Position papers

Committee Advice on the assessment of Pasteurised Akkermansia muciniphila as a novel food for use in supplements.

Reference number RP1468

Food Standards Agency (FSA) and Food Standards Scotland (FSS)

Regulated Product Dossier Assessment

Assessment finalised: 17th September 2024

Summary

An application was submitted to the Food Standards Agency (FSA) and Food Standards Scotland (FSS) in February 2022 from The Akkermansia Company SA, Belgium ("the applicant") for the authorisation of pasteurised *Akkermansia muciniphila* as a novel food. The novel food is derived from a strain of human gut bacteria and produced through fermentation and subsequent pasteurisation and lyophilisation. The resulting product is a powder intended for use in food supplements and Foods for Special Medical Purposes. The target population is the general population above twelve years old.

To support the FSA and FSS in their evaluation of the application, the Advisory Committee on Novel Foods and Processes (ACNFP) were asked to review the safety dossier and supplementary information provided by the applicant. Please note the Committee did not consider any potential health benefits or claims arising from consuming the food, as the focus of the novel food assessment is to ensure the food is safe, and not putting consumers at a nutritional disadvantage.

The Committee advised that the novel food, pasteurised *Akkermansia muciniphila,* was safe under the proposed conditions of use. The novel food is not considered to be nutritionally disadvantageous.

1. Introduction

1. The ACNFP assessed the food safety risks of the novel food, pasteurised *Akkermansia muciniphila* and its production in accordance with Article 7 of assimilated Commission Implementing Regulation (EU) 2017/2469. The regulatory framework and the guidance put in place by EFSA for full novel food applications has formed the basis and structure for the assessment (EFSA, 2016).

2. In February 2022, the FSA and the countries of GB received an application from The Akkermansia Company SA, Belgium ("the applicant") for the authorisation of pasteurised *Akkermansia muciniphila* as a novel food. The novel food is derived from a strain of human gut bacteria and produced through fermentation and subsequent pasteurisation and lyophilisation. The resulting product is a powder intended for use in food supplements and Foods for Special Medical Purposes (FSMPs). The target population is the general population above twelve years old.

3. Following the review by the ACNFP at their meeting in April 2023, further information was requested concerning the identification, production process, composition, specification, and proposed uses of the novel ingredient. The application was further reviewed in June and September 2023 and June 2024. The final advice from the Committee was agreed at the 167th ACNFP meeting, allowing the FSA and FSS to complete the risk assessment.

4. This Committee Advice Document (CAD) outlines the conclusions of the ACNFP assessment on the safety of pasteurised *Akkermansia muciniphila* as a novel food when used as an ingredient in food supplements and Foods for Special Medical Purposes (FSMPs).

2. Assessment

2.1 Identity of the novel ingredient

5. The novel food is a lyophilised (freeze-dried) powder consisting of pasteurised cells of the human gut bacterium *Akkermansia muciniphila* at less than ten (10 CFU) viable cells per gram. The bacterium was first isolated from the intestinal tract of a human and is conclusively described within the literature (Derrien *et al.* 2004).

6. The source material used to produce the novel food is *Akkermansia muciniphila* Muc^{T}, which is deposited in both the American Type Culture Collection (accession number = ATCC BAA-835) and at the Collection de l'institut Pasteur (accession

number = CIP 107961).

7. The identity of the source organism, *Akkermansia muciniphila* Muc^T (also referred to as *Akkermansia* or *A. muciniphila*), is verified through whole genome sequence analysis and alignment with genome sequence data from the National Centre for Biotechnology Information (NCBI). The source organism shares 99.96% and 99.98% (PacBio and Illumina, respectively) sequence homology with the NCBI whole genome sequence for *Akkermansia muciniphila*. The identity of the source organism is additionally verified through full 16S rRNA gene sequencing, in which it shares 100% sequence homology with the NCBI sequence for *Akkermansia muciniphila* 16S rRNA.

8. The taxonomic ranking of the source bacterium is defined below:

- Kingdom = *Bacteria*
- Phylum = *Verrucomicrobia*
- Class = *Verrucomicrobiae*
- Order = *Verrucomicrobiales*
- Family = *Verrucomicrobiaceae*
- Genus = *Akkermansia*
- Species = *Akkermansia muciniphila*
- Strain = *Akkermansia muciniphila* Muc^T (ATCC BAA-835)

9. An antimicrobial resistance study has been provided (Gueimonde, 2019), which assesses the susceptibility of *Akkermansia muciniphila* (ATCC BAA-835), along with five other *Akkermansia muciniphila* strains and one strain of *Akkermansia glycaniphila Pytt*, to a range of antimicrobial compounds. The study was performed in accordance with the European Food Safety Authority (EFSA) guidance on the assessment of bacterial susceptibility to antimicrobials of human and veterinary importance.

10. The results of the study indicate resistance to some of the compounds tested. However, it was considered that the antimicrobial resistance observed was intrinsic to the organism, since all five strains tested displayed the same resistance profile. This was further supported by evidence in the scientific literature.

11. The applicant provides a bioinformatic assessment of the whole genome sequence of *Akkermansia muciniphila* (ATCC BAA-835) with reference to known antimicrobial resistance genes. The whole genome sequence was first annotated using tools hosted by Pathosystems Resource Integration Centre (PATRIC), with subsequent searches for sequence homologies within the Comprehensive

Antibiotic Resistance Database (CARD) and the National Database of Antibiotic Resistant Organisms (NDARO); using the Basic Local Alignment Search Tool (BLAST) to align the sequences and identify any antimicrobial resistance genes that may be present.

12. Information was also provided on the publicly available literature on the *Akkermansia muciniphila* (ATCC BAA-835) genome, which includes a bioinformatic assessment of antibiotic resistance genes. The bioinformatic assessment indicates the absence of antibiotic resistance genes that would result in an undesirable phenotype. Additionally, no plasmids were identified in *Akkermansia muciniphila* (ATCC BAA-835).

13. The Committee concludes from the genome analysis presented that *Akkermansia muciniphila* (ATCC BAA-835) does not present a risk of horizontal transfer of antibiotic resistance genes. While the information indicates the presence of putative antibiotic resistance genes within the chromosome, they are not associated with predicted mobile genetic elements (MGEs).

14. Both a literature review and a bioinformatic assessment using the whole genome sequence of *Akkermansia muciniphila* (ATCC BAA-835) have been used to assess and identify possible virulence determinants (also called virulence factors) associated with the novel food product. Within the literature, there were some reports of sequence homology between proteins present in the *Akkermansia muciniphila* (ATCC BAA-835) proteome and putative virulence factors in several online databases. However, there are no reported cases, or reports which indicate that *Akkermansia muciniphila* (ATCC BAA-835) exhibits pathogenic behaviour.

15. Further to the literature review, a bioinformatic assessment of the whole genome sequence of *Akkermansia muciniphila* (ATCC BAA-835) has been provided to highlight possible virulence determinants and a bioinformatic assessment of pathogenicity. The online tool VirulenceFinder 2.0 was used to search for potential acquired virulence factors relating to *Escherichia coli* and its verocytotoxin subtypes (VTECs), and the online tool PathogenFinder 1.1 was used to predict the pathogenicity potential of *Akkermansia muciniphila* (ATCC BAA-835) to a human host. The presence of genetic elements encoding virulence factors was determined using BLAST to align *Akkermansia muciniphila* (ATCC BAA-835) amino acid sequences against putative virulence factors from PATRIC_VF, VFDB, and Victors online databases.

16. The assessment highlighted putative virulence factors which shared significant sequence homology with many proteins present in the *Akkermansia*

muciniphila (ATCC BAA-835) proteome. However, based on PathogenFinder 1.1 analyses, it was considered that the probability of being a human pathogen was predicted to be low (0.22%).

17. A bioinformatic assessment of the toxigenic potential of *Akkermansia muciniphila* (ATCC BAA-835) was conducted using BLAST to compare its amino acid sequences against a database of animal toxin proteins to predict their function. The search identified a single protein present in the proteome of *Akkermansia muciniphila* (ATCC BAA-835) which shares significant sequence homology with four animal venom proteins.

18. Two of the identified toxin proteins have targets in non-vertebrates and are therefore not a concern for humans. The other two toxin proteins, identified as alpha-latrotoxins, do have vertebrate targets. However, given that alpha-latrotoxins are homotetramers, the hypothetical protein from pasteurised *Akkermansia muciniphila* (341 amino acids) is unlikely to share protein function despite sharing sequence homology.

19. The analysis also identified the potential presence of ankyrin proteins which may have toxic effects. The degree of sequence homology to the protein in *Akkermansia muciniphila* does not meet the sequence homology criteria for a gene of potential concern according to EFSA guidance (EFSA, 2021). Therefore, the putative ankyrin protein identified in the *Akkermansia muciniphila* (ATCC BAA-835) genome is not expected to present a risk to consumers of the novel food as the protein is unlikely to share the toxic potential.

20. Following review, further information was sought surrounding the identification procedures used during production as part of the quality assurance measures. Information was provided that stated that 16s rRNA sequencing is conducted during multiple stages of the production process: during cell banking and after production of the pasteurised *Akkermansia* powder (final product) to assure the identity of the organism present.

2.2 Production Process

21. The novel food, pasteurised *Akkermansia muciniphila*, is produced through several steps which create a powdered final product. Initially, a bacterial cell culture is produced via resuscitation from a master cell bank, propagation via anaerobic fermentation, and subsequent heat-inactivation through pasteurisation.

22. The pasteurised cells are then concentrated and mixed with cryoprotectants prior to freeze-drying and mixing with a food grade excipient such as corn starch

or maltodextrin. The final total pasteurised cell concentration is stated to be at least $2.5x10^{10}$ non-viable bacterial cells per gram of powdered final product. The final product in powder form is stored in bulk in heat-sealed, three-layer, waterproof and airproof pouches at -18°C.

23. Management of the production process is demonstrated through a range of in-process analyses, which have been detailed throughout the production process. Both culture-based analyses (microscopy and colony morphology) and genetic analyses (16s rRNA and whole genome sequencing) are used initially to confirm the identity of the starting inoculum. Culture purity and viability are assessed following anaerobic fermentation through plating. Viability is checked again after pasteurisation to validate effectiveness of the heat-treatment. Total cell counts are performed throughout the production process using hemocytometry or flow cytometry.

24. A hazard analysis and critical control points (HACCP) declaration has been provided along with a schematic diagram of the critical control points (CCPs) identified. There are no concerns with how the product is produced or how the production process is managed by the applicant.

25. Further information was sought regarding hazard analysis throughout production and final product variability. Further information was also sought regarding the carryover of residue from production culture media. The response from the application regarding these potential hazards confirmed that there is minimal risk from carryover of production materials.

26. The food safety management plan which was provided characterised the potential hazards and described appropriate control measures.

2.3 Composition

27. To verify the compositional analysis provided was accurate and reliable, certification was provided to demonstrate that the laboratories were accredited to perform these analytical studies. Where in-house analysis was utilised, full methodology and supporting validation documentation were provided.

28. The final consumer product intended for market is an off-white to beige homogenous powder with a minimum total non-viable cell count of $2.5x10^{10}$ *Akkermansia muciniphila* cells per gram. Analyses of both total *Akkermansia muciniphila* cell counts and viable *Akkermansia muciniphila* cell counts have been performed on five independent batches using flow cytometry and plating methods, respectively. Species identification using 16s rRNA gene sequencing has

also been performed to confirm the identity of each of the five independent batches, all of which conform to the specification. Table 1 shows the results of the preliminary analysis of five batches of pasteurised *Akkermansia muciniphila* prior to the change in specification of the novel food. Further analysis of composition after the change in specification is provided in section 2.4, which demonstrates that the product is produced consistently.

Table 1: Analytical results for five independent representative batches of pasteurised *Akkermansia muciniphila.*

Parameter	**Original Specification**	**Batch 1**	**Batch 2**	**Batch 3**	**Batch 4**	**Batch 5**
Appearance (by visual observation)	Off-white to beige homogeneous powder	Conforms	Conforms	Conforms	Conforms	Conforms
Species identification (16S rRNA gene sequencing – Sanger Method)	*Akkermansia muciniphila*	Conforms	Conforms	Conforms	Conforms	Conforms
Total *Akkermansia muciniphila* cell count (flow cytometry A-Mansia method) (TFU/g powder) a	2.5×10^{10} to 2.5×10^{12}	2.23×10^{11}	1.78×10^{11}	1.13×10^{11}	9.34×10^{10}	9.94×10^{10}

Viable *Akkermansia muciniphila* cell count (plating method) (CFU/g powder)b	500	10	40	400	30	20

CFU = colony forming units; TFU = total fluorescent units. a) Limit of quantification = 1 x 10^5 TFU/g b) Limit of detection = 10 CFU/g powder

29. An assessment was conducted in accordance with the EFSA Guidance on technical requirements for regulated food and feed product applications to establish the presence of small particles including nanoparticles in the novel food (EFSA, 2021). The results show the particles in the number-size distribution for pasteurised *Akkermansia muciniphila* are well above the nano scale, with 50% of the cumulative distribution ranging between 50 and 100 µM and the smallest particle size detected being between 1 and 5 µM, confirming the final product is free from nanosized structures.

30. A proximate analysis of the final product has been provided. The applicant states this shows full characterisation of the product composition. The results show that the novel food is mostly comprised of carbohydrate and protein, with small amounts of lipid present. Proximate analysis data are displayed in Table 2.

31. Analyses for mycotoxins and polycyclic aromatic hydrocarbons (PAHs) in the final novel food product are omitted from the application. However, all raw materials used within the production process have undergone mycotoxin and PAH testing prior to use to monitor for environmental contamination. The results show the raw materials to be free of such contaminants and the production process does not introduce any mycotoxins or polycyclic aromatic hydrocarbons (PAHs).

Table 2: Proximate analysis of five independent representative batches of Pasteurised *Akkermansia muciniphila.*

Parameter and method	**Specification**	Batch 1	Batch 2	Batch 3	Batch 4	Batch 5

Water Activity (ISO 18787:2017)	≤0.43	0.165	0.280	0.353	0.321	0.285
Moisture Content/ Humidity (ISO 760:1978) (g/100g)	≤12	5.5	7.4	8.5	3.9	4.9
Crude Ash (Decree of 08/09/1977a) (g/100 g)	≤21	18.4	19.7	19.3	19.5	13.2
Total Carbohydrate (By Calculation) (g/100 g)	36 to 86	43.3	45.8	41.5	46.3	49.5
Energy (By Calculation) (kcal/100 g)	270 to 405	310	295	291	309	329
Energy (By Calculation) (kJ/100 g)	1,100 to 1,721	1,318	1,251	1,237	1,312	1,396
Protein (ISO 1871:2009) (g/100 g)	35	31.6	26.5	30.2	29.8	32.2
Total Fat (ISO 11085:2015) (g/100 g)	≤4	1.2	0.6	0.5	0.5	0.2

32. A heavy metals analysis of five independent batches has been provided. The results show that the levels of heavy metals (arsenic, cadmium, mercury, lead) consistently remain below the specified EU limits for final foods. Table 3 shows the heavy metal analysis for five independent batches of the novel food.

Table 3: Heavy metal analysis of five independent representative batches of Pasteurised *Akkermansia muciniphila.*

Parameter and methodology	EU Limit (final foods)	Specification	Batch 1	Batch 2	Batch 3	Batch 4	Batch 5
Arsenic (ICP-MS) (mg/kg)	0.3	0.3	0.25	0.22	0.23	0.18	0.17
Cadmium (ICP-MS) (mg/kg)	1.0	1.0	0.005	0.009	0.006	0.006	0.015
Mercury (ICP-MS) (mg/kg)	0.1	0.1	0.02	0.02	0.02	0.02	0.02
Lead (ICP-MS) (mg/kg)	3.0	3.0	0.04	0.043	0.038	0.04	0.02

33. Microbiological analysis of five individual batches has been provided. The product conforms to the limits set out within the specification of the novel food. Microbiological compositional data are displayed in Table 4.

Table 4: Microbiological analysis of five independent representative batches of Pasteurised *Akkermansia muciniphila.*

Parameter and Methodology	Specification	Batch 1	Batch 2	Batch 3	Batch 4	Batch 5
Aerobic mesophilic total count (NF EN ISO 4833-1) (CFU/g)	≤ 500	10	10	10	10	10

Sulphite reducing anaerobes (ISO 15213) (CFU/g)	≤ 50	10	10	10	40	10
Coagulase + staphylococci (NF EN ISO 6888-2) (CFU/g)	≤ 10	10	10	10	10	10
Enterobacteriaceae (ISO 21528-2) (CFU/g)	≤ 10	10	10	10	10	10
Bacillus cereus (NF EN ISO 7932) (CFU/g)	≤ 100	10	10	10	10	10
Listeria monocytogenes and *Listeria* spp. (ISO 11290-1:2017)	Absent in 25 g	Absent	Absent	Absent	Absent	Absent
Salmonella (ISO 6579-1:2017)	Absent in 25 g	Absent	Absent	Absent	Absent	Absent
Escherichia coli (NF ISO 16649-1)	Absent in 1 g	Absent	Absent	Absent	Absent	Absent
Yeasts (ISO 21527-2:2008) (CFU/g)	≤ 10	10	10	10	10	10
Moulds (ISO 21527-2:2008) (CFU/g)	≤ 10	10	10	10	10	10

2.4 Specifications

34. The specifications for the novel food are outlined within Tables 5 - 8. This provides details of all parameters which are being managed by the applicant and includes specifications for: physical characterisation and identity, proximate analysis, microbiological contaminants, and heavy metals. Details of methods of

analytical methods used were also provided within the application.

Table 5: Pasteurised *Akkermansia muciniphila* Characterisation and Identity Specifications.

Characterisation and Identity.

Parameter	Specification Value	Analytical Method
Appearance	Off-white to beige homogenous powder	Visual Observation
Species Identification	*Akkermansia muciniphila* ATCC BAA-835T	16S rRNA Gene Sequencing – Sanger Method
Total *Akkermansia muciniphila* Cell Count	$2.5x10^{10}$ to $2.5x10^{12}$ TFU/g	Flow Cytometry (A-Mansia Method)
Viable *Akkermansia muciniphila* Cell Count	10 CFU/g	Plating (A-Mansia Method – performed at Agrobio – ALE-PRO-111)

Table 6: Pasteurised *Akkermansia muciniphila* Proximate Analysis Specifications.

Proximate Analysis.

Parameter	Specification Value	Analytical Method
Water activity	≤0.43	NF ISO 21807:2004
Moisture content/humidity	≤12 g/100 g	Vacuum Drying Method

Protein	≤35 g/100 g	Kjeldhal Method or Dumas Method
Total Fat	≤4 g/100 g	Determined by extraction after hydrolysis
Crude Ash	≤21 g/100 g	Dry Ashing
Total Carbohydrate	36 - 86 g/100 g	By Calculation
Energy	270 to 405 kcal	By Calculation

Table 7: Pasteurised *Akkermansia muciniphila* Microbiological Specifications.

Microbiological Analyses.

Parameter	**Specification Value**	**Analytical Method**
Aerobic Mesophilic Total Count	≤500 CFU/g	NF EN ISO 4833-1
Sulphite Reducing Anaerobes	≤50 CFU/g	ISO 15213
Coagulase Positive *Staphylococci*	≤10 CFU/g	NF EN ISO 6888-2
Enterobacteriaceae	≤10 CFU/g	ISO 21528-2
Bacillus cereus	≤100 CFU/g	NF EN ISO 7932
Listeria	Absent in 25 g	ISO 11290-1:2017
Salmonella	Absent in 25 g	ISO 6579-1:2017

Escherichia coli	Absent in 1 g	NF ISO 16649-1
Yeasts	≤10 CFU/g	ISO 21527-2:2008
Moulds	≤10 CFU/g	ISO 21527-2:2008

Table 8: Pasteurised *Akkermansia muciniphila* Heavy Metal Specifications.

Heavy Metals

Parameter	Specification Value	Analytical Method
Arsenic	0.3 mg/kg	ICP-MS
Cadmium	1.0 mg/kg	ICP-MS
Mercury	0.1 mg/kg	ICP-MS
Lead	3.0 mg/kg	ICP-MS

Table footnotes: ATCC = American Type Culture Collection; CFU = colony forming units; CIP = Collection de l'Institut Pasteur; EN = European Standard; ISO = International Organization for Standardization; ICP-MS = inductively coupled plasma mass spectrometry; NF = norme francaise (French Standard); TFU = total fluorescent units

35. It was noted that initially the applicant sought a viable cell count of less than 500 CFU/g. This was considered higher than expected and the potential risks for vulnerable groups was explored with the applicant. It was explained that the variability of viable cell count (cfu/g) seen in the final product is due to limited selectivity of the culture medium used within the assay. The methodology has since been updated by the applicant to improve performance. Considering the methodological development, the specification has been adjusted to be as low as reasonably possible, conferring a viable cell count limit of less than 10 CFU/g. A further five batch analysis of total cell count and viable cell count representing

the changes in methodology and specification has been provided in Table 9:

Table 9: Analytical results for five independent representative batches of pasteurised *Akkermansia muciniphila.* (Batch numbers are confidential).

Parameter and method	**Specification**	**Batch 6**	**Batch 7**	**Batch 8**	**Batch 9**	**Batch 10**
Appearance (by visual observation)	Off-white to beige homogeneous powder	Conforms	NM	Conforms	Conforms	Conforms
Species identification (16S rRNA gene sequencing – Sanger Method)	*Akkermansia muciniphila*	NM	Conforms	Conforms	Conforms	Conforms
Total *Akkermansia muciniphila* cell count (flow cytometryAA-Mansia method) (TFU/g powder) a	2.5×10^{10} to 2.5×10^{12}	NM	1.8×10^{11}	1.8×10^{11}	2.16×10^{11}	2.1×10^{11}

Viable *Akkermansia muciniphila* cell count (plating method) (CFU/g powder)b	10	10	10	10	10	10

CFU = colony forming units; TFU = total fluorescent units; NM = Not Measured. a) Limit of quantification = 1 x 10^5 TFU/g b) Limit of detection = 10 CFU/g powder

36. The information provided is sufficient for the specification of the novel food, and appropriately characterises the novel food seeking authorisation.

2.5 Stability

37. A range of analytical data which characterises and describes the stability of pasteurised *Akkermansia muciniphila* over a 12-month period has been provided. The data provided includes real-time monitoring of the novel food at 25°C and 60% relative humidity for 12 months, and monitoring under accelerated conditions at 40°C and 75% relative humidity for 12 months.

38. The parameters measured in the stability studies include physiochemical and biochemical parameters (total *Akkermansia muciniphila* and viable *Akkermansia muciniphila* cell counts), and microbiological contaminants pertinent to the novel food. The results demonstrate the long-term stability of the novel food over a 12-month period, under both real-time and accelerated conditions.

2.6 History of Use

39. Literature evidence of consumption of the source organism is provided. There is evidence of detection of *Akkermansia muciniphila* cells in human breast milk, which is evidence of human consumption of the source organism from birth. Evidence is also brought forward which confirms the presence of *Akkermansia muciniphila* in the human gastrointestinal tract during infancy, from as young as one month old. The prevalence of *Akkermansia muciniphila* in the human gastrointestinal tract is stated to increase with age up to adulthood.

40. A study supplied by the applicant which describes the prevalence of *Akkermansia muciniphila* in samples taken from various human populations. A total of 96% of samples taken from adults and the elderly had detectable *Akkermansia muciniphila* cells. It was also detected in 16% of samples taken from 1 month old infants.

41. The applicant states that although this information evidences some form of consumption of *Akkermansia muciniphila* in the UK, the novel food seeking authorisation (pasteurised *Akkermansia muciniphila*) does not have a recorded history of consumption within the UK.

42. It was noted that pasteurised *Akkermansia muciniphila* was authorised in the EU in 2022. While not providing a history of consumption in the UK it provides some evidence of potential areas for consideration in the safety assessment of the novel food when used in a European population.

2.7 Proposed Use, Use Levels, and Anticipated Intake

43. Pasteurised *Akkermansia muciniphila* is intended for use in food supplements at up to $4x10^{10}$ cells per day, and in Foods for Special Medical Purposes (FSMPs) at a use level which is to be determined on an individual basis but no higher than the upper limit for food supplements. This novel food is not intended to replace any other food within the diet.

44. The target population for pasteurised *Akkermansia muciniphila* is adults (defined as 12 years old and above by the applicant), excluding sensitive populations (infants, young children, and pregnant or lactating women). The applicant states that products containing the novel food will be labelled accordingly to reflect these restrictions.

45. Following review, queries were raised on the choice of target population and whether any potential safety concerns had been identified for those under 18 who are still developing. No specific risks for the 12- to 17-year-olds were identified on the grounds that the organism is not absorbed and does not produce substances that can influence physiology. It was noted that there was no risk of colonisation by the source organism which could alter the gut microbiome.

46. The Committee noted that calculation of the combined exposure to pasteurised *Akkermansia muciniphila* from a range of sources was not possible as there is no data on the level of *Akkermansia* currently consumed from other food sources. However, as there are no other known, similar products on the market in the UK, over consumption was consider less likely on the basis that there is no

evidence that it is a significant part of the diet.

2.8 Absorption, Distribution, Metabolism and Excretion (ADME)

47. No ADME studies have been carried out on pasteurised *Akkermansia muciniphila*. However, it is not likely that the pasteurised cells of the microorganism are absorbed systemically as they are expected to be excreted in the faeces. This route of excretion and lack of absorption is explored within the publicly available literature; the conclusions regarding the ADME are based on the detection of *Akkermansia muciniphila* cells in faecal samples following oral ingestion. No significant changes are expected to occur in the human gut microbiome following consumption. This is evidenced through a clinical study involving pasteurised *Akkermansia muciniphila*.

48. Pasteurised *Akkermansia muciniphila* is therefore not expected to be absorbed systemically and no concerns are raised regarding the absorption, distribution, metabolism, and excretion of the novel food. The information presented did not indicate any areas of concern.

2.9 Nutritional Information

49. A summary of the proximate analysis of Pasteurised *Akkermansia muciniphila* can be found above within Table 2. While not expected to provide a significant contribution to the nutritional quality of the diet, consumption of pasteurised *Akkermansia muciniphila* is not nutritionally disadvantageous under the proposed conditions of use. It does not contain any antinutritional factors or influence nutrient intake.

50. Consumption of *Akkermansia muciniphila* has been shown to have no significant effect on the microbiota and abundance of other bacteria in the gut of rats. No specific concerns are raised regarding the nutritional information. The product was not considered nutritionally disadvantageous on the grounds that it would not replace other foods in the diet.

2.10 Toxicological Information

2.10.1 Genotoxicity

51. An *in vitro* bacterial reverse mutation assay (Brient, 2019a [unpublished]) and *in vitro* mammalian cell micronucleus test (Brient 2019b [unpublished]) were

performed on pasteurised *Akkermansia muciniphila*, following OECD 471 and OECD 487 guidelines, respectively, and the principles of GLP. These studies indicate that pasteurised *Akkermansia muciniphila* has no mutagenic, clastogenic or aneugenic effects and therefore the novel food is concluded to be non-genotoxic.

2.10.2 Subchronic Toxicology

52. A two-part preliminary 14-day dose rage-finding study (Bracken, 2019a [unpublished]) in Crl:wI(Han) rats was performed to evaluate potential short-term toxicity of the novel food and to select dose levels for use in the 90-day study. Part one of the study initially tested a dose of 1,500 mg/kg bw/day ($9.6x10^{10}$ cells/kg bodyweight/day) delivered by oral gavage in phosphate buffer saline once a day for 3 days. The second part of the study tested doses of either 0 mg/kg, 1125 mg/kg or 1500 mg/kg bw/day for 14 days. No adverse clinical effects relating to the novel food and no deaths were observed during the study.

53. A 90-day repeat dose toxicity study (Bracken MK, 2019b [unpublished]) was conducted to evaluate the sub-chronic toxicity of the novel food. Pasteurised *Akkermansia muciniphila* was administered to Crl:WI(Han) rats in sterile phosphate buffer solution via oral gavage once daily for 90 days. Control groups were dosed with the dosing vehicle only. Groups of 10 male and 10 female rats received by oral gavage 0 (control), 75, 375 or 1,500 mg/kg bw/day of the novel food for at least 90 days. The dosages corresponded to 0, 4.8×10^9, 2.4×10^{10} and 9.6×10^{10} cells (measured as TFU)/kg bw per day. The viable *Akkermansia muciniphila* cell count of the batch of the novel food as tested was 50 CFU/g of the test item. The study was conducted according to OECD 408 guideline and the principles of GLP.

54. No novel food related changes, adverse effects, deaths, or clinical signs were observed during the study. Some statistically significant, dose-related increases in absolute neutrophil count and total white blood cell count were observed in the high-dose group, although these changes were within the range of the historical control data and were therefore considered to be a result of natural biological variation. Statistically significant reductions in relative eosinophils for low- and mid-dose males were not considered to be related to the test item, as the differences were not observed in the high-dose group and therefore not dose dependent. The NOAEL was determined to be 1,500 mg/kg body weight per day ($9.6x10^{10}$ cells/kg body weight per day), the highest dose tested

55. A literature review on the toxicity of the *Akkermansia muciniphila* was carried out. The studies identified were consistent with the toxicological profile identified in the 90-day study. As such, no toxicological concerns were identified.

2.10.3 Human studies

56. A randomised, double-blind, placebo-controlled clinical trial (Depommier, 2019) has been conducted using the pasteurised *Akkermansia muciniphila* concerned in this application. The trial consisted of 32 male and female, clinically overweight or obese subjects that were split into groups, each of whom received either live or pasteurised *Akkermansia muciniphila* once daily for 12 weeks, at a dose level of $1x10^{10}$ cells/day. No adverse effects were observed across all measured parameters within the 12-week study.

2.10.4 Toxicology Summary

57. The Committee considered that the toxicological data did not identify risks to be managed. A NOAEL was identified at 1500 mg/kg/day (equivalent to $9.6x10^{10}$ cells/kg body weight per day) from the sub-chronic toxicology study. If the standard uncertainty factors of 200 (10 (interspecies variability) x 10 (intraspecies variability) x 2 (sub-chronic to chronic study duration), the safe level identified of 4.8×10^{8} cells/kg bw per day which is equivalent to 3.4×10^{10} cells/day for a 70 kg adult.

58. It was noted that a sub-chronic study was unlikely to capture wider effects that may be adverse given the novel food is a whole organism. However, when taking into consideration the human studies provided, the Committee noted that they confirm the results from the 90-day sub-chronic study. As such the human studies which indicated the novel food was well tolerated were considered a more appropriate basis for establishing the toxicological safety of the novel food.

2.11 Allergenicity

59. The novel food is comprised of 30% protein. However, there have been no reports of allergic reactions associated with pasteurised *Akkermansia muciniphila* within the literature and there were no reports during the 12-week human safety trials. As *Akkermansia muciniphila* is found endogenously within the human gut microbiome, the risk of allergic reactions occurring from consumption is therefore considered to be low.

60. It was noted that there was some carryover from the culture medium in the final product. Queries were raised with the applicant on whether any major allergens were present. It was confirmed that they were not. Therefore, based on this information and information on the organism the novel food is not expected to elicit allergic reactions.

3. Discussion

61. The application is for the authorisation of pasteurised *Akkermansia muciniphila* as an ingredient in food supplements at up to $4x10^{10}$ cells per day, and in Foods for Special Medical Purposes (FSMPs) at a use level which is to be determined on an individual basis but would not exceed that in food supplements.

62. Pasteurised *Akkermansia muciniphila* is a freeze-dried powder comprised of heat-inactivated whole-cells of the bacterium *Akkermansia muciniphila*. Less than 10 CFU per gram remain viable in the final novel food product. *Akkermansia muciniphila* is a naturally occurring/non-genetically modified gut bacterium which has been well-characterised within the literature and through several molecular analyses.

63. The strain of *Akkermansia muciniphila* used has been deposited in two culture banks and its identity is verified through implementation of full genome sequencing and 16S rRNA sequencing. Analysis of the organism for antimicrobial resistance, virulence, and pathogenicity has also been conducted. No safety concerns are raised regarding the identity of the source organism.

64. The production process for pasteurised *Akkermansia muciniphila* is well described by the applicant and management of the processes and controls are sufficiently protective of safety. The source of the novel food is the bacterium *Akkermansia muciniphila*. The working cell culture is propagated before the microbial cells are heat-inactivated through a pasteurisation process. The pasteurised cells are then concentrated and mixed with cryoprotectants prior to freeze-drying and mixing with a food grade excipient. The final cell concentration is stated to be at least $2.5x10^{10}$ non-viable bacterial cells per gram of powdered final product.

65. The compositional analyses of the product demonstrate that production is consistent and that the novel food falls within the specifications set out in the application. Microbiological analysis demonstrates the absence of potentially harmful microbes which are of significance to food safety, as well as demonstrating that the cell concentrations meet the required minimum

specification for the finished product.

66. The novel food is intended for use by the general population above 12 years of age, excluding pregnant and lactating women. Other sensitive populations have been excluded from the target consumer groups. The proposed daily dose of the novel food is below the NOAEL identified in the subchronic studies and below the tolerable dose identified in the human studies and is therefore considered to be appropriate.

67. Consumption of the novel food is also not expected to be nutritionally disadvantageous, as the novel food is not intended to provide significant nutrition or replace any other food within the diet.

68. The potential for allergic reactions from the novel food was explored. The novel food itself was considered to pose a low risk of eliciting allergic reactions. The risk from carryover of culture media in the production process was considered by the Committee and further information was sought. The risk of allergic reactions occurring as a result of media carry over was determined to be low as the final product does not contain any of the major allergens as defined in Annex II of assimilated Regulation 1169/2011 EU.

4. Conclusions

69. The ACNFP have undertaken the assessment of pasteurised Akkermansia muciniphila and concluded that the novel food is safe under the proposed conditions of use and does not pose a safety risk to human health. The anticipated intake levels and the proposed use was not considered to be nutritionally disadvantageous.

70. These conclusions were based on the information in the novel food dossier submitted by the applicant plus the supplementary information and could not have been reached without the following data claimed as proprietary by the applicant:

- Bacterial reverse mutation test Brient (2019a) [unpublished]
- In vitro mammalian cell micronucleus test Brient (2019b) [unpublished]
- Preliminary and 14-day dose range-finding toxicity study in rats Bracken (2019a) [unpublished]
- 90-Day toxicity study in rats Bracken (2019b) [unpublished]
- Method validation for 90-day study dose formulation analysis Jensen (2019) [unpublished]
- Antimicrobial resistance study Gueimonde (2019) [unpublished]

71. With thanks to the members of the ACNFP during the course of the assessment who were; Dr Camilla Alexander White, Dr Anton Alldrick, Ms Alison Austin, Dr Mark Berry, Professor George Bassel, Dr Christine Bosch, Professor Dimitris Charalampopoulos, Dr Meera Cush, Dr Cathrina Edwards, Professor Susan Fairweather-Tait, Dr Sophie Foley, Professor Paul Fraser, Dr Hamid Ghoddusi, Dr Andy Greenfield, Professor Wendy Harwood, Professor Huw D. Jones, Dr Kimon-Andreas Karatzas, Dr Ray Kemp, Dr Elizabeth Lund, Professor Harry J. McArdle, Rebecca McKenzie, Dr Lynn McIntyre, Professor Clare Mills, Dr Antonio Peña-Fernández, Dr Isabel Skypala, Dr Lesley Stanley, Professor Hans Verhagen, Dr Maureen Wakefield, and Professor Bruce Whitelaw.

References

Bracken MK, 2019b [unpublished]. Dated: 25 September 2019. Prepared by Citoxlab Scantox A/S, Lille Skensved, Denmark for A-Mansia Biotech S.A., Louvain-la-Neuve, Belgium. Study Title: Pasteurized Akkermansia muciniphila: 90-day oral (gavage) toxicity study in the rat: final report. Confidential. (Study No: 78367).

EFSA NDA Panel (EFSA Panel on Nutrition and Novel Foods), 2016. Guidance on the Preparation and Presentation of an Application for Authorisation of a Novel Food in the Context of Regulation (EU) 2015/2283. EFSA Journal 2016, 14(11): 4594. https://doi.org/10.2903/j.efsa.2016.4594. (opens in a new window)

EU, 2017. Commission Implementing Regulation (EU) 2017/2469 of 20 December 2017 laying down administrative and scientific requirements for applications referred to in Article 10 of Regulation (EU) 2015/2283 of the European Parliament and of the Council on novel foods. COMMISSION IMPLEMENTING REGULATION (EU) 2017/2469

Abbreviations

ACNFP	Advisory Committee on Novel Foods and Processes
ADME	Adsorption, Distribution, Metabolism, and Excretion
ATCC	The American Type Culture Collection
BLAST	Basic Local Alignment Search Tool

Bw	Body Weight
CAD	Committee Advice Document
CARD	Comprehensive Antibiotic Resistance Database
CCP	Critical Control Point
CIP	Collection de l’institut Pasteur
CFU	Colony Forming Unit
Crl:wl(Han)	Wistar Han IGS Rat
EFSA	European Food Safety Authority
EN	European Standard
EU	European Union
FSA	Food Standards Agency
FSMPs	Foods For Special Medical Purposes
FSS	Food Standards Scotland
g	Grams
GB	Great Britain
GLP	Good Laboratory Practice

HACCP	Hazard Analysis and Critical Control Points
ISO	International Organisation for Standardisation
kg	Kilograms
Ltd	Limited Company
mg	Milligrams
MGE	Mobile Genetic Elements
NCBI	National Centre for Biotechnology Information
NDARO	National Database of Antibiotic Resistant Organisms
NF	Norme Française
NOAEL	No Observed Adverse Effect Level
OECD	Organisation for Economic Co-operation and Development
PAH	Polycyclic Aromatic Hydrocarbon
rRNA	Ribosomal Ribonucleic AcidPATRIC Pathosystems Resource Integration Centre
TFU	Total Fluorescent Unit
UK	United Kingdom

VTEC	Verocytotoxin Subtype *Escherichia Coli*
µM	Micron