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Safety assessment of 2-methyloxolane as a food extraction solvent

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Abstract

The EFSA Panel on Food Contact Materials, Enzymes and Processing Aids (CEP) assessed the safety of 2-methyloxolane as an extraction solvent under the intended conditions of use and the maximum residue limits (MRLs) proposed by the applicant. 2-Methyloxolane is intended to be used in processes currently applying hexane for oil and protein extraction from plant sources or for extraction of food additives. The proposed MRLs for the following uses are: (i) 1 mg/kg in fat, oil or butter; (ii) 10 mg/kg in defatted protein products, defatted flour and other defatted solid ingredients; (iii) 1 mg/kg in food category 13 (foods intended for particular nutritional uses as defined by Directive 2009/39/EC); and (iv) 1 mg/kg for the extraction of food additives. The Panel calculated the dietary exposure with the highest potential maximum (95th percentile) for toddlers as 0.32 mg/kg body weight (bw) per day. Based on the available toxicological data, the Panel concluded that 2-methyloxolane was rapidly metabolised with a low bioaccumulation potential and does not raise a concern for genotoxicity. The Panel identified different no observed adverse effect levels (NOAELs) in a subchronic oral toxicity study in rats, an oral developmental toxicity study and an extended one-generation reproductive toxicity study, and a TDI of 1 mg/kg bw per day for 2-methyloxolane was derived based on the lowest identified NOAEL (100 mg/kg bw per day) for reproductive and developmental toxicity. This TDI was not exceeded in any of the population groups at the mean and 95th percentile exposure. The Panel concluded that the extraction solvent 2-methyloxolane does not raise a safety concern when used according to the intended conditions and at the proposed MRLs in the extracted foods or food ingredients.

Keywords: 2-methyloxolane, CAS No. is 96-47-9, extraction solvent, maximum residue limit, safety assessment

Requestor: European Commission

Question number: EFSA-Q-2021-00010

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Note: The opinion is published in accordance with article 39 of Regulation (EC) 178/2002. The following information is redacted to which EFSA awarded confidentiality status upon verification of justification provided by the applicant: information on the specification and level of impurities which do not influence the hazard classification; information on the method of manufacture and manufacturing process data of the solvent.

Panel members: José Manuel Barat Baviera, Claudia Bolognesi, Andrew Chesson, Pier Sandro Cocconcelli, Riccardo Crebelli, David Michael Gott, Konrad Grob, Claude Lambré, Evgenia Lampi, Marcel Mengelers, Alicja Mortensen, Gilles Rivière, Vittorio Silano (until 21 December 2020†), Inger-Lise Steffensen, Christina Tlustos, Henk Van Loveren, Laurence Vernis and Holger Zorn.

Amendment: One Panel member was missing from the author list. An editorial correction was carried out that does not materially affect the contents or outcome of this scientific output. To avoid confusion, the original version of the output has been removed from the EFSA Journal, but is available on request.

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1. Introduction

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

1.1.1. Background

The use of extraction solvents is regulated under Directive 2009/32/EC (recast)[1] on the approximation of the laws of the Member States on extraction solvents used in the production of foodstuffs and food ingredients.

An application has been introduced for the amendment of Annex I to Directive 2009/32/EC to authorise the use of 2-methyloxolane as an extraction solvent for the use in the production of various foodstuffs and food ingredients. Therefore, the European Commission asks the European Food Safety Authority (EFSA) to provide a scientific opinion on the safety of the use of 2-methyloxolane as an extraction solvent under the intended conditions of use and the proposed maximum residue limits (MRLs).

1.1.2. Terms of Reference

In accordance with Article 29(1)(a) of Regulation (EC) No 178/2002 the European Commission asks the European Food Safety Authority to provide a scientific opinion on the safety of use of 2-methyloxolane as an extraction solvent under the intended conditions of use and the proposed MRLs.

1.2. Interpretation of the Terms of Reference

The Panel has evaluated the safety of 2-methyloxolane when present as a solvent residue in lipid extracts, defatted protein products, or in extracted food additives (flavours, colours, antioxidants), at the MRLs proposed. The Panel did not evaluate aspects of the source materials used for the extracted or defatted food products or ingredients, the industrial processes applied, or the products coming from these processes.

2. Data and methodologies

2.1. Data

The applicant has submitted a dossier in support of its application for the authorisation of 2-methyloxolane as an extraction solvent for use in the production of various foodstuffs and food ingredients.

Additional information was provided by the applicant on 9 September 2021 during the assessment process in response to requests from EFSA sent on 3 June and 22 October 2021 (see 'Documentation provided to EFSA').

2.2. Methodologies

This opinion is formulated following the principles described in the EFSA Guidance on transparency with regard to scientific aspects of risk assessment (EFSA, 2009) and following the relevant existing guidance documents from the EFSA Scientific Committee.

The CEP Panel assessed the safety of 2-methyloxolane as an extraction solvent in line with the principles laid down in Directive 2009/32/EC and according to the common authorisation procedure for food additives, food enzymes and food flavourings set in Regulation (EC) No 1331/2008[2] and its implementation in Regulation (EU) No 234/2011[3]. There is no specific EFSA guidance document on extraction solvents, and where applicable, other relevant guidance documents have been followed (e.g. Guidance for submission for food additive evaluations; EFSA ANS Panel, 2012).

[1] Directive 2009/32/EC of the European Parliament and of the Council of 23 April 2009 on the approximation of the laws of the Member States on extraction solvents used in the production of foodstuffs and food ingredients (recast) (text with EEA relevance) - https://eur-lex.europa.eu/legal-content/EN/ALL/?uri=CELEX%3A32009L0032

[2] Regulation (EC) No 1331/2008 of the European Parliament and of the Council of 16 December 2008 establishing a common authorisation procedure for food additives, food enzymes and food flavourings (Text with EEA relevance) - https://eur-lex.europa.eu/legal-content/EN/ALL/?uri=CELEX%3A32008R1331

[3] Commission Regulation (EU) No 234/2011 of 10 March 2011 implementing Regulation (EC) No 1331/2008 of the European Parliament and of the Council establishing a common authorisation procedure for food additives, food enzymes and food flavourings (Text with EEA relevance) - https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A02011R0234-20210327

3. Assessment

3.1. Technical data

3.1.1. Identity of the substance[4]

2-Methyloxolane is an organic solvent also named tetrahydro-2-methylfuran or 2-methyltetrahydrofuran (MeTHF). The European Community (EC) name is tetrahydro-2-methylfuran. The Chemical Abstracts Service (CAS) and EC numbers are 96-47-9 and 202-507-4, respectively.

The molecular formula is $C_5H_{10}O$, the molecular mass 86.13 Da. 2-Methyloxolane has a melting point of −20°C, a boiling point of 78°C, a n-octanol/water partition coefficient (K_{ow}) of 1.85 at 25°C, a water solubility of 140 g/L and a relative density of 0.855 (20°C). It forms an azeotrope with 10.6% water. The auto-ignition temperature is 260°C. The substance is stable in a broad range of pH (1–14), but susceptible to peroxidation.

3.1.2. Specifications[5]

According to the applicant, the substance is > 99.9% pure. The applicant provided the specification for impurities that is controlled by gas chromatography with flame ionisation detection (GC-FID) for every 5-tonne shipment (Table 1).

Table 1: Specifications of the product as provided by the applicant (controlled for every 5-tonne shipment)

Parameters	Specification
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted](b)
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	

(b): In the dossier, the applicant used ppm and mg/kg as equivalent. The Panel decided not to use ppm in the opinion.

The applicant provided data generated by GC-FID on volatile organic compounds in seven shipments of 2-methyloxolane (Tables 2 and 3).

Table 2: Concentrations of impurities (mg/kg) in seven shipments of the substance (September–October 2017): substances with an individual specification

Substance	Shipment number							Max	LOD[(a)] for specified analytes
	1	2	3	4	5	6	7		
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]

[4] Technical dossier/sections 1.2.1 – 1.2.2/Annex TA-002-01 & Additional data (Sept 2021)/Annex TA-002-01rev12.
[5] Technical dossier/sections 1.2.3 – 1.2.4/Annexes TA-002-(01/02/03/04 & 07) & Additional data (Sept 2021)/ Annexes TA-002-01rev12 & 125492v2.

Substance	Shipment number							Max	LOD[(a)] for specified analytes
	1	2	3	4	5	6	7		
[redacted]									
[redacted]									
[redacted]									

Table 3: Concentrations of impurities (mg/kg) in seven shipments of the substance (September–October 2017): substances for which the sum must be below 0.1% to meet the specification for 2-methyloxolane

Substance	Shipment number							Max
	1	2	3	4	5	6	7	
[redacted]								
[redacted]								
[redacted]								
[redacted]								
[redacted]								
[redacted]								
[redacted]								
[redacted]								
[redacted]								
[redacted]								
[redacted]								

The Panel noted that for the results indicated as '0', the limit of detection (LOD) or limit of quantification (LOQ) was not provided.

The applicant also reported analytical results for [redacted] elements for three batches of 2-methyloxolane (Table 4). [redacted]

Table 4: Trace elements in three batches of 2-methyloxolane (indicated as < LOD or as actual value)

Element	Sample (μg/kg)		
	1	2	3
[redacted]			
[redacted]			
[redacted]			
[redacted]			
[redacted]			
[redacted]			
[redacted]			
[redacted]			
[redacted]			
[redacted]			
[redacted]			
[redacted]			
[redacted]			
[redacted]			
[redacted]			
[redacted]			

Element	Sample (μg/kg)		
	1	2	3
[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]

[redacted] in one batch is substantially higher than in the two others [redacted] but is not expected to be transferred to the 2-methyloxolane [redacted] The applicant provided analytical data for three more batches in which [redacted] and [redacted] were found. The applicant considered that the contamination with [redacted] was incidental and stated that a control [redacted] will be performed once a year on 3 batches.

The Panel noted that the LOD value for [redacted] reported in Table 4 is substantially higher than the specifications proposed by the applicant in the technical dossier [redacted].

Table 5: Specifications for elements given by the applicant

Element	Specification (mg/kg)
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]

3.1.3. Manufacturing process[6]

2-Methyloxolane is produced from lignocellulose biomass, more specifically, from pentosans (hemicelluloses). Pentosans are found in plant agricultural by-products, such as corn cobs, oat hulls, almond husks, bagasse, etc. According to the applicant, the produced 2-methyloxolane meets the proposed specifications regardless of the raw material source.

[redacted] 2-Methyloxolane is obtained with a purity superior to 99.9% [redacted] If pesticides were present in the starting material, the Panel expects that they would not be present in the final product due to the high efficiency [redacted]

[6] Technical dossier/section 1.2.3 & Additional data (Sept 2021).

Figure 1: Manufacturing process

3.1.4. Methods of analysis in food[7]

As there may be residual 2-methyloxolane in extracted foods, the applicant provided validated analytical methods to determine its residual amounts in lipophilic liquids (crude oil, refined oil) as well as in powders (plant defatted meals, plant protein isolate).

The analysis in oils was performed by head space (HS)-GC with desorption at 80°C. Quantification is for 1 to 10 mg 2-methyloxolane/kg refined oil and 50 to 1,000 mg/kg crude oil. Overall, the recoveries ranged between 81% and 118%. Repeatability and reproducibility were reported as < 20%.

For defatted plant meals, details on two validated analytical methods were submitted. The first uses HS-GC with desorption at 80°C. It was validated for 200 to 1,000 mg 2-methyloxolane/kg soybean and rapeseed meal. Recoveries were between 81 and 103%. Aqueous preparation of the dried samples, including ultrasonic treatment to aid dispersion, increased accuracy and recovery. To increase sensitivity, a validated HS-GC–mass spectrometry (MS) method was developed. The LOD was given as 1 mg/kg, with an accuracy of 70-90%.

A further HS-GC–MS method for the analysis of 2-methyloxolane in protein isolate was developed and validated to < 1 mg/kg. The study was Good Laboratory Practice (GLP) compliant and in accordance with the International Council for Harmonisation (ICH) validation guidelines. Detailed statistical parameters of the method depending on the applied concentration range were given.

Taking the anticipated residues and proposed maximum limits into account, the Panel considered that only those methods which cover the range of interest (0.1–100 mg/kg) are appropriate.

3.1.5. Peroxide formation[8]

2-Methyloxolane readily reacts with oxygen to form hydroperoxides (Figure 2). According to the applicant, it takes 4 days for non-stabilised, stirred and exposed to air 2-methyloxolane to reach [redacted]

[7] Technical dossier/sections 1.2.5.1/Annexes CPE 18/05/29059 (2-4/4); P1412_R01v02 (MeTHF in meals & flakes); KB003 (defatted soybean flakes); VR0044 (soy protein isolates).
[8] Technical dossier/section 1.2.3&1.5.2/Annex (TA-002-04/Applicant's Product Data & Handling Guidelines 2016).

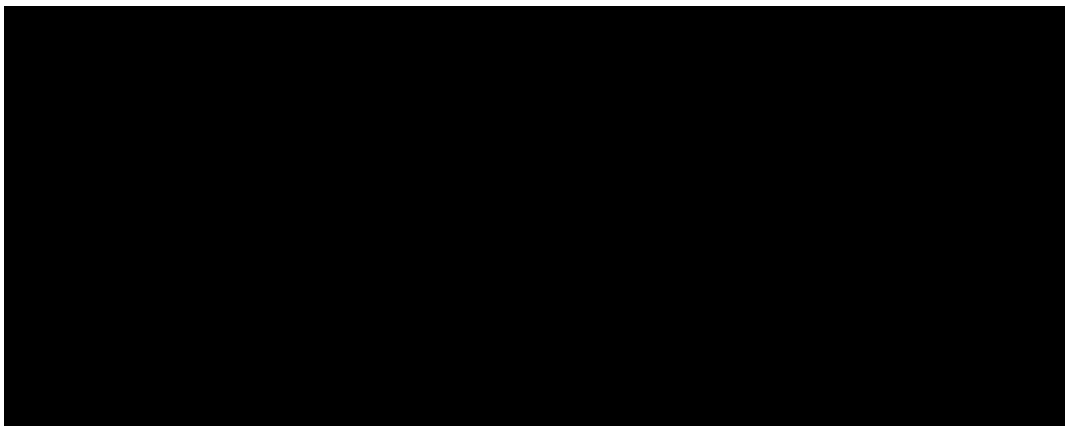

Figure 2: Formation of hydroperoxides and their decomposition

The formation of hydroperoxides can be largely inhibited by stabilisers like 3,5-di-*tert*-butyl-4-hydroxytoluene (BHT), tocopherols or water. According to the applicant, [redacted] BHT or [redacted] tocopherols are added to the product and it is packed under nitrogen. The applicant instructs users to test aged products for the peroxide content and to re-stabilise them prior to storage.

In continuous processes such as oil and protein extraction, the development of peroxides is largely prevented. Water and antioxidants (natural polyphenols and tocopherols) in the seeds inhibit the formation of peroxides. The solvent is recovered by distillation and immediately reused without drying. In the case of a plant shut down or solvent storage for more than 3 or 4 days, the applicant recommends re-stabilisation by addition of [redacted] tocopherol or storage under nitrogen. If the product has not been re-stabilised or stored under nitrogen, the applicant recommends the users to measure the peroxide content before reuse and, if that content is more than [redacted] to add edible oil to the solvent and purify it by distillation. The peroxide will concentrate in the edible oil and can be destroyed later.

The Panel noted that the applicant considered the possible formation of hydroperoxides in foods, taking the necessary measures to control and limit their formation. Moreover, the Panel considers that the effects of the peroxides on the food, if any, are minor compared to oxidation during the cooking practices, such as frying.

3.2. Information on existing authorisations and evaluations[9]

According to the applicant, the industrial scale production of 2-methyloxolane started in 2000. In the pharmaceutical industry, 2-methyloxolane has been proposed as a suitable replacement for tetrahydrofuran. It has been used as a process solvent for the production of pharmaceutical intermediates since 2007.

2-Methyloxolane has been registered under Regulation (EC) 1907/2006[10] of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH). It is registered for annual quantities between 1,000 and 10,000 tonnes as a solvent for chemical synthesis, including fine chemicals, agrochemicals, and pharmaceuticals.

[9] Technical dossier/section 1.2.6.

[10] Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), establishing a European Chemicals Agency, amending Directive 1999/45/EC and repealing Council Regulation (EEC) No 793/93 and Commission Regulation (EC) No 1488/94 as well as Council Directive 76/769/EEC and Commission Directives 91/155/EEC, 93/67/EEC, 93/105/EC and 2000/21/EC. OJ L 396, 30.12.2006, p. 1–849.

In the context of REACH, a number of hazards have been identified and classified in accordance with Regulation (EC) No 1272/2008[11] on the classification, labelling and packaging (CLP) of substances and mixtures (Table 6).

Table 6: Notified classification and labelling of 2-methyloxolane according to CLP criteria

Flammable liquid category 2	**H225: Highly flammable liquid and vapour**
Acute Toxicity category 4	H302: Harmful if swallowed
Skin Irritant category 2	H315: Causes skin irritation
Eye Damage category 1	H318: Causes serious eye damage

For the manufacture of pharmaceuticals, 2-methyloxolane was added to Class 3 (solvents with low toxic potential) in the International Council for Harmonisation (ICH) Q3C guideline on impurities: Residual Solvents (EMA, 2021).

3.3. Proposed uses and maximum residue limits[12]

2-Methyloxolane is intended to be used in processes currently applying hexane, in particular to extract lipids from oil-rich and protein-rich biomass (e.g. corn, sunflower seeds, rapeseeds, soybeans), as well as to extract natural aroma, flavours, antioxidants and colorants, particularly the lipophilic ones currently extracted with hexane (e.g. annatto, carotenoids, chlorophylls).

Therefore, the applicant proposes the following provisions for 2-methyloxolane to be included in Annex 1, Part II of the Directive 2009/32/EC on the approximation of the laws of the Member States on extraction solvents used in the production of foodstuffs and food ingredients (see Table 7).

The applicant also proposes to list 2-methyloxolane for extraction of food additives in Annex I, Part III of the Directive 2009/32/EC with an MRL of 1 mg 2-methyloxolane/kg food additive.

Table 7: MRLs for 2-methyloxolane proposed by the applicant to be added to Annex I, Part II, to Directive 2009/32/EC

Name	**Conditions of use (summary description of extraction)**	**MRLs in the extracted foodstuff or food ingredient**
2-methyloxolane	Production or fractionation of fat, oil or butter	1 mg/kg in the fat, oil or butter
	Preparation of defatted protein product, defatted flour, and other defatted solid ingredients	10 mg/kg in the food containing the defatted protein product, defatted flour or other defatted solid ingredients
	Defatted protein product, defatted flour, and other defatted solid ingredients for use in category 13 products[(a)]	1 mg/kg in the food of the category 13 products containing the defatted protein product, defatted flour or other defatted solid ingredients

MRL: maximum residue limit.
(a): Food category 13 includes foods intended for particular nutritional uses as defined by Directive 2009/39/EC.

The applicant noted that for sensory reasons, the residue limits in beverages or liquid foods have to be below 1 mg/kg. For fats and oils, organoleptic tests have shown that 5 mg/kg 2-methyloxolane is associated with an off-flavour. The applicant further indicated that residual levels of 2-methyloxolane below 1 mg/kg can be reached in liquids without difficulties by a standard refining process.

The applicant proposes the following residue limits related to the use in the following food categories (as described in Part D of Annex II to Regulation (EC) No 1333/2008 of the European Parliament and of the Council by establishing a Union list of food additives) (Table 8).

[11] Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006. OJ L 353, 31.12.2008, p. 1–1355.
[12] Technical dossier/section 1.2.7/Annexes ITERG/CPA 18/05/28870 (1-2/2) & Additional information (Sept & Oct 2021).

Table 8: MRLs of 2-methyloxolane in foods according to the Annex II to Regulation (EC) No 1333/2008

Food category Number	Food category Name	MRLs (mg/kg)
01.7.5	Processed cheese	1
01.8	Dairy analogues, including beverage whiteners	10
02.1	Fats and oils essentially free from water (excluding anhydrous milkfat)	1
02.2	Fat and oil emulsions mainly water-in-oil type	1
03	Edible ices	1
04.2	Processed fruits and vegetables	1
05.1	Cocoa and chocolate products as covered by Directive 2000/36/EC	10
05.1	Cocoa and chocolate products as covered by Directive 2000/36/EC, **only cocoa butter**	1
05.2	Other confectionery including breath freshening micro-sweets	10
05.4	Decorations, coatings and fillings, except fruit-based filings covered by category 4.2.4	1
06.3	Breakfast cereals	1
06.4	Pasta (of which potato gnocchi)	1
06.5	Noodles	10
07.1	Bread and rolls	10
07.2	Fine bakery wares	10
08.2	Meat preparations as defined by Regulation (EC) No 853/2004	10
08.3	Meat products	10
09.2	Processed fish and fishery products including molluscs and crustaceans	1
12.4	Mustard	1
12.6	Sauces	1
12.7	Salads and savoury based sandwich spreads	1
12.9	Protein products, excluding products covered in category 1.8	10
13.1.1	Infant formulae as defined by Commission Directive 2006/141/EC	1
13.1.2	Follow-on formulae as defined by Directive 2006/141/EC	1
13.1.3	Processed cereal-based foods and baby foods for infant and young children as defined by Commission Directive 2006/125/EC	1
13.1.4	Other foods for young children	1
13.2	Dietary foods for special medical purposes defined in Directive 1999/21/EC (excluding products from food category 13.1.5)	1
13.3	Dietary foods for weight control intended to replace total daily food intake or an individual meal (the whole or part of the total daily diet)	1
13.4	Foods suitable for people intolerant to gluten as defined by Commission Regulation (EC) No 41/2009	1
14.1.5	Coffee, coffee and chicory extracts, tea, herbal- and fruit-infusions; coffee substitutes, coffee mixes and mixes for 'hot beverages'	1
14.2.1	Beer and malt beverages	1
14.2.5	Mead	1
15.1	Potato-, cereal-, flour- or starch-based snacks	10
15.2	Processed nuts	1
17	Food supplements as defined in Directive 2002/45/EC of the European Parliament and of the Council excluding food supplements for infants and young children	1

MRL: maximum residue limit.

The applicant also proposes to use the solvent to extract food additives, with a residue level of 1 mg 2-methyloxolane/kg food additive. These uses were not considered in the exposure calculation (see Section 3.5.1).

3.4. Exposure data

3.4.1. Food consumption data used for exposure assessment

EFSA Comprehensive European Food Consumption Database

Since 2010, the EFSA Comprehensive European Food Consumption Database (Comprehensive Database) has been populated with national data on food consumption at a detailed level. Competent authorities in the European countries provide EFSA with data on the level of food consumption by the individual consumer from the most recent national dietary survey in their country (Guidance of EFSA on the 'Use of the EFSA Comprehensive European Food Consumption Database in Exposure Assessment', EFSA, 2011). The version of the Comprehensive database taken into account in this assessment was released in July 2021.[13]

The food consumption data gathered by EFSA were collected by different methodologies and, thus, direct country-to-country comparisons may not be appropriate. Depending on the food category and the level of detail used for exposure calculations, uncertainties could be introduced owing to possible subjects' underreporting and/or misreporting of the consumption amounts. Nevertheless, the EFSA Comprehensive Database includes the currently best available food consumption data across Europe.

Food consumption data from infants, toddlers, children, adolescents, adults and the elderly were used in the exposure assessment. For the present assessment, food consumption data were available from 41 different dietary surveys carried out in 22 European countries (Table 9).

Table 9: Population groups considered for the exposure estimates of 2-methyloxolane

Population	Age range	Countries with food consumption surveys covering more than 1 day
Infants	From more than 12 weeks up to and including 11 months of age	Bulgaria, Cyprus, Denmark, Estonia, Finland, France, Germany, Italy, Latvia, Portugal, Slovenia
Toddlers[(a)]	From 12 months up to and including 35 months of age	Belgium, Bulgaria, Cyprus, Denmark, Estonia, Finland, France, Germany, Hungary, Italy, Latvia, Netherlands, Portugal, Slovenia, Spain
Children[(b)]	From 36 months up to and including 9 years of age	Austria, Belgium, Bulgaria, Cyprus, Czechia, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Italy, Latvia, Netherlands, Portugal, Spain, Sweden
Adolescents	From 10 years up to and including 17 years of age	Austria, Belgium, Cyprus, Czechia, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Italy, Latvia, Netherlands, Portugal, Romania, Slovenia, Spain, Sweden
Adults	From 18 years up to and including 64 years of age	Austria, Belgium, Croatia, Cyprus, Czechia, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Netherlands, Portugal, Romania, Slovenia, Spain, Sweden
The elderly[(b)]	From 65 years of age and older	Austria, Belgium, Cyprus, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Netherlands, Portugal, Romania, Slovenia, Spain, Sweden

(a): The term 'toddlers' in the Comprehensive Database (EFSA, 2011) corresponds to 'young children' in Regulations (EC) No 1333/2008 and (EU) No 609/2013.
(b): The terms 'children' and 'the elderly' correspond, respectively, to 'other children' and the merge of 'elderly' and 'very elderly' in Comprehensive Database (EFSA, 2011).

Consumption records were codified according to the FoodEx2 classification system (EFSA, 2015). Nomenclature from the FoodEx2 classification system was linked to the food categorisation system (FCS) as presented in Annex II of Regulation (EC) No 1333/2008, part D, to perform the exposure assessments. In practice, the FoodEx2 food codes were matched to the FCS food categories.

[13] Available online: https://www.efsa.europa.eu/en/data-report/food-consumption-data

Food categories considered for the exposure assessment of 2-methyloxolane

The food categories in which the use of 2-methyloxolane is proposed were selected from the nomenclature of the EFSA Comprehensive Database (FoodEx2 classification system) at the most detailed level possible (up to FoodEx2 Level 7) (EFSA, 2015).

The restriction of only cocoa butter within the FC 05.1 (cocoa and chocolate products) as covered by Directive 2000/36/EC was considered and a level of 1 mg/kg was applied by selecting the FoodEx2 code related to cocoa butter.

3.5. Dietary exposure estimate[14]

3.5.1. Exposure to 2-methyloxolane from its application as an extraction solvent

The applicant estimated the potential exposure to 2-methyloxolane from its application as an extraction solvent using EFSAs food additives intake model (FAIM) at the proposed MRLs (Table 8).

As FAIM does not allow consideration of specific restrictions, the Panel estimated the exposure using FoodEx2 codes directly through the Comprehensive Database in order to consider the specific MRL for cocoa butter.

Dietary exposure to 2-methyloxolane was calculated by the Panel (Table 10) by multiplying concentrations of 2-methyloxolane per food category (Table 8) with their respective consumption amount per kilogram body weight for each individual in the Comprehensive Database. The exposure per food category was subsequently added to derive an individual total exposure per day. These exposure estimates were averaged over the number of survey days, resulting in an individual average exposure per day for the survey period. Dietary surveys with only 1 day per subject were excluded as they are considered not adequate to assess repeated exposure.

The exposure was estimated for all individuals per survey and per population group (Table 9), resulting in distributions of individual exposure per survey and population group. Based on these distributions, the mean and 95th percentile of exposure were calculated per survey and per population group. The 95th percentile of exposure was only calculated for those population groups with a sufficiently large sample size (EFSA, 2011).

Table 10: Summary of dietary exposure to 2-methyloxolane from its MRLs in six population groups (minimum-maximum across the dietary surveys, in mg/kg bw per day)

Estimated exposure (mg/kg bw per day)	Infants (12 weeks-11 months)	Toddlers (12–35 months)	Children (3–9 years)	Adolescents (10–17 years)	Adults (18–64 years)	The elderly (≥ 65 years)
Mean	0.04–0.12	0.05–0.18	0.06–0.14	0.03–0.08	0.03–0.06	0.02–0.05
95th percentile	0.10–0.21	0.10–0.32	0.12–0.27	0.06–0.16	0.06–0.11	0.05–0.09

MRL: maximum residue limit; bw: body weight.

At the proposed residue limits, the maximum estimated exposure was found to be for toddlers, with the mean exposure at 0.18 mg/kg body weight (bw) per day and the high exposure (95th percentile) at 0.32 mg/kg bw per day.

Main food categories across countries contributing to exposure to 2-methyloxolane

The main contributing food categories to the total mean exposure estimates were:

- For infants, FC 13.1 Foods for infants and young children (more specifically FC 13.1.1 Infant formulae as defined by Directive 2006/141/EC);
- For toddlers, FC 07.1 Bread and rolls;
- For children, FC 07.1 Bread and rolls, and FC 07.2 Fine bakery wares;
- For adolescents, adults and the elderly FC 07.1 Bread and rolls.

[14] Technical dossier/section 1.2.8 & Additional information (Sept 2021).

Uncertainty analysis

In accordance with the guidance provided in the EFSA opinion related to uncertainties in dietary exposure assessment (EFSA, 2007), the following sources of uncertainties have been considered and summarised in Table 11.

Table 11: Qualitative evaluation of influence of uncertainties on the dietary exposure estimate

Sources of uncertainties	Direction[(a)]
Consumption data: different methodologies/representativeness/underreporting/misreporting/no portion size standard	+/–
Methodology used to estimate high percentiles (95th) long-term (chronic) exposure based on data from food consumption surveys covering only a few days	+
Correspondence of proposed MRLs to the food items in the EFSA Comprehensive Database: uncertainties to which types of food categories the levels refer to	+/–
Concentration data: – MRLs applicable to all foods within the entire food category, whereas most probably not all food belonging to a food category will contain 2-methyloxolane	+
Exposure assessment scenario: – Exposure calculations based on the MRLs	+

MRL: maximum residue limit.
(a): +, uncertainty with potential to cause overestimation of exposure; –, uncertainty with potential to cause underestimation of exposure.

Residues of 2-methyloxolane may be present in the food categories presented in Table 8. For all food categories taken into account, it was assumed that 100% of the foods belonging to these food categories will contain 2-methyloxolane at the MRLs. Therefore, overall, the Panel considered that the uncertainties identified resulted in an overestimation of the exposure.

The foreseen use of 2-methyloxolane for the extraction of food additives is not included in the exposure assessment. However, the exposure estimates for the other foreseen uses corresponding to the MRLs in the extracted food and food ingredients are worst-case and it can therefore be assumed that they also cover the exposure to additives.

3.5.2. Exposure via other sources

The Panel noted that 2-methyloxolane is also used as a solvent for chemical synthesis including fine chemicals, agrochemicals, and pharmaceuticals. These other sources were not considered in the estimations from its use as an extraction solvent. This could result in an under-estimation.

The Panel noted that traces of 2-methyloxolane naturally occurs as a product of yeast of the human skin microbiota, *Malassezia furfur,* and it can also be found in several edible plants, such as watercress leaves.

3.5.3. Exposure to the impurities

For the exposure assessment, the Panel took into account that the solvent is usually re-used many times, recovered by distillation. Small amounts may be added to refill and compensate for losses. The consequences depend on the volatility of the impurities.

Organic compounds with a volatility from the matrix similar to that of 2-methyloxolane will evaporate at a similar rate and the concentration of the impurities will remain similar over many re-uses and so will the exposure. Compounds that are more volatile, like furan, will evaporate preferentially and the residues will be lower.

The amount of residues of compounds of lower volatility will be higher, but decrease in the recovered solvent, with the effect that over many re-uses the average exposure is the same.

Non-volatile impurities, such as metals, will remain completely in the product(s) obtained when the solvent is used the first time, hence absent in the solvent when it is re-used. For the first use, this is a considerable enrichment in the product, since the metals from the whole amount of solvent used remain in one or both fractions of the treated starting material (e.g. the oil and the meal). However, considering that the solvent is usually re-used many times and just small portions of fresh solvent are added to compensate for solvent losses during the recycle process, the Panel considers it more

appropriate to estimate exposure to metals on a long-term average basis. For a continuous re-use, average exposure can then be approximated by the initial concentration in the solvent.

The Panel calculated the possible exposure to organic impurities (Table 12) and [redacted] (Table 13).

Table 12: Summary of dietary exposure to an organic impurity if present at [redacted] (according to the specifications) in 2-methyloxolane in six population groups (minimum–maximum across the dietary surveys, in ng/kg bw per day)

Estimated exposure (ng/kg bw per day)	Infants (12 weeks-11 months)	Toddlers (12–35 months)	Children (3–9 years)	Adolescents (10–17 years)	Adults (18–64 years)	The elderly (≥ 65 years)
Mean	20–59	23–88	29–70	13–42	16–28	12–26
95th percentile	50–106	49–159	59–136	29–78	30–56	23–44

bw: body weight.

The Panel concluded that the exposure to impurities calculated from their concentrations at the specifications in the 2-methyloxolane does not raise a safety concern. This also applies to metals when present in concentrations higher than 2 to 3 orders of magnitude owing to the addition of fresh solvent.

Table 13: Summary of dietary exposure to [redacted] if present at [redacted] (according to the specifications) in 2-methyloxolane in six population groups (minimum–maximum across the dietary surveys, in ng/kg bw per day)

Estimated exposure (ng/kg bw per day)	Infants (12 weeks–11 months)	Toddlers (12–35 months)	Children (3–9 years)	Adolescents (10–17 years)	Adults (18–64 years)	The elderly (≥ 65 years)
Mean	0.002–0.006	0.002–0.009	0.003–0.007	0.001–0.004	0.002–0.003	0.001–0.003
95th percentile	0.005–0.011	0.005–0.016	0.006–0.014	0.003–0.008	0.003–0.006	0.002–0.004

bw: body weight.

3.6. Biological and toxicological data

3.6.1. Absorption, distribution, metabolism and excretion[15]

The applicant referred to one publication concerning an animal study on the disposition of 2-methyloxolane (Henderson et al., 2007).

The disposition of ^{14}C-2-methyloxolane was studied in rats and mice by measuring radioactivity levels in tissues and excreta after dosing. 2-Methyloxolane was administered orally to rats and mice at single doses of 1, 10 or 100 mg/kg bw, or intravenously at 1 mg/kg bw. The vehicle for the oral dose was water and 0.9% saline was used for intravenous administration. There were no overt signs of toxicity observed at any dose.

Based on recovery of radioactivity in excreta (other than faeces) and tissues (other than gastrointestinal tract), absorption of orally administered 2-methyloxolane was essentially complete (93–100%).

No specific tissue accumulated the radiolabel in either species. Following all oral doses in both species, the highest concentration of radioactivity at termination was in kidneys. Following intravenous dosing, the highest concentration was in liver. 2-Methyloxolane was rapidly metabolised and excreted with < 8% (mice) or 8-22% (rats) of the dose remaining in the body after 24 h (1 and 10 mg/kg bw) or 72 h (100 mg/kg bw).

The major route of excretion in mice was in urine followed by exhaled CO_2. In rats, the major route of excretion was exhaled CO_2 followed by urinary excretion. The excretion of exhaled volatile organic compounds (VOCs) was dose-dependent in both species; at lower doses exhaled VOCs represented 1-5% of the dose, but at the highest dose (100 mg/kg bw) this proportion was 14% (mice) and 27%

[15] Technical dossier/section 1.2.9.2.

(rats). Analysis of the VOCs exhaled at the high dose indicated that the increase was due to exhalation of the parent compound. There were two indications that mice may have a higher capacity to metabolise 2-methyloxolane than rats, (i) at all doses, mice had cleared a slightly higher percentage of the dose than rats by 24 h, and (ii) rats given the high dose, cleared a higher dose percentage as exhaled parent compound than mice did.

High-performance liquid chromatographic (HPLC) radiochromatograms revealed three highly polar compounds in the mouse urine and two polar compounds in the rat urine. Profiles were similar following oral or intravenous administration. These polar metabolites could not be identified. Levulinic acid (4-oxopentanoic acid), designated as a candidate urinary metabolite of 2-methyloxolane, could not be detected in urine.

In general, the described disposition of 2-methyloxolane is quite similar to the toxicokinetics of tetrahydrofuran (oxolane) in experimental animals and in humans that have been reviewed by Fowles et al. (2013).

The Panel considered that after oral dosing, 2-methyloxolane was almost completely absorbed. Following all oral doses in both mice and rats, the highest concentration of radiolabel at termination was in kidneys. Following intravenous dosing, the highest concentration was in liver. 2-Methyloxolane was rapidly metabolised with low bioaccumulation potential. Excretion was mostly via carbon dioxide exhalation and urine. Polar metabolites in urine could not be identified.

3.6.2. Acute oral toxicity[16]

The applicant referred to one publication (Deichmann and Gerarde, 1969) and provided one study report.

The study by Deichmann and Gerarde (1969) was poorly reported and therefore considered not useful for the assessment. In a study[17] conducted according to OECD TG 420 (OECD, 2002; fixed dose method) and in compliance with GLP, the acute oral LD_{50} in (female) rats was estimated using doses of 300 mg/kg bw (n = 5) and 2,000 mg/kg bw (n = 1). The one animal dosed orally with 2,000 mg/kg bw was killed due to severity of clinical signs 30 min after dosing. These signs included laboured respiration, decreased respiratory rate, hypothermia and pallor of the extremities, and the test animal was also comatose. Haemorrhage and epithelial sloughing of the non-glandular epithelium of the stomach, and clear fluid present in the stomach, were noted at necropsy. There were no signs of toxicity and no abnormalities were noted at necropsy in animals that had received the 300 mg/kg bw dose.

3.6.3. Short-term and subchronic toxicity[18]

3.6.3.1. Oral studies

The applicant referred to two publications.

In the publication by Antonucci et al. (2011), groups of male and female Sprague–Dawley rats were administered for 'approximatively 3 months' with only one dose of 26 mg/kg bw per day. There is limited information available for this study, which is therefore not useful for the assessment.

The publication by Parris et al. (2017) reports a GLP study with a protocol 'similar to the OECD 408 guideline', which was conducted to support the use of 2-methyloxolane as a solvent for pharmaceutical manufacture. Missing end points with respect to OECD TG 408 (OECD, 1998) were: serum T_4, T_3 or TSH measurements, sperm morphology and vaginal smears for oestrus cycle evaluation taken at termination. The Panel was not provided with the full report. The animals were administered 2-methyloxolane in water by gavage with doses of 0, 80, 250, 500 and 1,000 mg/kg bw per day for 13 weeks. Two additional groups of 5 rats/sex from the control group and the highest dose group of 1,000 mg/kg bw per day were maintained for a further 28-day recovery period.

There were no treatment-related mortalities. Occasional test-article related salivation was noted pre- and immediately post-administration at the two high doses in both sexes. At the dose of 1,000 mg/kg bw per day, a slight decrease in male weight gain was observed, which was not seen in females.

No significant ophthalmic or haematologic changes were reported.

[16] Technical dossier/section 1.2.9.3.
[17] Report no. 41205095 (Jan 2013; Acute oral toxicity in the rat-fixed dose method).
[18] Technical dossier/section 1.2.9.4.

A treatment-related increase in serum cholesterol was observed in both sexes at 1,000 mg/kg bw per day. Complete reversibility was reported at the end of the treatment-free period.

Treatment-related higher absolute and relative kidney weights (4–20% above control, relative to body and brain weights) were observed at the doses of 500 and 1,000 mg/kg bw in both sexes. There were no macroscopic or microscopic findings associated with these changes. Higher absolute and relative liver weights (9–32% above control, relative to body and brain weights) were observed in both sexes at the doses of 500 and 1,000 mg/kg bw per day. Histopathological examination revealed hepatocellular centrilobular hypertrophy only at the dose of 1,000 mg/kg bw per day. These findings were not observed at lower doses, in control animals and in the recovery groups.

Overall, considering the different reported effects, the authors identified a no observed adverse effect level (NOAEL) of 250 mg/kg bw per day. The Panel agreed with this conclusion and noted that some of the missing endpoints in the Parris et al. (2017) study were covered within the extended one-generation reproductive toxicity (EOGRT) study (see Section 3.6.6).

3.6.3.2. Inhalation study

The Panel noted that an inhalation study[19] was also provided by the applicant. This route of administration is usually considered as not relevant for assessing the oral toxicity. However, according to the authors, this study (conducted for REACH) was used to cover some of the end points which were not considered in the 13-weeks oral toxicity study by Parris et al. (2017), namely, functional observations battery (FOB), oestrus cycle monitoring, sperm analysis and T_3 and T_4 levels. Its main outcomes are summarised below.

The study was performed according to OECD TG 413 (OECD, 2018a) and GLP. The animals (Han Wistar rats) were exposed nose-only for 13 weeks at concentrations of 0, 2, 4.5 and 10 mg/L. It was noted that in a preliminary study, the dose of 19.7 mg/L induced severe clinical signs justifying early termination of this dose group.

For the highest dose group of 10 mg/L, no mortalities or clinical signs were reported, and there were also no histopathological changes in the CNS or peripheral nerves. Transient lower body weight gains were noted for males exposed to 10 mg/L, but terminal weights were within 5% of control values. Irregular oestrus cycles were seen in all treated groups and a shift in cycle length from 4 to 5 days was observed in females exposed to the highest concentration. There were no treatment-related histopathology findings, including in the thymus. In bronchoalveolar lavages, the cell counts, total protein and lactate dehydrogenase activities were not changed. The analysis of thyroid hormones (T_3 and T_4) was unchanged.

The authors noted that the percentage of normal spermatozoa was lower than expected in both control and treated groups; the majority of abnormal spermatozoa was decapitated. Slight changes compared with controls were observed for males exposed to 10 mg/L. Changes in percentage normal and total abnormal spermatozoa were not statistically significant, but tail abnormalities were statistically significantly higher in treated animals than in the control group. According to the authors, the relationship to treatment was uncertain as it may have been affected by body temperature effects consecutive to constraints during the nose only inhalation procedure.

Nevertheless, the Panel considered that the tail abnormalities in spermatozoa could be adverse and their consequence would be covered in the EOGRT study described below.

3.6.4. Genotoxicity[20]

3.6.4.1. *In vitro* assays

Gene mutations in bacteria

The genotoxicity of 2-methyloxolane (purity 99%) was investigated in *Salmonella* Typhimurium TA100, TA1535, TA97, TA98 strains (Ames test) according to OECD TG 471 (OECD, 1997a; National Toxicology Program (NTP), Study no. A41442). A pre-incubation method was conducted in the presence and absence of metabolic activation in a concentration range of 33–10,000 μg/plate (vehicle: water). The metabolic activation system included Aroclor-induced rat and hamster liver S9-mix (10% and 30%). Six concentrations of 2-methyloxolane were investigated in TA100 and TA98 strains (33, 100, 333, 1,000, 3,333, 10,000 μg/plate) and only five concentrations in TA1535 and TA98 strains (the

[19] Report no. YB26GY (Sept 2019; MeTHF: Toxicity Study by Inhalation Administration to Han Wistar Rats for 13 Weeks).
[20] Technical dossier/section 1.2.9.5.

lowest was excluded). No cytotoxicity was observed at any tested concentration. No increase in the mean number of revertant colonies was observed at any tested condition in any tester strain.

2-Methyloxolane was negative in the bacterial reverse mutation assay with and without metabolic activation.

Gene mutations in L5178Y mouse lymphoma cells

The mutagenic potential of 2-methyloxolane (purity 99.5%) was investigated in the *TK+/TK-* gene mutation assay in L5178Y mouse lymphoma cells. The study[21] was designed to be compatible with the OECD TG 476 (OECD, 1997b) and GLP. Two experiments were performed using the same protocol (same dose range in the presence and absence of metabolic activation), with the exception of the amount of rat liver S9-mix (1% vs. 2%) and the increased time of incubation (4 vs. 24 h) in the absence of metabolic activation in the second experiment. The doses of the test item (63.75, 127.5, 255, 510, 765, 1,020 μg/mL) were chosen based on the results of a preliminary cytotoxicity test.

No precipitation of the test item was observed at any dose level. No evidence of marked toxicity, as measured by relative suspension growth (% RSG) and relative total growth (% RTG) was observed in any experimental condition. 2-Methyloxolane did not induce any increase in the mutation frequency at any tested dose with or without metabolic activation in either experiment. No variation in the percentage of large and small colonies was observed in any experimental condition.

The test item did not induce any increase in mutation frequency at the *TK+/TK-* locus in L5178Y cells under the experimental conditions employed in this study.

In vitro *micronucleus test*

To evaluate the potential of 2-methyloxolane (purity 99.98%) to induce chromosomal damage, an *in vitro* micronucleus assay[22] was carried out in human peripheral blood lymphocytes according to OECD TG 487 (OECD, 2016) and following GLP. In a preliminary cytotoxicity test, the highest concentration recommended by the OECD guideline (10 mmol/L, equivalent to 860 μg/mL) did not induce relevant toxic effects. 2-Methyloxolane was then tested at 0, 26.88, 53.75, 107.5, 215, 430 and 860 μg/mL. No precipitation of the test item was observed in the cultures at the end of the exposure at any dose level in any exposure group. Three exposure conditions were used in two experiments: (1) 4 h without metabolic activation (S9-mix); (2) 24 h without S9-mix; (3) 4 h with 2% S9-mix. To induce binucleated cells, cytochalasin B (4 μg/mL) was added during the recovery period (24 h in all the exposure conditions). No toxicity was observed in any experimental condition. No increase in the micronucleus frequency was observed after treatment with 2-methyloxolane in any experimental condition.

The test item did not induce any increase in micronuclei in cultured human peripheral blood lymphocytes under the experimental conditions employed in this study.

3.6.4.2. Concluding remarks on genotoxicity

2-Methyloxolane did not induce gene mutations in bacteria either in the presence or the absence of metabolic activation (Ames test). Negative results were also reported in two well-conducted *in vitro* studies in mammalian cells: an *in vitro* micronucleus assay carried out in human peripheral blood lymphocytes and a gene mutation assay at the *TK+/-* locus in L5178Y mouse lymphoma cells.

Overall, the Panel considered that 2-methyloxolane does not raise a concern for genotoxicity.

3.6.5. Chronic toxicity and carcinogenicity[23]

No chronic or carcinogenicity studies on 2-methyloxolane were provided by the applicant.

3.6.6. Reproductive and developmental toxicity[24]

3.6.6.1. Developmental toxicity study[25]

Following a preliminary dose range finding study, developmental toxicity was investigated in a prenatal developmental toxicity study according to OECD TG 414 (OECD, 2018b) and GLP. Sprague-

[21] Report no. 41201924 (2013; Tetrahydro-2-methylfuran: L5178Y mouse lymphoma assay).
[22] Report no. PL39KF (2019; MeTHF: Micronucleus Test in Human Lymphocytes *in vitro*).
[23] Technical dossier/section 1.2.9.6.
[24] Technical dossier/section 1.2.9.7 & Additional data (Sept 2021).
[25] Reports no. QN59BK (June 2018; MeTHF: Preliminary Oral (Gavage) Pre-Natal Development Toxicity Study in the Rat) & no. FT37XK (June 2018; MeTHF: Oral (Gavage) Pre-Natal Development Toxicity Study in the Rat).

Dawley rats were administered 2-methyloxolane with arachis oil as vehicle by gavage between days 3 and 19 (inclusive) at doses of 0, 100, 300 and 1,000 mg/kg bw per day.

There were no mortality and no clinical signs of toxicity during the study.

No treatment-related effects on pre-implantation loss, number of implantations, embryo fetal survival, litter size or sex ratio were reported. There was a slight decrease in fetal weight at 1,000 mg/kg bw per day. Total placental weight, but not mean placental weight, was decreased at 1,000 mg/kg/day.

A statistically significant ($p < 0.05$) higher incidence of fetuses showing incomplete nasal ossification compared to control was observed (incidence: 8.3, 3.5, 8.2 and 15.1%, at 0, 100, 300 and 1,000 mg/kg per day, respectively), in the absence of any obvious effect on the overall pattern of ossification.

Overall, according to the authors, the findings observed for fetuses at 1,000 mg/kg bw per day are suggestive of a slight decreasing effect on fetal growth although survival and offspring development appeared unaffected.

The authors concluded that '1,000 mg/kg bw per day probably represents a NOAEL for fetal survival, growth and development of the conceptus. A maternal dosage of 300 mg/kg bw per day represents a clear no observed effect level (NOEL) for the survival, growth and development of the conceptus'.

The Panel agreed with the 300 mg/kg bw per day for maternal NOAEL but considered that the NOAEL for the fetus should also be 300 mg/kg bw per day.

3.6.6.2. Reproductive and developmental toxicity studies

In a preliminary (not GLP; dose range finding) study,[26] the test item (2-methyloxolane, 99.97% pure) was administered by gavage (vehicle: water) to groups of 10 male and female Sprague-Dawley rats for 8 days before and during mating, and for females throughout gestation until day 21 post-partum (p.p.) at doses of 0, 100, 300 and 1,000 mg/kg bw per day. After weaning, 3 groups of 10 male and 10 female rat pups (generation F1) received the test item (same dose levels) on days 22 to 28 p.p.

There were no mortalities during the study. At 1,000 mg/kg per day severe effects were reported on early pup survival, clinical signs and marked decreased body weight in pups. These signs were still present after weaning in the F1 generation. At 300 mg/kg bw per day, there was a significant increase in the number of pups found dead or cannibalised, and a marked increased number of pups with no milk in the stomach. No other signs were reported in the F1 generation.

Following this study, dose levels of 100, 250 and 625 mg/kg bw per day were selected for the EOGRT study[27]. This study was conducted according to OECD TG 443 (OECD, 2018c) and GLP. 2-Methyloxolane was administered daily by oral gavage at dose levels of 0, 100, 250 or 625 mg/kg bw per day to sexually-mature male and female rats (parental generation) starting 10 weeks before mating and continuously through mating, gestation and weaning of their pups (F1 generation). At weaning, the F1 generation was exposed to graduated doses of the test item and was assigned to cohorts of animals for reproductive/developmental toxicity (cohorts 1A and 1B), developmental neurotoxicity (cohort 2) or developmental immunotoxicity testing (cohort 3).

In the parental generation, no treatment-related mortality was observed. No effect on body weight, food consumption, oestrus cycle, mating, fertility, duration of gestation or number of implantation sites were observed. There was an increased number of females with difficulty to deliver (dystocia) at 625 mg/kg bw per day, which was considered adverse. However, in control females, it was also observed that dystocia was high compared to the background range. The same phenomenon was also observed in other groups as well as in other studies with delivery phases which were performed in the facility at the same time. Despite investigations having been conducted, no external confounding factors were identified and it was considered by the authors that this finding may represent evolving background of the rat. The authors considered that it was unclear how this may have influenced the study results. There were no adverse effects on haematology, coagulation, blood chemistry and urine analysis.

In the parental generation offspring (pre-weaning F1 pups), there was a non-statistically significant lower mean litter size at birth (11.5 compared to 12.6 in controls) at 625 mg/kg bw per day. At 250 mg/kg bw per day there was a lower live birth index (85.8% vs. 95.5% in controls), which was

[26] Report no. 46989 RSR (2019; Preliminary Reproduction and Developmental Toxicity Study by Oral Route (gavage) in Rats).
[27] Report no. 46990 RSR (2020; 2-methyloxolane - Extended One-Generation Reproductive Toxicity Study by Oral Route (Gavage) in Rats).

not statistically significant. These effects were considered treatment-related and adverse by the applicant and they identified a NOAEL of 100 mg/kg bw per day to be used for deriving a TDI. However, the Panel noted that the decreased live birth index may have been partly confounded by the dystocia reported in all the animals, including the controls.

In cohorts 1A and 1B, there were no test-item related deaths. There were no effects on body weight, sexual development, oestrus cycle or mating. At 250 mg/kg bw per day, a test item relationship with a statistically significant ($p < 0.05$) decreased female fertility index was found at 68.4% compared to 100% in controls ($p < 0.05$). The decrease in the live birth index observed in the parental generation was not repeated in the F1 generation. In F1 generation offspring (pre-weaning F2 pups), no effects were found on neo-natal deaths, survival index, clinical observations, body weight, macroscopic observations or sexual development. The Panel considered that a NOAEL of 100 mg/kg bw per day can be identified based on the decreased female fertility index.

In cohort 2, no adverse effects were reported on neurohistopathological evaluation, and there were no neurobehavioral effects (neuro startle tests, functional observation battery, motor activity) or sexual development at 250 mg/kg bw per day, the highest dose tested.

In cohort 3, no immunotoxic effects (anti-KLH IgM response) were reported at 250 mg/kg bw per day, the highest dose tested.

Thyroid hormone levels were measured in parental generation, F1 pups not selected for any cohorts, and in cohort 1A animals and F2 pups, and compared to controls. After treatment up to 625 mg/kg bw per day in F1 and F2 pups, there were no changes in T4 levels on day 22 p.p., and no changes at termination from the first 10 surviving males/group and the first 10 lactating females/group (parental and cohort 1A animals), as well as for measurements of T4 and thyroid stimulating hormone (TSH) levels.

Concerning sperm analysis, no effects were observed in parental generation males. In cohort 1A males at 625 mg/kg/day, there was a lower mean number of testicular spermatozoa heads and a lower daily spermatozoa production rate (−15% vs controls, $p < 0.05$). This finding was considered by the authors of the study to be treatment-related but not adverse as no effects were seen on the mean number of epididymal spermatozoa.

The different NOAELs identified by the Panel from the different cohorts and for the different endpoints of this EOGRT study were shown in Table 14.

3.6.7. Hypersensitivity, allergenicity and food intolerance[28]

2-Methyloxolane was not found to be a sensitiser in a local lymph node assay (LLNA) in mouse, in a study[28] conducted according to OECD TG 429 (OECD, 2010) and in compliance with GLP.

Table 14: NOAELs identified by endpoint in the EOGRT study

Endpoint	NOAEL (mg/kg bw per day)	Clinical observations
Systemic toxicity	250 (in males)	Hypoactivity, staggering gait, sudden startle and/or tremors at 625 mg/kg bw per day
	625 (in females)	No effects found before parturition
Reproductive and developmental toxicity	100	A decrease in female fertility index in cohorts 1A and 1B at 250 mg/kg bw per day
Developmental neurotoxicity	250	No effects (highest dose tested in cohort 2B)
Developmental immunotoxicity	250	No effects (highest dose tested in cohort 3)

NOAEL: no observed adverse effect level; EOGRT: extended one-generation reproductive toxicity; bw: body weight.

3.6.8. Impurities of potential toxicological relevance[29]

The Panel considered that among the impurities that are present in 2-methyloxolane preparations furan (classification mutagenic 2 and carcinogenic 1B) and 2-methylfuran are those with the highest hazardous properties. Both have the same lower confidence limit of the benchmark dose ($BMDL_{10}$) of 0.064 mg/kg bw per day for non-neoplastic effects and of 1.31 mg/kg bw per day for neoplastic effects (EFSA CONTAM Panel, 2017).

[28] Report no. 41205097 (2013; Tetrahydro-2-methylfuran: Local lymph node assay in the mouse).
[29] Technical dossier/section 1.2.8.2.

If assuming that their average concentration was at their maximum limits (0.05% according to the specifications), for the population with the highest exposure estimate (toddlers), the calculated MOEs at the mean exposure were 725 and 14,800 for non-neoplastic and neoplastic effects, respectively. The Panel considered that these MOEs do not indicate a safety concern.

3.7. Concluding remarks on toxicity

The Panel considered that the toxicity studies available were adequately conducted. Most of them were recent, conducted according to GLP and following the relevant OECD guidelines.

In a 13-week oral (gavage) study in the rat at 500 mg/kg bw per day, changes were limited to increased liver weight that were reversible after a 28-day recovery period, which suggests that they are adaptive. A conservative NOAEL of 250 mg/kg bw per day was identified.

2-Methyloxolane did not induce gene mutations in bacteria either in the presence or in the absence of metabolic activation (Ames test). Negative results were also reported in two well-conducted *in vitro* studies: an *in vitro* micronucleus assay carried out in human peripheral blood lymphocytes and a gene mutation assay at the *TK+/-* locus in L5178Y mouse lymphoma cells. Overall, the Panel considered that 2-methyloxolane does not raise a concern for genotoxicity.

No chronic or carcinogenicity studies on 2-methyloxolane were provided by the applicant.

For reproductive and developmental toxicity, one oral developmental toxicity and an EOGRT study were available. From the oral developmental toxicity study, the Panel identified the same NOAEL of 300 mg/kg bw per day for both maternal and fetal toxicity. From the EOGRT study, no neurodevelopmental or immunotoxicity effects were reported at 250 mg/kg bw per day (the highest dose tested); from the different cohorts included in the study, a conservative NOAEL of 100 mg/kg bw per day was identified based on a decreased fertility index in females at 250 mg/kg bw per day.

The Panel also noted that 2-methyloxolane was not a sensitiser in a LLNA in mice.

The Panel noted that exposure to the impurities with the highest hazardous potential, namely furan and 2-methylfuran, was associated with MOEs (725 and 14,800 for non-neoplastic and neoplastic effects, respectively, at the mean exposure) that did not indicate a safety concern.

Overall, the Panel considered that the available toxicity studies, in support of the application, can be used to identify different NOAELs, the lowest NOAEL being 100 mg 2-methyloxolane/kg bw per day for reproductive and developmental toxicity.

3.8. Risk characterisation

Using the NOAEL of 100 mg 2-methyloxolane/kg bw per day from the EOGRT study and applying an uncertainty factor of 100, the Panel established a TDI of 1 mg/kg bw per day for 2-methyloxolane.

The TDI of 1 mg/kg bw per day was not exceeded in any of the different population groups, at the mean or at the 95th percentile exposure, when 2-methyloxolane is used according to the intended conditions and respecting the proposed MRLs in the extracted food or food ingredient.

4. Conclusions

The Panel established a TDI of 1 mg/kg bw per day for 2-methyloxolane. This TDI was not exceeded in any of the population groups at the mean and the 95th percentile exposure, with the highest maximum calculated exposure for toddlers at 0.32 mg/kg bw per day.

The Panel concluded that the extraction solvent 2-methyloxolane does not raise a safety concern when used according to the intended conditions and at the proposed MRLs in the extracted food or food ingredient: (i) production or fractionation of fat, oil or butter (with an MRL of 1 mg/kg); (ii) preparation of defatted protein product, defatted flour, and other defatted solid ingredients (with an MRL of 10 mg/kg), (iii) including for use in food category 13 (foods intended for particular nutritional uses as defined by Directive 2009/39/EC, with an MRL of 1 mg/kg); and (iv) for the extraction of food additives (with an MRL of 1 mg/kg).

5. Documentation as provided to EFSA

1) Technical dossier 2-methyloxolane (Methyltetrahydrofuran, CAS 96-47-9). November 2020. Submitted by Pennakem Europa to the European Commission and received by EFSA in January 2021.
2) Additional info. September 2021. Submitted by Pennakem Europa.
3) Additional info. October 2021. Submitted by Pennakem Europa.

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Abbreviations

BHT	3,5-di-*tert*-butyl-4-hydroxytoluene
BMDL	lower confidence limit of the benchmark dose
Bw	body weight
CAS	Chemical Abstracts Service
CLP	classification labelling and packaging
[redacted]	[redacted]

EC	European Community
EOGRT	extended one generation reproductive toxicity
FAIM	EFSA's food additives intake model
FCS	food categorisation system
FEMA	Flavor Extract Manufacturers Association of the United States
FID	flame ionisation detection
FOB	functional observations battery
GC	gas chromatography
GLP	good laboratory practices
HS	head space
HPLC	high-performance liquid chromatography
ICH	International Council for Harmonisation
KLH	keyhole limpet hemocyanin
K_{ow}	n-octanol/water partition coefficient
LLNA	local lymph node assay
LOD	limit of detection
LOQ	limit of quantification
MeTHF	2-methyltetrahydrofuran
MOE	margin of exposure
MRL	maximum residue limit
MS	mass spectrometry
NOAEL	no observed adverse effect level
NOEL	no observed effect level
NTP	National Toxicology Program
p.p.	post-partum
REACH	registration, evaluation, authorisation and restriction of chemicals
RSG	relative suspension growth
RTG	relative total growth
T_3	triiodothyronine
T_4	thyroxine
TDI	tolerable daily intake
THF	tetrahydrofuran
TK	thymidine kinase
TSH	thyroid-stimulating hormone
VOC	volatile organic compounds