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Safety evaluation of long-chain glycolipids from *Dacryopinax spathularia*

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Abstract

The EFSA Panel on Food Additives and Flavourings (FAF) provides a scientific opinion on the safety of long-chain glycolipids from *Dacryopinax spathularia* (also called AM-1) as a food additive. AM-1 is a purified mixture of long-chain glycolipid congeners obtained by fermentation of the edible non-genetically modified fungus *Dacryopinax spathularia*. AM-1 glycolipids have very low oral bioavailability and overall available toxicology data do not demonstrate any adverse effects of the proposed food additive. Considering the available data set the Panel established an ADI of 10 mg/kg bw per day based on a range of NOAELs between 1,000 and 1,423 mg/kg bw per day (the highest doses tested), from the reproductive and a prenatal developmental toxicity studies in rats and 90-day studies in rat and dog. At the proposed maximum use levels, the exposure estimates ranged at the mean from 0.01 to 1.07 mg/kg bw per day and at the p95 from 0 to 3.1 mg/kg mg/kg bw per day. At the proposed typical use levels, the exposure estimates ranged at the mean from < 0.01 mg/kg bw per day to 0.23 mg/kg bw per day and at the p95 from 0 to 0.64 mg/kg bw per day. The Panel noted that the highest estimate of exposure of 3.1 mg/kg bw per day (in toddlers) is within the established ADI of 10 mg/kg bw per day and concluded that the exposure to long-chain glycolipids from *Dacryopinax spathularia* does not raise a safety concern at the uses and use levels proposed by the applicant.

Keywords: long-chain glycolipids, *Dacryopinax spathularia*, AM-1, food additive

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Summary

Following a request from the European Commission to the European Food Safety Authority (EFSA), the Panel on Food Additives and Flavourings (FAF) was asked to provide a scientific opinion on the safety of long-chain glycolipids from *Dacryopinax spathularia* proposed as a preservative food additive in certain beverages, in accordance with Regulation (EC) No 1331/2008 establishing a common authorisation procedure for food additives, food enzymes and food flavourings.

The present evaluation is based on the data on long-chain glycolipids from *Dacryopinax spathularia* in a newly submitted dossier by the applicant and additional information submitted by the applicant during the assessment process in response to a request by EFSA.

The proposed food additive is a purified mixture of long-chain glycolipid congeners obtained by fermentation of the edible basidiomycetes *Dacryopinax spathularia* using glucose and autolyzed yeast extract as feedstock (called AM-1).

The core structure of the AM-1 glycolipids is a hydroxylated long-chain fatty acid (C-26) with a glucopyranosyl-(1→2)-xylopyranosyl-(1→2)-xylopyranosyl trisaccharide moiety which is attached via glycosidic bond to the ω-hydroxy position. The glycolipids differ in the number of hydroxyl groups of the fatty acid backbone and their stereo- and regiochemistry as well as in the acylation pattern of the sugar unit, consisting mainly of acetyl and isovaleryl units.

The AM-1 glycolipid is produced by fermentation with *Dacryopinax spathularia* MUCL 53181 in high glucose medium containing also an extract of *Saccharomyces cerevisiae*. The production strain for the AM-1 glycolipid mixture is the non-genetically modified fungus *Dacryopinax spathularia* MUCL 53181.

Since no production organism remained in the final product, this manufacturing process does not raise a safety concern.

AM-1 glycolipids have very low oral bioavailability, rapid elimination and are mainly excreted in the faeces.

The toxicology data set comprised studies on acute toxicity, sub-chronic toxicity, genotoxicity and reproductive and development toxicity. Overall, available toxicology data did not demonstrate any adverse effects of the proposed food additive.

Considering the available data set, the Panel established an ADI of 10 mg/kg bw per day based on a range of NOAELs between 1,000 and 1,423 mg/kg bw per day (the highest doses tested), from reproductive and a prenatal developmental toxicity studies in rats and 90-day studies in rat and dog.

To assess the dietary exposure to long-chain glycolipids from *Dacryopinax spathularia*, the exposure was calculated based on the proposed maximum and typical use levels. At the proposed maximum use levels, the exposure estimates ranged at the mean from 0.01 to 1.07 mg/kg bw per day and at the p95 from 0 to 3.1 mg/kg mg/kg bw per day. At the proposed typical use levels, the exposure estimates ranged at the mean from < 0.01 mg/kg bw per day to 0.23 mg/kg bw per day and at the p95 from 0 to 0.64 mg/kg bw per day.

The Panel noted that the estimated long-term exposures are very likely conservative, as the exposure estimates assumed that all drinks belonging to the FC 14.1.4 Flavoured drinks, FC 14.1.5.2 Other and FC 14.2.1 Beer and malt beverages, restricted to "Only alcohol free" will contain long-chain glycolipids from *Dacryopinax spathularia*.

The Panel noted that the highest estimate of exposure of 3.1 mg/kg bw per day (in toddlers) is within the established ADI of 10 mg/kg bw per day.

Based on the toxicological database available, the Panel derived an ADI of 10 mg/kg bw per day for long-chain glycolipids from *Dacryopinax spathularia* and concluded that the exposure to long-chain glycolipids from *Dacryopinax spathularia* does not raise a safety concern at the uses and use levels proposed by the applicant.

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1. Introduction

The present scientific opinion deals with the safety evaluation of long-chain glycolipids from *Dacryopinax spathularia* proposed as a preservative food additive in certain beverages.

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

1.1.1. Background

The use of food additives is regulated under the European Parliament and Council Regulation (EC) No 1333/2008 on food additives.[1] Only food additives that are included in the Union list, in particular Annex II to that regulation, may be placed on the market and used in foods under conditions of use specification therein. Moreover, food additives shall comply with the specifications as referred to in Article 14 of that Regulation and laid down in Commission Regulation (EU) No 231/2012[2].

An application has been introduced for the authorisation of the use of long-chain glycolipids from *Dacryopinax spathularia* as a preservative in certain beverages. The request concerns in particular the food category 14.1.4 'Flavoured drinks', 14.1.5.2 'Others' and 14.2.1 'Beer and malt beverages' of Annex II to the Regulation (EC) No 1333/2008.

According to the applicant, long-chain glycolipids from *Dacryopinax spathularia* intended for use as a preservative, prolong the shelf-life by preventing growth of microorganisms (yeasts and moulds) and subsequent spoilage of beverages. They can complement or alternate with other preservation methods and also substitute currently authorised preservatives in beverages.

1.1.2. Terms of Reference

The European Commission request the European Food Safety Authority to perform a risk assessment and to provide a scientific opinion on the safety of the proposed use of long-chain glycolipids from *Dacryopinax spathularia* as a food additive, in accordance with Regulation (EC) No 1331/2008 establishing a common authorisation procedure for food additives, food enzymes and flavourings.[3]

1.2. Information on existing evaluations and authorisations

The long-chain glycolipids from *Dacryopinax spathularia*, named AM-1, is listed in the "*Generally-Recognised-As-Safe*" (GRAS) notice inventory for the intended condition of use (i.e. as a preservative in non-alcoholic beverages). In the GRAS notice, based on the toxicological data provided by the notifier, an Acceptable Daily Intake (ADI) of $\geq$ 10 mg/kg/bw for AM-1 is indicated (FDA, 2018).

2. Data and methodologies

2.1. Data

The applicant has submitted a dossier to support the safety evaluation of the present application on long-chain glycolipids from *Dacryopinax spathularia* proposed as a preservative food additive in certain beverages (Documentation provided to EFSA No. 1).

Following the request for additional data sent by EFSA on 23 September 2020, the applicant requested a clarification teleconference held on 21 October 2020, after which the applicant provided additional data on 22 December 2020 (Documentation provided to EFSA No. 2).

Following the request for additional data sent by EFSA on 24 February 2021 the applicant provided additional data on 25 February 2021 (Documentation provided to EFSA No. 3).

2.2. Methodologies

This opinion was formulated following the principles described in the EFSA Guidance of the Scientific Committee on transparency with regard to scientific aspects of risk assessment (EFSA

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

[2] Commission 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.

[3] Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December establishing a common authorisation procedure for food additives, food eznymes and food flavourings. OJ L 354, 31.12.2008.

Scientific Committee, 2009) and following the relevant existing Guidance documents from the EFSA Scientific Committee. The current '*Guidance for submission for food additive evaluation*' (EFSA ANS Panel, 2012) has been followed by the FAF Panel for evaluating the present application.

3. Assessment

3.1. Technical data

3.1.1. Identity of the proposed food additive

The proposed food additive, called AM-1, is a purified mixture of long-chain glycolipid congeners obtained by fermentation of the edible basidiomycete *Dacryopinax spathularia* using glucose and autolyzed yeast extract as feedstock. AM-1 has a total glycolipid content of ≥ 93%, expressed on a dry weight basis and as glycolipid sodium salts, determined according to a method which involves hydrolysis of AM-1, quantification of glucose and xylose by GC-MS, and back-calculation of total glycolipid content in the AM-1 sample based on the average molecular weight obtained from LC-MS analysis (Documentation provided to EFSA No. 2). The remaining 7% of the dry weight is comprised of; up to 3% residual protein (determined by Kjeldahl method); up to 2% total fat (by gravimetric determination) and 1.7–3.3% sodium (by atomic absorption spectroscopy determination).

Based on the information provided by the applicant, the core structure of the AM-1 glycolipids is a hydroxylated long-chain fatty acid (C-26) with a glucopyranosyl-(1→2)-xylopyranosyl-(1→2)-xylopyranosyl trisaccharide moiety which is attached via glycosidic bond to the ω-hydroxy position. The glycolipids differ in the number of hydroxyl groups of the fatty acid backbone and their stereo- and regiochemistry as well as in the acylation pattern of the sugar unit, consisting mainly of acetyl and isovaleryl units. 3-hydroxy-3-methylglutarate (HMG) can also be an acyl unit instead of isovalerate but additionally to the acetate (Documentation provided to EFSA No. 1). The molecular structures of the glycolipid congeners of AM-1 mixture and its composition have been elucidated by 1D and 2D nuclear magnetic resonance spectroscopy (NMR) and by high performance liquid chromatography-mass spectrometry (HPLC-MS), respectively. The applicant indicated that the spectroscopic and chromatographic data are available in the European patent application EP 2717691 and some were provided within the technical dossier submitted to EFSA. The chemical structures and names of the components of AM-1 are shown in Appendix A (Documentation provided to EFSA No. 2).

According to the applicant, in AM-1 there are three main structurally related glycolipid congeners, outlined in Figure 1.

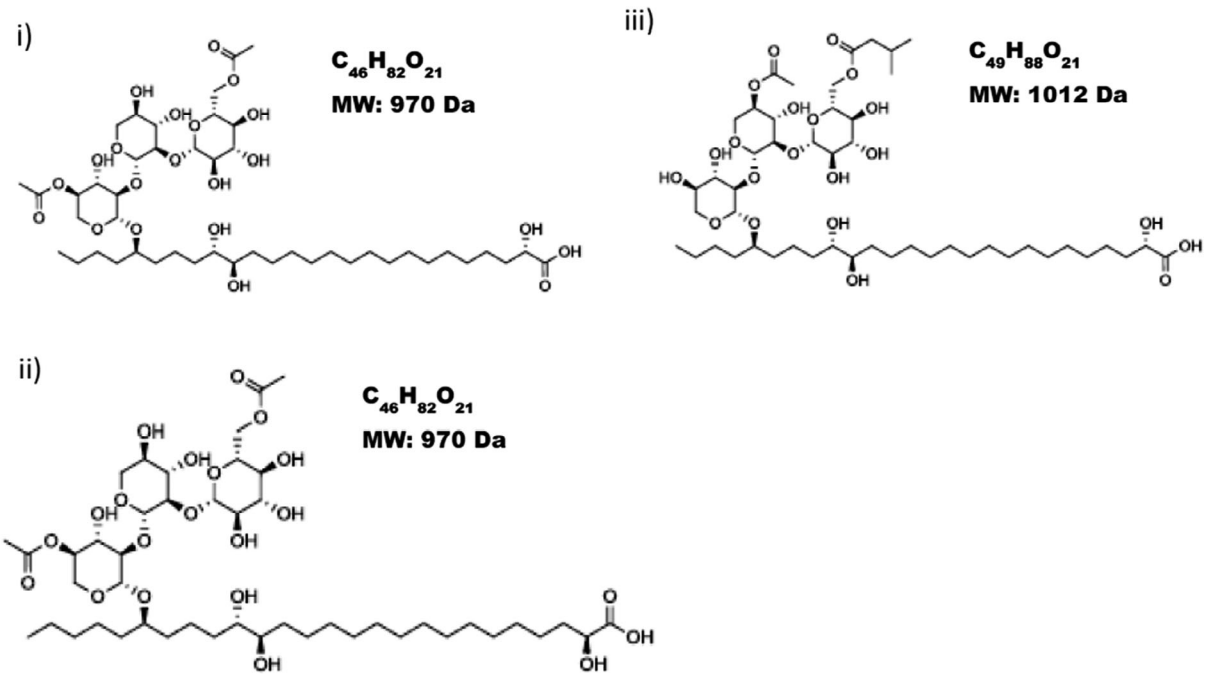

Figure 1: Chemical structures of the three main glycolipid components of AM-1

The applicant reported that to ensure that the proposed food additive possesses adequate and reliable product and quality properties, the glycolipid composition must be within certain ranges (see Table 1), set as threshold values by the applicant, these being minimum/maximum compositional limit values. To this end, an "*AM-1 pattern analysis*" is performed following an HPLC-MS method for the determination and normalisation of the glycolipid composition in AM-1 mixtures (Documentation provided to EFSA No. 2). For analysis, the glycolipids are divided into eight groups based on their molecular weights, MW = 886, 928, 954, 970, 1,012, 1,054, 1,072, 1,114 Da, and for each glycolipid group, the extracted ion chromatograms (EICs) are acquired and their relative total peak areas integrated. The percentage of each glycolipid group is calculated and then normalized by referencing to the reference batch. The normalised AM-1 glycolipid profile (called the '*pattern*' by the applicant) is presented in Table 1.

Table 1: Normalized glycolipid profile determined by HPLC-MS, as proposed by the applicant

MW [Da]	886	928	954	970	1,012	1,054	1,072	1,114
Threshold	Sum < 10%		< 20%	> 26%	> 20%	< 25%	Sum < 17%	

Calculation of the percentage of each glycolipid group (as expressed by the applicant):

[(normalized peak area of glycolipid group) × 100/(sum of all normalized peak areas)]

Glycolipids within MW group 886 and 928 Da are hydrolysis products, i.e. MW group 886 Da glycolipids have no acyl groups (non-acylated glycolipids) whereas MW group 928 Da glycolipids have one acetyl group left (mono-acetylated glycolipids). Glycolipids within the highest MW groups, i.e. MW 1,072 and 1,114 Da, are those congeners that are characterized by the presence of HMG as acyl groups.

3.1.2. Proposed specifications

The AM-1 specifications, as proposed by the applicant, are outlined in Table 2. In the brackets is given the related method of analysis, when applicable.

Table 2: Proposed specifications for long-chain glycolipids from *Dacryopinax spathularia* (AM-1)

Chemical name	Glycolipids from *Dacryopinax spathularia*
Trade name:	AM-1; Nagardo
CAS number	2205009-17-0
Definition	The naturally occurring glycolipids are obtained by bioconversion of glucose using a wild type strain of the edible sweet osmanthus ear mushroom (*Dacryopinax spathularia*). The solvent-free downstream process includes filtration and microfiltration to remove microbial cells, precipitation and washing with buffered water to purify. The product is pasteurized and spray-dried. The production process does not chemically modify the glycolipids or change their natural composition. The final product is a beige to light brown powder with at least 93% total glycolipid content (w/w based on dry weight).
Appearance (visual and olfactory assessment)	Beige to light brown powder; odor: weak, characteristic
Solubility (shake-flask at fixed concentration 10 g/L)	Complies
Turbidity (10 g/L in water, turbidity meter)	< 28 NTU
pH value (10 g/L in water, pH meter)	5.0–7.0
Water (Karl Fischer)	< 5% (w/w)

Total protein (Kjeldahl, N × 6.25)	< 3% (w/w)								
Total fat (Gravimetric)	< 2% (w/w)								
Sodium (AAS)	< 1.7–3.3% (w/w)								
Total glycolipids (GC-MS)	≥ 93% (w/w)								
Identity (HPLC-MS)	Having the same glycolipids (identified by molecular weight) in a similar ratio (quantified by peak areas). Integration of extracted ion chromatograms for eight groups of glycolipids identified by their molecular weight provides the relative composition, which must comply with the following thresholds:								
	MW [Da]	**886**	**928**	**954**	**970**	**1,012**	**1,054**	**1,072**	**1,114**
	Threshold	Sum < 10%		< 20%	> 26%	> 20%	< 25%	Sum < 17%	
Arsenic (ICP-MS)	≤ 1.00 mg/kg								
Cadmium (ICP-MS)	≤ 1.00 mg/kg								
Mercury (ICP-MS)	≤ 1.00 mg/kg								
Nickel (ICP-MS)	≤ 2.00 mg/kg								
Lead (ICP-MS)	≤ 2.00 mg/kg								
TAMC[(a)] (microbial enumeration)	≤ 100 CFU/g								
TYMC[(b)] (microbial enumeration)	≤ 10 CFU/g								
Salmonella spec. (microbial enumeration)	Absent in 25 g								

(a): Total aerobic microbial count.
(b): Total yeast/mould count.

The Panel noted that the glycolipids are obtained by bioconversion of glucose using a wild type strain of the edible sweet osmanthus ear mushroom (*Dacryopinax spathularia*) and its strain deposition number, i.e. MUCL 53181, should be included in the definition of the proposed food additive. In addition, the Panel observed that in the proposed definition "*to remove microbial cells* "can be omitted.

The Panel noted that the applicant provided analytical data on AM-1 glycolipids composition (EICs, peak areas and glycolipid patterns) for a number of batches (33), analysed by the HPLC-MS method mentioned in section 3.1.1. The glycolipid profile conformed with specifications of the food additive proposed by the applicant (see Table 2). With regard to the other proposed purity provisions for AM-1, i.e. content of total glycolipids, water, total protein, total fat and sodium, analytical results of five non-consecutive batches of AM-1 showed good agreement with the proposed specifications in Table 2.

The Panel noted that the provision on total content on glycolipids is sufficient to characterize the identity of the food additive and considered that the glycolipids profile is not necessary.

The Panel noted that glycolipid congeners within MW groups of 1072 and 1114 Da are acylated with HMG. HMG is a component of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA), which is an intermediate in primary metabolism; in particular, in the mevalonate and ketogenesis pathways as well as leucine degradation. According to the applicant, HMG could be considered as a naturally occurring constituent of human physiology. Commercial batches produced in 2019 and 2020 had an average content (w/w) of HMG-containing glycolipids of 9.3% and 6.4%, contributing for ca. 1.4% and 0.96% of HMG if fully released, respectively (Documentation provided to EFSA No.2). The Panel noted that the toxicological dataset for AM-1 (see section 3.4) supports the safety of HMG as a potential hydrolysis product, since the glycolipids contained congeners acylated with HMG. The Panel also noted that (Q)SAR analysis using the OECD Toolbox (4.2) did not reveal any structural alert for genotoxicity for HMG.

Regarding toxic elements, the applicant provided analytical data developed by Inductively Coupled Plasma Mass Spectrometry (ICP-MS), according to USP 233 method, on five non-consecutive batches

of AM-1 for levels of arsenic (As), cadmium (Cd), lead (Pb), mercury (Hg) and nickel (Ni). As ranged from 0.25 mg/kg to 0.67 mg/kg, Cd and Hg were found at 0.01 mg/kg only in two batches, Pb ranged from 0.10 mg/kg to 0.35 mg/kg, Ni ranged from 0.68 mg/kg to 1.84 mg/kg. The applicant stated that the proposed maximum limits (MLs) for such toxic elements (As ≤ 1 mg/kg, Cd ≤ 1 mg/kg, Hg ≤ 1 mg/kg, Ni ≤ 2 mg/kg and Pb ≤ 2 mg/kg, see Table 2) have been established based on the lowest technologically achievable limits and that they are supported by the data obtained during piloting and scale-up of AM-1 production. Particularly, the applicant indicated that nickel and lead MLs are specified at 2 mg/kg because AM-1 production is carried out in stainless steel bioreactors and traces of these elements are therefore unavoidable. The Panel noted that the limits proposed for Cd and Hg are high at 1.0 mg/kg given that they are both 100-times the highest values found for the 5 batches. The anticipated impact of these proposed specifications on the potential exposure to these elements is described in section 3.3.2 (Table 6). The Panel noted that there was no need to indicate the analytical methods for toxic elements (ICP-MS) and for sodium (AAS) in the proposed specifications.

Given that the proposed food additive is produced from a fungal organism (*Dacryopinax spathularia*), the presence of certain mycotoxins (aflatoxins, trichothecenes, ochratoxin, zearalenone, fumonisin B1 & B2, and deoxynivalenol) has been examined. None of the four batches analysed contained these mycotoxins at levels above their corresponding LOQs. The Panel noted that crude, freeze-dried fermentation AM-1 broth was tested rather than the purified final product. According to the applicant, this was done in order to avoid mycotoxins (if present) being washed out or their content being reduced in the course of the downstream manufacturing process. The Panel noted that the genome of the production organism *Dacryopinax spathularia* MUCL 53181 was sequenced and subjected to bioinformatic analysis. A total of 11 putative biosynthetic gene clusters for secondary metabolite synthesis were identified; none of the potential secondary metabolites shared any structural similarity to any known mycotoxin or virulence factor (in agreement with absence of any reports about mycotoxins in the taxonomic family (*Dacrymycetaceae*) of the basidiomycete producer strain in the scientific literature). The applicant considered that *Dacryopinax spathularia* does not produce mycotoxins or virulence factors and therefore that there is no need to set an EU specification for the presence of mycotoxins. The Panel concurs with this view.

The microbiological quality of the proposed food additive was confirmed by microbiological analyses of four non-consecutive commercial batches of AM-1 (all produced in 2019); for each of these batches, three individual samples were taken and analysed at an accredited laboratory. The results are in compliance with the proposed specifications (aerobic plate count < 10 cfu/g; moulds < 1 cfu/g; yeast < 1 cfu/g; coliforms < 1 cfu/g; *Escherichia coli* < 1 cfu/g; coagulase positive *Staphylococcus aureus* not detected/g; *Salmonella* species not detected/25 g; *Listeria* species and *L. monocytogenes* not detected/25 g; *Enterobacteriaceae* species not detected/1 g). The Panel considered that a maximum limit for *Enterobacteriaceae* (< 10 cfu/g) should be included in the specification as an indicator of good hygiene practise.

The proposed specification for solubility is described as "*complies*" and the method indicated is "*shake flask at fixed concentration 10 g/L*". The Panel noted that the shake-flask method formally involves adding an excess of solid to the solubility medium in the shaken flask, followed by analysis of the saturated solution to determine the solubility, and that procedure was not followed. Nevertheless, the solubility of the proposed food additive was studied using a visual assessment (see details of the determination in section 3.1.4) which is acceptable.

3.1.3. Manufacturing process

The applicant stated that the AM-1 manufacturing process uses certified food-grade materials.

AM-1 glycolipid is produced by fermentation of *Dacryopinax spathularia* MUCL 53181 in high glucose medium containing also an extract of *Saccharomyces cerevisiae*. The production is conducted in 2 stages: 1) production of the strain culture and fermentation; 2) isolation and purification, where the fermented broth is microfiltered to remove fungal cells, the glycolipids are precipitated by acids, washed and neutralized by sodium hydroxide solution and spray or freeze dried.

The absence of the production organism was confirmed in four independent batches, each sampled in triplicate. No colonies were observed by culturing of 1 g of each of the samples in non-selective medium at 28°C for 10 days.

3.1.3.1. Characterisation of the production organism

The production strain for the AM-1 glycolipid mixture is a non-genetically modified fungus *Dacryopinax spathularia* MUCL 53181. Taxonomic identification of *Dacryopinax spathularia* MUCL 53181 was performed by morphological characterization and by DNA sequencing of the 5.8S/ITS and 28S nuclear ribosomal DNA. These DNA sequences were identical to those in the *D. spathularia* strain CBS 197.63, which was obtained from the Centralbureau voor Schimmelcultures (CBS), Utrecht, The Netherlands.

D. spathularia MUCL 53181 has been deposited at the BCCM/MUCL culture collection (Belgium).

D. spathularia produces edible yellowish fruiting bodies, is known as an edible food (as documented for consumption in China), listed as edible in the 'Global list of wild fungi used as food' (Boa, 2004, Annex 3), India (Ao et al., 2016), Malaysia (Lee et al., 2009) and South Cameroon (Van Dijk, 2003). Recipes are available in Meuninck (2017).

The absence of antibiotics (fusidic acid $<$ 1 mg/L; penicillin G 1 mg/L; pleuromutilin 1 mg/L; tyromycin B 1 mg/L) was confirmed in 2 fermentation samples, in 4 batches of the end product produced on a pilot scale and in 4 batches of the end product produced on an industrial scale. Fermentation samples were freeze-dried and dissolved in water-acetonitrile (1:1 v/v) at 10.0 g/L and 1.0 g/L. The proposed food additive was also dissolved at 10.0 g/L and 1.0 g/L. HPLC-MS/UV/ELSD based analysis was used for the detection of antibiotics, with a LoD in the samples of $\leq$ 100 mg/kg (0.1 mg/g) or $\leq$ 0.01% of dry weight. The 4 named antibiotics were not detected in any of the samples.

3.1.4. Solubility and particle size

Water solubility

The glycolipids constituting the proposed food additive are biosurfactants. Therefore, the Panel agreed with the view of the applicant that the test for solubility in water based on the determination of the mass saturation concentration, as foreseen in the Technical Guideline 105 (OECD, 1995), is not applicable. As an alternative, the applicant determined the solubility in water at a fixed concentration of 10 g/L, i.e. 200 times higher than the proposed maximum use level in beverages. At this concentration, the water solubility of the AM-1 powder was confirmed visually and by measurement in a turbidity meter.

Particle size

Information about particle size distribution in six samples of the proposed food additive, measured by means of a laser diffraction analysis (LDA), demonstrated that 90% of the particles had an average diameter higher or equal than 14.9 μm. The smaller measured size in the powder was above 100 nm. Scanning electron microscopy (SEM) was used to examine the shape of the particles, the presence of aggregates and the dimensions of the smaller single particles. The observations revealed that the substance consists essentially of spherical particles with an average size that corresponds with the mean particle size determination by LDA. It was observed that the smaller particles have a diameter higher than 200 nm.

The Panel noted that the glycolipids are surfactants that are soluble in water at a concentration (10 g/L or higher) that is significantly higher than the proposed maximum use level in beverages (0.05 g/L; Table 2). Therefore, the Panel considered that the EFSA Guidance on Nanotechnology (EFSA Scientific Committee, 2018) is not applicable and the risk assessment of the proposed food additive should be done following the Guidance on Food Additive (2012).

3.1.5. Methods of analysis in food

An LC-MS method was developed and validated by the applicant for qualitative and quantitative determination of the proposed food additive in beverages. The method involves alkaline saponification prior to LC-MS analysis of the deacylated glycolipids. The applicant indicated that the accuracy of the method is ca. ± 15% and the method is suitable for AM-1 concentrations in beverages of 2–30 mg/L.

3.1.6. Stability, reaction and fate in food of the proposed food additive

The proposed food additive (AM-1) when stored as a dry powder in closed containers up to 40°C was shown to be stable for a least 36 months.

The applicant assessed the stability of the proposed food additive in liquid media by determining the extent of deacylation (hydrolysis of ester moieties) of the AM-1 mixture; the lower the ratio of deacylated glycolipids, the more stable the AM-1 mixture. The composition of glycolipids was determined by LC-MS analysis, i.e. via EIC analysis of the main components having nominal molecular weights of 886, 928, 954, 970, 1,012, 1,054, 1,072, 1,114 Da (see Section 3.1.1)

The stability of AM-1 was tested in aqueous solutions at concentrations of 25 μg/mL and 0.15%, 0.5%, 1.5% and 10% w/v using un-buffered demineralized water, at storage temperatures of 6°C, 20°C and 40°C. The rate of hydrolysis was monitored by LC-MS analysis, measuring the sum of hydrolysed and non-hydrolysed glycolipids at each time point. The rate of deacylation depended on the time and the temperature but was independent of the glycolipid concentration, indicating pseudo first-order reaction kinetics as anticipated for simple hydrolysis in water. The kinetic curves for each of the 5 concentrations tested showed a 30–50% loss after 36 months at 20°C, corresponding to a half-life ≥ 36 months. At 40°C there was a 75–90% loss after 36 months, corresponding to a half-life of ca. 8–10 months.

The applicant also provided a report describing the stability of AM-1, expressed as percentages of hydrolysis (i.e. 100% corresponding to a complete deacylation), in 110 commercially obtained beverages (carbonated soft drinks (CSDs), fruit drinks, enhanced waters, sport and energy, syrups, various ready-to-drink) stored at ambient temperature for 3 months. Carbonated soft drinks with low pH showed the highest degree of hydrolysis. The deacylation was observed being dependent on the pH and the cloudiness of the individual beverage, e.g. CSDs with low pH generally showed a higher degree of ester hydrolysis compared to cloudy fruit juices. Beverages with pH > 3.5 showed only low degrees of ester hydrolysis. At pH > 3 there was typically 15% or lower formation of non-acylated glycolipids after 3 months storage at ambient temperature, with a few 'outliers' at higher percentage hydrolysis values.

The stability of AM-1 was also tested in a commercial clear apple juice after a high temperature short time (HTST) treatment (117°C for 1 and 5 min). The stability of AM-1 (expressed as percentage) was calculated from the relative amount of hydrolysis products found by LC-MS analysis after the HTST treatment. The stability at all tested concentrations (3, 5 and 10 mg/L) was found to be higher than 98% or 95% after 1 or 5 min heat treatment, respectively.

Both in the experiments performed in aqueous solutions and those in the commercially beverages, the applicant indicated that the core glycolipid structures remained intact, i.e. no deglycosylation with subsequent decomposition into long-chain fatty acids and sugar units of the glycolipids was observed.

3.2. Proposed uses and use levels

Through the current application, an authorisation is sought with regards to the food categories listed in Table 3.

The Panel noted that the applicant has submitted proposed typical and maximum use levels of long-chain glycolipids from *Dacryopinax spathularia* (in mg/kg or mg/L) for three food categories according to Annex II of Regulation (EC) No 1333/2008, part D.

Table 3: Proposed uses and use levels of long-chain glycolipids from *Dacryopinax spathularia* in foods (Documentation provided to EFSA No. 1 and 2)

Food category number	Food category name	Restrictions/ exception	Proposed use level (mg/L)	
			Typical	Maximum
14.1.4	Flavoured drinks		10	50
14.1.5.2	Other		10	20
14.2.1	Beer and malt beverages	Only alcohol free	10	50

3.3. Exposure data

3.3.1. Food consumption data used for exposure assessment

EFSA Comprehensive European Food Consumption Database

Since 2010, the EFSA Comprehensive European Food Consumption Database (Comprehensive Database) has been populated with national data on food consumption at a detailed level. Competent

authorities in the European countries provide EFSA with data on the level of food consumption by the individual consumer from the most recent national dietary survey in their country (cf. Guidance of EFSA on the "Use of the EFSA Comprehensive European Food Consumption Database in Exposure Assessment" (EFSA, 2011). The version of the Comprehensive database taken into account in this assessment was published February in 2020.[4]

The food consumption data gathered by EFSA were collected by different methodologies and thus direct country-to-country comparisons may not be appropriate. Depending on the food category and the level of detail used for 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 40 different dietary surveys carried out in 23 European countries (Table 4).

Table 4: Population groups considered for the exposure estimates of long-chain glycolipids from *Dacryopinax spathularia*

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
Toddlers[(a)]	From 12 months up to and including 35 months of age	Belgium, Bulgaria, Cyprus, Denmark, Estonia, Finland, France, Germany, Italy, Latvia, 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, Italy, Latvia, 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, Italy, Latvia, Netherlands, Portugal, 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, 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, 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

(b): The terms 'children' and 'the elderly' correspond, respectively, to 'other children' and the merge of 'elderly' and 'very elderly' in Comprehensive Database (EFSA, 2011).

Consumption records were codified according to the FoodEx2 classification system (EFSA, 2015). Nomenclature from the FoodEx2 classification system was linked to the food categorisation system (FCS) as presented in Annex II of Regulation (EC) No 1333/2008, part D, to perform the exposure assessments. In practice, the FoodEx2 food codes were matched to the FCS food categories.

Food categories considered for the exposure assessment of long-chain glycolipids from *Dacryopinax spathularia*

The food categories in which the use of long-chain glycolipids from *Dacryopinax spathularia* is proposed were selected from the nomenclature of the EFSA Comprehensive Database (FoodEx2 classification system), at the most detailed level possible (up to FoodEx2 Level 7) (EFSA, 2015).

For the exposure assessment of long-chain glycolipids from *Dacryopinax spathularia* from its proposed use in FC 14.2.1 Beer and malt beverages, the restriction to "Only alcohol free" was considered.

[4] Available online: http://www.efsa.europa.eu/en/datexfoodcdb/datexfooddb.htm

3.3.2. Exposure to long-chain glycolipids from *Dacryopinax spathularia* from its proposed use as food additive

Estimate of exposure based on the Food Additives Intake Model (FAIM) template

The applicant has provided an estimate of exposure to long-chain glycolipids from *Dacryopinax spathularia* based on the output obtained using the FAIM model at the proposed maximum and typical use levels submitted.

The Panel decided not to use the estimate of exposure generated from the FAIM tool and provided by the applicant because of the level of aggregation of the data which were in the FAIM template resulted in an overly conservative estimates of the exposure. Indeed, with the FAIM tool, it is not possible to distinguish 1) between 14.1.5.1 and 14.1.5.2 and 2) between alcoholic and alcoholic free beverages in FC 14.2.1.

Therefore, to avoid any overly conservative estimates, the Panel decided to perform a more refined assessment, using the FoodEx2 Level categories.

Refined exposure assessment scenario

The Panel estimated the chronic dietary exposure to long-chain glycolipids from *Dacryopinax spathularia* for the following population groups: infants, toddlers, children, adolescents, adults and the elderly. Dietary exposure to long-chain glycolipids from *Dacryopinax spathularia* was calculated by multiplying concentrations of long-chain glycolipids from *Dacryopinax spathularia* per food category (Table 3) with their respective consumption amount per kilogram 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 exposure per survey and population group (Table 4). On the basis of these distributions, the mean and 95th percentile of exposure were calculated per survey and per population group. The 95th percentile of exposure was only calculated for those population groups with a sufficiently large sample size (EFSA, 2011). Therefore, in the present assessment, the 95th percentile of exposure for infants from France and Italy, for toddlers from Belgium and Italy and for adolescents from Estonia was not estimated. Detailed results per population group and survey are presented in Appendix B.

Exposure assessment to long-chain glycolipids from *Dacryopinax spathularia* was carried out by the FAF Panel based on the proposed typical and maximum use levels submitted by the applicant: 1) proposed maximum level exposure assessment scenario; and 2) proposed typical level assessment scenario.

Table 5: Summary of dietary exposure to long-chain glycolipids from *Dacryopinax spathularia* from its proposed use levels as a food additive in six population groups (minimum-maximum across the dietary surveys in mg/kg bw per day)

Estimated exposure (mg/kg bw per day)	Infants (12 weeks-11 months)	Toddlers (12–35 months)	Children (3–9 years)	Adolescents (10–17 years)	Adults (18–64 years)	The elderly (≥ 65 years)
Proposed maximum level exposure assessment scenario						
Mean	0.01–0.26	0.02–1.07	0.07–1.00	0.06–0.57	0.05–0.38	0.03–0.19
95th percentile	0.00*–0.88	0.10–3.10	0.33–2.16	0.31–1.33	0.22–1.32	0.12–0.61
Proposed typical level exposure assessment scenario						
Mean	< 0.01–0.12	< 0.01–0.23	0.02–0.22	0.01–0.13	0.01–0.10	0.01–0.09
95th percentile	0.00*–0.38	0.02–0.64	0.08–0.47	0.06–0.29	0.05–0.27	0.05–0.19

*: 95th percentile is null when there are less than 5% consumers.

At the proposed maximum use levels, the mean exposure to long-chain glycolipids from *Dacryopinax spathularia* from its use as a food additive ranged from 0.01 mg/kg bw per day in infants to 1.07 mg/kg bw per day in toddlers. The 95th percentile of exposure to long-chain glycolipids from

Dacryopinax spathularia ranged from 0 mg/kg bw per day in infants, to 3.1 mg/kg bw per day in toddlers.

At the proposed typical use levels, the mean exposure to long-chain glycolipids from *Dacryopinax spathularia* from its use as a food additive ranged from < 0.01 mg/kg bw per day in infants and toddlers to 0.23 mg/kg bw per day in toddlers. The 95th percentile of exposure to long-chain glycolipids from *Dacryopinax spathularia* ranged from 0 mg/kg bw per day in infants, to 0.64 mg/kg bw per day in toddlers.

Main food categories contributing to exposure to long-chain glycolipids from *Dacryopinax spathularia*

From both the *proposed maximum level exposure assessment scenario* and the *proposed typical level exposure assessment scenario*, the main contributing food categories to the total mean exposure estimates (Appendix C) were:

- FC 14.1.4 Flavoured drinks (83.5–100.0% for the proposed maximum levels and 65.4–100.0% for the proposed typical levels) for all population groups except the elderly;
- FC 14.1.5.2 Other (74.2–100.0% for the proposed maximum levels and 88.2–100.0% for the proposed typical levels) for all population groups.

Uncertainty analysis

In accordance with the guidance provided in the EFSA opinion related to uncertainties in dietary exposure assessment (EFSA Scientific Committee, 2007), the following sources of uncertainties have been considered and summarised in Table 6.

Table 6: Qualitative evaluation of influence of uncertainties on the dietary exposure estimate

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

(a): +, uncertainty with potential to cause overestimation of exposure; –, uncertainty with potential to cause underestimation of exposure.

Long-chain glycolipids from *Dacryopinax spathularia* is requested to be authorised in three food categories. For all food categories taken into account, it was assumed that 100% of the foods belonging to these food categories (considering their restrictions) will contain long-chain glycolipids from *Dacryopinax spathularia* at the typical or maximum proposed use levels. Therefore, overall, the Panel considered that the uncertainties identified resulted in an overestimation of the exposure to long-chain glycolipids from *Dacryopinax spathularia* in European countries considered in the EFSA Comprehensive database at both the typical and maximum proposed use levels.

Anticipated exposure to toxic elements from proposed specifications

The applicant provided lowest technically achievable levels for As (1.0 mg/kg), Pb (2.0 mg/kg), Ni (2.0 mg/kg), Cd (1.0 mg/kg) and Hg (1.0 mg/kg) in the proposed food additive (Documentation provided to EFSA No. 2) for the purpose of defining appropriate specifications. The potential exposure to the toxic elements from the use of the proposed food additive can be calculated by assuming

contamination of the additive may be up to the specifications limit values and then by calculation pro-rata to the estimates of exposure to the proposed food additive itself (Table 5). The outcome of such an exercise (Table 7) illustrates the health impact that would result if the maximum limits for toxic elements as proposed by the applicant were to be used.

Table 7: Risk assessment for toxic elements using the specifications proposed by the applicant based on their lowest technically achievable limits in the proposed food additive (documentation provided to EFSA no. 2)

Exposure to proposed additive (mg/kg bw per day)[(a)]	MOS/MOE for As at 1 mg/kg	MOS/MOE for Pb at 2.0 mg/kg	%age of the TWI for Cd at 1 mg/kg	%age of the TWI for Hg at 1 mg/kg	%age of the TDI for Ni at 2.0 mg/kg
3.1[(b)]	97–2,580	81	0.9%	0.5%	0.05%
0.64[(c)]	469–12,500	391	0.2%	0.1%	0.01%

(a): Data from Table 5 (Section 3.3.2), maximum exposure identified (95th percentile for toddlers).
(b): Proposed maximum use level exposure assessment scenario.
(c): Proposed typical use level exposure assessment scenario.

The resulting figures in both scenarios show that the exposure to toxic elements from the proposed uses of long-chain glycolipids from *Dacryopinax spathularia* as a food additive would not be of concern using the maximum limit values proposed by the applicant for these elements.

For arsenic the reference points are BMDL01 values (LB and UB) of 0.3 and 8 μg/kg b.w. per day from human epidemiological studies (EFSA CONTAM Panel, 2009). The reference points are based on carcinogenicity and so the MOS/MOE should be at least 10,000 (EFSA CONTAM Panel, 2005, 2009). The MOS/MOE values calculated can be lower than this value (Table 7). The MOS/MOE is calculated by dividing the reference point by the exposure estimate. The assessment of the uncertainty in the exposure showed a potential for overestimation of exposure for the proposed maximum use level (PMUL) exposure assessment scenario (see Table 5). Also, the calculations use the maximum limit for arsenic proposed by the business operator and so the actual content would be lower on average. Considering that the MOS/MOE might be underestimated by i) using the exposure estimates at the PMUL and by ii) using the proposed limit for arsenic, the MOS/MOE less than 10,000 was still considered adequate.

For lead the reference point is a BMDL01 of 0.5 μg/kg bw/day (EFSA CONTAM Panel, 2010). 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. In the opinion on lead (EFSA CONTAM Panel, 2010) it is 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 $BMDL_{01}$ (MOS/MOE lower than 1). The MOS/MOE is well above 1 (Table 7).

For cadmium, a TWI of 2.5 μg/kg bw has been established (EFSA CONTAM Panel, 2009) and the exposure to Cd would be only a small fraction of the TWI value (Table 7).

For mercury, a TWI of 4 μg/kg bw has been established (EFSA CONTAM Panel, 2012) and the exposure to Hg would be only a small fraction of the TWI value (Table 7).

For nickel, a TDI of 13 μg/kg bw has been established[5] (EFSA CONTAM Panel, 2020) and the exposure to Ni would be only a small fraction of the TDI value (Table 7).

3.4. Biological and toxicological data

The biological and toxicological studies were performed with test substance described as IMD AM-1 "glycolipid mixture derived from fermentation of glucose by *Dacryopinax spathularia*" (also referred to in some studies as IM 11). This substance was considered representative of long-chain glycolipids from

[5] Dietary exposure to nickel can also elicit acute effects for nickel-sensitised individuals. Concerning acute oral exposure to nickel, the EFSA CONTAM Panel selected a value of 4.3 μg Ni/kg bw as the reference point and considered that, with a margin of exposure (MOE) approach, an MOE of 30 or higher was indicative of a low health concern (EFSA CONTAM Panel, 2020). For the proposed food additive, an acute dietary exposure was identified by the FAF Panel as an adult drinking 2,333 mL of non alcoholic beer. This was the highest acute eating occasion observed at the P99 in the adult population and corresponded to 29.4 mL/kg bw. The proposed maximum use level of the additive for this food category is 50 mg/L (Table 3). At this use level and if the additive was contaminated at the proposed specification value for nickel of 2 mg/kg, acute exposure to nickel would be at a level giving an MOE of 1463, which does not give rise to a safety concern.

Dacryopinax spathularia under evaluation as food additive based on the production process and analytical data provided.

The toxicological studies submitted in support of the application were performed in compliance with GLP.

3.4.1. Absorption, distribution, metabolism and excretion

To investigate the stability of the test substance in the upper GI-tract, AM-1 was incubated for up to 8 h in simulated gastric fluid (SGF with pepsin) at different pH values (pH 2, 3.2 or 4.5) (Documentation provided to EFSA no. 4). Aliquots drawn at various time points were analyzed by HPLC-MS for glycolipid content and composition. After 1 h of incubation at pH 2, 3.2 or 4.5 glycolipid composition and content were unchanged (compared to blank without enzyme), and also after 8 h at pH 4.5. Samples of incubations at pH 3.2 and pH 2 for 8 h had lower glycolipid concentration; partial deacylation occurred (apparent as a higher portion of AM-1 glycolipids with lower acylation degree). Yet no other peaks/metabolites were detected which could be due to pepsin activity confirming that AM-1 is not a substrate for this enzyme. According to the study authors the slow, partial hydrolysis of acyl groups and, thus, change in the glycolipid composition after 8 h of incubation is in agreement with other experiments investigating the stability at acidic conditions (see Section 3.1.6). The Panel considered that the lower glycolipid concentrations observed could be also due to technical problems such as precipitation at low pH which affect the analytical recovery.

The Panel considered that the data on stability in simulated gastric fluid indicate that ingested AM-1 will pass mostly unchanged to the lower GI-tract.

Results of an experiment *in vitro* with simulated gastric fluid (see above) and from a small scale pharmacokinetic study with [^{13}C]-AM-1 in rats (Documentation provided to EFSA no.5 and 6) indicate that following ingestion, AM-1 passes mostly unchanged to the lower GI tract where predominantly its ester linkages and, partially its glycosidic linkages are hydrolysed by microflora in the lower intestine yielding glucose, xylose, acetic acid, isovaleric acid, and hydroxylated long chain fatty acids as major hydrolysis product of AM-1.

Another study was performed, in compliance with GLP, to investigate the pharmacokinetics, excretion balance, and tissue distribution of uniformly [^{14}C]-labelled AM-1 equivalents following single or repeated administration to Sprague Dawley rats, in comparison with [^{14}C]-LCFA (Documentation provided to EFSA n. 7; Bitzer et al., 2017a). Rats (aged 8 to 10 weeks) received equimolar doses of either [^{14}C]-AM-1 or [^{14}C]-LCFA via oral or intravenous administration followed by collection of biological samples at specified intervals.

The plasma concentration time courses in male and female rats after oral administration of the test materials (AM-1 at 100 mg/kg bw and LCFA at 46 mg/kg bw) indicate partial absorption, with [^{14}C] peak levels occurring earlier for LCFA (2 and 4 h) than for the AM-1 related radioactivity (8 and 24 h). The calculated oral bioavailability (F) for AM-1 equivalents was 10.7% for males and 12.7% for females. The Panel considered that these values are likely overestimates since they account largely for the hydrolysis products and further metabolites rather than the intact AM-1. Additional pharmacokinetic parameters (AUC, C_{max}, T_{max}, $T_{1/2}$, etc.) for this study and after i.v. application of test materials (at 1/10 of the oral dose) are also provided by the applicant. This information is considered to be of limited relevance since the composition of the circulating radioactivity was not studied, and metabolism can differ with application route.

In the study segment investigating disposition and excretion of [^{14}C]-AM-1 after single oral dosing (or following previous doses of unlabelled AM-1 for 14 days), the largest part of radioactivity was excreted with faeces: 73.7–82.6% of the dose (or 62.8–75.6%). Another part was captured in CO_2 traps for exhaled air, with 16.3–20.5% of the dose. This CO_2 is produced by systemic and possibly pre-systemic metabolism. Radioactivity recovered in urines was low in all groups ranging from 0.96–1.55%, and also low in carcasses (< 1%).

Results from whole body autography (QWBA) of rats sacrificed at specified intervals post dosing, also showed that [^{14}C]-AM-1 equivalents are predominantly present in the GI-tract; levels detected in most tissues were not higher than those detected in plasma.

Overall, these data show that AM-1 and its GI tract metabolites are largely eliminated in the faeces and only to a minor extent absorbed by the oral route. The pharmacokinetic, tissue distribution, and excretion balance data are consistent with an interpretation that following ingestion, AM-1 is partially hydrolyzed to its components, glucose, xylose, acetate, isovalerate and to LCFA. These results support

also the conclusion that systemic exposure to AM-1 or its metabolites would be low following oral ingestion and that any absorbed material is rapidly metabolised and exhaled.

3.4.2. Acute toxicity

An acute oral toxicity study was performed in female Wistar rats treated with IM11 (purity ≥ 90% suspended in sterile water) according to OECD TG 423 (OECD, 2001) (Documentation provided to EFSA No. 8). Two groups, each of three rats, received 2000 mg/kg bw test item by gavage. Throughout the 14-day observation period there were no deaths and no clinical signs - except for a transient piloerection at day 1 in two females. Body weight gain (13–22%) was within the normal range of variation for this strain. At necropsy, no specific findings were reported.

3.4.3. Sub-chronic toxicity

Rat

A 90-day oral study in CD rats exposed to IMD AM-1 via drinking water was performed according to OECD TG 408 (OECD, 1998) (Documentation provided to EFSA No. 9; Bitzer et al., 2017b). In the main study 20 rats/group per sex received 0%, 0.15%, 0.5% and 1.5% test item in drinking water (mean intake: 0.0, 132.5, 412.6, 1,200.5 mg/kg bw per day in males and 0.0, 165.5, 501.5 and 1,423.1 mg/kg bw per day in females, respectively) and in addition there were two 4-weeks recovery groups (10 rats/group per sex) with 0% and 1.5% test item.

No premature mortality was reported, except for a death of one female in the high dose group (at day 14 after blood withdrawal) which was considered incidental. Body weights of male rats treated with the highest dose were slightly decreased (5–7% vs controls) in weeks 1–6; the body weight gain from day 1–90 was 5.6% lower. A decreased food consumption was observed in females and males of the high dose groups in week 1 (by 13% vs controls). The drinking water consumption of both sexes decreased in the mid (up to 11%) and high dose (up to 22%) groups in the first two weeks. The authors suggested that this effect might be due to higher viscosity and/or a decreased palatability of the drinking water. Statistically significant changes in parameters measured in the neurological screening were without dose-response, not correlated with histopathological findings and well within the historical control data of the laboratory; therefore, the effects were not considered toxicologically relevant. The Panel noted that small reductions in red blood cell parameters (RBC, HBG, HCT) at several timepoints (days 14, 42 and 91) were consistent along with some increases in reticulocytes and were considered treatment-related findings in the high dose groups of female and male rats. A slight reduction of erythrocytes was also observed in the high dose males at the end of the recovery period. Also, statistically significant changes in clinical chemistry parameters in the mid and high dose groups of males at days 14, 42 and 91 were reported. Slight but consistent reductions of globulin and albumin were observed in the mid (day 42, 91) and high dose males along with a reduction in protein (days 14, 42 and 91) as well as in high dose females (day 14) which also showed an increase in the albumin/globulin ratio at day 42. A decrease in albumin was also observed in the recovery (high dose) group of males. In addition, for males a dose-dependent decrease in calcium (days 14, 42 and 91) and an increase of urea in blood (days 14, 42) was reported. In urinalysis slight increases in specific urinary gravity were observed in the high dose groups of females and males as well as reductions in the pH value and the urine volume in males. No toxicologically relevant test item-related findings were observed in clinical signs, ophthalmological examination, organ weights, macroscopic and histopathological findings.

Overall, the Panel noted that the observed dose-related effects were minor (below 10%) and mainly within the ranges of the historical control data of the laboratory. The minor reductions in body weight and body weight gain were most pronounced in the beginning of the treatment and not considered adverse. The reductions may be related to decreased food and water consumption. Minor dose-related changes in haematology and clinical chemistry parameters were not supported by histopathological findings. Therefore, the Panel derived a NOAEL of 1,201 mg/kg bw per day for males and 1,423 mg/kg bw per day for females, i.e. the highest doses applied in the study.

Dog

In a study, performed in compliance with GLP, sexually mature Beagle dogs were dosed with IMD AM-1 administered orally in capsules once daily for a minimum of 91 days. (Documentation provided to EFSA No. 10; Bitzer et al., 2017c). Three groups of dogs each consisting of 4 males and 4 females

received dosage levels of 150, 500, and 1,000 mg/kg/day respectively. A control group received empty capsules. All animals were euthanized after 91 or 92 days.

Clinical examinations were performed daily, individual body weights recorded weekly and individual food weights recorded daily. A modified functional observational battery was performed before dosing and during week 12. Clinical pathology parameters (haematology, coagulation, serum chemistry, and urinalysis) were analysed during weeks 2 and 6 and at necropsy. Ophthalmic examinations were performed during weeks 1 and 12. Complete necropsies were performed on all animals and selected organs were weighed. Selected tissues were examined microscopically from all animals.

There were no test substance-related clinical observations or effects on haematology, coagulation, serum chemistry, urinalysis, neurobehavioral parameters or organ weights. There were also no test substance-related ophthalmic, macroscopic or microscopic findings.

The only test substance-related changes observed were minimal effects on food consumption and cumulative body weight changes in the 1,000 mg/kg per day group females. The authors considered these minor effects non-adverse and thus considered the NOAEL to be 1,000 mg/kg per day, the highest dose tested, for both males and females. The Panel agreed with this conclusion.

3.4.4. Genotoxicity

Bacterial reverse mutation assay

The test substance, described as IM 11, was examined for its possible mutagenic activity in the bacterial reverse mutation test using the *Salmonella typhimurium* strains TA 1535, TA 1537, TA 98, TA 100 and the *Escherichia coli* strain WP2 uvrA, in the absence and presence of metabolic activation (S9-mix), in accordance with the OECD guideline 471 (Documentation provided to EFSA No.11; Bitzer et al., 2019).

The test substance was dissolved in dimethylsulphoxide (DMSO) and tested up to a maximum concentration of 5,000 μg/plate (plate incorporation method) or 5,000 μg/mL (pre-incubation method).

A first experiment was performed according to the plate-incorporation method. An increase in the number of revertants was observed in some strains, in the presence of a slightly more dense background lawn of bacterial growth. This finding was interpreted as the consequence of the growth stimulatory effect of the test material (possibly due to the presence of histidine). Therefore a second experiments was conducted according to the preincubation (treat and wash) method.

In this second experiment a more than 2-fold increase was observed in the TA1535 strain, however the increase was not concentration dependent and the negative control was at the lower end of the historical range. A third confirmatory experiment was therefore performed and was clearly negative. Therefore, considering that the increase found in the second experiment was not concentration-dependent or reproducible, this finding was not considered biologically relevant.

It is concluded that the test substance did not induce bacterial reverse mutations.

***In vitro* micronucleus assay**

The test substance, described as IM 11, was examined for its potential to induce micronuclei in cultured binucleated human lymphocytes from one donor (32 years old), in both the absence and presence of a metabolic activation system (S9-mix) in accordance with the OECD guideline 487 (Documentation provided to EFSA No. 12; Bitzer et al., 2019).

In the presence of metabolic activation (S9-mix) the treatment/recovery time was 4/20 h (pulse treatment) and in the absence of S9-mix, the treatment/recovery time was 4/20 (pulse treatment) and 20/28 h (continuous treatment). Cytotoxicity was calculated from the Cytokinesis-Block Proliferation Index (CBPI).

The treatment concentrations were selected on the basis of a dose range finding toxicity test. DMSO was used as solvent for the test substance and all the experiments were conducted in duplicate cultures. The following concentration levels were analysed: 700, 500 and 300 μg/mL (pulse treatment group with metabolic activation); 400, 200 and 100 μg/mL (pulse treatment group without metabolic activation); 900, 800 and 600 μg/mL (continuous treatment group without metabolic activation). At the maximum concentration the cytotoxicity level was in the range 52–59%.

The test substance did not induce statistically significant increases in the number of binucleated cells containing micronuclei in any experimental condition, when compared to the numbers found in the concurrent negative controls, while the positive controls performed as expected.

In vitro mammalian cell gene mutation test with L5178Y mouse lymphoma cells

The mutagenic activity of the test substance described as IMD AM-1 was evaluated in an *in vitro* mammalian cell gene mutation test on L5178Y mouse lymphoma cells (Documentation provided to EFSA No. 13; Bitzer et al., 2019).

The test was performed in the absence of S9-mix with 3 and 24-h treatment periods and in the presence of S9-mix with a 3 h treatment period in compliance with OECD guideline 490. The test substance was dissolved in dimethyl sulfoxide.

In the first experiment, the test substance was tested up to concentrations of 450 and 800 μg/ml in the absence and presence of S9-mix, respectively. The treatment time was 3 h. Relative total growth (RTG) was 15 and 18% in the absence and presence of S9-mix, respectively. In the second experiment, the test substance was tested up to concentrations of 500 μg/ml in the absence of S9-mix. The treatment time was 24 h. The RTG was 9% at highest concentration.

The test substance did not induce significant increases in the mutation frequency in any experimental conditions, both in the presence and in the absence of metabolic activation.

In vivo micronucleus test

The test substance described as IMD AM -1 was evaluated for its ability to induce micronuclei in the bone marrow when administered to ICR mice (Documentation provided to EFSA No. 14; Bitzer et al., 2019). The study was not fully compliant with OECD guideline 474.

Five animals per sex and experimental group were treated by gavage with 2.5, 5.0 and 10.0 g/kg body weight, in two administration with an interval of 24 h and were sacrificed by cervical dislocation 6 h after the last treatment. The negative control group received distilled water, while the positive control group was treated with cyclophosphamide at the dose of 40.0 mg/kg body weight, dissolved in distilled water.

At least 2,000 polychromatic erythrocytes per animal were examined for the incidence of micronuclei.

No effects on the induction of MNPCE were observed in mice treated with the test substance. The ratio between polychromatic erythrocytes and total red blood cells, analysed in 200 erythrocytes, was not affected by the treatment, therefore no evidence of target cell exposure was provided. The positive control substance cyclophosphamide induced a statistically significant increase in the incidence of micronuclei.

Overall, the test substance was negative in a bacterial reverse mutation test, in an *in vitro* micronucleus assay in human lymphocytes and in a gene mutation study in L5178Y mouse lymphoma cells. Therefore, the test substance does not raise a concern regarding genotoxicity.

3.4.5. Reproductive and developmental toxicity

IMD AM-1 was studied in a two-generation reproductive toxicity study in rats (Documentation provided to EFSA No. 15; Bitzer et al., 2018). This GLP study was designed in general accordance with the United States FDA Guidelines for Reproduction Studies (FDA, 2000a,b) and comparable to the OECD TG 416 (2001). Male and female Crl:CD(SD) rats (25/sex per group) were administered the test article, IMD AM-1, daily by oral gavage at dose levels of 0, 150, 500, and 1,000 mg/kg bw per day for the F0 and F1 generations. The concurrent control group received the vehicle (reverse osmosis-treated water). The dose volume for all groups was 10 mL/kg bw. There were no test article-related effects on F0 or F1 parental survival at any dosage level. Occasionally at the detailed physical examinations or daily examinations, and more frequently at 1–2 h following dose administration, a dose-related increase in incidence of rales and red and/or clear material around the nose and/or mouth were noted in the 150, 500, and 1,000 mg/kg bw per day group F0 and F1 males and females. The authors attributed the occurrence of transient rales 1-2 h following dose administration to the surfactant properties of the test substance combined with oral gavage dosing and considered it not to be toxicologically relevant. The Panel agreed with this view. In the F0 generation no test substance-related effects on body weight, body weight gain, food consumption, or food efficiency in the F0 generation were observed. During the first week of dosing of the males of the F1 generation a lower mean body weight gain was observed in the 150, 500, and 1,000 mg/kg bw per day group. No differences in body weight gain in these groups were seen throughout the remainder of the study. As a result of the initial lower mean body weights gains, mean body weights for F1 males were between 4.0% and 7.1% lower than the control group throughout the study. These differences were not considered adverse because the mean body weights in these groups at termination (PND 161) were

only 5.4% to 6.1% lower than the control group, demonstrating that the initial effects were ameliorated over the course of the generation. Furthermore, there were no test substance-related effects on food consumption or food efficiency for F1 males and females. In F1 females there was no effect on body weight or body weight gain in the groups dosed with the test substance. In both generations, no effect on mating and fertility, male copulation and female conception indices, oestrous cycle length, pre-coital intervals, gestation length, the process of parturition, and spermatogenesis parameters (motility, progressive motility, testicular and epididymal sperm concentration, sperm production rate, and the percentage of morphologically normal sperm) was observed in the test substance treated groups when compared to the control group. In the animals of both generations, no effect on organ weights, macroscopic or microscopic findings or on the F1 female primordial follicle counts were observed. In the F1 pups there were no effects on attainment of balanopreputial separation and vaginal patency or body weights at attainment of these developmental landmarks. For both generations, the number of pups born, live litter size on PND 0, sex ratio, or postnatal survival, pup weight were comparable in all groups. On PND 21 no macroscopic findings or differences in organ weight were seen at any dose level in the F1 and F2 pups. The Panel considered that the NOAEL for maternal and reproductive toxicity was 1000 mg/kg bw per day, the highest dose tested.

IMD AM-1 was studied in a prenatal developmental toxicity test (Documentation provided to EFSA No. 16; Bitzer et al., 2018). This GLP study was designed in general accordance with the United States FDA Guidelines for Developmental Toxicity Studies (FDA, 2000a) and comparable to the OECD TG 414 (2001). IMD AM-1, was administered by gavage to 4 groups of Crl:CD(SD) rats (24 females/group) once daily from Gestation Days (GD) 6–19. Dosage levels were 0, 150, 500, and 1,000 mg/kg bw per day administered at a dose volume of 10 mL/kg. The concurrent control group received the vehicle (reverse osmosis-treated water). All females survived to the scheduled necropsy on GD20. Rales was noted in a dose-dependent manner 1–2 h after dose administration in the 150, 500, and 1,000 mg/kg bw per day groups sporadically throughout the treatment period (GD 6–19). One to two hours after dose administration clear and/or red material around the nose and/or mouth were noted in the 500 and 1,000 mg/kg bw per day groups during GD 7–16. These findings were considered test article-related but not adverse because they generally did not persist to the daily examination the following day and there were no corresponding signs of systemic toxicity at any dosage level. Lower mean food consumption was noted during GD 6–9 and GD 15–20 in the high dose group and during GD 15–20 for the mid dose group. The authors considered these changes test substance-related but not adverse because the effects were transient and as no effects on mean body weight were observed in these groups throughout the treatment period. Furthermore, there were no test substance -related effects on body weight, body weight gain, gravid uterine weight, net body weight in the 150, 500, and 1,000 mg/kg bw per day groups or food consumption in the low dose group. At Caesarian section on GD 20, no differences between the control and the test substance treated groups were observed in number of implantations, resorptions, number of live and dead fetuses, fetal weight, macroscopic, visceral or skeletal abnormalities of the fetuses. The Panel considered that the NOAEL for developmental toxicity was 1,000 mg/kg bw per day, the highest dose tested.

3.4.6. Immunotoxicity, Hypersensitivity/allergy

The Panel noted that AM-1 may contain up to 3% total protein content associated with non-viable cells debris originating from the production organism, which is an edible fungus, that was not reported to induce allergic reactions. AM-1 does not contain any introduced protein that would be expected to result in an allergic reaction. In addition, no effects on the parameters that may be indicative of an immunotoxic or immunomodulatory effect were observed in the oral sub-chronic toxicity studies.

4. Discussion

The proposed food additive is a purified mixture of long-chain glycolipid congeners obtained by fermentation of the edible basidiomycetes *Dacryopinax spathularia* using glucose and autolyzed yeast extract as feedstock (called AM-1). The mixture has a total glycolipid content of not less than 93% w/w and the remaining 7% of the dry weight is comprised of: up to 3% of residual protein; up to 2% of total fat and of 1.7–3.3% of sodium.

The core structure of the AM-1 glycolipids is a hydroxylated long-chain fatty acid (C-26) with a glucopyranosyl-(1→2)-xylopyranosyl-(1→2)-xylopyranosyl trisaccharide moiety which is attached via glycosidic bond to the ω-hydroxy position. The glycolipids differ in the number of hydroxyl groups of the fatty acid backbone and their stereo- and regiochemistry as well as in the acylation pattern of the

sugar unit, consisting mainly of acetyl and isovaleryl units. 3-Hydroxy-3-methylglutarate (HMG) can also be an acyl unit. The glycolipid profile of AM-1 is analysed by HPLC-MS classifying the glycolipid congeners within eight groups based on their MW, i.e. MW 886, 928, 954, 970, 1,012, 1,054, 1,072 and 1,114 Da. Glycolipids within MW groups 886 and 928 Da are hydrolysis products (non-acylated and mono-acetylated glycolipids, respectively). Glycolipids within the highest MW groups, i.e. MW 1,072 and 1,114 Da, are those congeners acylated with HMG.

The Panel noted that the provision on total content of glycolipids is sufficient to characterize the identity of the food additive and considered that the glycolipids profile is not necessary.

Regarding the glycolipid congeners acylated with HMG (MW groups 1,072 and 1,114 Da), the Panel took note, as indicated by the applicant, that it is a component of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA), intermediate in primary metabolism; in particular, in the mevalonate and ketogenesis pathways as well as leucine degradation. As such, HMG is a naturally occurring constituent of human physiology. The Panel considered the provided toxicological dataset for AM-1 supporting the safety of HMG as potential hydrolysis product, since the glycolipids tested toxicologically contained even those congeners acylated with HMG (up to 1.5% w/w of HMG would be the calculated theoretical concentration based on the proposed maximum threshold of not less than 17% w/w for the two HMG-containing glycolipid groups). The Panel also noted that (Q)SAR analysis using the OECD Toolbox (4.2) did not reveal any structural alert for genotoxicity in this compound.

The Panel considered that the limits proposed for Cd and Hg are high at 1.0 mg/kg given that they are both 100-times the highest values found in 5 batches. The anticipated impact of these proposed specifications on the potential exposure to those elements does not give raise to safety concern (see Table 6).

The Panel considered that the proposed food additive is produced from an edible fungus (*Dacryopinax spathularia*) that does not produce detectable amounts of aflatoxins, trichothecenes, ochratoxin, zearalenone, fumonisin B1 & B2, and deoxynivalenol. None of the potential secondary metabolites predicted from a bioinformatic analysis of genome sequencing shared structural similarities to any known mycotoxin or virulence factor. The Panel considered that *Dacryopinax spathularia* does not produce mycotoxins or virulence factors and therefore there is no need to set an EU specification for the presence of mycotoxins.

The microbiological quality of the proposed food additive was confirmed by microbiological analyses of four non-consecutive commercial batches of AM-1. The results were in good compliance with the proposed specifications (see Table 2). The Panel considered that a maximum limit for *Enterobacteriaceae* (< 10 CFU/g) should be included in the EU specifications as indicator of good hygiene practice.

The Panel noted that an adequate dataset for investigating AM-1 bulk stability and stability in liquid media, including commercially obtained beverages (e.g. CSDs), was provided by the applicant. The parameter followed in the stability studies to judge the stability of the proposed food additive in liquid media was the extent of the deacylation in the AM-1 mixture. The deacylation was observed being dependent on the pH and the cloudiness of the individual beverage, e.g. CSDs with low pH generally showed a higher degree of ester hydrolysis compared to cloudy fruit juices. Beverages with pH > 3 showed typically 15%, or lower, formation of non-acylated glycolipids after 3 months storage at ambient temperature. In all the experiments performed in liquid media (aqueous solutions in unbuffered water and commercially beverages), the applicant indicated that the core glycolipid structures remained intact, i.e. no deglycosylation with subsequent decomposition into long-chain fatty acids and sugar units of the glycolipids was observed.

An *in vitro* experiment with AM-1 in simulated gastric fluid (at pH 2 or 3.2) showed no degradation after 1 h (and only minor losses after 8 h incubation due to partial deacylation) indicating that the test item will pass largely unchanged to the lower GI-tract. *In vivo* studies in rats (with [13C] and [14C] labelled test material) indicate that AM-1 is partially hydrolyzed, possibly by microflora, in the lower intestine to its components, glucose, xylose, acetate, isovalerate and hydroxylated long chain fatty acids. Data on the kinetics, excretion balance and from whole body autoradiography support the conclusion of a low oral bioavailability and rapid elimination of AM-1.

The toxicology data set comprised studies on acute toxicity, sub-chronic toxicity, genotoxicity and reproductive and development toxicity. Studies were performed using test substance which is considered by the Panel to be representative of the food additive.

Acute toxicity study in female rats showed no adverse effects at 2000 mg/kg bw.

Sub-chronic 90-day studies in rats and dogs did not reveal any adverse effects at any dose tested. Minor effects on feed consumption, body weights and body weight gains in both species as well as

minor changes in haematology and clinical chemistry parameters in rats were not considered adverse. The Panel identified NOAELs of 1,201 and 1,423 mg/kg bw per day for male and female rats respectively and 1,000 mg/kg bw per day for dogs, the highest doses tested in the respective studies.

Genotoxicity was investigated *in vitro* and *in vivo*. The test substance was negative in a bacterial reverse mutation test, in an *in vitro* micronucleus assay in human lymphocytes and in a gene mutation study in L5178Y mouse lymphoma cells. The test substance does not raise concern regarding genotoxicity.

Reproductive and development toxicity was studied in rats. In a two-generation reproductive toxicity study and a prenatal developmental toxicity study no signs of parental, reproductive or developmental toxicity were observed up to 1,000 mg/kg bw per day, the highest dose tested in both studies.

Overall, available toxicology data do not demonstrate any adverse effects for the proposed food additive.

Considering the available data set the Panel established an ADI of 10 mg/kg bw per day based on a range of NOAELs between 1,000 and 1,423 mg/kg bw per day (the highest doses tested), from the reproductive and a prenatal developmental toxicity studies in rats and 90-day studies in rats and dogs. Considering the absence of adverse effects in all studies and rapid elimination of test substance, the Panel considered it sufficient to apply an uncertainty factor of 100 and not deemed necessary to apply an additional time-correction factor of 2.

The Panel performed an exposure assessment to long-chain glycolipids from *Dacryopinax spathularia* based on the proposed use levels submitted by the applicant: 1) proposed maximum level exposure assessment scenario; and 2) proposed typical level assessment scenario.

At the proposed maximum use levels, the mean exposure to long-chain glycolipids from *Dacryopinax spathularia* from its use as a food additive ranged from 0.01 mg/kg bw per day in infants to 1.07 mg/kg bw per day in toddlers. The 95th percentile ranged from 0 mg/kg bw per day in infants and toddlers, to 3.1 mg/kg bw per day in toddlers.

At the proposed typical use levels, the mean exposure to long-chain glycolipids from *Dacryopinax spathularia* from its use as a food additive ranged from < 0.01 mg/kg bw per day in infants and toddlers to 0.23 mg/kg bw per day in toddlers. The 95th percentile ranged from 0 mg/kg bw per day in the infants, to 0.64 mg/kg bw per day in toddlers.

The Panel noted that the estimated long-term exposures based on this scenario are likely to be an overestimation, as the exposure estimates assumed that 100% of the foods belonging to the FC 14.1.4 Flavoured drinks, FC 14.1.5.2 Other and FC 14.2.1 Beer and malt beverages, restricted to "Only alcohol free" will contain long-chain glycolipids from *Dacryopinax spathularia* at the typical or maximum proposed use levels.

The Panel noted that the highest estimate of exposure of 3.1 mg/kg bw per day (in toddlers) is within the established ADI of 10 mg/kg bw per day.

5. Conclusions

Based on the toxicological database available, the Panel derived an ADI of 10 mg/kg bw per day for long-chain glycolipids from *Dacryopinax spathularia* and concluded that the exposure to long-chain glycolipids from *Dacryopinax spathularia* does not raise a safety concern at the uses and use levels proposed by the applicant.

6. Recommendations

The Panel recommends the European Commission to consider:

- introducing the strain deposition number, i.e. MUCL 53181, in the definition of the proposed food additive;
- including a maximum limit for *Enterobacteriaceae* (< 10 CFU/g) as indicator of good hygiene practise in the EU specifications for the proposed food additive.

7. Documentation as provided to EFSA (if appropriate)

1) Dossier 'Technical dossier for the application by LANXESS Deutschland GmbH to authorise the new food additive glycolipids, E 246 ("AM-1"). May 2020. Submitted by LANXESS Deutschland GmbH.

2) Additional information on 22nd December 2020. Submitted by LANXESS Deutschland GmbH in response to a request from EFSA.
3) Additional information on 25th February 2021. Submitted by LANXESS Deutschland GmbH in response to a request from EFSA.
4) IMD Natural Solutions, 2012. Stability of IMD AM-1 in simulated gastric fluid (SGF). Unpublished report. Submitted in response to a request from EFSA.
5) Pharmacelsus Project 2012IMD001, 2013. Pharmacokinetic evaluation of IMD AM-1 after intravenous and oral administration to rats and identification of components and major metabolites in plasma. Unpublished report. Submitted in response to a request from EFSA.
6) Pharmacelsus Project 2014IMD001/2014IMD001b, 2014. Excretion and orientating mass balance analysis of IMD AM-1 after oral administration to rats. Unpublished report. Submitted in response to a request from EFSA.
7) Charles River, Testing Facility Study No. WIL-294501, 2017. Pharmacokinetics, Excretion Balance, and Tissue Distribution of [14C]-AM-1 and [14C]-LCFA following Administration to Rats. Unpublished report. Submitted in response to a request from EFSA.
8) BSL BIOSERVICE Study No.: 115763, 2012. Acute oral toxicity (Acute Toxic Class Method) with IM-11. Unpublished report. Submitted in response to a request from EFSA.
9) LPT Report No. 29803, 2015. Repeated Dose 90-day oral toxicity study of IMD AM-1 in CD Rats – Administration via drinking water. Unpublished report. Submitted in response to a request from EFSA.
10) Charles River Testing Facility Study No. WIL-294503, 2017. A 3-month toxicity study of IMD AM-1 by oral administration (capsule) in male and female beagle dogs. Unpublished report. Submitted in response to a request from EFSA.
11) TNO Triskelion Report V9905/07, 2012. Bacterial reverse mutation test with IM 11 grade A. Unpublished report. Submitted in response to a request from EFSA.
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13) WIL Research Project 510377, 2016. Evaluation of the mutagenic activity of IMD AM-1 in an *in vitro* mammalian cell gene mutation test with L5178Y mouse lymphoma cells. Unpublished report. Submitted in response to a request from EFSA.
14) Zhejiang Academy of Medical Sciences Test Report Registration No.: Y20170081, 2017. Acute oral toxicity test, mammal RBC Micronucleus Test. Unpublished report. Submitted in response to a request from EFSA.
15) Charles River Testing Facility Study No. WIL-294508, 2018. A two-generation reproductive toxicity study of IMD AM-1 administered orally by gavage in rats. Unpublished report.
16) Charles River Testing Facility Study No. WIL-294507, 2017. An oral (gavage) developmental toxicity study of IMD AM-1 in rats. Unpublished report.

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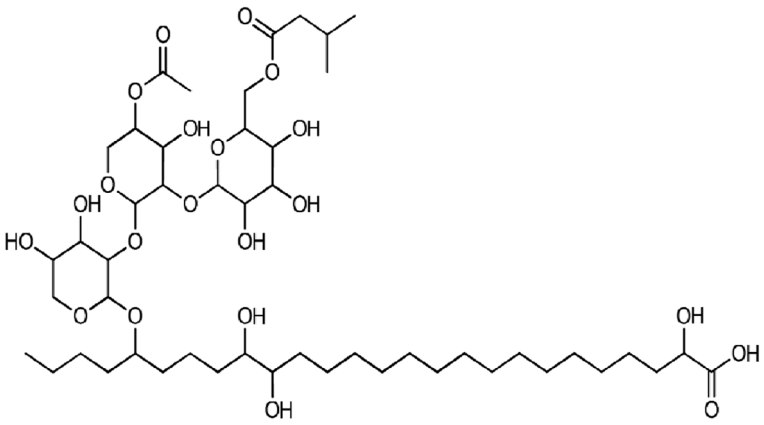
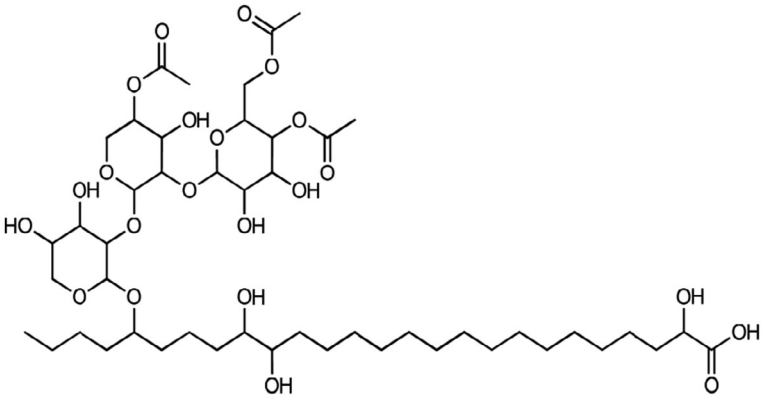
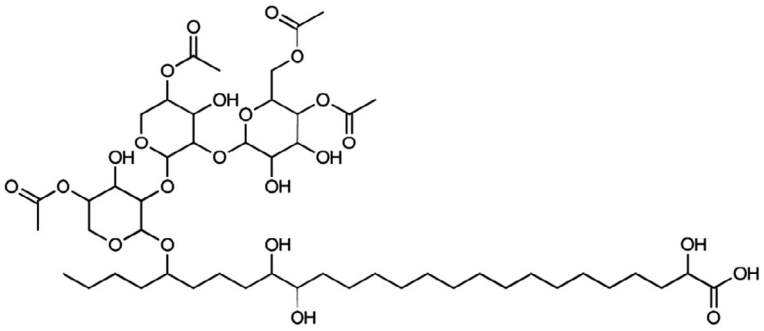
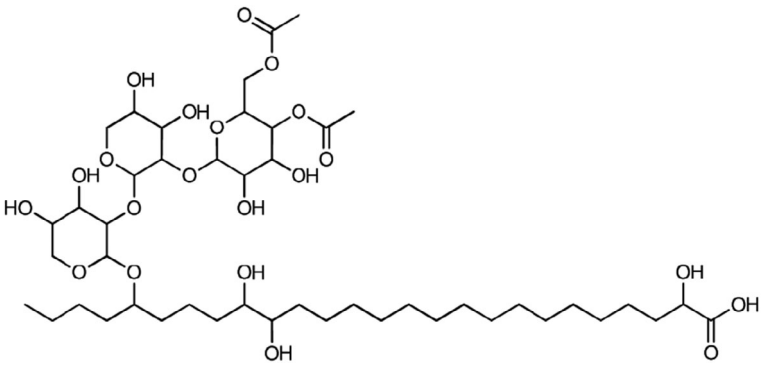
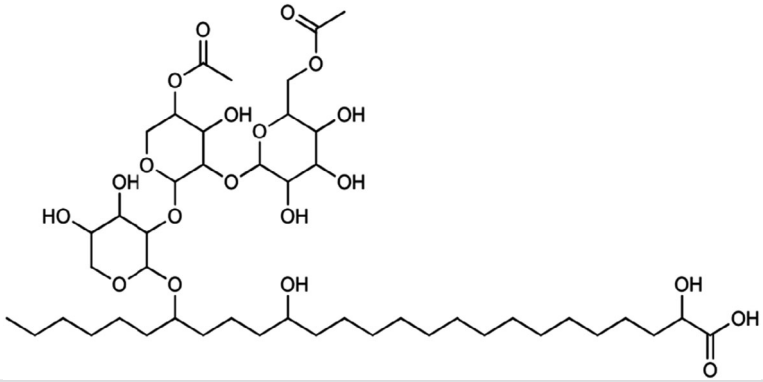
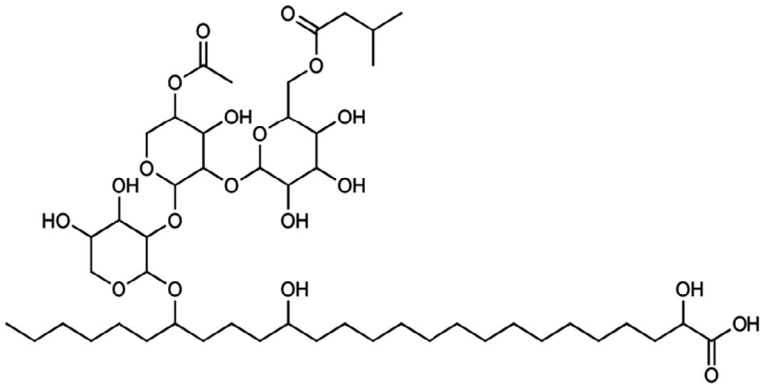
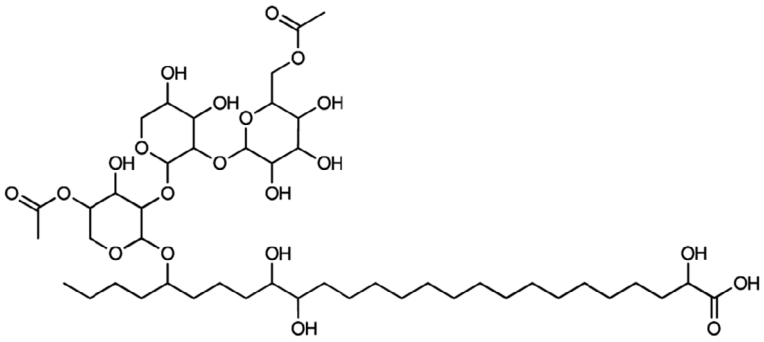
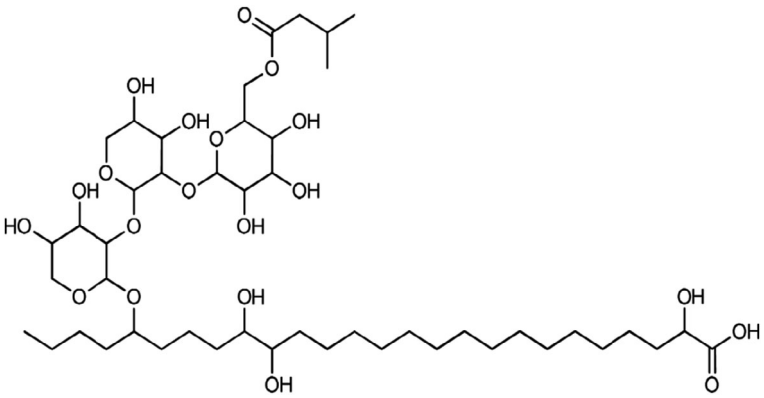
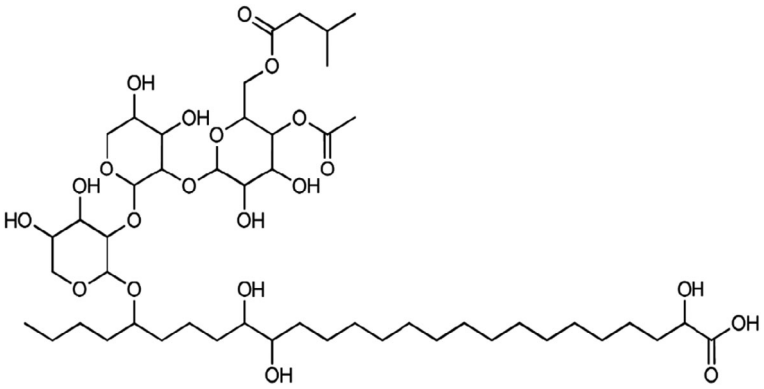
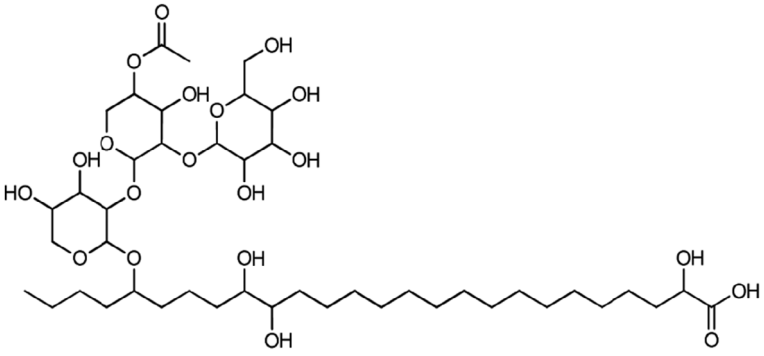
Abbreviations

ADI	acceptable daily intake
ADME	absorption, distribution, metabolism, excretion
FAF	EFSA Panel on Food Additives and Flavourings
FAIM	Food Additives Intake Model
FC	food category
FCS	food categorisation system
GLP	good laboratory practice
JECFA	Joint FAO/WHO Expert Committee on Food Additives
OECD	Organisation for Economic Co-operation and Development
LOD	limit of detection
MPL	maximum permissible limit
NOAEL	no observed adverse effect level
Qs	*quantum satis*

Appendix A – Main components of AM-1 mixture

Chemical name (CAS number when available)	Molecular structure
2,17,18-trihydroxyhexaco sanoic acid 22-O-[6-isovaleroylhexapyranosyl-(1→2)-5-acetylpentapyranosyl-(1→2)-pentapyranosid]-	
Glykenin – IIC (134528-36-2)	
	

Chemical name (CAS number when available)	Molecular structure
Glykenin IIIB or Glykenin IIIC 134528-37-3, 134479-71-3	
Antibiotic F 19848A 895129-04-1	
	

Chemical name (CAS number when available)	Molecular structure
Glykenin IVC or IVB 134479-72-4, 134528-38-4	   

Appendix B – Summary of total estimated exposure of long-chain glycolipids from *Dacryopinax spathularia* from their proposed use as food additives for the maximum and the typical level exposure assessment scenarios per population group and survey: mean and 95th percentile (mg/kg bw per day)

Appendix C – Main food categories contributing to exposure to long-chain glycolipids from *Dacryopinax spathularia* using the maximum proposed use level exposure assessment assessment (> 5% to the total mean exposure)

Appendix B and C can be found in the online version of this output ('Supporting information' section): https://doi.org/10.2903/j.efsa.2021.6609