Scientific Opinion 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

EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA)

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Scientific Opinion 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

EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA),
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Abstract

Following a request from the European Commission, the Panel on Dietetic Products, Nutrition and Allergies (NDA) was asked to deliver a scientific opinion 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. Behenic acid is produced from rapeseed–mustard seed variants prevalent in India, namely *Brassica juncea* (L.) Czern. (oriental mustard), *Brassica rapa* (L.) (brown/yellow Sarson or Toria), *Brassica napus* (L.) (rapeseed) and *Brassica nigra* (L.) W.D.J.Koch (black mustard). No human or animal allergenicity data were provided by the applicant for either behenic acid or the emulsifiers manufactured from behenic acid. Based on enzyme-linked immunosorbent assay (ELISA) data, the Panel considers that proteins and peptides may not be carried over into behenic acid after the two distillation steps reported in the manufacturing process in amounts beyond 1 mg/kg. The Panel notes that the maximum amount of mustard protein that could be consumed from emulsifiers manufactured from behenic acid (E 470a, E 471 and E 477) on a single occasion by an adult under the proposed conditions of use would be around 0.00119 mg, which is about 1,000 times lower than the protein doses reported to trigger allergic reactions in mustard-allergic individuals (around 1 mg). On the basis of the data presented, the Panel concludes that oral consumption of emulsifiers manufactured using behenic acid from mustard seeds (E 470a, E 471 and E 477) are unlikely to trigger an allergic reaction in susceptible individuals (i.e. mustard-allergic individuals) under the proposed conditions of use.

Keywords: behenic acid, emulsifiers, mustard, rapeseed, allergy, labelling exemption

Requestor: European Commission

Question number: EFSA-Q-2015-00844

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Summary

Following a request from the European Commission, the Panel on Dietetic Products, Nutrition and Allergies (NDA) was asked to deliver a scientific opinion 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.

Taking into account the well-documented reports of allergic individuals reacting to mustard, it is appropriate for the Panel to assess the likelihood of adverse reactions in allergic individuals consuming products derived from mustard.

The food allergen-derived preparation for which the exemption from labelling is requested is behenic acid. The food allergen-derived foodstuffs are emulsifiers (i.e. E 470a, E 471, E 472 (a, b, c and e), E 475 and E 477), which could be manufactured from behenic acid and be used as food additives.

Behenic acid is produced from rapeseed–mustard seed variants prevalent in India, namely *Brassica juncea* (L.) Czern. (oriental mustard), *Brassica rapa* (L.) (brown/yellow Sarson or Toria), *Brassica napus* (L.) (rapeseed) and *Brassica nigra* (L.) W.D.J.Koch (black mustard).

Sin a 1, Sin a 2, Sin a 3 and Sin a 4 are mustard allergens present in *Sinapis alba* (yellow mustard), which is not used for the production of behenic acid, whereas the relevant mustard allergen in *B. juncea* (oriental mustard) is Bra j 1. The major allergen of rapeseed (*B. napus*) is Bra n 1. The Panel notes that homologous proteins of Sin a 1 and Bra n 1 are also potentially allergenic for mustard-sensitive individuals.

A detailed description of the manufacturing process of behenic acid and the emulsifiers thereof was provided by the applicant. Following a request by the European Food Safety Authority (EFSA), 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 Panel considers that the presence of mustard proteins in behenic acid cannot be excluded on the basis of the manufacturing process only.

The Panel notes that the maximum use level estimated by the applicant for adults for the combined intake of E 470a, E 471 and E 477 (1,400 mg/day, assumed to be on a single occasion in a conservative approach) is similar to that reported in the literature for E 475 (1,174 mg), and can be considered the highest estimate for all population groups. The Panel also notes that E 472a–e and E 475, which are currently not manufactured with behenic acid, have not been considered by the applicant in the intake estimate.

No human or animal allergenicity data were provided by the applicant for either behenic acid or the emulsifiers manufactured from behenic acid.

Three different batches of behenic acid were tested using an enzyme-linked immunosorbent assay (ELISA) test for mustard seed proteins with a limit of quantification (LOQ) of 1 mg/kg. The assay was developed using seed proteins from *S. alba* and *B. nigra*. The assay has been shown to detect seed proteins from *S. alba* and *B. nigra*, but also from *B. juncea*, using positive standards with known amounts of seed proteins from these three species of mustard. The Panel considers that the assay was appropriate for testing the presence of residual mustard allergens in samples of behenic acid. Mustard allergens were not detected in any of the batches. The results of a passive sensitisation histamine release test (HR-test) applied to six batches of behenic acid provided only limited evidence that residual mustard in behenic acid may be < 0.03 mg/kg.

Considering that the highest amount of emulsifiers ingested per person on a single occasion is 1,400 mg for adults, that behenic acid constitutes 25–85% of the final emulsifier, and that the typical amount of an emulsifier in a food item is maximum 1% on a weight basis, the applicant calculated the maximum amount of mustard protein that could be consumed from emulsifiers manufactured from behenic acid on a single occasion by an adult using the limit of detection (LOD) of 0.03 mg/kg from the passive sensitisation HR-test. However, the Panel considers that using the LOQ of 1 mg/kg from the ELISA assay would be more appropriate, as follows:

$$(1{,}400\,\text{mg} \times 85 \times 1\,\text{mg/kg}) : (100 \times 1{,}000{,}000) = 0.00119\,\text{mg mustard proteins.}$$

The Panel considers that mustard allergens may not be carried over into behenic acid after the two distillation steps reported in the manufacturing process in amounts beyond 1 mg/kg. No human or animal data on the allergenicity of either behenic acid or the emulsifiers manufactured thereof *in vivo* were provided by the applicant. The Panel notes, however, that the maximum amount of mustard

protein that could be consumed from emulsifiers manufactured from behenic acid (E 470a, E 471 and E 477) on a single occasion by an adult under the proposed conditions of use would be around 0.00119 mg, which is about 1,000 times lower than the protein doses reported to trigger allergic reactions in mustard-allergic individuals (around 1 mg).

On the basis of the data presented, the Panel concludes that oral consumption of emulsifiers (E 470a, E 471 and E 477) manufactured using behenic acid from mustard seeds is unlikely to trigger an allergic reaction in susceptible individuals (i.e. mustard-allergic individuals) under the proposed conditions of use.

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1. Introduction

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

1.1.1. Background

Annex II to Regulation (EU) No 1169/2011 (hereafter 'the Regulation') includes an European Union (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 indicated. This list has been established on the basis of the scientific opinions of the European Food Safety Authority (EFSA).[1] According to the latter, those substances are considered as part of the most common food allergens and there is sufficient evidence to support their inclusion into the list.

In order to ensure better information to consumers and to take account of the most recent scientific progress and technical knowledge, Article 21 paragraph 2 of the Regulation requires the Commission to systemically re-examine, and where necessary, update the list in Annex II, in accordance with Article 51 of the Regulation.

Pursuant to Article 5 of the same legislation, any Union measure in the field of food information law which is likely to have an effect on public health shall be adopted after consultation of EFSA on the basis of Article 29 of Regulation (EC) No 178/2002[2]. The update of the list may also consist in the deletion of food allergens for which it has been scientifically established that it is impossible to cause adverse reactions. To this end, the interested parties may communicate to the Commission studies establishing that certain products derived from substances listed in Annex II are unlikely to trigger adverse reactions in individuals.

The EFSA's Panel on Dietetic Products, Nutrition and Allergies (NDA) had delivered a scientific opinion on scientific and technical guidance for the preparation and presentation of such applications. It is confirmed that this request is in line with this guidance.[3]

1.1.2. Terms of Reference

In accordance with Article 29 (1) (a) of Regulation (EC) No 178/2002, the European Commission requests the EFSA to evaluate the scientific data submitted by the *DuPont Nutrition Biosciences Aps* in the context of the re-examination of the Annex II to Regulation (EU) No 1169/2011 as requested in Article 21(2) of the latter.

EFSA is requested to issue an opinion on the information provided, and particularly to consider the likelihood of adverse reactions triggered in susceptible individuals by the consumption of the following ingredients/substances used under the conditions specified by the applicant: behenic acid used in the manufacturing of emulsifiers, namely,

E 470a: sodium, potassium and calcium salts of fatty acids
E 471: mono- and diglycerides of fatty acids
E 477: propane-1,2-diol esters of fatty acids

The following emulsifiers are currently not manufactured with behenic acid, and are therefore not included in the calculation of intake. However, new applications requiring the technological properties of an emulsifier made with behenic acid may evolve.

E 472a: acetic acid esters of mono- and diglycerides of fatty acids
E 472b: lactic acid esters of mono- and diglycerides of fatty acids
E 472c: citric acid esters of mono- and diglycerides of fatty acids
E 472e: mono- and diacetyl tartaric acid esters of mono- and diglycerides of fatty acids
E 475: polyglycerol esters of fatty acids

[1] http://www.efsa.europa.eu/EFSA/Scientific_Opinion/opinion_nda_04_en1,1.pdf
[2] 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.
[3] http://www.efsa.europa.eu/en/efsajournal/doc/3417.pdf

2. Data and methodologies

2.1. Data

Notification from DuPont Nutrition Biosciences Aps on behenic acid to be used in the manufacturing of emulsifiers pursuant to Article 21(2) of Regulation (EU) No 1169/2011 for permanent exemption from labelling, presented in a common and structured format as outlined in the Guidance on the preparation and presentation of applications pursuant to Article 6 Paragraph 11 of Directive 2000/13/EC, as amended.[4] As outlined in that guidance document, it is the duty of the applicant to provide all pertinent scientific data in the application (published and unpublished, data in favour and not in favour) relative to the assessment of the likelihood of adverse reactions being triggered in sensitive individuals by the oral consumption of the food allergen-derived preparation/foodstuff(s) under the proposed conditions of use.

2.2. Methodologies

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 pursuant to Article 6 Paragraph 11 of Directive 2000/13/EC, as amended.[4]

3. Assessment

3.1. Introduction

The mustard plant belongs to the Brassicaceae (formerly Cruciferae) family. White/yellow (*Sinapis alba* (L.), black (*Brassica nigra* (L.) W.D.J.Koch) and brown/oriental mustard (*Brassica juncea* (L.) Czern.) are the main types of mustard seeds used in cuisine and food processing. Mustard powder commercially available is usually a mixture of ground white and black mustard seeds. White and brown seeds are blended to make the English style mustard. White mustard seeds are the main ingredient in North American mustard, while the brown seeds are mainly used in Europe and China. Black mustard is mostly used in Indian cuisine. Mustard oil is also widely used as an edible oil and as a flavouring agent in India (EFSA NDA Panel, 2014).

Data on the prevalence of mustard sensitisation, clinical mustard allergy and the incidence of severe allergic reactions to mustard in the general population are lacking. However, the potential severity of mustard allergy has been described by several authors. Anaphylactic reactions have been reported in 2% of children and in up to 48% of adults with confirmed mustard allergy (EFSA NDA Panel, 2014).

Taking into account the well-documented reports of allergic individuals reacting to mustard, it is appropriate for the Panel to assess the likelihood of adverse reactions in allergic individuals consuming products derived from mustard.

The present opinion is based on 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 can be used for the synthesis of several emulsifiers (i.e. E 470a, E 471, E 472 (a, b, c and e), E 475 and E 477). Emulsifiers are food additives permitted for use in a number of food categories.

3.2. Food allergen-derived preparation

The food allergen-derived preparation for which the exemption from labelling is requested is behenic acid. Behenic acid is a fraction of fully hydrogenated refined mustard seed oil (a product of mustard).

The majority of behenic acid used for the manufacture of food emulsifiers is imported from India. It is obtained from refined, bleached and deodorised oil of rapeseed or mustard origin, which can include oil from the mustard strains *B. juncea* (L.) Czern. (oriental mustard) and *B. nigra* (L.) W.D.J.Koch

[4] EFSA NDA Panel (EFSA Panel on Dietetic Products, Nutrition and Allergies), 2013. Guidance on the preparation and presentation of applications pursuant to Article 6 Paragraph 11 of Directive 2000/13/EC, as amended. EFSA Journal 2013; 11(10): 3417, 17 pp. doi:10.2903/j.efsa.2013.3417

(black mustard), but also from the rapeseed strains *Brassica rapa* (L.) (brown/yellow Sarson or Toria) and *Brassica napus* (L.) (rapeseed). The Panel notes that both mustard and rapeseed share the presence of allergens belonging to the 2S albumin family, which is a complex protein fraction characterised by multiple isoforms.

3.3. Conditions of use

The applicant specifies that behenic acid will not be consumed as such, but rather used in the manufacturing of the following emulsifiers:

E 470a: sodium, potassium and calcium salts of fatty acids
E 471: mono- and diglycerides of fatty acids
E 477: propane-1,2-diol esters of fatty acids.

The applicant also specifies that other emulsifiers could be manufactured in the future by using behenic acid, such as:

E 472a: mono- and diglycerides of fatty acids esterified with acetic acid
E 472b: mono- and diglycerides of fatty acids esterified with lactic acid
E 472c: mono- and diglycerides of fatty acids esterified with citric acid
E 472e: mono- and diglycerides of fatty acids esterified with mono- and diacetyltartaric acid
E 475: polyglycerol esters of fatty acids.

3.4. Anticipated intake/exposure

The emulsifiers made with behenic acid are permitted for use in a number of food categories. According to the applicant, the final content of behenic acid in the emulsifier is 25–85%, depending on the type of emulsifier manufactured, and the typical amount of an emulsifier in a food item is 1% maximum on a weight basis.

The applicant claims that, although E 470a, E 471 and E 477 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.

E 470a and E 471 belong to the group I additives according to Regulation (EC) No 1129/2011[5] and may be used in a wide range of food categories at *quantum satis* level. E 470a is predominantly used in edible ice creams with a typical use level of 500 mg/kg. E 471 is mostly used in fat and oil emulsions including spreads and liquid emulsions at typical use levels of 5,000 mg/kg, and in decorations, coatings and fillings, except fruit based fillings, at typical use levels of 7,500 mg/kg.

E 477 is used according to maximum and typical use levels depicted in Table 1.

Table 1: Maximum and typical use levels of E 477

Food category	Maximum use levels	Typical use level
Fat and oil emulsions including spreads and liquid emulsions	Only fat emulsions for baking purposes: 10,000 mg/kg	5,000 mg/kg
Fine bakery wares	5,000 mg/kg	2,500 mg/kg

A rough estimate of the daily combined dietary exposure to E 470a, E 471 and E 477 has been calculated by the applicant using the Food Additive Intakes Model. This approach is based on the assumption 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 results for different age groups according to use level are depicted in Table 2.

[5] Commission Regulation (EU) No 1129/2011 of 11 November 2011 amending Annex II to Regulation (EC) No 1333/2008 of the European Parliament and of the Council by establishing a Union list of food additives. OJ L 295, pp. 1–177.

Table 2: Combined intake of E 470a, E 471 and E 477 for different age groups according to use level

	Total estimated exposure (mg/kg bw per day)							
	Maximum permitted levels				Use levels			
	Range for mean across dietary surveys		Range for highest level across dietary surveys		Range for mean across dietary surveys		Range for high level across dietary surveys	
	Min	Max	Min	Max	Min	Max	Min	Max
Toddlers (1 to < 3 years)	2.56	29.53	8.42	84.12	2.01	11.28	7.86	25.49
Children (3 to < 10 years)	6.85	24.67	11.56	63.01	4.32	11.42	10.46	25.87
Adolescents (10 to <18 years)	3.53	12.58	6.76	29.02	1.84	6.98	6.10	15.17
Adults (18 to < 65 years)	1.82	7.16	4.99	20.49	1.36	3.57	4.59	9.65
Elderly (65 to < 75 years)	2.02	7.14	4.76	23.04	1.10	4.56	3.66	9.64

bw: body weight.

The estimated combined intake of E 470a, E 471 and E 477 at the maximum permitted levels could lead to a maximum total exposure of 84 mg/kg body weight (bw) per day for toddlers and 20 mg/kg bw per day for adults. Using a reference body weight 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, it could be assumed that these doses could be consumed on a single occasion. The Panel notes that the intake for these population subgroups with the maximum use levels is much lower, i.e. 25.5 mg/kg bw per day for toddlers and 9.6 mg/kg bw per day for adults, respectively (Table 2).

It has been reported (Vin et al., 2013) that the highest tier 3 estimates of daily dietary intake of E 475 in France, Ireland, Italy and the United Kingdom showed a 97.5th percentile intake of 97.8 mg/kg bw per day in children and of 9.5 mg/kg bw per day in adults, while the median exposure was estimated to be 7.1 and 3.3 mg/kg bw per day, respectively. Using a reference body weight of 12 kg for toddlers and of 70 kg for adults, the highest amount ingested per person per day for the 97.5th percentile would be 1,174 mg for children and 665 mg for adults.

The Panel notes that the maximum use level estimated for adults by the applicant for the combined intake of E 470a, E 471 and E 477 of 1,400 mg is similar to that reported in the literature for E 475, and can be considered the highest estimate for all population groups. The Panel also notes that E 472a–e and E 475, which are currently not manufactured with behenic acid, have not been considered by the applicant in the intake estimate.

3.5. Characteristics of the food allergen-derived preparation/foodstuff(s)

3.5.1. Food allergen-derived preparation (behenic acid)

3.5.1.1. General specifications

Behenic acid is produced from rapeseed–mustard seed variants prevalent in India, namely *B. juncea* (L.) Czern. (oriental mustard), *B. rapa* (L.) (brown/yellow Sarson or Toria), *B. napus* (L.) (rapeseed) and *B. nigra* (L.) W.D.J.Koch (black mustard).

Behenic acid (docosanoic acid; $C_{22}H_{44}O_2$, C22:0) (CAS No: 112-85-6) is produced as a light solid with a minimum 85% purity and insoluble in water. The chemical and physical data are reported in Table 3.

Table 3: Chemical and physical data for behenic acid

Chemical and physical properties	Unit	Specific range	Analytical methods
Acidic value	mg KOH/g	162–168	ISO 660, 1996
Iodine value	g/100 g	Max. 2	ISO 3961, 1996
Nickel	%	Max. 0.1	
Fatty acid distribution	%		Gas chromatography ISO 5508, 1990/5509, 1978
≤ C18		Max. 5	
C20		Max. 12	
C22:0 behenic acid		Min. 85	
C22:1 erucic acid		Max. 0.5	
> C22		Max. 4	
Boiling point	°C	> 270[(a)]	
Melting point	°C	Approx. 75[(b)]	

ISO: International Organization for Standardization.
(a): The boiling point of pure behenic acid is 306°C.
(b): The melting point of pure behenic acid is 80°C.

The product, analysed by gas chromatography, is also shown to contain other fatty acid lower homologues (≤ C_{18}, max 5%), such as stearic acid (octadecanoic acid; $C_{18}H_{36}O_2$), and arachidic acid (eicosanoic acid, $C_{20}H_{40}O_2$ max 12%), as well as higher homologues (> C_{22}, max 4%).

3.5.1.2. Manufacturing process

Rapeseed–mustard seeds are crushed in an expeller at the oil mill and the crude oil obtained is refined, bleached and deodorised at the refinery. The fully refined oil is hydrolysed under high pressure steam at high temperature to obtain the free fatty acids. The monounsaturated erucic acid ((*Z*)–docos-13-enoic acid; $C_{22}H_{42}O_2$, C22:1) is separated by fractional distillation under vacuum and hydrogenated using a nickel catalyst under high pressure and heating conditions to produce behenic acid, which is then purified by distillation under vacuum.

The manufacturing process conforms to the British Retail Consortium (BRC) Global Standard of Food Safety (Issue 6, July 2011) and the ISO 9001:2008 Management Standards.

The Panel notes that, following a request by EFSA, 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 Panel considers that the presence of mustard proteins in behenic acid cannot be excluded on the basis of the manufacturing process only.

3.5.1.3. Allergen specifications

The applicant states that, since behenic acid is made in part from mustard seeds, relevant allergens are the mustard allergens Sin a 1, Sin a 2, Sin a 3, Sin a 4 and Bra j 1. The Panel notes that Sin a 1, Sin a 2, Sin a 3 and Sin a 4 are mustard allergens present in *S. alba* (yellow mustard), which is not used for the production of behenic acid, whereas the relevant mustard allergen in *B. juncea* (oriental mustard) is Bra j 1. However, Bra j 1 is a 2S seed storage albumin similar to Sin a 1 in structure and amino acid composition (González de la Peña et al., 1991), with an identity of 86% and a sequence similarity of 91%. Bra j 1 and Sin a 1 have also been reported to share a homologous epitope, so that antibodies reacting to one type of mustard may also react to other types (Monsalve et al., 1993).

The major allergen of rapeseed (*B. napus*), Bra n 1 (Monsalve et al., 1997), is a close homologue of Sin a 1, with 92% identity and 94% sequence similarity. The antigenic properties of Bra n 1 were studied in comparison with Sin a 1 by using sera from individuals sensitised to mustard and rapeseed proteins, showing that these two proteins share common antigenic and allergenic determinants. Cross-reactivity between the proteins of rape and mustard seeds has been reported (Meding, 1985; Widstrom and Johansson, 1986; Monreal et al., 1992).

The Panel notes that homologous proteins of Sin a 1 and Bra n 1 are also potentially allergenic for mustard-sensitive individuals.

3.5.1.4. Stability

No stability data has been provided by the applicant on the food allergen-derived preparation (behenic acid).

3.5.2. Food allergen-derived foodstuffs (emulsifiers)

3.5.2.1. General specifications

The specifications for each emulsifier identified by its E number (E 470a, E 471 and E 477) are given in Regulation 231/2012 laying down the specifications for food additives listed in Annexes II and III to Regulation (EC) No 1333/2008 of the European Parliament and of the Council.

3.5.2.2. Manufacturing process

The Panel notes that in foods, emulsifiers are widely used as additives in a variety of food products to control and improve texture. Glycerol and oils or fats (triglycerides) are the raw materials used for the production of mono- and diglycerides. The oils and fats employed in emulsifier production are widely used throughout the food industry, including but not limited to palm, sunflower, rapeseed and soy. As an alternative to triglycerides, the corresponding fatty acids can also be used. Monoglycerides can be further esterified with acids, i.e. tartaric acid, lactic acid, citric acid or acetic acid, to produce other emulsifiers with a range of functional properties. According to the applicant, other emulsifiers for food use that can be based on behenic acid are polyglycerol esters and propylene glycol esters.

The functional properties of emulsifiers can be controlled through the raw materials used. An emulsifier made from behenic acid can, according to the applicant, be used for example in food products to control fat crystallisation, which is important in specific products where recipes have been developed to avoid *trans*-fatty acids, such as vegetable spreads.

The applicant states that, depending on the type of emulsifier manufactured, the final content of behenic acid in the emulsifier is 25–85%. The applicant also states that the manufacturing process conforms to the BRC Global Standard of Food Safety (Issue 6, July 2011) and the ISO 9001: 2008 Management standards.

3.5.2.3. Allergen specifications

No data was provided by the applicant on any emulsifier.

3.5.2.4. Stability

Stability of the emulsifiers was not considered by the applicant.

3.6. Scientific data on allergenicity

3.6.1. Human intervention studies

No human intervention studies on the food allergen-derived preparation (behenic acid) or the food allergen-derived foodstuff(s) (or the emulsifiers) have been provided.

3.6.2. Human observational studies

No human observational studies (e.g. case reports) of allergic reactions to the food allergen-derived preparation (behenic acid) or the food allergen-derived foodstuff(s) (or the emulsifiers) have been provided.

3.6.3. Animal studies

No animal studies with the food allergen-derived preparation (behenic acid) or the food allergen-derived foodstuff(s) (the emulsifiers) have been provided.

3.6.4. *In vitro* studies on the food allergen-derived preparation (behenic acid)

3.6.4.1. ELISA assays

The applicant states that two samples of behenic acid of mustard origin were tested with an ELISA assay for residual mustard allergens. The limit of detection (LOD) is reported to be 2.5 mg/kg. The method for protein extraction from the oil is well described. However, the antibodies were raised

against a generic mustard allergen which was not better specified, and therefore the Panel did not consider the results of this test in the context of this application.

Three different batches of behenic acid were tested using an ELISA test for mustard seed protein with a limit of quantification (LOQ) of 1 mg/kg. The assay was developed using seed proteins from *S. alba* and *B. nigra*. The assay has been shown to detect seed proteins from *S. alba* and *B. nigra*, but also from *B. juncea*, by using positive standards with known amounts of seed proteins from these three species of mustard. The Panel considers that, although the method for protein extraction from the oil is not specified in the application (claimed as proprietary by the company manufacturing the ELISA test), the information provided on the method of analysis is sufficient to conclude that this assay is appropriate for testing the presence of residual mustard allergens in behenic acid samples complying with the specifications given in Section 3.5.1. Mustard allergens were not detected in any of the batches.

3.6.4.2. Passive sensitisation histamine release test (HR-test)

The biological activity of allergens can also be determined by their efficacy to activate passively sensitised stripped basophils by measuring release of histamine from the sensitised cells. The Panel notes that the results are dependent on the serum used for passive sensitisation of the stripped basophils. Thus, the serum should contain immunoglobulin E (IgE) in high concentrations against the relevant allergens in the extract.

Among 37 sera screened for mustard reactivity, the applicant reports that three sera fulfilled the criteria for sensitising capacity (releasing $>$ 10 ng histamine/mL at a concentration of 1 ng mustard standard extract/mL). Buffy coats used for passive sensitisation were collected at the Blood Bank (National University Hospital of Copenhagen). Since the Blood Bank has a general ethical approval to hand out buffy coats making sure that the blood donors are anonymous, the applicant refers that it was not possible to characterise blood donors regarding their clinical history of mustard allergy.

The study plan included testing by passive sensitisation of stripped basophils using sera with high reactivity against mustard and subsequent challenge of the passively sensitised basophils with a freshly made extract from *S. alba* (L.) (yellow mustard) seeds. Results are expressed as the release of histamine in ng/mL and the biological activity is expressed as the lowest concentration of test material inducing $>$ 10 ng histamine release/mL. The method is used routinely and validated by an accredited laboratory according to EN ISO/IEC 17025: 2005, and has an in-house assay for measuring histamine release from basophils obtained from blood bank buffy coat.

The test was applied to six different batches of behenic acid. The LOD was 30 pg dry mustard seed/mg sample, which is equal to 0.03 mg/kg. Mustard allergens were not detected in any of the batches. The applicant concluded that the behenic acid has less than 0.03 mg/kg mustard residue and that the content of the actual mustard protein is even less, since the normal protein content of mustard is about 26% (Remington, 2013).

The Panel notes that behenic acid is produced from *B. juncea/B. nigra* and not from *S. alba* seeds, and that the major mustard allergen in *B. juncea* seeds is Bra j 1, whereas in *S. alba* seeds are Sin a 1, Sin a 2, Sin a 3 and Sin a 4. However, the Panel also notes that Bra j 1 and Sin a 1 have a similar primary structure and share a homologous epitope (González de la Peña et al., 1991; Monsalve et al., 1993, 2001). Therefore, the Panel considers that, since the reference material used in this study was not the most appropriate for testing the allergenicity of behenic acid *in vitro*, the results of the HR-test provide only limited evidence that residual mustard in behenic acid may be $<$ 0.03 mg/kg.

3.7. Exposure to the food allergen (mustard)

Considering that the highest amount of emulsifiers ingested per person on a single occasion is 1,400 mg for adults (Section 3.4), that behenic acid constitutes 25–85% of the final emulsifier, and that the typical amount of an emulsifier in a food item is maximum 1% on a weight basis, the applicant calculated the maximum amount of mustard protein that could be consumed from emulsifiers manufactured from behenic acid on a single occasion by an adult using the LOD of 0.03 mg/kg from the passive sensitisation HR-test (Section 3.6.4). However, the Panel considers that using the LOQ of 1 mg/kg from the ELISA assay would be more appropriate, as follows:

$$(1{,}400\,\text{mg} \times 85 \times 1\,\text{mg/kg}) : (100 \times 1{,}000{,}000) = 0.00119\,\text{mg mustard proteins.}$$

The Panel notes that the maximum amount of mustard protein that could be consumed from emulsifiers manufactured from behenic acid on a single occasion by an adult under the proposed

conditions of use would be around 0.00119 mg. The Panel also notes that protein doses triggering allergic reactions in mustard-allergic individuals have been reported to be are around 1 mg (EFSA NDA Panel, 2014).

4. Conclusions

The Panel considers that mustard allergens may not be carried over into behenic acid after the two distillation steps reported in the manufacturing process in amounts beyond 1 mg/kg. No human or animal data on the allergenicity of either behenic acid or the emulsifiers manufactured thereof *in vivo* were provided by the applicant. The Panel notes, however, that the maximum amount of mustard protein that could be consumed from emulsifiers manufactured from behenic acid (E 470a, E 471 and E 477) on a single occasion by an adult under the proposed conditions of use would be around 0.00119 mg, which is about 1,000 times lower than the protein doses reported to trigger allergic reactions in mustard-allergic individuals (around 1 mg).

On the basis of the data presented, the Panel concludes that oral consumption of emulsifiers (E 470a, E 471 and E 477) manufactured using behenic acid from mustard seeds is unlikely to trigger an allergic reaction in susceptible individuals (i.e. mustard-allergic individuals) under the proposed conditions of use.

Documentation provided to EFSA

1) Notification on behenic acid from mustard seed protein to be used in the manufacturing of emulsifiers E 470a, E 471, E 472, E 475 and E 477 pursuant to Article 21(2) of Regulation (EU) No 1169/2011 – for permanent exemption from labelling. August 2015. Submitted by DuPont Nutrition Biosciences Aps.
2) Additional information submitted by the applicant upon EFSA's request on 20 September 2016.

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Abbreviations

BRC	British retail consortium
bw	body weight
CAS	Chemical Abstracts Service

ELISA	enzyme-linked immunosorbent assay
HR-test	histamine release test
IgE	immunoglobulin E
ISO	International Organization for Standardization
LOD	limit of detection
LOQ	limit of quantification
NDA	Panel on Dietetic Products, Nutrition and Allergies