Adopted: 24 July 2024

DOI: 10.2903/j.efsa.2024.8985

efsa JOURNAL

SCIENTIFIC OPINION

Safety evaluation of curdlan as a food additive

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Abstract

The EFSA Panel on Food Additives and Flavourings (FAF) provides a scientific opinion on the safety of curdlan as a new food additive used as firming and gelling agent, stabiliser, thickener. Curdlan is a high molecular weight polysaccharide consisting of β-1,3-linked glucose units, produced by fermentation from *Rhizobium radiobacter biovar* 1 strain NTK-u. The toxicological dataset consisted of sub-chronic, chronic and carcinogenicity, reproductive and developmental toxicity studies as well as genotoxicity. In vivo data showed that curdlan is not absorbed as such but is extensively metabolised by the gut microbiota into CO_2 and other innocuous compounds. Curdlan was not genotoxic and was well-tolerated with no overt organ-specific toxicity. Effects observed at very high doses of curdlan, such as decreased growth and increased cecum weight, are common for indigestible bulking compounds and therefore considered physiological responses. In a combined three-generation reproductive and developmental toxicity study, decreased pup weight was observed during lactation at 7500 mg curdlan/kg body weight (bw) per day, the highest dose tested. The Panel considered the observed effects as treatment-related and adverse, although likely secondary to nutritional imbalance and identified a conservative no observed adverse effect level (NOAEL) of 2500 mg/kg bw per day. Despite the limitations noted in the dataset, the Panel was able to conclude applying the margin of exposure (MOE) approach. Given that curdlan and its break-down products are not absorbed and that the identified adverse effect is neither systemic nor local, no adjustment factor was deemed necessary. Thus, an MOE of at least 1 was considered sufficient. The highest exposure estimate was 1441 mg/kg bw per day in toddlers at the 95th percentile of the proposed maximum use level exposure assessment scenario. The Panel concluded that there is no safety concern for the use of curdlan as a food additive at the proposed uses and use levels.

KEYWORDS

CAS number 54724-00-4, gut microbiota, INS No.: 424, *Rhizobium radiobacter biovar* 1, stabiliser, β-1,3-glucan

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SUMMARY

Following a request from the European Commission to the European Food Safety Authority (EFSA), the Panel on Food Additives and Flavourings (FAF) was asked to provide a scientific opinion on the safety of curdlan proposed for use as a food additive (used as a firming agent, gelling agent, stabiliser and thickener), in accordance with Regulation (EC) No 1331/2008 establishing a common authorisation procedure for food additives, food enzymes and food flavourings.

The present evaluation is based on the data submitted by the applicant in a dossier and on additional information provided during the assessment process in response to requests from EFSA.

The proposed food additive is a high molecular weight polysaccharide consisting of β-1,3-linked glucose units, without other linkages or branching. It is produced by pure-culture fermentation from a non-pathogenic and non-toxicogenic *Rhizobium radiobacter biovar 1 strain NTK-u* (also named as *Agrobacterium radiobacter)*. Crude curdlan is then isolated through several steps, which include alkaline dissolution and crystallisation in methanol. The crystallised solution is subjected to purification steps, dried, milled and sieved to obtain the final product. The production organism, *Rhizobium radiobacter*, qualifies for the QPS approach for safety assessment.

Based on scanning electron microscopy (SEM) and energy-dispersive X-ray (EDX) spectroscopy data, and the polysaccharide nature of curdlan having the capacity to disperse and form gels in water (> 55°C) the Panel concluded there is no concern with regard to the potential presence of small particles, including nanoparticles, in the proposed food additive under the proposed conditions of use and considered that curdlan can be assessed following the conventional risk assessment, i.e. EFSA Guidance for submission for food additive evaluations (EFSA ANS Panel, 2012).

The Panel noted that the information provided on five batches of the proposed food additive showed that curdlan is produced according to the method described above and within the proposed specifications. Additionally, the absence of viable cells and of residual DNA from the production strain in the end product were demonstrated.

Concerning the proposed EU specifications, the Panel recommends referring to the fermenting microorganism with the common name *R. radiobacter* in the entry 'Definition'. In addition, the Panel recommends changing the word 'solution' into 'suspension' or 'dispersion' and report that curdlan is insoluble in water and ethanol. In addition, the method for the assessment of 'Solubility in alkali' should be reported. The Panel also recommends introducing the description of 'Gel formation'. As described in the manufacturing process, curdlan is crystallised in methanol and residues of this solvent may result in the final product. The applicant proposed to set a maximum level for residual methanol of 10 mg/kg. The Panel agreed with this proposal and recommended including limits for residual methanol in the proposed EU specifications.

Regarding stability of the proposed food additive, the Panel agreed with applicant's recommended shelf-life period of 3 years from the date of manufacture, on condition that curdlan is stored in tightly closed containers and away from moisture and light. No specific experimental data on the reaction and fate in food of curdlan were provided by the applicant. However, based on data from the literature and tests in model and real food systems provided by the applicant, the Panel considered curdlan to be stable under the proposed conditions of use in food.

Dietary exposure to curdlan was estimated with FAIM (version 2.1) using proposed maximum and typical use levels of curdlan in 25 food categories (FCs).

At the proposed maximum use levels, the mean exposure to curdlan from its proposed use as a food additive ranged from 30 to 744 mg/kg body weight (bw) per day in infants and toddlers, respectively. The 95th percentile exposure to curdlan ranged from 126 to 1441 mg/kg bw per day in the same two population groups. At the proposed typical use levels, the mean and 95th percentiles exposure were 15 to 383 mg/kg bw per day and 61 to 889 mg/kg bw per day, respectively, for the same two population groups.

The main FCs contributing to the total mean exposure estimates for both proposed use levels were 07.1 'Bread and rolls', 07.2 'Fine bakery wares' and 04.2 'Processed fruit and vegetables'. Additionally, for children and adolescents, food category 05.1 'Cocoa and chocolate products as covered by Directive 2000/36/EC' contributed greatly to exposure.

Overall, the Panel considered that the uncertainties identified resulted in an overestimation of the dietary exposure to curdlan at both the proposed maximum and typical use levels.

Additionally, the potential dietary exposure to arsenic and lead from their presence in curdlan as impurities was estimated by assuming that they are present up to a certain limit value, and then by calculating pro-rata to the estimates of exposure to curdlan itself. The potential exposure to arsenic and lead was compared against their relevant reference points (RP). The Panel performed a risk assessment for two concentration scenarios (Table 9), and the resulting figures indicate that in both scenarios (i) and (ii), the potential exposure to inorganic arsenic (assuming that any arsenic in curdlan corresponds to the element in the inorganic form rather than organic form) may be substantial and may raise a concern. Whereas the potential exposure to lead according to both scenarios does not give rise to a concern, with exception for toddlers at 95th percentile of the exposure estimates of curdlan in scenario (i) where the MOE was deemed insufficient. Taking into account the calculations performed by the Panel and the fact that the proposed food additive is not the only potential dietary source of toxic elements, the Panel recommended to lower the proposed specification limits for arsenic and lead. The Panel considered that the choice of maximum limits for toxic elements in the specifications is in the remit of risk manager(s).

The Panel considered that intact curdlan is unlikely to be absorbed in animals and that data in humans indicated no systemic availability of curdlan or its break-down products. It was demonstrated to be extensively metabolised by the gut microbiota and largely excreted as CO_2. There are no data on the identity and/or concentration of intermediate metabolites. Despite the shortcomings identified in the submitted absorption, distribution, metabolism, excretion (ADME) studies

and the lack of direct evidence to prove the metabolic pathway of curdlan, the Panel considered that it is likely that curdlan is broken down into constituent sugars, which are then converted into CO_2 or other innocuous substances.

The toxicological dataset comprised studies on sub-chronic, chronic and carcinogenicity, reproductive and developmental toxicity studies as well as genotoxicity studies. In addition, data concerning immunotoxicity and one study in humans were provided. Overall, the available data did not demonstrate adverse effects of the proposed food additive. The observed effects regarded gastrointestinal symptoms (e.g. soft stools, increased cecum weight), in particular at high doses, which can be attributed to the indigestible nature of the substance and the consequent bulking effect. Curdlan is not genotoxic, and no carcinogenic effects were observed.

In the combined three-generation reproductive and developmental toxicity study, effects on pup weight were observed at 7500 mg curdlan/kg bw per day, the highest dose tested. The Panel considered the observed effects as treatment-related and adverse, although they could derive from maternal nutritional deficiencies, and therefore identified a no observed adverse effect level (NOAEL) of 2500 mg curdlan/kg bw per day based on reduced pup weight during lactation. One human study was provided but considered by the Panel too limited to be used for risk assessment.

Overall, the Panel considered that the available data have certain limitations (e.g. in reporting, characterisation of the test substance) which do not allow to derive an acceptable daily intake (ADI). Nonetheless, the limitations do not prevent the Panel from reaching a conclusion regarding the safety of the use of the proposed food additive curdlan by applying a margin of exposure (MOE) approach.

Taking into account that (i) the production organism, *Rhizobium radiobacter*, qualifies for the QPS approach for safety assessment; (ii) at the proposed maximum use levels, the 95th percentile exposure was up to 1441 mg/kg bw per day in toddlers; (iii) the ADME database demonstrated that, in humans, curdlan and its breakdown products are not absorbed and that in animals curdlan is extensively metabolised by the gut microbiota into carbon dioxide and the remainder is excreted in faeces; (iv) curdlan does not raise a concern for genotoxicity; (v) curdlan is not carcinogenic; (vi) the reduced pup weight in the combined three-generation reproductive and developmental toxicity study, although likely secondary to nutritional imbalance, was considered adverse, leading to the identification of a conservative NOAEL of 2500 mg/kg bw per day; considering also that the observed adverse effect is neither systemic nor local, the Panel considered that no adjustment factor is needed. Thus, an MOE of at least 1 was considered sufficient.

Therefore, the Panel concluded that there is no safety concern for the use of curdlan as a food additive at the proposed uses and use levels.

1 | INTRODUCTION

The present scientific opinion deals with the evaluation of the safety of curdlan, proposed for use as a food additive in several food categories to be used as a firming agent, gelling agent, stabiliser and thickener.

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

1.1.1 | Background

The use of food additives is regulated under the European Parliament and Council Regulation (EC) No 1333/2008[1] on food additives. Only food additives that are included in the Union list, in particular in Annex II to that regulation, may be placed on the market as such and used in foods under the conditions of use specified therein.

An application has been introduced for the authorisation of the use of curdlan (INS No. 424), which is a high molecular weight polysaccharide, as a stabiliser in several food categories. The substance is derived by glucose fermentation from a strain of *Agrobacterium radiobacter biovar*, strain NTK-u.

The interest in the use of curdlan lays in its specific physicochemical properties that distinguish it from other stabilisers.

1.1.2 | Terms of Reference

In accordance with Article 29(1)(a) of Regulation (EC) 178/2002[2] the European Commission asks the European Food Safety Authority to perform a risk assessment and to provide a scientific opinion on the safety of use of curdlan as a food additive in different categories of food, in accordance with Regulation (EC) No 1331/2008[3] establishing a common authorisation procedure for food additives, food enzymes and food flavourings.

1.2 | Information on existing authorisations and evaluations

Curdlan is currently listed as a firming agent, gelling agent, stabiliser and thickener in the Codex Alimentarius General Standard for Food Additives (GSFA) (FAO, online), approved for use in several food categories, under Codex Alimentarium Identification number (INS no.) 424.

Curdlan has been evaluated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) at its 57th meeting. JECFA concluded that data did not indicate any safety concerns for curdlan and an acceptable daily intake (ADI) of 'not specified' was established (JECFA, 2002).

The Panel noted that the applicant has also provided information on existing uses, authorisations and evaluations of curdlan in other countries (United States, Japan, Taiwan and South Korea) (Documentation provided to EFSA No. 1).

2 | DATA AND METHODOLOGIES

2.1 | Data

The applicant has submitted a dossier in support of its application for the authorisation of curdlan as a new food additive for the proposed uses in several food categories (Documentation provided to EFSA No. 1) and additional information provided during the assessment process in response to requests from EFSA (Documentation provided to EFSA No. 2–7).

2.2 | Methodologies

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

The current 'Guidance for submission for food additive evaluations' (EFSA ANS Panel, 2012) was followed by the FAF Panel for evaluating the present application.

[1]Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives. OJ L 354, 31.12.2008, p. 16–33.

[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, p. 1–24.

[3]Regulation (EC) No 1331/2008 of the European Parliament and of the Council of 16 December 2008 establishing a common authorisation procedure for food additives, food enzymes and food flavourings. OJ L 354, 31.12.2008, pp. 1–6.

In animal studies, when the test substance was administered in the feed or in drinking water, but doses were not explicitly reported by the authors as mg/kg bw per day based on actual feed or water consumption, the daily intake is calculated by the Panel using the relevant default values. In case of rodents, the values as indicated in the EFSA Scientific Committee Guidance document (EFSA Scientific Committee, 2012) are applied. In the case of other animal species, the default values by JECFA (2000a) are used. In these cases, the dose was expressed as 'equivalent to mg/kg bw per day'. If a concentration in feed or drinking water was reported and the dose in mg/kg bw per day was calculated (by the authors of the study report or the Panel) based on these reported concentrations and on reported consumption data for feed or drinking water, the dose was expressed as 'equal to mg/kg bw per day'. When in human studies (adults aged above 18 years) the dose of the test substance administered was reported in mg/person per day, the dose in mg/kg bw per day is calculated by the Panel using a body weight of 70 kg as default for the adult population as described in the EFSA Scientific Committee Guidance document (EFSA Scientific Committee, 2012).

3 | ASSESSMENT

3.1 | Technical data

3.1.1 | Identity of the proposed food additive

This opinion deals with the safety evaluation of the proposed food additive curdlan (INS No. 424).

Based on the information provided by the applicant, curdlan is a high molecular weight linear polysaccharide with thermal gelling properties. It consists of D-glucose units linked by β-(1- > 3) bonds without other linkages or branching (see Figure 1). Curdlan is produced by fermentation using a non-pathogenic and non-toxicogenic strain of *Rhizobium radiobacter* biovar 1, also named *Agrobacterium radiobacter* biovar 1 (*A. radiobacter* biovar 1).

Curdlan is described as a white to nearly white powder, odourless to almost odourless and tasteless. It is neutral (non-ionic) and soluble in alkaline solutions at pH ≥ 12 and in dimethylsulphoxide (DMSO). It is insoluble in water, alcohol (ethanol and methanol) and most other organic solvents. It can be dispersed in cold water as a suspension. The applicant stated that curdlan can form both low- and high-set gels (Documentation provided to EFSA No. 1). *'High-set'* gels of curdlan are irreversible gels formed upon heating in aqueous suspension at temperatures above 80°C. *'Low-set'* gels are reversible gels formed when curdlan is heated in aqueous suspension at temperatures from 55°C to 65°C and then cooled at ≤ 40°C.

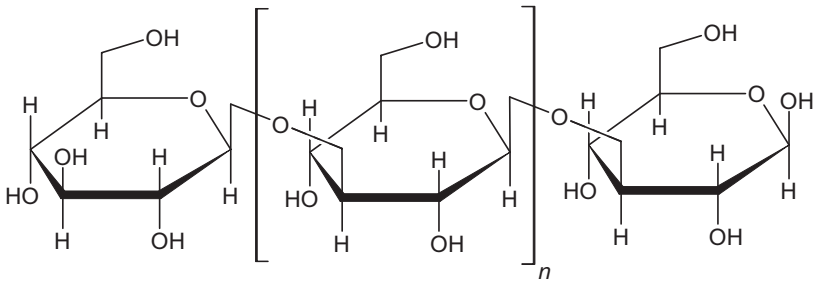

FIGURE 1 Structural formula of curdlan, as reported by the applicant.

Several analyses, e.g. infrared spectroscopy (IR), gas chromatography (GC), thin layer chromatography (TLC), etc. were performed on curdlan in order to investigate its structural identity. The IR spectrum of a batch of curdlan showed an absorption band at 890 cm^{-1} that is characteristic of β-linked polysaccharides (Documentation provided to EFSA No. 1). Upon request from EFSA, a new IR analysis was conducted on a commercially representative batch of curdlan (Documentation provided to EFSA No. 5). The new IR data demonstrated that the identity is consistent with the reference IR spectrum provided in the initial submission (Documentation provided to EFSA No. 1).

3.1.2 | Proposed specifications

The applicant provided the product specifications and informed that the proposed food additive is manufactured within its proposed specifications (Documentation provided to EFSA No. 1–6). The Panel noted that the JECFA Specifications for 'curdlan' are available, and a comparison between the proposal from the applicant and the current JECFA specifications (2006) is presented in Table 1.

TABLE 1 Specifications as proposed by the applicant for 'curdlan', and for 'curdlan' as set in the JECFA Monographs 1 (2006) (Documentation provided to EFSA No. 1–6).

	Specifications as proposed by the applicant for 'curdlan'	JECFA specifications for 'curdlan' (JECFA, 2006)
Synonyms	(1 → 3)-ß-D-Glucan	β-1,3-glucan; INS No.: 424
Definition	Curdlan is a high molecular weight polysaccharide consisting of β-1,3-linked glucose units, produced by pure-culture fermentation from a non-pathogenic and non-toxicogenic *Agrobacterium radiobacter biovar* 1 strain NTK-u. Curdlan consists of β-(1,3)-linked glucose residues and has the unusual property of forming an elastic gel upon heating in its aqueous solution	Curdlan is a high molecular weight polysaccharide consisting of β-1,3-linked glucose units, produced by pure-culture fermentation from a non-pathogenic and non-toxicogenic strain of *Agrobacterium biovar* 1 (identified as *Alcaligenes faecalis* var. myxogenes at the time of discovery) or *Agrobacterium radiobacter*. Curdlan consists of β-(1,3)-linked glucose residues and has the unusual property of forming an elastic gel upon heating its aqueous suspension
CAS number	54724-00-4	54724-00-4
Chemical formula	$H{\cdot}(C_6H_{10}O_5)_n.OH$	$(C_6H_{10}O_5)_n$
Average molecular weight	Not less than 1.4×10^6 Da	
Assay (calculated as anhydrous glucose)	Not less than 80%	Not less than 80%
Description	Odourless or almost odourless, white to nearly white powder	Odourless or almost odourless, white to nearly white powder
Functional uses		Firming agent, gelling agent, stabiliser, thickener
Characteristics		
Identification	Positive	
Solubility		Insoluble in water and ethanol
Solubility in alkali	Passes test	Passes test Suspend 0.2 g of the sample in 5 mL of water, add 1 mL of 3 N sodium hydroxide and shake. The sample dissolves
Gel formation		Heat a 2% aqueous suspension of the sample in a boiling water bath for 10 min and cool. A firm gel forms
Precipitate formation with cupric tartrate	Passes test	Passes test
Purity		
Gel strength	Not less than 600 g/cm^2	Not less than 600 g/cm^2 (2% aqueous suspension)
pH (1% aqueous suspension)	Between 6.0 and 7.5	6.0–7.5
Loss on drying	Not more than 10%	Not more than 10% (60° for 5 h, in vacuum)
Residue on ignition	Not more than 6%	
Sulfated ash	Not more than 6%	Not more than 6%
Nitrogen	Not more than 0.3%	Not more than 0.3%
Heavy metals (as Pb)	Not more than 20 mg/kg	
Lead	Not more than 0.5 mg/kg	Not more than 0.5 mg/kg Determine using an atomic absorption technique appropriate to the specified level
Arsenic	Not more than 2 mg/kg	
Microbiological criteria		
Total plate count	Not more than 1000 cfu/g	Not more than 1000 cfu/g
Coliform Bacteria	Negative in 1 g	
Escherichia coli	Negative in 1 g	Negative in 1 g

The applicant submitted data for different parameters from the analyses of up to nine samples of curdlan (Documentation provided to EFSA No. 1–6). Based on the data submitted, the Panel considered that the proposed food additive is consistently produced and compliant with the proposed specifications, as outlined in Table 1.

The Panel noted that in the entry 'Definition' of the proposed Specifications, curdlan is reported to be produced from *Agrobacterium radiobacter biovar* 1 strain NTK-u. The Panel noted that *Agrobacterium radiobacter* (*A. radiobacter*)is commonly known as *Rhizobium radiobacter*. Hence, the Panel recommends referring to the fermenting bacterium with the common name *Rhizobium radiobacter* (*R. radiobacter*).

Additionally, the Panel noted that in the 'Definition', it is reported that curdlan forms '*an elastic gel upon heating in its aqueous solution*'. In line with the JECFA Specifications and based on the solubility data submitted by the applicant showing that curdlan is insoluble in water, the Panel recommends changing the word 'solution' into 'suspension' or 'dispersion'. The Panel also noted that the entry 'Solubility' should report that curdlan is insoluble in water and ethanol, as reported in the technical dossier (Documentation provided to EFSA No 1–6) and in the JECFA Specifications. In addition, the Panel noted that the entry 'Solubility in alkali' should contain the description of the method employed to assess this parameter, in line with the JECFA Specifications.

The Panel recommends introducing the description of 'Gel formation' in the proposed specifications.

Regarding the toxic elements, following an additional data request from EFSA, the applicant provided analytical data on the content of arsenic (As) and lead (Pb) in five samples (from four batches) of the proposed food additive (Documentation provided to EFSA No. 6). The analyses were performed by an external laboratory using inductively coupled plasma–mass spectrometry (ICP–MS), with method DIN EN 15763. The certificates of analyses were provided, along with the limits of quantification (LOQ), which were 0.01 mg/kg for both As and Pb. As was below the LOQ in all samples whereas the levels of Pb ranged from 0.04 to 0.19 mg/kg ($n = 5$).

In addition, the applicant proposed the entry of 'Heavy metals (as Pb)' to be included in the specifications for the food additive. The Panel does not agree with this proposal and considers this terminology as obsolete. In addition, the Panel noted that no data were provided for the analysis of mercury and cadmium. However, given the nature of the production process, the purification steps that are used, and that the substrate to produce curdlan is glucose, the Panel considers it unlikely that the proposed food additive would be appreciably contaminated with these two toxic elements.

Regarding the entry of 'Sulfated ash', following an additional data request from EFSA, the applicant analysed the elemental profile of the ash residues (Documentation provided to EFSA No. 5). The analyses were performed on three batches through X-ray spectroscopy and ICP–MS. Results indicated that the majority of the ash residue consists of oxygen, carbon and sodium with lesser amounts of phosphorus, sulphur and potassium. Tests were conducted by an external laboratory and the certificates of analysis were provided. As regards sulphated ash, the samples were in line with the proposed specifications.

According to the reported manufacturing process, curdlan is crystallised in methanol and residues of this solvent may be present in the final product. The applicant measured the residual level of methanol in five batches of curdlan. This resulted in a level of 10 mg/kg (Documentation provided to EFSA No. 1), which was also proposed by the applicant as a maximum level for residual methanol in the specifications. The Panel agreed with this proposal (Documentation provided to EFSA No. 1–3) and recommended including a limit for residual methanol in the proposed specifications of 10 mg/kg. The Panel also noted that the value of 10 mg/kg corresponds to the value set in the Directive 2009/32/EC of the European Parliament and of The Council of 23 April 2009,[4] for the 'Maximum residue limits (for methanol) in the extracted foodstuff or food ingredient'.

Up to nine samples of curdlan were analysed for the presence of potential microbiological contaminants. Total plate count and total coliforms and individual types of microorganisms, including *E. coli*, were not detected in the tested samples (Documentation provided to EFSA No. 1–6). In detail, the total (aerobic) plate count was < 1000 colony forming units (CFU)/g, coliform bacteria and *E. coli* were absent in 1 g. All the microbiological tests were supported by the certificate of analyses. The microbiological specification parameters and limits proposed by the applicant were considered by the Panel to be in line with the data provided.

In the proposed specifications, the applicant reported that 'not more than 0.3%' of nitrogen is expected to be found in the food additive. The Panel noted that the certificates of analysis support this specification limit. The applicant informed that nitrogen in the final product derives from the cell culture medium. Nitrogen was determined by the Kjeldahl method (USP), which is an indirect measurement that does not distinguish between protein nitrogen and non-protein nitrogen such as amino acids, amides, ammonium salts, etc. (Mæhre et al., 2018). Taking into account the raw materials and processing aids used to manufacture curdlan, the Panel considered that the nitrogen in the final product results from simple salts and is not expected to raise concerns regarding allergenicity.

The applicant was requested to demonstrate the absence of DNA from the production organism, in compliance with requirements of section 1.3.4.2 of EFSA's Scientific Guidance 2021 (EFSA CEF Panel, 2021). The applicant replied submitting a study performed on nine samples of curdlan. No amplification signal indicative of contamination with genomic DNA was reported in any of the test samples, thus confirming the absence of DNA from the production organism in the final product. The test was performed by an external laboratory and the study report was submitted to EFSA (Documentation provided to EFSA No. 6).

3.1.2.1 | *Particle size and particle size distribution*

Despite that no specifications on particle size were proposed, the applicant stated that the particle size of curdlan, as measured using a laser diffraction (LD) method, ranges between 30 and 500 μm, wherein the majority of curdlan was in the region of ~ 100–200 μm (Documentation provided to EFSA No. 2).

The Panel noted that LD methods are not considered suitable to investigate the presence of nanosized particles as these methods do not allow to accurately measure the size of the constituent particles as required by the Guidance on Particle-TR

[4]Directive 2009/32/EC of the European Parliament and of the Council of 23 April 2009 on the approximation of the laws of the Member States on extraction solvents used in the production of foodstuffs and food ingredients. OJ L 141, 6.6.2009, p. 3–11.

(EFSA Scientific Committee, 2021) and are prone to bias for polydisperse materials (Mech, Rauscher, Babick, et al., 2020; Mech, Rauscher, Rasmussen, et al., 2020; Rauscher et al., 2019).

Following a further EFSA request, the applicant provided results from scanning electron microscopy (SEM) analysis coupled with Energy Dispersive X-Ray spectrometry (EDX) on five batches of pristine (i.e. as produced) curdlan material (Documentation provided to EFSA No. 7) dispersed in toluene by vortexing. The sample preparation and method of analysis were described in detail. The particle size distribution, shape and elemental composition of the material were analysed. Three independent number-based particle size distributions referring to length, width and thickness and their descriptive parameters were provided. The applicant reported that the constituent particles of curdlan contains large irregular polyhedron-shaped or spheroidal particles with entangled structure, with many small particles on the surface or embedded within the holes. The surface of larger particles appears to be irregular because of their tangled structure, but at high magnifications the surface is only slightly rippled. Smaller particles have different shapes (spheroidal, globular or polyhedral) and they are either embedded or laid on top of the larger ones.

Quantitative analysis of SEM images revealed that in three batches of curdlan, the median (d50) particle size ranged from 161 to 243 nm, 117 to 174 nm and 85 to 97 nm in length, width and thickness, respectively. In the remaining two batches, the median particle size ranged from 272 to 397 nm, 178 to 251 nm and 108 to 132 nm in length, width and thickness, respectively.

The applicant concluded that pristine curdlan (as produced) contains a fraction of small particles, including nanoparticles, as defined in the EFSA Guidance on Particle-TR (EFSA Scientific Committee, 2021).

The Panel noted a high polydispersity in the size of constituent particles of curdlan among the analysed products, with observed particles ranging from as small as 22 nm to as large as 450 μm.

The applicant further provided the results of a study aimed at assessing the potential presence of small particles, including nanoparticles, in curdlan mimicking the realistic conditions of use in food (Documentation provided to EFSA No. 7). Gels obtained from five distinct batches of curdlan were investigated via SEM–EDX. Specifically, for each analysed batch two curdlan concentrations (4% and 10% w/w) and two gelling temperatures (60°C for low-set gels and 90°C for high-set gels) were tested. The applicant declared that the gel preparation followed protocols designed to replicate the actual conditions of curdlan use in food applications. After gel formation, small sections of each gel were excised with a scalpel and deposited onto a clean silicon wafer to expose either the surface or cross-section of the gel. Subsequently, samples underwent vacuum drying and gold-sputter coating for SEM–EDX imaging, with images randomly acquired from both areas. No evident differences were noted between the appearance of the gel on the surface and in the cross-section, resulting in random image collection without further distinction. To detect the presence of small particles, samples were observed thoroughly using increasing magnifications up to x80000 and collecting images of the objects identified in the samples. The applicant reported that in all tested conditions the curdlan gel is smooth, and some clusters of gelled material of various sizes and shapes, forming ripples or lumps were detected but no well-defined particles attributable to pristine particles of curdlan were found.

The Panel observed that SEM images of both low-set and high-set curdlan gels at concentrations of 4% and 10% displayed predominantly smooth and uniform textures with occasional accumulations, appearing as lumps or aggregations.

Additionally, the applicant provided an SEM–EDX study of curdlan in water at a concentration of 0.5% w/w aiming at investigating the curdlan gel at the typical use level of curdlan in food (Documentation provided to EFSA No. 7). Five batches of the product, along with two gelling temperatures (60°C for low-set conditions and 90°C for high-set conditions), were employed to explore the behaviour of curdlan particles under realistic use conditions. Following the preparation process, regardless of the heating condition and batch tested, all mixtures exhibited two distinct phases: a gelled portion and an aqueous fraction. To investigate the potential presence of small particles within these two fractions, all samples underwent comprehensive SEM–EDX analysis at varying magnifications up to x120,000.

According to the applicant the gelled fractions of the mixtures appeared homogeneous, with micrometric or submillimetric clumps of material without well-defined particles which could be attributed to pristine curdlan. On the contrary, the aqueous phases revealed regions containing small circular objects uniformly dispersed throughout the sample, with sizes falling within the nano range. The applicant stated that the SEM–EDX data, coupled with particle morphology, suggested that small spheroidal objects in the aqueous phase could potentially be artefacts resulting from the recrystallisation of inorganic salts during sample air-drying. Further investigations by applying TEM–EDX were conducted on the aqueous phase after vacuum-drying of the samples. The change of the drying technique resulted in the formation of irregular or branched crystallisations surrounded by spheroidal particles, with their size decreasing as the distance from the main crystals increased. The applicant stated that this characteristic pattern is commonly observed when solutions containing inorganic salts are dried, strongly suggesting that the small spheroidal particles found in the aqueous phase of curdlan mixtures were likely artefacts stemming from the drying process rather than actual curdlan particles. Moreover, TEM–EDX analysis revealed that these spheroidal particles were likely composed of phosphates, as indicated by their EDX spectra showing the presence of oxygen and phosphorus, along with calcium, sodium or magnesium (Documentation provided to EFSA No. 7).

The applicant concluded that considering the collected data, irrespective of the heating condition employed (i.e. low-set or high-set) or the fraction constituting the curdlan mixture in water (i.e. gelled part or aqueous phase), no curdlan-based nanoparticles were detected at a concentration of 0.5% w/w, corresponding to real use conditions (Documentation provided to EFSA No. 7).

The Panel noted that the applicant conducted comprehensive studies aimed at analysing curdlan in gel form mimicking the realistic conditions and levels of use in food. The Panel highlights that currently no standardised methods are available

to determine the presence of small particles, including nanoparticles, in the pristine form or when used in food of polysaccharide thickening and gelling agents. Thus, some uncertainties remain in the provided measurements. Nevertheless, the Panel considered that the provided data indicates that curdlan gels, both low-set and high-set at 0.5% concentration, do not contain small particles including nanoparticles of pristine curdlan. SEM images reveal small lumps of material within a smooth film, which were confirmed through EDX data analysis to be recrystallised salts.

Overall, based on the data provided, and the polysaccharide nature of curdlan having the capacity to disperse and form gels in water (see Section 3.1.1), the Panel considered that the presence of a fraction of small particles including nanoparticles of pristine curdlan in food at the proposed uses and use levels can be excluded, given the conditions of use.

Therefore, the Panel concluded there is no concern with regard to the potential presence of small particles, including nanoparticles, in the proposed food additive at the proposed uses and use levels and considered that curdlan can be assessed following the conventional risk assessment, i.e. EFSA Guidance for submission for food additive evaluations (EFSA ANS Panel, 2012).

3.1.3 | Manufacturing process

3.1.3.1 | *Identity of the raw materials and processing aids*

The list of raw materials and processing aids used for producing curdlan was provided, along with the respective CAS numbers. In addition, the applicant submitted the specifications of the individual raw materials, and informed that they are all suitable for pure-culture fermentation (Documentation provided to EFSA No. 1).

3.1.3.2 | *Description of the manufacturing process*

Curdlan is produced by pure-culture fermentation of *R. radiobacter biovar* 1 strain NTK-u in high glucose media. The production process occurs in two main stages: (1) production of the strain culture and fermentation and (2) purification. Crude curdlan is purified through several steps, which include alkaline dissolution and crystallisation in methanol. The crystallised solution is subjected to purification steps, i.e. centrifugation, washing and centrifugation once more. Then the product is dried, milled and sieved to obtain the final product. Monitoring steps are applied throughout the manufacturing process to ensure that the final product meets the internal specifications.

3.1.3.3 | *Characterisation of the production organism*

According to the applicant, the production strain of curdlan, i.e. *R. radiobacter* biovar 1 strain NTK-u, was taxonomically identified by analysing a 438 bp sequence of the 16S rDNA. *R. radiobacter* (synonym *A. radiobacter*) is present in soil. The production strain was deposited in the American Type Culture Collection (ATCC) as *Alcaligenes faecalis* subsp. *myxogenes* under the strain designation NTK-u (IFO 13140, NCIB 12233) (ATCC 21680) by Takeda Chemical Industries, Ltd.2 (ATCC, 2016) (Documentation provided to EFSA No. 1).

The production strain was obtained by chemical mutagenesis. It is characterised by the capacity to form the gel of (1–3)-β-D-glucan.

Several studies showed no cytotoxicity or pathogenicity of *R. radiobacter* strain NTK-u. After incubating HeLa cells with the bacterial culture supernatant, no cytotoxicity was observed (Documentation provided to EFSA No. 1). No significant effects were observed in male ICR-JCL mice (8/group) given a single oral dose (10 or 20 g/kg body weight) suspension of dead cells with regards to clinical observations and mortality, nor were any changes observed macroscopically upon autopsy examination (Documentation provided to EFSA No. 1). Similarly, an oral suspension of viable cells (10^{9-10}) for 2 days or a single dose via intravenous, intraperitoneal or intracerebral injection in ddY-SLC strain male mice did not induce any pathogenic effects (Documentation provided to EFSA No. 1).

The Panel noted that the applicant provided information on the taxonomic classification of the *R. radiobacter (*synonym *A. radiobacter)* biovar 1 (Table 2), and on the taxonomic characteristics of strain NTK (Table 3).

TABLE 2 Taxonomic classification of the *Rhizobium radiobacter* (synonym *Agrobacterium radiobacter*) biovar 1 strain NTK-u as provided by the applicant (Documentation provided to EFSA No. 1).

Kingdom	Bacteria
Phylum	Proteobacteria
Class	Alphaproteobacteria
Order	Rhizobiales
Family	Rhizobiacae
Genus	*Rhizobium/Agrobacterium*
Species	*Rhizobium radiobacter/Agrobacterium radiobacter*
Strain	NTK-U

TABLE 3 Taxonomic characteristics of strain NTK-u as provided by the applicant (Documentation provided to EFSA No. 1).

Morphological characteristics	
Shape	Short rods (singly or paired, with occasional chains of three or branching)
Size	0.3–0.5 μm × 0.8–1.0 μm
Motility	Motile
Gran staining	Negative
Spore	Not produced
Physiological characteristics	
Temperature for growth	15–40°C (optimum 28–32°C)
pH for growth	4.7–9.7 (optimum 6–7)
Relation of oxygen	Aerobic
Methyl red test	Negative
Taxonomic characteristics	
Voges-Proskauer test	Negative
Indole	Not produced
Hydrogen sulphide	Not produced
Ammonia	Slightly produced
Nitrates	Reduced to nitrites
Catalase	Produced
Citric acid	Slightly utilised
Ammonium salts, nitrates, urea	Utilised as nitrogen sources
Utilisation of carbon sources	Glucose, sucrose, galactose, mannose, xylose, maltose, ribose, sorbitol, succinate and fumarate

3.1.3.4 | *Safety of the production strain*

Rhizobium radiobacter, synonym of *A. radiobacter*, qualifies for the QPS approach for safety assessment and was considered as safe with the qualification 'for production purposes only' (EFSA BIOHAZ Panel, 2024).

Upon request from EFSA, the applicant provided the results of a minimum inhibitory concentration (MIC) analysis (Documentation provided to EFSA No. 4). For several antibiotics, the results show resistance above the cut off values indicated as reference values for *Enterobacteriaceae* in the EFSA FEEDAP Guidance (2018). The reasoning of the applicant that these resistances would be of an intrinsic nature cannot be accepted. The cited references are irrelevant to demonstrate this issue.

To follow up on these findings, the applicant was requested to analyse the whole genome sequence (WGS) for the presence of antimicrobial resistance genes following EFSA's statement on the requirements for whole genome sequence analysis of microorganisms intentionally used in the food chain (EFSA, 2021). The presence of antimicrobial resistance genes in the genome of the production strain was analysed on the WGS by the 'Resistance Gene Identifier' software towards the Comprehensive Antibiotic Resistance Database (CARD) (Documentation provided to EFSA No. 5). Besides the presence of [redacted] and a gene encoding a [redacted] were also discovered. [redacted]

3.1.3.5 | *Absence of viable cells of the production strains in the end product*

The absence of viable cells of the production strain in the final product was confirmed by the applicant in three different production batches of curdlan, each tested in triplicate (Documentation provided to EFSA No. 8). To this end, 10 g of product from each sample were dispersed in 500 mL of 0.9% NaCl; 50 mL of this dispersion, corresponding to 1 g of product, was inoculated on non-selective and selective agar and the plates were incubated for 6 days at 28°C. No colonies of the production strain were produced. A positive control was included.

3.1.3.6 | *Absence of DNA of the production strain in the end product*

The absence of DNA of the production strain *R. radiobacter* strain NTK-u was confirmed in nine samples of the end product by polymerase chain reaction, using primers that would amplify a fragment of 572 bp [redacted] with an LOD lower than 10 ng of spiked DNA/g product (Documentation provided to EFSA No. 8).

3.1.4 | Methods of analysis in food

The applicant reported information on a method for the determination of curdlan in food, based on the use of exo-β-1,3-glucanase, as described by Kusui et al. (1993). This method is based on the use of the purified enzyme exo-β-1,3-glucanase, derived from the fungus *Trametes sanguinea*, to hydrolyse curdlan into D-glucose monomers. Exo-β-1,3-glucanase is an enzyme specific for cleaving β-1,3-glucans into glucose and does not hydrolyse other β-linked glucans. First, the food matrix to be analysed is repeatedly washed in water to remove hydrophilic substances including any glucose originally present in the food product, then curdlan is extracted into aqueous solution with sodium hydroxide. The extracted curdlan is hydrolysed to its glucose monomers using the purified exo-β-1,3-glucanase, subsequently the released glucose units are quantified using a glucose oxidase assay by detecting the absorbance at 530 nm.

The applicant applied the above method to model systems containing known levels of added curdlan (i.e. gelled curdlan, mixture of curdlan and starch and curdlan in homogenate of ham, with addition levels from 12.5% to 90% w/w), and to a number of food products (i.e. pressed ham, canned orange jelly, dry dessert mixes, biscuits and dried noodles, with addition levels of 0.22–5.25% w/w). The recovery of the method was ≥ 92% for these samples. Control samples with no added curdlan tested blank.

3.1.5 | Stability of the substance, and reaction and fate in food

The Panel noted that the applicant has provided information on the stability of the proposed food additive for up to 6 years, under standard storage conditions at room temperature. However, the applicant recommended a shelf-life period of 3 years from the date of manufacture, on condition that curdlan is stored in tightly closed containers and away from moisture and light. The stability of curdlan is claimed to be supported by a study performed at room temperature for up to 6 years. Three batches of curdlan were stored in polyethylene bags, within carton boxes, for the duration of the stability study. Sampling was performed regularly up to six years to assess the stability concerning the physicochemical and microbiological parameters. The only notable change over time was an increase in the loss on drying; however, samples remained within the specification limits of not more than 10% (i.e. 9.57 ± 0.6%; $n = 3$). Based on these results, curdlan is stable under the tested storage conditions, for the 3 years of shelf-life.

However, the Panel noted that the raw data supporting the above stability test were not provided by the applicant, since only tables showing conformity of curdlan to the internal specifications were submitted to EFSA (Documentation provided to EFSA No. 1).

Given the analyses for curdlan performed by the applicant in several models and real food systems (Section 3.1.4), and the evidence from the literature submitted by the applicant, the Panel considered curdlan to be stable under the proposed conditions of use in food.

3.2 | Proposed uses and use levels

Through the current application, an authorisation is sought with regards to the food categories listed in Table 4.

The Panel noted that the applicant has submitted proposed typical and maximum use levels of curdlan (in mg/kg or mg/L) for 25 food categories according to Annex II of Regulation (EC) No 1333/2008, part D.

TABLE 4 Proposed uses and use levels for curdlan (in mg/kg or mg/L) (Documentation provided to EFSA No. 6).

Food category number	Food category name	Restrictions or exception	Proposed used level (mg/L or mg/kg as appropriate)	
			Typical	Maximum
01.7.5	Processed cheese		30,000	40,000
02.2	Fat and oil emulsions mainly of type water-in-oil		30,000	50,000
03	Edible ices		10,000	30,000
04.2	Processed fruit and vegetables		30,000	50,000
05.1	Cocoa and chocolate products as covered by Directive 2000/36/EC		30,000	30,000
05.2	Other confectionery including breath refreshening microsweets (with and without added sugar)		20,000	50,000
05.3	Chewing gum		5000	10,000
05.4	Decorations, coatings and fillings, except fruit-based fillings covered by category 4.2.4	Curdlan is intended for use in fillings only	5000	5000
06.3	Breakfast cereals		10,000	20,000

(Continues)

TABLE 4 (Continued)

Food category number	Food category name	Restrictions or exception	Proposed used level (mg/L or mg/kg as appropriate)	
			Typical	Maximum
06.4	Pasta		10,000	20,000
06.5	Noodles		10,000	20,000
06.6	Batters		5000	5000
07.1	Bread and rolls		10,000	20,000
07.2	Fine bakery wares		10,000	20,000
08.2	Processed meat		10,000	20,000
08.3	Meat products		5000	20,000
09.2	Processed fish and fishery products including molluscs and crustaceans		10,000	30,000
10.2	Processed eggs and egg product		20,000	20,000
12.4	Mustard		30,000	30,000
12.5	Soups and broths		5000	10,000
12.6	Sauces		10,000	30,000
12.7	Salads and savoury based sandwich spread		10,000	30,000
12.9	Protein products, excluding products covering in category 1.8		70,000	80,000
15.1	Potato-, cereal-, flour- or starches-based snacks		5000	10,000
16	Desserts excluding products covered in category 1, 3 and 4		10,000	30,000

3.3 | Exposure data

3.3.1 | Food consumption data used for exposure assessment

EFSA Comprehensive European Food Consumption Database

To assess whether the proposed uses and use levels (Table 4) pose a possible health concern, the potential chronic dietary exposure to curdlan was calculated by the Panel using the proposed use levels with the Food Additive Intake Model (FAIM; version 2.1)[5] (Documentation provided to EFSA No. 6).

FAIM contains food consumption data from the EFSA Comprehensive European Food Consumption Database (Comprehensive Database). Since 2010, this database has been populated with national data on food consumption at a detailed level. Competent authorities in the European countries provide EFSA with data on the level of food consumption by the individual consumer from the most recent national dietary survey in their country (cf. Guidance of EFSA on the 'Use of the EFSA Comprehensive European Food Consumption Database in Exposure Assessment' (EFSA, 2011)). The version of the Comprehensive database taken into account in the exposure assessment was published in November 2023.[6]

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

Food consumption data from infants, toddlers, children, adolescents, adults and the elderly were used in the exposure assessment with FAIM. For the present assessment, food consumption data were available from 43 different dietary surveys carried out in 22 European countries (Table 5).

[5]https://www.efsa.europa.eu/sites/default/files/applications/FAIM-instructions.pdf.
[6]https://www.efsa.europa.eu/en/data-report/food-consumption-data.

TABLE 5 Population groups considered for the exposure estimates of curdlan with FAIM.

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

[a]The term 'toddlers' in the Comprehensive Database (EFSA, 2011) corresponds to 'young children' in Regulations (EC) No 1333/2008 and (EU) No 609/2013.[7]
[b]The terms 'children' and 'the elderly' correspond, respectively, to 'other children' and the merge of 'elderly' and 'very elderly' in Comprehensive Database (EFSA, 2011).

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

Food categories considered for the exposure assessment of curdlan

All food categories in which the use of curdlan was proposed, were considered in FAIM. For the food category (FC) 05.4 'Decorations, coatings and fillings, except fruit-based fillings covered by category 4.2.4', the applicant proposed the restriction 'intended for use in fillings only' (see Table 4). In FAIM, it is not possible to distinguish fillings from other foods within this food category, and therefore, the whole food category was considered in the exposure assessment at the level of 5000 mg/kg (proposed maximum/typical level for fillings).

3.3.2 | Exposure to curdlan from its proposed use as food additive

The applicant provided an estimate of the dietary exposure to curdlan based on FAIM. However, the Panel noted that the applicant (1) did not correctly apply the proposed use levels to the food categories (sub-categories missing), and (2) did not perform an exposure assessment using the proposed typical use levels.

For these reasons, the Panel updated the estimates of the exposure using FAIM with the proposed maximum and proposed typical use levels. The summary of the results per population group is provided in Table 6. Detailed results per population group and survey are presented in Annex A.

TABLE 6 Summary of dietary exposure to curdlan from its proposed use levels as a food additive in six population groups (minimum–maximum across the dietary surveys in mg/kg bw per day), across all proposed food categories.

Estimated exposure (mg/kg bw per day)	Infants (12 weeks-11 months)	Toddlers (12–35 months)	Children (3–9 years)	Adolescents (10–17 years)	Adults (18–64 years)	The elderly (≥ 65 years)
Proposed maximum level exposure assessment scenario						
Mean	30–476	150–744	229–545	125–283	81–212	67–19
95th percentile	126–935	381–1441	423–951	238–537	162–363	138–378
Proposed typical level exposure assessment scenario						
Mean	15–293	76.7–383	122–289	65–151	42–106	34–102
95th percentile	61–525	198–889	244–592	125–299	84–187	72–202

[7]Regulation (EU) No 609/2013 of the European Parliament and of the Council of 12 June 2013 on food intended for infants and young children, food for special medical purposes, and total diet replacement for weight control and repealing Council Directive 92/52/EEC, Commission Directives 96/8/EC, 1999/21/EC, 2006/125/EC and 2006/141/EC, Directive 2009/39/EC of the European Parliament and of the Council and Commission Regulations (EC) No 41/2009 and (EC) No 953/2009 Text with EEA relevance. OJ L 181, 29.6.2013, p. 35–56.

At the proposed maximum use levels, the mean exposure to curdlan ranged from 30 mg/kg bw per day in infants to 744 mg/kg bw per day in toddlers. The 95th percentile exposure to curdlan ranged from 126 mg/kg bw per day in infants, to 1441 mg/kg bw per day in toddlers.

At the proposed typical use levels, the mean exposure to curdlan ranged from 15 mg/kg bw per day in infants to 383 mg/kg bw per day in toddlers. The 95th percentile exposure to curdlan ranged from 61 mg/kg bw per day in infants, to 889 mg/kg bw per day in toddlers.

Main food categories contributing to exposure to curdlan using the proposed use levels

Using the maximum proposed use levels, the main food categories contributing to the total mean exposure estimates for all population groups were FCs 07.1 'Bread and rolls' and 07.2 'Fine bakery wares'. For all population groups, with the exception of adolescents, FC 04.2 'Processed fruit and vegetables' was also a main contributor to the exposure to curdlan. Additionally, for children and adolescents, FC 05.1 'Cocoa and chocolate products as covered by Directive 2000/36/EC contributed greatly to the exposure.

Using the typical proposed use levels, the main food category contributing to the total mean exposure estimates for all population groups was FC 04.2 'Processed fruit and vegetables'. FCs 07.1 'Bread and rolls' and 07.2 'Fine bakery wares' were main contributors to the exposure for all population groups, with the exception of infants. Additionally, for children and adolescents, FC 05.1 'Cocoa and chocolate products as covered by Directive 2000/36/EC contributed greatly to the exposure.

Annex A indicates all the contributing food categories by population groups.

Uncertainty analysis

In accordance with the guidance provided in the EFSA opinion related to uncertainties in dietary exposure assessment (EFSA, 2007), the following sources of uncertainties were considered and summarised in Table 7.

TABLE 7 Qualitative evaluation of influence of uncertainties on the dietary exposure estimate.

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

[a]+, uncertainty with potential to cause overestimation of exposure; –, uncertainty with potential to cause underestimation of exposure.

Curdlan is requested to be authorised in 25 food categories. For all food categories considered, it was assumed that 100% of the foods belonging to these food categories will contain curdlan at the typical or at the maximum proposed use levels. As it is not likely that this will be the case in practice, the Panel considered overall that the uncertainties identified resulted in an overestimation of the exposure to curdlan in European countries available in the EFSA Comprehensive database at both the typical and the maximum proposed use levels.

3.3.3 | Anticipated exposure to toxic elements from the use of the proposed food additive

As indicated in Section 3.1.2 (Table 1), curdlan may contain arsenic and lead as impurities. The potential exposure to these impurities from the use of curdlan can be calculated by assuming that they are present up to a certain limit value, and then by calculating pro-rata to the estimates of exposure to curdlan itself. Considering the proposed maximum level exposure assessment scenario, the highest estimated mean and 95th percentile exposure were 744 and 1441 mg/kg bw per day, for toddlers, respectively (Table 6).

The level of impurities in the food additive combined with these estimated exposure levels to curdlan could result in an exposure which can be compared with the reference points (RP) in Table 8. It is assumed that any arsenic in curdlan corresponds to the element in the inorganic form rather than organic form. Consequently, the RP for inorganic arsenic was used for the comparison (Table 8).

TABLE 8 Reference points for impurities present in curdlan.

Impurity/constituent/RP	Basis/reference
Lead (Pb)/0.5 μg/kg bw per day ($BMDL_{01}$)	The reference point is based on a study demonstrating perturbation of intellectual development in children with the critical response size of 1 point reduction in IQ. The EFSA CONTAM Panel mentioned that a 1-point reduction in IQ is related to a 4.5% increase in the risk of failure to graduate from high school and that a 1-point reduction in IQ in children can be associated with a decrease of later productivity of about 2%. A risk cannot be excluded if the exposure exceeds the $BMDL_{01}$ (MOE lower than 1) (EFSA CONTAM Panel, 2010)
Inorganic arsenic (iAs)/0.06 μg/kg bw per day ($BMDL_{05}$)	The reference point is based on a benchmark dose lower confidence limit ($BMDL_{05}$) of 0.06 μg/kg bw per day identified for skin cancer. The reference point is considered to cover lung cancer, bladder cancer, skin lesions, ischemic heart disease, chronic kidney disease, respiratory disease, spontaneous abortion, stillbirth, infant mortality and neurodevelopmental effects. An MOE of 1 would correspond to the exposure level that is associated with a 5% increase relative to the background incidence for skin cancer, based on the available data. An MOE of 1 raises a health concern Because there are no precedents in EFSA for identification of a MOE of low concern, when using a BMDL derived from human cancer data the CONTAM Panel decided not to determine a value for an MOE of low concern (EFSA CONTAM Panel, 2024)

Abbreviations: BMDL, benchmark dose (lower confidence limit); BW, body weight; MOE, margin of exposure; RP, reference point.

The risk assessment of the undesirable impurities helps to determine whether there could be a possible health concern if these impurities would be present at a certain level in the proposed food additive. The assessment is then performed by calculating the MOE (margin of exposure) by dividing the RP (i.e. BMDL, Table 8) by the exposure estimate (Table 6).

The Panel assessed the risk that would result if these toxic elements were present in curdlan according to two concentration scenarios: (i) considering their presence at the proposed specification limits and (ii) considering the data reported at the rounded up highest measured value (i.e. for lead) and, in the absence of any measured value, at the LOQ (i.e. for arsenic), modulated by the Panel by applying a factor of 10 to allow flexibility with respect to representativeness, homogeneity and differing analytical methods.

The outcome of the risk assessment of the FAF Panel (Table 9) illustrates the health impact that could result if these toxic elements are present in curdlan at the proposed specification limits or at the modulated/highest levels.

TABLE 9 Risk assessment for arsenic and lead present in curdlan as impurities according to two concentration scenarios, using the reference points in Table 8.

Exposure to curdlan (mg/kg bw/day)	(i) considering the presence of toxic elements at the proposed specification limits for curdlan	
	MOE for Pb at 0.5 mg/kg	**MOE for iAs at 2 mg/kg**
743.5[a]	1.3	0.04
1441.4[b]	0.7	0.02
	(ii) considering the presence of toxic elements at the rounded up highest measured value for Pb and for As at the LOQ modulated by the Panel by applying a factor of 10	
Exposure to curdlan (mg/kg bw/day)	**MOE for Pb at 0.2 mg/kg**	**MOE for iAs at 0.1 mg/kg**
743.5[a]	3.4	0.8
1441.4[b]	1.7	0.4

Abbreviations: As, arsenic; BW, body weight; LOQ, limit of quantification; MOE, margin of exposure; Pb, lead.

[a]Highest mean exposure among different population groups (proposed maximum use level exposure assessment scenario–toddlers [Table 6]).

[b]Highest 95th percentile exposure among different population groups (proposed maximum use level exposure assessment scenario–toddlers [Table 6]).

The resulting figures (Table 9) indicate that for arsenic the calculated MOEs were considered to be insufficient, irrespective of whether these were based on the proposed specification limit (scenario i) or on the modulated/highest levels (scenario ii). For lead, the Panel noted that the MOE was deemed insufficient for the 95th percentile exposure based on the proposed specification limit.

Taking into account the calculations performed by the Panel (Table 9) and the fact that the proposed food additive is not the only potential dietary source of toxic elements, the Panel recommended to lower the proposed specification limits for arsenic and lead. The Panel considered that the choice of maximum limits for toxic elements in the specifications is in the remit of risk manager(s). The numbers used here were merely taken to support the risk assessment of these toxic elements as presented above.

3.4 | Biological and toxicological data

Within the application dossier, unpublished study reports as well as scientific publications considered by the applicant relevant to the safety assessment of curdlan were submitted.

The Panel noted that in the studies provided by the applicant, the materials named PS 13140 and gelled PS 13140 refer to 'curdlan' and 'gelled curdlan', respectively. The batch numbers used were described but no specifications or certificate of analyses were available. 'Gelled curdlan' was prepared by heating a 10% suspension of 'curdlan' in distilled water (i.e. 100 g of curdlan in 1 kg of gelled curdlan) to 90°C for 15 min and then cooling at room temperature.

Based on the nature of the material, the information on its metabolic fate and the dose levels used in the old studies (very high doses up to 15% and 40% in the diet, for curdlan and gelled curdlan respectively), the Panel considered that detailed information on the tested material in comparison to the proposed food additive was unnecessary as the hazards would not differ significantly and an evaluation based on these old studies would be relevant.

3.4.1 | Absorption, distribution, metabolism and excretion (ADME)

The Panel noted that the applicant submitted results from two in vivo labelling studies aimed at assessing the absorption of curdlan in rats and humans (Documentation provided to EFSA No. 1). Upon request from EFSA, the applicant provided a rationale for the adequacy of the information on the metabolic fate of curdlan (Documentation provided to EFSA No. 2 and 3).

After oral administration of ^{14}C-radiolabelled curdlan, faecal excretion was a major route (~ 7.7%–38% of radiolabelled dose) of elimination, while only minor amounts were excreted in the urine (< 4% of radiolabelled dose). Most of the ^{14}C-radiolabel was found as radiolabelled carbon dioxide (~ 39%–89% of the radiolabelled dose) being in the air of the metabolic cage and trapped in the potassium hydroxide (KOH) (Takeda Chemical Industries, Ltd., 1972a in Documentation provided to EFSA No. 1). Pre-treatment of rats with tetracycline reduced the amount of radiolabelled carbon dioxide found in the metabolic cage (~three-fold decrease) and increased the amount of total ^{14}C-radiolabel excreted in the faeces (~ 2.6-fold increase). According to the applicant, this suggests the involvement of the gut microbiota in the metabolism of curdlan to carbon dioxide and therefore the CO_2 in the metabolic cage comes from the gut of the animals. The Panel agreed to this interpretation of the data.

Similar findings were reported in a human study conducted on 4 healthy male volunteers given a single oral dose of ^{14}C-curdlan with and without pre-treatment with antibiotic regimen for 3 days. ^{14}C-curdlan was received as gelatine capsule of 512.2 mg containing 20 μCi. Blood samples were obtained at 0, 1, 2, 4, 6, 8 and 24 h. Faecal collections were obtained on days 0, 1, 2 and 3. No radioactivity was found in the blood samples collected following administration of ^{14}C–curdlan indicating no systemic availability of curdlan or break-down products. Faecal excretion was reported to be the main pathway of elimination and the gut microbiota were responsible for metabolism to carbon dioxide, despite incomplete faecal collection which led to low total recovery of administered radiolabel (Takeda Chemical Industries Ltd., 1975c in Documentation provided to EFSA No. 2).

Curdlan was demonstrated to be nutritionally inert as a carbohydrate or energy source in male Sprague Dawley rats given 1, 2, 3 and 6.56 g curdlan/day for 7 or 8 days in a carbohydrate-free basal diet. No change in body weight of curdlan treated animals was observed compared to control animals receiving the carbohydrate-free basal diet, in contrast to rats receiving sucrose or starch diets whose body weight increased (Takeda Chemical Industries, Ltd., 1974a in Documentation provided to EFSA No. 1). Enlarged caecum and increased caecum contents were observed in the curdlan-treated rats along with three- to five-fold increased amount of faeces. According to the applicant, this effect is likely due to a normal pass-through process in the gastrointestinal tract due to the poor absorption of the polymerised β-(1- > 3)-linked glucose residues that contribute to the indigestible nature of curdlan and is not considered a toxicological effect. Furthermore, when curdlan was given as a suspension to fasted male Sprague–Dawley rats, no increase in fasting levels of either hepatic glycogen or blood glucose was reported.

Similar findings were also reported in a study performed in male Sprague–Dawley rats (Takeda Chemical Industries, Ltd., 1974b in Documentation provided to EFSA No. 1) where curdlan administered at concentrations of 1, 2 and 4 g/day in the diet for 7 days did not induce a dose-dependent increase in net body weight compared to the control. On the basis of these findings, the applicant concluded that curdlan is not systemically absorbed and is considered a nutritionally inert compound.

The Panel considered that intact curdlan is unlikely to be significantly absorbed based on the limited amount of urinary radioactivity in all but particularly in the antibiotic studies. In addition, in the human study no radioactivity was found in blood.

Furthermore, the Panel considered that curdlan is extensively metabolised by the gut microbiota and the majority ends up as carbon dioxide. However, there is no direct information on any intermediate metabolites. Based on the comparative feeding studies and absence of effects on blood glycogen and glucose, the Panel can infer that there is no release of usable sugar moieties.

There is some evidence that the balance of metabolism varies from carbon dioxide to faecal elimination with increasing doses over a 100-fold range (0.4–40 mg/kg bw) and of significant variability in gut microbiota metabolism.

The Panel noted that the applicant concluded that curdlan was broken down into constituent sugars that were metabolically converted into CO_2.

The Panel noted that the studies are old and have very limited numbers of either humans or animals in the groups together with poor recoveries in some studies.

Despite these shortcomings and the lack of direct evidence provided details of the different steps of the metabolic pathway for conversion of curdlan to carbon dioxide, the Panel considered that the explanation provided by the applicant is acceptable.

3.4.2 | Acute toxicity

No acute toxicity studies were submitted in the dossier. However, the Panel noted that one study (Aomori & Tanida, 1968) was reported in the JECFA evaluation (JECFA, 2000a, 2000b). In that evaluation, an LD_{50} of > 10,000 mg/kg bw in mice and rats following oral administration was reported. No abnormalities or deaths were observed in both species. However, it is noted that the origin of the test item and its purity was unknown and that the study was not performed according to GLP or current OECD TGs.

3.4.3 | Short-term and subchronic toxicity

No short-term studies were provided.

Rats

In a 90-day paired feeding study in CD rats, curdlan was fed in the diet at a level of 0% or 15% (equal to 0 or 15,000 mg/kg bw per day) to 25 males and 25 females per group. Only clinical signs and body weights were recorded. No changes in clinical signs were observed and none of the rat died. Treated male rats gained slightly more body weight as compared to male controls. In females, body weight gain was similar in the treated group and controls (Takeda Chemical Industries Ltd., 1976d in Documentation provided to EFSA No. 1). Because of the small number of endpoints investigated, the Panel considered this study as limited.

Male and female Sprague–Dawley rats (15/sex/group) were fed a basal diet with 0%, 5%, 10% or 20% (equal to 0, 4400, 9000 or 19,100 mg/kg bw per day for males and 0, 5500, 12,400 or 24,300 mg/kg bw per day for females) curdlan for 12 weeks. Clinical observations, body weight gain, food intake, food efficiency, blood biochemistry, urinalysis, organ weights and gross and microscopic pathology did not demonstrate differences among the groups, except for body weight gain in male rats which decreased dose-dependently (down to −18%), while food intake increased (up to +15%) and for caecum weight (empty and full) which increased dose-dependently (Takeda Chemical Industries Ltd., 1972b in Documentation provided to EFSA No. 1).

Dogs

Beagle dogs (4/sex/group) were fed 0, 1, 5 or 15% curdlan (equivalent to 0, 200, 1250 or 3750 mg/kg bw per day) or 40% gelled curdlan (equivalent to 10,000 mg gelled curdlan/kg bw per day corresponding to 1000 mg curdlan/kg bw per day) for 1 year. Soft stools were observed more frequently in dogs fed with 15% curdlan or 40% gelled curdlan compared to the control. Gross and microscopical evidence of mild acute irritation of the small intestine was observed in dogs fed with 1% and 15% curdlan, but not in the 5% group. This was also observed in the 40% gelled curdlan treated group. The authors considered this lesion as compound related, however the Panel noted that there was no dose–response. A significant compound related increase in weight of the empty and full caecum was observed in dogs fed with curdlan at 3750 mg/kg bw/day. No other compound-related effects were observed (Takeda Chemical Industries Ltd., 1975a in Documentation provided to EFSA No. 1).

3.4.4 | Genotoxicity

The applicant provided in vitro and in vivo genotoxicity studies on curdlan. The Panel noted that the production organism, *R. radiobacter*, qualifies for the QPS approach for safety assessment, so no concern is expected for potential secondary metabolites from the fermentation process.

In vitro studies

Bacterial reverse mutation test

In the reverse mutation assay (plate incorporation method) with *S. typhimurium* TA1535, TA 1537, TA 1538, TA98 and TA100, curdlan was not mutagenic at a concentration range up to 5000 µg/plate, both in the absence and presence of rat liver S9 metabolic activation (Inveresk Research International Ltd., 1994a in Documentation provided to EFSA No. 1) in compliance with GLP and in accordance with the version of the OECD TG 471 in force when the study was performed (OECD, 1983a). The Panel noted that tester strains *S.* Typhimurium TA102 and *E. coli* WP2uvrA were not used in this study. Negative results were also observed in a separate repeat experiment, at the same concentration range both in the absence and presence of rat liver S9 metabolic activation. Slight toxicity to the bacteria, indicated by reduction of the background lawn, was noted in the absence of S9 mix at 5000 µg/plate in all strains except TA 1538. No precipitation of the test material was observed.

In vitro mammalian cell gene mutation test

Curdlan was tested for mutagenic activity in the mouse lymphoma L5178Y assay both in the absence and presence of S9 metabolic activation (Inveresk Research International Ltd., 1994c in Documentation provided to EFSA No. 1). The study was performed in compliance with GLP and in accordance with the OECD TG 476 (OECD TG, 1997). The cells were treated for 4 h both with and without metabolic activation. Curdlan gave no indication of a mutagenic effect up to 5000 μg/mL; this concentration produced some cytotoxicity.

In vitro mammalian chromosomal aberration test

Curdlan was evaluated for clastogenic potential in an in vitro chromosomal aberration test in Chinese hamster ovary (CHO) cells. Cell cultures were treated with the test material in the absence and presence of S9 metabolic activation up to a maximum concentration of 5000 μg/m. The conduct of the study was compatible with the OECD test guideline available at that time (OECD 473, 1983b) (Inveresk Research International Ltd., 1994d in Documentation provided to EFSA No. 1).

Three experiments were performed. In all the experiments cells were treated for 6 h in the presence of S9 or 22 h in the absence of S9. Cells were harvested 24 h post treatment (in experiment 1, 2 and 3) or 48 h post treatment (only in experiment 2). Colcemid was added 2 h before the harvesting. Two hundred cells were analysed from two independent cultures for each treatment concentration for the presence of structural aberrations. Numerical aberrations were quantified as a percentage of aneuploidy, polyploidy and endoreduplicated cells.

The response of the positive control cultures was weak in the first experiment, in particular in the presence of metabolic activation. In the following two experiments the positive controls performed as expected.

A slight sporadic increase in structural aberration was reported in a single culture at an intermediate treatment concentration, however, as this increase was not confirmed in the duplicate culture nor at higher concentrations, it was considered of no biological relevance. No other increase in structural aberrations were observed in any experimental condition. No indication of numerical aberrations was reported.

Despite some deviations from the current OECD guideline, this study indicates the absence of clastogenic potential of the test substance.

Moreover, considering that aneuploid cells were separately scored and that the harvesting time of 48 h post-treatment allows the observation of cells after the second division, these data are sufficient also to exclude a possible aneugenic activity of the test item.

In vivo study

In vivo micronucleus test

Curdlan was assessed for its genotoxicity in an in vivo micronucleus test in mice (Inveresk Research International Ltd., 1994b in Documentation provided to EFSA No. 1). The study was performed according to GLP and in line with the OECD test guideline available at that time (OECD TG 474, 1983c). Curdlan was administered orally (by gavage) to five animals per sex per dose level up to a maximum dosage of 2000 mg/kg bw, given twice at a 24-h interval and 1000 polychromatic erythrocytes per animal were analysed. The test item did not induce micronuclei, however the Panel noted that no bone marrow toxicity (alteration of PCE/NCE ratio) was detected, therefore no evidence of target cell exposure was provided. However, no exposure was expected due to lack of absorption as demonstrated in the kinetic data (see Section 3.4.1).

Overall summary of genotoxicity data

In summary, curdlan was not genotoxic in vitro in a bacterial reverse mutation assay, in a gene mutation test and in a chromosomal aberration test. The Panel noted that the experimental design of the in vitro chromosomal aberration test allowed also to exclude a concern for potential aneugenicity. Based on the in vitro genotoxicity data provided, an in vivo follow-up was not required. Overall, the Panel considered that curdlan does not raise a concern for genotoxicity.

3.4.5 | Chronic toxicity and carcinogenicity

Mice

In an 18-months chronic toxicity study, curdlan was fed at 0%, 1%, 5% or 15% (equivalent to 0, 1500, 7500 or 22,500 mg/kg bw per day) and at 40% gelled curdlan (equivalent to 60,000 mg gelled curdlan/kg bw per day corresponding to 6000 mg curdlan/kg bw per day) to CD-1 mice (100/sex/group). Following 12 and 18 months of study, five males and five females from each group were necropsied and selected tissues and organs were collected for histopathology. No changes considered related to treatment were seen in general behaviour, appearance, growth, gross observations or histopathology in all groups (Takeda Chemical Industries Ltd., 1976c in Documentation provided to EFSA No. 1).

Rats

In a 2-year chronic toxicity study in CD rats (60/sex/group), curdlan was fed via the diet at levels of 0%, 1%, 5% or 15% (equivalent to 0, 500, 2500 or 7500 mg/kg bw per day) or at level of 40% gelled curdlan (equivalent to 20,000 mg gelled curdlan/kg bw per day corresponding to 2000 mg curdlan/kg bw per day). Rats were observed daily for clinical signs and body weights and food consumption were recorded weekly. Ophthalmoscopy (all animals), haematology, clinical biochemistry and urinalyses were conducted in five rats/sex/group at 3, 6, 12, 18 and 24 months. No changes to be considered compound related were seen in general behaviour and appearance. Rats of the 7500 mg/kg bw per day group consumed less food and gained less weight as compared with controls (–11 and –10% final body weight in males and females, respectively). No compound related changes were seen in ophthalmoscopy, haematology and biochemistry or urinalysis for all groups. No gross or histopathological lesions attributable to curdlan were observed. No tumorigenic effect was observed at any feeding level. An increase in weight of the empty and full caeca at 7500 mg/kg bw per day was the only organ weight effect attributed to curdlan. Increased mean absolute weights of empty caeca was also observed in the 40% gelled curdlan treated group (Takeda Chemical Industries Ltd., 1976b in Documentation provided to EFSA No. 1).

Charles River rats (60/sex/group) obtained from the first mating (F_{1a}) of a multigeneration reproductive toxicity study were fed via the diet at levels of 0%, 1%, 5% or 15% curdlan (equivalent to 0, 500, 2500 or 7500 mg/kg bw per day) or at level of 40% gelled curdlan (equivalent to 20,000 mg gelled curdlan/kg bw per day corresponding to 2000 mg curdlan/kg bw per day) for 124–127 weeks. The rats were observed daily for clinical signs and body weights and food consumption were recorded weekly. Ophthalmoscopic examinations, haematology and clinical biochemistry and urinalysis were conducted at 3, 6, 12, 18 and 24 months and prior to termination of the study (Takeda Chemical Industries Ltd., 1976g in Documentation provided to EFSA No. 1). No changes considered compound related were seen in general behaviour and appearance, ophthalmology, haematology, biochemistry or urinalysis. Soft stools were observed. Occasionally, the weight of full or empty caeca were increased and considered by the authors to be related to treatment. Histopathological examination revealed a statistically significantly ($p < 0.05$) increase in number of endometrial polyps in the uterus of females of the 7500 mg/kg bw per day curdlan group. The authors considered these lesions as possibly compound related. The Panel, however, does not agree with this conclusion since endometrial polyps occur spontaneously rather frequently and the background incidence in control rats showed a large variation.

Overall, curdlan was well-tolerated with no overt organ-specific toxicity. The effects upon consumption of high doses of curdlan were related to gastrointestinal symptoms (i.e. soft stool, increased caeca weights), which were attributed to a bulking effect based on the consumption of high doses of indigestible curdlan which is not readily absorbed but is either excreted via the faeces or undergoes fermentation by the gut microbiota. The Panel considered that these effects are not adverse because they are due to physiological responses both in curdlan and gelled curdlan treated groups. The Panel identified a NOAEL for chronic toxicity of curdlan of 7500 mg/kg bw per day, the highest dose tested.

3.4.6 | Reproductive and developmental toxicity

Reproductive toxicity studies

Curdlan was tested in a combined three-generation reproductive and developmental toxicity study in rats (the latter is dealt with in section Developmental Toxicity) (Takeda Chemical Industries Ltd., 1976g in Documentation provided to EFSA No. 1). Groups of 20 males and 40 females were fed for up to 100 days of age when mating started, during the mating, gestation and lactation period during all generations, with diets containing curdlan at levels of 0, 1, 5 and 15% (equivalent to 0, 500, 2500 or 7500 mg curdlan/kg bw per day) or 40% gelled curdlan (equivalent to 20,000 mg gelled curdlan/kg bw per day corresponding 2000 mg curdlan/kg bw per day). No mortality or adverse effects were observed. Body weight and food consumption of the males were incidentally lower in the 7500 mg curdlan/kg bw group during the study. No effects on organ weights and histopathological changes were observed for the F_2 parental animals and F_3 pups. No compound related differences were seen between control and treated rats with respect to fertility, gestation or viability indices during the study. F_{1A} pup weights were only measured on post-natal day (PND) 0, 4 and 21, F_{1B} on PND 0, 4, 14 and 21. For the other litters, pup weights were measured on PND 0, 4, 7, 10, 14, 17 and 21. F_{1A}, F_{1B}, F_{2A} and F_{3A} pup weights of the 7500 mg curdlan/kg group were statistically significantly lower at PND 21. In addition, $F2_A$ pup weights for the 7500 mg/kg group were statistically significantly decreased on PND 4, 7, 10, 14 and 17 and for F_{3A} pups from PND 7 onwards. Furthermore, F_{1B} pup weight of the 2500 mg curdlan/kg bw group was statistically significantly decreased on PND 14. Pup birth weights (PND 0) were not affected. By contrast, F_{2B} pup birth weight (PND 0) was significantly higher than control at 7500 mg/kg.

Two additional studies were performed to clarify the effects seen on pup weight.

Curdlan was tested in an one-generation reproductive study in weanling Charles River CD rats ($n = 20$ male and 40 females per group) (Takeda Chemical Industries Ltd., 1976e in Documentation provided to EFSA No. 1) to determine if withdrawal of the test compound from the dams during the lactation period (only exposure during the premating, mating and gestation period) could help to clarify the results of a previous three generation reproductive study (Takeda Chemical Industries Ltd., 1976g in Documentation provided to EFSA No. 1). The animals were fed diets containing curdlan at levels of 0%, 5% and 15% (equivalent to 0, 2500 or 7500 mg curdlan/kg bw per day) or 40% gelled curdlan (equivalent to 20,000 mg gelled curdlan/kg bw per day, corresponding to 2000 mg curdlan/kg bw per day). The parental animals were allowed

to produce two litters. No compound related effects were observed in the parental animals, the reproductive performance was not affected. Adverse effects in offspring (appearance, survival and pup weight) of both generated litters were not observed. The study authors stated that lack of effect (on pup weight during lactation), when the test compound was not present in the mothers' feed during lactation, supports the hypothesis that the decrease in pup weight observed in the study above described was due to the pup eating from the mothers feed and not due to the pups receiving mother's milk, nor the direct effect on the pups in utero. However, the Panel noted that the pup weight in the three-generation reproductive toxicity study was also decreased before PND 12, which is by the Panel considered as the time when pups start eating solid feed.

Curdlan was tested in another one-generation reproductive study in weanling Charles River CD rats ($n = 20$ male and 40 females per group except for the control group $n = 40$ males and 80 females per group) (Takeda Chemical Industries Ltd., 1976f in Documentation provided to EFSA No. 1). The aim of the study was to determine if offspring, from females treated with curdlan or gelled curdlan would gain weight at a normal rate during lactation if the pups were nursed by untreated control females. This study would explain the effects observed in the previous three-generation reproductive and developmental toxicity study (Takeda Chemical Industries Ltd., 1976g in Documentation provided to EFSA No. 1). Pups of untreated control dams were nursed by treated foster dams. Furthermore, the Panel noted that during the lactation period no group was included in which control dams nursed control pups. The parental animals were fed with diets containing at levels of 0%, 5% or 15% curdlan (equivalent to 0, 2500 or 7500 mg curdlan/kg bw per day) or 40% gelled curdlan (equivalent to 20,000 mg gelled curdlan/kg bw per day corresponding to 2000 mg curdlan/kg bw per day). No treatment related clinical signs were observed during the study. Body weight change was comparable for the control and the treated groups except for the male rats of the 7500 mg curdlan/kg bw group. Compared to the control animals, the animals of this group gained slightly less weight. Food consumption group of rats fed with diet containing curdlan at 15% (7500 mg curdlan/kg bw per day) was less compared to the control. No effect was observed on reproductive parameters. In this study, the offspring of dams fed with curdlan or gelled curdlan showed significant reductions in weight during lactation when nursed by their natural mothers. This was observed in the F_{1A} litters for each dose level but only for the 7500 mg curdlan/kg bw group in the F_{1B} litters. When the pups of treated dams were transferred to control dams the effect on pup weight was markedly reduced in the F_{1A} litters and it was absent in the F_{1B} litters. When control pups were suckled by treated foster mothers kept on diet added 15% curdlan (equivalent to 7500 mg/kg bw per day) they demonstrated significant reduction in body weight (F_{1A} litters and F_{1B} litters). Furthermore, the Panel noted that in this study the effects on pup weight were not observed at birth and started in most cases not before PND 10.

On request of the Panel, the applicant provided a further explanation on the effect on curdlan on the nutritional composition of breast milk (Documentation provided to EFSA No. 4).

The applicant considered that, based on the ADME data (see Section 3.4.1), curdlan would not be present in the breast milk. In addition, it was noted that the combination of replacement of nutrients in the diet with curdlan and decreased feed consumption by the dams during lactation (as reported by the study investigators; statistical significance not reported) may have been sufficient to have an effect on the energy intake by the dams, which may in turn have had an effect on the energy content of the milk as a contributing factor in the reduction of the body weight of pups during lactation. The Panel noted that no data on the amount, composition or impurities in the milk were provided.

Overall, taking into account the studies described above, the Panel considered that the lack of difference in pup body weight at birth (PND 0), together with other endpoints investigated in the study (early and late resorptions, fetal external, soft tissue and skeletal examinations), indicated no embryofetal adverse effects. There were also no treatment-related effects on fertility or gestation endpoints at any dose in any generation or cohort.

Treatment-related differences in pup body weight were seen during lactation at various stages (PND 4, 10, 14, 21) in all generations (Takeda Chemical Industries Ltd., 1976g in Documentation provide to EFSA No. 1). The Panel noted that pups gradually shift from milk to solid feed after PND 10 (when incisor eruption begins); therefore, possible contributors to the pup body weight difference include (i) maternal nursing behaviour (ii) and/or milk quality/quantity resulting from the nutritional imbalance of the diet consumed by the dams as well as (iii) reduced palatability/quality of feed containing curdlan.

Treatment-related decreases in pup weights during lactation were less than 20% at 7500 mg/kg bw per day, not entirely consistent and did not persist to adulthood. Nonetheless, the Panel considered that reduced pup weight on PND 21 at the dietary dose level of 7500 mg/kg bw per day could not be dismissed and therefore considered it adverse. Thus, the Panel identified a NOAEL of 2500 mg/ kg bw per day, based on reduced pup weight during lactation at 7500 mg/kg bw per day.

Developmental toxicity studies

Rats

Curdlan was tested in a combined three-generation reproductive toxicity and developmental study in rats (the full study description can be found in the section Reproductive Toxicity) (Takeda Chemical Industries Ltd., 1976g in Documentation provided to EFSA No. 1). Animals were fed with diets containing curdlan at levels of 0%, 1%, 5% or 15% (equivalent to 0, 500, 2500 or 7500 mg curdlan/kg bw per day) or 40% gelled curdlan (equivalent to 20,000 mg gelled curdlan/kg bw per day corresponding to 2000 curdlan mg/kg bw per day). On GD 13, a C-section was performed on F2 pregnant females ($n = 5–9$ per group). The authors stated that no compound related effects were observed. However, the Panel noted that the number of dams per group was low. On GD 20, a C-section was performed on F2 pregnant females ($n = 5–9$ per group). The authors

stated that no compound related effects were observed. However, the Panel noted that the number of dams per group was low and therefore no conclusion can be drawn concerning developmental toxicity of curdlan from this study.

Rabbits

A study was performed in inseminated Dutch Belted rabbits ($n = 15–22$ does per group) administered by gavage with 0, 1000, 2000 or 5000 mg curdlan/kg bw per day or with gelled curdlan at a dose level of 20,000 mg/kg bw per day (corresponding to 2000 mg curdlan/kg bw per day) from GD 6–18. Rabbits which received the 5000 mg/kg bw per day were given divided doses twice daily (Takeda Chemical Industries Ltd., 1974c in Documentation provided to EFSA No. 1). C-sections were performed on GD 28. None of the control but, 3, 1, 3, 16 does of the 1000, 2000 or 5000 mg curdlan/kg bw per day or of the gelled curdlan/kg bw per day levels, respectively died during the study. C-sections were performed on GD 28. The authors stated that the high number of deaths in the group receiving the gelled curdlan was due to mechanical occlusion of the pharynx by the gel rather than any toxicity of the curdlan. No difference in clinical observation and body weights were seen between the curdlan- treated and the control groups. No difference in number of corpora lutea, implantation sites, number of dead or resorbed fetuses, litters aborted, live fetuses and live fetal weights were observed. The fetal pathology as assessed by external, visceral and skeletal examinations was comparable between the groups.

Overall, the Panel considered that no effects were observed on maternal and developmental toxicity based on a prenatal developmental toxicity study in rabbits up to 5000 mg curdlan/kg bw per day (Takeda Chemical Industries Ltd., 1974c in Documentation provided to EFSA No. 1).

3.4.7 | Immunogenicity/Immunotoxicity

Several in vitro studies have demonstrated that curdlan can modulate signalling pathways of the immune system in animal and human cell systems including mast cells, dendritic cells, macrophages, neutrophils, myeloid cells and leukocytes. While some of the studies did not show any curdlan induced immune related changes, others report that curdlan may modulate the production and secretion of a range of proteins involved in the signalling pathways in a variety of cells of the immune system. Curdlan was also shown to have a potentiating effect on histamine release and regulate the expression of various receptors involved in the activation of dendritic cells and macrophages in a number of publications submitted by the applicant (Apetrei et al., 2010; Barbosa-Lorenzi et al., 2017; Călugăru et al., 2009; Cypryk et al., 2014; Higashi et al., 2010; Holck et al., 2007; Juul-Madsen et al., 2010; Kankkunen et al., 2010; Kataoka et al., 2002; Kim et al., 2016; Kumar et al., 2009; Min et al., 2012; Noss et al., 2013; Rand et al., 2013; Rui et al., 2016; Soltani et al., 2012; Sonck et al., 2010; Sonck et al., 2011; van Eijk et al., 2010; Yamasaki et al., 2014).

In vivo the immunological potential of curdlan was investigated in mice and rats in comparison to known immunogenic compounds including dextran and alum-precipitated bovine serum albumin (Takeda Chemical Industries Ltd., 1974d in Documentation provided to EFSA No. 1). Contrary to the known immunogenic compounds curdlan did not induce production of reactive antibodies in the passive haemagglutination reaction in mice given a single intravenous injection of 10 µg curdlan nor upon intraperitoneal injection of 10 µg curdlan in male Wistar rats twice a week for 4 weeks. Similarly, no effects were observed in male Wistar rats fed up to 5 g curdlan/kg bw per day.

In order to further assess the immunological potential of curdlan in vivo, data of the existing subchronic and chronic studies with curdlan were examined to determine whether there were any significant biological relevant changes on immune-related parameters.

In the 3-month study in rats, some curdlan-induced changes of immune-related parameters such as a dose dependent decrease serum total protein and platelet count, increased serum albumin: globulin ratio and a decrease in absolute thymus weights were observed. However, these effects were minor and not expected to be of significant biological or toxicological relevance (Takeda Chemical Industries, Ltd., 1972b in Documentation provided to EFSA No. 1).

The 18-months chronic toxicity study of curdlan in mice demonstrated inconsistent changes in platelets, leucocytes and albumin: globulin ratios that did not follow a dose response and were not observed consistently in both sexes (Takeda Chemical Industries Ltd., 1976c in Documentation provided to EFSA No. 1). Furthermore, no consistent effects were observed upon histological examination relative to the control group.

In the 2-year chronic toxicity study of curdlan in rats, significant changes were reported in haematological parameters such as neutrophils and lymphocytes (Takeda Chemical Industries Ltd., 1976b in Documentation provided to EFSA No. 1). However, these changes did not show a consistent dose–response effect and remained within historical control ranges. Further, these changes did not lead to overt haematological effects, nor were there any consistent histopathological changes within the immune organs.

Lastly, in the 1-year toxicity study of curdlan conducted in dogs (Takeda Chemical Industries Ltd., 1975a in Documentation provided to EFSA No. 1) sporadic changes were observed in leucocytes that did not follow a dose response relationship nor did it lead to any overt haematological effects. Furthermore, histopathological changes in the immune organs were not observed.

Overall, several in vitro studies demonstrate that curdlan may be involved in the modulation of signalling pathways of the immune system in animal and human cell systems. However, these in vitro findings are not consistent with results from several in vivo studies in animals (Takeda Chemical Industries, Ltd., 1972b; Takeda Chemical Industries, Ltd., 1974d; Takeda

Chemical Industries Ltd., 1975a; Takeda Chemical Industries Ltd., 1976a, 1976b, 1976c in Documentation provided to EFSA No. 1) wherein no significant effects to the immune system were observed following ingestion of curdlan. This clearly demonstrates that the in vitro effects of curdlan do not translate into immunologic effects at the physiological level when assessed in vivo. The reason for this is not clear but the fact that curdlan is not absorbed to any significant extent and thus not systemically available is probably an important factor.

The Panel considered that in light of the absence of biologically relevant effects on the immune system in vivo, curdlan is not of concern with respect to immunotoxicity.

3.4.8 | Human studies

There is one study in which the tolerance to curdlan in humans has been assessed. In a 28-day double-blind study (Takeda Chemical Industries Ltd., 1975b in Documentation provided to EFSA No. 1), 12 healthy male subjects were randomised to receive two milkshakes per day either with or without curdlan under a graduated dosing regimen. The subjects were initially given 6 to 7 g curdlan/day on Day 1, doses were then gradually increased to 25 g curdlan by Day 5, 32 g curdlan/day from Days 6 to 14, 33 to 34 g/day for the next 6 days, after which point the maximum dose (50 g/day, i.e. 715 mg/kg bw per day) was given for the remainder of the study (Days 21–28).

No differences were reported in body weight, blood pressure and pulse rate, nor were changes observed in haematological or biochemical measures in any of the subjects. The only minor adverse effects were flatulence in three subjects and diarrhoea in two of the flatulence subjects were observed. One other subject complained of constipation. No other effects were reported. These effects are likely due to the indigestible nature of curdlan that can lead to a bulking effect.

The Panel considered this study too limited to be used for risk assessment.

4 | DISCUSSION

The present opinion deals with the safety evaluation of curdlan, which is a high molecular weight polysaccharide composed of a linear polymer of β-(1→3)-linked glucose residues, with thermal gelling properties.

Curdlan is obtained following fermentation of glucose with a non-pathogenic and non-toxicogenic strain of *R. radiobacter* biovar 1 strain NTK-u (synonym *A. radiobacter*), which has a QPS status (EFSA BIOHAZ Panel, 2024). *R. radiobacter biovar* 1 strain NTK-u is added to a fermentation medium (high in glucose), and is allowed to produce crude curdlan, which is subsequentially isolated and purified. Curdlan is then dried, milled and sieved to obtain the final product.

The Panel noted that the information provided on five batches of the proposed food additive showed that curdlan is produced according to the method described in Section 3.1.3 and within the proposed specifications.

Concerning the entry 'Definition' of the proposed specifications, the Panel recommends referring to the fermenting microorganism with the common name *R. radiobacter*. In addition, the Panel recommends changing the word 'solution' into 'suspension' or 'dispersion'. The Panel also noted that the entry 'Solubility' should report that curdlan is insoluble in water and ethanol.

Analytical data on the levels of arsenic and lead were provided by the applicant for five samples of the proposed food additive. Arsenic was reported in all samples as below the LOQ of 0.01 mg/kg, whereas for lead the levels ranged from 0.04 to 0.19 mg/kg. The Panel noted that no data were provided for mercury and cadmium. However, given the nature of the production process, the degree of purity of the raw materials and processing aids used, the purification steps that are used, and that glucose is the substrate for the production of curdlan, the Panel considers it unlikely that mercury and cadmium would be present in the proposed food additive.

The Panel performed the risk assessment that would result if lead and arsenic were present in curdlan at two different concentration scenarios (Table 9), and the resulting figures indicate that for arsenic the calculated MOEs in both scenarios were considered to be insufficient. Whereas for lead, the Panel noted that the MOE was deemed insufficient for the 95th percentile exposure based on the proposed specification limit (Section 3.3.3).

As described in the manufacturing process, curdlan is crystallised in methanol and residues of this solvent may be present in the final product. The applicant proposed to set a maximum level for residual methanol of 10 mg/kg. The Panel agreed with this proposal and recommended including a limit for the residual methanol in the proposed specifications.

The applicant provided results of quantitative SEM analysis of pristine (as produced) curdlan powder. The analysis revealed that the pristine proposed food additive contains a fraction of small particles including nanoparticles. Furthermore, the applicant provided results of the SEM–EDX analysis of low-set and high-set curdlan gels at concentrations of 0.5%, 4% and 10%, mimicking realistic uses and use levels of the proposed food additive.

The Panel noted that currently no standardised methods are available to identify small particles including nanoparticles, in the polysaccharides and thickening agents in the pristine form or as used in food.

The Panel noted that the provided data indicates that curdlan gels, both low-set and high-set at a concentration of 0.5%, 4% and 10% w/w, do not contain small particles including nanoparticles of pristine curdlan. SEM images of gels at 0.5% w/w reveal small lumps of material within a smooth film, which were confirmed through EDX data analysis to be recrystallised salts (see Section 3.1.2.1). These findings demonstrate that these particles are not attributable to pristine curdlan particles.

Overall, based on the data provided, and the polysaccharide nature of curdlan having the capacity to disperse and form gels in water (see Section 3.1.1), the Panel considered that the presence of a fraction of small particles including nanoparticles of pristine curdlan in food at the proposed uses and use levels can be excluded, given the conditions of use.

Therefore, the Panel concluded there is no concern with regard to the potential presence of small particles, including nanoparticles, in the proposed food additive under the proposed conditions of use and considered that curdlan can be assessed following the conventional risk assessment, as outlined in EFSA Guidance for submission for food additive evaluations (EFSA ANS Panel, 2012).

With regards to the storage stability, curdlan was tested under standard conditions at room temperature. The applicant recommended a shelf-life period of 3 years from the date of manufacture, on condition that curdlan is stored in tightly closed containers and away from moisture and light. The Panel agrees with this recommendation.

Dietary exposure to curdlan was estimated with FAIM (version 2.1) using proposed maximum and typical use levels of curdlan in 25 food categories.

At the proposed maximum use levels, the mean exposure to curdlan from its proposed use as a food additive ranged from 30 to 744 mg/kg bw per day in infants and toddlers, respectively. The 95th percentile exposure to curdlan ranged from 126 to 1441 mg/kg bw per day in the same two population groups.

At the proposed typical use levels, the mean exposure to curdlan from its proposed use as a food additive ranged from 15 to 383 mg/kg bw per day in infants and toddlers, respectively. The 95th percentile exposure to curdlan ranged from 61 to 889 mg/kg bw per day in these two population groups.

The main food categories contributing to the total mean exposure estimates were FCs 07.1 'Bread and rolls, 07.2 'Fine bakery wares' and 04.2 'Processed fruit and vegetables'. Additionally, for children and adolescents, FC 05.1 'Cocoa and chocolate products as covered by Directive 2000/36/EC' contributed greatly to the exposure.

Overall, the Panel considered that the uncertainties identified resulted in an overestimation of the dietary exposure to curdlan in European countries considered in the EFSA Comprehensive database at both the typical and the maximum proposed use levels.

According to the data provided by the applicant, the Panel considered that intact curdlan is unlikely to be absorbed in animals and that data in humans indicated no systemic availability of curdlan or break-down products. It was demonstrated to be extensively metabolised by the gut microbiota and largely excreted as CO_2. There are no data on the identity or amounts of intermediate metabolites. Despite the shortcomings identified in the submitted ADME studies and the lack of direct evidence to prove the metabolic pathway of curdlan, the Panel considered that it is likely that curdlan is broken down into constituent sugars, which are then converted into CO_2 or other innocuous substances.

The toxicological data submitted consisted of sub-chronic, chronic and carcinogenicity, reproductive and developmental toxicity studies as well as genotoxicity studies. In addition, data concerning immunotoxicity and one study in humans were provided. The dataset shows that curdlan is generally well-tolerated with no overt organ-specific toxicity. The effects observed regarded gastrointestinal symptoms (e.g. soft stools, increased cecum weight), in particular at high doses, which can be attributed to the indigestible nature of curdlan and the consequent bulking effect. Curdlan was not genotoxic, and no carcinogenic effects were observed. In the combined three-generation reproductive and developmental toxicity study, decreased pup weight was observed at 7500 mg curdlan/kg bw per day, the highest dose tested. The Panel considered the observed effect as treatment-related and adverse, therefore identified a NOAEL of 2500 mg curdlan/kg bw, based on reduced pup weight during lactation. One human study was provided but considered by the Panel as too limited to be used for risk assessment.

The available data are considered to have certain limitations (e.g. in reporting, in the characterisation of the test substance) which do not allow to derive an ADI. Nonetheless, these limitations do not prevent the Panel from reaching a conclusion regarding the safety of the use of the proposed new food additive curdlan by applying the MOE approach.

For the risk assessment, the Panel took into consideration that:

- the production organism, *R. radiobacter*, qualifies for the QPS approach for safety assessment;
- at the proposed maximum use levels, the 95th percentile exposure was up to 1441 mg/kg bw per day in toddlers;
- the ADME data demonstrated that, in humans, curdlan and its break-down products are not absorbed, and that curdlan is extensively metabolised in animals by the gut microbiota into carbon dioxide and that the remainder is excreted in faeces;
- curdlan does not raise a concern for genotoxicity;
- curdlan is not carcinogenic; and
- the reduced pup weight in the combined three-generation reproductive and developmental toxicity study, although likely secondary to nutritional imbalance, was considered adverse, resulting in the identification of a conservative NOAEL of 2500 mg/kg bw per day.

Given that curdlan and its break-down products are not absorbed and that the identified adverse effect is neither systemic nor local, the Panel considered that no adjustment factor is needed. Thus, an MOE of at least 1 is considered sufficient.

5 | CONCLUSIONS

The Panel concluded that there is no safety concern for the use of curdlan as a food additive at the proposed uses and use levels.

6 | DOCUMENTATION PROVIDED TO EFSA

1. Dossier 'Application for the Authorisation of curdlan (INS No. 424) as a Food Additive in the European Union'. September 2016. Submitted by MC Food Specialties Inc. The following toxicological unpublished study reports[8] were submitted:

   - Inveresk Research International Ltd., 1994a. Curdlan (Lot No FU11S). Testing for mutagenicity activity with *Salmonella typhimurium* TA 1535, TA1537, TA 1538, TA 98 and TA 100. IRI Project No 754834. Scotland. Report No 10239. Unpublished study report.
   - Inveresk Research International Ltd., 1994b. Curdlan (Lot No FU11S). Micronucleus test in bone marrow of CD-1 mice. IRI Project No 754860. Scotland. Report No 10239. Unpublished study report.
   - Inveresk Research International Ltd., 1994c. Curdlan (FU11S) Mouse lymphoma mutation assay. IRI Project No 754876. Scotland. Report No 10269. Unpublished study report.
   - Inveresk Research International Ltd., 1994d. Curdlan (lot no. FU11S) chromosomal aberrations assay with Chinese hamster ovary cells in vitro (OECD protocol). IRI Project No. 754855. Scotland. Report No.10337. Unpublished study report.
   - Takeda Chemical Industries, Ltd., Biological Research Laboratories, 1972a. Metabolism of Polysaccharide 13,140 in the rat. Japan. Unpublished study report.
   - Takeda Chemical Industries, Ltd., Biological Research Laboratories, 1972b. Oral three month toxicity study of polysaccharide 13,140 in rats. Japan. Unpublished study report.
   - Takeda Chemical Industries, Ltd., Biological Research Laboratories, 1974a. Caloric inertness of Polysaccharide 13140 in the rat. Japan. Unpublished study report.
   - Takeda Chemical Industries, Ltd., Biological Research Laboratories, 1974b. Nutritional Study on Polysaccharide 13140 in the rat. Japan. Unpublished study report.
   - Takeda Chemical Industries Ltd., 1974c. Teratology study of PS 13140 in rabbits. Japan. Report No. 295–01316. Unpublished study report.
   - Takeda Chemical Industries, Ltd., Central Research Laboratories, 1974d. Immunogenicity of Polysaccharide 13140 in rats and mice. Japan. Unpublished study report.
   - Takeda Chemical Industries Ltd., 1975a. One year feeding study of PS 13140 in dogs. Japan. Report No. 295–0088. Unpublished study report.
   - Takeda Chemical Industries Ltd., 1975b. Human tolerance study of PS 13140. Japan. Report No. 295–024. Unpublished study report.
   - Takeda Chemical Industries Ltd., 1976a. Lifetime feeding study of PS 13140 in rats. Japan. Report No. 295–01130. Unpublished study report.
   - Takeda Chemical Industries Ltd., 1976b. Two year feeding study of PS 13140 in rats. Japan. Report No. 295–01418. Unpublished study report.
   - Takeda Chemical Industries Ltd., 1976c. Lifetime carcinogenic study of PS 13140 in mice. Japan. Report No. 295–0105. Unpublished study report.
   - Takeda Chemical Industries Ltd., 1976d. Ninety day paired feeding study of PS 13140 in rats. Japan. Report No. 295–0185. Unpublished study report.
   - Takeda Chemical Industries Ltd., 1976e. Single generation reproduction study of PS 13140 in rats. Japan. Report No. 295–019. Unpublished study report.
   - Takeda Chemical Industries Ltd., 1976f. Single generation reproduction study of PS 13140 in rats. Japan. Report No. 295–020. Unpublished study report.
   - Takeda Chemical Industries Ltd., 1976g. Multigeneration reproduction study of PS 13140 in rats. Japan. Report No. 295–0124. Unpublished study report.

2. Additional information submitted by MC Food Specialties Inc. following a request from EFSA. September 2017. The following unpublished study report was submitted8:

   - Takeda Chemical Industries Ltd., 1975c. Metabolism study of PS 13140 in humans. Japan. Report No. 295–023. Unpublished study report.

[8]All these unpublished study reports are the same considered in JECFA (2000b).

3. Additional information submitted by MC Food Specialties Inc. following a request from EFSA. January 2018.
4. Additional information submitted by MC Food Specialties Inc. following a request from EFSA. January 2019.
5. Additional information submitted by Intertek Health Science Inc. on behalf of Mitsubishi Corporation Life Sciences Limited (formerly MC Food Specialties Inc.) in response to a request from EFSA. April 2020.
6. Additional information submitted by Mitsubishi Corporation Life Sciences Limited (formerly MC Food Specialties Inc.) in response to a request from EFSA. January 2024.
7. Additional information submitted by Mitsubishi Corporation Life Sciences Limited (formerly MC Food Specialties Inc.) in response to a request from EFSA. March 2024.

ABBREVIATIONS

ADI	acceptable daily intake
ADME	absorption, distribution, metabolism, excretion
ANS	EFSA Panel on Food Additives and Nutrient sources added to Food
ATCC	American Type Culture Collection
BMDL	benchmark dose lower bound
bp	base pairs
bw	bodyweight
BWC	body weight change
CAS	Chemical Abstracts Service
CARD	Comprehensive Antibiotic Resistance Database
CFU	colony forming unit
CHO	Chinese hamster ovary
CI	confidence interval
CONTAM	EFSA Panel on Contaminants in Food Chain
d50	median particle size
DMSO	dimethylsulphoxide
EDX	with Energy Dispersive X-Ray spectrometry
FAF	EFSA Panel on Food Additives and Flavourings
FAIM	Food Additives Intake Model
FC	food category
FEEDAP	EFSA Panel on Additives and Products or Substances used in Animal Feed
GC–MS	Gas Chromatography–Mass Spectrometry
GD	Gestational day
GI	gastrointestinal
GLP	Good Laboratory Practice
GSFA	Codex Alimentarius General Standard for Food Additives
HBGV	health-based guidance value
ICP–MS	Inductively Coupled Plasma–Mass Spectrometry
INS	International Numbering System for Food Additives
IQ	intelligence quotient
IR	Infrared Spectroscopy
JECFA	Joint FAO/WHO Expert Committee on Food Additives
LD	laser diffraction
LD50	Median lethal dose
LOD	limit of detection
LOQ	limit of quantification
MIC	minimum inhibitory concentration
MOE	margin of exposure
MPL(s)	maximum permitted level(s)
NCE	normochromatic erythrocytes
NOAEL	no observed adverse effect level
OECD	TG Organisation for Economic Co-operation and Development Testing Guidelines
P95	95th percentile
PCE	polychromatic erythrocytes
PND	post-natal day
PS	polysaccharide
RP	reference point
SC	Scientific Committee
SCF	Scientific Committee on Food
SEM	scanning electron microscopy
TEM	transmission electron microscopy
TG	test guideline

TLC	thin layer chromatography
TR	technical requirements
WGS	whole genome sequencing

CONFLICT OF INTEREST

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

REQUESTOR

European Commission

QUESTION NUMBER

EFSA-Q-2017-00024

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REFERENCES

Aomori, T., & Tanida, M. (1968). Preliminary acute toxicity of polysaccharide 13140 in mice and rats. Takeda Chemical Industries, Ltd., Biological Research Laboratories. Japan. Unpublished study report.

Apetrei, N. S., Călugăru, A., Bădulescu, M. M., Lupu, A. R., Moscovici, M., Mocanu, G., Mihai, D., Szegli, G., & Cremer, L. (2010). The effects of some Curdlan derivatives on Dectin-1 expression and cytokine production in human peripheral blood mononuclear cells. *Roumanian Archives of Microbiology and Immunology*, *69*(2), 61–66.

Barbosa-Lorenzi, V. C., Peyda, S., Scheynius, A., Nilsson, G., & Lunderius-Andersson, C. (2017). Curdlan induces selective mast cell degranulation without concomitant release of LTC4, IL-6 or CCL2. *Immunobiology*, *222*, 647–650. https://doi.org/10.1016/j.imbio.2016.12.001

Călugăru, A., Cremer, L., Lupu, A. R., Bădulescu, M. M., Apetrei, N. S., Moscovici, M., Mocanu, G., Mihai, D., Kerek, F., & Szegli, G. (2009). Recognition and modulation of Dectin-1 and TLR-2 receptors by curdlan derivatives and purified natural extracts. *Roumanian Archives of Microbiology and Immunology*, *68*(3), 119–124.

Cypryk, W., Öhman, T., Eskelinen, E.-L., Matikainen, S., & Nyman, T. A. (2014). Quantitative proteomics of extracellular vesicles released from human monocyte-derived macrophages upon β-glucan stimulation. *Journal of Proteome Research*, *13*, 2468–2477. [plus supplementary data]. https://doi.org/10.1021/pr4012552

EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), Koutsoumanis, K., Allende, A., Alvarez-Ordóñez, A., Bolton, D., Bover-Cid, S., Chemaly, M., De Cesare, A., Hilbert, F., Lindqvist, R., Nauta, M., Nonno, R., Peixe, L., Ru, G., Simmons, M., Skandamis, P., Suffredini, E., Cocconcelli, P. S., Fernández Escámez, P. S., … Herman, L. (2024). Update of the list of qualified presumption of safety (QPS) recommended microbiological agents intentionally added to food or feed as notified to EFSA 20: Suitability of taxonomic units notified to EFSA until March 2024. *EFSA Journal*, 22(7), e8882. https://doi.org/10.2903/j.efsa.2024.8882

EFSA (European Food Safety Authority). (2007). Scientific opinion of the Scientific Committee related to uncertainties in dietary exposure assessment. *EFSA Journal*, *5*(1), 438. https://doi.org/10.2903/j.efsa.2007.438

EFSA (European Food Safety Authority). (2011). Use of the EFSA comprehensive European food consumption database in exposure assessment. *EFSA Journal*, *9*(3), 2097. https://doi.org/10.2903/j.efsa.2011.2097

EFSA (European Food Safety Authority). (2015). The food classification and description system FoodEx 2 (revision 2). *EFSA Supporting Publication*, EN-804. https://doi.org/10.2903/sp.efsa.2015.EN-804

EFSA (European Food Safety Authority). (2021). EFSA statement on the requirements for whole genome sequence analysis of microorganisms intentionally used in the foodchain. *EFSA Journal*, *19*(7), 6506. https://doi.org/10.2903/j.efsa.2021.6506

EFSA ANS Panel (EFSA Panel on Food Additives and Nutrient Sources). (2012). Guidance for submission for food additive evaluations. *EFSA Journal*, *10*(7), 2760. https://doi.org/10.2903/j.efsa.2012.2760

EFSA CEP Panel (EFSA Panel on Food Contact Materials, Enzymes and Processing Aids), Lambré, C., Barat Baviera, J. M., Bolognesi, C., Cocconcelli, P. S., Crebelli, R., Gott, D. M., Grob, K., Lampi, E., Mengelers, M., Mortensen, A., Rivière, G., Steffensen, I.-L., Tlustos, C., Van Loveren, H., Vernis, L., Zorn, H., Glandorf, B., Herman, L., … Chesson, A. (2021). Scientific Guidance for the submission of dossiers on food enzymes. *EFSA Journal*, *19*(10), 6851. https://doi.org/10.2903/j.efsa.2021.6851

EFSA CONTAM Panel (EFSA Panel on Contaminants in the Food Chain). (2010). Scientific opinion on Lead in food. *EFSA Journal*, *8*(4), 1570. https://doi.org/10.2903/j.efsa.2010.1570

EFSA CONTAM Panel (EFSA Panel on Contaminants in the Food Chain), Schrenk, D., Bignami, M., Bodin, L., Chipman, J. K., del Mazo, J., Grasl-Kraupp, B., Hogstrand, C., Hoogenboom, L. R., Leblanc, J.-C., Nebbia, C. S., Nielsen, E., Ntzani, E., Petersen, A., Sand, S., Vleminckx, C., Wallace, H., Barregård, L., Benford, D., … Schwerdtle, T. (2024). Update of the risk assessment of inorganic arsenic in food. *EFSA Journal*, *22*(1), e8488. https://doi.org/10.2903/j.efsa.2024.8488

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Rychen, G., Aquilina, G., Azimonti, G., Bampidis, V., Bastos, M. L., Bories, G., Chesson, A., Cocconcelli, P. S., Flachowsky, G., Gropp, J., Kolar, B., Kouba, M., López-Alonso, M., López Puente, S., Mantovani, A., Mayo, B., Ramos, F., Saarela, M., … Galobart, J. (2018). Guidance on the characterisation of microorganisms used as feed additives or as production organisms. *EFSA Journal*, *16*(3), 5206. https://doi.org/10.2903/j.efsa.2018.5206

EFSA Scientific Committee. (2009). Guidance of the scientific committee on transparency in the scientific aspects of risk assessments carried out by EFSA. Part 2: General principles. *EFSA Journal*, *7*(7), 1051. https://doi.org/10.2903/j.efsa.2009.1051

EFSA Scientific Committee. (2012). Guidance on selected default values to be used by the EFSA scientific committee, scientific panels and units in the absence of actual measured data. *EFSA Journal*, *10*(3), 2579. https://doi.org/10.2903/j.efsa.2012.2579

EFSA Scientific Committee, More, S., Bampidis, V., Benford, D., Bragard, C., Halldorsson, T., Hernández-Jerez, A., Bennekou, S. H., Koutsoumanis, K., Lambré, C., Machera, K., Naegeli, H., Nielsen, S., Schlatter, J., Schrenk, D., Silano (deceased), V., Turck, D., Younes, M., Castenmiller, J., … Schoonjans, R. (2021). Guidance on technical requirements for regulated food and feed product applications to establish the presence of small particles including nanoparticles. *EFSA Journal*, *19*(8), 6769. https://doi.org/10.2903/j.efsa.2021.6769

FAO (Food and Agriculture Organization of the United Nations) and WHO (World Health Organization). (online). GSFA Online Updated up to the 42nd Session of the Codex Alimentarius Commission (2019), FOOD ADDITIVE DETAILS, Curdlan (424). https://www.fao.org/gsfaonline/additives/details.html?id=307

Higashi, T., Hashimoto, K., Takagi, R., Mizuno, Y., Okazaki, Y., Tanaka, Y., & Matsushita, S. (2010). Curdlan induces DC-mediated Th17 polarization via Jagged1 activation in human dendritic cells. *Allergology International*, *59*(2), 161–166. https://doi.org/10.2332/allergolint.09-oa-0103

Holck, P., Sletmoen, M., Stokke, B. T., Permin, H., & Norn, S. (2007). Potentiation of histamine release by microfungal (1→3)- and (1→6)-β-D-glucans. *Basic & Clinical Pharmacology & Toxicology*, *101*, 455–458. https://doi.org/10.1111/j.1742-7843.2007.00140.x

JECFA (Joint FAO/WHO Expert Committee on Food Additives). (2000a). Guidelines for the preparation of toxicological working papers for the joint FAO/WHO expert committee on food additives. Geneva, Switzerland.

JECFA (Joint FAO/WHO Expert Committee on Food Additives). (2000b). Evaluation of certain food additives and contaminants. Fifty-third meeting of the joint FAO/WHO expert committee on food additives. No. 44. World Health Organization.

JECFA (Joint FAO/WHO Expert Committee on Food Additives). (2002). Evaluation of certain food additives and contaminants. Fifty-seventh report of the joint FAO/WHO expert committee on food additives. No. 909. World Health Organization, Geneva, Switzerland. https://iris.who.int/bitstream/handle/10665/42578/WHO_TRS_909.pdf;jsessionid=B23C793046F13845026B68E8D43AB5DC?sequence=1

JECFA (Joint FAO/WHO Expert Committee on Food Additives). (2006). Monograph 1. Combined compendium of food additive specifications. https://www.fao.org/food/food-safety-quality/scientific-advice/jecfa/jecfa-additives/detail/en/c/416/

Juul-Madsen, H. R., Norup, L., & Nygaard Lærke, H. (2010). Modulation of the immune response of porcine neutrophils by different β-glucan kuspreparations. *Livestock Science*, *133*, 249–252. https://doi.org/10.1016/j.livsci.2010.06.078

Kankkunen, P., Teiril, L., Rintahaka, J., Alenius, H., Wolff, H., & Matikainen, S. (2010). (1,3)-β-Glucans activate both dectin-1 and NLRP3 inflammasome in human macrophages. *Journal of Immunology*, *184*, 6335–6342. https://doi.org/10.4049/jimmunol.0903019

Kataoka, K., Muta, T., Yamazaki, S., & Takeshige, K. (2002). Activation of macrophages by linear (1→3)-β-D-glucans: Implications for the recognition of fungi by innate immunity. *Journal of Biological Chemistry*, *277*, 36825–36831. https://doi.org/10.1074/jbc.M206756200

Kim, H. S., Park, K. H., Lee, H. K., Kim, J. S., Kim, Y. G., Lee, J. H., Kim, K. H., Yun, J., Hwang, B. Y., Hong, J. T., Kim, Y., & Han, S.-B. (2016). Curdlan activates dendritic cells through dectin-1 and toll-like receptor 4 signaling. *International Immunopharmacology*, *39*, 71–78. https://doi.org/10.1016/j.intimp.2016.07.013

Kumar, H., Kumagai, Y., Tsuchida, T., Koenig, P. A., Satoh, T., Guo, Z., Jang, M. H., Saitoh, T., Akira, S., & Kawai, T. (2009). Involvement of the NLRP3 inflammasome in innate and humoral adaptive immune responses to fungal β-glucan. *Journal of Immunology*, *183*, 8061–8067 [plus supplementary data]. https://doi.org/10.4049/jimmunol.0902477

Kusui, S., Ohnishi, K., Nishimura, S., & Morita, T. (1993). An enzymatic determination of polysaccharide 13140 (Curdlan type polysaccharide) in food. Takasago Plant, Takeda Chemical Industries, Ltd.:1–5, Aioimachi, Takasago-cho, Takasago, Hyogo.

Mæhre, H. K., Dalheim, L., Edvinsen, G. K., Elvevoll, E. O., & Jensen, I. J. (2018). Protein determination-method matters. *Foods*, *7*(1), 5. https://doi.org/10.3390/foods7010005

Mech, A., Rauscher, H., Babick, F., Hodoroaba, V. D., Ghanem, A., Wohlleben, W., Marvin, H., Weigel, S., Brüngel, R., & Friedrich, C. M. (2020). The NanoDefine methods manual. Part 1: The NanoDefiner framework and tools, EUR29876 EN. Publications Office of the European Union, Luxembourg. https://doi.org/10.2760/55181

Mech, A., Rauscher, H., Rasmussen, K., Babick, F., Hodoroaba, V. D., Ghanem, A., Wohlleben, W., Marvin, H., Brüngel, R., & Friedrich, C. M. (2020). The NanoDefine methods manual. Part 2: Evaluation of methods, EUR 29876 EN. Publications Office of the European Union, Luxembourg. https://doi.org/10.2760/071877

Min, L., Isa, S. A. B. M., Fam, W. N., Sze, S. K., Beretta, O., Mortellaro, A., & Ruedl, C. (2012). Synergism between curdlan and GM-CSF confers a strong inflammatory signature to dendritic cells. *Journal of Immunology*, *188*, 1789–1798. https://doi.org/10.4049/jimmunol.1101755

Noss, I., Doekes, G., Thorne, P. S., Heederik, D. J. J., & Wouters, I. M. (2013). Comparison of the potency of a variety of β-glucans to induce cytokine production in human whole blood. *Innate Immunity*, *19*, 10–19. https://doi.org/10.1177/1753425912447129

OECD (Organisation for Economic Co-operation and Development). (1983a). Test No. 471: Bacterial Reverse Mutation Test. OECD Guidelines for the Testing of Chemicals, Section 4. OECD Publishing, Paris. https://doi.org/10.1787/9789264071247-en

OECD (Organisation for Economic Co-operation and Development). (1983b). Test No. 473: In vitro Mammalian Chromosome Aberration Test. OECD Guidelines for the Testing of Chemicals. OECD Publishing, Paris. https://doi.org/10.1787/9789264264649-en

OECD (Organisation for Economic Co-operation and Development). (1983c). Test No. 474: Mammalian Erythrocyte Micronucleus Test. OECD Guidelines for the Testing of Chemicals. OECD Guidelines for the Testing of Chemicals, Section 4. OECD Publishing, Paris. https://doi.org/10.1787/9789264264762-en

OECD (Organisation for Economic Co-operation and Development). (1997). Test No. 476: In Vitro Mammalian Cell Gene Mutation Tests using the Hprt and xprt genes. OECD Guidelines for the Testing of Chemicals, Section 4.

Rand, T. G., Robbins, C., Rajaraman, D., Sun, M., & Miller, J. D. (2013). Induction of Dectin-1 and asthma-associated signal transduction pathways in RAW 264.7 cells by a triple-helical (1,3)-β-D glucan, curdlan. *Archives of Toxicology*, *87*, 1841–1850. [plus supplementary tables]. https://doi.org/10.1007/s00204-013-1042-4

Rauscher, H., Mech, A., Gibson, N., Gilliland, D., Held, A., Kestens, V., Koeber, R., Linsinger, T., & Stefaniak, E. (2019). Identification of nanomaterials through measurements, EUR 29942 EN. Publications Office of the European Union, Luxembourg. https://doi.org/10.2760/053982

Rui, K., Tian, J., Tang, X., Ma, J., Xu, P., Tian, X., Wang, Y., Xu, H., Lu, L., & Wang, S. (2016). Curdlan blocks the immune suppression by myeloid-derived suppressor cells and reduces tumor burden. *Immunologic Research*, *64*, 931–939. https://doi.org/10.1007/s12026-016-8789-7

Soltani, M. H., Kalantari, T., Karimi, M. H., Erfani, N., & Kamali Sarvestani, E. (2012). Evaluation of the immunomodulatory effect of curdlan on maturation and function of mouse spleen-derived dendritic cells. *Iranian Journal of Immunology*, *9*, 168–174.

Sonck, E., Devriendt, B., Goddeeris, B., & Cox, E. (2011). Varying effects of different β-glucans on the maturation of porcine monocyte-derived dendritic cells. *Clinical and Vaccine Immunology*, *18*, 1441–1446. https://doi.org/10.1128/CVI.00080-11

Sonck, E., Stuyven, E., Goddeeris, B., & Cox, E. (2010). The effect of β-glucans on porcine leukocytes. *Veterinary Immunology and Immunopathology*, *135*, 199–207. https://doi.org/10.1016/j.vetimm.2009.11.014

van Eijk, M., Voorn-Brouwer, T., Scheij, S. S., Verhoeven, A. J., Boot, R. G., & Aerts, J. M. F. G. (2010). Curdlan-mediated regulation of human phagocyte-specific chitotriosidase. *FEBS Letters*, *584*, 3165–3169. https://doi.org/10.1016/j.febslet.2010.06.001

Yamasaki, T., Ariyoshi, W., Okinaga, T., Adachi, Y., Hosokawa, R., Mochizuki, S., Sakurai, K., & Nishihara, T. (2014). The dectin 1 agonist curdlan regulates osteoclastogenesis by inhibiting nuclear factor of activated T cells cytoplasmic 1 (NFATc1) through Syk kinase. *Journal of Biological Chemistry*, *289*, 19191–19203. https://doi.org/10.1074/jbc.M114.551416

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: EFSA FAF Panel (EFSA Panel on Food Additives and Flavourings), Andreassen, M., Aquilina, G., Bastos, M. L., Boon, P., Fallico, B., FitzGerald, R., Frutos Fernandez, M. J., Grasl-Kraupp, B., Gundert-Remy, U., Gürtler, R., Houdeau, E., Kurek, M., Louro, H., Morales, P., Passamonti, S., Barat Baviera, J. M., Degen, G., Gott, D., … Castle, L. (2024). Safety evaluation of curdlan as a food additive. *EFSA Journal*, *22*(9), e8985. https://doi.org/10.2903/j.efsa.2024.8985

ANNEX A

Exposure data and estimates

Annex A can be found in the online version of this output (in the 'Supporting information' section): https://doi.org/10.2903/j.efsa.2024.8985