeOx, and there was no apparent toxicity to the bacteria. Assays with appropriate positive control substances were run in parallel and expected increases in mutation incidence were observed.

Seifried et al (2006) published a compilation of the results of two decades of genotoxicity testing of chemicals for the National Cancer Institute by a contract research laboratory. Chemicals were tested on test strains *Salmonella enterocolitica* var. typhimurium TA98, TA100, TA 1535, TA1537, and TA1538, in the presence or absence of S9 mix for metabolic activation. Little detail is provided in the paper but 2-MeOx, under the synonym 2-methyltetrahydrofuran, is reported to be negative for genotoxicity at doses ranging from 100 to 10000 µg/plate.

### *3.4.5.2* In vitro *Mammalian Cell Gene Mutation Tests*

*L517LY Mouse Lymphoma Assay of 2-MeOx (Harlan 2013) Regulatory status: GLP; equivalent to OECD Test Guideline 476, method B17 of Commission Regulation (EC) No. 440/2008, US-EPA OPPTS 870.530 Guideline, and METI/MHLW (Japan) guidelines.*

For both the dose range-finding experiment (Experiment 1) and the definitive experiment (Experiment 2) the vehicle and negative control article was R0 Medium. Positive control articles were ethylmethanesulphonate for tests in the absence of S9 mix for metabolic activation, and cyclophosphamide for tests in the presence of S9 mix.

In Experiment 1, mouse lymphoma cells were treated with the test article (purity 99.5%) at concentrations ranging from 3.99 to 1020 µg/mL, with negative and positive control tests conducted in parallel. Tests at all concentrations were conducted in duplicate, with four-hour exposure periods. No precipitation or cytotoxicity was observed.

On the basis of these results, the definitive assay was conducted using six concentrations ranging from 63.75 to 1020 µg/mL, with negative and positive control tests conducted in parallel. Exposure was for four hours in the presence of S9 mix and 24 hours in the absence of S9 mix.

No increase in frequency of mutations was observed associated with the test article at any concentration, when compared to the negative control. The expected increases in frequency of mutations was observed in the positive control tests, confirming the validity of the assay. It was concluded that the test article was non-mutagenic under the conditions of this test.

The publication by Siefried et al (2006), which is a compilation of the results of a large number of genotoxicity assays of chemicals conducted for the National Cancer Institute by a contract research laboratory, reports that 2-MeOx produced negative results in a mouse lymphoma assay at concentrations of 1500 to 5000 µg/mL in the absence of S9 mix. Results were reported as inconclusive in the presence of S9 mix. There are no further details provided concerning the latter result.

### *3.4.5.3 Mammalian Micronucleus Tests*

In vitro *micronucleus test of 2-MeOx human lymphocytes (Envigo 2019).Regulatory status: GLP, compliant with OECD Test Guideline 487.*

The report of this study was provided to FSANZ on a CCI basis and therefore full details cannot be described. A preliminary cytotoxicity established that the highest concentration recommended by the OECD guideline (10 mmol/L, equivalent to 860 µg 2-MeOx/mL) did not

induce toxic effects. Accordingly, concentrations used in the definitive assay were 20, 26.88, 53.75, 107.5, 215, 430 and 860 µg/mL. No precipitation of the test item was observed at any dose level.

Exposure to 2-MeOx included 4 h without S9-mix, 24 h without S9-mix and 4 h with S9-mix. Binucleated cells were created by addition of cytochalasin B to inhibit cytoplasmic division, and micronuclei were counted. No increase in the micronucleus frequency was observed after treatment with 2-MeOx. Significant increases in the incidence of micronuclei were observed with positive control articles, confirming the validity of the assay.

Antonucci et al (2011) briefly described a micronucleus assay conducted on bone marrow cells harvested at termination of a three-month subchronic toxicity study of 2-MeOx in Sprague Dawley rats described in section 3.4.2. Positive control cells from rats that had been treated with mitomycin C were included among the coded slides prepared for evaluation. No increase in frequency of micronuclei was observed in cells from negative control rats or rats treated with 2-MeOx for three months. It was concluded that 2-MeOx was non-clastogenic and non-aneugenic to human lymphocytes *in vitro*.

#### *3.4.5.4* In vitro *chromosomal aberration test*

Antonucci et al (2011) briefly described an *in vitro* chromosomal aberration test conducted using human lymphocytes, in accordance with OECD Guideline 473. Test concentrations of 2-MeOX (purity unspecified) were up to 10.7 nM, and assays were conducted with and without the addition of S9 mix for metabolic activation. Human lymphocytes in cell culture were treated with 2-MeOx with and without S9 mix for 4 h, and for 21 h without S9 mix. There was no evidence of cytotoxicity and no evidence of significant increase in the frequency of chromosomal aberrations. Appropriate positive control assays were run in parallel to confirm the validity of the assay.

## 3.5 Potential for allergenicity

No case reports of allergy or intolerance to oral exposure to 2-MeOx were located by literature search.

## 3.6 Safety assessments by international agencies or other national government agencies

The European Food Safety Authority (EFSA) Panel on Food Contact Materials, Enzymes and Processing Aids assessed the safety of 2-MeOx as a food extraction solvent and published their assessment in 2022. A Tolerable Daily Intake (TDI) of 1 mg/kg bw/day was derived from the lowest NOAEL, 100 mg/kg bw/day, which was identified in the developmental and reproductive study in rats (Charles River 2020b) described in Section 3.4.4. The NOAEL was identified based on a decrease in group mean female fertility index. EFSA applied a total Uncertainty Factor of 100 but did not specify individual Uncertainty Factors.

EFSA noted the potential for furan and/or 2-methylfuran to be present as contaminants in 2-MeOx, and calculated Margins of Exposure (MOEs) of 725 and 14,800 for non-neoplastic and neoplastic effects, respectively, at the mean exposure. EFSA concluded that these MOEs did not indicate a safety concern. The ADI derived by FSANZ is the same level as EFSA's TDI and therefore MOEs would be of similar orders of magnitude. FSANZ concurs with EFSA's conclusion that these MOEs do not indicate a safety concern.

## 3.7 Safety assessment discussion and conclusion

2-MeOx has been used in pharmaceutical production since 2007 and was recently approved by EFSA for use as a food extraction solvent up to a TDI of 1 mg/kg bw/day (EFSA 2022).

Toxicokinetic studies in rats and mice show that 2-MeOx is rapidly absorbed and excreted in these species and does not accumulate in any organ. Excretion is via the urine and by exhalation.

The acute oral $LD_{50}$ of 2-MeOx in the female Wistar rat is in the range 300-2000 mg/kg bw. A three-month repeat-dose oral gavage study in rats, at doses up to 1000 mg/kg bw/day, identified a NOAEL of 250 mg/kg bw/day, based on minimal to mild reversible hepatic centrilobular hypertrophy at the highest dose. A developmental toxicity study in rats, at doses up to 1000 mg/kg bw/day, resulted in identification of a NOAEL for maternal toxicity of 1000 mg/kg bw/day, and a NOAEL for fetal toxicity of 1000 mg/kg bw/day. An extended reproductive toxicity study of 2-MeOx in rats, at doses up to 625 mg/kg bw/day, identified a paternal NOAEL of 250 mg/kg bw/day, based on adverse clinical observations at the highest dose, and a NOAEL for reproductive and developmental toxicity of 100 mg/kg bw/day on the basis of decrease in group mean live birth index in P generation females dosed with ≥250 mg/kg bw/day. Thus, the lowest NOAEL identified in animal studies was 100 mg/kg bw/day.

A range of *in vitro* and *in vivo* genotoxicity assays, including bacterial reverse mutation assays, mammalian gene mutation tests, micronucleus tests and a chromosomal aberration test, have been conducted. Collectively, these support the conclusion that 2-MeOx does not show genotoxic potential.

No long-term or carcinogenicity studies of 2-MeOx were submitted or located by literature search. FSANZ concludes that 2-MeOx is unlikely to be a carcinogen because it is not genotoxic and no lesions likely to lead to neoplasia by a nongenotoxic mechanism were observed in the three-month repeat-dose study conducted in rats.

No case reports of allergy or intolerance attributable to oral exposure to 2-MeOx were located.

There is potential for ethanol, furan and 2-methylfuran to be present as contaminants, but only at trace amounts that are not considered to be of safety concern.

The lowest NOAEL identified in animal studies, 100 mg/kg bw/day, is suitable for use for derivation of an ADI for 2-MeOx. FSANZ considers it appropriate to apply an Uncertainty Factor of 10 for extrapolation from animal studies to human hazard, and an Uncertainty Factor of 10 to allow for variability in sensitivity within the human population. An Uncertainty Factor for extrapolation from subchronic studies to chronic toxicity is not considered to be necessary because there is no evidence that a chronic study is required. The total Uncertainty Factor is therefore 100, from which an ADI of 1.0 mg/kg bw/day is derived.

# 4 Dietary exposure assessment

## 4.1 Introduction and purpose

The applicant requested permission to use 2-MeOx as an extraction solvent processing aid and proposed a MPL for 2-MeOx of 20 mg/kg in foods, with the exception of infant formula products (this includes infant formula, follow on formula and special purpose formula) and foods for infants, which were requested to have a lower MPL of 5 mg/kg.

When used at the requested MPL of 5 mg/kg, it was found the estimated 90$^{th}$ percentile exposure to 2-MeOx to exceed the ADI of 1 mg/kg bw/day for infants aged 3 months and exclusively formula-fed. As a result of this, the MPL for infant formula products was reduced from 5 mg/kg to 3 mg/kg. During the assessment FSANZ sought confirmation from the applicant on the MPL they were requesting for formulated supplementary foods for young children (FSFYC) regulated under Standard 2.9.3 of the Code. The applicant requested it align with the MPL requested for foods for infants (i.e. 5 mg/kg). For the purpose of dietary exposure assessment, the applicant provided a list of food categories that are most likely to be processed using 2-MeOx (see Table F.1-1 and Table F.7-2 in the application for details) while stating that 2-MeOx is not intentionally added to any food.

The purpose of the dietary exposure assessments were to estimate the levels of chronic dietary exposures to 2-MeOx for the Australian and New Zealand populations. Chronic dietary exposure estimates are used to represent the long term, usually life-long, dietary exposure for the population from the range of foods containing the chemical of interest.

## 4.2 Approaches to estimating dietary exposures

Dietary exposure assessment requires data on the concentration of the chemical of interest in the food requested and food consumption data. Dietary exposure assessments at FSANZ are conducted using a tiered approach. The first assessment is conducted using the most conservative assumptions and the least amount of resources, with refinements made following this assessment if needed. A detailed discussion of the FSANZ methodology and approach to conducting dietary exposure assessments is set out in *Principles and Practices of Dietary Exposure Assessment for Food Regulatory Purposes* (FSANZ 2009).

The safety assessment did not identify any population sub-groups or at-risk groups for which there were specific safety considerations or where separate chronic dietary intake estimates were needed. However, as all foods may contain 2-MeOx residues, including foods consumed by different consumer sub-groups, separate dietary exposure assessments were conducted to estimate exposures from general purpose foods and special purpose food categories using appropriate dietary exposure assessment methods. These methods were:

1. a budget method calculation for consumers of general purpose foods
2. model diets for consumers of infant formula products and FSFYC (or 'toddler milks')
3. a deterministic calculation for consumers of food for special medical purposes (FSMP).

The dietary exposure assessment was conducted to make the most realistic estimation of dietary exposure to 2-MeOx as possible. However, where uncertainties in the data existed, conservative assumptions were generally used to ensure that the estimated dietary exposure was not an underestimation of exposure. Assumptions and limitations in the dietary exposure assessments are provided under each method used.

The ADI of 1.0 mg/kg bw/day confirmed in the FSANZ safety assessment for this application was used for risk characterisation purposes.

***Concentrations of 2-MeOx***

The food categories used in the dietary exposure assessment and the proposed MPLs are listed in Table 3.

***Table 3*** The food categories and the proposed MPLs used in the dietary exposure assessment

| Food | Maximum permitted level (mg/kg) |
|---|---|
| General purpose foods (including formulated meal replacements and formulated supplementary foods) | 20 |
| Food for special medical purposes | 20 |
| Infant formula (as prepared or ready-to-feed) | 3 |
| Follow on formula (as prepared or ready-to-feed) | 3 |
| Infant formula products for special dietary use (as prepared or ready-to-feed) | 3 |
| Formulated supplementary foods for young children (as prepared or ready-to-feed) | 5 |
| Foods for infants | 5 |

### 4.2.1 Estimating dietary exposure from general purpose foods

The budget method calculation was used as a 'worse-case scenario' approach to estimating likely levels of dietary exposure to 2-MeOx from all general purpose foods, assuming 2-MeOx will remain in final foods at the highest proposed MPL (20 mg/kg).

The budget method is a valid screening tool for estimating the theoretical maximum daily intake (TMDI) of a food additive (Douglass et al 1997). Whilst the budget method was originally developed for use in assessing food additives, it is also appropriate to use for estimating the TMDI for processing aids (FAO/WHO 2020), for instance 2-MeOx as an extraction solvent processing aid for this application. This method is used by international regulatory bodies and the FAO/WHO Joint Expert Committee on Food Additives (JECFA) (FAO/WHO 2021) for dietary exposure assessments for processing aids. The calculation is based on physiological food and liquid requirements, the processing aid concentration in foods and beverages, and the proportion of foods and beverages that may contain the processing aid. The TMDI can then be compared to an ADI or a NOAEL for risk characterisation purposes.

In this budget method calculation, FSANZ made the following assumptions that are conservative and reflective of a first tier in estimating dietary exposure (FAO/WHO 2009):

- the maximum physiological requirement of solid foods (including milk) is 50 g/kg body weight/day. This is the standard level used in a budget method calculation where there is a potential for the processing aid to be present in baby foods or general purpose foods that would be consumed by infants (Hansen 1966).

- the maximum physiological requirement for liquids is 100 mL/kg body weight/day. This is the standard level used in a budget method calculation.

- 12.5% of solid foods and 25% of non-milk beverages are processed, based on commonly used default proportions noted in FAO/WHO 2009.

- the maximum 2-MeOx residue level in solid foods (including milk) and non-milk beverages is 20 mg/kg.

Based on these assumptions, FSANZ calculated the TMDI of 2-MeOx to be 0.63 mg/kg bw/day (approximately 65% of the ADI). As the calculated TMDI is less than 100% of the ADI, no further refinement to the dietary exposure assessment was required in accordance with FSANZ's tiered approach for conducting dietary exposure assessments (FSANZ 2009). The calculated TMDI will be an overestimate of the dietary exposure to 2-

MeOx given the conservatisms in the budget method. This includes that it was assumed that 2-MeOx remains in all solid foods and non-milk beverages at the proposed MPL (20 mg/kg) whilst noting that 2-MeOx is not intentionally added to any food.

As a conservative method, the budget method calculation covers exposures from formulated meal replacements (FMR) and formulated supplementary foods (FSF) given they are a supplement to a normal diet and not a total diet replacement.

### 4.2.2 Estimating dietary exposures for infants aged one year and below

As the list of food categories that are most likely to be processed using 2-MeOx includes infant formula products and FSFYC (Table F.1-1 and Table F.7-2 in the application), separate dietary exposures were estimated for infants aged 3 months, 9 months and 12 months.

The population groups that were considered for this dietary exposure assessment were:

- infants aged 3 months – representing exclusively formula-fed infants
- infants aged 9 months – representing infants who consume food as well as follow on formula
- infants aged 12 months – representing infants who consume food as well as FSFYC.

There are no national consumption data for Australia and New Zealand population groups aged less than 2 years. Therefore, model diets were constructed and used for the estimation of exposure to 2-MeOx from infant formula products and FSFYC. The same model diets were used for Australia and New Zealand.

As the 3 month, 9 month and 12 month old infant model diets are based on mean food consumption amounts only, a distribution of food consumption was not available. Therefore, 90$^{th}$ percentile (P90) dietary exposures were estimated to be double the mean exposure as per the calculation used by FSANZ (WHO 1985).

The energy contents of infant formula, follow on formula and FSFYC were required for the calculation of the amount of these foods in the model diets. AUSNUT is the latest nutrient dataset published for Australian foods. In this dataset, the energy content of *Infant formula, 6-12 months, prepared with water* is 264 kJ/100 g, and the energy content of *Toddler milk, regular, prepared with water* is 269 kJ/100 g (FSANZ 2016).

Details of the infant groups assessed, composition of the model diets and assumptions used are provided below.

***Construction of the model diets***

The model diets were constructed for infants following the methodology explained in the supporting document 1 (SD1) of A1155 (FSANZ 2018).The details are provided below.

- *Infants aged 3 months*

The model diet was constructed based on the recommended energy intake for a three-month-old boy (343 kJ/kg bw/day) and the 50$^{th}$ percentile weight (6.4 kg) for the same age and sex (FSANZ 2018). The entire energy requirement (100%) in the 3 month old infant diet was derived from infant formula.

- *Infants aged 9 months*

By the age of 9 months, infants consume a mixed diet of solids and follow on formula / human milk. The model diet was constructed based on recommended energy intakes for a 9 month old boy (330 kJ/kg bw/day), the 50$^{th}$ percentile weight (8.9 kg) for the same age and sex and the proportion of milk and solid foods in the diet for a 9 month old infant (FSANZ 2018). It was assumed that 50% of energy intake was derived from follow on formula and 50% from solid foods and other fluids.

- *Infants aged 12 months*

Infants aged under 12 months should be breastfed or fed a commercial infant/follow on formula as their main drink, not cow's milk (National Health and Medical Research Council (Australia) 2013). From 12 months of age, infants can drink cow's milk (or other appropriate substitute). 'Toddler milk' / FSFYC is not a requirement for healthy children (National Health and Medical Research Council (Australia) 2013). Infants aged 12 months consume a mixed diet. The model diet was constructed based on the recommended energy intake for a 12 month old boy (335 kJ/kg bw/day) at the 50$^{th}$ percentile weight (9.6 kg) for the same age and sex (FSANZ 2018). It was assumed that 35% of energy intake was derived from FSFYC as their sole source of milk and 65% from solid foods and other fluids.

A set of model diets was not established for infants consuming infant formula products for special dietary uses as the energy and/or fluid requirements can vary depending on the medical conditions of the infant. Additionally, the energy content of the various infant formula products for special dietary uses can be variable. The assessment of A1155 included an examination of products, including formulas for premature infants, formulas for use by infants with inborn errors of metabolism, and formulas for use by infants with severe food allergies, which found the range of energy contents was 269 – 415 kJ/100 g (FSANZ 2018). If an infant consuming infant formula products for special dietary uses has similar energy requirements to those used in the model infant diets and their specific formula has a similar energy content to that used in the model diets, then their exposure to 2-MeOx is anticipated to be similar to that outlined in the assessment for this application. If an infant consuming infant formula products for special dietary uses has similar energy requirements to those used in the model infant diets and their specific formula has a higher energy content to that used in the model diets, then their exposure to 2-MeOx is anticipated to be similar to or lower than that estimated in this assessment. Further to these considerations, infants consuming infant formula products for special dietary uses are generally under medical and dietetic supervision given their specific needs. Short term dietary exposures to food additives in excess of those estimated may be of a lesser priority than medical and dietetic considerations in their overall case management.

***Assumptions and limitations of the dietary exposure assessment***

Assumptions and limitations in the dietary exposure assessments included:

- infants aged 3 months are exclusively fed infant formula.
- infants aged 9 months consume follow on formula in amounts that meet 50% of their energy requirements, with the other 50% of energy requirements obtained from consuming solid foods and other fluids.
- infants aged 12 months consume FSFYC in amounts that meet 35% of their energy requirements, with the other 65% of energy requirements obtained from consuming solid foods and other fluids.
- consumption of foods as outlined in the model diets represent current food consumption amounts for Australian and New Zealand children aged 3 months, 9 months and 12 months.

- 100% of infant formula and follow on formula contain 2-MeOx at an MPL of 3 mg/kg
- 100% of FSFYC contain 2-MeOx at an MPL of 5 mg/kg.

***Estimation of dietary exposure for 3 month, 9 month and 12 month old infants from infant formula /follow on formula / FSFYC***

Based on the assumptions and the variables (e.g. energy requirements, body weight and energy content of foods) stated above, dietary exposures from infant formula, follow on formula and FSFYC were estimated for 3-month, 9-month and 12-month old infants, expressed as mg/kg bw/day and %ADI (Table 4).

***Table 4*** Estimated dietary exposure to 2-MeOx for infants aged 3 months, 9 months and 12 months from infant formula / follow on formula / FSFYC

| Item | Units | Infant groups assessed | | |
|---|---|---|---|---|
| | | 3 months | 9 months | 12 months |
| Recommended energy intake* | kJ/kg bw/day | 343 | 330 | 335 |
| 50th percentile body weight# | kg | 6.4 | 8.9 | 9.6 |
| Recommended energy intake | kJ/day | 2195 | 2937 | 3216 |
| Amount of infant formula required to meet 100% of energy requirements$ | g/day | 830 | n/a | n/a |
| Amount of follow on formula required to meet 50% of energy requirements$ | g/day | n/a | 555 | n/a |
| Amount of FSFYC required to meet 35% of energy requirements$ | g/day | n/a | n/a | 420 |
| Mean dietary exposure to 2-MeOx from the milk source | g/kg bw/day | 0.39 | 0.19 | 0.22 |
| | %ADI^ | 40 | 20 | 20 |
| P90 dietary exposure to 2-MeOx from the milk source& | g/kg bw/day | 0.78 | 0.37 | 0.44 |
| | %ADI^ | 80 | 35 | 45 |

*(United Nations University et al 2004)

#(World Health Organization 2006)

n/a not applicable

$The energy content of Infant formula, 6-12 months, prepared with water (264 kJ/100 g) was used for infant formula and follow on formula (FSANZ 2016). The energy content of FSFYC/Toddler milk, regular, prepared with water (269 kJ/100 g) was used for FSFYC (FSANZ 2016).

&P90 dietary exposure is equal to double of the mean dietary exposure.

^ADI-1.0 mg/kg bw/day.

The estimated mean and P90 dietary exposures from infant formula for infants aged 3 months are 0.39 mg/kg bw/day and 0.78 mg/kg bw/day respectively. As infants aged 3 months were considered 'exclusively formula-fed', these are the total estimated exposures to 2-MeOx from the diet.

The estimated mean and P90 dietary exposures from follow on formula for the infants aged 9 months are 0.19 mg/kg bw/day and 0.37 mg/kg bw/day respectively. Similarly, the estimated mean and P90 dietary exposures from FSFYC for the infants aged 12 months are 0.22 mg/kg bw/day and 0.44 mg/kg bw/day respectively. These are the estimated exposures to 2-MeOx from the source of milk in their diet.

As 9-month and 12-month old infants consume foods in addition to follow on formula and FSYC respectively, additional calculations were used to estimate the amounts of foods for infants or general purpose foods that could be safely consumed, assuming the proposed MPLs of 2-MeOx in these foods as provided in Table 5.

***Estimation of consumption amounts of foods for infants and general purpose foods for infants aged 9 months and 12 months***

As infants aged 9 months and 12 months consume a mixed diet (including foods for infants and general purpose foods), additional calculations were used to estimate the amount of solid foods and other fluids that could be consumed without exceeding the ADI of 1 mg/kg bw/day.

For these estimations, it was assumed that:

- 50% of the energy requirement for 9-month old infants comes from solid foods and other fluids
- 65% of the energy requirement for 12-month old infants comes from solid foods and other fluids
- 100% of foods for infants are processed and contain 2-MeOx at an MPL of 5 mg/kg
- 12.5% of general purpose foods are processed (the same percentage used for the solid foods in the budget method calculation) and contain 2-MeOx at an MPL of 20 mg/kg.

The results revealed that:

- infants aged 9 months could consume up to 1447 g of food for infants, or up to 2894 g of general purpose foods, in addition to 555 g of follow on formula per day before exceeding the ADI.
- infants aged 12 months could consume up to 1500 g of food for infants, or up to 3000 g of the general purpose foods, in addition to 420 g of FSFYC per day before exceeding the ADI.

### 4.2.3 Estimating dietary exposures from food for special medical purposes (FSMP)

FSMP include both foods that are, and are not, very low energy foods. As consumption of FSMP that are very low energy foods is different to FSMP that are not very low energy foods, these foods are considered separately in any dietary exposure assessment. For this assessment, FSMP that are manufactured in the form of very low energy food products are referred to as VLED (Very Low Energy Diets) (described as VLED hereafter), and FSMP that are not very low energy food products are referred to as ‘other FSMP’. The requested maximum level of 2-MeOx is 20 mg/kg for both FSMP types.

#### *4.2.3.1 VLED*

VLED are used as a total diet replacement for the dietary management of overweight and obesity for a prescribed duration of typically no longer than twelve weeks (FSANZ 2021). During the intensive phase of the program, daily consumption typically consists of three VLED (various product types), a minimum of 2 litres of water, two cups of low-starch vegetables, one teaspoon of vegetable oil and additional low joule beverages. VLED is not recommended for pregnant, nursing, lactating women or use by infants, children, adolescents and elderly people.

Given relatively short-term consumption (no longer than twelve weeks) in comparison to the long term consumption considered for the chronic dietary exposure estimates, and considering the budget method calculation covers this type of exposure as a conservative method, the dietary exposure to 2-MeOx from VLED was not conducted for this application.

#### *4.2.3.2 Other FSMP*

Nationally representative consumption data for other FSMP, which in this case are formulated food products intended to be used under medical supervision, are not available because they were outside the scope of food consumption data collected through the Australian or New Zealand National Nutrition Surveys. As FSMP may be developed to perform a wide range of medical functions the composition and consumption amount of other FSMP will be different for each consumer. The requirements for any person taking other FSMP may vary with medical and nutritional needs and is administered under medical supervision.

Other FSMP may be consumed orally and/or used for enteral tube feeding. A literature search identified that recommended feeding of other FSMP for an adult ranges from 12 kcal/kg bw/day to 30 kcal/kg bw/day (Compher et al 2022; Stroud et al 2003). In a conservative approach, this higher energy intake (30 kcal/kg bw/day) was used for the dietary exposure assessment for adults.

The Australian and New Zealand Paediatric Critical Care Nutrition Support Guidelines recommend a minimum protein requirement of 1.5 g/kg bw/day for children up to 18 years of age (AuSPEN 2023). Online product information indicates the minimum protein content of several other FSMP suitable as a sole source of nutrition for children is 30 g/L[3]. These two variables (protein requirement and minimum protein content) were used in estimating the dietary exposure for children. To estimate dietary exposure to 2-MeOx, deterministic exposure assessments were conducted for adults and children in a tiered approach assuming they consume other FSMP as a sole source of nutrition.

Assumptions made in the dietary exposure assessment included:

- other FSMP are a sole source of nutrition
- the density of all other FSMP is 1.03 g/mL (similar to regular fat cows' milk)
- all other FSMP are a standard feed containing energy content of 1 kcal/mL
- energy intake for adults is 30 kcal/kg bw/day (Stroud et al 2003)
- the minimum protein content of other FSMP is 30 g/L
- protein requirement for children up to 18 years is 1.5 g/kg bw/day (AuSPEN 2023)
- all other FSMP contain 2-MeOx at the proposed MPL (20 mg/ kg)
- there are no other contributions to 2-MeOx exposure, for example through the consumption from general purpose foods containing 2-MeOx.

The estimated dietary exposures to 2-MeOx from other FSMP for adults and children were calculated and expressed as mg/kg bw/day and the %ADI (Table 5 and Table 6).

***Table 5*** Estimated dietary exposure to 2-MeOx for adults from other FSMP

| Energy intake (kcal/kg bw/day)* | Energy content of other FSMP (kcal/mL) | Proposed maximum level (mg/ kg) | Estimated exposure | |
|---|---|---|---|---|
| | | | mg/kg bw/day | % ADI |
| 30 | 1 | 20 | 0.62 | 60 |

*Stroud et al (2003)

[3] https://www.nestlemedicalhub.com/products; https://www.abbottnutrition.com/product-guides

***Table 6*** Estimated dietary exposure to 2-MeOx for children up to 18 years of age from other FSMP

| Protein requirement (g/kg bw/day)* | Minimum protein content of FSMP (g/L) | Proposed maximum level (mg/ kg) | Estimated exposure | |
|---|---|---|---|---|
| | | | mg/kg bw/day | % ADI |
| 1.5 | 30 | 20 | 1.00 | 100 |

*AuSPEN 2023

At the proposed MPL of 2-MeOx (20 mg/kg), the estimated dietary exposures from other FSMP for adults and children are 0.62 mg/kg bw/day and 1.00 mg/kg bw/day respectively.

# 5 Discussion and risk characterisation

Using a first-tier budget method approach, the estimated dietary exposure to 2-MeOx from general purpose foods (including formulated meal replacements and formulated supplementary foods) is approximately 65% of the ADI. Due to the conservative assumptions inherent in the budget method, this is likely an overestimation of dietary exposure, and no further refinement of the exposure estimate was required.

For 3-month old infants exclusively fed infant formula the estimated mean and 90$^{th}$ percentile dietary exposure to 2-MeOx is 40% and 80% of the ADI respectively. For 9-month old infants the estimated mean and 90$^{th}$ percentile dietary exposures to 2-MeOx from follow on formula are 20% and 35% of the ADI, respectively, and for 12-month old infants the estimated mean and 90$^{th}$ percentile dietary exposure to 2-MeOx from FSFYC is 20% and 45% of the ADI, respectively.

As 9-month old and 12-month old infants consume solid food and other fluids in addition to follow on formula/FSFYC, additional calculations were used to estimate the amounts of foods for infants and general purpose foods that could be consumed without exceeding the ADI. For 9 month-old infants, these amounts (1447 g – 2894 g), when compared to the amounts of foods reported for 9-month old infants in other studies, were well above estimated or actual food consumption amounts. In the 25$^{th}$ Australian Total Diet Study (ATDS), a model diet was constructed in which the patterns of consumption of a two year old children from the 2011-12 National Nutrition and Physical Activity Survey were scaled down and used to determine the solids and other fluids portion of the 9-month old infant's diet, with certain foods (e.g. nuts, coffee, tea, honey and alcohol) removed because they are not recommended for 9-month old infants (FSANZ 2019). In the 25$^{th}$ ATDS, the total mean consumption of all food groups (except infant formulas) for respondents aged 9-months was approximately 300 g per day (FSANZ 2019). For respondents to the Melbourne Infant Feeding, Activity and Nutrition Trial (InFANT) program[4], mean consumption of all foods excluding infant and toddler formulae and human breast milk, across the 3 days of the study was approximately 590 g/day (Campbell et al 2008, 2013). In the 2016 New Zealand Total Diet Study (NZTDS), simulated typical diets were used to derive mean daily food consumption for different age–gender cohorts in the New Zealand population including infants (6–12 months) and toddlers (1–3 years) (MPI 2018). In this study, the total mean consumption for simulated typical diets (excluding water and infant/follow-on formula) for infants and toddlers were approximately 550 g per day and 1100 g per day respectively.

[4] As part of the Melbourne Infant Feeding, Activity and Nutrition Trial (InFANT) program, three day Multi-pass 24 hour recalls were collected from parents when their infants were 9 months old (mid-intervention) and 20 months old (post-intervention) (Campbell et al 2013). For infants in the control group, food consumption data were coded into food groups using AUSNUT 2007 (FSANZ 2008) and aggregated data were provided to FSANZ.

For child and adult consumers of other FSMP, estimated dietary exposures of 2-MeOx were 60% of the ADI and 100% of the ADI, respectively. These estimates are, however, overestimates given the conservative assumptions used in the calculation. For instance, it was assumed that all other FSMP contain 2-MeOx at the proposed MPL (20 mg/ kg) to be representative of the worst-case scenario.

# 6 Conclusions from the risk and technical assessment

Based on the safety and dietary exposure assessments, there are no safety concerns associated with use of 2-MeOx as an extraction solvent at the proposed MPLs.

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