Adopted: 30 January 2025

DOI: 10.2903/j.efsa.2025.9267

efsa JOURNAL

SCIENTIFIC OPINION

# Re-evaluation of pullulan (E 1204) as a food additive and new application for its extension of use

**EFSA Panel on Food Additives and Flavourings (FAF) | Laurence Castle | Monica Andreassen | Gabriele Aquilina | Maria Lourdes Bastos | Polly Boon | Biagio Fallico | Reginald Fitzgerald | Maria Jose Frutos Fernandez | Bettina Grasl-Kraupp | Ursula Gundert-Remy | Rainer Gürtler | Eric Houdeau | Marcin Kurek | Henriqueta Louro | Patricia Morales | Sabina Passamonti | José Manuel Barat Baviera | Gisela Degen | David Gott | Jean-Charles Leblanc | Peter Moldeus | Ine Waalkens-Berendsen | Detlef Wölfle | Jaime Aguilera Entrena | Gabriele Gagliardi | Agnieszka Mech | Concepción Medrano-Padial | Simone Lunardi | Ana Maria Rincon | Camilla Smeraldi | Alexandra Tard | Laura Ruggeri**

**Correspondence:** fip@efsa.europa.eu

The declarations of interest of all scientific experts active in EFSA's work are available at https://open.efsa.europa.eu/experts

## Abstract

The present opinion deals with the re-evaluation of pullulan (E 1204) when used as a food additive and with the new application on the extension of use to several food categories. Pullulan (E 1204) is obtained by fermentation of a food-grade hydrolysed starch with non-genetically modified *Aureobasidium pullulans* [redacted]. Based on the available information, the Panel considered that the manufacturing process of pullulan (E 1204) using this microorganism does not raise a safety concern. The Panel confirmed that pullulan (E 1204) is of no concern for genotoxicity. In vitro, pullulan (E 1204) is broken down by salivary and pancreatic amylase and intestinal iso-amylase and it is further metabolised to short chain fatty acids in the colon by fermentation. Human adult volunteer studies suggested that effects of pullulan (E 1204) are similar to the effects of other poorly digestible carbohydrate polymers including modified celluloses and that mild undesirable gastrointestinal symptoms (i.e. abdominal fullness, flatulence, bloating and cramping) may occur at doses of 10 g pullulan per day and greater. The Panel compared the dose of 10 g pullulan per day with the dietary exposure estimates to pullulan (E 1204) in its currently permitted uses and considering the proposed changes to the currently permitted uses. The Panel concluded that there is no need for a numerical ADI for pullulan (E 1204) and there is no safety concern for the currently reported uses and use levels. Additionally, the Panel concluded that the exposure estimates considering the proposed changes to the currently permitted uses and use levels of pullulan (E 1204) are of no safety concern. The estimates for dietary exposure to pullulan (E 1204) indicate that individuals with a high level of exposure, principally coming from food supplements, may experience mild gastrointestinal symptoms at the currently reported uses and use levels.

**KEYWORDS**

*Aureobasidium pullulans*, E 1204, extension of use, food additive, pullulan, re-evaluation

# CONTENTS

## SUMMARY

The present opinion deals with the re-evaluation of pullulan (E 1204) when used as a food additive and with a new application on the extension of use of pullulan (E 1204) in several food categories.

Pullulan (E 1204) is currently authorised as a food additive in the EU in accordance with Annex II, Part E, to Regulation (EC) No 1333/2008 at maximum permitted levels (MPLs) equal to QS in two food categories (FCs) (5.2 'Other confectionery including breath freshening microsweets' and 17.1 'Food supplements supplied in a solid form, excluding food supplements for infants and young children'), with restrictions.

EFSA has evaluated pullulan (E 1204) in 2004 (EFSA AFC Panel, 2004) intended to be used in the production of capsule shells and of coated tablets for the preparation of dietary supplements and as a matrix for edible flavoured films (breath fresheners), concluding that '*on the basis that pullulan is similar to other poorly digested carbohydrates and that the current proposed usage levels are below the level likely to cause abdominal fullness (10 g per person per day), the Panel consider that the expected intakes of pullulan would not present any concern when used as a food additive in the proposed uses and at the usage levels requested*'.

JECFA evaluated the safety of pullulan (INS 1204) as a food additive in 2005 and later in 2011 (JECFA, 2006, 2011a) and published a specifications monograph in 2011 (JECFA, 2011b). Based on the available data, JECFA concluded that pullulan is a substance of low toxicity with an acceptable daily intake (ADI) 'not specified'. A toxicological monograph was not prepared.

According to the definition given in Commission Regulation (EU) No 231/2012, pullulan is defined as a '*Linear, neutral glucan consisting mainly of maltotriose units connected by -1,6 glycosidic bonds. It is produced by fermentation from a food-grade hydrolysed starch using a non-toxin-producing strain of Aureobasidium pullulans. After completion of the fermentation, the fungal cells are removed by microfiltration, the filtrate is heat-sterilised and pigments and other impurities are removed by adsorption and ion exchange chromatography*'.

The present re-evaluation applies to pullulan (E 1204) obtained by fermentation of a food-grade hydrolysed starch with non-genetically modified *Aureobasidium pullulans* ([redacted]). Information on the manufacturing process of pullulan (E 1204) obtained by fermentation of a food-grade hydrolysed starch with non-genetically modified *A. pullulans* ([redacted]) was submitted and evaluated. Based on the detailed information on the characterisation of the microorganism and the demonstration of the absence of viable cells in pullulan (E 1204), the Panel considered that the manufacturing process of pullulan (E 1204) using this microorganism does not raise a safety concern. However, in order to better describe the manufacturing processes evaluated in the current assessment, the Panel recommended modifying the definition of the food additive in the Commission Regulation (EU) 231/2012 by specifying that pullulan (E 1204) is produced by fermentation from a food-grade hydrolysed starch using *A. pullulans* [redacted], followed by several purification steps and drying.

The Panel emphasised that the present re-evaluation does not apply to pullulan (E 1204) produced by other manufacturing processes (e.g. different microorganisms, strains, different carbohydrates sources, purifications steps). The reason is that this would be considered as a significant change in the production methods which would require an assessment in accordance with relevant legislation (Regulation (EC) No 1331/2008).

With regard to toxic elements, one interested business operator (IBO) provided analytical data on the levels of arsenic (As), lead (Pb), mercury (Hg) and cadmium (Cd) in commercial samples of pullulan (E 1204). The Panel noted that no information on the lowest technologically achievable levels for the toxic elements in E 1204 was provided by the IBOs. The Panel noted that among the potential inorganic impurities tested, only lead has defined limit value in EU specifications for E 1204, and that lead and arsenic were quantified in some of the analysed samples. The Panel performed the risk assessment that would result if these elements were present in E 1204 at the maximum measured value (for arsenic and lead), at the reported values (for mercury and cadmium), and at the current maximum limit in the EU specifications (for lead). The Panel noted that for arsenic, the calculated margin of safety (MOE) values would give rise to concern at the measured values. For the other toxic elements, the concentrations considered for the calculation of their potential exposure do not give rise to safety concerns (Table A.3).

The Panel recommended to include limits for arsenic and to lower the EU specification limit for lead, taking into account (i) the results of the calculations performed by the Panel; (ii) the fact that the food additive is not the only potential dietary source of toxic elements; and that (iii) the maximum limits should be established based on actual levels in the commercial food additive. Taking into account that pullulan (E 1204) is produced from food-grade hydrolysed starch and undergoes to some purification steps, systematic contamination by cadmium and mercury is not anticipated and the Panel did not see a need to recommend additional specification limits for cadmium and mercury. In addition, the results of the calculations performed by the Panel (Table A.3) do not give rise to concern.

According to the data provided, secondary metabolites of concerns (i.e. mycotoxins and Aureobasidin A) were not detected in the tested samples of pullulan.

The Panel noted that the stability tests provided by the IBO show a decrease in viscosity at low pH (2–3). Regardless, based on the submitted information and the physicochemical characteristics of pullulan (E 1204), the Panel noted that the additive is expected to be stable under a wide range of temperature and pH conditions and does not interact with food.

Although the water solubility test provided (results > 300 g/L) was not in line with the Guidance on Particle-TR, the Panel considered that, based on the information in the literature, pullulan (E 1204) can be considered as highly soluble in water (above the 33.3 g/L criterion). Therefore, the Panel concluded there is no concern with regard to the potential presence of small particles, including nanoparticles, in pullulan (E 1204) at its intended uses and use levels.

Dietary exposure to pullulan (E 1204) was estimated according to different exposure scenarios as described in Table 5, to address both its re-evaluation and the proposed changes to the currently permitted uses. Currently, pullulan (E 1204) is an authorised food additive in the EU in two food categories (FC 5.2 'Other confectionery including breath freshening microsweets' and FC 17.1 'Food supplements supplied in a solid form, excluding food supplements for infants and young children') at QS.

Following the call for data for the re-evaluation, one IBO provided EFSA with current use levels for FC 17.1 only. To calculate the dietary exposure in the context of the re-evaluation of pullulan (E 1204), the Panel used the 'food supplements consumers only' scenario, as described in the approach for the refined exposure assessment of food additives under re-evaluation published by EFSA ANS Panel (2017): (i) only consumers of food supplements were considered; and (ii) it was assumed that these consumers will be exposed to pullulan (E 1204) at the highest reported use level in food supplements, i.e. 277,777 mg/kg for FC 17.1. The highest mean and 95th percentile chronic exposure to pullulan (E 1204) among consumers calculated following this approach were 62 and 219 mg/kg bw per day, respectively, in adolescents.

In the present assessment, an application requesting changes to the currently permitted use of pullulan (E 1204) with new uses and maximum and typical use levels (Table 3) was also evaluated. To calculate the dietary exposure to pullulan (E 1204) deriving from the proposed changes to the currently permitted uses, using the 'food supplement consumers only' scenario, the Panel considered the highest level provided by an IBO for FC 17.1 (i.e. 277,777 mg/kg), as well as the proposed maximum use level for FC 17.2 (60,000 mg/kg) and either the maximum or typical use levels proposed by the applicant for all the other FCs. The highest mean and 95th percentile chronic exposure among consumers were 65 and 224 mg/kg bw per day, respectively, in adolescents.

The Panel noted that the outcome of the exposure calculations for the 'food supplements consumers only' scenarios for both the re-evaluation and extension of use are almost identical because of the predominant contribution of solid food supplements (FC 17.1) to the overall exposure estimates.

Overall, the Panel considered that the exposure to pullulan (E 1204) from its use as a food additive was overestimated in all scenarios (see Table 9).

The Panel confirmed that pullulan (E 1204) is of no concern for genotoxicity. In vitro, pullulan (E 1204) is broken down by salivary and pancreatic amylase and intestinal iso-amylase and it is further metabolised to short chain fatty acids in the colon by fermentation. Toxicity studies showed that pullulan has effects in the gastrointestinal tract (increased relative weights of the stomach, small intestine, large intestine and caecum). The newly submitted 13-week rat study confirmed the effect on the caecum. This effect is commonly seen when high doses of unabsorbed, fermentable carbohydrates are ingested and is considered to be an adaptive response without toxicological relevance. The Panel identified a NOAEL of 7.9 g/kg bw per day for male rats and 9.7 g/kg bw per day for female rats. This study confirmed the conclusion drawn by the AFC Panel in 2004 that administration of pullulan in rats up to 9 weeks caused mainly local effects in the gastrointestinal tract and there is no evidence of systemic effects.

Human adult volunteer studies suggested that effects of pullulan (E 1204) are similar to the effects of other poorly digestible carbohydrate polymers including modified celluloses. In addition, human volunteer studies have reported mild undesirable gastrointestinal symptoms (i.e. abdominal fullness, flatulence, bloating and cramping) at doses of 10 g pullulan per day and greater (corresponding to 143 mg/kg bw per day, for 70 kg adult).

The Panel compared the dose of 10 g pullulan per day with the dietary exposure estimates to pullulan (E 1204) in its currently permitted uses and considering the proposed changes to the currently permitted uses. For the re-evaluation in its currently permitted uses, the mean exposure estimates to pullulan (E 1204) calculated for consumers of food supplements only, was at the maximum 4 g/person per day in adolescents and adults (adult bw 70 kg, adolescents bw 53 kg). Similar exposure estimates were estimated for the same dietary exposure scenario, applied to the proposed changes to the currently permitted uses. When considering the changes to the currently permitted uses for the general population, the levels were well below the value of 10 g/person per day associated with the undesirable symptoms both at the mean and the high-level consumers. The value of 10 g/person per day was instead exceeded in adults and adolescents at 95th percentile estimated for food supplements consumers only (both for currently permitted uses and proposed changes to the currently permitted uses), with maximum levels reaching approximately 12–13 g/person per day.

The Panel concluded that there is no need for a numerical ADI for pullulan (E 1204) and there is no safety concern for the currently reported uses and use levels. Additionally, the Panel concluded that the exposure estimates considering the proposed changes to the currently permitted uses and use levels of pullulan (E 1204) are of no safety concern.

The estimates for dietary exposure to pullulan (E 1204) indicate that individuals with a high level of exposure, principally coming from food supplements, may experience mild gastrointestinal symptoms at the currently reported uses and use levels.

Furthermore, the Panel recommended to the European Commission:

- Revising the definition of the food additive in the EU specifications as 'produced by fermentation from a food-grade hydrolysed starch using *Aureobasidium pullulans* [redacted] ([redacted])';
- Including CAS No 9057-02-7 in the EU specifications;
- Lowering the current limits for lead (Pb) in the EU specifications for Pullulan (E 1204) in order to ensure that it will not be a significant source of exposure to lead in food;
- Introducing a maximum limit for arsenic (As) in the EU specifications for Pullulan (E 1204) in order to ensure that it will not be a significant source of exposure to arsenic in food.

# 1 | INTRODUCTION

The present opinion deals with the re-evaluation of pullulan (E 1204) when used as a food additive and with a new application on the extension of use of pullulan (E 1204) in several food categories.[1]

## 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[2] on food additives. Only food additives that are included in the Union list in Annex II to that Regulation, may be placed on the market and used in foods under the conditions of use specified therein. Only food additives included in the Union list in Annex III may be used in food additives, in food enzymes, in food flavourings and in nutrients under the conditions of use specified therein.

Pullulan (E 1204), a polysaccharide produced from a yeast, is currently authorised as a food additive in the European Union in accordance with Annex II to Regulation (EC) 1333/2008 for its use in breath freshening microsweets in the form of films within the food category 05.2 "Other confectionery including breath freshening microsweets" and in food supplements in capsule and tablet form within the food category 17.1 "Food supplements supplied in a solid form, excluding food supplements for infants and young children". Both provisions authorise the use of pullulan (E 1204) at *quantum satis*.

The above authorisation is based on the EFSA scientific opinion issued in 2004 which assessed the safety of pullulan as a new food additive and concluded that the expected intakes of pullulan would not present any concern when used as a food additive in the proposed uses and at the usage levels requested, however, if higher levels of use or other uses were to be requested then more data might be required.

The Directorate-General for Health and Food Safety has received an application for the extension of use of pullulan (E 1204) to several food categories. The application was presented to the Member States and a clarification from the applicant was needed as regards the food categories for which the extension of use was requested. The applicant clarified that the extension of the use is requested as follows:

*Annex II to Regulation (EC) No 1333/2008*

| Food category | MPL mg/kg |
|---|---|
| 1.2 Unflavoured fermented milk products, including natural unflavoured buttermilk (excluding sterilised buttermilk) non-heat treated after fermentation | 2 |
| 1.4 Flavoured fermented milk products including heat-treated products | |
| 1.5 Dehydrated milk as defined by Directive 2001/114/EC | |
| 1.6.1 Unflavoured pasteurised cream (excluding reduced fat creams) | |
| 1.6.2 Unflavoured live fermented cream products and substitute products with a fat content of less than 20% | |
| 1.6.3 Other creams | |
| 1.7.1 Unripened cheese excluding products falling in category 16 | |
| 12.9 Protein products, excluding products covered in category 1.8 | |
| 13.2 Dietary foods for special medical purpose defined in Directive 1999/21/EC (excluding products from food category 13.1.5) | |
| 13.3 Dietary foods for weight control diets intended to replace total daily food intake or an individual meal (the whole or part of the total daily diet) | |
| 14.1.2 Fruit juices as defined by Directive 2001/112/ECC and vegetable juices | |
| 14.1.3 Fruit nectars as defined by Directive 2001/112/EC and vegetables nectars and similar products | |
| 14.1.4 Flavoured drinks | |
| 14.1.5.1 Coffee, coffee extracts | |
| 14.1.5.2 Other | |
| 1.8 Dairy analogues, including beverage whiteners | 14 |
| 2.1 Fats and oils essentially free from water (excluding anhydrous milkfat) | 20 |
| 2.2.1 Butter and concentrated butter and butter oil and anhydrous milkfat | |
| 2.2.2 Other fat and oil emulsions including spreads as defined by Council Regulation (EC) no 1234/2007 and liquid emulsions | |
| 13.4 Foods suitable for people intolerant to gluten as defined by Regulation (EC) No 41/2009 | |

(Continues)

[1] https://open.efsa.europa.eu/questions/EFSA-Q-2020-00517.

[2] 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.

(Continued)

| Food category | MPL mg/kg |
|---|---|
| 3 Edible ices | 8 |
| 5.1 Cocoa and chocolate products as covered by Directive 2000/36/EC | 4 |
| 5.2 Other confectionery including breath refreshening microsweets | 140 |
| 5.3 Chewing gum | 300 |
| 6.7 Pre-cooked or processed cereals | 6400 |
| 10.2 Processed eggs and egg products | |
| 17.1 Food supplements supplied in a solid form, excluding food supplements for infants and young children | 60,000 |
| 17.2 Food supplements supplied in a liquid form, excluding food supplements for infants and young children | |

*Note:* The food categories were grouped based on the same maximum use level requested.

*Annex III to Regulation (EC) No 1333/2008*

Section A of Part 5 to be used at *quantum satis* as a carrier of nutrients.

Taking into account the application requesting the use in a wide range of food categories and the EFSA's conclusions on the safety of pullulan (E 1204) in 2004, it is appropriate to proceed first with the safety re-evaluation of this food additive, in accordance with the procedure set in Regulation (EC) No. 257/2010,[3] before addressing the safety of the proposed extension of use.

### 1.1.2 | Terms of Reference

The European Commission requests the European Food Safety Authority to provide a scientific opinion on the safety of pullulan (E 1204) in accordance with the Regulation (EU) No 257/2010 as part of the programme for the re-evaluation of food additives already permitted in the European Union before 20 January 2009 and on the safety of the proposed extension of use of pullulan (E 1204) to the food categories listed above in accordance with Regulation (EC) No 1331/2008[4] establishing a common authorisation procedure for food additive, food enzymes and food flavourings.

## 1.2 | Information on existing authorisation and evaluations

Pullulan (E 1204) is currently authorised as a food additive in the European Union in accordance with Annex II to Regulation (EC) 1333/2008 for its use in breath freshening microsweets in the form of films within the food category 05.2 'Other confectionery including breath freshening microsweets' and in food supplements in capsule and tablet from within the food category 17.1 'Food supplements supplied in a solid form, excluding food supplements for infants and young children'. Both provisions authorise the use of pullulan (E 1204) at *quantum satis* (QS). Specific purity criteria have been defined in Commission Regulation (EU) No 231/2012.[5]

The EFSA Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food (AFC Panel) evaluated pullulan (E 1204) in 2004 (EFSA AFC Panel, 2004) in the production of capsule shells and of coated tablets for the preparation of dietary supplements and as a matrix for edible flavoured films (breath fresheners). The pullulan product under consideration was named PI-20 and was characterised by an average molecular weight (MW) of 200,000 Daltons (Da).

The AFC Panel noted that the toxicological database was limited and many of the available studies provided no information on the type of pullulan used as test material. Of those that did, the majority used a material with a MW of about 50,000 Da. In vitro studies suggested that PI-20 is broken down into smaller polymers (of around 70,000 Da) by salivary and pancreatic amylases. In vitro and in vivo experiments suggested that it may be completely fermented to short chain fatty acids (SCFA) in the colon, however there is no clear in vivo evidence of the complete fermentation and on whether the rate of fermentation depends on the size of the polymer. Subchronic (9–62 week) oral toxicity studies in the rat indicated that pullulan is of low toxicity. Dietary toxicity studies in rats to which pullulan was administered up to 9 weeks suggested that pullulan has local effects in the gastrointestinal tract but provided no evidence of systemic effects. At dietary concentrations of 1% pullulan (around 0.5 g pullulan/kg bw per day) and greater the following effects were reported: increased relative weights of the stomach, small intestine, large intestine and caecum, and evidence of changes in the size and shape of intestinal pouches (the haustra coli) in the intestinal mucosa. Limited evidence indicated a decrease in severity of effects with time of administration, suggesting possible adaptation. No adequate chronic toxicity, carcinogenicity, reproductive

[3]Commission Regulation (EU) No 257/2010 of 25 March 2010 setting up a programme for the re-evaluation of approved food additives in accordance with Regulation (EC) No 1333/2008 of the European Parliament and of the Council on food additives. OJ L 80, 26.3.2010, p. 19–27.

[4]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, p. 1–6.

[5]Regulation (EU) No 231/2012 of 9 March 2012 laying down specifications for food additives listed in Annexes II and III to Regulation (EC) No 1333/2008 of the European Parliament and of the Council. OJ L 83, 22.3.2012, p. 1–295.

and developmental studies were available. Human volunteer studies have reported mild gastrointestinal symptoms (abdominal fullness) at doses of 10 g pullulan per day and greater. The AFC Panel calculated that the estimated exposure using specified worst-case assumptions (daily intake of 12 tablets and a packet of breath freshening films) in adults would be around 23% of the dose of 10 g pullulan, and 46% in children. Pullulan was considered to hold similarities to a number of other poorly digestible carbohydrate polymers including modified celluloses.

The AFC Panel concluded that '*on the basis that pullulan is similar to other poorly digested carbohydrates and that the current proposed usage levels are below the level likely to cause abdominal fullness, the Panel considers that the expected intakes of pullulan would not present any concern when used as a food additive in the proposed uses and at the usage levels requested*'.

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) first evaluated pullulan (INS 1204) at its 65th meeting in 2006 and concluded that the current uses of pullulan as a food additive and the studies on its safety provided sufficient information to establish an ADI 'not specified' (JECFA, 2006).

The safety of pullulan when used as a low digestible carbohydrate/dietary fibre in various types of food was later evaluated by JECFA during its 74th meeting in 2011, focussing on the safety of the estimated intake of pullulan resulting from the proposed use levels without assessing the efficacy of pullulan used as a dietary fibre (JECFA, 2011a). The specifications monograph was also revised at that occasion (JECFA, 2011b). Based on the available data, JECFA concluded that pullulan is a substance of low toxicity and confirmed the previously established ADI 'not specified'. A toxicological monograph was not prepared.

Pullulan has been extensively used as a food ingredient in Japan (EFSA AFC Panel, 2004).

# 2 | DATA AND METHODOLOGIES

## 2.1 | Data

In accordance with Regulation (EU) No 257/2010, EFSA launched a public call for data[6] in 2022. The Panel based its assessment on the information submitted by two IBOs in reply to this public call for data (Documentation provided to EFSA No 3, 4), information from previous evaluations and additional literature published since the last evaluation (from January 2003 to October 2024), as foreseen under point (e) of Article 4 of Regulation (EU) No 257/2010.

A request for extension of use of pullulan (E 1204) to 26 food categories was also considered in this assessment.[7] The original application dossier was submitted in 2020 (Documentation provided to EFSA No 1). Additional information was submitted in April 2021 (Documentation provided to EFSA No 2), May 2024 (Documentation provided to EFSA No 5), in reply to EFSA's requests for clarification.

The EFSA Comprehensive European Food Consumption Database (Comprehensive Database) and the Mintel's Global New Products Database (GNPD) were used to estimate the dietary exposure to pullulan (E 1204).

## 2.2 | Methodologies

This opinion was formulated following the principles described in the EFSA Guidance 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 FAF Panel assessed the safety of pullulan (E 1204) as a food additive in line with the principles laid down in Regulation (EU) 257/2010 and in the Guidance for submission for food additive evaluations in 2012 (EFSA ANS Panel, 2012).

In the context of this re-evaluation, the Panel took also into account the 'Conceptual framework for the risk assessment of certain food additives re-evaluated under Commission Regulation (EC) No 257/2010' (EFSA ANS Panel, 2014), as well as the 'Approach followed for the refined exposure assessment as part of the safety assessment of food additives under re-evaluation' (EFSA ANS Panel, 2017).

# 3 | ASSESSMENT

## 3.1 | Technical data

### 3.1.1 | Identity and specifications of pullulan (E 1204)

According to the definition given in Regulation (EU) No 231/2012, pullulan is defined as '*Linear, neutral glucan consisting mainly of maltotriose units connected by α-1,6 glycosidic bonds*'. The structural formula of pullulan (E 1204) is presented in Figure 1.

[6]Available online: https://www.efsa.europa.eu/en/call/call-technical-and-toxicological-data-pullulan-e-1204-authorised-food-additive-eu.

[7]Available online: https://open.efsa.europa.eu/questions/EFSA-Q-2021-00278?search=pullulan.

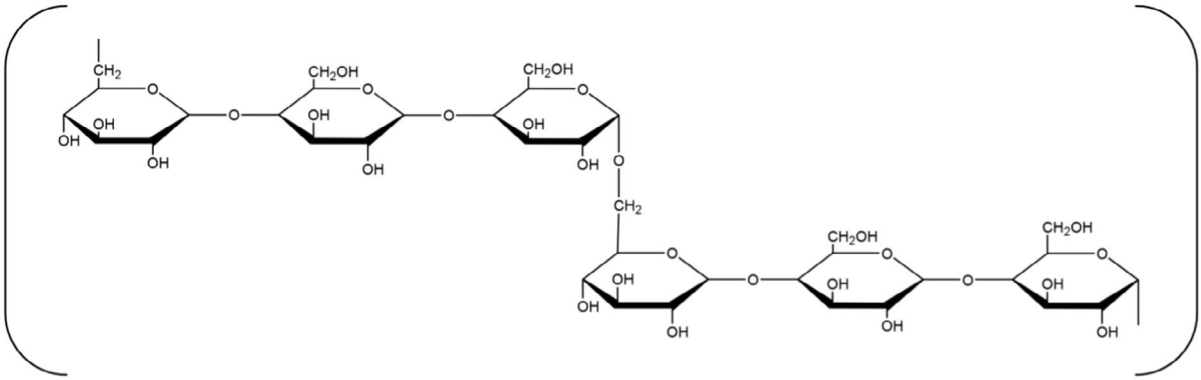

**FIGURE 1** Structural formula of pullulan E 1204 (EFSA AFC Panel, 2004).

The following information on the manufacturing process is included in the current EU specifications '*It is produced by fermentation from a food-grade hydrolysed starch using a non-toxin-producing strain of A. pullulans. After completion of the fermentation, the fungal cells are removed by microfiltration, the filtrate is heat-sterilised and pigments and other impurities are removed by adsorption and ion exchange chromatography*'.

The Panel noted that the current definition of the microorganism used to produce pullulan (E 1204) '*It is produced by fermentation from a food-grade hydrolysed starch using a non-toxin-producing strain of A. pullulans*' does not indicate the specific production strain. According to information provided by the IBO, the production strain of *A. pullulans* used is [redacted] (Documentation provided to EFSA No 3).

The Panel also noted that JECFA updated the definition of the production organism outlined in the specification monograph on pullulan (JECFA, 2011b) from '*a non-toxin-producing strain of A. pullulans*' to '*a non-toxigenic strain of A. pullulans*'.

According to the information provided by the IBO, the filtrate resulting from the previous processing steps is finally concentrated by evaporation, dried and crushed (Documentation provided to EFSA No 3).

Specifications for pullulan (E 1204), as laid down in Commission Regulation (EU) No 231/2012, are listed in Table 1. Table 1 also report the specifications for pullulan as reported by JECFA (JECFA, 2011b).

**TABLE 1** Specifications for pullulan (E 1204), according to Commission Regulation (EU) No 231/2012 and as provided by the JECFA specifications monograph on pullulan (JECFA, 2011b).

| | Commission Regulation (EU) No 231/2012 | JECFA (2011b) |
|---|---|---|
| **Synonyms** | | INS No. 1204 |
| **Definition** | Linear, neutral glucan consisting mainly of maltotriose units connected by α-1,6 glycosidic bonds. It is produced by fermentation from a food-grade hydrolysed starch using a non-toxin-producing strain of *Aureobasidium pullulans*. After completion of the fermentation, the fungal cells are removed by microfiltration, the filtrate is heat-sterilised and pigments and other impurities are removed by adsorption and ion exchange chromatography | Linear, neutral glucan consisting mainly of maltotriose units connected by α-1,6 glycosidic bonds. It is produced by fermentation from a food-grade hydrolysed starch using a non-toxigenic strain of *Aureobasidium pullulans*. After completion of the fermentation, the fungal cells are removed by microfiltration, the filtrate is heat-sterilised and pigments and other impurities are removed by adsorption and ion exchange chromatography |
| CAS No | | 9057-02-7 |
| Einecs | 232-945-1 | |
| Chemical name | | |
| Chemical formula | $(C_6H_{10}O_5)_n$ | $(C_6H_{10}O_5)_n$ |
| Assay | Not less than 90% of glucan on the dried basis | Not less than 90% of glucan on the dried basis |
| **Description** | White to off-white odourless powder | White to off-white odourless powder |
| **Identification** | | |
| Solubility | Soluble in water, practically insoluble in ethanol | Soluble in water, practically insoluble in ethanol |
| pH | 5–7 (10% solution) | 5–7 (10% solution) |
| Precipitation with polyethylene glycol 600 | Add 2 mL of polyethylene glycol 600 to 10 mL of a 2% aqueous solution of pullulan. A white precipitate is formed | Add 2 mL of polyethylene glycol 600 to 10 mL of a 2% aqueous solution of pullulan. A white precipitate is formed |
| Depolymerisation with pullulanase | Prepare two test tubes each with 10 mL of a 10% pullulan solution. Add 0.1 mL pullulanase solution having activity 10 units/g to one test tube and 0.1 mL water to the other. After incubation at about 25°C for 20 min, the viscosity of the pullulanase-treated solution is visibly lower than that of the untreated solution | Prepare two test tubes each with 10 mL of a 10% pullulan solution. Add 0.1 mL pullulanase solution having activity 10 units/g (refer to pullulanase activity, under Methods for enzyme preparations in Volume 4) to one test tube and 0.1 mL water to the other. After incubation at about 25° for 20 min, the viscosity of the pullulanase-treated solution is visibly lower than that of the untreated solution |

**TABLE 1** (Continued)

| | Commission Regulation (EU) No 231/2012 | JECFA (2011b) |
|---|---|---|
| Viscosity | 100 to 180 $mm^2/s$ (10% w/w aqueous solution at 30°C) | 100–180 $mm^2/s$ (10% w/w aqueous solution at 30°C) |
| **Purity** | | |
| Loss on drying | Not more than 6% (90°C, pressure not more than 50 mmHg, 6 h) | Not more than 6% (90°C, pressure not more than 50 mm Hg, 6 h) |
| Mono-, di- and oligosaccharides | Not more than 10% expressed as glucose | Not more than 10% (expressed as glucose) |
| Lead | Not more than 1 mg/kg | Not more than 1 mg/kg |
| **Microbiological criteria** | | |
| Yeast and moulds | Not more than 100 colonies per gram | Not more than 100 CFU/g |
| Coliforms | Absent in 25 g | Negative in 25 g |
| *Salmonella* spp. | Absent in 25 g | Negative in 25 g |

No IBO has proposed changes to the current EU specifications for pullulan (E 1204).

The Panel noted that the CAS number 9057-02-7 is not included in the specifications for E 1204 laid down by Commission Regulation (EU) No 231/2012.

Following the EFSA call for data, two IBOs provided data and information to support the re-evaluation of pullulan E 1204. Technical data regarding identity and specifications for E 1204 were provided by one IBO (Documentation provided to EFSA No 3).

One IBO provided analytical on 10 commercial samples of E 1204 (Documentation provided to EFSA No 3). In all tested samples of E 1204, the purity of pullulan, determined by calculation according to the JECFA monograph (2011b), exhibited a range between 92% and 99%. The pH and viscosity of the food additive were in the range of 5.5–6.0 and 124–168 $mm^2/s$, respectively. The loss on drying was up to 2%. The content of mono, di- and oligosaccharides ranged from 1% to 8%. Yeast and moulds were not detected. Coliforms and *Salmonella* ssp. were both absent in 25 g of sample. Concentration of lead (Pb) was below 1 mg/kg in all tested samples. Overall, the analytical results confirmed that the tested material complies with the specifications outlined in Regulation (EU) No 231/2012.

Supplementary results of analysis were provided for additional 18 commercial samples of E 1204, including protein content, sulphated ash, arsenic (As) and total heavy metals (Documentation provided to EFSA No 3). Sulphated ash was in the range of 0%–0.2%. Proteins were not detected. As and total heavy metals were consistently below 1 and 5 mg/kg in the tested samples, respectively.

Additional data on microbiological parameters were provided by the IBO (Documentation provided to EFSA No 3). In all tested samples of E 1204, the tested microbiological parameters were lower than the detection limits, with the exception of flat sour spores that gave values up to 7 colony forming unit (CFU/g).

According to the IBO, there is no evidence from the scientific literature suggesting that *A. pullulans* produces mycotoxins. Nonetheless, the levels of mycotoxins were analysed by high-performance liquid chromatography (HPLC) in five samples of E 1204 (Documentation provided to EFSA No 3). In all tested samples of E 1204, the levels of mycotoxins were below the limits of detection (LODs) (2 µg/kg for aflatoxin B1, B2, G1 and G2; 10 µg/kg for zearalenone; 50 µg/kg for sterigmatocystin; 2 µg/kg for ochratoxin).

The presence of Aureobasidin A, a secondary metabolite that can be produced by certain strains of *A. pullulans*, was assessed by means of antimicrobial activity tests on five commercial samples of E 1204, using *Saccharomyces cerevisiae* as a sensitive tester strain (Documentation provided to EFSA No 3). No antimicrobial activity was detected in any of the tested samples.

The IBO provided information on the MW distribution of E 1204. The analysis was performed by gel permeation chromatography (GPC) of 10 commercial samples of E 1204 (Documentation provided to EFSA No 3). The MW of pullulan ranged from 310 to 420 kDa.

The Panel considered the data sufficient to demonstrate that the analysed samples meet the current specifications outlined in Regulation (EU) No 231/2012 for E 1204.

However, the Panel recommends a revision of the specifications. In particular, the description should include indications on the specific strain of the microorganism used for the production of the food additive (*A. pullulans* [redacted]).

*Particle size distribution*

No data on particle morphology and size distribution were provided. Based on the results of water solubility tests, for which water solubility of pullulan was much higher than the threshold included in the EFSA Scientific Committee Guidance on Particle -TR (i.e. solubility in water of E 1204 above 33.3 g/L), the IBO decided not to perform any further assessment of the fraction of small particles, including nanoparticles, for the food additive E 1204.

*Solubility*

Information on water solubility of pullulan (E 1204) was provided by the IBO (Documentation provided to EFSA No 3) supporting the re-evaluation of this food additive.

The IBO provided results of water solubility tests for three samples of E 1204 performed according to the Japanese Pharmacopoeia Seventeenth Edition, General Notice 30 (Documentation provided to EFSA No 3). Under these conditions, the solubility in water at 20°C for three samples of E 1204 was found to be 357 g/L, 323 g/L and 357 g/L, respectively.

The Panel noted that the performed solubility test was not in line with the Guidance on Particle-TR, which foresees the use of OECD TG 105 with an additional step of ultrafiltration, or an equivalent method (EFSA Scientific Committee, 2021). Based on the information from the literature (Sugimoto, 1978; Tsujisaka & Mitsuhashi, 1993) and given the nature of this substance, the Panel considered nonetheless that the solubility of pullulan (E 1204) is substantially higher than the value of 33.3 g/L proposed as a criterion to decide whether an additional assessment for the fraction of small particles, including nanoparticles, is needed according to the EFSA Guidance particle-TR (EFSA Scientific Committee, 2021).

Even though the water solubility test provided by the applicant was not compliant with the requirements of the EFSA Scientific Committee Guidance on Particle-TR (EFSA Scientific Committee, 2021), the Panel concurred with the IBO's approach based on the information available in the literature on the solubility of pullulan. Therefore, the Panel concluded there is no concern with regard to the potential presence of small particles, including nanoparticles, in pullulan used as food additive at the proposed uses and use levels and considered that pullulan (E1204) can be assessed following the conventional risk assessment, i.e. EFSA Guidance for submission for food additive evaluations (EFSA ANS Panel, 2012).

*Toxic elements*

With regard to toxic elements, one IBO provided analytical data on the levels of arsenic (As), lead (Pb), mercury (Hg) and cadmium (Cd) in commercial samples of pullulan (E 1204) (Documentation provided to EFSA No 3). Details of the analytical data provided are available in Appendix A. The Panel noted that no information on the lowest technologically achievable levels for the toxic elements in E 1204 was provided by the IBOs.

The Panel noted that among the potential inorganic impurities tested, only lead has defined limit value in EU specifications for E 1204, and that lead and arsenic were quantified in some of the analysed samples.

The Panel performed the risk assessment that would result if these elements were present in E 1204 at the maximum measured value (for arsenic and lead), at the reported values (for mercury and cadmium) and at the current maximum limit in the EU specifications (for lead). The outcome of the risk assessment for these scenarios is presented in Table A.3, Appendix A.

The Panel noted that for arsenic, the calculated MOE values would give rise to concern at the measured values. For the other toxic elements, the concentrations used for the calculation of their potential exposure do not give rise to safety concerns (Table A.3).

The Panel recommended to include limits for arsenic and to lower the EU specification limit for lead, taking into account: (i) the results of the calculations performed by the Panel (Table A.3); (ii) the fact that the food additive is not the only potential dietary source of toxic elements; and that (iii) the maximum limits should be established based on actual levels in the commercial food additive.

Taking into account that pullulan (E 1204) is produced from food-grade hydrolysed starch and undergoes to some purification steps, systematic contamination by cadmium and mercury is not anticipated and the Panel did not see a need to recommend additional specification limits for cadmium and mercury. In addition, the results of the calculations performed by the Panel (Table A.3) do not give rise to concern.

If the European Commission decides to revise the current limits in the EU specifications, the values in Table A.3 and Appendix A could be considered.

The Panel noted that the choice of maximum limits for toxic elements in the EU specifications is in the remit of risk management.

### 3.1.2 | Manufacturing process

*Identity of the raw materials and processing aids*

The list of raw materials and processing aids used for producing pullulan was provided (Documentation provided to EFSA No 3).

*Description of the manufacturing process*

Following the EFSA call for data, one IBO provided information on the manufacturing process of pullulan (E 1204) (Documentation provided to EFSA No 3). Information submitted in the application dossier for the extension of use of pullulan (E 1204) was also considered (Documentation provided to EFSA No 1).

According to the IBO, the manufacturing process of E 1204 uses food-grade raw materials and processing aids and is conducted under conditions of good manufacturing practices (Documentation provided to EFSA No 1, 3). Pullulan is a polysaccharide obtained from the fermentation of a starch syrup, used as a substrate, in a broth of a non-GM strain of *A. pullulans* ([redacted]), under appropriate conditions of pH, temperature and oxygen.

After the completion of the fermentation stage, the mixture undergoes microfiltration or, alternatively, centrifugation to separate the fungal cells from the other components. If needed, [redacted]. Once the cells have been removed, the preparation undergoes heat sterilisation to guarantee microbial stability. This is followed by decolorisation [redacted] to remove pigments and other impurities, deionisation on [redacted] to eliminate ionic compounds, concentration of the product, a second treatment [redacted], and then filtration. Finally, the filtrate is concentrated by evaporation to the desired solid content, dried, pulverised and packed (Documentation provided to EFSA No 3).

The IBO highlighted that the treatment [redacted] is intended to remove organic compounds, such as melanin and protein, while the [redacted] treatments eliminate compounds like organic acids. Volatile compounds, such as ethanol, volatilise during the final evaporation and drying steps (Documentation provided to EFSA No 3).

Overall, the Panel considered that the process has been adequately described by the applicant and it does not raise any concern.

*Characterisation of the production organism*

Following the EFSA call for data, one IBO submitted information on the production microorganism of pullulan (E 1204) (Documentation provided to EFSA No 3). Additional information was provided in the context of the extension of use of E 1204 (Documentation provided to EFSA No 5).

The food additive E 1204 is produced using the non-genetically modified *A. pullulans* [redacted] which is deposited at the National Institute of Technology and Evaluation (NITE) Biological Resource Center (Japan), with [redacted]. *A. pullulans* is considered a filamentous fungus based on recent taxonomic insights (EFSA, 2021). The production strain was identified as *A. pullulans* by phylogenetic analysis based on the sequence of the 18S rRNA gene showing close relationship (> 99% sequence identity) with the type strain *A. pullulans* CBS 584.75. The production strain was obtained after different steps of conventional mutagenesis using chemical agents and irradiation (Documentation provided to EFSA No 3, 5).

The Panel recommends revising the specifications to explicitly identify the production strain of *A. pullulans*, [redacted], used for pullulan production.

*Absence of viable cells of the production strain in the end product*

Following the EFSA call for data, one IBO submitted information on the absence of viable cells of the production strain in E 1204 (Documentation provided to EFSA No 3). The initial information was not considered adequate, and additional data were submitted upon EFSA request (Documentation provided to EFSA No 5). The absence of viable cells of the production strain in the food additive was demonstrated in four independent batches of the final product analysed in triplicate. For this, 10 g of product was dissolved in 90 mL of sterile $H_2O$ and 10 times 1 mL was mixed with selective agar medium and poured in plates. Plates were incubated for 8 days at 27°C. No colonies of the production strain were produced. Positive controls by adding vegetative cells and spores to the product samples were included in the study showing the recovery on the plate.

### 3.1.3 | Method(s) of analysis in food

Following the EFSA call for data, one IBO submitted information on the method of analysis of the food additive pullulan (E 1204) in food (Documentation provided to EFSA No 3).

Pullulan can be extracted from food matrices in 78% ethanol solution and then precipitated according to the Prosky method (Prosky et al., 1988). Alternatively, pullulan can be extracted from food matrices in water and subsequently precipitated with methanol in the presence of potassium chloride. Afterwards, the obtained precipitate is dissolved in 0.02 M acetate buffer (pH 6) to an approximately 5% solution. Pullulanase (22 U/g pullulan) is added, and the mixture is incubated at 40°C for 24 h. The solution is then desalted and adjusted to a concentration of about 3%. Hence, maltotriose is determined by HPLC-RI (HPLC-Refractive Index) in the dissolved precipitate before and after pullulanase treatment. Pullulan content is calculated by difference.

The Panel considered the described method adequate to determine pullulan in food.

### 3.1.4 | Stability, reaction and fate in food

Following the EFSA call for data, one IBO submitted information on the stability of the food additive pullulan (E 1204) as such (Documentation provided to EFSA No 3). At least five samples were tested for stability under normal conditions

(temperature ranging 10–30°C, relative humidity (RH) 40%–65%) and accelerated conditions (temperature 40°C, RH 75%). Tested parameters were the assay (as defined in Table 1), loss on drying, pH, viscosity, microbiological parameters.

Additional tests were conducted under severe conditions to test the effects of different temperatures (30°C; 60°C) and RH (< 50%; 80%) on the chosen parameters; the effects of different pH (2–10) while boiling on viscosity; the effects of different RH (33%–97%) on change of water content (Documentation provided to EFSA No 3).

According to the stability data submitted, E 1204 was found to be stable throughout the entire testing period under normal storage conditions (24 months) and accelerated storage conditions (2 months).

Following the stability tests under harsh conditions, the viscosity of the food additive was observed to decrease significantly at pH 2–3 but remained stable at pH 7 during 2 h of boiling at 100°C. The pH remained unaffected by heating at that temperature. The results also demonstrated that the water content of the food additive may vary according to RH.

The Panel considered that the stability of the food additive as such was sufficiently demonstrated.

According to the IBO, E 1204 is not expected to chemically interact with nutrients given the absence of chemically reactive groups (Documentation provided to EFSA No 3).

The Panel concurs with the IBO's argumentation.

## 3.2 | Authorised use and use levels

Pullulan (E 1204) is used in the production of capsule shells and of coated tablets for the preparation of dietary supplements and as a matrix for edible flavoured films (breath fresheners) (Documentation provided to EFSA No 1).

Maximum levels of pullulan (E 1204) have been defined in Annex II to Regulation (EC) No 1333/2008 on food additives, as amended. In this document, these levels are named maximum permitted levels (MPLs).

Currently, pullulan (E 1204) is an authorised food additive in the EU in two food categories (FCs) at QS. Table 2 lists the food categories with their restrictions/exceptions that are permitted to contain added pullulan (E 1204) and the corresponding MPLs as defined in Annex II to Regulation (EC) No 1333/2008.

**TABLE 2** MPLs of pullulan (E 1204) in food categories according to Annex II to Regulation (EC) No 1333/2008.

| Food category number | Food category name | Restrictions/exceptions | MPL (mg/kg) |
|---|---|---|---|
| 5.2 | Other confectionery including breath freshening microsweets | Only breath freshening microsweets in the form of films | QS |
| 17.1 | Food supplements supplied in a solid form, excluding food supplements for infants and young children | Only food supplements in capsule and tablet form | QS |

Abbreviations: MPL, maximum permitted level; QS, *quantum satis*.

Pullulan (E 1204) is not listed in Annex III of Regulation (EC) No 1333/2008.

## 3.3 | Proposed changes to the currently permitted uses and use levels

The current opinion considers the proposed inclusion of pullulan (E 1204) in Annex II to Regulation (EC) No 1333/2008 on food additives, for its use as a food additive in additional food categories[8] to those already authorised. The changes to the currently permitted uses also include a proposal for maximum use levels for the two food categories that are already authorised at QS. Table 3 summarises the proposed changes to the currently permitted uses and use levels.

**TABLE 3** Proposed extension of use for pullulan (E 1204) and proposed maximum and typical use levels of (mg/kg) in food categories according to Annex II of Regulation (EC) No 1333/2008 (Documentation provided to EFSA No 1).

| Food category number | Food category name | Proposed maximum use level (mg/kg) | Proposed typical use level (mg/kg) |
|---|---|---|---|
| 1.2 | Unflavoured fermented milk products, including natural unflavoured buttermilk (excluding sterilised buttermilk) non-heat treated after fermentation | 2 | 1 |
| 1.4 | Flavoured fermented milk products including heat-treated products | 2 | 1 |
| 1.5 | Dehydrated milk as defined by Directive 2001/114/EC | 2 | 1 |
| 1.6.1 | Unflavoured pasteurised cream (excluding reduced fat creams) | 2 | 1 |

[8] https://open.efsa.europa.eu/questions/EFSA-Q-2020-00517.

**TABLE 3** (Continued)

| Food category number | Food category name | Proposed maximum use level (mg/kg) | Proposed typical use level (mg/kg) |
|---|---|---|---|
| 1.6.2 | Unflavoured live fermented cream products and substitute products with a fat content of less than 20% | 2 | 1 |
| 1.6.3 | Other creams | 2 | 1 |
| 1.7.1 | Unripened cheese excluding products falling in category 16 | 2 | 1 |
| 1.8 | Dairy analogues, including beverage whiteners | 14 | 7 |
| 2.1 | Fats and oils essentially free from water (excluding anhydrous milkfat) | 20 | 10 |
| 2.2.1 | Butter and concentrated butter and butter oil and anhydrous milkfat | 20 | 10 |
| 2.2.2 | Other fat and oil emulsions including spreads as defined by Council Regulation (EC) no 1234/2007 and liquid emulsions | 20 | 10 |
| 3 | Edible ices | 8 | 4 |
| 5.1 | Cocoa and chocolate products as covered by Directive 2000/36/EC | 4 | 2 |
| 5.2[a] | Other confectionery including breath refreshening microsweets | 140 | 70 |
| 5.3 | Chewing gum | 300 | 150 |
| 6.7 | Pre-cooked or processed cereals | 6400 | 3200 |
| 10.2 | Processed eggs and egg products | 6400 | 3200 |
| 12.9 | Protein products, excluding products covered in category 1.8 | 2 | 1 |
| 13.2 | Dietary foods for special medical purpose defined in Directive 1999/21/EC (excluding products from food category 13.1.5) | 2 | 1 |
| 13.3 | Dietary foods for weight control diets intended to replace total daily food intake or an individual meal (the whole or part of the total daily diet) | 2 | 1 |
| 13.4 | Foods suitable for people intolerant to gluten as defined by Commission Regulation (EC) No 41/2009 | 20 | 10 |
| 14.1.2 | Fruit juices as defined by Directive 2001/112/EC and vegetable juices | 2 | 1 |
| 14.1.3 | Fruit nectars as defined by Directive 2001/112/EC and vegetables nectars and similar products | 2 | 1 |
| 14.1.4 | Flavoured drinks | 2 | 1 |
| 14.1.5.1 | Coffee, coffee extracts | 2 | 1 |
| 14.1.5.2 | Other | 2 | 1 |
| 17.1[a] | Food supplements supplied in a solid form, excluding food supplements for infants and young children | 60,000 | 30,000 |
| 17.2 | Food supplements supplied in a liquid form, excluding food supplements for infants and young children | 60,000 | 30,000 |

[a]The use of E 1204 for the food category is currently authorised, with restrictions, at QS (see Table 2).

The applicant also requested the inclusion of pullulan (E 1204) in section A, Part 5 of Annex III to Commission Regulation (EU) No 1333/2008 at QS as a food additive in nutrients. Following EFSA's request for additional information on this request, the applicant clarified that pullulan (E 1204) is proposed to be used to formulate capsule and microcapsules at a dose of 2% relative to the maximum amount of the ingredient (Documentation provided to EFSA No 2). The applicant confirmed that the proposed use of pullulan (E 1204) in nutrients is intended in the same food categories as listed in Table 3, and that the proposed maximum use levels cover both the use of pullulan (E 1204) as a food additive (under Annex II to Regulation (EC) No 1333/2008) and the carry-over from its use as a food additive in nutrients (under section A, Part 5 of Annex III to Regulation (EC) No 1333/2008).

## 3.4 | Exposure assessment

The Panel assessed the dietary exposure to pullulan (E 1204) as part of the re-evaluation of the food additive, alongside the evaluation of the proposed changes to the currently permitted uses. Distinct dietary exposure estimates were performed by the Panel for each of the two scopes.

### 3.4.1 | Exposure data

#### Concentration data

Pullulan (E 1204) is currently authorised QS in two food categories (see Table 2). To assess the dietary exposure to this food additive in the context of the re-evaluation, concentration data (use levels and/or analytical data) are required. To obtain these data, EFSA issued in 2022 a public call for data on pullulan (E 1204)7. In response to this public call for data, updated information on the actual use levels of this additive in foods was made available to EFSA by one IBO, namely Food Supplements Europe (FSE) (Documentation provided to EFSA No 4). No analytical data were made available by the Member States.

#### Summarised data on reported use levels in foods provided by IBO

FSE provided EFSA with 28 use levels of pullulan (E 1204) in food supplements (Documentation provided to EFSA No 4). A summary of the data is listed in Table A1 in Annex A.

#### Summarised data extracted from the Mintel's Global New Products Database

Mintel's Global New Products Database (GNPD)[9] was used for checking the labelling of food and beverage products and food supplements for pullulan (E 1204) within the EU's food market between January 2019 and July 2024. This database contains the required ingredient information on the label.[10]

According to Mintel's GNPD, E 1204 was labelled on 63 products. These products all belong to 'Vitamins & Dietary Supplements' ($n = 63$). These represent 0.51% of the products belonging to 'Vitamins & Dietary Supplements' that entered the market during this time period (Annex A, Table A2).

#### Food consumption data used for exposure assessment

*EFSA Comprehensive European Food Consumption Database*

Since 2010, the EFSA Comprehensive European Food Consumption Database (Comprehensive Database)[11] 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 December 2022 and its linkage with the food classification system[12] was updated in November 2023.

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. For the present assessment, food consumption data were available from 43 different dietary surveys carried out in 22 European countries[13] (Table 4).

[9]Available online (accessed July 2024) https://www.mintel.com/.

[10]Mintel's GNPD is an online database which monitors new introductions of packaged goods in the market worldwide. It contains information of over 4.7 million food and beverage products of which more than 1.4 million are or have been available on the European food market. Mintel started covering EU's food markets in 1996, currently having 24 out of its 27 member countries and Norway presented in the database. Mintel's GNPD does not contain information from the food markets of Cyprus, Luxembourg and Malta.

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

[12]Food categories in Part D of Annex II to Regulation (EC) No 1333/2008 on Food Additives.

[13]Not all Member States provided consumption information for all population groups, and in some cases the same country provided food consumption data from more than one consumption survey. In most cases, when, for one country and age class, different dietary surveys were available, only the most recent was used. However, when two national surveys from the same country gave a better coverage of the age range than using only the most recent one, both surveys were kept. For details on each survey, see Annex A.

**TABLE 4** Population groups considered for the exposure to pullulan (E 1204).

| 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, the Netherlands, Portugal, Slovenia, Spain |
| Children[b] | From 36 months up to and including 9 years of age | Austria, Belgium, Bulgaria, Cyprus, Czechia, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Italy, Latvia, 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.[10]
[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).

Since 2018, all consumption records in the Comprehensive Database have been codified according to the FoodEx2 classification system (EFSA, 2015). Nomenclature from the FoodEx2 classification system was linked to the food categorisation system of Annex II of Regulation (EC) No 1333/2008, part D, to perform the exposure assessments of food additives. In practice, the FoodEx2 food codes were matched to the food categories.

During the assessment of the proposed changes to the currently permitted uses of use for pullulan (E 1204), the Panel noted that the applicant provided exposure assessments for the proposed food categories using the Food Additive Intake Model 1.1 (FAIM) (Documentation provided to EFSA No 1). Because this tool is unable to perform the 'food supplement consumers only' exposure assessment scenario, and because a former version of the tool was used for the calculations, the Panel performed exposure assessments for the general population (food supplements not considered) and for consumers of food supplements ('food supplement consumers only' scenario).

### Food categories considered for the exposure assessment of pullulan (E 1204)

*Food categories considered for the exposure assessment of pullulan (E 1204) in the context of the re-evaluation*

The food category (FC 17.1 'Food supplements supplied in a solid form, excluding food supplements for infants and young children') for which concentration data of pullulan (E 1204) were provided was selected from the nomenclature of the EFSA Comprehensive Database (FoodEx2 classification system), at the most detailed level possible (up to FoodEx2 Level 1) (EFSA, 2015). Since no use of pullulan (E 1204) in FC 5.2 'Other confectionery including breath refreshening microsweets' was reported by an IBO, this food category was not considered. This is confirmed by the information from the Mintel database (Annex A, Table A2).

Pullulan (E 1204) is authorised in FC 17.1 'Food supplements supplied in a solid form, excluding food supplements for infants and young children'. For this reason, in the context of the re-evaluation, no exposure was calculated for these two population groups.

As exposure to a food additive via food supplements may deviate largely from that via food, and the number of food supplement consumers may be low depending on populations and surveys, the Panel estimated the exposure to pullulan (E 1204) using a 'food supplements consumers only' approach.

The exposure assessment does not consider the restriction 'capsules and tablets' for FC 17.1 indicated in the Regulation (EC) 1333/2008.

*Food categories considered for the exposure assessment of pullulan (E 1204) in the context of the proposed changes to the currently permitted uses*

Food categories for which an extension of use of pullulan (E 1204) was proposed were selected from the nomenclature of the EFSA Comprehensive Database (FoodEx2 classification system), at the most detailed level possible (up to FoodEx2 Level 4) (EFSA, 2015). This included also the two food categories that are already authorised but for which maximum use levels were proposed to replace the current use at QS (see Table 2).

For the assessment of the proposed changes to the currently permitted uses of pullulan (E 1204), the Panel considered the maximum and typical use levels as proposed by the applicant for all the food categories in which use is proposed, with some exceptions for the assessment for the general population. For the reason explained above, the exposure assessment for the general population did not consider FCs 17.1 'Food supplements supplied in a solid form, excluding food supplements for infants and young children' and 17.2 'Food supplements supplied in a liquid form, excluding food supplements for infants and young children'.

The Panel also performed two 'food supplement consumers only' exposure assessment scenarios to estimate the dietary exposure to the additive via its proposed use in food supplements. Both scenarios were performed for consumers of food supplements and considered: (i) the highest use level reported by the IBO for pullulan (E 1204) in food supplements in solid form (FC 17.1) based on current uses (i.e. 277,777 mg/kg); (/ii) maximum use level proposed by the applicant for food supplements in liquid form (FC 17.2) as indicated in Table 3 (i.e. 60,000 mg/kg); and (iii) either the maximum or typical use levels as proposed by the applicant for the remaining food categories as listed in Table 3.

Eating occasions belonging to the FC 13.2 'Dietary foods for special medical purposes defined in Directive 1999/21/EC (excluding products from food category 13.1.5)', FC 13.3 'Dietary foods for weight control diets intended to replace total daily food intake or an individual meal (the whole or part of the total daily diet)' and FC 13.4 'Foods suitable for people intolerant to gluten as defined by Commission Regulation (EC) No 41/2009' are very diverse and were reclassified under food categories in accordance to their main component (e.g. gluten-free pasta reclassified as pasta).

### 3.4.2 | Exposure estimates

For this opinion, the exposure to pullulan (E 1204) was estimated using different exposure assessment scenarios (Table 5) for the current permitted uses as part of the re-evaluation and for the proposed uses following the application for the extension of use.

For the assessment of the proposed changes to the currently permitted uses of pullulan (E 1204), the Panel considered: (i) the highest level reported by the IBO for pullulan in food supplements in solid form (FC 17.1) based on current uses (277,777 mg/kg); (ii) maximum use level proposed by the applicant for food supplements in liquid form (FC 17.2) (60,000 mg/kg) as indicated in Table 3; (iii) extended uses and use levels proposed by the applicant as listed in Table 3 (Documentation provided to EFSA No 1) for the remaining food categories. This approach was considered to reflect the situation in which the current use at QS remains applicable for food category 17.1.

**TABLE 5** Summary table of the exposure assessment scenarios.

| Table | Framework | Population groups considered | Concentration data used | Food categories considered |
|---|---|---|---|---|
| Table 6 | Re-evaluation | Food supplements consumers only | Highest use level as reported by an IBO for food supplements in solid form (277,777 mg/kg) | Food supplements in solid form (FC 17.1) |
| Table 7 | Extension of use | General population | Maximum use levels proposed by the applicant for all food categories considered (see Table 3)<br>Typical use levels proposed by the applicant for all food categories considered (see Table 3) | All food categories proposed by the applicant, except food supplements (FCs 17.1 and 17.2) |
| Table 8 | | Food supplements consumers only | • FC 17.1 at the highest use level reported by an IBO (277,777 mg/kg)<br>• FC 17.2 at the maximum proposed use level proposed by the applicant (60,000 mg/kg)<br>• Other food categories at the maximum use levels proposed by the applicant (see Table 3)<br>• FC 17.1 at the highest use level reported by an IBO (277,777 mg/kg)<br>• FC 17.2 at the maximum proposed use level proposed by the applicant (60,000 mg/kg)<br>• Other food categories at the typical use levels proposed by the applicant (see Table 3) | All food categories proposed by the applicant (see Table 3) |

Abbreviations: FC, food category; IBO, interested business operator.

Dietary exposure to pullulan (E 1204) was calculated by multiplying the use levels for each food category with their respective consumption amount per kilogram of body weight for each individual in the Comprehensive Database. The exposure per food category was subsequently added to derive an individual total exposure per day. These exposure estimates were averaged over the number of survey days, resulting in an individual average exposure per day for the survey period. Dietary surveys with only 1 day per subject were excluded as they are considered as not adequate to assess repeated exposure.

This was carried out for all individuals per survey and per population group, resulting in distributions of individual average exposure per survey and population group. Based on these distributions, the mean and 95th percentile of exposure were calculated per survey and per population group. High percentile exposure was only calculated for those population groups where the sample size was sufficiently large to allow calculation of the 95th percentile of exposure (EFSA, 2011).

Considering the current authorisation of pullulan (E 1204) in food supplements and the proposed request for a modification of use of pullulan (E 1204) in food supplements, the 'food supplements consumers only scenario' (EFSA ANS Panel, 2017) was performed (i.e. only the consumers of food supplements are considered while still taking into account their whole diet). Consumers of food supplements are only a small subset of the total study population in some dietary surveys. Not considering the whole population avoids 'diluting' the exposure with lower exposure levels of non-consumers of food supplements and the estimates will thus reflect the potential exposure to pullulan (E 1204) of consumers of food supplements.

Exposure assessment to pullulan (E 1204) was carried out by the Panel based on different sets of use levels and considering different population groups, following different scenarios (see Table 5).

Annex A, Table A3 summarises the use levels of pullulan (E 1204) used in the exposure assessment scenarios.

### Re-evaluation under Regulation (EU) 257/2010: Exposure estimates to pullulan (E 1204) based on the currently authorised uses

*Food supplements consumers only scenario*

For the re-evaluation of pullulan (E 1204) in its currently permitted uses, only use levels for food supplements (FC 17.1) were available (see Section 3.4.1). As exposure via food supplements may deviate largely from that via food, and the number of food supplement consumers may be low depending on population groups and surveys, the Panel calculated the exposure to pullulan (E 1204) using the food supplements consumers only scenario, covering the following population groups: children, adolescents, adults and the elderly. This scenario considers only the use of pullulan (E 1204) in solid food supplements (FC 17.1) at their maximum level reported by an IBO, i.e. 277,777 mg/kg. The Panel appreciates that this assumption might result in an overestimation of the dietary exposure via food supplements (see Section 3.4.4, Table 9).

The exposure results are presented in Table 6.

**TABLE 6** Summary of the dietary exposure to pullulan (E 1204) using the food supplements consumers only scenario, in four population groups (minimum–maximum across the dietary surveys expressed in mg/kg bw per day and number of surveys in brackets).

| | Children (3–9 years) | Adolescents (10–17 years) | Adults (18–64 years) | The elderly (≥ 65 years) |
|---|---|---|---|---|
| **Highest use level reported by the IBO (277,777 mg/kg for FC 17.1)** | | | | |
| Mean | 7–35 (12) | 2–62 (14) | 2–40 (15) | 1–60 (15) |
| 95th percentile | 17–139 (8) | 15–219 (6) | 17–174 (10) | 14–68 (7) |

The mean exposure to pullulan from its use as a food additive calculated for the food supplements consumers only scenario ranged from 1 mg/kg bw per day the elderly, to 62 mg/kg bw per day in adolescents. The 95th percentile of exposure to pullulan ranged from 14 mg/kg bw per day in the elderly to 219 mg/kg bw per day in adolescents.

Detailed results per population group and survey are presented in Annex A, Table A4.

### New application under EU Reg. 1331/2008: Exposure estimates to pullulan (E 1204) based on the proposed extension of use and use levels

*Dietary exposure estimates for the general population*

The exposure assessment was performed for the general population, considering all food categories proposed by the applicant for the changes to the currently permitted uses (see Table 3), excluding food supplements in solid and liquid forms (FCs 17.1 and 17.2).

The exposure assessment considered the maximum and typical use levels as proposed by the applicant (see Table 3) (Documentation provided to EFSA 1, 3).

The results of the assessment for the general population are reported in Table 7.

**TABLE 7** Summary of the dietary exposure to pullulan (E 1204) from its proposed maximum/typical use levels, in six population groups (minimum–maximum across the dietary surveys expressed in 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 use level exposure assessment scenario for the general population** | | | | | | |
| Mean | 0.01–8 | 0.04–11 | 0.1–7 | 0.04–4 | 0.02–3 | 0.02–4 |
| 95th percentile | 0.03–52 | 0.1–40 | 0.2–26 | 0.1–12 | 0.1–10 | 0.03–10 |
| **Proposed typical use level exposure assessment scenario for the general population** | | | | | | |
| Mean | < 0.01–4 | 0.02–5 | 0.04–3 | 0.02–2 | 0.01–1 | 0.01–2 |
| 95th percentile | 0.02–26 | 0.05–20 | 0.1–13 | 0.05–6 | 0.03–5 | 0.02–5 |

At the proposed maximum use levels, the mean exposure to pullulan (E 1204) ranged from 0.01 mg/kg bw per day in infants to 11 mg/kg bw per day in toddlers. The 95th percentile of exposure to pullulan ranged from 0.03 mg/kg bw per day in infants and the elderly to 52 mg/kg bw per day in infants.

At the proposed typical use levels, the mean exposure to pullulan (E 1204) ranged from < 0.01 mg/kg bw per day in infants to 5 mg/kg bw per day in toddlers. The 95th percentile of exposure to pullulan ranged from 0.02 mg/kg bw per day in infants and the elderly to 26 mg/kg bw per day in infants.

Detailed results per population group and survey are presented in Annex A, Table A5.

*Dietary exposure estimates for food supplements consumers only*.

As exposure via food supplements may deviate largely from that via food, and the number of food supplement consumers may be low depending on populations and surveys, the Panel also calculated the exposure to pullulan (E 1204) according to the food supplements consumers only scenario, covering the following population groups: children, adolescents, adults and the elderly.

This scenario considered the highest level provided by an IBO for FC 17.1 (i.e. 277,777 mg/kg), as well as the proposed maximum use level for FC 17.2 (60,000 mg/kg) and either the maximum or typical use levels proposed by the applicant for all the other FCs.

The dietary exposure estimates for food supplements consumers only are reported in Table 8.

**TABLE 8** Summary of the dietary exposure to pullulan (E 1204) for the food supplements consumers only scenario, considering FC 17.1 at the highest reported use level, FC 17.2 at the proposed maximum use level and all other food categories at the proposed maximum or typical use levels, in four population groups (minimum–maximum across the dietary surveys expressed in mg/kg bw per day and number of surveys in brackets).

| | Children (3–9 years) | Adolescents (10–17 years) | Adults (18–64 years) | The elderly (≥ 65 years) |
|---|---|---|---|---|
| **Scenario considering FC 17.1 at the highest reported use level, FC 17.2 at the proposed maximum level and proposed maximum use levels for all other food categories** | | | | |
| Mean | 9–39 (12) | 4–65 (14) | 5–42 (15) | 1–62 (15) |
| 95th percentile | 24–160 (8) | 19–224 (6) | 21–189 (10) | 15–72 (7) |
| **Scenario considering FC 17.1 at the highest reported use level, FC 17.2 at the proposed maximum level and proposed typical use levels for all other food categories** | | | | |
| Mean | 9–37 (12) | 3–65 (14) | 3–41 (15) | 1–61 (15) |
| 95th percentile | 23–156 (8) | 18–224 (6) | 18–189 (10) | 15–70 (7) |

Abbreviations: FC, food category; IBO, interested business operator.

In the scenario considering FC 17.1 at the highest reported use level, FC 17.2 at the proposed maximum level and proposed maximum use levels for all other food categories, the mean exposure to pullulan (E 1204) ranged from 1 mg/kg bw per day in the elderly to 65 mg/kg bw per day in adolescents. The 95th percentile of exposure to pullulan (E 1204) ranged from 15 mg/kg bw per day in the elderly to 224 mg/kg bw per day in adolescents.

In the scenario considering FC 17.1 at the highest reported use level, FC 17.2 at the proposed maximum level and proposed typical use levels for all other food categories, the mean exposure to pullulan (E 1204) ranged from 1 mg/kg bw per day in the elderly to 65 mg/kg bw per day in adolescents. The 95th percentile of exposure to pullulan (E 1204) ranged from 15 mg/kg bw per day in the elderly to 224 mg/kg bw per day in adolescents.

The Panel noted that the outcome of the exposure calculations for the 'food supplements consumers only' scenarios for both the re-evaluation and extension of use are almost identical because of the predominant contribution of solid food supplements (FC 17.1) to the overall exposure estimates.

Detailed results are reported in Annex A, Table A6.

### 3.4.3 | Main food categories contributing to exposure to pullulan

The main food category contributing to the exposure to pullulan (E 1204) calculated for the general population was FC 10.2 'Processed egg and egg products', for all population groups. To a lower extent, FC 06.7 'pre-cooked and processed cereals' contributed also to the total mean exposure of all population groups. Additionally, FC 02.1 'Fats and oils essentially free from water (excluding anhydrous milkfat)' contributed for infants, toddlers and adults while FC 05.2 'Other confectionery including breath refreshening microsweets' contributed to the exposure for toddlers, children, adolescents and adults (see Annex A, Table A7).

The main food category contributing to the exposure to pullulan (E 1204) calculated for food supplements consumers only was FC 17.1 'Food supplements supplied in a solid form, excluding food supplements for infants and young children' (see Annex A, Table A8).

### 3.4.4 | Uncertainty analysis

Uncertainties in the exposure assessment of pullulan (E 1204) have been described above. In accordance with the guidance provided in the EFSA opinion related to uncertainties in dietary exposure assessment (EFSA, 2007), the following sources of uncertainties have been considered and summarised in Table 9.

**TABLE 9** Qualitative evaluation of influence of uncertainties on the dietary exposure estimate.

| Sources of uncertainties | Direction[a] |
|---|---|
| Consumption data:<br>• 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 | + |
| Uncertainty in possible national differences in use levels of food categories | +/– |
| Food categories selected for the exposure assessment for all the scenarios considered: foods belonging to food categories 13.2, 13.3 and 13.4 were considered through reclassification under other food categories in accordance with their main component | – |
| Food categories included in the exposure assessment for the 'food supplement consumers only' scenario for the re-evaluation (Table 6):<br>• no data were received for the other relevant food category (5.2) that was therefore not considered in the exposure estimates ($n = 1/2$ total number of food categories) | – |
| Concentration data used in the 'food supplements consumers only' scenario for the re-evaluation (Table 6):<br>• use levels were considered applicable to all food supplements in solid form within the entire food category, whereas on average 0.5% of the supplements, belonging to a food supplements category, were labelled to contain pullulan (E 1204) according to Mintel's GNPD | + |
| Concentration data used in the general population scenario (Table 7) and 'food supplement consumers only' scenario for the extension of use (Table 8):<br>• proposed maximum/typical use levels considered applicable to all foods within the entire food category, whereas it is not likely that pullulan (E 1204) will be added as a food additive to all foods belonging to a proposed food category | + |
| Methodology used in the 'food supplement consumers only' scenario for the re-evaluation (Table 6):<br>• do not consider the restriction 'only food supplements in capsule and tablet form' indicated for FC 17.1. | + |
| • considered only the highest use level submitted by an IBO for FC 17.1 | + |
| Methodology used in the 'food supplements consumers only' exposure assessment scenarios for the extension of use (Table 8):<br>• exposure calculations performed considered only the highest use level submitted by an IBO for FC 17.1<br>• exposure calculations performed considered only the maximum use level proposed by the applicant for FC 17.2<br>• exposure calculations do not consider the restriction 'only food supplements in capsule and tablet form' indicated for FC 17.1 | <br>+<br>+<br>+ |

Abbreviations: FC, food category; IBO, interested business operator.

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

Overall, the Panel considered that the uncertainties identified resulted in an overestimation of the exposure to pullulan (E 1204) across all evaluated scenarios.

## 3.5 | Biological and toxicological data

In 2004, the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food concluded the following:

'The toxicological database is limited. The pullulan product under consideration is PI-20, with an average molecular weight of 200,000 Daltons (Da). Many of the available studies provided no information on the type of pullulan used. Of those that did, the majority used a material with a molecular weight of about 50,000 Da. In vitro studies suggest that PI-20 is broken down into smaller polymers (of around 70,000 Da) by salivary and pancreatic amylases (Okada et al., 1990). In vitro

and in vivo experiments suggest that it may be fermented to short chain fatty acids in the colon. It is assumed to be completely fermented but in vivo evidence for this is unclear and there are no data to indicate whether the rate of fermentation depends on the size of the polymer. No adequate chronic toxicity studies are available nor are there data on carcinogenicity, reproductive toxicity or developmental toxicity. Subchronic (9-62 week) studies in the rat indicate that pullulan is of low toxicity. Pullulan is a soluble carbohydrate polymer that is poorly digested by intestinal enzymes. Studies in which pullulan was administered in the diet to rats for up to 9 weeks suggest that pullulan has local effects in the gastrointestinal tract but provided no evidence of systemic effects. Increased relative weights of the stomach, small intestine, large intestine and caecum and evidence of changes in the size and shape of intestinal pouches (the haustra coli) in the intestinal mucosa were reported at dietary concentrations of 1% pullulan (around 0.5 g pullulan/kg bw/day) and greater. Limited evidence indicated a decrease in severity of effects with time of administration, suggesting possible adaptation. Human volunteer studies have reported mild gastrointestinal symptoms at doses of 10 g pullulan per day and greater (i.e. approximately 0.17 g/kg bw per day for a 60 kg individual). At 10 g the only reported gastrointestinal symptom was abdominal fullness. The estimated exposure in adults using the specified worst-case assumptions (12 tablets and a packet of breath freshening films) would be around 23% of this amount. If the same worse case assumptions were applied to children weighing 30 kg, exposure expressed per kg body weight would be 46% of this amount. Pullulan has similarities to a number of other poorly digestible carbohydrate polymers including modified celluloses. In 1992 the Scientific Committee on Food (SCF) reviewed five modified celluloses. The SCF noted that modified celluloses are practically non-absorbed, are of low toxicity and do not possess carcinogenic properties. The SCF considered "the observed gastrointestinal effects in feeding studies were related to the physical effects of the bulk and hydrophilic properties of the material" and traditional toxicological evaluation procedures were not considered to be appropriate (SCF, 1994, 1999)'.

The Panel noted that the three rat feeding studies submitted in the context of the 2004 AFC Panel assessment (Oku et al., 1979, 1982; Katayama-Sugawa et al., 1993) are not suitable for the derivation of a reference point.

Biological and toxicological studies were also reviewed by JECFA in a subsequent evaluation completed in 2011 (JECFA, 2011a). The following paragraphs summarise the JECFA evaluation.

Data on the digestibility of pullulan as specified were available only from an in vitro digestibility study (Okada et al., 1990) and a pilot study in five human volunteers (Richards & Higashiyama, 2004). In this human study, pullulan exhibited a glycaemic index of less than 20% on average in comparison with maltose as the reference carbohydrate. Low digestibility of pullulan was also indicated by the occurrence of caecal enlargement in rats fed diets containing 5% or 10% pullulan for 13 weeks (Sommer et al., 2003).

Studies in humans and laboratory animals in which the glycaemic and insulinemic responses to oral administration of pullulan were measured indicate that the higher the relative molecular mass of the pullulan, the lower its digestibility by mammalian intestinal enzymes (Kern, 2011; Knapp et al., 2008; Knapp et al., 2010; Spears, Karr-Lilienthal, & Fahey Jr, 2005; Spears, Karr-Lilienthal, Grieshop, et al., 2005a; Wolf et al., 2003).

Pullulan is degraded by a large variety of bacterial species (Domań-Pytka & Bardowski, 2004). A study in dogs showed that daily consumption of 2 or 4 g of pullulan (relative molecular mass, 100,000 Da) for 14 days increased the ileal bifidobacteria and lactobacilli concentration and the faecal lactobacilli concentration (Spears, Karr-Lilienthal, Grieshop, et al., 2005c). No effects of pullulan on nutrient intake or faecal characteristics were noted. Pullulans with low relative molecular masses (average MW of 6300) appear to be more readily fermented than pullulans with high relative molecular masses (average MW of 100,000) (Spears et al., 2007).

Pullulan (relative molecular mass not reported) administered to rats in the diet at a concentration of 10% for 2 weeks (equivalent to 5000 mg/kg bw per day) and subsequently at a concentration of 5% for 10 weeks (equivalent to 2500 mg/kg bw per day) caused increased mass of caecal contents and increased levels of short chain fatty acids, indicating that unabsorbed pullulan is fermented in the large intestine. No effects of pullulan on food intake, body composition, calcium retention or bone calcium content were observed (Weaver et al., 2010).

In a study in humans, in which a single oral dose of 25 g of pullulan conforming to the existing specification was given to 15 subjects, the results of a questionnaire revealed no statistically significant treatment-related differences in intensity or frequency of gastrointestinal symptoms, such as nausea, abdominal cramping, abdominal distension and flatulence (Kern, 2011).

Similarly, in a human study with 35 subjects, a dose of 15 g pullulan did not evoke gastrointestinal symptoms (Peters et al., 2011). In comparison with maltodextrin, pullulan of high relative molecular mass (486,000 Da), consumed by humans at a dose of 6 g twice per day for a period of 14 days, induced small increases in gastrointestinal symptoms, such as bloating, cramping, flatulence and borborygmi (Stewart et al., 2010). In this study, no differences were found between pullulan and maltodextrin in fasting plasma levels of triglycerides, cholesterol, glucose, insulin, C-reactive protein, ghrelin or laxation.

Colonic fermentation of pullulan leads to the formation of hydrogen, which is exhaled. In human studies, an increase of the breath hydrogen expiration was observed after consumption of 15–25 g of pullulan, particularly long chain pullulans with a relative molecular mass of 100,000–200,000 Da (Peters et al., 2011; Wolf et al., 2003). No pullulan could be detected in faeces of humans who had consumed 10 g pullulan (relative molecular mass, 50,000 Da) per day over a period of 2 weeks, indicating that it is fully metabolised in the intestine (Yoneyama et al., 1990).

Following EFSA public calls for data, a 13-weeks repeated dose toxicity study in rats in compliance with Good Laboratory Practice (GLP) was provided as reply to the call for data by an IBO (Sommer et al., 2003). The Panel noted that this study had been already evaluated by JECFA in its first evaluation of pullulan in 2005 (JECFA, 2006).

A few relevant publications were identified from the literature searches by the Panel and assessed in the Sections 3.5.4 and 3.5.8 below.

### 3.5.1 | Absorption, distribution, metabolism and excretion

No new relevant information was found in the literature search.

### 3.5.2 | Acute toxicity

No new relevant information was found in the literature search.

### 3.5.3 | Short-term and subchronic toxicity

In a 13-weeks toxicity study, groups of 10 Wistar rats of each sex were fed diets containing different concentrations of Pullulan PI-20 (purity 93.7%, MW 200,000; units not provided) (0%, 2.5%, 5% and 10%, equal to 1955, 4064 and 7914 mg/kg bw per day in males and 2519, 4880 and 9674 mg/kg bw per day in females) for 13 weeks (Sommer et al., 2003). The rats were monitored daily for clinical signs of toxicity, and their body weights and food consumption were recorded regularly. Grip strength and locomotor activity were measured in Week 13. At the end of the treatment period, urine samples were collected for analysis, and blood samples were taken to assess standard haematological and clinical chemical parameters. After the animals were euthanised, their organs and tissues were examined visually, and microscopic examinations were performed. No treatment-related clinical signs or mortalities were observed during the study. The body weights and food intakes did not differ between the treated groups and the control groups. Grip strength and locomotor activity showed no relevant treatment-related effects. Changes in the haematological and clinical chemical parameters did not show a dose–response relationship or were within the reference values. Therefore, the observed changes were not considered attributable to the treatment. Urine volumes were increased in females of the mid- and high-dose groups. Water consumption was not measured. Apart from the caecum, organ weights were not affected by the treatment. The weight of the empty caecum was significantly increased in males and females of the high-dose group and in males of the mid-dose group. The weight of the full caecum was increased in males of the high-dose group. However, apart from caecal enlargement, no other treatment-related macroscopic observations were found during necropsy. The histopathological examination of the liver, kidneys and gross lesions did not reveal any treatment-related changes. The only observed effect in rats receiving 5% and 10% pullulan in the diet was caecal enlargement. This effect is commonly seen when high doses of unabsorbed, fermentable carbohydrates are ingested and is considered to be an adaptive response without toxicological relevance. Therefore, the No Observed Adverse Effect Level (NOAEL) for pullulan was determined to be 10% in the diet, which is equal to a daily dose of 7.9 g/kg bw per day for male rats and 9.7 g/kg bw per day for female rats.

These 13 weeks study confirmed the conclusion drawn by the AFC Panel in 2004 from an earlier study, that administration of pullulan in rats for up to 9 weeks caused effects in the gastrointestinal tract and that there is no evidence of systemic effects.

### 3.5.4 | Genotoxicity

The AFC Panel had evaluated in 2004 the available genotoxicity data which was negative but of limited relevance and concluded that 'considering the structure and molecular weight of pullulan, genotoxicity is not expected'.

A single publication was retrieved in the literature (Yuzbasioglu et al., 2021), reporting an in vitro micronucleus (MN) assay and an in vitro comet assay in human lymphocytes on Pullulan (Sigma-Aldrich, Steinheim, Germany, purity not reported), with negative results. However, due to major shortcomings of the assays, the reported results could not be used in the overall assessment of genotoxicity.

In particular, in the MN assay the Panel noted the following deviations from the OCED test guideline 487: (1) the substance was not tested in the presence of metabolic activation; (2) cells were treated 24 h after the start of the culture, instead of 48 h as recommended by OECD 487; (3) the only treatment time used was 48 h, while the OECD TG 487 recommends a short treatment of 3–6 h and a long treatment of 1.5–2.0 normal cell cycle length (usually around 24 h for human lymphocytes). Regarding the in vitro comet assay the Panel noted that this test is not validated for regulatory purposes and no OECD test guideline is currently available. Moreover, the treatment time applied was only 30 min and no cytotoxicity was reported, therefore it is not possible to assess the genotoxicity after longer treatment times. Overall, the relevance of these results is considered limited.

The Panel noted that the available data set is not aligned with current requirements for genotoxicity hazard identification. However, noting that pullulan is a polysaccharide, the Panel considered that based on its structure, no concern for genotoxicity is expected. Moreover, the purity of the additive is considered sufficiently high and there is no uncharacterised fraction in the food additive, hence the presence of potentially genotoxic fermentation by-products is not expected.

The Panel overall considered that there are no indications for concerns on genotoxicity.

### 3.5.5 | Chronic toxicity and carcinogenicity

No new relevant information was found in the literature search.

### 3.5.6 | Reproductive and developmental toxicity

No new relevant information was found in the literature search.

### 3.5.7 | Hypersensitivity, allergenicity and food intolerance

No new relevant information was found in the literature search.

### 3.5.8 | Human studies

The Panel assessed four publications that became available after the first evaluation by the AFC Panel in 2004.

In the study of Wolf et al., 2003, the glycaemic and breath hydrogen responses and the gastrointestinal tolerance to pullulan (MW = 100,000; units not provided) was tested by ingestion of 50g pullulan (corresponding to 714 mg/kg bw per day), compared with 50 g maltodextrose in two separate 3-h meal tolerance tests in healthy subjects ($n = 36$; 22 men, 14 women) aged 18–75 years (mean ± SEM, 45 ± 2 years). It was confirmed that pullulan was digested slowly (slow rise of blood glucose levels). It was concluded that pullulan attenuated the postprandial glycaemic excursion compared with an equivalent maltodextrin challenge. Pullulan increased breath hydrogen excretion and the incidence of gastrointestinal intolerance symptoms (flatulence), indicating that a portion of pullulan was not fully digested and absorbed, consistent with the data on its glycaemic index and the residual fraction underwent intestinal fermentation.

Spears, Karr-Lilienthal, Grieshop, et al. (2005a), determined the postprandial glycaemic response of nondiabetic healthy adult human subjects ($n = 34$; 19 men, 15 women, aged 20–39 years) in two separate 3-h meal tolerance tests. Test beverages contained low-molecular weight pullulan (MW = 6300; units not provided) or maltodextrin (50 g of carbohydrates in 474 mL of beverage, corresponding to 714 mg/kg bw per day of pullulan), which is a rapidly digestible starch that normally elicits a high glycaemic response. Low-molecular weight pullulan appeared to be rapidly digested and elicited an elevated glycaemic response in healthy humans similar to that of maltodextrin. However, blood glucose concentrations remained elevated in subjects consuming low-molecular weight pullulan at 150 and 180 min postprandial compared with maltodextrin, indicating that a portion of low-molecular weight pullulan is more slowly digested and absorbed. The low breath hydrogen response of low-molecular weight pullulan indicated a high small intestinal digestibility, leaving only a small amount of carbohydrate available for fermentation in the colon.

In the study of Stewart et al. (2010), healthy adult human subjects (10 males, mean age 32 ± 5 years; 10 females, mean age 38 ± 4 years) consumed 12 g of dietary fibres or placebo per day for 14 days, (divided into two 6-gram doses, each mixed into 177 mL of an apple sauce, corresponding to 171 mg/kg bw of fibre per day) in a single-blind crossover design. The dietary fibres were pullulan (MW = 486,000; units not provided), resistant starch, soluble fibre dextrin, soluble corn fibre and maltodextrin as a control. Minor symptoms, i.e. bloating, cramping, flatulence, stomach noises and gastrointestinal (GI) score were significantly affected by treatment, with all fibres inducing a modest increase in GI symptoms compared to the control. The number of stools collected in 4 days, stool weight, total stool output in 4 days and investigator-evaluated stool consistency did not differ among treatments. In all treatments serum lipid values were near or within the recommended ranges for triglycerides (< 150 mg/dL), total cholesterol (< 200 mg/dL), LDL cholesterol (< 100 mg/dL) and HDL cholesterol (> 40 mg/dL). Pullulan, in a dose (12 g/day for 14 days) did not significantly alter bowel function or serum markers of coronary heart disease, diabetes or satiety.

In the study of Klosterbuer et al. (2012), healthy adult human subjects (10 males and 10 females, mean age 29 ± 8 years) consumed five treatments of 25 g of fibres in a randomised, crossover design. The dietary fibres were soluble corn fibre (SCF) or resistant starch (RS) alone or in combination with 5g of pullulan (MW not indicated) (SCF + P and RS + P) mixed with 250 mL in water (corresponding to 71 mg/kg bw per day of pullulan). The AUC insulin was significantly higher after SCF compared to SCF + P. The addition of 5 g of pullulan to the RS treatment (RS + P) resulted in lower AUC for both glucose and insulin compared to control. The addition of pullulan to the RS meal contributed to the reduction in the glycaemic and insulinemic response.

These studies indicated that low-molecular weight pullulan is digested slowly (slow rise of blood glucose levels).

Overall, high-molecular weight pullulan increased breath hydrogen excretion and the incidence of gastrointestinal intolerance symptoms (flatulence), indicating that a portion of pullulan is not fully absorbed. On the other hand, the relatively low breath hydrogen response of low-molecular weight pullulan indicated a high small intestinal digestibility, leaving only a small amount carbohydrate available for fermentation in the colon. Reducing the molecular weight of pullulan appears to make it more available for rapid enzymatic digestion in the small intestine.

If consumed in a dose of 12 g/day pullulan in humans did not significantly alter bowel function or serum markers of coronary heart disease, diabetes or satiety, but it may cause minor gastrointestinal effects (i.e. bloating, cramping, flatulence, stomach noises).

The results of the studies above described did not change the conclusion reached by the AFC Panel in 2004 that, based on human volunteer studies, mild gastrointestinal symptoms can occur at doses of 10 g pullulan per day and greater.

## 4 | DISCUSSION

The current EFSA assessment addresses the request from the European Commission to provide a scientific opinion on (i) the safety of pullulan (E 1204) in accordance with the Regulation (EU) No 257/2010 as part of the programme for the re-evaluation of food additives already permitted in the European Union before 20 January 2009 and (ii) on the safety of the proposed extension of use of pullulan (E 1204) to the food categories listed in section 3.6 in accordance with Regulation (EC) No 1331/2008 establishing a common authorisation procedure for food additives, food enzymes and food flavourings.

Pullulan (E 1204) is currently authorised as a food additive in the EU in accordance with Annex II, Part E, to Regulation (EC) No 1333/2008 at MPLs equal to QS in two food categories (FCs) (5.2 'Other confectionery including breath freshening microsweets' and 17.1 'Food supplements supplied in a solid form, excluding food supplements for infants and young children'), with restrictions.

EFSA has evaluated pullulan (E 1204) in 2004 (EFSA AFC Panel, 2004) intended to be used in the production of capsule shells and of coated tablets for the preparation of dietary supplements and as a matrix for edible flavoured films (breath fresheners), concluding that *'on the basis that pullulan is similar to other poorly digested carbohydrates and that the current proposed usage levels are below the level likely to cause abdominal fullness (10 g per person per day), the Panel consider that the expected intakes of pullulan would not present any concern when used as a food additive in the proposed uses and at the usage levels requested'*.

JECFA evaluated the safety of pullulan (INS 1204) as a food additive in 2005 and later in 2011 (JECFA, 2011a, 2006) and published a specifications monograph in 2011 (JECFA, 2011b). Based on the available data, JECFA concluded that pullulan is a substance of low toxicity with an ADI 'not specified'. A toxicological monograph was not prepared.

According to the definition given in Commission Regulation (EU) No 231/2012, pullulan is defined as a '*Linear, neutral glucan consisting mainly of maltotriose units connected by -1,6 glycosidic bonds. It is produced by fermentation from a food-grade hydrolysed starch using a non-toxin-producing strain of A. pullulans. After completion of the fermentation, the fungal cells are removed by microfiltration, the filtrate is heat-sterilised and pigments and other impurities are removed by adsorption and ion exchange chromatography*'.

The present re-evaluation applies to pullulan (E 1204) obtained by fermentation of a food-grade hydrolysed starch with non-genetically modified *A. pullulans* ([redacted]). Information on the manufacturing process of pullulan (E 1204) obtained by fermentation of a food-grade hydrolysed starch with non-genetically modified *A. pullulans* ([redacted]) was submitted and evaluated. Based on the detailed information on the characterisation of the microorganism and the demonstration of the absence of viable cells in pullulan (E 1204), the Panel considered that the manufacturing process of pullulan (E 1204) using this microorganism does not raise a safety concern. However, in order to better describe the manufacturing processes evaluated in the current assessment, the Panel recommended modifying the definition of the food additive in the Commission Regulation (EU) 231/2012 by specifying that pullulan (E 1204) is produced by fermentation from a food-grade hydrolysed starch using *A. pullulans* [redacted], followed by several purification steps and drying.

The Panel emphasised that the present re-evaluation does not apply to pullulan (E 1204) produced by other manufacturing processes (e.g. different microorganisms, strains, different carbohydrates sources, purifications steps). The reason is that this would be considered as a significant change in the production methods which would require an assessment in accordance with relevant legislation (Regulation (EC) No 1331/2008).

With regard to toxic elements, one IBO provided analytical data on the levels of arsenic (As), lead (Pb), mercury (Hg) and cadmium (Cd) in commercial samples of pullulan (E 1204). The Panel noted that no information on the lowest technologically achievable levels for the toxic elements in E 1204 was provided by the IBOs. The Panel noted that among the potential inorganic impurities tested, only lead has defined limit value in EU specifications for E 1204, and that lead and arsenic were quantified in some of the analysed samples. The Panel performed the risk assessment that would result if these elements were present in E 1204 at the maximum measured value (for arsenic and lead), at the reported values (for mercury and cadmium), and at the current maximum limit in the EU specifications (for lead). The Panel noted that for arsenic, the calculated MOE values would give rise to concern at the measured values. For the other toxic elements, the concentrations considered for the calculation of their potential exposure do not give rise to safety concerns (Table A.3).

The Panel recommended to include limits for arsenic and to lower the EU specification limit for lead, taking into account (i) the results of the calculations performed by the Panel; (ii) the fact that the food additive is not the only potential dietary source of toxic elements; and that (iii) the maximum limits should be established based on actual levels in the commercial food additive. Taking into account that pullulan (E 1204) is produced from food-grade hydrolysed starch and undergoes to some purification steps, systematic contamination by cadmium and mercury is not anticipated and the Panel did not see a need to recommend additional specification limits for cadmium and mercury. In addition, the results of the calculations performed by the Panel (Table A.3) do not give rise to concern.

According to the data provided, secondary metabolites of concerns (i.e. mycotoxins and Aureobasidin A) were not detected in the tested samples of pullulan.

The Panel noted that the stability tests provided by the IBO show a decrease in viscosity at low pH (2–3). Regardless, based on the submitted information and the physicochemical characteristics of pullulan (E 1204), the Panel noted that the additive is expected to be stable under a wide range of temperature and pH conditions and does not interact with food.

Although the water solubility test provided (results > 300 g/L) was not in line with the Guidance on Particle-TR, the Panel considered that, based on the information in the literature, pullulan (E 1204) can be considered as highly soluble in water (above the 33.3 g/L criterion). Therefore, the Panel concluded there is no concern with regard to the potential presence of small particles, including nanoparticles, in pullulan (E 1204) at its intended uses and use levels.

Dietary exposure to pullulan (E 1204) was estimated according to different exposure scenarios to address both its re-evaluation and the proposed changes to the currently permitted uses. Currently, pullulan (E 1204) is an authorised food additive in the EU in two food categories (FC 5.2 'Other confectionery including breath freshening microsweets' and FC 17.1 'Food supplements supplied in a solid form, excluding food supplements for infants and young children') at QS.

Following the call for data for the re-evaluation, one IBO provided EFSA with current use levels for FC 17.1 only. To calculate the dietary exposure in the context of the re-evaluation of pullulan (E 1204), the Panel used the 'food supplements consumers only' scenario, as described in the approach for the refined exposure assessment of food additives under re-evaluation published by EFSA ANS Panel (2017): (i) only consumers of food supplements were considered; and (ii) it was assumed that these consumers will be exposed to pullulan (E 1204) at the highest reported use level in food supplements, i.e. 277,777 mg/kg for FC 17.1. The highest mean and 95th percentile chronic exposure to pullulan (E 1204) among consumers calculated following this approach were 62 and 219 mg/kg bw per day, respectively, in adolescents.

In the present assessment, an application requesting changes to the currently permitted use of pullulan (E 1204) with new uses and maximum and typical use levels was also evaluated. To calculate the dietary exposure to pullulan (E 1204) deriving from the proposed changes to the currently permitted uses, using the 'food supplement consumers only' scenario, the Panel considered the highest level provided by an IBO for FC 17.1 (i.e. 277,777 mg/kg), as well as the proposed maximum use level for FC 17.2 (60,000 mg/kg) and either the maximum or typical use levels proposed by the applicant for all the other FCs. The highest mean and 95th percentile chronic exposure among consumers were 65 and 224 mg/kg bw per day, respectively, in adolescents.

The Panel noted that the outcome of the exposure calculations for the 'food supplements consumers only' scenarios for both the re-evaluation and extension of use are almost identical because of the predominant contribution of solid food supplements (FC 17.1) to the overall exposure estimates.

Overall, the Panel considered that the exposure to pullulan (E 1204) from its use as a food additive was overestimated in all scenarios.

The Panel confirmed that pullulan (E 1204) is of no concern for genotoxicity. In vitro, pullulan (E 1204) is broken down by salivary and pancreatic amylase and intestinal iso-amylase and it is further metabolised to short chain fatty acids in the colon by fermentation. Toxicity studies showed that pullulan has effects in the gastrointestinal tract (increased relative weights of the stomach, small intestine, large intestine and caecum). The newly submitted 13-week rat study confirmed the effect on the caecum. This effect is commonly seen when high doses of unabsorbed, fermentable carbohydrates are ingested and is considered to be an adaptive response without toxicological relevance. The Panel identified a NOAEL of 7.9 g/kg bw per day for male rats and 9.7 g/kg bw per day for female rats. This study confirmed the conclusion drawn by the AFC Panel in 2004 that administration of pullulan in rats up to 9 weeks caused mainly local effects in the gastrointestinal tract and there is no evidence of systemic effects.

Human adult volunteer studies suggested that effects of pullulan (E 1204) are similar to the effects of other poorly digestible carbohydrate polymers including modified celluloses. In addition, human volunteer studies have reported mild undesirable gastrointestinal symptoms (i.e. abdominal fullness, flatulence, bloating and cramping) at doses of 10 g pullulan per day and greater (corresponding to 143 mg/kg bw per day, for 70 kg adult).

The Panel compared the dose of 10 g pullulan per day with the dietary exposure estimates to pullulan (E 1204) in its currently permitted uses and considering the proposed changes to the currently permitted uses. For the re-evaluation in its currently permitted uses, the mean exposure estimates to pullulan (E 1204) calculated for consumers of food supplements only, was at the maximum 4 g/person per day in adolescents and adults (adult body weight 70 kg, adolescents body weight 53 kg). Similar exposure estimates were estimated for the same dietary exposure scenario, applied to the proposed changes to the currently permitted uses. When considering the changes to the currently permitted uses for the general population, the levels were well below the value of 10 g/person per day associated with the undesirable symptoms both at the mean and the high-level consumers. The value of 10 g/person per day was instead exceeded in adults and adolescents at 95th percentile estimated for food supplements consumers only (both for currently permitted uses and proposed changes to the currently permitted uses), with maximum levels reaching approximately 12–13 g/person per day.

## 5 | CONCLUSIONS

The Panel concluded that there is no need for a numerical ADI for pullulan (E 1204) and there is no safety concern for the currently reported uses and use levels. Additionally, the Panel concluded that the exposure estimates considering the proposed changes to the currently permitted uses and use levels of pullulan (E 1204) are of no safety concern.

The estimates for dietary exposure to pullulan (E 1204) indicate that individuals with a high level of exposure, principally coming from food supplements, may experience mild gastrointestinal symptoms at the currently reported uses and use levels.

## 6 | RECOMMENDATIONS

The Panel recommends the European Commission to consider:

- Revising the definition of the food additive in the EU specifications as 'produced by fermentation from a food-grade hydrolysed starch using Aureobasidium pullulans [redacted]';
- Including CAS No 9057-02-7 in the EU specifications;
- Lowering the current limits for lead (Pb) in the EU specifications for Pullulan (E 1204) in order to ensure that it will not be a significant source of exposure to lead in food;
- Introducing a maximum limit for arsenic (As) in the EU specifications for Pullulan (E 1204) in order to ensure that it will not be a significant source of exposure to arsenic in food.

## 7 | DOCUMENTATION PROVIDED TO EFSA

1. Regal B.V. on behalf of NAGASE (Europa) GmbH, October 2020. Application for extension of use of the food additive pullulan.
2. Regal B.V. on behalf of NAGASE (Europa) GmbH, April 2021. Additional information to EFSA-Q-2020-00517.
3. Regal B.V. on behalf of NAGASE (Europa) GmbH, July 2022. Pullulan (E1204) call for data EFSA-Q-2021-00278.
4. Food Supplements Europe, July 2022. Call for data on pullulan (E 1204) EFSA-Q-2021-00278.
5. Regal B.V. on behalf of NAGASE (Europa) GmbH, May 2024. Additional information to EFSA-Q-2020-00517.

### ABBREVIATIONS

| | |
|---|---|
| ADI | acceptable daily intake |
| AFC | Panel on Food additives, Flavourings, Processing Aids and Materials in contact with Food |
| ANS | Panel on Food Additives and Nutrient Sources added to Food |
| BMDL | Benchmark dose (lower confidence interval) |
| bw | body weight |
| CAS | Chemical Abstract Service |
| CFU | colony forming unit |
| CONTAM | Panel on Contaminants in the Food Chain |
| FAIM | Food Additives Intake Model |
| FC | food category |
| FCS | food categorisation system |
| FDA | Food and Drug Administration |
| FSE | Food Supplements Europe |
| GI | gastrointestinal |
| GLP | Good Laboratory Practice |
| GPC | gel permeation chromatography |
| HBGV | health-based guidance value |
| HPLC | high-performance liquid chromatography |
| IBO | interested business operator |
| JECFA | Joint FAO/WHO Expert Committee on Food Additives |
| LOD | limit of detection |
| Mintel's GNPD | Mintel's Global New Products Database |
| MN | micronucleus |
| MOE | margin of safety |
| MPLs | maximum permitted levels |
| MW | molecular weight |
| NOAEL | no observed adverse effect level |
| OECD | Organisation for Economic Co-operation and Development |
| *qs* | quantum satis |
| RH | relative humidity |
| RP | reference point |
| RS | resistant starch |
| SCF | Scientific Committee on Food |
| SCF | soluble corn fibre |

| | |
|---|---|
| SCFA | short chain fatty acids |
| TG | Test Guideline |
| TWI | tolerable weekly intake |

## ACKNOWLEDGEMENTS
The Panel wishes to thank: Sam Vermeiren for the preparatory work on this scientific output.

## REQUEST
European Commission

## QUESTION NUMBERS
EFSA-Q-2020-00517, EFSA-Q-2021-00278



## PANEL MEMBERS
Monica Andreassen, Gabriele Aquilina, Maria Lourdes Bastos, Polly Boon, Biagio Fallico, Reginald Fitzgerald, Maria Jose Frutos Fernandez, Bettina Grasl-Kraupp, Ursula Gundert-Remy, Rainer Gürtler, Eric Houdeau, Marcin Kurek, Henriqueta Louro, Patricia Morales, Sabina Passamonti and Laurence Castle.

## NOTE
The full opinion will be published in accordance with Article 12(3) of Regulation (EC) No 1331/2008 once the decision on confidentiality will be received from the European Commission.

## LEGAL NOTICE
Relevant information or parts of this scientific output have been blackened in accordance with the confidentiality requests formulated by the applicant pending a decision thereon by the European Commission. The full output has been shared with the European Commission, EU Member States and the applicant. The blackening will be subject to review once the decision on the confidentiality requests is adopted by the European Commission.

## REFERENCES
Domań-Pytka, M., & Bardowski, J. (2004). Pullulan degrading enzymes of bacterial origin. *Critical Reviews in Microbiology*, *30*(2), 107–121. https://doi.org/10.1080/10408410490435115

EFSA (European Food Safety Authority). (2007). 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 Ppublications*, *12*(5), EN-804. https://doi.org/10.2903/sp.efsa.2015.EN-804

EFSA AFC Panel (EFSA Panel on Food Additives and Nutrient Sources Added to Food). (2004). Opinion of the Scientific Panel on food additives, flavourings, processing aids and materials in contact with food (AFC) on a request from the commission related to pullulan PI-20 for use as a new food additive. *EFSA Journal*, *2*(9), 85. https://doi.org/10.2903/j.efsa.2004.85

EFSA ANS Panel (EFSA Panel on Food Additives and Nutrient Sources added to Food), Mortensen, A., Aguilar, F., Crebelli, R., Di Domenico, A., Dusemund, B., Frutos, M. J., Galtier, P., Gott, D., Gundert-Remy, U., Lambré, C., Lindtner, O., Moldeus, P., Mosesso, P., Parent-Massin, D., Oskarsson, A., Stankovic, I., Waalkens-Berendsen, I., Woutersen, R. A., … Leblanc, J.-C. (2017). Statement on approach followed for the refined exposure assessment as part of the safety assessment of food additives under re-evaluation. *EFSA Journal*, *15*(10), 5042. https://doi.org/10.2903/j.efsa.2017.5042

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 ANS Panel (EFSA Panel on Food Additives and Nutrient Sources). (2014). Opinion of the scientific panel on food additives, Flavourings, processing aids and materials in contact with food on a request from the commission related to pullulan PI-20 for use as a new food additive. *EFSA Journal*, *85*, 1–32. https://doi.org/10.2903/j.efsa.2004.85

EFSA CONTAM Panel (EFSA Panel on Contaminants in the Food Chain). (2009). Scientific opinion on cadmium in food. *EFSA Journal*, 7(3), 980. https://doi.org/10.2903/j.efsa.2009.980

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). (2012). Scientific Opinion on the risk for public health related to the presence of mercury and methylmercury in food. *EFSA Journal*, 10(12), 2985. https://doi.org/10.2903/j.efsa.2012.2985

EFSA CONTAM Panel (EFSA Panel on Contaminants in the Food Chain). (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 Scientific Committee. (2009). Guidance of the scientific committee on transparency in the scientific aspects of risk assessment carried out by EFSA. Part 2: General principles. *EFSA Journal*, *7*(11), 1051. https://doi.org/10.2903/j.efsa.2009.1051

EFSA Scientific Committee. (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

JECFA (Joint FAO/WHO Expert Committee on Food Additives). (2006). Evaluation of certain food additives. Sixty-fifth report of the Joint FAO/WHO Expert Committee on Food Additives, WHO Technical Report Series, No 934. https://www.who.int/publications/i/item/9241209348

JECFA (Joint FAO/WHO Expert Committee on Food Additives). (2011a). Evaluation of certain food additives and contaminants. Seventy-fourth report of the Joint FAO/WHO Expert Committee on Food Additives, WHO Technical Report Series, No 966. https://www.who.int/publications/i/item/9789241660655

JECFA (Joint FAO/WHO Expert Committee on Food Additives). (2011b). Specifications Monograph prepared by the meeting of the Joint FAO/WHO Expert Committee on Food Additives. Compendium of Food Additive Specifications, 74th Meeting. https://www.fao.org/4/i2358e/i2358e00.pdf

Katayama-Sugawa, Y., Kondou, F., & Katayama, M. (1993). Changes in colon mucosa of rats fed pullulan, Polydextrose and pectin. *Nippon Eiyo Shokuryo Gakkaishi*, *46*(4), 325–332. https://doi.org/10.4327/jsnfs.46.325

Kern, M. (2011). Metabolic responses to ingestion of pullulan. Unpublished study report to Hayashibara Co., Ltd, Japan.

Klosterbuer, A. S., Thomas, W., & Slavin, J. L. (2012). Resistant starch and pullulan reduce postprandial glucose, insulin, and GLP-1, but have no effect on satiety in healthy humans. *Journal of Agricultural and Food Chemistry*, *60*(48), 11928–11934. https://doi.org/10.1021/jf303083r

Knapp, B. K., Parsons, C. M., Bauer, L. L., Swanson, K. S., & Fahey, G. C., Jr. (2010). Soluble fiber dextrins and pullulans vary in extent of hydrolytic digestion in vitro and in energy value and attenuate glycemic and insulinemic responses in dogs. *Journal of Agricultural and Food Chemistry*, *58*(21), 11355–11363. https://doi.org/10.1021/jf102397r

Knapp, B. K., Parsons, C. M., Swanson, K. S., & Fahey, G. C., Jr. (2008). Physiological responses to novel carbohydrates as assessed using canine and avian models. *Journal of Agricultural and Food Chemistry*, *56*(17), 7999–8006. https://doi.org/10.1021/jf801042b

Okada, K., Yoneyama, M., Mandai, T., Aga, H., Sakai, S., & Ichikawa, T. (1990). Digestion and fermentation of pullulan. *Nippon Eiyo Shokuryo Gakkaishi*, *43*(1), 23–29. https://doi.org/10.4327/jsnfs.43.23

Oku, T., Konishi, F., & Hosoya, N. (1982). Mechanism of inhibitory effect of unavailable carbohydrate on intestinal calcium absorption. *The Journal of Nutrition*, *112*(3), 410–415. https://doi.org/10.1093/jn/112.3.410

Oku, T., Yamada, K., & Hosoya, N. (1979). Effects of pullulan and cellulose on the gastrointestinal tract of rats. *Eiyo to Shokuryo*, *32*(4), 235–241. https://doi.org/10.4327/jsnfs1949.32.235

Peters, H. P., Ravestein, P., van der Hijden, H. T., Boers, H. M., & Mela, D. J. (2011). Effect of carbohydrate digestibility on appetite and its relationship to postprandial blood glucose and insulin levels. *European Journal of Clinical Nutrition*, *65*(1), 47–54. https://doi.org/10.1038/ejcn.2010.189

Prosky, L., Asp, N. G., Schweizer, T. F., DeVries, J. W., & Furda, I. (1988). Determination of insoluble, soluble, and total dietary fiber in foods and food products: Interlaboratory study. *Journal of the Association of Official Analytical Chemists*, 71(5), 1017–1023.

Richards, A. B., & Higashiyama, T. (2004). Glycemic index of pullulan (PI-20). Unpublished study report from Hayashibara International, Japan.

SCF (Scientific Committee on Food). (1994). *Reports of the Scientific Committee for Food (32nd Series)* (p. 3). European Commission, Food Science and Techniques. ISBN:92-826-6662-x.

SCF (Scientific Committee on Food). (1999). Opinion of Ethylhydroxyethyl cellulose (addendum to the "Opinion on re-evaluation of five modified celluloses" of 13 March 1992, expressed on 24 March 1999). Available online https://food.ec.europa.eu/document/download/17e91acb-62e6-496f-9a30-3f58ed2dfcc1_en?filename=sci-com_scf_out29_en.pdf (Accessed February 2025).

Sommer, E. W., Flade, D., Gretener, P., & Mehrbass, D. (2003). Pullulan PI-20. 13-week oral (feeding) toxicity study in the Wistar rat. Unpublished report for Hayashibara Co. Ltd, Japan, 2003 (RCC Study No. 842710).

Spears, J. K., Karr-Lilienthal, L. K., Bauer, L. L., Murphy, M. R., & Fahey, G. C., Jr. (2007). In vitro fermentation characteristics of selected glucose-based polymers by canine and human fecal bacteria. *Archives of Animal Nutrition*, *61*(1), 61–73. https://doi.org/10.1080/17450390601117017

Spears, J. K., Karr-Lilienthal, L. K., & Fahey, G. C., Jr. (2005). Influence of supplemental high molecular weight pullulan or gamma-cyclodextrin on ileal and total tract nutrient digestibility, fecal characteristics, and microbial populations in the dog. *Archives of Animal Nutrition*, *59*(4), 257–270. https://doi.org/10.1080/17450390500216993

Spears, J. K., Karr-Lilienthal, L. K., Grieshop, C. M., Flickinger, E. A., Wolf, B. W., & Fahey, G. C., Jr. (2005a a). Glycemic, insulinemic, and breath hydrogen responses to pullulan in healthy humans. *Nutrition Research*, *25*(12), 1029–1041. https://doi.org/10.1016/j.nutres.2005.09.011

Spears, J. K., Karr-Lilienthal, L. K., Grieshop, C. M., Flickinger, E. A., Wolf, B. W., & Fahey, G. C., Jr. (2005c). Pullulans and gamma-cyclodextrin affect apparent digestibility and metabolism in healthy adult ileal cannulated dogs. *The Journal of Nutrition*, *135*(8), 1946–1952. https://doi.org/10.1093/jn/135.8.1946

Stewart, M. L., Nikhanj, S. D., Timm, D. A., Thomas, W., & Slavin, J. L. (2010). Evaluation of the effect of four fibers on laxation, gastrointestinal tolerance and serum markers in healthy humans. *Annals of Nutrition & Metabolism*, *56*(2), 91–98. https://doi.org/10.1159/000275962

Sugimoto, K. (1978). Pullulan production and applications. *Journal of Fermentation Association Japan*, *36*(2), 98–108.

Tsujisaka, Y., & Mitsuhashi, M. (1993). Pullulan. In *Industrial gums (3rd Ed.)* (pp. 447–460). Academic Press.

Weaver, C. M., Martin, B. R., Story, J. A., Hutchinson, I., & Sanders, L. (2010). Novel fibers increase bone calcium content and strength beyond efficiency of large intestine fermentation. *Journal of Agricultural and Food Chemistry*, *58*(16), 8952–8957. https://doi.org/10.1021/jf904086d

Wolf, B. W., Garleb, K. A., Choe, Y. S., Humphrey, P. M., & Maki, K. C. (2003). Pullulan is a slowly digested carbohydrate in humans. *The Journal of Nutrition*, *133*(4), 1051–1055. https://doi.org/10.1093/jn/133.4.1051

Yoneyama, M., Okada, K., Mandai, T., Aga, H., Sakai, S., & Ichikawa, T. (1990). Effects of pullulan intake in humans. *Journal of the Japanese Society of Starch Science*, *37*(3), 123–127. https://doi.org/10.5458/jag1972.37.123

Yuzbasioglu, D., Mamur, S., Avuloglu-Yilmaz, E., Erikel, E., Celebi-Keskin, A., & Unal, F. (2021). Evaluation of the genotoxic and antigenotoxic effects of exopolysaccharide pullulan in human lymphocytes in vitro. *Mutation Research. Genetic Toxicology and Environmental Mutagenesis*, *870*, 503391. https://doi.org/10.1016/j.mrgentox.2021.503391

**How to cite this article:** EFSA FAF Panel (EFSA Panel on Food Additives and Flavourings), Castle, L., 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., … Ruggeri, L. (2025). Re-evaluation of pullulan (E 1204) as a food additive and new application for its extension of use. *EFSA Journal*, *23*(3), e9267. https://doi.org/10.2903/j.efsa.2025.9267

# APPENDIX A

## Exposure calculations to toxic elements (As, Pb, Hg, Cd) from the use of the proposed food additive

Following the EFSA call for data, one IBO provided information on the levels of arsenic (As), lead (Pb), mercury (Hg), cadmium (Cd) in samples of pullulan (E 1204) (Documentation provided to EFSA No 3).

Levels of Pb and As were determined in 20 commercial samples of E 1204. For the tested samples of E 1204, the levels of Pb and As were always below the internal specifications indicated by the applicant (1 mg/kg for Pb, 2 mg/kg for As). The highest measured values were 0.09 mg/kg for Pb and 0.03 mg/kg for As. Levels of Cd and Hg were determined in two samples of E 1204. For the two samples of E 1204 levels of Cd and Hg were reported as below 0.005 and 0.01 mg/kg, respectively, without indicating if these values are reporting limits, LODs or LOQs (Documentation provided to EFSA No 3). The potential exposure to these toxic elements from the use of pullulan (E 1204) can be calculated by assuming that the impurities are present in the food additive at the reported level, and then by calculation pro-rata to the estimates of exposure to the food additive itself.

With regard to the dietary exposure to E 1204, the Panel considered the estimates resulting from the exposure assessment reported in Table 8. The selected scenario considered the highest level provided by an IBO for FC 17.1 (i.e. 277,777 mg/kg), as well as the proposed maximum use level for FC 17.2 (60,000 mg/kg) and the maximum use levels proposed by the applicant for all the other FCs. This scenario was chosen being the more protective. For the current assessment, the highest exposure levels for the mean and 95th percentile among the different population groups were considered, i.e. 65 and 224 mg/kg bw per day, for adolescents.

Since the exposure assessment scenario considered followed the 'food supplements consumers only' approach, the population groups 'infants' and 'toddlers' are not represented.

The potential level of the toxic elements in the food additive combined with the estimated exposure levels to E 1204, presented in Table 8, results in exposure estimates that can be compared with the following reference points (RPs) or health-based guidance values (HBGV) (Table A.1). It is considered that any mercury or arsenic in the food additive corresponds to the element in the inorganic form rather than organic form. Consequently, the HBGV for inorganic mercury (iHg) and RP for inorganic arsenic (iAs) were used for comparison.

**TABLE A.1** Reference points/health-based guidance value for toxic elements potentially present in pullulan (E 1204).

| Element HBGV/RP (μg/kg bw/day or/w) | Basis/reference |
|---|---|
| Inorganic arsenic (iAs)/0.06 μg/kg bw per day (BMDL05) | The reference point is based on a benchmark dose lower confidence limit (BMDL05) 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, ischaemic 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.<br>Because there are no precedents in EFSA for identification of an 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.<br>EFSA CONTAM Panel (2024) |
| 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).<br>EFSA CONTAM Panel (2010) |
| Inorganic mercury (iHg)/4 μg/kg bw per week (TWI) | The HBGV was set using kidney weight changes in male rats as the pivotal effect. Based on the $BMDL_{10}$ of 0.06 mg/kg bw per day, expressed as mercury, and an uncertainty factor of 100 to account for inter- and intraspecies differences, with conversion to a weekly basis and rounding to one significant figure, a TWI for inorganic mercury of 4 μg/kg bw per week, expressed as mercury was established.<br>EFSA CONTAM Panel (2012) |
| Cadmium (Cd)/2.5 μg/kg bw per week (TWI) | The derivation of the reference point is based on a meta-analysis to evaluate the dose–response relationship between selected urinary cadmium and urinary beta-2-microglobulin as the biomarker of tubular damage recognised as the most useful biomarker in relation to tubular effects. A group-based $BMDL_5$ of 4 μg Cd/g creatinine for humans was derived. A chemical-specific adjustment factor of 3.9 was applied to account for human variability in urinary cadmium within each dose-subgroup in the analysis resulting in a reference point of 1.0 μg Cd per g creatinine. In order to remain below 1 μg Cd/g creatinine in urine in 95% of the population by age 50, the average daily dietary cadmium intake should not exceed 0.36 μg Cd/kg bw, corresponding to a weekly dietary intake of 2.5 μg Cd/kg bw.<br>EFSA CONTAM Panel (2009) |

Abbreviations: BMDL 01, benchmark dose (lower confidence limit); HBGV, health-based guidance value; MOE, margin of exposure; RP, Reference point; TWI, tolerable weekly intake.

The risk assessment of impurities helps determine whether there could be a possible health concern if these impurities would be present at certain level in the food additive. The assessment is then performed by calculating the MOE (margin of exposure) by dividing the reference point (e.g. BMDL Table A.1) by the exposure estimate (Table 8), or by estimating the contribution of the use of E 1204 to the HBGV (expressed as percentage of the HBGV).

The Panel noted that no proposal for the lowest achievable levels for arsenic, lead, cadmium and mercury in E 1204 was provided by the IBO. The Panel noted that among the potential inorganic impurities tested, only lead has defined limit value in EU specifications for E 1204, and lead and arsenic were quantified in some of the analysed samples.

The Panel assessed the risk that would result if these toxic elements were present in the food additive E 1204 (i) at the current limit in the EU specifications (only available for Pb); (ii) at the highest measured values reported in commercial samples of As and Pb; (iii) in absence of measured values, at the reported values for Hg and Cd.

Toxic elements concentrations used for the calculation of their potential exposure from the use of the food additive are reported in Table A.2.

**TABLE A.2** Toxic elements concentrations (mg/kg) in E 1204 used for the calculation of their potential exposure from the use of the food additive.

| Toxic element concentrations in E 1204 | Pb | Hg | Cd | As |
|---|---|---|---|---|
| **(i) Current limits in the EU specifications for E 1204 (mg/kg)** | 1 | – | – | – |
| **(ii) Highest measured values** | 0.09 | – | – | 0.03 |
| **(iii) Reported values** | – | 0.01 | 0.005 | – |

The outcome of the risk assessment is presented in Table A.3. The Panel emphasised that the choice of the maximum limit values as well as other considerations, such as on multiple sources of exposure to conclude on the maximum limits for toxic elements in the specifications, is in the remit of risk management. The numbers used here are merely taken to support the risk assessment of these toxic elements as presented below.

**TABLE A.3** Risk assessment for toxic elements from the use of E 1204.

| **(i) Considering the presence of toxic elements at the current limits of the EU specifications for E 422 (commission regulation (EU) No 231/2012), where available** | | | | |
|---|---|---|---|---|
| **Exposure to E 1204 (mg/kg bw per day)** | **MOE for Pb at 1 mg/kg** | **% of the TWI for Hg** | **% of the TWI for Cd** | **MOE for As** |
| 65[a] | 8 | – | – | – |
| 224[b] | 2 | – | – | – |
| **(ii) Considering the presence of toxic elements at the highest measured values reported in commercial samples of E 1204, where available** | | | | |
| **Exposure to E 1204 (mg/kg bw per day)** | **MOE for Pb at 0.09 mg/kg** | **% of the TWI for Hg** | **% of the TWI for Cd** | **MOE for As at 0.03 mg/kg** |
| 65[a] | 87 | – | – | 31 |
| 224[b] | 25 | – | – | 9 |
| **(iii) Considering the presence of toxic elements at the reported values[c] in commercial samples of E 1204, where available** | | | | |
| **Exposure to E 1204 (mg/kg bw per day)** | **MOE for Pb** | **% of the TWI for Hg at 0.01 mg/kg** | **% of the TWI for Cd at 0.005 mg/kg** | **MOE for As** |
| 65[a] | – | 0.1 | 0.1 | – |
| 224[b] | – | 0.4 | 0.3 | – |

[a]Highest exposure level among the different population groups at the mean (Table 8).

[b]Highest exposure level among the different population groups at the 95th percentile (Table 8).

[c]Since it was not indicted if the values correspond to a LOD, LOQ or reporting limit, the modulation factor was not applied.

For iAs, the BMDL is within the range of the mean dietary exposure estimates for adults in Europe (EFSA CONTAM Panel, 2024) and therefore any additional exposure may be critical. Taking into account the calculations performed by the Panel (Table A.3), and the fact that the food additive is not the only potential dietary source of arsenic, the Panel considered that a limit should be introduced for arsenic in the specifications for E 1204.

Considering the results of the exposure to lead, the Panel noted that its presence in E 1204 at the measured value or at the maximum permitted level set in EU specification, would not give rise to concern. Nevertheless, given the discrepancy between the maximum permitted levels indicated in Regulation (EU) No 231/2012 and the measured values in commercial samples of E 1204, and given the fact that the food additive is not the only potential dietary source of lead, the current maximum limit in the specifications for lead should be lowered, based on actual concentration of lead in the food additive.

Taking into account that pullulan is produced from food-grade hydrolysed starch and undergoes to some purification steps, systematic contamination by cadmium and mercury is not anticipated and the Panel did not see a need to recommend additional specification limits for cadmium and mercury. In addition, the results of the calculations performed by the Panel (Table A.3) do not give rise to concern.