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Safety of nisin (E 234) as a food additive in the light of new toxicological data and the proposed extension of use

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Abstract

The present scientific opinion deals with the evaluation of the safety of nisin (E 234) in the light of new toxicological data and with the proposed extension of use in unripened cheese and heat-treated meat products. Nisin (E 234) is currently an authorised food additive in the EU under Annex II of Regulation (EC) 1333/2008 for use in several food categories. The safety of nisin (E 234) as a food additive has been evaluated in 2006 by the EFSA Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food, where an acceptable daily intake (ADI) of 0.13 mg/kg body weight (bw) was confirmed as previously established by Scientific Committee on Food (SCF). In addition to the studies previously evaluated by EFSA in 2006, the Panel considered in the present opinion, data from a new subchronic toxicity study. No adverse effects were observed in a repeated dose oral toxicity study in which rats were administered nisin A for 90 days. A no observed adverse effect level (NOAEL) of 225 mg nisin A/kg bw per day, the highest dose tested, was identified for this study. Using this NOAEL, an ADI of 1 mg nisin A/kg bw per day for nisin (E 234) was calculated applying a default uncertainty factor of 200 for extrapolation of subchronic to chronic exposure and inter- and intra-species variability. The Panel calculated exposure estimates for both the current and the proposed uses based on the data available in the EFSA Comprehensive Database. The Panel considered that the overall exposure estimate was below the new ADI for nisin A for all population groups. The Panel concluded that the proposed extension of use of nisin (E 234) as a food additive in unripened cheese (at maximum level of 12 mg/kg) and in heat-treated meat products (at maximum level of 25 mg/kg) would not be of safety concern.

Keywords: food additive, nisin, E 234, extension of use

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Summary

The European Commission asked the European Food Safety Authority (EFSA) to provide a scientific opinion as regards the safety of nisin (E 234) as a food additive in the light of the new toxicological data and on the proposed extension of use of nisin (E 234) to the categories 01.7.1 Unripened cheese excluding products falling in category 16 and 08.3.2 Heat-treated meat products in accordance with Regulation (EC) No 1331/2008 establishing a common authorisation procedure for food additives, food enzymes and flavourings.

The Panel on Food Additives and Nutrient Sources Added to Food (ANS) considered that in the current risk assessment, the possible induction of antimicrobial resistance (AMR) linked to exposure to nisin (E 234) and its possible modulating effect on the gut microbiota have deliberately not been considered, as it was deemed to fall outside the remit of the ANS Panel and the scope of the current mandate.

Nisin (E 234) is currently an authorised food additive in the European Union (EU) under Annex II of Regulation (EC) 1333/2008 for use in several food categories. Nisin (nisin A) is a polypeptide composed of 34 amino acids and belongs to class I bacteriocins. Bacteriocins are proteins/peptides naturally produced by bacteria to inhibit the growth of other bacteria. Nisin A is produced via fermentation by *Lactococcus lactis* subsp. *lactis*.

Nisin (E 234) was previously assessed in 2006 for its use as a food additive by the former EFSA Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food (AFC), where an acceptable daily intake (ADI) of 0.13 mg/kg body weight (bw) was confirmed as previously established by Scientific Committee on Foods (SCF). The authorisation of food additive was based on an evaluation of nisin A by the former EFSA AFC Panel. Therefore, this opinion refers to nisin A only.

In addition to the studies previously evaluated by EFSA in 2006, the Panel considered data from a new subchronic toxicity study made available since then. The Panel evaluated a new 90-day study in F344/DuCrlCrij rats. In this study, the effects of nisin preparation at dietary levels of 0%, 0.2%, 1% or 5% were investigated. The test substance used in the study was Nisaplin®, a commercial preparation of nisin A. According to the authors, nisin A at dietary levels up to 5% for 90 days caused no adverse effects in both sexes of rats. The Panel agreed with this conclusion. The no observed adverse effect level (NOAEL) was determined to be the 5% diet. Considering that the nisin preparation used contained 7.5% nisin A, the 5% diet corresponds to average daily intakes of 224.7 mg nisin A/kg bw per day for males and 239 mg nisin/kg bw per day for females. A NOAEL of 225 mg nisin A/kg bw per day is identified for this study.

The Panel considered that the toxicological database was sufficient to derive an ADI using the NOAEL of 225 mg nisin A/kg bw per day from the data in Hagiwara et al. (2010), which was the highest dose tested. An ADI of 1 mg nisin A/kg bw per day for nisin (E 234) was calculated applying a default uncertainty factor of 200 for extrapolation of subchronic to chronic exposure and inter- and intra-species variability.

The Panel calculated exposure estimates for both the current and the proposed uses based on the data available in the EFSA Comprehensive Database. The Panel considered that the overall exposure estimate was below the new ADI of 1 mg/kg bw per day for nisin A for all population groups.

The Panel concluded that, on the basis of the new ADI established for nisin A the proposed extension of use as a food additive (E 234), in unripened cheese (at maximum level of 12 mg/kg) and in heat-treated meat products (at maximum level of 25 mg/kg) would not be of safety concern.

However, the Panel recommended to evaluate separately the risk of inducing AMR to nisin (E 234) in pathogenic bacteria through its use as a food additive. This should be assessed in relation to the potential use of nisin (E 234) as an antimicrobial drug in humans and domestic animals.

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1. Introduction

Nisin (E 234) is currently an authorised food additive in the European Union (EU) under Annex II of Regulation (EC) 1333/2008 for use in several food categories. The authorisation of food additive was based on an evaluation of nisin A by the former EFSA Panel on Food additives, Flavourings, Processing Aids and Materials in Contact with Food (AFC). Therefore, the present evaluation referred to nisin A only.

The present scientific opinion deals with the evaluation of the safety of nisin (E 234) in the light of new toxicological data and with the proposed extension of use in unripened cheese and heat-treated meat products.

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

1.1.1. Background

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

Nisin (E 234) is currently an authorised food additive in the European Union under Annex II to Regulation (EC) 1333/2008 for use in some foods (*clotted cream*, *mascarpone*, ripened and processed cheese and cheese products, pasteurised liquid eggs and semolina and tapioca puddings and similar products).

In 2006 the former Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Foods (AFC Panel) issued an opinion on the safety of use of nisin (E 234) as a food additive,[2] also addressing the issue of antimicrobial resistance. The AFC Panel concluded that the Acceptable Daily Intake (ADI) of 0.13 mg/kg bw per day previously set by the Scientific Committee on Food remained valid.

Subsequently, EFSA assessed safety of the request for the extension of use and modification of the manufacturing process[3] and concluded that the ADI is also valid for the food additive nisin produced using an alternative manufacturing process based on fermentation of a sugar medium and that the additional use of nisin in liquid eggs is not safety concern.

The Health and Food Safety Directorate-General has received a request from DuPont Nutrition Biosciences ApS for (i) a modification of the conditions of use, (ii) a re-evaluation of the safety, including a revision of the ADI as appropriate and (iii) a modification of the specifications, of nisin (E 234).

The Health and Food Safety Directorate-General considers appropriate to consult EFSA as regards:

i) The amendment of Annex II to Regulation (EC) 1333/2008 to extend the use of nisin to category 01.7.1 Unripened cheese excluding products falling in category 16[4] at a maximum use level (ML) of 12.5 mg/kg and category 8.3.2 Heat-treated meat products at ML of 25 mg/kg.
ii) A re-evaluation of the safety of Nisin (E 234) as a food additive taking into account new toxicity data that have become available.

1.1.2. Terms of Reference

The European Commission requests the European Food Safety Authority to provide a scientific opinion on the safety of nisin (E 234) as a food additive in the light of the new toxicological data and on the proposed extension of use of nisin (E 234) to the categories 01.7.1 Unripened cheese excluding products falling in category 16 and 08.3.2 Heat-treated meat products in accordance with Regulation (EC) No 1331/2008 establishing a common authorisation procedure for food additives, food enzymes and flavourings.[5]

[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, pp. 16–33.
[2] EFSA Journal 2006; 314, 16 pp.
[3] EFSA Journal 2006; 314b, 8 pp.
[4] Nisin is currently authorised for use in category 01.7.1 but its use is limited to *mascarpone* at 10 mg/kg.
[5] OJ L 354, 31.12.2008, p. 1.

1.1.3. Interpretation of Terms of Reference

The Panel is aware that in the current risk assessment the possible induction of antimicrobial resistance (AMR) linked to exposure to nisin (E 234) and its possible modulating effect on the gut microbiota have deliberately not been considered, as it was deemed to fall outside the remit of the ANS Panel and the scope of the current mandate.

In several recent studies in rodents, some other food additives such as emulsifiers have been reported to alter the gut microbiota, to promote gut inflammation, obesity and to impair glycaemic control (Swidsinski et al., 2009a,b; Renz et al., 2012; Merga et al., 2014; Cani and Everard, 2015; Chassaing et al., 2015; Romano-Keeler and Weitkamp, 2015; Lecomte et al., 2016; Chassaing et al., 2017).

The Panel noted that, even though some of these effects are not systematically studied in toxicity studies performed according to toxicity testing guidelines, they would be investigated on a case by case basis if indicated by the results of the general toxicity testing as recommended in the Guidance for submission of food additives (EFSA ANS Panel, 2012). The Panel considered that additional studies will be needed to show the relevance of the effects seen in rodents for human health.

The current risk assessment was performed to address specific questions on safety of nisin (E 234) as a food additive in the light of the new toxicological data and on the proposed extension of its use.

1.2. Information on existing evaluations and authorisations

In 1990, the Scientific Committee on Food (SCF) evaluated the safety of nisin and established an ADI of 0.13 mg/kg body weight (bw) (SCF, 1992).

Nisin (E 234) was assessed in 2006 for its use as a food additive by the former AFC Panel, also taking into account previous evaluations and authorisations (EFSA ANS Panel, 2006a).

In 2013, the FAO/WHO Joint Expert Committee on Food Additives (JECFA) at its 77th meeting established an ADI of 0–2.0 mg/kg bw per day for nisin based on a new 13-week subchronic toxicity rat feeding study (Hagiwara et al., 2010). The ADI was established by applying a safety factor of 100. According to the applicant, nisin A was the test substance used in all the referred studies assessed by the different Authorities.

The Panel noted that the applicant has provided information on existing uses, authorisations and evaluations in the EU and in other countries (Australia, New Zealand, Japan and USA).

According to the applicant, nisin (INS 234) is also listed in the *Codex Alimentarius* General Standard for Food Additives (GSFA, Codex STAN 192-1995) approved for use in several food categories.

2. Data and methodologies

2.1. Data

The applicant has submitted a dossier in support of its application for the safety evaluation and extension of the approved uses of nisin (E 234) in the EU ('Documentation provided to EFSA' No. 1).

The Panel noted that the data provided by the applicant are specifically related to nisin A, in line with the data considered in previous evaluations by the AFC Panel (EFSA ANS Panel, 2006a,b).

The EFSA Comprehensive European Food Consumption Database was used to estimate dietary exposure.

2.2. Methodologies

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

The current 'Guidance for submission for food additive evaluations' (EFSA ANS Panel, 2012) has been followed by the ANS Panel for assessing the safety and the proposed extension of use of nisin (E 234) in unripened cheese and heat-treated meat products.

Dietary exposure to nisin (E 234) from its proposed extension of use as a food additive was estimated combining the food consumption data available within the EFSA Comprehensive European Food Consumption Database with the proposed use levels provided by the applicant.

3. Assessment

3.1. Technical data

3.1.1. Identity of the substance

According to EFSA ANS Panel (2006a,b), nisin (nisin A) is a polypeptide composed of 34 amino acids and belongs to class I bacteriocins. Bacteriocins are proteins/peptides naturally produced by bacteria to inhibit the growth of other bacteria which is produced via fermentation by *Lactococcus lactis* subsp. *lactis.*

3.1.2. Specifications

The specifications for Nisin (E 234) as defined in Commission Regulation (EU) No 231/2012 are presented in Table 1.

Table 1: Nisin (E 234) specifications defined in Commission Regulation (EU) No 231/2012

Definition	Nisin consists of several closely related polypeptides produced by strains of *Lactococcus lactis* subsp. *lactis*
EINECS	215-807-5
Chemical formula	$C_{143}H_{230}N_{42}O_{37}S_7$
Molecular weight	3,354.12 Da
Assay	Nisin concentrate contains not less than 900 units per mg in a mixture of non-fat milk solids and a minimum sodium chloride content of 50%
Description	White powder
Purity	
Loss on drying	Not more than 3% (102–103°C to constant weight)
Arsenic	Not more than 1 mg/kg
Lead	Not more than 1 mg/kg
Mercury	Not more than 1 mg/kg

EINECS: European INventory of Existing Commercial chemical Substances.

The Panel noted that the applicant provided analytical results of five independently manufactured batches of Nisaplin® (according to the applicant Nisaplin® is a commercial preparation of nisin A, containing 1,000 IU/mg) to demonstrate the compliance with nisin (E 234) specifications ('Documentation provided to EFSA' No. 1). The Panel noted that 1 IU would correspond to 0.025 μg of nisin and therefore 1 μg of nisin is equivalent to 40 IU (JECFA, 2013).

Based on the information provided by the applicant, the Panel considered that the Nisaplin® is consistently manufactured within the defined specifications for Nisin (E 234).

The high-performance liquid chromatographic (HPLC) method used for the assay is validated against the resazurin assay. The resazurin assay is performed as described in the JECFA monograph for nisin (JECFA, 1969, 2013).

Particle size and particle size distribution

Although nisin (E 231) is described as a white powder, the particle size is not defined in the current EU specifications (see Section 3.1.2).

3.1.3. Manufacturing process

The applicant has provided information on the manufacturing process used for the production of the food additive Nisaplin®.

Nisaplin® is produced via fermentation of a sugar-based medium with added yeast extract by nisin producing strains of *Lactococcus lactis* subsp. *lactis*.

After fermentation, producer cells are removed by membrane filtration and the resulting filtrate is concentrated via ultrafiltration. The concentrate is precipitated with sodium chloride, recovered by centrifugation and spray-dried. The dried concentrate is then standardised with sodium chloride and pin milled to produce the commercial nisin preparation with a potency of 1,000 IU nisin A/mg.

The manufacturing process currently used by the applicant to produce Nisaplin® has been already evaluated by EFSA (EFSA ANS Panel, 2006a). EFSA considered the manufacturing process conformed to the specifications for E 234 (EFSA ANS Panel, 2006a).

According to the applicant, there are no impurities identified for the food additive nisin (E 234). Nisin complies with the purity criteria defined for E 234 in Regulation (EU) 231/2012 laying down specifications for food additives.

3.1.4. Stability of the substance, and reaction and fate in food

The Panel noted that the applicant provided literature studies to describe stability and reaction and fate in food of nisin (E 234).

Nisin residual levels were unaffected in cottage cheese (pH of 4.6–4.7) after storage at 20°C for 7 days (Ferreira and Lund, 1996).

Davies et al. (1997) observed only 10–32% nisin loss in ricotta-type cheese after 10 weeks at 6–8°C.

Higher nisin retention in foods will occur at lower storage temperatures and lower pH. Nisin shows increased solubility at low pH and becomes less soluble at higher pH values. Similarly, nisin is most stable in acidic conditions, and the stability will decrease at pH 3–7 during heat treatment (Davies and Delves-Broughton, 1999).

Nisin solutions showed to withstand autoclaving at 121°C for 15 min at pH 3.0–3.5 with less than 10% activity loss. However, more than 90% decrease in activity was verified at pH 1 and pH 7. Pasteurisation temperatures have less impact on nisin activity. During the manufacturing of processed cheese at pH 5.6–5.8, 20% nisin loss was verified. Applying temperatures of 80–105°C for 5–10 min resulted in 20–30% initial nisin loss in a cheese spread with a pH of 5.6–5.8. After storage for 30 weeks, the nisin retention in the cheese spread decreased with the increase in storage temperature, from 80% of storage at 20°C, to 60% at 25°C and 40% at 30°C (Delves-Broughton, 1998; Davies and Delves-Broughton, 1999).

Nisin was rapidly inactivated in raw ground beef due to potential interaction with meat components (as proteases and glutathione). Nisin is stable when it is in contact with raw ground meat for 15 min at 4°C and then heated at 71°C. However for longer periods (30 min and more) in the same conditions, only liposome-encapsulated nisin was stable upon thermal treatment keeping the activity of nisin during food processing and storage (Boualem et al., 2013).

3.2. Proposed uses and use levels

The applicant has submitted an application to extend the use of nisin (E 234) as a food additive at maximum levels as displayed in Table 2.

Table 2: Maximum Permitted Levels (MPLs) of nisin (E 234) in foods according to the Annex II of Regulation (EC) No 1333/2008 and proposed uses and use levels.

Food category	Restrictions/ exceptions	Authorised MPL (mg/kg or mg/L as appropriate)	Proposed maximum levels of use (mg/kg or mg/L as appropriate)
1.6.3 Other cream	Only clotted cream	10	
1.7.1 Unripened cheese excluding products falling in category 16	Only mascarpone	10	12.5
1.7.2 Ripened cheese		12.5	
1.7.5 Processed cheese		12.5	
1.7.6 Cheese products (excluding products falling in category 16)	Only ripened and processed products	12.5	
8.3.2 Heat-treated meat products		–	25
10.2 Processed eggs and egg products	Only pasteurised liquid egg	6.25	
16 Desserts excluding products covered in category 1, 3 and 4	Only semolina and tapioca puddings and similar products	3	

MPL: maximum permitted level.
–: not currently authorised.

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, 2011a). New consumption surveys added in the Comprehensive database were also taken into account in this assessment.[6]

The food consumption data gathered by EFSA were collected by different methodologies and thus direct country-to-country comparisons should be interpreted with caution. 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 represents the best available source of food consumption data across Europe at present.

Food consumption data from the following population groups: infants, toddlers, children, adolescents, adults and the elderly were used for the exposure assessment. For the present assessment, food consumption data were available from 33 different dietary surveys carried out in 19 European countries (Table 3).

Table 3: Population groups considered for the exposure estimates of nisin (E 234)

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, Denmark, Finland, Germany, Italy, UK
Toddlers	From 12 months up to and including 35 months of age	Belgium, Bulgaria, Denmark, Finland, Germany, Italy, Netherlands, Spain, UK
Children[(a)]	From 36 months up to and including 9 years of age	Austria, Belgium, Bulgaria, Czech Republic, Denmark, Finland, France, Germany, Greece, Italy, Latvia, Netherlands, Spain, Sweden, UK
Adolescents	From 10 years up to and including 17 years of age	Austria, Belgium, Cyprus, Czech Republic, Denmark, Finland, France, Germany, Italy, Latvia, Spain, Sweden, UK
Adults	From 18 years up to and including 64 years of age	Austria, Belgium, Czech Republic, Denmark, Finland, France, Germany, Hungary, Ireland, Italy, Latvia, Netherlands, Romania, Spain, Sweden, UK
The elderly[(a)]	From 65 years of age and older	Austria, Belgium, Denmark, Finland, France, Germany, Hungary, Ireland, Italy, Romania, Sweden, UK

(a): The terms 'children' and 'the elderly' correspond, respectively, to 'other children' and the merge of 'elderly' and 'very elderly' in the Guidance of EFSA on the 'Use of the EFSA Comprehensive European Food Consumption Database in Exposure Assessment' (EFSA, 2011a).

Consumption records were codified according to the FoodEx classification system (EFSA, 2011b). Nomenclature from the FoodEx classification system has been linked to the food categorisation system (FCS) as presented in Annex II of Regulation (EC) No 1333/2008, part D, to perform exposure estimates. In practice, the FoodEx food codes were matched to the FCS food categories.

3.3.2. Exposure to nisin (E 234) 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 the exposure to nisin (E 234) based on the output obtained using the FAIM model (version 1) ('Documentation provided to EFSA' no. 1).

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

The Panel decided to not use the estimate exposure generated from the FAIM tool version 1 and provided by the applicant since a new version of the FAIM tool (version 2)[7] have been made available since the receipt of the current application.

The Panel therefore decided to perform a new estimate exposure using the FAIM tool (version 2). The results of the estimate exposure are reported in Tables 4 and 5.

Table 4: Summary of dietary exposure to nisin (E 234) from its use as a food additive in the MPLs scenario and in the refined exposure scenarios, in six population groups (minimum-maximum across the dietary surveys in μg/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)
Current MPLs						
Mean	1–28	5–35	5–24	3–12	2–9	1–9
High level	2–94	23–94	12–73	9–29	6–26	5–22
Current MPLs and proposed maximum extension of uses						
Mean	4–59	28–163	23–128	19–69	18–57	15–50
High level	33–164	96–230	49–238	41–143	37–118	29–97

bw: body weight.

From the MPLs scenario, mean exposure to nisin (E 234) from its use as a food additive ranged from 1 μg/kg bw per day in infants and the elderly to 35 μg/kg bw per day in toddlers. The 95th percentile of exposure to nisin (E 234) ranged from 2 μg/kg bw per day in infants to 94 μg/kg bw per day in toddlers.

From the MPLs and proposed use levels scenario, mean exposure to nisin (E 234) from its use as a food additive ranged from 4 μg/kg bw per day in infants to 163 μg/kg bw per day in toddlers and from 29 μg/kg bw per day in the elderly to 238 μg/kg bw per day in children at the high exposure level.

Table 5: Main food categories contributing to total exposure to nisin (E 234) calculated with MPLs and proposed use levels across dietary surveys using FAIM (version 2). Results are shown as range of contribution (%) and number of surveys (> 5% to the total mean exposure)

FC Number	Food Category name	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)
01.6	Cream and cream powder	5.3–26.0 (2)	15.3 (1)	5.6–10.1 (4)	5.5–8.6 (3)	5.2–10.1 (3)	10.1–11.2 (2)
01.7.1	Unripened cheese excluding products falling in category 16	17.5–47.2 (3)	10.9–28.5 (8)	5.6–24.7 (13)	6.3–25.5 (6)	6.0–26.9 (9)	5.8–21.8 (7)
01.7.2	Ripened cheese	5.4–39.2 (4)	5.3–20.5 (9)	5.6–16.0 (12)	5.7–25.6 (11)	5.4–25.4 (13)	5.1–24.5 (11)
01.7.5	Processed cheese	6.8–40.9 (2)	5.4–20.7 (2)	5.2–7.6 (3)	5.7 (1)	–	7.3 (1)
08.3	Meat products	19.1–75.2 (6)	44.5–85.6 (10)	52.1–92.1 (18)	55.4–92.5 (17)	51.8–92.1 (17)	45.6–93.9 (14)

[7] Available online: http://www.efsa.europa.eu/en/applications/foodingredients/tools

FC Number	Food Category name	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)
16	Desserts excluding products covered in category 1, 3 and 4	5.2 (1)	5.1–11.3 (3)	5.2–10.3 (3)	–	–	–

bw: body weight.

The main food categories that contributes to total exposure to nisin (E 234) calculated with MPLs and proposed use levels is category 8.3 Meat products in all population groups.

The Panel considered that there are uncertainties in the exposure estimate due to some food categories which are not available in FAIM tool (version 2). The food categories that are not included in the FAIM tool (version 2) are:

- 1.6.3 'other cream', category 1.6 'cream and cream powder' was used
- 1.7.6 'cheese products'
- 8.3.2 'heat-treated meat products', category 8.3 'meat products' was used

The Panel considered that overall there is an overestimation in the results.

3.4. Biological and toxicological data

3.4.1. Absorption, distribution, metabolism and excretion

According to the studies already evaluated by EFSA in the previous scientific opinion (EFSA ANS Panel, 2006a), nisin (E 234) is inactivated by human saliva, pancreatin and α-chymotrypsin (Claypool et al., 1966; Heinemann and Williams, 1966; Jarvis and Mahoney, 1969). However, the Panel noted that in a later study nisin was not inactivated by human saliva (Tong et al., 2010).

Bernbom et al. (2006) demonstrated that nisin A is degraded or inactivated in the gastrointestinal tract. The authors detected nisin fragments in the faeces of rats by competitive enzyme-linked immunosorbent assay (ELISA) 24 h after feeding the rats with 60 mg nisin A. The Panel also noted that the authors detected residual antimicrobial activity in the faeces of rats receiving nisin A.

After simulated gastrointestinal digestion, intact nisin A was not present in the system. Nisin fragments were detected by reversed phase high performance liquid chromatography and mass spectroscopy and some of these nisin fragments demonstrated low antibacterial activity against *Lactococcus lactis* HP in agar diffusion activity assays (Gough et al., 2017).

3.4.2. Acute toxicity

No new acute toxicity data were available since the previous EFSA evaluation in 2006.

3.4.3. Short-term and subchronic toxicity

In addition to the studies previously evaluated by EFSA in 2006, the applicant provided data from two new subchronic toxicity studies.

Hagiwara et al. (2010) evaluated the effects of nisin preparation at dietary levels of 0%, 0.2%, 1% or 5% in F344/DuCrlCrij rats (10 males and 10 females per group) in a 90-day study performed in compliance with good laboratory practice (GLP). The reference group was fed sodium chloride (NaCl) at a dietary level of 3.712%, which was the same NaCl level as the one in the 5% nisin diet. The test substance used in this study was Nisaplin®, a commercial preparation of nisin A, with a potency of 3,000 IU/mg and a sodium chloride content of 74.23%. According to the information provided by the applicant, the preparation used contained 7.5% nisin A. The average daily test material intakes in the 0%, 0.2%, 1.0% and 5% nisin A preparation group for males were equal to 0, 117, 568 or 2,996 mg/kg bw per day and for females 0, 129, 638 or 3,187 mg/kg bw per day. Average intakes of NaCl of the 3.7% NaCl diet were 2,196 mg/kg bw per day for males and 2,423 mg/kg bw per day for females.

There were no deaths observed during the 90 days or any clinical signs of treatment-related abnormality in either treated or reference (NaCl) group. No statistically significant changes in body

weight were found in either sex fed with 5% nisin A compared to the control group. An increase in body weight was sporadically noted in males fed with 1% nisin A, which was not dose-dependent and considered to have no toxicological significance.

An increase in water consumption and absolute and relative kidney weight, as well as in sodium and chloride levels in the urine were noted in both sexes fed with 5% nisin A diet and in the reference group fed with 3.7% NaCl. These observations were considered to be related to the NaCl intake, which was similar in the 5% nisin A diet and reference group. A decrease in potassium levels was detected in the urine of males receiving the 5% nisin A diet. An increase in water consumption was also noted in both sexes fed with 1% nisin A. The urine levels of sodium, chloride and potassium in the urine also varied in both sexes fed with the 1% nisin A diet and in males fed with the lower nisin A concentration (0.2%).

No treatment-related effects were noticed on ophthalmoscopy, haematology, clinical chemistry and necropsy.

Histopathological examination revealed squamous cell hyperplasia of the forestomach limiting ridge were observed in all animals fed with 5% nisin A and 3.7% NaCl diets. This histopathological change in the forestomach was also observed at low incidence for the groups fed with lower nisin concentration. These alterations were considered to be related to the sodium chloride intake. Moreover, the forestomach lesions are not considered a relevant toxicological endpoint for humans, due to the absence of this organ in humans.

The authors concluded that nisin A at dietary levels up to 5% for 90 days caused no adverse toxicological effects in rats. The Panel agreed with this conclusion. The no observed adverse effect level (NOAEL) was determined to be the 5% diet (equal to 2,996 mg/kg bw per day for males, 3,187 mg kg bw per day for females). Considering that the nisin preparation used contained 7.5% nisin A, the 5% diet corresponds to average daily intakes of 224.7 mg nisin/kg bw per day for males and 239 mg nisin/kg bw per day for females. A NOAEL of 225 mg nisin/kg bw per day is identified for this study, which was the highest dose tested.

Reddy et al. (2011) studied the effect of nisin in male Holtzman rats (10 animals per group). Purified nisin (commercial nisin with 2.5% nisin, $\geq$ 1,000 IU nisin/mg) dissolved in distilled water was administered by oral gavage for 13 weeks at a dose levels of 10, 25 or 50 mg/kg bw per day to groups of 10 animals. Control animals received only the vehicle (0.9% saline). No treatment-related adverse effects were observed among the groups. The NOAEL of the study was 50 mg/kg bw per day, the highest dose tested. However, this study showed similar limitations as the previous study of Reddy et al. (2004). Therefore, the Panel considered that the study cannot be used for hazard characterisation.

The Panel considered that the toxicological database is sufficient to derive an ADI using the NOAEL of 225 mg nisin/kg bw per day using the data in Hagiwara et al. (2010).

3.4.4. Genotoxicity

The Panel noted that the applicant submitted results from *in vitro* and *in vivo* tests aimed at assessing the genotoxicity potential of nisin ('Documentation provided to EFSA' no. 1).

The Panel noted that the applicant did not provide any new data in addition to those already considered in the AFC Panel in 2006. The Panel agreed with the conclusion of the AFC that there was no concern with respect to the genotoxic potential of nisin.

3.4.5. Chronic toxicity and carcinogenicity

The Panel considered that the studies already reported by EFSA (EFSA ANS Panel, 2006a, 2006b) (Frazer et al., 1962; Shtenberg and Ignat'ev, 1970) are limited and not adequate for hazard characterisation.

3.4.6. Reproductive and developmental toxicity

Reproductive toxicity

No reproductive toxicity data were available since the previous EFSA evaluation in 2006.

Developmental toxicity

Gupta et al. (2008) investigated the developmental toxicity of nisin in mature female pregnant Holtzman rats. Nisin was purified from a commercial nisin preparation (2.5% nisin, $\geq$ 1,000 IU/mg).

Mated females were divided into three groups (20 animals per group) were administered nisin orally at doses of 10, 25 or 50 mg nisin/kg bw per day from gestation day (GD) 6–15.

There were no treatment-related effects noticed on maternal mortality and morbidity or any clinical signs derived from nisin administration at necropsy. The treated animals did not show any clinical signs of toxicity. Maternal body weights and consumption of food and water were not affected by nisin intake. No treatment-related effect was observed on implantations, resorptions and gravid uterine weights at all the doses tested. No external changes were observed in the fetuses at any tested dose. Detailed skeletal examination revealed no abnormalities in any fetuses from the treated groups, nor visceral malformations. Litter size, fetal body weights, fetal sex ratio, postnatal survival, growth and development did not reveal any treatment-related effect on F1 progeny growth and reproductive performance. The authors concluded that daily administration of nisin up to 50 mg/kg bw per day during organogenesis did not cause developmental toxicity in rats. The Panel noted that the reporting of the study was limited and in general restricted to the high-dose group. Furthermore, the number and use of the control groups is not clear.

Overall, an adequate dietary three-generation reproduction toxicity study in rats was performed according to GLP with Nisaplin® (Report APL1/801028, 1981 reported by EFSA ANS Panel, 2006a, 2006b). The Panel identified from this study a NOAEL 5% Nisaplin® in the diet (equivalent to 62.5 mg nisin/kg bw per day) for maternal, reproductive and developmental toxicity. Furthermore, limited studies (two reproductive studies, Frazer et al., 1962 and Reddy et al., 2004) and one prenatal developmental study, Gupta et al., 2008) did not show reproductive or developmental effects.

3.5. Discussion

The present scientific opinion deals with the evaluation of the safety of nisin (E 234) in the light of new toxicological data and with the proposed extension of use in unripened cheese and heat-treated meat products. The authorisation of food additive was based on an evaluation of nisin A by the former EFSA AFC Panel. Therefore, this opinion refers to nisin A only.

Nisin (E 234) is currently an authorised food additive in the EU under Annex II of Regulation (EC) 1333/2008 for use in several food categories.

The safety of nisin (E 234) as a food additive has been evaluated in 2006 by the EFSA Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food, where an ADI of 0.13 mg/kg bw was confirmed as previously established by Scientific Committee on Foods (SCF, 1992).

In 2013, JECFA established an ADI of 0–2.0 mg/kg bw per day for nisin (E 234) based on a new 13-week subchronic toxicity rat feeding study (Hagiwara et al., 2010). The ADI was established by applying a safety factor of 100.

According to the studies already evaluated by EFSA in the previous assessment (EFSA ANS Panel, 2006a, 2006b), absorption of the intact nisin is unlikely since it is inactivated by proteolytic enzymes. Furthermore, the Panel considered that there is no concern for genotoxicity.

The Panel noted that the application did not contain a comprehensive review of the information on nisin stability in the intestine or in simulated intestinal fluids. Although these data may address the uncertainties on residual nisin activity and the microbiome, the Panel noted that these would not alter the conclusions on the terms of reference.

No adverse effects on toxicological parameters were observed in a repeated dose oral toxicity study in which rats were administered nisin A for 90 days. A NOAEL of 225 mg nisin/kg bw per day is identified for this study, which was the highest dose tested (Hagiwara et al., 2010).

The Panel considered that the toxicological database is sufficient to derive a new ADI using the NOAEL of 225 mg nisin A/kg bw per day from the data in Hagiwara et al. (2010). An ADI of 1 mg nisin A/kg bw per day for nisin (E 234) was calculated applying a default uncertainty factor of 200 for extrapolation of subchronic study and inter- and intra-species variability (EFSA, 2012).

The Panel calculated exposure estimates for both the current and the proposed uses based on the data available in the EFSA Comprehensive Database. From the use of nisin (E 234) as a food additive, the calculated exposure ranges from 4 μg/kg bw per day in infants to 163 μg/kg bw per day in toddlers at the mean exposure level and from 29 μg/kg bw per day in elderly to 238 μg/kg bw per day in children at the high exposure level. The main food category that contributes to total exposure to nisin (E 234) from both the current and the proposed uses is category 8.3 Meat products in all population groups.

The Panel considered that the overall exposure estimate was below the new ADI of 1 mg/kg bw per day for nisin A for all population groups.

4. Conclusions

The Panel concluded that, on the basis of the new ADI of 1 mg/kg bw per day established for nisin A, the proposed extension of use as a food additive (E 234), in unripened cheese (at maximum level of 12 mg/kg) and in heat-treated meat products (at maximum level of 25 mg/kg) would not be of safety concern.

5. Recommendations

The Panel recommended that the risk of inducing AMR to nisin (E 234) in pathogenic bacteria through its use as a food additive be evaluated separately.

This should be assessed in relation to the potential use of nisin (E 234) as an antimicrobial drug for in humans and domestic animals infectious disease.

Documentation provided to EFSA

1) Dossier 'Application for modification of the conditions of use of nisin (E 234)'. 11 March 2016. Submitted by DuPont Nutrition Biosciences ApS.

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Abbreviations

ADI	acceptable daily intake
AFC	Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Foods
AFC Panel	EFSA Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food
AMR	antimicrobial resistance
ANS Panel	EFSA Panel on Food Additives and Nutrient Sources added to Food
bw	body weight
EINECS	European INventory of Existing Commercial chemical Substances
ELISA	enzyme-linked immunosorbent assay
FAIM	Food Additives Intake Model
FCS	food categorisation system
GD	gestation day
GLP	good laboratory practice
GSFA	General Standard for Food Additives
HPLC	high-performance liquid chromatography

JECFA	The FAO/WHO Joint Expert Committee on Food Additives
ML	maximum use level
MPL	maximum permitted level
MS	mass spectroscopy
NOAEL	no observed adverse effect level
OECD	Organisation for Economic Co-operation and Development
SCF	Scientific Committee on Food