SCIENTIFIC OPINION

EFSA Journal

ADOPTED: 24 November 2021

doi: 10.2903/j.efsa.2022.6998

Safety of hydrothermally treated kernels from edible *Jatropha curcas* L. (Chuta) as a novel food pursuant to Regulation (EU) 2015/2283

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Abstract

Following a request from the European Commission, the EFSA Panel on Nutrition, Novel Foods and Food Allergens (NDA) was asked to deliver an opinion on hydrothermally treated kernels from edible *Jatropha curcas* (Chuta) as a novel food (NF) pursuant to Regulation (EU) 2015/2283. Although *Jatropha curcas* is generally considered a toxic plant due to the presence of phorbol esters (PEs), edible varieties exist in Central America. The applicant has developed a breeding programme for an edible cultivar and proposes the kernels from this cultivar as an NF as whole kernels or fragments thereof to be used as a snack or as a food ingredient. Procedures are in place to avoid commingling with non-edible kernels, with the last steps being the analytical control of PEs concentrations in all produced batches. The Panel considers that the production process of the NF is sufficiently described and that the information provided on the composition of the NF is sufficient for its characterisation. Components of the NF were tested for genotoxicity applying the standard *in vitro* test battery and no genotoxic concerns have been identified. In a conservative scenario for exposure to PEs from the NF, it was assumed that all kernels contain PEs at the level of detection of the analytical method. When comparing the estimated maximum exposure to PEs with a reference point from a subchronic study in pigs, a margin of exposure $\geq$ 900 is obtained, which is considered sufficiently large. The presence of anti-nutritional factors does not pose safety concerns as they are within the ranges found in vegetables. The Panel concludes that the NF is safe under the proposed conditions of use.

Keywords: edible *Jatropha curcas* kernels, Chuta, phorbol esters, hydrothermal treatment, anti-nutritional factors, novel food, safety

Requestor: European Commission

Question number: EFSA-Q-2018-00542

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Declarations of interest: The declarations of interest of all scientific experts active in EFSA's work are available at https://ess.efsa.europa.eu/doi/doiweb/doisearch.

Acknowledgements: The Panel wishes to thank the Working Group on Compendium of Botanicals, Eirini Kouloura and Petra Gergelova for the support provided to this scientific output.

Suggested citation: EFSA NDA Panel (EFSA Panel on Nutrition, Novel Foods, Food Allergens), Turck D, Bohn T, Castenmiller J, De Henauw S, Hirsch-Ernst KI, Maciuk A, Mangelsdorf I, McArdle HJ, Naska A, Pelaez C, Pentieva K, Siani A, Thies F, Tsabouri S, Vinceti M, Cubadda F, Frenzel T, Heinonen M, Marchelli R, Neuhäuser-Berthold M, Poulsen M, Prieto Maradona M, Schlatter JR, van Loveren H, Colombo P and Knutsen HK, 2022. Scientific Opinion on the safety of hydrothermally treated kernels from edible *Jatropha curcas* L. (Chuta) as a novel food pursuant to Regulation (EU) 2015/2283. EFSA Journal 2022;20(1):6998, 26 pp. https://doi.org/10.2903/j.efsa.2022.6998

ISSN: 1831-4732

The EFSA Journal is a publication of the European Food Safety Authority, a European agency funded by the European Union.

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1. Introduction

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

On 29 August 2016, the company JatroSolutions GmbH submitted a request to the German Federal Office of Consumer protection and Food Safety (BVL) in accordance with Article 4 of Regulation (EC) 258/1997[1] to place on the EU market edible *Jatropha curcas* L. kernels (Chuta®) as a novel food ingredient.

Pursuant to article 35(1) of Regulation (EU) 2015/2283[2] any request for placing a novel food on the market within the Union submitted to a Member State in accordance with Article 4 of Regulation (EC) 258/1997 of the European Parliament and of the Council concerning novel foods and novel foods ingredients and for which the final decision has not been taken before 1 January 2018, shall be treated as an application submitted under Regulation (EU) 2015/2283.

In accordance with Article 10(3) of Regulation (EU) 2015/2283, the European Commission asks the European Food Safety Authority to provide a scientific opinion by carrying out the assessment for edible *Jatropha curcas* L. kernels (Chuta®) as a novel food.

2. Data and methodologies

2.1. Data

The safety assessment of this novel food (NF) is based on data supplied in the application, information provided by the EFSA Working Group on Compendium of Botanicals and information submitted by the applicant following EFSA requests for supplementary information.

During the assessment, the Panel identified additional data which were not included in the application (Panigrahi et al., 1984; Makkar and Becker, 1997; Garcia Estepa et al., 1999; Haas et al., 2002; Aregheore et al., 2003; Chivandi et al., 2006; Goel et al., 2007; Makkar et al., 2008; Rakshit et al., 2008; Martin-Cabrejas et al., 2009; Schlemmer et al., 2009; Shah and Sanmukhani, 2010; Chomchai et al., 2011; Baldini et al., 2014a; Langrand et al., 2015; Li et al., 2015; Gupta et al., 2016; Pal et al., 2017; Sabolová et al., 2017; Süvari et al., 2017; Vagadia et al., 2017; White, 2017; Faria-Machado et al., 2019; Nematollahi et al., 2020).

Administrative and scientific requirements for NF applications referred to in Article 10 of Regulation (EU) 2015/2283 are listed in the Commission Implementing Regulation (EU) 2017/2469[3].

A common and structured format on the presentation of NF applications is described in the EFSA guidance on the preparation and presentation of an NF application (EFSA NDA Panel, 2016). As indicated in this guidance, it is the duty of the applicant to provide all of the available (proprietary, confidential and published) scientific data (including both data in favour and not in favour) that are pertinent to the safety of the proposed NF.

This NF application includes a request for protection of proprietary data in accordance with Article 26 of Regulation (EU) 2015/2283. The data requested by the applicant to be protected comprise: compositional data including nutritional information and allergens, biological and process contaminants, management of Chuta cultivation, shelf-life of the NF, phorbol esters analytical methods, procedures for the verification of phorbol esters content, hydrothermal treatment, molecular markers, anticipated intake and toxicological information.

2.2. Methodologies

The assessment follows the methodology set out in the EFSA guidance on NF applications (EFSA NDA Panel, 2016) and the principles described in the relevant existing guidance documents from the EFSA Scientific Committee. The legal provisions for the assessment are laid down in Article 11 of Regulation (EU) 2015/2283 and in Article 7 of the Commission Implementing Regulation (EU) 2017/2469.

[1] Regulation (EC) 258/97 of the European Parliament and of the Council of 27 January 1997 concerning novel foods and novel food ingredients. OJ L 43, 14.2.1997, pp. 1–6.

[2] Regulation (EU) 2015/2283 of the European Parliament and of the Council on novel foods, amending Regulation (EU) No 1169/2011 of the European Parliament and of the Council and repealing Regulation (EC) No 258/97 of the European Parliament and of the Council and Commission Regulation (EC) No 1852/2001 (2013/0435 (COD). OJ L 327, 11.12.2015, pp. 1–22.

[3] Commission Implementing Regulation (EU) 2017/2469 of 20 December 2017 laying down administrative and scientific requirements for applications referred to in Article 10 of Regulation (EU) 2015/2283 of the European Parliament and of the Council on novel foods. OJ L 351, 30.12.2017, pp. 64–71.

The information provided by the EFSA Working Group on Compendium of Botanicals is based on an extensive literature search on *Jatropha curcas*, following a search strategy and standard operating procedure as described by Dibusz and Vejvodova (2020).

This assessment concerns only the risk that might be associated with consumption of the NF under the proposed conditions of use and is not an assessment of the efficacy of the NF with regard to any claimed benefit.

Dietary intake to the NF was estimated based on the proposed uses and use levels (see Section 3.8).

3. Assessment

3.1. Introduction

The NF which is the subject of the application is the hydrothermally treated *Jatropha curcas* L. kernels (Chuta®), hereinafter referred as 'Chuta' or 'Chuta kernel'. Chuta kernels are obtained from edible *Jatropha curcas* cultivars (called 'EdibleNut' cultivars) developed in the context of JatroSolutions breeding programme. The applicant intends to market the NF as a whole food (consumed as a 'snack' similar to other nuts) or as a food ingredient (whole kernels or fragments thereof, excluding flour) in selected food categories. The proposed target population is the general population (adolescents and adults).

According to the definition as reported in Article 3.2 of NF regulation (EU) 2015/2283 for *Jatropha curcas* L. kernels (Chuta) the following applies:

'food consisting of, isolated from or produced from plants and their parts' (iv).

3.2. Identity of the NF

The NF is obtained from edible *Jatropha curcas* cultivars. *Jatropha curcas* L. is a plant belonging to the Euphorbiaceae family (order Malpighiales) and has seeds rich in oil. It is distributed in wild or semi-cultivated stands in many tropical regions across Africa, Southeast Asia and especially Central and North America (Aregheore et al., 1998; Martinez-Herrera et al., 2012a; Vera-Castillo et al., 2014). The *Jatropha curcas* fruits are round to oval fruits that contain one to four black oval seeds (approximately 2 cm long and 1 cm wide). After removal of the black shell, one white/beige kernel per seed is obtained with a weight of 0.4–0.5 g (Makkar et al., 1998a,b, 2011).

Although *Jatropha curcas* kernels are toxic because of the presence of phorbol esters (PEs), the possibility of using the protein and oil-rich kernels as animal feed following detoxification procedures has been studied (Devappa et al., 2010b; He et al., 2011; Francis et al., 2013; Montes et al., 2014; Vera-Castillo et al., 2014). After extraction of the oil for biodiesel production, kernel meals intended for the diet of farm animals and in aquaculture are detoxified by means of thermal and chemical treatments to decrease anti-nutrients and PEs content (Makkar and Becker, 1999; Goel et al., 2007; Kumar et al., 2011).

An edible variety of *Jatropha curcas* is also available and known since centuries by local populations in Mexico (see Section 3.7). From a phenotypical and compositional point of view, this edible cultivar is similar to the non-edible varieties (Makkar and Becker, 1997; Makkar et al., 2011; Valdes-Rodríguez et al., 2013) but contains PEs at concentrations below limits of detection (LOD) by different analytical methods (LOD as low as 0.026 μg PEs/g kernel by HPLC-UV) (Makkar et al., 1997; Martínez-Herrera et al., 2010; Devappa et al., 2013; Baldini et al., 2014b; Faria-Machado et al., 2019).

The NF in the present assessment is the hydrothermally treated kernel from an edible cultivar of *Jatropha curcas* L. (Chuta).

3.3. Production process

The applicant reported that quality control checkpoints and principles of Hazard Analysis and Critical Control Point (HACCP) are applied in the production process.

Chuta kernels are obtained from *J. curcas* plants grown from 'EdibleNut' planting material developed within the breeding programme of JatroSolutions GmbH. Farmers located in tropical or subtropical areas characterised by adequate climatic factors for Chuta production are using 'EdibleNut' planting material that is verified for quality, identity and genetic purity based on the use of molecular markers. The molecular marker used by the applicant targets a mutation in a single genetic locus that has been identified as coding for PEs biosynthesis (King et al., 2013). The mutation in this genetic

region prevents PEs biosynthesis as suggested by PEs concentrations below the LOD of the analytical method applied (0.1 μg PEs/g kernel by HPLC-UV) (He et al., 2011; King et al., 2013).

Once the plantlets reach the appropriate size, they are planted at adequate density into prepared soils. Fertilisation is done considering the nutritional conditions of the soil and the nutritional requirements of the plant. Plant protection is carried out including the concepts of integrated pest management and good agricultural practices. Fruits are allowed to ripen naturally and are harvested manually or semi-mechanically once they have reached a suitable maturity condition. To ensure traceability of the product, information on location, farm, parcel and block, where Chuta fruits were harvested, is included in the accompanying documents and/or the label. Farmers are requested to respect strict procedures to avoid any possibility of commingling with other *Jatropha* plants or seeds.

During post-harvest handling, the fruits are cleaned and mechanically de-husked by machines calibrated according to fruit size and moisture content to extract the seeds. The shelled seeds are then dried generally by solar or forced air-drying. Afterwards, Chuta seeds are sorted by size and cleaned to remove debris and other residues. In case the subsequent processing steps are not performed immediately, the seeds are packed in hermetically sealed bags and stored in a dry, cool, aerated and closed place. The applicant stated that traceability is ensured in every step and that to implement the quality management measures in the farms a 'harvesting' and a 'post-harvest' officer per farm are appointed.

Before proceeding further with the production process, analytical controls are carried out. Specifically, particular attention is paid and specific measures are in place to prevent possible commingling with non-edible (i.e. due to the presence of PEs) *Jatropha curcas* plants and kernels (see Section 3.4.2). The applicant stated that every batch of Chuta kernels will undergo, among others, PEs analytical control according to a specific procedure for appropriate sampling (see Appendix A) and applying a validated analytical method. From each batch of seeds, a number of incremental samples (from 10 up to 100) have to be taken depending on the size of the lots (from 0.1 up to 500 tons) to form an aggregate sample (with a minimum weight of 3.5 kg that may increase proportionally to the number of incremental samples to be taken). Five laboratory samples extracted from each aggregate sample will be de-shelled, ground and analysed for PEs according to the validated method. Only batches in which PEs are undetectable in all five samples will be further processed. The seeds from suitable batches are de-shelled mechanically. Whole kernels and broken kernel fragments are separated. Afterwards, kernels are processed through a hydrothermal treatment (at temperature $> 120°C$) to reduce anti-nutrient content and the microbiological load, and according to the applicant at the same time improving flavour and texture of the kernels. After the hydrothermal treatment, Chuta kernels are packed in airtight non-transparent polypropylene sacks.

The applicant stated that the de-shelling of seeds and hydrothermal treatments of kernels will be performed exclusively in European processing facilities.

The Panel considers that the production process is sufficiently described.

3.4. Compositional data

3.4.1. Proximate analysis

The NF is the hydrothermally treated kernels of edible *Jatropha curcas*. Kernels are primarily constituted of fats (approximately 60%) and proteins (25%), the remaining components being fibre, ash and carbohydrates (see Table 1). The moisture content is generally limited to $< 1\%$.

The applicant provided results of the analysis of a total of six batches of the NF obtained from kernels received from three different countries (Cameroon, Paraguay and Mexico) to also account for possible variability due to different environmental and agronomic conditions (Senger et al., 2017).

Table 1: Batch to batch analysis of the NF: proximate analysis

Parameter	Batch 1 (Cameroon)	Batch 2 (Paraguay)	Batch 3 (Mexico)	Batch 4 (Mexico)	Batch 5 (Mexico)	Batch 6 (Paraguay)	Method of analysis
Moisture (%)	0.3	0.6	0.3	1.1	1.1	0.9	ASU L 06.00-3
Total fat (%)	58.9	61.1	57.7	60.5	60.5	53.8	ASU L 06.00-6, gravimetric after extraction

Parameter	Batch 1 (Cameroon)	Batch 2 (Paraguay)	Batch 3 (Mexico)	Batch 4 (Mexico)	Batch 5 (Mexico)	Batch 6 (Paraguay)	Method of analysis
– Saturated FA (%)	12.0	12.0	9.7	12.2	12.0	10.5	ISO 5508/5509 GC-FID
Carbohydrates (%)	3.3	2.1	1.4	3.6	3.9	7.1	Calculated parameter
– Total sugars (%)	2.0	2.1	1.4	2.4	2.5	1.7	Enzymatic methods
Total fibre (%)	7.4	8.1	8.5	8.2	8.5	7.6	ASU L 00.00-18, gravimetric
Total protein (%)	26	25	28	21.6	21.1	25.9	ASU L 06.00-7, Kjeldahl
Ash (%)	4.1	4.4	5.4	5.0	4.9	4.7	ASU L 06.00-4, gravimetric

FA: fatty acids; ASU: Official collection of analytical methods according to § 64 LFGB (German Food, Commodities and Feed Code); ISO: International Organization for Standardization; GC-FID: gas chromatography-flame ionisation detection.

Analyses were conducted in an accredited laboratory according to standard methods (i.e. ASU, ISO).

The Panel considers that the information provided on proximate analysis is sufficient for characterising the NF.

In addition to the proximate analysis, the applicant provided detailed analyses of fatty acid and amino acid composition, sugars, minerals and anti-nutritional factors in the NF that are reported in Section 3.10.

3.4.2. Phorbol esters, contaminants, microbiological and process contaminants

Phorbol esters

PEs represent the potential major hazard in the use of *Jatropha* kernels (Makkar et al., 1998b; Martínez-Herrera et al., 2012a; Vera-Castillo et al., 2014). Data on concentrations of PEs from three batches of *Jatropha curcas* kernels and three batches of Chuta kernels have been submitted in the original dossier. A non-validated HPLC-UV method (modified from Devappa et al., 2010b; refined by Montes et al., 2013) has been used initially by the University of Hohenheim with an LOD of 0.05 mg PEs/g (expressed as 12-O-tetradecanoylphorbol-13-acetate (TPA) equivalent). While the three Chuta batches showed PEs content below the LOD, the three non-edible *Jatropha* kernel batches contained values from 1.8 to 9.2 mg PE/g. From literature data, in flour obtained from edible *Jatropha* seeds collected from a few regions in the Mexican area (e.g. Veracruz) known for the presence of edible *Jatropha* plants, PEs were not detectable. According to Makkar et al. (1998a,b), concentrations of PEs in non-edible *Jatropha* kernels across the world (e.g. Nicaragua, Nigeria) ranged from 2.2 to 2.7 mg/g kernel, while for the edible Mexican variety concentrations were not detectable (< 0.01 mg/g LOD expressed as TPA equivalent) or ranged from 0.01 up to 0.11 mg/g, depending on the location. Makkar et al. (2011) reported values of 2.79 mg/g as an average for non-edible kernels while undetectable levels for the edible varieties were noted (< 3 μg/g sample as TPA equivalent). Martínez-Herrera et al. (2010) have noted undetectable levels in the edible varieties, with levels ranging from 0.6 to 4.1 mg/g for the non-edible variety instead (same analytical method than Makkar et al., 1998b). A content of 2.45 mg PEs/g of seed (Baldini et al., 2014a) and 2–6 mg/g of kernel (Devappa et al., 2010b) has been also reported for non-edible kernels.

Upon request from EFSA, a more sensitive analytical method was developed by the applicant and validated by an accredited laboratory for the detection of PEs in Chuta kernels. According to these new UHPLC-UV and UHPLC-MS methods (the latter used for identification of PEs peaks, if any), the applicant stated that an LOD of 0.75 μg PEs/g kernel (as TPA equivalent) was obtained. Concentration of PEs in analysed Chuta kernels was below the LOD.

In addition, the applicant performed a sensitivity test for the detection of PEs by intentionally commingling of *Jatropha* kernels (containing 1.5 mg PEs/g) in Chuta kernels at a proportion of 1:1,000 using this analytical method. Based on the sampling protocol, which varies according to the size of the batch, a total of five samples of material obtained from ground aggregate samples were analysed. The presence of PEs using this methodology was evident in all five samples. Intentional commingling at a

proportion corresponding to half of a *Jatropha* kernel in 1,000 Chuta kernels (i.e. 1:2,000) did not result in a fully reliable detection of PEs after applying the same sampling protocol as described above. On the basis of these experiments and accounting for the uncertainty in the LOD, the Panel uses a twofold higher concentration than the LOD when estimating exposure to PEs from the NF (see Section 3.8.4).

Contaminants and microbiological parameters

Additional parameters including contaminants (heavy metals), mycotoxins and microbiological quality are reported in Table 2.

Table 2: Batch to batch analysis of the NF: contaminants and microbiological quality

Parameter	Batch 1	Batch 2	Batch 3	Method of analysis
Contaminants				
Arsenic (mg/kg)	< 0.05	< 0.05	0.06	ASU L 00.00-135 - ICP-MS
Lead (mg/kg)	< 0.02	< 0.02	< 0.02	ASU L 00.00-135 - ICP-MS
Cadmium (mg/kg)	< 0.02	< 0.02	< 0.02	ASU L 00.00-135 - ICP-MS
Mercury (mg/kg)	< 0.005	< 0.005	0.009	ASU 00.00-19/4
Aflatoxin B1 (μg/kg)	< 0.2	< 0.2	< 0.2	DIN EN 14123 - HPLC-PCD
Aflatoxin B2 (μg/kg)	< 0.2	< 0.2	< 0.2	DIN EN 14123 - HPLC-PCD
Aflatoxin G1 (μg/kg)	< 0.2	< 0.2	< 0.2	DIN EN 14123 - HPLC-PCD
Aflatoxin G2 (μg/kg)	< 0.2	< 0.2	< 0.2	DIN EN 14123 - HPLC-PCD
Sum B1, B2, G1, G2 (μg/kg)	< 0.8	< 0.8	< 0.8	DIN EN 14123 - HPLC-PCD
Deoxynivalenol (DON) (μg/kg)	< 20	< 20	< 20	PV-18-Fusarium (LC-MS/MS)
HT-2 Toxin (μg/kg)	< 10	< 10	< 10	PV-18-Fusarium (LC-MS/MS)
T-2 Toxin (μg/kg)	< 10	< 10	< 10	PV-18-Fusarium (LC-MS/MS)
Zearalenone (ZEA) (μg/kg)	< 10	< 10	< 10	PV-18-Fusarium (LC-MS/MS)
Ochratoxin A (OTA) (μg/kg)	< 0.5	< 0.5	< 0.5	DIN EN 14132
Microbiology (CFU/g)				
Mesophilic total aerobic count (CFU/g)	< 10	< 10	< 10	ISO 4833
Yeasts (CFU/g)	< 10	< 10	< 10	ISO 7954
Moulds (CFU/g)	< 10	< 10	< 10	ISO 7954
Enterobacteriaceae (CFU/g)	< 10	< 10	< 10	ISO 21528-2
Coagulase-positive *Staphylococcus* (CFU/g)	< 10	< 10	< 10	ISO 6888-1
Bacillus cereus, presumptive (CFU/g)	< 10	< 10	< 10	ASU L 00.00-33
Salmonella spp. (in 25 g)	ND	ND	ND	ISO 6579
Listeria monocytogenes (CFU/g)	< 10	< 10	< 10	ISO 11290-2

ND: not detected; HPLC-PCD: high-performance liquid chromatography-post-column derivatisation; ICP-MS: inductively coupled plasma-mass spectrometry; CFU: colony forming unit; LC-MS/MS: liquid chromatography with tandem mass spectrometry; ISO: International Organization for Standardisation; DIN: German Institute for Standardisation; ASU: official collection of analytical methods according to § 64 LFGB (German Food, Commodities and Feed Code).

Results referring to the mycotoxins and microbiological contaminants have been obtained from three representative NF batches collected after a 6-month storage period (vacuum packaging at 20°C in a cabinet).

Analyses on chemical contaminants were performed according to requirements of the Regulation (EC) No 1881[4]/2006. Certificates are available and analyses were performed in an accredited laboratory according to standard validated methods.

Pesticide residues were analysed according to a multiscreen method for dry foodstuffs with high carbohydrate/protein content according to the accredited method ASU L 00.00-115 QuEChERS (Quick Easy Cheap Effective Rugged Safe) with LC-MS/MS (liquid chromatography-tandem mass spectrometry) and GC-MS/MS (gas chromatography-tandem mass spectrometry). All values were below the limit of quantification.

[4] Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. OJ L 364, 20.12.2006, pp. 1–33.

Process contaminants

The hydrothermal treatment (with temperature above 120°C) may lead to the production of harmful by-products. The applicant provided experimental data on acrylamide, furan, chloropropanols (i.e. 3-MCPD) and glycidyl fatty acid esters (FAE) concentrations from three batches of the NF.

Concentrations found in the NF were in many occasions below limits of detection. Results obtained in an additional single batch of untreated kernels are also reported for comparative purposes (Table 3).

Table 3: Heat-induced contaminants in raw and hydrothermally treated Chuta kernels

	mg/kg fat			µg/kg	
	3-MCPD and glycidyl FAE (expressed as 3-MCPD)[(a)]	**3-MCPD FAE (expressed as 3-MCPD)[(a)]**	**glycidyl FAE (expressed as glycidol)[(a)]**	**Acrylamide[(b)]**	**Furan[(c)]**
NF batch A	< 0.20	0.12	< 0.10	31	< 200
NF batch B	< 0.20	0.11	< 0.10	26	< 200
NF batch C	< 0.20	< 0.10	< 0.10	< 0.10	< 200
Raw Chuta batch	< 0.20	0.18	< 0.10	< 0.10	< 200

(a): DGF C-VI18 (10) (GC-MS).
(b): PV-212-Acryl (LC-MS/MS).
(c): Validated in house GC-MS.

The Panel notes that for 3-monochloropropane diol (3-MCPD) and glycidyl FAE, no data from comparable foods are currently available. When considering 3-MCPD, the maximum level (ML) for 'hydrolysed vegetable protein' and 'soy sauce' is set at 20 µg/kg. For the sum of 3-MCPD and 3-MCPD fatty acid esters, expressed as 3-MCPD, an ML in 'vegetable fats as an ingredient in food' is set at 1.25 mg/kg (Regulation (EC) No 1881/2006).

The 3-MCPD FAE concentrations were up to about 0.2 mg/kg fat in the NF, while for glycidyl FAE levels lower than 0.1 mg/kg fat were recorded. The concentrations were similar in the batch with raw kernels.

Levels of acrylamide according to Commission Recommendation (EU) 2019/1888[5] should be monitored in roasted nuts. Concentrations of acrylamide in the NF were rather low (maximum of 31 µg/kg) in comparison to other nuts: concentrations in roasted nuts and seeds have been reported to range from 33 to 251 µg/kg (Nematollahi et al., 2020) or from 21 to 271 µg/kg with levels depending on temperature (Süvari et al., 2017).

Furans were not detected (< 200 µg/kg NF) in any of the analysed batches.

To summarise, although data are limited, there are no indications of substantial formation of process contaminants.

The Panel considers that the information provided on PEs, contaminants, mycotoxins and microbiological quality is sufficient for characterising the NF.

3.5. Stability

The applicant provided experimental data after 6-month storage of the NF. The Panel notes that the applicant did not provide data from the same batches over time (no results at time 0). Data from a total of four batches (three hydrothermally treated batches and one untreated) after 6-month storage in vacuum packaging at 20°C have been provided. From the results recorded, no safety concerns have been identified.

In addition, in consideration of the relevant fat content and upon request from EFSA, the applicant provided data from an accelerated stability test to determine oxidative resistance (*Oxipres*) performed at high temperatures (70–110°C) and oxygen pressure (5–6 bar) to stimulate lipid oxidation and assess oxidative resistance (Trojáková et al., 2001; Sabolová et al., 2017). The results indicated that the NF showed a high resistance towards fat oxidation. According to the measured induction points of

[5] Commission Recommendation (EU) 2019/1888 of 7 November 2019 on the monitoring of the presence of acrylamide in certain foods. OJ L 290/31, 11.11.2019, pp. 31–33.

the *Oxipres* and applying the Arrhenius equation, the applicant estimated the oxidative stability to be at least 23 months at temperatures ≤ 25°C.

The Panel considers that, also given the low moisture content of the NF (around 1% and always < 3%), formation of possible degradation products or microbial activity is unlikely. Data after 6 months of storage assessing microbiological activity support this statement (see Section 3.4.2).

A shelf-life of 1 year was proposed by the applicant.

The Panel considers that the data provided sufficient information with respect to the proposed stability of the NF.

3.6. Specifications

The specifications of the NF are indicated in Table 4. The applicant stated that each batch of the NF will be subjected to analytical quality controls to ensure that all product specifications are met, including physico-chemical parameters, organoleptic characteristics, microbial counts, concentrations of heavy metals and pesticide residues.

To exclude health risk from exposure to PEs from commingling of the NF with non-edible *Jatropha* kernels, an accurate procedure for sampling and analysis of PEs as described in the production process (Sections 3.3 and 3.4.2) must be followed for each NF batch. Only batches with PEs levels below the LOD can be fully processed and considered for food use.

Table 4: Specifications of the NF

Description: hydrothermally treated kernels from edible *Jatropha curcas* varieties	
Parameter	**Specification**
Physico-chemical parameters	
Moisture (%)	≤ 3
Total fat (%)	54–61
Total protein (%)	21–32
Carbohydrates (%)	1–7
Total fibre (%)	6–10
Ash (%)	3–5
Contaminants	
Phorbol esters (PEs) (μg TPA eq/g kernel)[(a)]	≤ 0.75
Lead (mg/kg)	≤ 0.20
Cadmium (mg/kg)	≤ 0.20
Sum aflatoxins B1, B2, G1, G2 (μg/kg)	≤ 4
Microbiological	
Total aerobic microbial count (CFU/g)	< 1,000
Total yeast/moulds count (CFU/g)	< 100
Enterobacteriaceae (CFU/g)	< 10
Salmonella spp.	Not detected in 25 g
Listeria monocytogenes (CFU/g)	≤ 100

An accurate procedure for sampling and analysis of PEs as described in the production process (Sections 3.3, 3.4.2 and Appendix A) must be followed for each NF batch. Only batches with concentrations of PEs below the LOD can be fully processed. TPAeq: 12-O-tetradecanoylphorbol-13-acetate equivalent; CFU: colony forming unit; UHPLC-UV-MS: ultra-high-performance liquid chromatography coupled to ultraviolet spectrophotometry and mass spectrometry.
(a): Validated UHPLC-UV-MS method for detection of PEs peaks.

The Panel notes that *Listeria monocytogenes* should be part of the specification parameters to contribute to the safety of the NF as foreseen by Regulation (EC) No 2073/2005[6].

The Panel considers that the information provided on the specifications of the NF is sufficient and does not raise safety concerns.

[6] Commission Regulation (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs. OJ L 338, 22.12.2005, pp. 1–30.

3.7. History of use of the NF and of its source

3.7.1. History of use of the source

Jatropha curcas is a tropical and subtropical plant that grows in the wild and is widely distributed. The non-edible variety is also semicultivated in Central and South America, Africa and Southeast Asia. In the last decades, the non-edible *Jatropha* kernels were used to extract oil to be used as a biofuel, while exhausted cakes were considered as possible source of proteins for use in feed after decontamination of PEs and reduction of anti-nutritional factors by different procedures (Makkar et al., 2008, 2012; Kumar et al., 2011; Wang et al., 2011; Contran et al., 2013). The Panel noted that *Jatropha* seeds are listed as a 'harmful botanical impurity' in Directive 2002/32/EC[7] on undesirable substances in animal feed where it is stated that *Jatropha* seeds and fruits and their processed derivatives may only be present in feed materials and compound feed 'in trace amounts not quantitatively determinable'.

EFSA has assessed the human and animal health risk related to use of detoxified *Jatropha* kernel meal (EFSA CONTAM Panel, 2015).

3.7.2. History of use of the NF

A *Jatropha* variety considered to be 'PEs-free' is grown or cultivated in some regions of Mexico (e.g. state of Veracruz) and used to prepare a variety of traditional dishes. It is consumed always after seed roasting or cooking as such or more commonly ground and used as a constituent of dishes. Roasting or heating procedures are needed to make kernels more tasteful and digestible and particularly to decrease the content of some heat-labile anti-nutritional components (see Section 3.10).

Specifically in the Totonaca culture, that started about 1500 B.C., the consumption of roasted seeds of the edible *Jatropha* variety (named 'piñon manso' and its seed 'xuta') is in its cooking tradition (Aregheore et al., 1998; Makkar et al., 2011; Martinez-Herrera et al., 2012a; Valdes-Rodriguez et al., 2013; Vera-Castillo et al., 2014; Osuna-Canizalez et al., 2015). However, although even recipes are available, only very limited information on the level of intake is available.

The NF has no history of use in Europe.

3.8. Uses and use levels and anticipated intake

3.8.1. Target population

The target population proposed by the applicant for the consumption of the NF is the general population (adolescents and adults). However, as the NF is intended to be used as an ingredient in standard food categories, it cannot be excluded that the NF can be consumed by other groups of the population. Therefore, the safety data and the exposure assessment shall cover all population groups (Commission Implementing Regulation (EU) 2017/2469[3], article 5(6)).

3.8.2. Proposed uses and use levels

The NF is proposed to be used as a snack or as an ingredient in several food products (e.g. mixed breakfast cereals, dried fruits and cereal bars). These food products, defined using the FoodEx2 hierarchy,[8] and the maximum use levels are reported in Table 5. The NF in the European market is intended to be used as a whole food similar to the way peanuts are consumed.

Table 5: Food categories and maximum use levels intended by the applicant

FoodEx2 level	FoodEx2 CODE	Food category	Maximum use level (g NF/100 g)
4	A0DBS	Peanuts and similar	100
4	A00FA	Cereal bars mixed	5
4	A00EL	Mixed breakfast cereals	5

[7] Directive 2002/32/EC of the European Parliament and of the Council of 7 May 2002 on undesirable substances in animal feed. OJ L 140, 30.05.2002, pp. 10–22.

[8] FoodEx2 is a standardised food classification and description system https://www.efsa.europa.eu/en/data/data-standardisation

FoodEx2 level	FoodEx2 CODE	Food category	Maximum use level (g NF/100 g)
3	A0FOL	Candied or sugar preserved nuts	100
3	A01QF	Mixed dried fruits	5

3.8.3. Anticipated intake of the novel food

EFSA performed an assessment of the anticipated daily intake of the NF based on the applicant's proposed uses and maximum proposed use levels (Table 5), using individual data from the EFSA Comprehensive European Food Consumption Database (EFSA, 2011). The lowest and highest mean and 95th percentile anticipated daily intakes of the NF (on an mg/kg body weight (bw) basis), among the EU dietary surveys, are presented in Table 6. The estimated daily intake of the NF for each population group from each EU dietary survey is available in the Excel file annexed to this scientific opinion (under supporting information).

Table 6: Intake estimate resulting from the use of the NF as an ingredient in the intended food categories at the maximum proposed use levels

Population group	Age (years)	Mean intake (mg/kg bw per day)		P95th intake (mg/kg bw per day)	
		Lowest[(a)]	Highest[(a)]	Lowest[(b)]	Highest[(b)]
Infants	< 1	0	2	0	0
Young children[(d)]	1 to < 3	0	13	0	76
Other children	3 to < 10	0	21	0	153
Adolescents	10 to < 18	0	29	0	127
Adults[(c)]	≥ 18	3	44	0	301

bw: body weight.
(a): Intakes are assessed for all EU dietary surveys available in the food comprehensive database on 03/08/2020. The data relate to a period in which UK was still a Union Member State. The lowest and the highest averages observed among all EU surveys are reported in these columns.
(b): Intakes are assessed for all EU dietary surveys available in the food comprehensive database on 03/08/2020. The data relate to a period in which UK was still a Union Member State. The lowest and the highest P95th observed among all EU surveys are reported in these columns (P95th based on < 60 individuals are not considered).
(c): Includes elderly, very elderly, pregnant and lactating women.
(d): Referred as 'toddlers' in the EFSA food consumption comprehensive database (EFSA, 2011).

The Panel notes that the highest estimated 95th percentile intake (i.e. 301 mg/kg bw per day in adults) on the basis of 22 dietary surveys covered by the EFSA Food Consumption Database is corresponding to a regular daily intake of about 21 g of kernels (equivalent to 40–50 kernels) for an adult of 70 kg bw (EFSA Scientific Committee, 2012).

Based on the characteristics of the NF, the Panel assumes that the NF consumption when used as a snack is similar to that of peanuts.

3.8.4. Estimate of exposure to undesirable substances

The presence of PEs represents the potential major hazard for the consumption of Chuta kernels (see Section 3.4.2). The Panel notes that a possible exposure to the PEs can be related to the accidental presence of non-edible *Jatropha* kernels as a result of commingling with the Chuta kernels (see Sections 3.3, 3.4.2 and 4) or can be due to a minimal amount of PEs (below the LOD of the analytical method) present in the NF.

Assuming that all the kernels contain an amount of PEs that is at the LOD, an estimate of the theoretical exposure to the PEs can be made (Table 7). To account for uncertainties in the analytical method in these estimations, it is considered appropriate to correct the LOD by a factor of two (1.5 instead of the 0.75 μg PEs/g de-shelled kernel (as TPA equivalent) as reported in the specifications) (see Section 3.4.2).

Table 7: Possible maximum exposure to PEs from P95 NF intake with concentration of PEs of 1.5 μg/g kernel (twofold the LOD in the specifications)

	NF intake (P95) (mg/kg bw per day)	Possible PEs intake from P95 consumption of the NF (μg/kg bw per day)
Young children (1 to < 3 years)	76	0.11
Other children (3 to < 10 years)	153	0.23
Adolescents (10 to < 18 years)	127	0.19
Adults (≥ 18 years)	301	0.45

bw: body weight.

3.9. Absorption, distribution, metabolism and excretion (ADME)

The applicant did not provide any studies or information pertaining to the ADME characteristics of the NF.

3.10. Nutritional information

The major components of the NF are lipids and proteins. Total fat comprises approximately 60% of the NF with the relative proportion of unsaturated fatty acids accounting for around 80% of total fat, while proteins account for about 25% (see Section 3.4.1).

3.10.1. Fatty acids and amino acids composition, sugars and minerals

The applicant has provided detailed analyses of fatty acids and amino acid composition, sugars and minerals in the NF. The fatty acid composition (Table 8) showed that mono- and- poly-unsaturated fatty acids are the major components.

Table 8: Fatty acid composition (fatty acid % of total fat) of three batches of raw Chuta kernels

Fatty acid	Mean	Range
Myristic acid (C14:0)	0.24	0.18–0.29
Palmitic acid (C16:0)	11.52	11.19–11.73
Palmitoleic acid (C16:1)	0.53	0.48–0.57
Stearic acid (C18:0)	6.44	5.26–7.98
Oleic acid (C18:1)	34.83	30.70–38.10
Linoleic acid (C18:2)	45.89	41.57–50.21
α-Linolenic acid (C18:3)	0.21	0.18–0.23
Arachidic acid (C20:0)	0.19	0.18–0.20
Total saturated fatty acids	18.39	17.48–19.55
Total unsaturated fatty acids	81.46	80.33–82.37
Total mono-unsaturated fatty acids	35.36	31.25–38.58
Total poly-unsaturated fatty acids	46.10	41.75–50.43

Data from three raw batches of Chuta – internal method.

The amino acid profile was assessed for three batches (Table 9).

Table 9: Amino acid composition of three batches of the NF

Amino acid	Results (g/100 g)		Amino acid	Results (g/100 g)	
	Mean	Range		Mean	Range
Isoleucine	1.26	1.11–1.50	Glycine	1.79	1.01–3.10
Histidine	0.85	0.56–1.40	Serine	2.51	1.29–4.70

Amino acid	Results (g/100 g)		Amino acid	Results (g/100 g)	
	Mean	Range		Mean	Range
Leucine	1.75	1.57–2.00	Threonine	1.04	0.80–1.50
Lysine	0.78	0.46–1.30	Tyrosine	0.88	0.64–1.20
Methionine	0.36	0.33–0.40	Proline	1.13	0.97–1.40
Phenylalanine	1.43	1.09–2.00	Aminobutyric acid	< 0.05	< 0.05
Valine	1.54	1.36–1.90	Carnosine	< 0.05	< 0.05
Alanine	1.54	1.16–2.20	Hydroxylysine	< 0.05	< 0.05
Arginine	2.37	1.97–3.20	Hydroxyproline	< 0.05	< 0.05
Aspartic acid	2.61	2.11–3.50	Ornithine	< 0.5	< 0.5–1.10
Cystine	< 0.05	< 0.05	Taurine	< 0.05	< 0.05
Glutamic acid	3.98	3.55–4.60			

Mean of three batches, LC-MS/MS.

Carbohydrates do not constitute a major component of the kernels (approximately 4%, ranging from 1.4 to 7.1 g/100 g kernel), the most represented sugar being sucrose.

A similar or higher (i.e. phosphorus) content of minerals (Table 10) is noted in comparison to other nuts (Ros, 2010; White, 2017).

Table 10: Content of minerals from three batches of the NF

Parameter	mg/100 g NF	
	Mean	Range
Calcium	215	202–232
Phosphorus	1141	986–1363
Magnesium	548	488–600
Potassium	890	814–1017
Sodium	3	2–4
Zinc	5	4–6

According to standard accredited methods, rounded figures.

Hazelnuts, Brazil nuts and macadamia nuts have similar fat levels while only peanuts possess similar protein values (Ros, 2010). The Panel noted that the fatty acid (Chuta kernels) and amino acid profiles (NF) are similar to those of other nuts (Maguire et al., 2004; Senger et al., 2017).

3.10.2. Anti-nutritional factors

Non-edible and edible varieties of *Jatropha curcas* possess similar levels of anti-nutrients (Makkar et al., 1998b; Devappa et al., 2010b; Francis et al., 2013; Vera-Castillo et al., 2014). The applicant submitted data from three batches of raw Chuta kernels and three hydrothermally treated kernels (the NF) documenting the concentration of anti-nutrient compounds, such as phytic acid (myoinositol hexaphosphoric acid, IP6), trypsin inhibitors (TI) and lectin (i.e. curcin). The Panel noted that results are referring to different batches and not to the same batch before and after the hydrothermal treatment.

Anti-nutrients are widely reported to be present at various concentrations in cereals and legumes. For phytic acid, a wide variability of contents in vegetables is reported. In Chuta kernels batches (raw and hydrothermally treated), the phytates (analysed with HPLC according to Zeller et al. (2015) and expressed as IP6) showed values ranging from 3.7% to 4.6% irrespective of heat treatment. Values around 2.2% and up to 5.8% in whole wheat flour and in wheat brans, respectively, are reported (Garcia Estepa et al., 1999). Other authors reported ranges from 1.1% to 8.7% in wheat bran and germs and rice bran, while phytic acid was ranging from 1% to 5.4% in oilseeds (e.g. sunflowers and soybean), from 0.61% to 2.38% in beans, from 0.17% to 4.47% in peanuts and from 0.35% to 9.42% in almonds (Schlemmer et al., 2009; Gupta et al., 2015; EFSA NDA Panel, 2019). Since phytates are heat stable, the content is not influenced by thermal treatments (Martínez-Herrera et al., 2006; Pal et al., 2017).

For trypsin inhibitors, the inhibitory activity (TIA, mg/g dry matter) is measured. In the available literature, a maximum of about 42 mg/g dry matter in raw lentil flours (Pal et al., 2017), a range of 16–27 mg/g in whole soybean (Vagadia et al., 2017) or up to 12.5 and 48.2 mg/g in peas and raw soybeans, respectively (Gilani et al., 2012), were reported. TIA was ranging from 18.4 to 27.5 mg/g dry matter in a variety of *Jatropha* kernels from different origin (non-edible kernels included) (Makkar et al., 1997). It is known that TIA can be decreased by heat treatment (Schlemmer et al., 2009; Martínez-Herrera et al., 2010; Gilani et al., 2012; Mada et al., 2012; Vagadia et al., 2017). The applicant has provided data from three raw Chuta kernels and three 'roasted' Chuta kernels (considered representative of the NF), in duplicate (according to Smith et al., 1980). Results provided by the applicant indicated that heat-treated kernels have at least halved TIA levels when compared to the raw ones (average of about 14.3 vs. 34.0 mg trypsin inhibited/g defatted kernel).

Lectins are carbohydrate-binding glycoproteins; specifically, in *Jatropha* kernels, the ribosome-inactivating protein (RIP) type 1, namely curcin, is found (Makkar et al., 2012). Type 1 RIP is characterised by low toxicity, especially when compared to type 2 RIPs (e.g. ricin) (EFSA CONTAM Panel, 2008; Devappa et al., 2010b; Wu and Sun, 2012). Curcin concentrations are assessed by the applicant according to a specific qualitative method based on SDS-gel electrophoresis followed by mass spectrometry (SDS-gel electrophoresis and nano-LC-ESI-MS/MS). Heat treatments removed curcin peptides from the NF in the five tested batches since no levels were detected in hydrothermally treated kernels (< 0.05 mg curcin/g protein), while in other five untreated Chuta kernels, an average of 4.83 mg curcin/g protein (4.35–5.58) was found. This is in agreement with the published literature that reports lectin activity in vegetables and legumes (e.g. peanuts, lentils, beans) disappearing after heating or boiling (Aregheore et al., 1998; Martin-Cabrejas et al., 2009; Embaby, 2011; Martinez-Herrera et al., 2012a).

Saponins are present in a variety of plants as well as in *Jatropha* kernels. It is reported that in both non-edible and edible *Jatropha* cultivars levels are similar, ranging from 1.8 and 3.4% in kernel meal (Makkar et al., 1997; Devappa et al., 2010b) to 2.1–2.9% in defatted *Jatropha* seeds (Martínez-Herrera et al., 2006). Saponins found in *Jatropha* kernels are reported to be non-haemolytic and with limited toxicity (Devappa et al., 2010). Heat treatment only marginally decreased saponin levels (Makkar et al., 1998b; Mada et al., 2012). Saponin content is reported to be higher in soybean meal than in *Jatropha* meals (Martínez-Herrera et al., 2006). The applicant did not perform any additional investigation on saponins.

It is anticipated that the hydrothermal treatment of Chuta kernels will minimise or inactivate the content of specific anti-nutritional components. The presence of anti-nutritional substances does not appear to be at levels higher than those recorded in cereals, legumes or nuts. In addition, the mean estimated chronic intake of the NF is limited to a few grams per day (Section 3.8.3).

The Panel considers that consumption of the NF is not nutritionally disadvantageous.

3.11. Toxicological information

The applicant provided eight *in vitro* genotoxicity studies on the NF and *Jatropha* kernels. These studies which were claimed proprietary by the applicant are listed in Table 11.

Table 11: List of toxicological studies with the NF and *Jatropha* kernels provided by the applicant

Test material	Reference	Type of study
NF oil	Unpublished study report (2021a)	Bacterial reverse mutation test (Ames test)
Jatropha kernel oil	Unpublished study report (2021b)	
NF defatted meal	Unpublished study report (2021c)	
Jatropha kernel defatted meal	Unpublished study report (2021d)	
NF oil	Unpublished study report (2021e)	*In vitro* mammalian cell micronucleus test
Jatropha kernel oil	Unpublished study report (2021f)	
NF defatted meal	Unpublished study report (2021g)	
Jatropha kernel defatted meal	Unpublished study report (2021h)	

At least six PEs are present in *Jatropha* seeds (Haas et al., 2002; Goel et al., 2007). In a previous assessment performed by the CONTAM Panel (EFSA CONTAM Panel, 2015), a read-across comparison

with the structural analogue TPA, a well-known non-genotoxic tumour promoter, was performed. The analysis suggested that PEs present in *Jatropha* seeds have similar, but also additional, structural alerts relevant to genotoxicity when compared to TPA. The phorbol esters, like TPA, are reported to mimic the action of diacyl glycerol, activator of protein kinase C (PKC), which regulates different signal transduction pathways. Interference with the activity of PKC affects a number of processes including phospholipid and protein synthesis, enzyme activities, DNA synthesis, phosphorylation of proteins, cell differentiation and gene expression (Goel at al., 2007; Devappa et al., 2010b; EFSA CONTAM Panel, 2015).

3.11.1. Genotoxicity: studies conducted with the NF and *Jatropha* kernels

The potential genotoxicity of the NF was investigated in a bacterial reverse mutation test and an *in vitro* mammalian cell micronucleus test (Unpublished study report, 2021a,c,e,g). For comparative purposes, the same investigations were also performed with non-edible *Jatropha* kernels (Unpublished study report, 2021b,d,f,h) (Table 11). Oil extracted from kernels and the remaining defatted meals from both Chuta and *Jatropha* were considered. These studies were conducted in compliance with OECD principles of Good Laboratory Practice (GLP) (OECD, 1998a) and in accordance with the OECD test guidelines No 471 and 487 of 2020 and 2016, respectively.

The assessment of the mutagenic potential of the NF (unpublished study reports, 2021a–d) was performed with histidine-dependent auxotrophic mutants of *Salmonella typhimurium*, strains TA98, TA100, TA102, TA1535 and TA1537 that were exposed to the NF and *Jatropha* oil emulsified in acetone at concentrations up to 5 μL/plate (stock emulsion of 50 ± 5 mL/L) either in the presence or absence of liver microsomal fractions (S9). No reproducible or dose-related increases in revertant colony numbers over control counts were observed with any of the strains following exposure to NF oil or *Jatropha* oil at any concentration (irrespective of the presence or absence of S9). No evidence of toxicity towards the bacterial test strains was obtained following exposure to the NF oil. Therefore, under the experimental conditions applied, the NF oil and the *Jatropha* oil were non-mutagenic at concentrations up to 5 μL/plate, in the absence or presence of metabolic activation.

The supernatant obtained from the centrifugation of a stock suspension of the defatted kernel meals (concentration of 50 g/L in sterile demineralised water followed by dilutions) caused the formation of a slimy coating on the plates that prevented the counting of the colonies, at any dilution. Therefore, due to interferences of the test substance with the test system, no evaluation of revertant colonies and no mutagenicity assessment was performed on the defatted kernel meals (from both Chuta and *Jatropha*).

In the *in vitro* mammalian cell micronucleus test (unpublished study reports, 2021e–h), taking into consideration characteristics of the test item (i.e. the oil) that was insoluble in all tested solvents, concentrations up to 1.25 μL/mL as emulsion in acetone were tested in cultured human peripheral blood lymphocytes in the presence or absence of metabolic activation (S9 fraction). No cytotoxicity and no solid precipitates were observed (0.31, 0.63 and 1.25 μL/mL), however, the presence of an oily film at all concentrations tested was noted. No statistically significant increases in the number of binucleate cells containing micronuclei both after 4-h treatment in the presence of S9 mix or following 23-h treatment in the absence of S9 were recorded. The NF oil and the *Jatropha* oil did not show any evidence of clastogenicity or aneugenicity in the absence and presence of metabolic activation.

When the defatted kernel meals were assessed, the same approach as in the bacterial reverse mutation test was followed. For the NF, as well as for *Jatropha* defatted meals, neither a statistically significant nor a biologically relevant increase in the number of binucleated cells containing micronuclei in human lymphocytes at the three evaluated concentrations (2.5%, 5% and 10%) was observed. Under the experimental conditions applied, the NF did not show any evidence of clastogenicity or aneugenicity.

Based on the results of these studies, the Panel considers that there are no concerns regarding genotoxicity of the NF.

3.11.2. Subacute toxicity

The applicant did not perform any study with the NF. However, publications with descriptions of studies conducted in a few animal species (e.g. rats and fish) with test items derived from edible *Jatropha curcas* (i.e. defatted meals) or from a non-edible variety of *Jatropha curcas* after detoxification treatment (i.e. defatted and detoxified meals) have been provided and are summarised below.

– Studies conducted with edible *Jatropha* kernels

Studies performed with edible *Jatropha curcas* defatted meals were carried out in rats (Panigrahi et al., 1984; Makkar and Becker, 1999; Martinez-Herrera et al., 2012b). Although with limitations (e.g. test item not fully representative of the NF), these studies are considered as supportive for the current assessment and are summarised in Table 12.

Table 12: *In vivo* studies with edible *Jatropha curcas* meals

Reference	Type of study	Strain/species	Dose and exposure/route of administration
Makkar and Becker (1999)	Nutritional quality study (7 days)	Sprague Dawley (7 male rats/ group)	Diets with 10% protein (casein, non-heated and heated defatted edible *Jatropha* meal)
Martinez-Herrera et al. (2012b)	Nutritional quality study (28 days)	Wistar rats (10/group, 5 males and 5 females)	Rats were fed ad libitum (10% protein) and feed intake was recorded daily: Diet 1) Protein free, 2) with casein, 3) Edible *J. curcas* defatted flour heat-treated, 4) Diet 3 + 1% lysine added, 5) Diet 3 + 500 phytase units
Panigrahi et al. (1984)	Nutritional quality study (5/7-week)	Wistar rats (4 sex/group)	Arm with oil 15% of diet (pure maize oil, pure *Jatropha* oil, mix 1:1 and 4:1) Arm with raw and roasted defatted *Jatropha* meals providing 48% of crude protein in the diet

In the 7-day feeding study in SD rats (7 male rats/group), casein, unheated and heated defatted *Jatropha* meal were used in diets to provide 10% protein. At the end of the 7 days, protein efficiency ratio (PER)[9] for the unheated *Jatropha* diet was clearly lower than the one with casein (1.29 vs. 3.52) and also feed intake and body weight were affected (21 and 23% lower, respectively). Heated *Jatropha* diet only marginally affected PER (3.02 vs. 3.52), feed intake and body weight (7% lower, considered related to a reduced protein utilisation) (Makkar and Becker, 1999).

The 28-day rat study (Martinez-Herrera et al., 2012b) was conducted using a set of diets containing heat-treated (121°C for 15 min) defatted flour of edible *Jatropha* kernels with different combinations of enzymes (see Table 12). Rats were fed ad libitum and feed intake was recorded daily. The PER in the *Jatropha* diets was lower than that of casein diet (2.07), especially in the pure *Jatropha* diet (1.37, diet 3), however still within standard levels for cereals and legumes (1.2 and 1.4 for maize and soybeans, respectively). The authors reported no adverse effects at the end of the 28-day study.

Finally, one study in Wistar rats investigated the toxicity of kernel oil (mixed with maize oil in various proportions and added to the feed) and kernel meal (roasted and non-roasted) from a non-toxic genotype of *Jatropha* (grown in the Veracruz, region of Mexico). Food consumption and body weight were lower, especially in male rats fed with meals. This was considered to be a consequence of the low palatability of the feed. No indications of toxicity were found when a diet containing the *Jatropha* oil or defatted meals providing 48% of crude protein was fed to rats for 5–7 weeks (Panigrahi et al., 1984).

– Studies conducted with non-edible *Jatropha* kernels (detoxified and defatted meals)

Some studies were carried out in different animal species using the detoxified non-edible *Jatropha* meal after oil extraction. Those studies were performed in fish, shrimp, rat and pig and all were of short duration (< 28 days) and rather focused on growth performance and feed utilisation generally with limited assessment of toxicological endpoints (Aregheore et al., 2003; Chivandi et al., 2006; Rakshit et al., 2008; Kumar et al., 2011; Wang et al., 2011; Makkar et al., 2012). In these studies, detoxified *Jatropha* meals obtained after solvent extraction of oil from non-edible *Jatropha curcas* plants intended for biofuel production were used. This approach resulted in defatted feed with approximately 60% of protein content, limited amount of fat and some residual levels of PEs. The Panel was of the view that the test material used in these studies, although based on *Jatropha curcas* kernels, was not representative of the NF because of the different final composition and detoxification treatments that have taken place. In addition, the duration of the studies was short. Therefore, these studies were not used in the assessment of the NF.

3.11.3. Subchronic toxicity

No subchronic toxicity studies performed with edible *Jatropha* kernels were reported.

[9] PER = fresh body mass gain (g)/crude protein fed (g).

The Panel considers that the available data set for the assessment of the NF is limited. However, data referring to the toxicity of PEs from *Jatropha* are of relevance.

With reference to the toxicity of PEs, the Panel noted that an NOAEL (no observed adverse effect level) of 0.4 mg/kg bw per day was identified in a subchronic study in pigs by EFSA CONTAM Panel (2015). This study (described by Li et al., 2015) was specifically designed to investigate the use of defatted *Jatropha* kernel meal to replace soybean meal in the diet of growing pigs. The main goal was to measure growth performances (feed intake, average daily body weight gain, gain-to-feed ratio); however, several safety endpoints were also considered (haematological and biochemical investigations, weight of selected organs and histopathology of liver and kidney).

A total of 144 pigs were used, divided in six groups of 12 pigs/sex each. Post-mortem investigations were performed on two pigs sex/group. The study duration was 79 days, feed consumption was measured daily and body weight every 2 weeks. Clinical pathology investigations were performed on study days 14, 28, 56 and 79. While haematology was rather complete (data available only for day 79), biochemical investigations were limited to two hepatic parameters, ALP and ALT, since liver has been identified as the main target.

The *Jatropha* kernel meals were obtained after oil extraction, steam treatment and solvent removal. The final meal contained 0.11 mg/g of PEs and was used to replace from 0% (control) up to 75% of soybean meal protein in the diet. In the six treatment groups, the corresponding amounts of PEs in the diet were 0, 2.75, 5.50, 8.25, 11.00 and 13.75 mg/kg diet for 0%, 15%, 30%, 45%, 60% and 75% protein replacement, respectively.

Growth performances were clearly impaired at concentrations of PEs of $\geq$ 8.25 mg/kg diet (decreased food consumption and growth) while some changes in haematological parameters (mainly lower haemoglobin concentration) at 11.00 and 13.75 mg/kg diet were recorded. In the same two dose groups, an increase in ALP and a decrease in ALT were noted throughout the study.

No changes in organ weights (heart, liver, spleen, lung, kidney and pancreas) were reported. At histopathology, no changes were noted in kidneys. Signs of steatosis or hepatic lipidosis and leucocyte infiltration were recorded at concentrations of $\geq$ 5.50 mg/kg diet (reported as 'mild') and signs of 'degeneration and necrosis' at concentrations of $\geq$ 8.25 mg/kg diet.

An NOAEL was not mentioned by the authors, however, at the concentration of 5.50 mg PEs/kg diet no effects on growth performance were noted. The next concentration of 8.25 mg PEs/kg diet 'did not alter haemato-immunological and pathological parameters but reduced growth performance'. The concentration of 5.50 mg PEs/kg diet was identified as an NOAEL by the CONTAM Panel, corresponding approximately to 0.4 mg PEs/kg bw per day (EFSA CONTAM Panel, 2015).

3.12. Human data

The applicant provided no human data with the NF that were relevant for the safety assessment.

If a non-edible kernel is ingested, clinical symptoms include burning and pain in the mouth or throat and vomiting and this intoxication is generally self-limiting. However, it is reported that sometimes dizziness, nausea, abdominal pain and severe diarrhoea may occur and a few cases of more severe symptoms are also reported, mainly in children due to ingestion of a number of kernels (Devappa et al., 2010a; Shah and Sanmukhani, 2010; Chomchai at al., 2011; EFSA CONTAM Panel, 2015; Langrand et al., 2015; Gupta et al., 2016).

3.13. Allergenicity

This NF has a protein content of up to 32%, according to the proposed specifications. There is little information available on the allergenic potential of *Jatropha curcas* from the literature. The applicant indicated the absence of any report regarding allergic reactions from Mexican areas where people are consuming edible *Jatropha* kernels. However, Maciel et al. (2009) studied the allergenic properties of a non-edible type of *J. curcas* and identified a potentially allergenic 2S albumin, named Jat c 1. This potential allergen was shown to cross-react with the 2S albumin from castor bean. Subsequently, Crespo et al. (2016) characterised the IgE-binding regions of this specific allergen. It is also noted that *Jatropha* kernels contain trypsin inhibitors that are part of the prolamin superfamily that includes the major allergens from tree nuts (EFSA NDA Panel 2014).

Finally, the applicant provided ELISA (peanuts, almonds, pistachios, macadamia nuts and Brazil nuts) and PCR (cashew, hazelnuts and pecans) analysis for the detection of food allergens[10] potentially present in the NF and all gave negative results. However, while the ELISA data are informative on IgE binding of allergens and potential cross-reactive proteins, the PCR analyses do not allow to assess potential cross-reactivities between the NF and the common allergenic foods tested.

The Panel considers that, given the protein content of the NF (20–30 g/100 g), allergic reactions (cross-reactivity and/or primary sensitisation) to the NF are possible.

4. Discussion

The NF is the hydrothermally treated kernels (Chuta), from edible *Jatropha curcas* cultivars (EdibleNut). The applicant intends to market the NF as a whole food, consumed as a snack similar to other nuts or added as an ingredient as kernel or fragments thereof in foods such as cereal bars, breakfast cereals or mixed with dried fruits.

The target population proposed by the applicant for the consumption of the NF is the general population (adolescents and adults).

The potential presence of PEs is the major hazard in the consumption of *Jatropha* kernels (see Sections 3.4.2 and 3.11). The Panel noted that the edible cultivar used in production of the NF is phenotypically undistinguishable from the *Jatropha curcas* varieties that are extensively used for biofuel production and that contain variable levels of PEs. Therefore, the Panel considers it particularly crucial that the process ensures that only the selected cultivar ('non-PE' producing plants) within the applicant's breeding programme is used. The entire production process must ensure that mixing with non-edible kernels does not occur. To control this, all batches must be tested for concentrations of PEs with appropriate sensitive methods prior to being marketed.

The analytical method and the procedures intended for detection of PEs were demonstrated to be able to recognise at least a single non-edible kernel with a content of 1.5 mg PEs/g kernel (PEs content in kernels from non-edible varieties can range from 0.6 up to about 10 mg/g) when mixed with 1000 edible kernels. It is noted that only whole kernels or their fractions are consumed (not flour).

The applicant demonstrated the validity of molecular markers used to check that seeds to be used by the farmers are of the genotype of the edible *Jatropha curcas* cultivars. Although it is plausible that PEs biosynthesis does not occur in these edible plants, low PE levels (below the LOD) might be still present. The Panel made a theoretical exposure calculation to PEs possibly present in the NF (Section 3.8.4) and took into account uncertainties in the analytical method, by considering an LOD corrected with a factor of two (1.5 instead of the 0.75 μg PEs/g de-shelled kernel (as TPA equivalent) as reported in the specifications). Margins of exposure to the NOAEL (no observed adverse effect level) of 0.4 mg PEs/kg bw per day as identified by EFSA CONTAM Panel (2015) are reported in Table 13.

Table 13: Possible exposure to PEs from high (P95) consumption of the NF and estimated margins of exposure (MOE) across age groups

Age group	Possible PEs intake from P95 consumption of the NF (μg/kg bw per day)	MOE
Young children	0.11	3,600
Other children	0.23	1,700
Adolescents	0.19	2,100
Adults	0.45	900

In its scientific opinion in 2015, the CONTAM Panel stated that due to limitations in the toxicological data set and the characteristic of PEs (protein kinase C activation and structural alert for genotoxicity), an MOE (margin of exposure) of 400 would not be sufficient to conclude that the human risk is low. On the basis of the new data generated in the genotoxicity tests performed with the NF and with the

[10] 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, amending Regulations (EC) No 1924/2006 and (EC) No 1925/2006 of the European Parliament and of the Council, and repealing Commission Directive 87/250/EEC, Council Directive 90/496/EEC, Commission Directive 1999/10/EC, Directive 2000/13/EC of the European Parliament and of the Council, Commission Directives 2002/67/EC and 2008/5/EC and Commission Regulation (EC) No 608/2004; J L 304, 22.11.2011, p. 18.

non-edible *Jatropha* kernels, the Panel concludes that no genotoxic concerns have been identified. In addition, the calculated MOEs according to a conservative exposure scenario were 900 or higher. The Panel considers that these margins are sufficient to ensure a safe consumption of the NF.

The presence of anti-nutrient components in the NF is within the ranges found in cereals, legumes and nuts and do not pose safety concerns. The Panel considers that, taking into account the composition of the NF and the proposed conditions of use, its consumption is not nutritionally disadvantageous.

Due to the protein content and possible cross-reactivity, allergic reactions to the NF are possible.

5. Conclusions

The Panel concludes that the NF, the hydrothermally treated *Jatropha curcas* L. kernels from an edible cultivar (Chuta), is safe under the proposed conditions of use.

5.1. Protection of Proprietary data in accordance with Article 26 of Regulation (EU) 2015/2283

The Panel could not have reached the conclusion on the safety of the NF under the proposed conditions of use without the data claimed as proprietary by the applicant: management of Chuta cultivation and use of molecular markers, compositional data including nutritional information and allergens, biological and process contaminants information, analytical methods for PEs detection, procedures for verification of PEs content, toxicological information (*in vitro* genotoxicity studies).

6. Steps taken by EFSA

1) On 19/10/2018 EFSA received a letter from the European Commission with the request for a scientific opinion on the safety of edible *Jatropha curcas* (Chuta) as a novel food. Ref. Ares (2018)5373912.
2) On 19/10/2018, a valid application on edible *Jatropha curcas* (Chuta) as a novel food, which was submitted by JatroSolutions GmbH, was made available to EFSA by the European Commission through the Commission e-submission portal (NF 2018/0177) and the scientific evaluation procedure was initiated.
3) On 18/01/2019, EFSA requested the applicant to provide additional information to accompany the application and the scientific evaluation was suspended.
4) On 18/06/2019, additional information was provided by the applicant through the Commission e-submission portal and the scientific evaluation was restarted.
5) On 26/07/2019, EFSA requested the applicant to provide additional information to accompany the application and the scientific evaluation was suspended.
6) On 15/05/2020, additional information was provided by the applicant through the Commission e-submission portal and the scientific evaluation was restarted.
7) On 08/06/2020, EFSA requested the applicant to provide further clarifications to the additional information provided.
8) On 16/07/2020, additional clarifications were provided by the applicant through the Commission e-submission portal.
9) On 06/08/2020, EFSA requested the applicant to provide further clarifications to the additional information provided.
10) On 16/10/2020, additional clarifications were provided by the applicant through the Commission e-submission portal.
11) On 06/11/2020, EFSA requested the applicant to provide further clarifications to the additional information provided.
12) On 27/11/2020, additional clarifications were provided by the applicant through the Commission e-submission portal and the scientific evaluation was restarted.
13) On 12/02/2021, EFSA requested the applicant to provide additional information to accompany the application and the scientific evaluation was suspended.
14) On 31/08/2021, additional information was provided by the applicant through the Commission e-submission portal.
15) On 20/09/2021, EFSA requested the applicant to provide further clarifications to the additional information provided.

16) On 29/09/2021 additional information was provided by the applicant through the Commission e-submission portal and the scientific evaluation was restarted.
17) During its meeting on 24/11/2021, the NDA Panel, having evaluated the data, adopted a scientific opinion on the safety of hydrothermally treated kernels from edible *Jatropha curcas* (Chuta) as a novel food as a NF pursuant to Regulation (EU) 2015/2283.

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Abbreviations

ADME	absorption, distribution, metabolism and excretion
ALP	alkaline phosphatase
ALT	alanine transaminase
ASU	official collection of analytical methods according to § 64 LFGB (German Food, Commodities and Feed Code)
B.C.	before Christ
BVL	German Federal Office of Consumer Protection and Food safety
bw	body weight
CFU	colony forming units
CONTAM Panel	EFSA Panel on Contaminants
DIN	German Institute for Standardisation
DNA	deoxyribonucleic acid
DON	deoxynivalenol
ELISA	enzyme-linked immunosorbent assay
ESI	electrospray ionisation
FA	fatty acids
FAE	fatty acid esters
GC-FID	gas chromatography - flame ionisation detection
GC-MS/MS	gas chromatography - tandem mass spectrometry
GLP	good laboratory practice
HACCP	hazard analysis and critical control points
HPLC-UV	high-performance liquid chromatography - ultraviolet
ICP-MS	inductively coupled plasma-mass spectrometry
IP6	inositol hexaphosphate
ISO	International Organization for Standardisation
LC-MS/MS	liquid chromatography - tandem mass spectrometry
LFD	lateral flow device
LFGB	German Food, Commodities, and Feed Code
LOD	limit of detection
MCPD	monochloropropane diol
ML	maximum level
MOE	margin of exposure
ND	not detected
NDA Panel	EFSA Panel on Nutrition, Novel Foods and Food Allergens

NOAEL	no observed adverse effect level
NF	Novel food
OECD TG	Organisation for Economic Co-operation and Development Test Guideline
OTA	ochratoxin A
PCD	post-column derivatisation
PCR	polymerase chain reaction
PEs	phorbol esters
PER	protein efficiency ratio
PKC	protein kinase C
QuEChERS	Quick Easy Cheap Effective Rugged Safe
RIP	ribosome-inactivating proteins
SD	Sprague Dawley
SDS	sodium dodecyl sulfate
TI	trypsin inhibitor
TIA	trypsin inhibitory activity
TPA	12-O-tetradecanoylphorbol-13-acetate
UHPLC-MS	ultra high-performance liquid chromatography - mass spectrometry
UHPLC-UV	ultra high-performance liquid chromatography - ultraviolet
ZEA	Zearalenone

Appendix A – Phorbol esters analytical control: procedures for appropriate samplings

Batch weight (tons)	Weight or number of sublots	Number of incremental samples
≥ 500	100 tons	100
> 100 and < 500	5 sublots	100
> 10 and ≤ 100	5 sublots	100
> 5.0 and ≤ 10	–	80
> 1.0 and ≤ 5.0	–	60
> 0.1 and ≤ 1.0	–	30
≤ 0.1	–	10

Each sublot shall be sampled separately. Aggregate samples are composed by a minimum of 10 incremental samples. The minimum amount of an aggregate sample shall be 3.5 kg. This amount may increase proportionally according to the number of incremental samples taken.