SCIENTIFIC OPINION

Scientific Opinion on the safety of a "novel chewing gum base (Rev-7®)" as a Novel Food ingredient[1]

EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA)[2, 3]

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

Following a request from the European Commission, the EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA) was asked to deliver a scientific opinion on the safety of a 'novel chewing gum base (Rev-7®)'. The novel food ingredient (NI) is a synthetic polymer intended to be used in the formulation of chewing gum, with the aim of reducing its adhesive properties compared with conventional chewing gum. It consists of branched polymers of monomethoxypolyethylene glycol (MPEG) grafted onto polyisoprene-graft-maleic anhydride (PIP-g-MA), and unreacted MPEG (<35 %). The NI and its main ingredients have no history of use in foods within the EU. A detailed specification has been provided, including the toxicologically relevant compounds potentially present in the end product. The applicant intends to use a maximum of 8 % Rev-7® in the formulation of chewing gums. The maximum daily intake was calculated to occur in male teenagers at a level of 1.16 g/person per day. The specification sets limits for monomer residues, (heavy) metals, solvent residues, additives (BHT and lactic acid), and other impurities from the starting materials. The theoretical maximum exposures to these substances are below established safety limits. A maximum of 50 mg/kg for MPEG oligomers of <1000 Da has been set in the specification. The calculated maximum intake of MPEG oligomers is <0.058 mg. No genotoxicity or toxicity data on MPEG oligomers were provided, but because of its structural similarity to low molecular weight PEG, the exposure to MPEG oligomers causes no concern at the proposed use level. The toxicological data show that Rev-7® has low oral toxicity in rats after short-term administration (28 days) and is not genotoxic. Rev-7® is unlikely to cause food allergic reactions. The Panel concludes that the novel ingredient Rev-7® is safe at the proposed conditions of use and the proposed levels of intake.

KEY WORDS

Chewing gum, Rev-7®, polymer, monomethoxy polyethylene glycol, polyisoprene-graft-maleic anhydride, oligomer.

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SUMMARY

Following a request from the European Commission, the EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA) was asked to deliver a scientific opinion on the safety of a 'novel chewing gum base (Rev-7®)'.

The novel food ingredient, Rev-7®, is a synthetic polymer manufactured by the company Revolymer Ltd., intended to be used in the formulation of chewing gum base, the insoluble component of chewing gum. The aim is to give chewing gum reduced adhesive properties making it easier to remove from a variety of surfaces.

Rev-7® consists mainly of branched polymers of monomethoxypolyethylene glycol (MPEG) grafted onto polyisoprene-graft-maleic anhydride (PIP-g-MA), and unreacted MPEG (<35 % by weight). The production process entails the reaction of the two polymers MPEG and PIP-g-MA in a solvent-free system. No purification step is carried out after synthesis.

A detailed specification for Rev-7® has been provided including the toxicologically relevant low molecular weight compounds potentially present in the end product. The applicant also supplied specifications for the two starting materials since many of the low molecular weight substances in Rev-7® would have originated from these. Analytical data provided by accredited laboratories for four non-consecutive production batches of Rev-7® showed all of them to be within specification.

The Panel considers that the specification of Rev-7® and description of the production process are satisfactory.

The stability of Rev-7® has been demonstrated through accelerated and ambient stability studies and its shelf life has been set at three years.

The applicant has estimated intake levels of chewing gum for children, teenagers and adults based on UK National Diet and Nutrition Surveys. At the 95th percentile teenagers aged 12-18 years of age are estimated to consume 14.0 g/person per day and this represents the worst-case scenario. The applicant intends to use a maximum of 8 % Rev-7® in the formulation of chewing gums. The applicant has used a consumption level of 14.5 g/person per day (equivalent to five sticks of chewing gum) so that the worst-case intake of Rev-7® for teenagers would be 1.16 g/person per day.

Rev-7® has virtually no nutritional value as the chewing gum base is rarely swallowed. However, low molecular weight components (<1000 Da) could migrate from the polymer product during chewing. The applicant established that the monomers isoprene, maleic anhydride and ethylene oxide, oligomers, residues of the solvents hexane and methanol, the additives butylated hydroxytoluene and lactic acid, the metals aluminium, lithium and nickel, heavy metals and the substances 1:4 dioxane, formaldehyde, ethylene glycol, diethylene glycol, monoethylene glycol methyl ether, diethylene glycol methyl ether and triethylene glycol methyl ether are liable to be present in the novel ingredient. The applicant has compared the maximum limits for these substances in the specification with the reference values given in legislation or other literature. The applicant developed a MALDI-TOF (matrix assisted laser desorption/ionisation – time of flight)-based technique to estimate the amount of MPEG of <1000 Da in the raw material; the average value from four batches was <71 mg/kg. Since in the production process MPEG is less than 50 % of the raw materials, the applicant has set a maximum of 50 mg/kg in the specification for Rev-7®. At the calculated worst-case intake of the novel ingredient of 1.16 g/person per day, the intake of MPEG oligomers <1000 Da would be very low (<0.058 mg) and would approximate to the lowest level suggested in the SCF Guidelines for the safety assessment of substances to be used in Food Contact Materials. The Panel is satisfied that these substances do not raise concern at the levels given in the specification.

The applicant provided results from a 28-day feeding trial conducted in rats with a maximum dose of 6.9 g Rev-7®/kg bw per day without adverse effect. The applicant has carried out two *in vitro* and one *in vivo* genotoxicity studies. The Rev-7® extracts are not genotoxic.

The applicant has demonstrated that the concentration anticipated of maleic anhydride, a known skin sensitiser, in Rev-7® is insufficient to have skin sensitising potential. Given the nature of the novel ingredient, the Panel considers it unlikely that Rev-7® will induce food allergic reactions in the population.

The Panel concludes that the novel ingredient Rev-7® is safe at the proposed conditions of use and the proposed levels of intake.

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BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

On 10 October 2007, the company Revolymer Ltd. submitted a request under Article 4 of the Novel Food Regulation (EC) N° 258/97 to place on the market a 'novel chewing gum base' as a novel food ingredient.

On 23 April 2009, the competent authorities of the Netherlands forwarded to the Commission their initial assessment report, which came to the conclusion that the 'novel food chewing gum base' meets the criteria for acceptance as a novel food.

On 30 April 2009, the Commission forwarded the initial assessment report to the other Member States. Several of the Member States submitted comments or raised objections.

The concerns of a scientific nature raised by the Member States can be summarised as follows:

- Chemical specifications of all component parts of this novel ingredient (NI) have yet to be set out.
- Test data for verifying the specification are incomplete. In particular, the final specification of the molecular weight of MPEG and the data on molecular weight distribution cannot be verified. Information on the places of analyses and laboratory accreditations is lacking for some of the test results provided.
- The estimated intakes calculated by the applicant significantly underestimate the consumption of chewing gum and, consequently, the NI. Consumption by children, who would be likely to be amongst the highest consumers (by kilogram body weight) and may also be more likely to swallow chewing gum, is of particular concern. Consumption in adults is likely to be particularly high in specific sub-groups, including individuals who regularly consume chewing gum when dieting.
- An *in vitro* study showed that MPEG is released from chewing gum when chewing is simulated. There are uncertainties as to the actual amount that is released. Given that the molecular weight of MPEG is close to the threshold of 1000 Dalton, which is regarded as the threshold of toxicological concern, exact and verifiable data on the percentage of MPEG with a molecular weight of <1000 Dalton are indispensable.
- Studies of the behaviour and/or stability of the NI when it is consumed were carried out solely in respect of the release of MPEG. Other potential changes to the polymer were not examined.
- PEG is close to MPEG but not the same, thus the ADI determined for PEG cannot apply to MPEG without a specific assessment of the latter.
- This evaluation of the novel chewing gum base should follow the procedure for the risk assessment of food contact materials which requests that toxicological data on substances with a molecular weight of <1000 Dalton, which migrate into food, must be provided in relation to their levels in food.
- The NI contains oligomers that are not completely identified. There are no long-term toxicity studies. A large proportion of the population may be exposed to the NI. Thus, additional genotoxicity tests should be carried out. The *in vitro* genotoxicity test provided is not sufficient to rule out mutagenic and clastogenic effects on mammalian cells.
- Human studies to determine the fate of the NI during transit through the human gastrointestinal (GI) tract (e.g. effect of digestive enzymes, bacterial degradation, different pH conditions) are

lacking. REV-7® is a novel polymer that exhibits different adhesion properties to existing gum products. The effects of GI conditions on the visco-elastic nature of the polymer, which could lead to an increased risk of intestinal obstruction, particularly in children, should be considered.

- Reassurance as to potential effects of Rev-7® on the digestion or absorption of any nutrients or other bioactive components of food is lacking.

In consequence, EFSA is asked to carry out the additional assessment and to consider the elements of a scientific nature in the comments raised by the other Member States in accordance with Article 7, paragraph 1 of Regulation (EC) No 258/97.

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

In accordance with Article 29 (1) (a) of Regulation (EC) No 178/2002, the European Food Safety Authority is asked to carry out the additional assessment for a 'novel chewing gum base (Rev-7®)' as food ingredient in the context of Regulation (EC) N° 258/97.

EFSA is asked to carry out the additional assessment and to consider the elements of a scientific nature in the comments raised by the other Member States.

ASSESSMENT

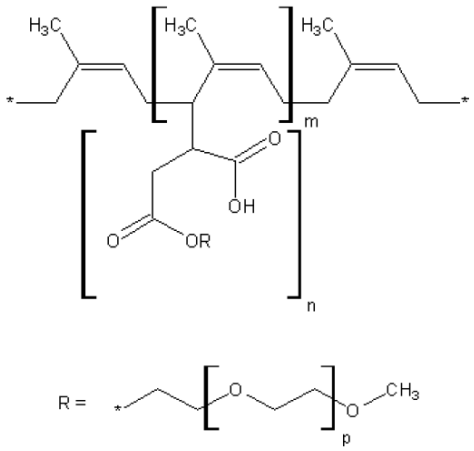
In accordance with the Commission Recommendation 97/618/EC, the 'novel chewing gum base (Rev-7®)' is allocated to Class 1.2, a food or food ingredient that is 'a single chemically defined substance or a mixture of these which is not obtained from plants, animals or microorganisms that have been genetically modified. The source of the NF has no history of food use in the Community'. The assessment of the safety of this novel food ingredient is based on data supplied in the original application, the initial assessment by the competent authority of the Netherlands, the concerns and objections of the other Member States and the responses of the applicant. The data are required to comply with the information required for the novel foods of Class 1.2, i.e. structured schemes I, II, III, IX, XI, XII and XIII of the Commission Recommendation 97/618/EC. In the text these structured schemes are listed 1 to 7. This assessment concerns only risk that might be associated with consumption and is not an assessment of the efficacy of the 'novel chewing gum base (Rev-7®)' with regard to any claimed benefit.

1. Specification of the Novel Food (NF)

The Novel Food ingredient (NI), Rev-7®, is a synthetic polymer manufactured by the company Revolymer Ltd. (Cosgrove et al., 2006; Patent number WO2006016179). Rev-7® is intended to be used as an ingredient in the formulation of chewing gum with the aim of giving chewing gums reduced adhesive properties compared with conventional chewing gums, thus making used gums easier to remove from a variety of surfaces.

Rev-7® mainly consists of branched polymers of monomethoxypolyethylene glycol (MPEG) grafted onto polyisoprene-graft-maleic anhydride (PIP-g-MA), and unreacted MPEG (<35 % by weight). According to the applicant, the molecular weight distribution or polydispersity index for Rev-7® will typically be 1.20 – 1.30.

MPEG groups are chemically bound on the PIP backbone via the maleic anhydride moiety. The molecular structure of the branched polymer is shown in Figure 1.



where m = 366 to 1,248, n = 3 to 15 and p = 44 to 50

Figure 1: Molecular structure of MPEG grafted PIP-g-MA.

PIP-g-MA is an elastomeric polymer, predominantly used in the manufacture of adhesive materials (Kuraray Co., Ltd., 2003). The maleic anhydride content is 1.2 % of the total weight of PIP-g-MA. The applicant indicates that the molecular weight of the PIP-g-MA material used for the production of the NI will range from 25,000 – 85,000 Daltons and the molecular weight distribution will be in the range 1.20 – 1.30.

MPEG is a commercially available polyethylene glycol (PEG) with one of the two terminal hydroxy groups capped by a methyl ether group. According to the applicant, the molecular weight of MPEG materials used for the production of the NI will range from 2,000 – 5,000 Daltons and the molecular weight distribution will be around 1.04.

The specification for REV-7® has been developed throughout the Novel Food evaluation process. A detailed specification was viewed by the Dutch competent authority to be of critical importance, as no further purification takes place after the synthesis of the NI. Thus, the applicant expanded the initial specification in order to reflect the possibility of components in the starting materials being present in the end product. Following the move to full-scale production (~150 kg batches), the applicant also requested changes to the specification, in order to take account of some variations in the measured impurity levels of certain contaminants (hexane, formaldehyde, butylated hydroxytoluene (BHT) and free MPEG). The specification of the NI accepted by the Panel is shown in Table 1.

According to the specification, Rev-7® contains <35 % free MPEG, <15 µmol/g residual maleic anhydride, and <5 % moisture content. In addition, the specification includes limits for monomer and oligomer residues, (heavy) metals, solvent residues, additives (BHT and lactic acid), and a number of impurities from the starting materials.

Analytical results from four non-consecutive full-scale production batches of Rev-7® provided by the applicant show them to be all within specification. For those contaminants that originate from the raw materials and are not increased during the production process, the applicant calculates the levels in Rev-7® from those measured in the raw material multiplied by the ratio of material added in the process e.g. BHT, which is present only in PIP-g-MA. The applicant has provided specification sheets for both PIP-g-MA and MPEG.

The applicant has provided the names and accreditations of all the laboratories that carried out the analyses that confirmed that the product complied with the specification.

Table 1: Specification for Rev-7®

		Method of analysis
Physical Properties		
Polydispersity index	<1.4	GPC
Free MPEG content	<35 %	GPC or HPLC
Residual anhydride	<15 µmol/g	FT-IR
Moisture content	<5 %	Karl Fisher
Colour	Off-white	Visual
Chemical Properties		
Monomer and Oligomer residues		
Isoprene	<0.05 mg/kg	GC
Ethylene Oxide	<0.2 mg/kg	GC
Free Maleic Anhydride	<0.1 %	GPC or GC
Total Oligomers (<1000 Daltons)	<50 mg/kg	GPC or MALDI-TOF
Solvent Residues		
Hexane	<8 mg/kg	GC
Methanol	<2 mg/kg	GC
Additives		
BHT	<800 mg/kg	GC
Lactic acid	<430 mg/kg	GC
Heavy metals		
Total Heavy Metals	<0.5 mg/kg	ICP-MS
Metals		
Aluminium	<3 mg/kg	ICP-MS
Lithium	<0.5 mg/kg	ICP-MS
Nickel	<0.5 mg/kg	ICP-MS
Microbiology		
Aerobic Colony Count	<1 x 10^3 cfu/g	Standard methodology
Yeasts	<100 cfu/g	
Moulds	<100 cfu/g	
Fungi	<100 cfu/g	
Enterobacteriaceae	<10 cfu/g	
E. coli	<10 cfu/g	
Staphylococcus aureus	<20 cfu/g	
Pseudomonas spp.	<20 cfu/g	
Salmonella spp.	Not detected (per 25g)	
Impurities from Raw Materials		
Ethylene Glycol	<200 mg/kg	GC
Diethylene Glycol	<30 mg/kg	GC
Monoethylene glycol methyl ether	<3 mg/kg	GC
Diethylene glycol methyl ether	<4 mg/kg	GC
Triethylene glycol methyl ether	<7 mg/kg	GC
1,4-Dioxane	<2 mg/kg	GC
Formaldehyde	<10 mg/kg	HPLC or UV

Stability analysis

The stability of Rev-7® was evaluated by the applicant in a 12-week accelerated stability study by storing samples from three batches under controlled conditions at 40 °C and 75 % relative humidity. In addition, samples were stored under ambient (laboratory) conditions and were analysed after approximately 2 and 3 years, respectively. Samples were tested for moisture content, microbiological contamination and relative amounts of free MPEG (polymer degradation).

In both experiments the moisture content of the polymer slightly increased upon storage; however, the water content remained below the limit given in the specification.

No pathogenic bacteria were detected in any of the stored Rev-7® samples. The levels of yeasts and moulds reached a maximum of 90 cfu/g and the total aerobic colony count reached a maximum of 1.8 x 10^3 cfu/g after 12 weeks in the accelerated stability study in one of the samples. This was above the specification of < 1.3 x 10^3 cfu/g. In the other two samples values were <50 cfu/g. The high value was attributed to poor sampling.

The applicant analysed the stability of the MPEG grafted PIP-g-MA polymer in Rev-7® for potential degradation of the MPEG chains from the PIP-g-MA backbone using gel permeation chromatography (GPC). The relative GPC peak areas of the MPEG grafted PIP-g-MA polymer and the free MPEG were compared. Under the accelerated storage conditions, there was a 6 % decrease of the ratio of Rev-7® : free MPEG; in the Rev-7® samples kept under ambient conditions for up to three years this ratio remained unchanged. In addition, the GPC chromatograms did not show changes in retention times of individual components or appearance of new peaks.

The batches were also tested for formaldehyde, methyl glycol ethers, ethylene and diethylene glycols, and 1,4-dioxane after three years and all values were within the specification.

The applicant intends to allocate a shelf life of three years from manufacture to Rev-7® batches.

The Panel has no concerns regarding the specification and the stability of Rev-7®.

2. Effect of the production process applied to the NF

Rev-7® is produced using a solvent-free reaction in which PIP-g-MA and MPEG are placed in a mixer in an approximate stoichiometric ratio of 1.5 to 1.0 and heated to induce a reaction. The reaction time and temperature are confidential. During the mixing process, the MPEG grafts onto the PIP-g-MA by formation of an ester bond between the maleic anhydride component of the PIP-g-MA polymer and the hydroxyl group of MPEG. The disappearance of the anhydride group, as the MPEG opens the ring of the anhydride and becomes chemically linked to the PIP-g-MA, is monitored using Fourier Transform Infrared Spectroscopy (FT-IR).

The finished product is a mixture of branched polymers consisting of MPEG grafted onto PIP-g-MA, and free, unreacted MPEG. If necessary, water is added at the end of the reaction to remove the excess of maleic anhydride by hydrolysis. No purification step is undertaken after synthesis. The polymer is extruded into the required form for gum base manufacture.

The initial dossier from the applicant described the pilot-scale production process and provided information on the scale-up procedures, including process controls. The applicant has confirmed that the full-scale process for the manufacture of Rev-7® does not differ significantly from the pilot-scale production process. A process flow diagram for the full-scale process has been supplied for HACCP requirements.

The Panel has no concerns regarding the production process.

3. History of the organism used as a source

The NI and its main ingredients have no history of use in foods within the EU.

The applicant notes that synthetic polymers and natural polyisoprenes are commonly used as gum base ingredients by chewing gum manufacturers. The applicant refers to a report from the UK Food Advisory Committee concerning the safety of synthetic gum bases (UK Food Advisory Committee, 1994) and to the 5th edition of the Food Chemicals Codex (IoM, 2003), which lists the authorised polymers and their specification. Synthetic polyisoprene is not permitted as a synthetic polymer for chewing gum bases.

Polyethylene glycols are synthetic polymers identified by a number approximating to their corresponding molecular weight, e.g. PEG 2000. They are widely used in the pharmaceutical industry as a coating agent for tablets and capsules, as a film-coating agent for food supplements, as excipients in sweetener based tablets and as carrier solvents for sweeteners. In the US, PEGs with a mean molecular weight of 200 to 9500 are permitted for use as a direct, multipurpose food additive. In the EU, PEGs 400-8000 are authorised as carriers for sweeteners (E1521, Directive 95/2/EC[4]). MPEGs are structurally very similar to PEGs, the only difference regards the methylation of one of the two terminal hydroxyl groups in MPEG. Considering the molecular weight of the employed MPEG 2000, the presence of the methoxy group at the end of the long polymer chain as sole difference, it is not expected to be of significant impact on the grafted MPEG compared to PEG. However, the Panel notes that no history of use in food has been reported for MPEGs.

4. Anticipated intake/extent of the use of the NF

Rev-7® is to be used as an ingredient in chewing gum base material. A chewing gum base is typically a mixture of substances that form the (insoluble) basis of the chewing gum, to which other ingredients, such as soluble sweeteners and flavourings, are added. Flavourings and sweeteners typically constitute 50 to 70 % by weight of the final chewing gum product (Douglas, 2006). The applicant indicated that the level of Rev-7® in chewing gum would range from 3 to maximum 8 % by weight.

The applicant originally estimated Rev-7® intake levels on the basis of chewing gum commercial data (Wrigley company data; Datamonitor, 2002; Leatherhead, 2003) and Codex data regarding average daily use of chewing gum in the EU (JECFA, 2006a). Based on the highest estimated intake of chewing gum of 4.8 g per day for adult heavy consumers, and a higher level of incorporation of Rev-7® of 15 %, the applicant had initially estimated a maximum exposure level of 0.72 g of Rev-7® per day.

In response to those Member States that considered that this figure significantly underestimated consumption by chewing gum users, the applicant reviewed the estimate using recent data from the UK National Diet and Nutrition Survey (NDNS) (Table 2).

[4] European Parliament and Council Directive No 95/2/EC of 20 February 1995 on food additives other than colours and sweeteners, OJ L 61, 18.3.1995, p. 1-63.

Table 2: Estimates of daily intakes of chewing gum by users only, from UK NDNS data

Population group	Age (years)	Chewing gum consumption (g / person per day)		
		Mean	90th percentile	95th percentile
Children	4 - 11	2.10	4.86	6.00
Teenagers	12 - 18	3.25	6.86	14.00
Adults	19 - 64	1.71	2.86	8.57

The applicant produced three scenarios corresponding to consumptions of 1, 3, and 5 pieces of chewing gum per day, deemed to represent approximate occasional, mean, and upper level consumption. The calculations have been based on the higher weight commercial chewing gums, i.e. 2.9 g for traditional "stick gum" type. At 5 servings per day, the estimated total weight of chewing gum consumed is 14.5 g, corresponding to an estimated exposure level of 1.16 g of Rev-7® per day for the highest level of incorporation of 8 % (Table 3).

Table 3: Daily Intake Estimates for Rev-7® at 8 % incorporation in chewing gums

	Chewing gum consumption		
	1 piece of chewing gum (2.9 g)	3 pieces of chewing gum (8.7 g)	5 pieces of chewing gum (14.5 g)
Rev-7® intake (g)	0.23	0.70	1.16

5. Nutritional information on the NF

The applicant states that Rev-7®, as other synthetic gum bases, has no nutritional value as it is not digested and absorbed by the human body.

In response to Member States' concerns as regards impairment of the digestion or absorption of nutrients or other bioactive components of food in case the chewing gum is swallowed, the applicant noted that chewing gums are typically consumed without other foods and that occurrence of swallowing is normally low. The applicant concluded that any significant impact of Rev-7® on the absorption of nutrients is unlikely.

In response to Member States' concerns as regards potential changes in the visco-elastic properties of chewing gum containing Rev-7® in case it should be swallowed, the applicant commissioned a study to characterise rheological properties of the NI during transit in the GI tract (Titoria, 2009, unpublished). A chewing gum containing Rev-7® (7.8 % by weight) was compared to three commercial chewing gums. Chewing gum samples were introduced to an *in vitro* simulator of the human intestinal microbial ecosystem, which mimics the conditions of the GI tract, and were then subjected to small deformation (non-destructive) or large deformation (destructive) techniques.

After application of small deformation, the chewing gum containing Rev-7® reverted to the original rheological properties, whilst one commercial gum became harder and the other two commercial gums became softer. Large deformation rheological measurements indicated a behaviour of the chewing gum containing Rev-7® similar to the three commercial gums, in terms of increased hardness and reduction of stickiness. The applicant concludes that the changes that would occur to chewing gum containing Rev-7® during transit through the GI tract would fall in between a typical commercial range and that there would be no additional risk of intestinal obstruction.

Based on the information provided on the composition and the proposed maximum use level, the Panel considers the consumption of Rev-7® in chewing gum as not nutritionally disadvantageous.

6. Microbiological information on the NF

Microbiological limits are set in the product specification (Table 1). The applicant notes that microbiological contamination of the product is unlikely, because of the high temperatures involved in the production (above sterilising temperature) and the application of HACCP procedures.

The Panel has no concerns with regard to the microbiological safety of Rev-7®.

7. Toxicological information on the NF

7.1. Toxicological information on individual constituents

7.1.1. Polymers

7.1.1.1. PIP-g-MA

According to the applicant, no toxicological information on PIP-g-MA is available. There is toxicological information on polyisoprene, which indicates that the oral LD_{50} in rats is more than 2,000 mg/kg bw (Kuraray Co., Ltd., 2003). However, the full report is not available.

7.1.1.2. Free MPEG content

According to the specification, the NI contains up to a maximum of 35 % of free MPEG. The applicant argues that most of the MPEG polymers will not be released from the chewing gum during normal use.

An *in vitro* study of the degradation effects of simulated saliva and gastric juices and the consequent release of free MPEG was carried out. Samples of chewing gum containing 12 % Rev-7® (32 % free MPEG) were placed in an artificial masticator and "chewed" for 60 minutes in artificial 'saliva'. The simulated 'saliva' was then analysed by gel permeation chromatography (GPC) for free MPEG. Ten samples were analysed. The average amount of free MPEG released was 0.059 +/-0.010 mg/g of chewing gum (0.156 % released). However, results were highly variable (min: 0.024; max: 0.107 mg/g). In order to evaluate the effect of gastric juices, samples of chewing gum containing 12 % Rev-7® were stirred overnight in simulated 'gastric juices'. Free MPEG in the 'gastric juices' were then measured using GPC. The average amount of free MPEG released was 0.23 +/- 0.02 mg/g of chewing gum (0.605 %) (min: 0.129; max: 0.335 mg/g). Considering the estimated intake level of 1.16 g/day of Rev-7® for a high consumer (i.e. 14.5 g chewing gum with a 8 % incorporation), the level of exposure to free MPEG would amount to 0.62 mg in 'saliva' and 2.43 mg of free MPEG in 'gastric juices' in case the chewing gum is swallowed. Thus, the highest exposure level to free MPEG for high consumers of Rev-7® would be 3.05 mg/day. This is considering all five pieces of chewing gum are swallowed.

In response to Member States who questioned the reliability of the results obtained by this simulation, the applicant provided a further assessment of a worst-case scenario where all free MPEG is assumed to be ingested and available to the body. Considering the maximal level of 35 % of free MPEG set in the specification and the highest estimated daily consumption of 14.5 g of chewing gum at the level of incorporation of 8 %, the calculated MPEG exposure amounts to 406 mg. The applicant noted that the 28-day study in rats (described in section 7.2.2) showed no indication of adverse effects up to the

highest dose of Rev-7® tested of approximately 6.9 g/kg bw/day, equivalent to 414 g Rev-7® per day (for a 60 kg consumer) containing 132.5 g free MPEG (considering 32 % of MPEG in the batch used for the study), a margin of >300.

The Panel considers that under normal circumstances where the chewing gum base is not (or only occasionally) swallowed the amount of free MPEG consumed does not present a safety risk.

7.1.2. Low Molecular Weight components

7.1.2.1. Monomer and oligomer residues

Rev-7® may contain monomers and oligomers derived from the synthesis of the starting materials, PIP-g-MA and MPEG. Therefore, the specification for Rev-7® includes limits for the levels of isoprene, free maleic anhydride, ethylene oxide, isoprene oligomers and MPEG oligomers.

Isoprene is classified as possibly carcinogenic for humans (IARC, 1999). However, in low concentrations, it is present as a normal metabolite in biological systems. The applicant provided data demonstrating that isoprene is not detected in Rev-7® above the detection limit of 0.05 mg/kg. This is the limit chosen for the product specification. At this maximum level, the theoretical maximum exposure scenario would amount to 0.058 µg/day. This is low in relation to natural exposure to isoprene, including endogenous isoprene typically 10-48 µg/L in blood (NTP, 2008).

In line with the SCF recommendations (2002a) to restrict ethylene oxide as an impurity below its current limit of detection, and the EFSA opinion on the safety of polyethylene glycol (PEG) used as a film coating agent in food supplements (2006a), the maximum limit for ethylene oxide in Rev-7® is set at 0.2 mg/kg. At this maximum amount, the theoretical maximum exposure scenario corresponds to a daily intake of 0.23 µg.

The maximal limit for free maleic anhydride is set at 0.1 %. At the maximal level set in the specification, there would be a maximum exposure scenario of 1.16 mg maleic anhydride per day. The group tolerable daily intake (TDI) for maleic anhydride and maleic acid is set at 0.5 mg/kg bw as maleic acid, corresponding to 30 mg for a 60 kg person (SCF, 1986).

According to SCF guidelines for the safety assessment of polymers to be used in food contact materials, the fraction with a molecular weight <1000 Daltons is deemed to be toxicologically relevant (SCF, 2001). In response to Member States' concerns as to the composition and safety of the oligomeric fraction of Rev-7®, the applicant undertook quantification and composition analysis of the fraction below 1000 Daltons.

GPC analysis of PIP-g-MA showed that the lowest detectable molecular weight species in Rev-7® is ~ 5000 Da. The applicant took the view that, given the high molecular weight of the polyisoprene polymer and its inherent stability, it is unlikely that any of the oligomer present would come from the polyisoprene component. The oligomeric fraction would rather come from the MPEG 2000 starting material.

The applicant commissioned an analysis to determine the oligomer content below 1000 Da in MPEG 2000 by a MALDI-TOF (matrix assisted laser desorption/ ionisation – time of flight) technique. The lowest molecular weight species in MPEG 2000 was ~ 1200 Da. By comparing the summation of the mass/charge (m/z) x intensity values for the peaks below 1000 Da to the summation of the m/z x intensity values for the entire distribution, the maximum percentage (by mass) of the MPEG below 1000 Da was estimated to be 71 mg/kg (average of four batches). This calculation is likely to overestimate the oligomeric fraction.

Based on this value, the applicant proposes to consider a maximum limit of 100 mg/kg for the MPEG raw material and, because of its dilution in the manufacture of Rev-7®, the product specification will be <50 mg/kg for total oligomers below 1000 Da.

One Member State suggested that for oligomers of MPEG <1000 Da, the amount in Rev-7® should be considered in the light of the SCF Guidelines on the safety assessment of substances to be used in Food Contact Materials (SCF, 2001). The amount of MPEG <1000 Da in 1.16 g of Rev-7® consumed per person per day as a worst case scenario would be <0.058 mg which approximates to the lowest migration level, for which a limited toxicology data set is required in the SCF Guidelines. The applicant did not provide genotoxicity or toxicity data on MPEG <1000 Da but because of its structural similarity to low molecular weight PEG, the Panel considers that this low level of migration of MPEG <1000 Da from the chewing gum base causes no concern at the proposed use level.

7.1.2.2. Solvent residues

Hexane and methanol are used for the synthesis of the starting materials PIP-g-MA and MPEG. Thus, Rev-7® may contain residues of these solvents.

The specification limit for hexane is set at 8 mg/kg. The applicant calculated that this would relate to a maximum of 0.64 mg of hexane per kg of chewing gum. According to the applicant, this complies with the maximum amount of hexane permitted as extraction solvent used in foodstuffs and food ingredients of 1 mg/kg set in Directive 2009/32/EC[5]; the Directive lays down maximum hexane levels for various types of food: 1 mg/kg in fat, oil and cocoa butter, and higher standards for certain named defatted products. Chewing gum is not among the named products, but the hexane content cited by the applicant is less than the lowest standard set in the Directive.

For methanol, the specification limit is set at 2mg/kg. This corresponds to a maximum exposure scenario of 2.3 µg per day. The maximum permitted in foodstuff is of 10 mg/kg (Directive 2009/32/EC[5]).

7.1.2.3. Additives

The starting materials PIP-g-MA and MPEG may contain the antioxidant BHT. Therefore, a specification limit for Rev-7® is set at 800 mg/kg. The applicant indicates that BHT is allowed in chewing gums up to 400 mg/kg in combination with other anti-oxidants (Codex guidelines[6]). At the proposed maximum level in the specification, the level of residual BHT from Rev-7® would be of maximum 64 mg/kg chewing gum, in compliance with Codex guidelines for combined antioxidants.

The applicant also set a maximum level for lactic acid (from MPEG) of 430 mg/kg. Lactic acid is an authorised additive (E270). JECFA considered it not necessary to establish an ADI for this substance (JECFA, 1974).

7.1.2.4 Metals

The overall limit for heavy metals is set at 0.5 mg/kg. This corresponds to a maximum exposure scenario of 0.58 µg per day. This is well below the safety limits determined for antimony (TDI: 360 µg/day; WHO, 2003), arsenic ($BMDL_{01}$: 0.3 - 8 µg/kg bw per day; EFSA, 2009a), mercury (TWI: 0.7 - 1.6 µg/kg; EFSA, 2004), lead (BMDL: 0.5-1.5 µg/kg bw; EFSA, 2010) and cadmium (TWI: 2.5 µg/kg bw; EFSA, 2009b).

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

[6] Codex general Standard for Food Additives (GSFA) Online Database, Chewing gum (05.3), Butylated hydroxytoluene (BHT), INS No 321.

The applicant indicates that Rev-7® may also contain traces of aluminium, lithium and nickel, derived from the starting material PIP-g-MA and set specification limits for these three metals. For aluminium, the specification limit is set at 3 mg/kg. According to the maximum exposure scenario, this corresponds to a theoretical maximum daily intake of 3.5 µg, well below the TWI level of 60 mg/week (EFSA, 2008). For lithium, the specification limit is set at 0.5 mg/kg. According to the maximum exposure scenario, this corresponds to a theoretical maximum daily intake of 0.58 µg, well below the proposed TDI of 0.6 mg/day (EFSA, 2006b). For nickel, the specification limit is set at 0.5 mg/kg. According to the maximum exposure scenario, this corresponds to a theoretical maximum daily intake of 0.58 µg, well below the proposed TDI of 3 mg/day proposed by Baars et al. (2001).

7.1.2.5. Other impurities derived from the starting materials

Mono- and diethylene glycol, and 1,4-dioxane are by-products of the PEG synthesis (EFSA, 2006a). For mono- and diethylene glycol, the maximum levels in the Rev-7® specification are set at 200 mg/kg and 30 mg/kg respectively. The theoretical maximum exposure scenario for these substances would amount to 0.23 mg and 0.03 mg per day respectively. These levels are well below the group TDI of 30 mg/day set for both substances (SCF, 2002b).

The applicant has limited the level of 1,4-dioxane permitted in Rev-7® to 2 mg/kg. This would correspond to a maximum exposure scenario of 2.3 µg per day. The applicant indicates that 1,4-dioxane level should be kept as low as technically measurable (SCF, 2002b).

The specification of MPEG includes limits for the ethylene glycol methyl ethers: monoethylene glycol, diethylene glycol and triethylene glycol methyl ethers. This was reflected by the applicant in the final specification of Rev-7®, where maximum levels permitted are 3, 4 and 7 mg/kg respectively. The corresponding maximum exposure scenarios are of 3.5, 4.6 and 8.1 µg/day respectively. The applicant carried out a search of the literature and did not find any tolerable daily intake values or NOAEL for these compounds. The applicant used the Threshold of Toxicological Concern (TTC) approach to determine safe daily intake levels for these substances and determined that an intake below 90 µg per day for each of these substances would not constitute a safety concern.

The final specification limits the formaldehyde content to 10 mg/kg. The corresponding maximum exposure scenario is of 11 µg/day. The applicant indicates that it is well below the TDI of 150 µg/kg bw per day in drinking water (WHO, 1996).

The Panel accepts this approach and has no concerns relating to the safety of low molecular weight substances present in Rev-7® at the levels given in the specification.

7.2. Studies undertaken with the NI

7.2.1. Genotoxicity studies

Tests on gene mutations in bacteria were conducted in accordance with OECD Guideline 471 (OECD, 1997a). Using five strains of *Salmonella enterica* var. Typhimurium (TA98, TA100, TA1535, TA1537 and TA102) Rev-7®, dissolved in tetrahydrofuran, was not mutagenic with and without metabolic activation (S9) up to the highest tested concentration of 5 mg/plate (Ballantyne, 2008, unpublished; Farber et al., 2010).

Rev-7® was also tested for genotoxic activity using L5178Y $TK^{+/-}$ mouse lymphoma cells with and without metabolic activation (S9) (Kraft et al., 2008, unpublished; Farber et al., 2010). The tests were conducted in accordance with OECD Guideline 476 (OECD, 1997b). Rev-7® was extracted in a cell culture medium (RPMI + 3 % horse serum) at a weight/volume ratio of 0.2 g/mL. Rev-7® was tested using a 4-h exposure at levels up to 86.4 % of the extracted material, with and without S9 and no

increase of the mutation frequency was observed. In a second experiment, the negative result obtained in the presence of S9 was confirmed up to a level of 100 % of the extract. Without S9, using an exposure period of 24-h at levels up to 95 % of the extract, a dose-related increase in the mutation frequency was observed at the two highest tested levels. At these levels, the relative total growth was reduced, indicating cytotoxicity of the test material. According to the study report, colony sizing indicated potential clastogenic effects. The authors concluded that Rev-7® was mutagenic in this assay. However, considering internationally agreed criteria (Moore et al., 2007), the Panel concludes that the results are inconclusive.

Rev-7® potential genotoxicity was further investigated through a mammalian erythrocyte micronucleus test using mice (Hofman-Hüther et al., 2008, unpublished; Farber et al., 2010). Rev-7® was extracted in 0.9 % NaCl (polar) and Cottonseed Oil (non-polar) at a weight/volume ratio of 0.2 g/mL. The concentrations tested were 20, 50 and 100 % extract concentrations (polar and non-polar). The volume administered intraperitoneally was 10 mL/kg bw. Peripheral blood samples were collected for micronuclei analysis 44h and 68h after application of the test extracts. The procedures used in this study were in accordance with OECD Guideline 474 (OECD, 1997c). In a preceding toxicity study, the animals treated with polar extracts showed no signs of systemic toxicity. Animals treated with non-polar extracts at 50 and 100 % concentrations showed signs of systemic toxicity. No biologically relevant increase in the number of micronucleated erythrocytes was found after treatment with the Rev-7® extracts. The Panel concludes that the Rev-7® extracts were not genotoxic under the conditions of the assay.

7.2.2. Repeated-dose oral toxicity

The applicant has undertaken a 28-day feeding study in rats with Rev-7® (not further specified/characterised) (Lowe et al., 2008, unpublished; Farber et al., 2010), which was conducted in accordance with OECD Guideline 407 (OECD, 2008). Four groups of Sprague-Dawley rats (10/sex/group) received a diet containing 0 (Group 1), and approximately 30 (Group 2), 50 (Group 3) or 80 (Group 4) mg of Rev-7®/kg diet. The mean overall daily dose of Rev-7® in male rats was 0, 2,394, 4,160 and 6,879 mg/kg bw/day for Groups 1-4 respectively. For the same dietary concentrations, the mean overall daily intake of Rev-7® in female rats was 0, 2,352, 4,182 and 6,844 mg/kg bw/day, respectively.

During the treatment period no mortality occurred and daily observations, as well as weekly detailed observations of the animals, did not reveal clinically relevant effects. Ophthalmoscopic examinations at the end of the treatment period showed that all eyes were normal. Body weight, body weight gain, food consumption and food efficiency were comparable in all groups. No relevant differences between groups were observed in Functional Observational Battery (FOB) and motor activity tests. Haematology analysis showed single statistically significant differences only in female animals of the low- and mid-dose groups compared with the control group, which were not dose-related and thus considered incidental. No differences were found in coagulation and urinalysis parameters. Clinical chemistry analysis showed a higher total protein level in males of the high-dose group and a higher blood urea nitrogen level in females of the high-dose group, as well as a lower calcium level in females of the mid- and high-dose groups.

Since there were no changes in related parameters, which might indicate specific organ toxicity, and no histopathological changes in microscopic examinations of organs and tissues at necropsy, the observed differences were not considered toxicologically relevant. Organ weight determinations showed a lower absolute mean liver weight in males of the mid-dose group and lower mean relative spleen weights (in relation to brain weight) in females of the low-dose group, which were both regarded as incidental findings. It is concluded that there were no indications of adverse effects up to the highest tested dose of approximately 6.9 g/kg bw/day.

8. Allergenicity

The applicant referred to recent studies that indicated skin-sensitising potential for PIP-g-MA (Shimamura, 2006), the parent compound of Rev-7®. The applicant attributed the skin sensitisation potential of PIP-g-MA to the maleic anhydride grafts reacting with skin proteins. The applicant provided results from follow-up research in which the sensitising capacity of Rev-7® was assessed in the Local Lymph Node Assay (Dreher, 2007; Farber et al., 2010). In a first study using Rev-7® containing 9 µmol/g residual graft maleic anhydride at concentrations of 10, 25 and 50 % weight/volume ratio, stimulation indices of 1.44, 1.98 and 1.09 respectively were found. In a second study using Rev-7® with a higher content of 21 µmol/g residual graft maleic anhydride, stimulation indices of 1.16, 1.12 and 1.20 respectively were found at concentrations 5, 10 and 25 % weight/volume ratio. There was no dose-response relationship, and all values were below the threshold level of 3.0, which is considered as the indicator of the potential to cause skin sensitisation. Maleic anhydride itself is a sensitizer, but the amount of free maleic anhydride in Rev-7® is less than 1 %, and for this reason the formulation of Rev-7® with free maleic anhydride is not considered as sensitising. The Panel considers that the food ingredient is unlikely to cause skin sensitisation.

The Panel also notes that no information concerning potential food allergenic properties of the novel ingredient was provided. Given the nature of Rev-7®, the Panel considers it unlikely that the food ingredient will induce food allergic reactions in the population.

DISCUSSION

The specification for Rev-7® shown in Table 1 has been developed during the assessment process and analytical data from pilot plant and full scale production batches have confirmed that the novel ingredient conforms to the specification. The analytical methods, the laboratories carrying out the analyses and their accreditation have been described by the applicant. In particular the molecular weights of the polymers and their distribution have been confirmed.

There were concerns from Member States that the estimated intake of chewing gum at the 95th percentile of 4.8 g/person/day was too low. The applicant has now used data from the UK NDNS to determine a worst case scenario (95th percentile for teenagers) of 14.0 g/person/day and has used the weight of five pieces of gum totalling 14.5 g containing 8 % Rev-7® to recalculate the amount of Rev-7® ingested as 1.16 g/person/day (as opposed to 0.72 g at 15 % incorporation in the initial dossier). The Panel accepts this recalculated value.

Some concern was expressed by Member States with regard to children being more likely to swallow chewing gum than older groups and that for all consumers there needed to be evidence that the viscoelastic properties of the novel polymer would not cause intestinal obstruction particularly in children. Information was provided to show that even if all the chewing gum is swallowed (an unlikely scenario) at a worst case of five chewing gums per day, there would be a large safety factor (>300) compared with the maximum amount of Rev-7 consumed by rats in a 28-day feeding study with no observed adverse effects. The applicant has demonstrated that the viscoelastic properties of chewing gum made with Rev-7® are not unusual when compared with traditional chewing gums. The applicant also stressed that chewing gum is not normally swallowed and is not eaten with other foods so that interference with digestion or absorption of nutrients is unlikely.

The novel ingredient has virtually no nutritional value as it is not, or rarely, swallowed. However, low molecular weight components (<1000 Da) could migrate from the polymer product during chewing. The applicant established that the monomers isoprene, maleic anhydride and ethylene oxide, oligomers, residues of the solvents hexane and methanol, the additives butylated hydroxytoluene and lactic acid, the metals aluminium, lithium and nickel, heavy metals and the substances 1,4-dioxane, formaldehyde, ethylene glycol, diethylene glycol, monoethylene glycol methyl ether, diethylene glycol methyl ether and triethylene glycol methyl ether were liable to be present. The applicant has

compared the maximum limits for these substances in the specification and has shown them to be below reference values given in legislation or other literature. The Panel considers that these substances do not cause concern at the level given in the specification.

There was some concern expressed that the amount of MPEG with a molecular weight of <1000 Da, and therefore of toxicological concern, should be quantified. The applicant developed a MALDI-TOF method and used it to estimate the amount of MPEG of <1000 Da in the polymer. The value was <71 mg/kg in the MPEG and less in the novel ingredient, because there is <50 % MPEG used in the preparation of Rev-7®. The applicant has set a limit of <100 mg/kg in the raw material and <50 mg/kg in the novel ingredient for total oligomers of <1000 Da.

One Member State suggested that for oligomers of MPEG <1000 Da the amount in Rev-7® should be considered in the light of the SCF Guidelines on the safety assessment of substances to be used in Food Contact Materials (SCF, 2001). The amount of MPEG <1000 Da in 1.16 g of Rev-7® consumed per person per day as a worst case scenario would be <0.058 mg which approximates to the lowest migration level, for which a limited toxicology data set is required. The applicant did not provide genotoxicity or toxicity data on MPEG <1000 Da but because of its structural similarity to low molecular weight PEG the Panel considers that this level of migration of MPEG <1000 Da from the chewing gum base causes no concern at the proposed use level.

The toxicological data show that Rev-7® has low oral toxicity in rats after short-term administration (28 days), is not genotoxic and is unlikely to cause food allergic reactions in the population.

The applicant has demonstrated using the Local Lymph Node Assay that the amount of free maleic anhydride in Rev-7® is insufficient to have skin-sensitising potential. Taking into consideration the chemical nature of the novel food ingredient, the Panel considers it unlikely to induce food allergic reactions in the population.

On the basis of all the data provided the Panel concludes that Rev-7® can safely be used as an ingredient of chewing gum at a maximum of 8 %, provided it meets the specification described.

CONCLUSIONS

The Panel concludes that the novel food ingredient Rev-7® is safe at the proposed conditions of use and the proposed levels of intake.

DOCUMENTATION PROVIDED TO EFSA

1. Dossier "Application for Novel Foods Approval Rev-7® Polymer for Low Tack Gum Base" received on 07 July 2010. Submitted by Revolymer Ltd. on 10 October 2007. Additional data were provided 19 November 2010 and 17 December 2010.

2. Letter from the European Commission to the European Food Safety Authority with the request for an opinion on the safety of a 'novel chewing gum base (Rev-7®)'. SANCO E6/AK/bs Ref. Ares (2010)390452, dated 2 July 2010.

3. Initial assessment report carried out by the Netherlands: Synthetic chewing gum base ingredient (Rev-7®), Assessment of safety for consumer, in accordance with European regulation 258/97 concerning novel foods and novel food ingredients, Novel Foods Unit, the Minister of Health, Welfare and Sport of the Netherlands.

4. Member States' comments and objections.

5. Response by the applicant to the initial assessment report and the Member States' comments and objections.

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GLOSSARY AND ABBREVIATIONS

ADI	Acceptable daily intake
BHT	Butylated hydroxytoluene
BMDL	Benchmark dose lower confidence limit
GC	Gas chromatography
GPC	Gel permeation chromatography
NI	Novel ingredient
FOB	Functional observational battery
FT-IR	Fourier transform infrared spectroscopy
GI	Gastrointestinal
ICP-MS	Inductively coupled plasma mass spectrometry
HPLC	High performance liquid chromatography
MALDI-TOF	Matrix assisted laser desorption/ionisation – time of flight
MPEG	Monomethoxypolyethylene glycol
NDNS	National diet and nutrition survey
NOAEL	No observed adverse effect level
PEG	Polyethylene glycol
PIP-g-MA	Polyisoprene-graft-maleic anhydride
TDI	Tolerable daily intake
TTC	Threshold of toxicological concern
TWI	Tolerable weekly intake