SCIENTIFIC OPINION

efsa JOURNAL

ADOPTED: 31 August 2023

doi: 10.2903/j.efsa.2023.8240

Re-evaluation of behenic acid from mustard seeds to be used in the manufacturing of certain emulsifiers pursuant to Article 21(2) of Regulation (EU) No 1169/2011 – for permanent exemption from labelling

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Abstract

Following a request from the European Commission, the Panel on Nutrition, Novel Foods and Food Allergens (NDA) was asked to review a scientific assessment related to a notification from DuPont Nutrition Biosciences Aps on behenic acid from mustard seeds to be used in the manufacturing of certain emulsifiers pursuant to Article 21(2) of Regulation (EU) No 1169/2011 – for permanent exemption from labelling. The EC requested EFSA to consider comments raised by the German authorities in relation to: (a) the maximum amount of mustard protein that could be consumed from the emulsifiers manufactured from behenic acid (E470a, E471 and E477) on a single occasion and (b) the minimal observed eliciting dose (MOED) triggering allergic reactions in mustard-allergic individuals. The maximum amount of mustard protein content in behenic acid was re-assessed in view of new analytical data provided by the applicant. Intake estimates by the EFSA ANS Panel for E471 (adults) were used as a proxy for the combined intake (E470a, E471 and E477). Food challenge data and systematic reviews thereof deriving population minimal observed eliciting dose distributions for mustard protein were used to calculate the MOED and estimate the risk. The margin of exposure between the MOED (0.26 mg mustard protein) and the maximum amount of mustard protein that could be consumed from the emulsifiers on a single occasion (0.00895475 mg) is 29. It is predicted that between 0.1% and 1% of the mustard allergic population would react with mild objective symptoms to that dose. Overall, the assessment is conservative, particularly in relation to the exposure. Based on the information and data available, the NDA Panel concludes that it is extremely unlikely ($\leq$ 1% probability) that oral consumption of emulsifiers to be manufactured using behenic acid from mustard seeds (i.e. E470a, E471 and E477) will trigger an allergic reaction in mustard-allergic individuals under the proposed conditions of use.

Keywords: behenic acid, emulsifiers, mustard, food allergy, minimal observed eliciting dose, labelling exemption

Requestor: European Commission

Question number: EFSA-Q-2022-00042

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Declaration of interest: If you wish to access the declaration of interests of any expert contributing to an EFSA scientific assessment, please contact interestmanagement@efsa.europa.eu.

Acknowledgements: The Panel on Nutrition, Novel Foods and Food Allergens (NDA) wishes to thank the members of the Working Group on Food Allergy Stefaan de Henauw, Rosangela Marchelli, Dominique Turck and Hendrik Van Loveren, and the EFSA staff Ionut Craciun and Silvia Valtueña Martínez, for the support provided to this scientific output. The Panel also wishes to thank Carmen Peláez for her contribution as member of the NDA Panel until June 2023.

Suggested citation: EFSA NDA Panel (EFSA Panel on Nutrition, Novel Foods and Food Allergens), Turck, D., Bohn, T., Castenmiller, J., Hirsch-Ernst, K. I., Knutsen, H. K., Maciuk, A., Mangelsdorf, I., McArdle, H. J., Naska, A., Pentieva, K., Siani, A., Thies, F., Tsabouri, S., Vinceti, M., Marchelli, R., Van Loveren, H., Valtueña Martínez, S., & de Henauw, S. (2023). Re-evaluation of behenic acid from mustard seeds to be used in the manufacturing of certain emulsifiers pursuant to Article 21(2) of Regulation (EU) No 1169/2011 – for permanent exemption from labelling. *EFSA Journal, 21*(9), 1–27. https://doi.org/10.2903/j.efsa.2023.8240

ISSN: 1831-4732

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1. Introduction

This assessment follows the scientific principles outlined in the 'Protocol for the re-evaluation of behenic acid from mustard seeds to be used in the manufacturing of certain emulsifiers pursuant to Article 21(2) of Regulation (EU) No 1169/2011 – for permanent exemption from labelling', which was endorsed by the EFSA Panel on Nutrition, Novel Foods and Food Allergens (NDA) on 29 June 2022 at its 126th Plenary meeting (Annex A).

1.1. Background and Terms of Reference as provided by the requestor

1.1.1. Background

Annex II to Regulation (EU) No 1169/2011[1] (hereafter "the Regulation") includes the EU list of food substances or products known as likely to trigger adverse reactions in sensitive individuals, and therefore their presence in foods must be always indicated.

The Regulation also requires the Commission to systemically re-examine, and where necessary, update the list in Annex II, after consulting EFSA. Following EFSA's opinion, the list included in the Annex II can then be amended.

The update of the list may also consist in the deletion of food allergens for which it has been scientifically established that it is unlikely to cause adverse reactions. To this end, the interested parties may communicate to the Commission studies applying to certain products derived from substances listed in Annex II that are not likely to trigger adverse reactions in individuals.

In this context, on 22 September 2015, *DuPont Nutrition Biosciences Aps* submitted to the Commission, an application for the exemption of behenic acid from mustard seeds to be used in the manufacturing of certain emulsifiers from the obligation to be labelled as an allergen, in accordance with Articles 9(1)(c) and 21 read together with Annex II to the Regulation.

On 25 October 2016, EFSA adopted its scientific opinion related to behenic acid from mustard seeds to be used in the manufacturing of certain emulsifiers pursuant to Article 21(2) of Regulation (EU) No 1169/2011 for permanent exemption from labelling, following a request from the Commission.

On 9 October 2019, in the context of the Member States Working Group under Regulation (EU) No 1169/2011, the Commission submitted for discussion a draft measure exempting behenic acid from the labelling requirements established for food allergens by Regulation (EU) No 1169/2011, which was based on the above-mentioned EFSA scientific opinion. During the discussion, the German authorities raised concerns as to the correctness of certain data on which the EFSA's conclusions were based. In particular, it was pointed out that the estimations of the daily exposure for the substances in question should be revised in view of the EFSA opinions published between 2017 and 2018 concerning the use of behenic acid from mustard seeds in the manufacture of certain additives.[2] The German authorities also challenged the value of 1 mg for the protein dose triggering allergic reactions in mustard-allergic individuals.

Therefore, in the light of more recent scientific knowledge available, it is considered necessary to request EFSA to update its scientific opinion from 2016 on the non-allergenicity of behenic acid from mustard seeds to be used in the manufacturing of certain emulsifiers.

1.1.2. Terms of Reference

In accordance with Article 29(1)(a) of Regulation (EC) 178/2002[3], the European Commission asks EFSA:

- To update the EFSA scientific opinion[4] related to a notification from DuPont Nutrition Biosciences Aps on behenic acid from mustard seeds to be used in the manufacturing of certain emulsifiers, pursuant to Article 21(2) of Regulation (EU) No 1169/2011, for permanent exemption from labelling.

[1] Regulation (EU) No 1169/2011 of the European Parliament and of the Council of 25 October 2011 on the provision of food information to consumers; OJ L 304, 22.11.2011, p. 18.

[2] EFSA ANS Panel scientific opinion on E471: https://www.efsa.europa.eu/en/efsajournal/pub/5045 EFSA ANS Panel Scientific opinion on E470a and E470b: https://www.efsa.europa.eu/en/efsajournal/pub/5180 EFSA FAF Panel Scientific opinion on E477: https://www.efsa.europa.eu/en/efsajournal/pub/5497

[3] Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. OJ L 31, 1.2.2002, p. 1–24.

[4] EFSA Journal 2016;14(11):4631.

- In elaborating its opinion, EFSA is requested to consider the attached observations made by the German authorities, as well as data used for the EFSA scientific opinions with regard to E470, E471 and E477 issued by ANS and FAF Panels in the context of the re-evaluation of the safety of the substances in question as food additives.

1.2. Interpretation of the Terms of Reference

The Panel interprets this mandate as a re-evaluation of behenic acid from mustard seeds to be used in the manufacturing of certain emulsifiers pursuant to Article 21(2) of Regulation (EU) No 1169/2011 related to a notification from *DuPont Nutrition Biosciences Aps*, for permanent exemption from labelling. The re-assessment concerns:

a) the maximum amount of mustard protein that could be consumed from emulsifiers manufactured from behenic acid (E470a, E471 and E477) on a single occasion under the proposed conditions of use,
b) the available scientific data on the allergenicity of mustard, in particular regarding the minimal eliciting dose triggering allergic reactions in mustard-allergic individuals and
c) the margin of exposure between the minimal eliciting dose of mustard protein triggering allergic reactions in mustard-allergic individuals and the maximum amount of mustard protein that could be consumed from emulsifiers manufactured from behenic acid on a single occasion under the proposed conditions of use.

1.3. Additional information

1.3.1. Observations made by the German authorities

The written observations made by the German authorities (English translation) can be found in Annex A (Appendix A).

1.3.2. Additional information requested to the applicant

DuPont Nutrition Biosciences Aps (at present wholly owned subsidiary of International Flavors and Fragrances, IFF) was informed by EFSA about the present re-evaluation and given the opportunity to provide any new information/data available regarding:

1) The general specifications, manufacturing process, allergen specifications (including the protein content analysed by e.g. the Kjeldahl method) and stability of behenic acid to be used in the manufacturing of emulsifiers E470a, E471 and E477.
2) The manufacturing process, allergen specifications and stability of emulsifiers E470a, E471 and E477 manufactured from behenic acid.
3) Scientific data on allergenicity of the food allergen-derived preparation (behenic acid) and/or the food allergen-derived foodstuff(s) (the emulsifiers).

1.4. Definition of terms

In the context of this opinion, the following terms will be used as defined below:

Behenic acid. Denotes behenic acid extracted from mustard seeds.
Emulsifiers. Refers to emulsifiers manufactured using behenic acid from mustard seeds.
Food allergen-derived preparation (e.g. behenic acid) means a product derived from a food allergen (e.g. mustard).
Food allergen-derived foodstuffs are foods/ingredients (e.g. emulsifiers) manufactured using a food allergen-derived preparation (e.g. behenic acid).
Minimal eliciting dose (MED). Minimal dose of an allergen (expressed as the amount of total protein) triggering allergic reactions with either objective or subjective symptoms in food allergic individuals.
Minimal observed eliciting dose (MOED). Minimal dose of an allergen (expressed as the amount of total protein) triggering allergic reactions with objective symptoms in food allergic individuals.

2. Data and methodologies

This assessment follows the scientific principles outlined in the 'Protocol for the re-evaluation of behenic acid from mustard seeds to be used in the manufacturing of certain emulsifiers pursuant to Article 21(2) of Regulation (EU) No 1169/2011 – for permanent exemption from labelling', which was endorsed by the EFSA Panel on Nutrition, Novel Foods and Food Allergens (NDA) on 29 June 2022 at its 126th Plenary meeting (Annex A). The protocol depicts the data and methodologies to be used in the assessment depending on the additional information/data that could be provided by the applicant.

The applicant provided additional information on 5 July 2022 and asked for an extension of deadline up to 15 September 2022 to complete protein analyses on behenic acid samples. On 21 September 2022, the applicant re-submitted the same information as provided on 5 July 2022 but no data on protein analyses of behenic acid samples or any other data that could be used in the re-valuation of this application. The applicant submitted additional information on allergen specifications for behenic acid on 12 December 2022 and replied to additional questions raised by EFSA (letter to the applicant sent on 22 December 2022) on 31 March 2023.

2.1. Data

In addition to the original application evaluated by EFSA in 2016 (EFSA NDA Panel, 2016), the applicant provided the following information/data upon EFSA's request:

a) The applicant clarified that mustard seeds-derived behenic acid is currently not being used in the manufacturing process of any emulsifier, as there is no commercial interest in emulsifiers that are labelled as allergens and the company is awaiting a decision from the European Commission on the exemption from allergen labelling before doing so.
b) Data on protein analysis of behenic acid using the Kjeldahl method and an enzyme-linked immunosorbent assay (ELISA) method have been provided (two batches).
c) No publications related to the allergenicity of behenic acid from mustard seeds were retrieved by the applicant in PubMed or ScienceDirect when generally searching for 'behenic acid allergic rection' or 'behenic acid allergenicity'.

No new information/data was provided by the applicant on:

a) The general specifications, manufacturing process or stability of behenic acid to be used in the manufacturing of emulsifiers E470a, E471 and E477.
b) The manufacturing process, allergen specifications and stability of emulsifiers E470a, E471 and E477 manufactured from behenic acid, as no emulsifiers are currently manufactured using behenic acid from mustard seeds.
c) Scientific data on allergenicity of the food allergen-derived preparation (behenic acid) and/or the food allergen-derived foodstuff(s) (the emulsifiers).

Considering the reply from the applicant, the following data will be used for the present assessment:

1) The maximum amount of mustard protein content in behenic acid will be re-assessed by considering the new analytical data provided by the applicant.
2) Levels of behenic acid intended for use in the manufacturing of the emulsifiers (i.e. in amounts from 25% to 85% on a weight basis, as stated by the applicant).
3) Intake estimates for emulsifier E471 as reported by the EFSA ANS Panel (2017) under the refined estimated exposure assessment scenario, and in particular the brand-loyal scenario, as a proxy for the combined intake of emulsifiers to be manufactured from behenic acid.
4) Available food challenge data on mustard (Morisset et al., 2003; Figueroa et al., 2005) and systematic reviews thereof deriving population minimal observed eliciting dose distributions for mustard protein (Houben et al., 2020; Remington et al., 2020).

2.2. Methodologies

The methodologies used for the assessment are explained in the protocol. The conceptual framework is given in Figure 1.

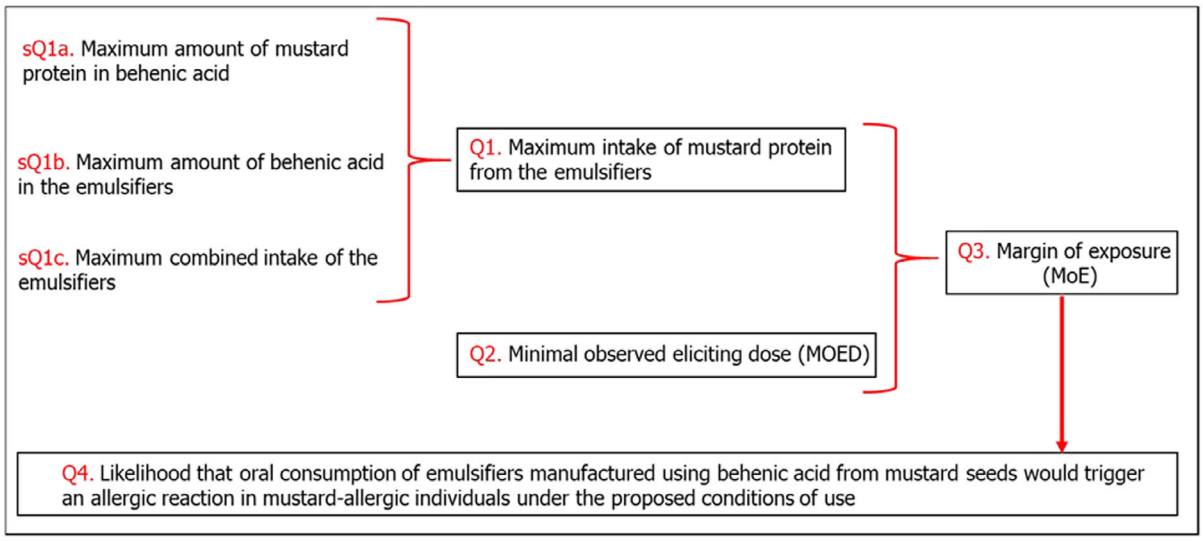

Figure 1: Conceptual framework for the assessment. Q: question; sQ: sub-question

The internal and external validity of the food challenge studies with mustard (Section 3.4) was assessed with a risk of bias (RoB) tool developed for that purpose (Appendix A.1), as none of the existing RoB tools for the appraisal of human studies was considered appropriate to capture the features and potential risk of bias associated to this specific type of study design. The tool has been developed with features from the UK National Institute for Clinical Excellence (NICE) methodologic checklist for cohort studies, which was taken as a basis;[5] the Quality in Prognosis studies tool (QUIPS; Hayden et al., 2013); and the Office of Health Assessment and Translation (OHAT) from the US National Toxicology Program tool (OHAT-NTP, 2019) for the only purpose of ensuring consistency in the appraisal of internal and external validity across studies.

The general approach of the NDA Panel for the evaluation of applications on food ingredients or substances with known allergenic potential is outlined in the Guidance on the preparation and presentation of applications for exemption from mandatory labelling of food allergens and/or products thereof pursuant to Article 21 (2) of Regulation (EU) No 1169/2011 (EFSA NDA Panel, 2021).

A draft opinion was endorsed by the NDA Panel on 24 May 2023 and was open for public consultation from 12 June to 23 July 2023. The draft opinion has been amended in view of the comments received, which have all been addressed and are published in a technical report (Annex B).

3. Assessment

3.1. Introduction

Prevalence data on mustard allergy have been generally reported in selected populations, either in patients recruited at hospital or in atopic individuals (FAO and WHO, 2022). Mustard allergy was found to be the fourth leading cause of food allergy after eggs, peanuts and cow's milk in France in a selected population of children and adolescents with 'food hypersensitivity' based on skin prick tests (SPT), specific serum IgE and single-blind placebo-controlled food challenges (Rancé et al., 1999). No recent data have been published to confirm the importance of mustard allergy in France (FAO and WHO, 2022).

Prevalence data on mustard allergy in the general (unselected) European population with food challenge confirmation are missing. Recent data collected in the context of the EU-funded EuroPrevall project among adults in six European countries (Switzerland, Spain, Poland, The Netherlands, Iceland and Greece) suggest that the prevalence of probable allergy to mustard seeds, based on symptoms plus specific IgE-sensitisation, is very low. In all countries, the prevalence approached zero based on complete case analysis and only in Poland reached 0.03% based on imputed data (Lyons et al., 2019).

[5] National Institute for Health and Care Excellence. The social care guidance manual – process and methods. Published on 30 April 2013 and updated in July 2016. Appendix D. Available online: https://www.nice.org.uk/process/pmg10/chapter/appendix-d-methodology-checklist-cohort-studies#notes-on-use-of-methodology-checklist-cohort-studies [Accessed: 11 October 2022].

In the FAO/WHO meeting report on the review and validation of CODEX Alimentarius priority allergens list for labelling purposes (FAO and WHO, 2022), a combination of three criteria (prevalence, severity of symptoms and potency) was used to score and classify food allergens for that purpose. The FAO/WHO Expert Committee proposed not to include mustard in the global priority list for allergen labelling based on the following reasons:

a) Prevalence: very low ($< 0.5\%$ in one region OR $< 0.1\%$ in all regions) based on grade 2 evidence (probable allergy in unselected populations).
b) Severity of symptoms: mustard causes at least 5–10% of all anaphylaxis reactions to food only in one CODEX region (France), but a lower proportion in other CODEX regions.
c) Potency: Although the overall assessment of potency is considered as high, the data quantity available is poor ($n < 40$ individuals) and overlaps with the 95% CI of medium-potency allergens.

The FAO/WHO Expert Committee recommends mustard not to be listed as a global priority allergen but may be kept on a list of allergens for regional consideration.

Considering the well-documented reports of allergic individuals reacting to mustard, and that mustard is an allergen subject to mandatory labelling in the EU, the Panel assesses the likelihood of adverse reactions in allergic individuals consuming products derived from mustard.

3.2. Context of the assessment

This scientific opinion is a re-assessment of a dossier submitted by DuPont Nutrition Biosciences Aps for permanent exemption from labelling of behenic acid to be used in the manufacturing of emulsifiers, pursuant to Article 21(2) of Regulation (EU) No 1169/2011. Behenic acid (docosanoic acid $C_{22}H_{44}O_2$, C22:0) is obtained from a fraction of fully hydrogenated refined mustard seed oil and could be used for the synthesis of several emulsifiers, namely E470a, E471 and E477, as stated by the applicant, and possibly E472 (a, b, c and e) and E475. Emulsifiers are food additives permitted for use in several food categories.

The dossier was first evaluated by EFSA in 2016 (EFSA NDA Panel, 2016). Information and data on the general specifications, manufacturing process, allergen specifications and conditions of use of behenic acid to be used in the manufacturing of certain emulsifiers (from 25% to 85% by weight) can be found in the EFSA opinion, as well as the results of *in vitro* tests (ELISA assays and passive sensitisation histamine release test) on allergenicity. No data were provided on the residual total protein content of behenic acid. Instead, the applicant argued that proteins and peptides are unlikely to be carried over into the distillate following the two sequential distillation steps described in the manufacturing process of behenic acid. The NDA Panel considered that the presence of mustard proteins in behenic acid could not be excluded based on the manufacturing process only (EFSA NDA Panel, 2016).

The applicant has now clarified that behenic acid has not been used yet in the manufacturing of any emulsifier, and therefore no data were provided in the original application regarding the general specifications, manufacturing process, allergen specifications or allergenicity tests of these food additives.

3.3. Maximum intake of mustard protein from the emulsifiers (Q1)

3.3.1. Maximum amount of mustard protein in behenic acid (sQ1a)

In the previous evaluation, the limit of quantification (LOQ) of the ELISA assay used by the applicant to assess mustard allergens in behenic acid (undetected in all samples tested) was taken as a proxy for the maximum amount of mustard protein content in behenic acid (EFSA NDA Panel, 2016). The LOQ was ≤ 1 mg/kg, expressed as mustard seed protein.

The assay was developed using seed proteins from *Sinapis alba* L. and *Brassica nigra* (L.) W. D. J. Koch. The assay has been shown to detect seed proteins from *S. alba* and *B. nigra*, but also from *B. uncea*, by using positive standards with known amounts of seed proteins from these three species of mustard. Although the method for protein extraction from the oil was not specified in the application (claimed as proprietary by the company manufacturing the ELISA test), the information provided on the method of analysis was sufficient to conclude that the assay was appropriate for testing the presence of residual mustard allergens in behenic acid samples complying with the specifications given in the application. Mustard allergens were not detected in any of the three different batches of behenic acid that were tested.

Upon EFSA's request, the applicant has provided additional analyses on two more batches of behenic acid. Total residual nitrogen in behenic acid was analysed using a modified version of the Kjeldahl method (protocol provided to EFSA – claimed as proprietary by the applicant) and total protein was calculated by multiplying the nitrogen content by a factor of 6.25. The total protein content was below the LOQ (≤ 0.1 g/100 g, equivalent to ≤ 1,000 mg/kg) of the method in both samples. The Panel notes the high LOQ of the method.

In addition, the two samples of behenic acid were analysed with an ELISA method based on polyclonal antibodies, with a LOQ of ≤ 2 mg/kg expressed as 'mustard' in the certificate of analysis. The protocol used for protein extraction (claimed as confidential by the applicant) has been provided upon EFSA's request and is considered by the NDA Panel as appropriate for the current assessment.

The LOQ of the ELISA has been estimated to be ≤ 0.5 mg/kg expressed as mustard seed protein in the certificate of analysis by using the protein content in mustard seed (ground) from the US Department of Agriculture (USDA) database[6] (26%) as conversion factor. Therefore, the Panel assumes that the LOQ of the ELISA of ≤ 2 mg/kg is expressed as mustard seeds. Mustard allergens were below the limit of detection in both samples. The Panel notes that the USDA database used a conversion factor for protein from nitrogen of 5.4 to account for non-protein nitrogen. The Panel also notes that, as claimed by the applicant, the ELISA method used for this re-evaluation is more sensitive for the detection of mustard seed proteins (LOQ ≤ 0.5 mg/kg) than the kit used for the previous submission (LOQ ≤ 1 mg/kg) (EFSA NDA Panel, 2016).

The Panel notes that the protein content in mustard seeds depends on the species, and that higher protein content has been reported in the literature for *S. alba* L. (between 30% and 37%) than for *B. juncea* (L.) Czern (between 26% and 32%) or *B. nigra* (23%), using a conversion factor for protein from nitrogen of 6.25, which may overestimate the protein content in mustard seeds (Aboulfadl et al., 2011; Sharma et al., 2019). The Panel also notes that *S. alba* L. is not used in the manufacturing of behenic acid (EFSA NDA Panel, 2016). Therefore, the Panel considers that assuming a protein content in mustard seeds of 26% is appropriate for the purpose of this opinion, and that the LOQ of the ELISA ≤ 0.5 mg/kg expressed as mustard seed protein is an appropriate proxy for the mustard protein content in behenic acid.

3.3.2. Maximum amount of behenic acid in the emulsifiers (sQ1b)

The applicant proposed that behenic acid could be used in the manufacturing of the emulsifiers in amounts from 25% to 85% on a weight basis depending on the emulsifier and its intended use.

The Panel notes that the maximum amount of behenic acid in the emulsifiers is 85% on weight basis. The Panel also notes that this amount could be lower depending on the emulsifier and intended use.

3.3.3. Maximum combined intake of the emulsifiers (sQ1c)

In the previous evaluation (EFSA NDA Panel, 2016), the applicant claimed that, although E470a, E471 and E477 can be used in a variety of foods, there are only few applications where the functional properties of emulsifiers manufactured with behenic acid are needed. On that basis, a rough estimate of the daily combined dietary exposure to E470a, E471 and E477 was calculated by the applicant using the Food Additive Intakes Model. This approach assumes that an individual is a high consumer of one food category only and an average consumer of all the remaining food categories. The method consists of adding the highest level of exposure from one food category (calculated for consumers only) to the mean exposure values for the remaining categories (calculated for the total population) (EFSA, 2015).

The combined intake of E470a, E471 and E477 estimated by the applicant at the maximum permitted levels could lead to a maximum total exposure to the emulsifiers of 84 mg/kg body weight (bw) per day for toddlers and 20 mg/kg bw per day for adults. Using a reference bw of 12 kg for toddlers and of 70 kg for adults, the highest amount of emulsifiers ingested per person per day would be 1,008 mg for toddlers and 1,400 mg for adults. As a conservative approach, the Panel assumed that these doses could be consumed on a single occasion. The combined intake of E470a, E471 and E477 estimated by the applicant for these population subgroups with the maximum reported use levels was much lower, i.e. 25.5 mg/kg bw per day for toddlers and 9.6 mg/kg bw per day for adults, respectively.

The NDA Panel noted that the maximum intake of 1,400 mg as estimated for adults by the applicant for the combined intake of E470a, E471 and E477 was similar to that reported in the

[6] https://fdc.nal.usda.gov/fdc-app.html#/food-details/170929/nutrients

literature for E475 (Vin et al., 2013), and thus 1,400 mg was considered the highest estimate for all population groups (EFSA NDA Panel, 2016).

Between 2017 and 2018, EFSA re-evaluated the safety of E470a (EFSA ANS Panel, 2018), E471 (EFSA ANS Panel, 2017) and E477 (EFSA FAF Panel, 2018). In that context, intakes of each of these emulsifiers were assessed by considering use levels in foods as declared by food manufacturers in an open call for data. As explained in the protocol (Annex A), intakes of E471 (EFSA ANS Panel, 2017) will be used as a proxy for the combined intake of emulsifiers manufactured from behenic acid E470a, E471 and E477 because (see Annex A for details):

a) intake estimates for E471 are several times higher than for either E470a or E477 and
b) the use of E471 in combination with E470a, E477 or both, is rare, as assessed through the Mintel Global New Products Database (GNPD).

Table 1 depicts intake data for the emulsifier E471 (mg/kg bw per day) as estimated by the EFSA ANS Panel (2017) for all population groups except infants < 16 weeks of age, for whom intakes of E471 from infant formula were estimated by the EFSA FAF Panel (2021). The Panel notes, however, that intake estimates for that population group in mg/kg bw per day would lead to lower absolute daily intakes of E471 as compared to other population groups owing to their lower bw, and thus are not appropriate for use in the present assessment.

The exposure assessment of E471 was carried out by the ANS Panel based on two different sets of concentration data:

1) *Regulatory maximum level exposure assessment scenario*. It considers maximum permitted levels for all food categories as set down in EU legislation or the maximum reported use levels as provided to EFSA by the food industry for categories where E471 is authorised as *quantum satis*;
2) *Refined exposure assessment scenario*, which considers reported use levels for all food categories. For this set of concentration data, two refined exposure estimates are calculated:
   a) *The brand-loyal scenario*, which assumes long-term exposure to the maximum reported use levels for the main contributing food category at individual level and to the mean of the typical reported use levels for the remaining food categories.
   b) *The non-brand-loyal scenario*, which assumes long-term exposure to E471 at the mean of the typical reported use levels for all food categories.

Table 1: Summary of dietary exposure to the emulsifier E471 from its use as a food additive in the 'regulatory maximum level exposure assessment scenario' and in the 'refined exposure assessment scenarios', in six population groups (minimum/maximum across the dietary surveys in mg/kg bw per day)

Population group	**Infants**		**Toddlers**		**Children**		**Adolescents**		**Adults**		**Older adults**	
Reference body weight	**5 kg**		**12 kg**		**23 kg**		**52 kg**		**70 kg**		**70 kg**	
Age	12 weeks-11 months		12–35 months		3–9 years		10–17 years		18–64 years		≥ 65 years	
	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max
1. Regulatory maximum levels exposure assessment scenario												
Mean	149	432	118	417	74	376	36	270	53	179	50	185
P95	384	845	291	837	168	761	86	562	110	387	100	360
2. Refined estimated exposure assessment scenario												
2.a. Brand-loyal scenario												
Mean	30	179	30	247	45	252	23	184	36	**129**	37	137
P95	72	519	61	620	124	557	64	416	74	**301**	70	313
2.b. Non-brand-loyal scenario												
Mean	24	58	24	69	21	60	11	42	10	26	9	28
P95	59	111	50	128	52	124	27	89	21	58	18	54

Legend to Table 1. Sources: EFSA ANS Panel (2017) for E471 intake data and EFSA Scientific Committee (2012) for reference body weights. P95: 95th percentile. Values in bold are those used for the current assessment.

Daily intake estimates of emulsifiers using the refined estimated exposure assessment according to a brand-loyal scenario is considered by the Panel as the most suitable for the present re-evaluation. Although daily intake estimates per kg bw are higher for toddlers (young children) than for any other population group, absolute intake estimates for adults, which are the highest owing to their higher bw, are used as reference for the assessment.

According to this refined estimated exposure assessment brand-loyal scenario, the maximum mean level of exposure to E471 for adults is 129 mg/kg bw per day, and the maximum 95th percentile is 301 mg/kg bw per day. Assuming a bw of 70 kg for adults, this corresponds to an exposure of 9,030 mg per person per day for mean levels of consumption and of 21,070 mg per person per day for high levels of consumption.

The main food categories contributing to the intake of E471 were bread and rolls and fine bakery wares. Overall, the ANS Panel concluded that EFSA intake estimates for E471 could have been underestimated in all exposure scenarios. The complete uncertainty analysis of the intake estimates can be found in Section 3.4.5 of the EFSA ANS Panel (2017) opinion.

In the context of allergenicity risk assessments, relevant intakes are absolute amounts of the allergenic food consumed on a single occasion, and thus relevant intakes could be lower than daily doses depending on the number of meals consumed during the day. However, as a conservative approach, the Panel will assume in this case that daily intakes could be consumed on a single occasion, particularly considering that daily intakes may have been underestimated by EFSA based on use and use levels data provided by food business operators in the public call for data EFSA ANS Panel (2017).

3.3.4. Conclusions (Q1)

The maximum amount of mustard protein that could be consumed from the emulsifiers on a single occasion under the proposed conditions of use is calculated as follows:

$$\text{Maximum intake of mustard protein from the emulsifiers} = \text{maximum intake of the emulsifiers on a single occasion} \times \text{maximum content of behenic acid in the emulsifiers} \times \text{maximum content of mustard protein in behenic acid},$$

where daily intakes of E471 in adults are used as a proxy for the combined intakes of E471 and E470a, E477 on a single occasion (Section 3.3.3) and the LOQ of 0.5 mg/kg from the ELISA (Section 3.3.1) is taken as a proxy for the maximum content of mustard protein in behenic acid. This results in:

$$\text{Maximum intake of mustard protein from the emulsifiers} = (21,070\ \text{mg} \times 85 \times 0.5\ \text{mg/kg})/(100 \times 1,000,000) = 0.00895475\ \text{mg mustard protein}.$$

3.4. Minimal observed eliciting doses for mustard protein (Q2)

One of the objectives of the EFSA grant GP/EFSA/AFSCO/2017/03: 'Detection and quantification of allergens in foods and minimum eliciting doses in food allergic individuals' (Mills et al., 2023), was to collect and curate historic and published oral food challenge data, as well as new oral food challenge data available to clinical centres as part of the diagnosis of food allergy, for data-poor allergens, including mustard. In that context, no new challenge data for mustard have been identified, in addition to the studies already available to EFSA for the previous evaluation (EFSA NDA Panel, 2014, 2016).

One single-blind placebo-controlled food challenge (SBPCFC) reported in two publications (Rancé et al., 2000; Rancé et al., 2001) and two double-blind placebo-controlled food challenge (DBPCFC) studies (Morisset et al., 2003; Figueroa et al., 2005) documenting mustard allergy and anaphylactic reactions to mustard have been identified.

The SBPCFC conducted in France (Rancé et al., 2000; Rancé et al., 2001) was carried out in a hospital where – at the time the study was conducted – all atopic children attending the hospital as outpatients with a clinical history suggesting food allergy, systematically underwent a mustard SPT. Regarding the study population, it was reported by the authors that 'from April 1997 to April 1999, 36 consecutive patients with a positive SPT, selected from the 3600 mustard SPT performed (1%), were

enrolled in the study'. These 36 patients participating in the study (14 females and 22 males) had an average age of 5.5 years (range from 10 months to 15 years). A mustard seed powder containing both *S. alba* and *B. juncea* was used for the SPT. Children underwent a SBPCFC with a mustard and a placebo challenge administered 4 h apart. Increasing doses of mustard powder (dose progression: 1, 5, 10, 20, 50, 100, 250 and 500 mg) were masked in stewed apple and given every 20 min. Fifteen subjects (42% of those tested) reacted to the food challenge with objective symptoms (i.e. urticaria, rhino-conjunctivitis, angioedema, eczema) within a few minutes to 2 h after the last dose (criteria for diagnosis of IgE-mediated food allergy). The cumulative reactive dose varied from 1 to 936 mg. The mean cumulative reactive dose was 153 mg. Two patients reacted to the first dose of 1 mg mustard powder, corresponding to about 0.26 mg of mustard protein. No reactions to placebo were observed.

For the second study conducted in France (Morisset et al., 2003), 30 subjects (age 3–20 years; two adults) with suspected food allergy to mustard seeds (i.e. reported urticaria and angioedema, atopic dermatitis, 'asthma', abdominal pain or bouts of diarrhoea upon consumption during the clinical interview) were recruited based on a positive SPT to mustard. SPTs were performed with four different mustard preparations, and specific serum IgE to mustard was determined (radioallergosorbent test (RAST)). A total of 24 subjects underwent a DBPCFC with increasing doses (10, 30, 100, 300 and 900 mg) of a mustard seasoning given every 20 min up to a total cumulative dose of 1340 mg seasoning. The mustard seasoning provided by the food industry (33.6% *B. juncea* mustard seeds) was masked with a cola drink. The vehicle was used as placebo. Mustard and placebo were given in a randomised order 24-h apart. Six subjects were tested with a SBPCFC (first mustard and then placebo) to reduce follow-up. Among the seven patients reacting to the challenge, four did to a DBPCFC and three to a SBPCFC. Among those tested, 17% reacted to the DBPCFC and 50% to the SBPCFC. No information is provided for the placebo challenge. The MOED (based on the cumulative eliciting dose on DBPCFC) was 40 mg of mustard seasoning (rhinitis, urticaria). It is reported in the paper that 40 mg of mustard seasoning is 'equivalent to 13.5 mg of mustard seeds, roughly equivalent to 0.8 mg of proteins (mustard seasoning is considered to contain 6% of proteins)'. The Panel notes, however, that 6% of 40 mg is 2.4 mg protein, and that the protein content of mustard seed powder is about 26%, corresponding to 3.5 mg mustard protein.

In a DBPCFC (Figueroa et al., 2005) conducted in the Canary Islands (Spain), 38 mostly adult subjects (mean age 21.9 $\pm$ 8.6 years; age range 3–39 years) suspected of mustard allergy based on clinical history (immediate adverse reactions related to mustard ingestion) and positive SPT to mustard were recruited. Of these, four (11%) had reported systemic anaphylaxis after mustard ingestion. Fourteen patients were not tested with DBPCFC because of severe reactions or denial of consent. The remaining 24 patients underwent DBPCFCs with a commercial mustard sauce mixed in vanilla-lemon flavoured yoghurt to mask its strong taste and placebo (vehicle), provided in a randomised order 2 h apart. The mustard sauce contained water, *S. alba* seeds (14% w/v), vinegar, salt, turmeric, paprika and cloves. Increasing doses of mustard sauce (80, 240, 800, 2,400 and 6,480 mg) were administered at 15-min intervals until symptoms appeared or a cumulative dose of 10 g of mustard sauce was reached. Four subjects (17%) reacted with objective symptoms (urticaria, conjunctivitis, angioedema and 'bronchial asthma', and systemic anaphylaxis, one case of each) and 10 subjects (42%) with subjective symptoms (oral allergy syndrome). There were no reactions to placebo. The MOED (urticaria) and the minimal eliciting dose (MED) were both 44.8 mg of mustard (cumulative eliciting doses), corresponding to 11.7 mg of mustard protein.

The appraisal of the internal and external validity of the above-mentioned human studies can be found in Appendix A.2 and the heatmap in Appendix A.3.

The Panel notes that, in the above-mentioned studies, the lowest observed adverse effect level (LOAEL) for objective symptoms was 1 mg of mustard powder, corresponding to about 0.26 mg of mustard protein, which was also the first dose tested in the study by Rancé et al. (2000); Rancé et al. (2001). The Panel also notes, however, that the study was a SBPCFC, and that blinding of participants to the test food may have failed owing to difficulties in masking the taste of mustard. In the other two studies available (Morisset et al., 2003; Figueroa et al., 2005), the LOAEL for objective symptoms was higher (3.5 mg and 11.7 mg of mustard protein, respectively), and none of the patients reacted to the first dose tested. However, in one study the selection of patients eligible for the challenge was unclear (Morisset et al., 2003) and in another study most sensitive subjects were excluded from the challenge (Figueroa et al., 2005), questioning the external validity of these studies. In all cases, limited information was available regarding the exposure assessment (i.e. how the amount of the test food and the consistency across test days and tested patients was ensured).

The full range of population eliciting dose (EDp) values (and 95%CIs) for objective symptoms (i.e. doses of total protein from an allergenic food (ED) that are predicted to elicit mild objective symptoms in a certain percentage (p) of the food allergic population) for 14 priority allergenic foods (including mustard) and recommendations for use in risk characterisation have been published ((Houben et al., 2020); methodology described in (Remington et al., 2020)).

The aim in these publications was to use only individual data from DBPCFCs, except for infants and very young children. Individual data were collected and assessed in terms of discrete dose and cumulative dose datasets and expressed in mg of total protein from the allergenic food. Individuals were left-censored if they reacted with objective symptoms to the first challenge dose, while individuals were right-censored if they failed to respond with objective symptoms to the uppermost challenge dose but did have clear histories of allergic reactions upon consumption of the offending food. Sources of threshold data for mustard used in the publications (Houben et al., 2020); (Remington et al., 2020) are shown in Table 2.

Table 2: Sources of threshold data for mustard

Study	Country	First dose tested (mg mustard protein)	Total no. with objective symptoms	Right-censored[(a)]	Left-censored[(b)]	Adults	Children
Figueroa et al. (2005)	Spain	2.92	14	10	0	9	5
Morisset et al. (2003)	France	0.876	4	0	0	0	4
Rancé et al. (2001)	France	0.2608	15	0	2	0	15
Total			**33**	**10**	**2**	**9**	**24**

Legend to Table 2: Source: Remington et al. (2020) – Supplemental material 1.
(a): Number of right-censored individuals; subjects who completed the food challenge without experiencing objective symptoms to the largest dose (NOAEL = highest challenge dose; LOAEL set to infinity).
(b): Number of left-censored individuals; subjects who reacted with objective symptoms to the first dose of the food challenge (NOAEL set to zero; LOAEL = lowest challenge dose).

The Panel notes that, whereas only data from the four patients reacting to the DBPCFC (and not from those reacting to the SBPCFC) in the study by Morisset et al. (2003) were included, data from the 15 patients reacting to the SBPCFC in the study by Rancé et al. (2001) were included (as reported in Rancé et al., 2000), seven of which were older than 5 years of age. The Panel also notes that the 10 patients reacting with subjective symptoms in the study by Figueroa et al. (2005) were right-censored even if not tested for the highest dose. In addition, mustard was considered a 'data poor' allergen, since only data from 33 patients were available (half of which right- or left-censored) while a sample size of 60 or larger is recommended for obtaining stable EDp estimates (Houben et al., 2020).

Individual studies were combined per allergen and analysed with Bayesian Stacked Parametric Survival methods with Frailty Components and Interval Censored Failure Times as described (Wheeler et al., 2021), and the whole range of EDp (and 95% CIs) was derived (Houben et al., 2020). An extract of such values for mustard is shown in Appendix B.

Using data from Houben et al. (2020), the FAO/WHO Expert Committee (FAO and WHO, 2022) noted that the 95% CIs for one or both the mustard ED10 and ED50 estimates overlap with the 95% CIs for cashew, celery, egg, hazelnut, lupin, milk, peanut, sesame, walnut and wheat. Thus, while the potency decision is labelled as 'high' for mustard, there is a large level of overlap of EDp estimates between mustard and the foods designated 'medium potency'. The FAO/WHO Expert Committee also noted that the highest discrete doses of mustard in the three studies providing individual data for dose distribution modelling were relatively low (ranging from about 80 mg mustard protein in Morisset et al. (2003) to about 235 mg mustard protein in Figueroa et al. (2005) compared to other food-challenge protocols for common food allergens (ending above 1,000 mg of protein). This low dosing scheme for mustard resulted in a high proportion of right-censored results which could have impacted the resulting dose distribution, particularly in the ED50 range and above when compared to other foods. The NDA Panel agrees with these considerations.

For a dose of mustard protein of 0.26 mg (the LOAEL for objective symptoms in the study by Rancé et al. (2000); Rancé et al. (2001)), it is predicted that between 3% and 4% of the mustard allergic population would react with mild objective symptoms,[7] both in discrete and cumulative dose datasets (i.e. between 0.1 and 9% of the mustard allergic population considering the 95% CI; see Appendix B).

The Panel considers that 0.26 mg of mustard protein can be used as the MOED (LOAEL) for this assessment.

3.5. Margin of exposure (Q3) and estimation of the risk

The MoE between the MOED of mustard protein triggering allergic reactions in mustard-allergic individuals and the maximum amount of mustard protein that could be consumed from emulsifiers manufactured from behenic acid on a single occasion under the proposed conditions of use is calculated as follows:

$$\text{MoE} = \text{MOED} \div \text{maximum intake of mustard protein from emulsifiers manufactured from behenic acid on a single occasion} = 0.26 \text{ mg mustard protein} \div 0.00895475 \text{ mg mustard protein} = 29.$$

This means that mustard allergic individuals should consume 29 times the maximum intake of mustard protein from emulsifiers manufactured from behenic acid on a single occasion (i.e. the highest P95 for adults) to reach MOED.

As described in the protocol (Annex A), an alternative approach to the LOAEL and MoE to estimate the risk of adverse reactions to behenic acid in mustard allergic individuals under the proposed conditions of use is to use all available data from food challenge studies in mustard allergic individuals (rather than the LOAEL from a single study) and the population minimal eliciting dose (EDp) distributions derived from them. Using data from the published EDp distributions (Houben et al., 2020), it is predicted that between 0.2% (discrete dose dataset) and 0.3% (cumulative dose dataset) of the mustard allergic population (between 0.1% to 1%, considering the 95% CI) would react with mild objective symptoms to the maximum intake of mustard protein from emulsifiers manufactured from behenic acid on a single occasion (i.e. 0.00895475 mg mustard protein).

3.6. Uncertainty assessment

Uncertainties related to the exposure

Mustard seed protein was not detected in three batches of behenic acid (LOQ $\leq$ 1 mg/kg mustard seed protein, data submitted in the original application [EFSA NDA Panel, 2016]) and mustard was not detected in two batches of behenic acid (LOQ $\leq$ 2 mg/kg mustard seeds; data submitted by the applicant for the current assessment; Section 3.3.1). Whereas a 26% protein content in mustard seeds has been assumed to recalculate the LOQ of the ELISA for use in the present assessment (LOQ $\leq$ 0.5 mg/kg mustard seed protein), both lower and higher values have been reported in the literature for the mustard seed species used to manufacture behenic acid (*B. juncea* (L.) Czern between 26% and 32%, *B. nigra* (23%); Section 3.3.1). Since mustard seed protein was undetected in all samples of behenic acid tested, the 'true' mustard protein content of behenic acid should be lower than the LOQ of the ELISA used for this assessment.

Overall, the ANS Panel concluded that EFSA intake estimates for E471 could have been underestimated in all exposure scenarios (see complete uncertainty analysis of the intake estimates in Section 3.4.5 of the EFSA ANS Panel, 2017 opinion). In addition, intake estimates for E471 alone could underestimate the combined intake of E470a, E471 and E477. However, the following additional assumptions will lead to an overall overestimation of the exposure in this assessment:

a) assuming the maximum content of behenic acid for all emulsifiers and intended uses, which could range from 25% to 85% depending on the emulsifier and intended use (Section 3.3.2);
b) assuming that all E471 in the market will be manufactured using behenic acid manufactured from mustard seeds;

[7] It is not expected that mustard allergic individuals would react with anaphylaxis to these levels of intake. No cases of anaphylaxis were reported in the studies by Rancé et al. (2000) or Morisset et al. (2003). The only case of anaphylaxis was reported in the study by Figueroa et al. (2005) at a cumulative dose of 156.8 mustard, corresponding to 40.95 mg mustard protein.

c) the extrapolation of the maximum intake estimates of E471 for adults (highest P95 across countries) to all population groups and countries (Section 3.3.3) and.
d) the assumption that daily intakes could be consumed on a single occasion (i.e. in a single meal), considering that the main food categories contributing to the intake of E471 were bread and rolls and fine bakery wares (EFSA ANS Panel, 2017 and Section 3.3.3).

The Panel considers that, overall, the assessment is conservative in relation to the exposure.

Uncertainties related to the hazard characterisation

Few uncertainties have been identified in relation to the hazard characterisation with a clear impact on the risk assessment (i.e. leading to an over- or underestimation of the risk).

Sampling uncertainty, and particularly the exclusion of most sensitive adults from the DBPCFC in the study by Figueroa et al. (2005), could lead to an underestimation of the risk (i.e. patients with most severe symptoms could react to lower doses of mustard protein) when using EDp distributions, but not when using the MoE approach using the LOAEL.

On the other hand, uncertainties related to accuracy and precision of the outcome in human challenge studies (Rancé et al., 2000; Rancé et al., 2001; Morisset et al., 2003), in particular lack of clarity about the protocols used for recording and rating objective/subjective symptoms and the use of SBPCFC data (risk of overdiagnosis if outcome assessors are aware of the food tested), could lead to an overestimation of the risk, both when using the LOAEL and EDp distributions to estimate such risk. This is also the case for uncertainties related to the true protein content in mustard seeds used for the food challenges, which is assumed to be 26% by weight. Higher values have been reported in the literature for the mustard seed spices used in the human studies (*B. juncea* (L.) Czern between 26% and 32%; *S. alba* L. between 32% and 37%; Section 3.3.1), which could lead to an underestimation of the LOAEL in the study by Rancé (the LOAEL could be higher) and to a shift of the EDp distributions for mustard to the left, leading to an overall overestimation of the risk.

Most uncertainties identified in relation to the hazard characterisation might have led to both over- and underestimation of the risk. These include uncertainties related to:

a) the true amount of mustard seeds/powder used for the food challenges. The three human studies available were deemed to be at high risk of bias in relation to the exposure, but the scarce information provided in relation to the mustard protein content in the food challenges and on how/when this was ascertained by analytical methods did not allow to predict the direction of the bias);
b) missing human challenge studies (none published since 2005). Publication bias cannot be assessed.
c) external validity (challenge studies only in France and Spain). It is unclear whether the prevalence of mustard allergy in the general population is higher or lower as compared to other European countries, or whether mustard allergic subjects in these countries have different sensitivities to the exposure.
d) methodological uncertainty in the derivation of EDp using Stacked Parametric Survival methods. As discussed in Section 3.4, there are several limitations in the dataset used to derive EDp distributions for mustard protein (low number of patients, data from SBPCFC included, 10 patients reacting with subjective symptoms and not tested for the highest dose were right censored, 2 patients reacting to the lowest dose tested were left-censored). The direction of the bias that this could introduce in EDp distributions is difficult to assess.

The Panel considers that, overall, the assessment is conservative in relation to the hazard characterisation.

Overall uncertainty

Table 3 provides a summary of the uncertainties in relation to the risk assessment, highlighting the main sources of uncertainty and indicating an estimate of whether the respective source of uncertainty might have led to an over- or underestimation of the exposure or the resulting risk.

Table 3: Summary of the qualitative evaluation of the impact of uncertainties on the risk assessment

Sources of uncertainty	Direction[(a)]
Exposure	
Uncertainties related to the true protein content in mustard seeds used to manufacture behenic acid (assumed to be 26%)	–/+
Mustard seed protein was not detected in 3 batches of behenic acid (LOQ ≤ 1 mg/kg mustard seed protein [EFSA NDA Panel, 2016]) and mustard was not detected in 2 batches of behenic acid (LOQ ≤ 0.5 mg/kg mustard seed protein)	+
Assumed maximum content of behenic acid for all emulsifiers and intended uses (85%)	+
Intake estimates for E471 (EFSA ANS Panel, 2017)	–
Intake of E471 as a proxy for the combined intake of all emulsifiers to be manufactured from behenic acid	–
Assumed that all E471 in the market will be manufactured using behenic acid	+
Extrapolation of maximum intake estimates of E471 (P95) for adults to all population groups and countries	+
Extrapolation of daily intakes to intakes on a single occasion	+
Hazard characterisation	
Uncertainties related to the true amount of mustard seeds/powder used for the food challenges	–/+
Uncertainties related to the true protein content in mustard seeds used for the food challenges (assumed to be 26% by weight)	+
Uncertainties related to accuracy and precision of the outcome (protocols for the recording and rating of objective/subjective symptoms not reported; SBPCFC)	+
Sampling uncertainty (most sensitive adults removed from the food challenge; unclear selection process in children in one study)	–
Uncertainties related to missing human challenge studies (none published since 2005)	–/+
Uncertainties related to external validity (challenge studies only in France and Spain)	–/+
Methodological uncertainty in the derivation of EDp using Stacked Parametric Survival methods	–/+

(a): + = uncertainty with potential to cause over-estimation of exposure/risk; – = uncertainty with potential to cause under-estimation of exposure/risk.

The Panel notes the uncertainties in the risk assessment and considers that, overall, the assessment is conservative, particularly in relation to the exposure assessment.

3.7. Likelihood of adverse reactions in susceptible individuals under the proposed conditions of use (Q4)

To reach a conclusion on the likelihood of adverse reactions to behenic acid in mustard allergic individuals under the conditions of use proposed by the applicant, an informal expert knowledge elicitation (EKE) was conducted among the members of the WG on Food Allergy. WG members were asked to express the above-mentioned likelihood in qualitative (probability term) and quantitative (subjective probability range) terms using as starting point the approximate probability scale recommended for use by the EFSA Scientific Committee (2018). Differences among experts were resolved through discussion and conclusions were reached by consensus, by considering the following:

a) The MoE is 29, several times > 1 (Section 3.5)
b) It is predicted that between 0.1% and 1% of the mustard allergic population would react with mild objective symptoms to the maximum intake of mustard protein from emulsifiers manufactured from behenic acid that could be consumed on a single occasion (i.e. 0.00895475 mg mustard protein) (Section 3.5)
c) Overall, the assessment is conservative, particularly in relation to the exposure assessment (Section 3.6).

Taking into account the evidence available and related uncertainties, the WG on Food Allergy considered that it is extremely unlikely (≤ 1% probability) that oral consumption of emulsifiers manufactured using behenic acid from mustard seeds (i.e. E470a, E471 and E477) would trigger an allergic reaction in susceptible individuals (i.e. mustard-allergic individuals) under the proposed

conditions of use (see [EFSA NDA Panel, 2016] for general specifications, manufacturing process and allergen specifications).

4. Conclusions

Based on the information and data provided by the applicant, and on the food challenge data currently available for mustard allergic individuals, the Panel concludes that it is extremely unlikely (≤ 1% probability) that oral consumption of emulsifiers to be manufactured using behenic acid from mustard seeds (i.e. E470a, E471 and E477) will trigger an allergic reaction in mustard-allergic individuals under the proposed conditions of use.

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Abbreviations

ANS Panel	The EFSA Panel on Food Additives and Nutrient Sources added to Food
bw	Body weight
CI	Confidence interval
DBPCFC	Double-blind placebo-controlled food challenge
ED	Eliciting dose
EDp	Population eliciting dose
EKE	Expert Knowledge Elicitation
ELISA	Enzyme-linked immunosorbent assay
FAF Panel	The EFSA Panel on Food Additives and Flavourings
FAO	Food and Agriculture Organization
IFF	International Flavors and Fragrances
IgE	Immunoglobulin E
LCI	Lower Confidence Interval
LOAEL	Lowest observed adverse effect level
LOQ	Limit of quantification
MED	Minimal eliciting dose
Mintel GNPD	Mintel Global New Products Database
MoE	Margin of exposure
MOED	Minimal observed eliciting dose
NDA Panel	The EFSA Panel on Nutrition, Novel Foods and Food Allergens
NICE	National Institute for Clinical Excellence
NOAEL	No observed adverse effect level

NTP	National Toxicology Program
OHAT	Office of Health Assessment and Translation
Q	Question
QUIPS	Quality in Prognosis studies
P95	Ninety-fifth percentile
RAST	Radioallergosorbent test
RoB	Risk of bias
SBPCFC	Single-blind placebo-controlled food challenge
SC	Scientific Committee
sQ	Sub-question
SPT	Skin prick test
UCI	Upper Confidence Interval
USDA	United States Department of Agriculture
WG	Working Group
WHO	World Health Organization
w/v	Weight-per-volume

Appendix A – Risk of bias tool and appraisal of food challenge studies

A.1. Risk of bias tool used for the appraisal of food challenge studies

A	Selection bias[1]	Rating options
A1	Adequate description of inclusion and exclusion criteria	Yes/No/Unclear/Partially/Not applicable
A2	Adequate methods used to identify and recruit the study population	
A3	Adequate participation in the study by eligible persons (≥ 80%)	
Based on your answers to the above, in your opinion was selection bias present?		Low/unknown-unclear/high risk of bias
B	**Performance bias[2]**	
B1	A clear definition or description of the diagnostic intervention is provided, including a food challenge protocol	Yes/No/Unclear/Partially/Not applicable
B2	The method and setting of the diagnostic intervention is the same for all study participants (those reacting and those not reacting to the food challenge) AND the subjects undergoing the food challenge received the same care and support when receiving the test food and placebo	
B3	Participants were kept 'blind' to the order in which the test food and placebo were administered	
B4	Outcome assessors were kept 'blind' to the order in which the test food and placebo were administered	
Based on your answers to the above, in your opinion was performance bias present?		Low/unknown-unclear/high risk of bias
C	**Attrition bias[3]**	
C1	Adequate participation in the food challenge by eligible persons	Yes/No/Unclear/Partially/Not applicable
C2	Adequate availability of outcome data for eligible persons participating in the food challenge (e.g. results available for all subjects participating in the challenge, not only for those reacting to the challenge)	
C3	Availability of outcome data is the same for the test food and placebo (e.g. results equally available for the test food AND placebo)	
Based on your answers to the above, in your opinion was attrition bias present?		Low/unknown-unclear/high risk of bias
D	**Detection bias – outcome[4]**	
D1	The study had an appropriate length of follow-up (up to 2 h after the food challenge) and subjects were followed for an equal length of time when receiving the test food and when receiving placebo	Yes/No/Unclear/Partially/Not applicable
D2	The study used a precise definition of outcome (clear rules to asses and rate symptoms, clear rules to stop the challenge)	
D3	A valid and reliable method was used to determine the outcome (to assess and rate symptoms)	
D4	Outcome assessors were kept 'blind' to participants' exposure to the intervention and to other important confounding factors	
Based on your answers to the above, in your opinion was detection bias present in relation to the outcome?		Low/unknown-unclear/high risk of bias
E	**Detection bias – exposure[5]**	
E1	The test and placebo foods are well described (origin, type, amount, preparation, etc), so that the amount of allergenic food is reported or it can be estimated/calculated	Yes/No/Unclear/Partially/Not applicable

E2	There is confidence on the amount of food allergen provided on each test session	
E3	There is confidence that all tested subjects received the desired amount of food allergen on each test session	
E4	Initial dose, dose intervals and dose range are appropriate for diagnosis of food allergy/to detect the minimal eliciting dose	
Based on your answers to the above, in your opinion was detection bias present in relation to the exposure?		Low/unknown-unclear/high risk of bias
F	**Overall assessment of internal validity – Are the study results obtained with the food challenge reliable regarding diagnosis of mustard allergy and minimal eliciting dose?**	++/+/−[6]
G	**Overall assessment of external validity – Are the study results externally valid (i.e. generalisable to the whole mustard-allergic population)? Consider participants, interventions, settings, comparisons and outcomes**	++/+/−[6]

1: It applies to the selection of individuals potentially allergic to mustard that are eligible for the food challenge, and not to the participation of eligible subjects to the challenge (evaluated under attrition bias).
2: Systematic differences in the care provided when administering the test food and placebo.
3: Systematic differences between eligible subjects that received and not received the food challenge, and between subjects (and treatments) with available and not available outcome data.
4: Bias in relation to how outcomes are ascertained, diagnosed or verified.
5: Bias in how the exposure is ascertained or verified and in how dose and dose intervals are appropriate for diagnosis of food allergy and for the calculation of the minimal eliciting dose.
6: ++ All or most of the checklist criteria have been fulfilled, where they have not been fulfilled the conclusions are very unlikely to alter, + Some of the checklist criteria have been fulfilled, where they have not been fulfilled or not adequately described, the conclusions are unlikely to alter, − Few or no checklist criteria have been fulfilled and the conclusions are likely or very likely to alter.

A.2. Appraisal of food challenge studies

Figueroa (Figueroa et al., 2005)

A	**Selection bias**	**Rating**	**Rationale for rating**
A1	Adequate description inclusion/exclusion	Partially	Immediate adverse reactions related to mustard ingestion required. No exclusion criteria mentioned
A2	Adequate methods	Yes	Immediate adverse reaction to mustard suggestive of being IgE-mediated + positive SPT to mustard extract
A3	Adequate participation	Yes	Every patient reporting immediate adverse reactions related to mustard ingestion, suggestive of being IgE-mediated, and showing positive SPT to a mustard extract, was recruited
Was selection bias present? RoB:		Low	
B	**Performance bias**	**Rating**	**Rationale for rating**
B1	Clear definition diagnostic intervention	Yes	Food challenges are well described as well as the SPT methodology and IgE assays
B2	Same method and care for test food and placebo	Yes	One challenge was performed with mustard and the other one with placebo with the same increasing dose pattern and time intervals. Both challenges were performed 2 h apart
B3	Blinding of participants	Yes	It was a DBPCFC where the order of the intervention was randomised and tests were done to ensure that patients could not guess whether assigned to test food or placebo
B4	Blinding of assessors	Yes	DBPCFC with order of treatment randomised
Was performance bias present? RoB:		Low	

C	Attrition bias	Rating	Rationale for rating
C1	Adequate participation by eligible persons	No	10/34 (29%) patients did not undergo DBPCFC because of either severe symptoms (n = 4) or denial of consent
C2	Adequate availability of outcome data	Yes	Outcome data available for all participants in the food challenge
C3	Results equally available for the test and placebo	Yes	Outcome data available for all participants for the test food and the placebo
Was attrition bias present? RoB:		High	
D	**Detection bias – outcome**	**Rating**	**Rationale for rating**
D1	Appropriate length of follow-up	Yes	Described in methods
D2	Precise definition of outcome	Unclear	Unclear whether diagnosis was based on subjective or objective symptoms, unclear rules to stop challenge
D3	Valid method for outcome assessment	Unclear	Reference made to guidelines which are not specific on this point
D4	Blinding of outcome assessors	Yes	Clear blinding to the intervention; unclear blinding to other confounding factors (e.g. pre-challenge STP or sIgE)
Was detection bias present in relation to the outcome? RoB:		Unknown/ unclear	
E	**Detection bias – exposure**	**Rating**	**Rationale for rating**
E1	Amount of allergenic food known	Yes	Information is given in the methods section
E2	Amount consistent across sessions	Unclear	Only mentioned that preparation of challenges was done in the allergy laboratory on the same morning
E3	Amount consistent across individuals	Unclear	Unclear how this was ascertained
E4	Initial dose, dose intervals and dose range appropriate for diagnosis/minimal eliciting dose	No	10 subjects reacted with subjective symptoms to different doses below the highest cumulative dose, 10 subjects did not react at the highest cumulative dose, none reacted to the first dose tested
Was detection bias present in relation to the exposure? RoB:		High	
F	**Overall assessment of internal validity**	–	Unclear or high risk of detection bias (exposure and outcome)
G	**Overall assessment of external validity**	–	Patients with most severe symptoms were excluded from the challenge

RoB: Risk of bias.

Morisset (Morisset et al., 2003)			
A	**Selection bias**	**Rating**	**Rationale for rating**
A1	Adequate description inclusion/exclusion	Partially	30 subjects (2 adults) suspected of food allergy based on objective and subjective symptoms suggestive of mustard allergy. Inclusion based on positive SPT (clinical symptoms not sufficient). No exclusion criteria mentioned
A2	Adequate methods	Unclear	Unclear the population from which subjects were recruited (number and characteristics of screened individuals to select participants)
A3	Adequate participation	Unclear	Unclear how many subjects reported symptoms in the interview, how many were interviewed, and from those reporting symptoms, how many underwent the SPT
Was selection bias present? RoB:		Unknown/unclear	

B	Performance bias	Rating	Rationale for rating
B1	Clear definition diagnostic intervention	Partially	Food challenge, SPT methodology and IgE assays described, but no reference to the methodology used to perform the food challenge
B2	Same method and care for test food and placebo	Partially	One challenge was performed with mustard and the other with placebo, with the same increasing dose pattern and time intervals. Both challenges were performed 24 h apart. It is assumed that in the DBPCFC the order was randomised. SBPCFC in 6 subjects (convenience)
B3	Blinding of participants	Yes	DBPCFC in 24 subjects. SBPCFC in 6 subjects (mustard first)
B4	Blinding of assessors	No	It is assumed that patients, not outcome assessors, were kept blind to the intervention in SBPCFC
Was performance bias present? RoB:		Unknown/unclear	
C	**Attrition bias**	**Rating**	**Rationale for rating**
C1	Adequate participation by eligible persons	Yes	All eligible subjects participated in the food challenge
C2	Adequate availability of outcome data	Yes	Outcome data available for all participants
C3	Results equally available for the test and placebo	Partially	No information for the placebo
Was attrition bias present? RoB:		Unknown/unclear	
D	**Detection bias – outcome**	**Rating**	**Rationale for rating**
D1	Appropriate length of follow-up	No	Placebo and mustard challenges performed 24 h apart. No information about the maximum time post-challenge at which symptoms should develop for diagnosis of mustard allergy. An 8-h delayed reaction was considered as diagnostic
D2	Precise definition of outcome	No	Unclear whether diagnosis of mustard allergy was based on objective or subjective symptoms, and whether the severity of symptoms was considered and how. Slight symptoms close to routine irritative signs of mustard testing were considered 'positive' for patient 27, and delayed symptoms for patient 30
D3	Valid method for outcome assessment	Unclear	Unclear how outcome assessment was done
D4	Blinding of outcome assessors	Unclear	Not mentioned. Assumed NOT for SBPCFC
Was detection bias present in relation to the outcome?		High	
E	**Detection bias - exposure**	**Rating**	**Rationale for rating**
E1	Amount of allergenic food known	Yes	Clear description of the test food and placebo. Type (seasoning) origin (*Brassica juncea* seed), content (33.6% of seasoning), masked in Cola, which was used as placebo. 40 mg of seasoning equivalent to 13.5 mg of mustard seeds, roughly equivalent to 0.8 mg of proteins (mustard seasoning is considered to contain 6% of proteins)
E2	Amount consistent across sessions	Unclear	Little information provided about the preparation of the test material and quality control procedures
E3	Amount consistent across individuals	No	No information provided. Seasoning provided by food industry, no analysis done and no information about the allergen context across batches used

E4	Initial dose, dose intervals and dose range appropriate for diagnosis/ minimal eliciting dose	Partially	No individual reacted to the first dose tested, but 3 reacted to the highest cumulative dose and the eliciting dose was not reported for two patients tested with SBPCFC. Unclear whether higher doses could have increased the number of diagnosed patients/ shifted the minimal eliciting dose curve to the left
Was detection bias present in relation to the exposure?		High	
F	**Overall assessment of internal validity**	–	Unclear or high risk of performance and detection bias (exposure and outcome)
G	**Overall assessment of external validity**	–	Selection bias cannot be judged

RoB: Risk of bias.

Rancé (Rancé et al., 2000; Rancé et al., 2001)

A	**Selection bias**	**Rating**	**Rationale for rating**
A1	Adequate description inclusion/exclusion	Yes	3600 SPTs between 1997 and 1999 systematically performed if history of food allergy. 36 consecutive patients with a positive mustard SPT were included. No exclusion criteria mentioned
A2	Adequate methods	Yes	All atopic children attending the hospital as outpatients with a clinical history suggesting food allergy underwent a mustard SPT
A3	Adequate participation	Yes	It can be assumed that all patients seen in the outpatient clinic with a positive mustard SPT within the 2-year time-frame were included, but not indicated if any refused
Was selection bias present? RoB:		Low	
B	**Performance bias**	**Rating**	**Rationale for rating**
B1	Clear definition diagnostic intervention	Yes	Methodology for food challenges, SPT and IgE assays described
B2	Same method and care for test food and placebo	Yes	One challenge was performed with mustard and the other with placebo with the same increasing dose pattern and time intervals. Both challenges were performed 4 h apart
B3	Blinding of participants	No	It was a SBPCFC and participants were able to recognise the taste of mustard. It was indeed not possible to mask the taste of mustard, young children being not able to swallow capsules
B4	Blinding of assessors	Unclear	If participants are not blinded, it is difficult to keep assessors blinded
Was performance bias present? RoB:		High	
C	**Attrition bias**	**Rating**	**Rationale for rating**
C1	Adequate participation by eligible persons	Yes	The 36 eligible children having a positive mustard SPT participated in the challenge
C2	Adequate availability of outcome data	Yes	Outcome data available for all participants
C3	Results equally available for the test and placebo	Yes	Outcome data available for the test food for people reacting and not reacting to the food challenge. No participant reacted to placebo
Was attrition bias present? RoB:		Low	

D	Detection bias – outcome	Rating	Rationale for rating
D1	Appropriate length of follow-up	Yes	Placebo and mustard challenges were performed 4 h apart, and only symptoms developed within a few minutes to 2 h after the last dose were considered for the diagnosis of food allergy
D2	Precise definition of outcome	Unclear	Not mentioned in the paper. General reference to food challenge standards available at the time
D3	Valid method for outcome assessment	Unclear	Not mentioned in the paper. General reference to food challenge standards available at the time
D4	Blinding of outcome assessors	Unclear	Contradictory information about whether the study was a SBPCFC (Rance, 2000) or a DBPCFC (Rance, 2001). No information about blinding of assessors
Was detection bias present in relation to the outcome? RoB:		Unclear/unknown	
E	**Detection bias – exposure**	**Rating**	**Rationale for rating**
E1	Amount of allergenic food known	Partially	The placebo is not described. Fresh extracts (mustard seed powder including *Sinapis alba* and *Brassica juncea*, 1: 10 w/v) were used for SPT. It is assumed that the food challenge was conducted with the same mustard powder
E2	Amount consistent across sessions	Partially	The amount of food provided on each test session is well described but no information is available on how this was verified. No food analysis is mentioned
E3	Amount consistent across individuals	No	If fresh extracts were used also for the food challenge, it could be assumed they were made daily. No information about ensuring the same amount of food allergen in all test sessions is provided
E4	Initial dose, dose intervals and dose range appropriate for diagnosis/ minimal eliciting dose	Partially	Authors report that the highest dose tested (500 mg) was higher than the dose habitually consumed and sufficient. Dose-intervals seem appropriate. However, two patients reacted with urticaria, i.e. an objective symptom, to the first dose tested (1 mg)
Was detection bias present in relation to the exposure? RoB:		High	
F	**Overall assessment of internal validity**	–	Unclear or high risk of performance and detection bias (exposure and outcome)
G	**Overall assessment of external validity**	+	Low risk of selection bias. Only children

RoB: Risk of bias.

A.3. Risk of bias heatmap

	Risk of bias domain	Figueroa	Morisset	Rancé
A	Selection bias	Low risk of bias	Unclear/unknown risk of bias	Low risk of bias
B	Performance bias	Low risk of bias	Unclear/unknown risk of bias	High risk of bias
C	Attrition bias	High risk of bias	Unclear/unknown risk of bias	Low risk of bias
D	Detection bias-outcome	Unclear/unknown risk of bias	High risk of bias	Unclear/unknown risk of bias
E	Detection bias-exposure	High risk of bias	High risk of bias	High risk of bias
F	**Overall internal validity**	–	–	–
G	**Overall external validity**	–	–	+

Appendix B – Population eliciting dose (ED) values from model averaged population threshold dose distributions for mustard, in mg total mustard protein with 95% lower (LCI) and upper confidence intervals (UCI)

Discrete dose datasets			
	Value	**LCI**	**UCI**
ED00.1	0.003	0.001	0.3
ED00.2	0.008	0.001	0.5
ED00.3	0.01	0.002	0.6
ED00.4	0.02	0.003	0.7
ED00.5	0.03	0.003	0.7
ED00.6	0.03	0.004	0.8
ED00.7	0.04	0.005	0.9
ED00.8	0.05	0.006	1.0
ED00.9	0.06	0.008	1.0
ED01.0	0.07	0.009	1.1
ED02.0	0.1	0.03	1.8
ED03.0	0.2	0.05	2.4
ED04.0	0.3	0.07	3.0
ED05.0	0.4	0.1	3.6
ED06.0	0.6	0.1	4.3
ED07.0	0.7	0.2	5.0
ED08.0	0.8	0.2	5.7
ED09.0	0.9	0.2	6.5
ED10.0	1.1	0.3	7.3
Cumulative dose datasets			
	Value	**LCI**	**UCI**
ED00.1	0.002	0.000	0.2
ED00.2	0.005	0.001	0.3
ED00.3	0.008	0.002	0.4
ED00.4	0.01	0.002	0.5
ED00.5	0.02	0.003	0.6
ED00.6	0.02	0.003	0.6
ED00.7	0.03	0.004	0.7
ED00.8	0.03	0.005	0.8
ED00.9	0.04	0.005	0.8
ED01.0	0.05	0.006	0.9
ED02.0	0.1	0.02	1.6
ED03.0	0.2	0.04	2.3
ED04.0	0.3	0.06	3.1
ED05.0	0.5	0.09	3.9
ED06.0	0.6	0.1	4.8
ED07.0	0.8	0.2	5.8
ED08.0	0.9	0.2	6.9
ED09.0	1.1	0.2	8.0
ED10.0	1.3	0.3	9.2

Reproduced and adapted from Houben et al. (2020) – Supplemental material Table S1 and S2.
Abbreviations: ED, eliciting dose; LCI, lower confidence interval; UCI, upper confidence interval. ED00.1 = predicted ED for the 0.1% of the mustard allergic population: ED01.0 = predicted ED for the 1% of the mustard allergic population.

Annexes

The Annex can be found in the online version of this output, under the Section 'Supporting information', at: https://efsa.onlinelibrary.wiley.com/doi/full/10.2903/j.efsa.2023.8240#support-information-section

Annex A: Protocol for the re-evaluation of behenic acid from mustard seeds to be used in the manufacturing of certain emulsifiers pursuant to Article 21(2) of Regulation (EU) No 1169/2011 – for permanent exemption from labelling

Annex B: Technical report: Outcome of the public consultation on the draft scientific opinion on the re-evaluation of behenic acid from mustard seeds to be used in the manufacturing of certain emulsifiers pursuant to Article 21(2) of Regulation (EU) No 1169/2011 – for permanent exemption from labelling