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FINAL ASSESSMENT REPORT

APPLICATION A565

USE OF NISIN IN PROCESSED MEAT PRODUCTS

For Information on matters relating to this Assessment Report or the assessment process generally, please refer to <http://www.foodstandards.gov.au/standardsdevelopment/>

Executive Summary

An Application (A565) was received on 23 June 2005 from Danisco Australia Pty Ltd, submitted by Axiome Pty Ltd, seeking to amend Schedule 1 of Standard 1.3.1 – Food Additives, of the *Australia New Zealand Food Standards Code* (the Code) to include limits for nisin of 12.5 mg/kg in processed meat products, specifically for:

- processed meat, poultry and game products in whole cuts or pieces; and
- processed comminuted meat, poultry and game products.

Nisin (INS 234) is a bacteriocin produced by certain strains of *Lactococcus lactis*, a non-pathogenic grade bacterium that occurs in milk. Currently, a range of foods are permitted to contain nisin including cream products (up to 10 mg/kg) and flour products (up to 250 mg/kg). In addition, other foods are permitted to contain nisin at GMP (Good Manufacturing Practice), including: cheese and cheese products; oil emulsions; tomato products; fruit and vegetable preparations; liquid egg products; tomato juices; beer and related products; dairy and fat-based desserts; dips and snacks; and sauces and toppings, mayonnaises and salad dressings.

In assessing the proposed extended use of nisin into processed meat products, Food Standards Australia New Zealand (FSANZ) considered the potential of nisin to induce antimicrobial resistance. As part of these considerations FSANZ sought advice from the Expert Advisory Group on Antimicrobial Resistance (EAGAR) of the National Health and Medical Research Council (NHMRC). The EAGAR concluded that nisin was unlikely to induce antimicrobial resistance under the proposed conditions of use.

The dietary modelling of nisin-containing foods, including for the proposed new range of foods, indicated that consumers would not exceed the Acceptable Daily Intake (ADI) even for the high intake consumers (i.e. 95th percentile intake levels). These results do not raise any public health and safety concerns at the levels proposed by the Applicant for nisin.

The Applicant provided data demonstrating that the presence of nisin would inhibit the growth of certain spoilage bacteria and *Listeria monocytogenes*, although the effect of nisin will be dependent upon the composition of the meat product. The effects of nisin are also likely to be enhanced when used with other food preservation techniques. On this basis and given the limited spectrum of activity of nisin, FSANZ considered that there is technological justification for the use of nisin in processed meat products and that it will be a potentially useful component of food preservation systems for processed meat production.

FSANZ has identified two regulatory options for this Application:

- Option 1: maintain the *status quo* approach; no change to Standard 1.3.1; or
- Option 2: vary Standard 1.3.1 to include limits for nisin in processed meat products.

On balance, there is likely to be an overall benefit to consumers and industry from the approval of limits for nisin and its use in processed meat products. There is unlikely to be a significant impact on government compliance agencies as a result of the potential use of nisin in processed meat products.

Purpose

The purpose of this Application is to seek an amendment to Standard 1.3.1 – Food Additives of the Code to include a limit of 12.5 mg/kg for nisin in processed meat products.

Decision

Standard 1.3.1 – Food Additives be amended to include limits of 12.5 mg/kg for nisin in processed meat products.

Reasons for Decision

This variation is proposed for the following reasons.

- The variation to the Code is consistent with the section 18 objectives of the *Food Standards Australia New Zealand Act 1991* (FSANZ Act). In particular, it does not raise any public health and safety concerns, including any potential for development of antimicrobial resistance. The relevant assessments are based on the best available scientific evidence and the use of nisin in processed meat products may assist in promoting an efficient and internationally competitive food industry.
- Use of nisin in processed meats is technologically justified and it will be a potentially useful component of food preservation systems for processed meat production. Based upon data provided by the Applicant, the presence of nisin would inhibit the growth of Gram-positive bacteria, and its effects are likely to be enhanced when used with other food preservation techniques.
- The regulation impact assessment concluded that the benefits of the potential use of nisin in processed meats outweigh any costs associated with its use.
- To achieve what the Application seeks, there are no alternatives that are more cost-effective than a variation to Standard 1.3.1 – Food Additives.

Consultation

The Initial Assessment Report was circulated for a round of public comment from 9 August 2006 until 20 September 2006. Nine submissions were received and none of these submissions objected to the further assessment of nisin addition to processed meat products.

The Draft Assessment Report was circulated for a round of public comment from 8 August 2007 until 19 September 2007 and eight submissions were received. One of these submitters opposed the Application, another submitter raised issues for further consideration by FSANZ and a third submitter did not object to further consideration of the Application. The remaining five submitters supported the incorporation of a limit for nisin in processed meat products, although one of these submitters raised an issue in relation to the draft variation. FSANZ has taken the submitters' comments into account in preparing the Final Assessment of this Application and the issues raised in submissions have been addressed (See Section 9.1).

Nisin is typically added to foods as part of a food additive formulation which may contain allergens such as milk or soy constituents. The presence of these allergens in processed meats is either currently declared, or would be required to be declared if the addition of nisin-containing formulations added allergens to processed meats that were not previously present.

FSANZ will investigate whether the existing available information about the presence of allergens in food from food additive formulations is sufficient and will consider whether additional information may be required to improve awareness in the area of allergens in food from food additive formulations.

Amendment to the Draft Variation after Consultation

The intent is for the limit for nisin to apply to all processed meat products under item 8.2 and item 8.3 in Schedule 1 of Standard 1.3.1. The draft variation circulated for public comment included the words ‘commercially sterile canned cured meat’ as part of the item 8.2 heading. If this draft variation had been approved, it would have had the unintended effect of restricting the nisin limit to commercially sterile canned cured meat, and other processed meat products standardised under item 8.2 would not have been permitted to contain nisin. To address this FSANZ has amended the draft variation to delete the words ‘commercially sterile canned cured meat’ from the heading to item 8.2, thereby ensuring that the nisin limit applies to all processed meat products under item 8.2 and item 8.3 in Schedule 1 of Standard 1.3.1. Attachment 1 includes the amended draft variation.

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INTRODUCTION

An Application (A565) was received on 23 June 2005 from Danisco Australia Pty Ltd, submitted by Axiome Pty Ltd, seeking to amend Schedule 1 of Standard 1.3.1 – Food Additives, of the Code. The Application sought to include limits for nisin, a preservative, to a maximum level of 12.5 mg/kg in the following food categories in Schedule 1 of Standard 1.3.1:

- 8.2 – Processed meat, poultry and game products in whole cuts or pieces.
- 8.3 – Processed comminuted meat, poultry and game products.

The Applicant claims that although various preservatives are currently permitted for use in processed meat products (mainly nitrites/nitrates, and sorbic acid), they are not completely effective and spoilage is not uncommon. Processed meats are also prone to post-processing contamination during slicing and packaging operations, and in retail operations and the Applicant claims that the presence of nisin will mitigate the impacts of this contamination in processed meat products.

1. Background

1.1 Current Standards

Nisin (International Numbering System INS 234) is a naturally occurring bacteriocin produced by certain strains of *Lactococcus lactis*, a non-pathogenic bacterium that occurs in milk. Currently, a range of foods are permitted to contain nisin including cream products (up to 10 mg/kg) and flour products (up to 250 mg/kg). In addition, other foods are permitted to contain nisin at GMP (Good Manufacturing Practice), including: cheese and cheese products; oil emulsions; tomato products; fruit and vegetable preparations; liquid egg products; tomato juices; beer and related products; dairy and fat-based desserts; dips and snacks; and sauces and toppings, mayonnaises and salad dressings. The Applicant wishes to include limits for nisin as a food additive (a preservative) in processed meat products.

1.2 Technological Purpose

Nisin is a small heat stable peptide belonging to a group of bacteriocins known as lantibiotics, which are produced by different genera of Gram-positive bacteria. Nisin is active against a wide range of Gram-positive vegetative bacteria, and particularly bacterial spore-formers, including *Bacillus*, *Clostridium* and *Lactobacillus*, as well as the Gram-positive pathogen *Listeria monocytogenes*. Nisin is, however, ineffective against Gram-negative bacteria, yeasts and moulds.

The Applicant has claimed that nisin is very effective in preventing or delaying the growth of Gram-positive bacteria in processed meat products. These bacteria are associated with processed meat products and have a relatively high tolerance to reduced water activity¹, refrigeration temperatures, low pH and the presence of nitrate and phosphate emulsifying salts. Furthermore, while the temperatures used in processing are sufficient to kill most bacteria, they are not effective against heat-resistant spores.

¹ Water activity is the amount of water in a food or beverage that is available to micro-organisms.

Processed meats are also prone to post-processing contamination during slicing and packaging operations, and in retail operations (delicatessens). Growth of these spoilage organisms may shorten the shelf-life of processed meat products, even at refrigeration temperatures.

1.3 History of Use

Nisin has been used for over 50 years as a food preservative and is currently approved in a number of countries for use in a range of foods, including processed cheese products, flour products and certain dairy based products (e.g. dips). A formulation containing nisin has been sold under the trade name of *Nisaplin*®.

Nisaplin® contains approximately 2.5% nisin, the balance consisting of salt and milk and milk solids derived from the fermentation of a modified milk medium by nisin producing strains of *L. lactis*. The Applicant indicates that the nisin containing formulation could be added to meat mix, or meat products could be dipped in the formulation solution or casings pre-treated by dipping in a solution of the nisin containing formulation.

1.4 Regulatory Status

1.4.1 Codex Standards

Nisin is currently included as a preservative in the following Codex Standards:

- Codex General Standard for Named Variety Processed Cheese and Spreadable Processed Cheese (Ref: Codex Standard A-8(a))
- Codex General Standard for Processed Cheese and Spreadable Processed Cheese (Ref: Codex Standard A-8(b))
- Codex General Standard for Processed Cheese Preparations, Processed Cheese Food and Processed Cheese Spread (Ref: Codex Standard A-8(c))
- Codex General Standard for Cheese (Ref: Codex Standard A-6)

The maximum level of nisin permitted in all of these standards is 12.5 mg/kg.

Nisin is currently under consideration for inclusion in the Codex Alimentarius General Standard for Food Additives for use in a wide range of foods including meat and meat products (including poultry and game) at a maximum level of 500 mg/kg.

1.4.2 International Legislation

Nisin is approved as a preservative in specific foods in a number of countries and jurisdictions including the European Union (e.g. 12.5 mg/kg in cheese), USA (see below), China (500 mg/kg in meat products) and MERCOSUR countries (Argentina, Brazil, Paraguay, Venezuela and Uruguay) (e.g. 12.5 mg/kg in cheese).

In the USA, a GRAS (generally recognized as safe) notice response was issued on April 20, 2001 (GRN 000065) about nisin as an antimicrobial agent for use on casings for frankfurters and on cooked meat and poultry products².

² <http://www.cfsan.fda.gov/%7E7Erdb/opa-g065.html>

2. The Issue

Food additives, including preservatives, are required to undergo a pre-market safety assessment before limits are included in Standard 1.3.1. Limits for nisin currently exist in Standard 1.3.1 for a number of foods. This Application seeks to include limits for nisin in processed meat products so that it may be present in these foods. This requires a consideration of:

- the safety of increased or varied dietary exposure to nisin; and
- the technological justification for nisin in processed meat products.

3. Objectives

The objective of this assessment is to determine whether it is appropriate to amend the Code to include limits for nisin in processed meat products. This assessment is carried out in order to ensure that the amendment to the standard is technologically justified while ensuring that public health and safety is protected. In developing or varying a food regulatory measure, FSANZ is required by its legislation to meet three primary objectives which are set out in section 18 of the FSANZ Act. These are:

- the protection of public health and safety;
- the provision of adequate information relating to food to enable consumers to make informed choices; and
- the prevention of misleading or deceptive conduct.

In developing and varying standards, FSANZ must also have regard to:

- the need for standards to be based on risk analysis using the best available scientific evidence;
- the promotion of consistency between domestic and international food standards;
- the desirability of an efficient and internationally competitive food industry;
- the promotion of fair trading in food; and
- any written policy guidelines formulated by the Ministerial Council.

4. Key Assessment Questions

For this Application, FSANZ has considered the following key questions:

- What are the possible public health and safety consequences of increasing or varying the dietary exposure to nisin by permitting it in processed meat products?
- What level of nisin is technologically justified in processed meat products?

RISK ASSESSMENT

5. Risk Assessment Summary

5.1 Safety Standard

In 1992, the European Union (EU) Scientific Committee for Food (SCF) allocated an Acceptable Daily Intake (ADI) of 0.13 mg nisin/kg bodyweight based on the second highest dose used in a multigenerational reproduction study. In 2006, the European Food Safety Authority (EFSA) reviewed the same study and concluded that the No Observed Effect Level (NOEL) was 62.5 mg/kg bodyweight/day. FSANZ has assessed the original data of this study and found no consistent evidence to indicate adverse effects associated with the ingestion of nisin even at the highest dose tested (62.5 mg/kg bw/day). After reviewing all the available data, FSANZ has used the NOEL of 62.5 mg/kg bodyweight/day and applied a 100-fold safety factor to establish an ADI of 0-0.625 mg/kg bodyweight for nisin.

Ingested nisin is inactivated by digestive enzymes in a similar way to other dietary proteins or peptides and has therefore no effect on the colonic microflora. Based on the absence of any appreciable systemic exposure there are unlikely to be any safety issues associated with the presence of nisin in food. A full report on the safety of nisin is provided at **Attachment 2**.

5.2 Dietary Exposure

If the requested limits were approved, processed meat products (including processed poultry and game products) would be the highest potential contributor to nisin exposures for all population groups. Other major contributors to estimated nisin exposures would be beer and cream products.

The estimated dietary exposures to nisin, as a percent of the ADI, are included in the Dietary Exposure Assessment Report at **Attachment 3**.

Based upon the limits proposed by the Applicant and taking into account current dietary exposure, the estimated mean dietary exposures to nisin were 1% of the ADI for Australians aged two years and above and New Zealanders aged 15 years and above, and 3% of the ADI for Australians aged 2-6 years. Estimated 95th percentile dietary exposures for consumers of nisin under this scenario were lowest for New Zealanders aged 15 years and above at 5% of the ADI and highest for Australian children aged 2-6 years at 10% of the ADI.

5.3 Risk Characterisation

Estimated dietary exposures to nisin were compared to the ADI of 0-0.625 mg/kg bw/day for risk characterisation purposes. Estimated dietary exposures for all population groups assessed were well below the ADI for both mean and 95th percentile consumers of nisin. These results do not raise any public health and safety concerns with the dietary exposure to nisin at the proposed levels of use.

5.3.1 Potential Allergenicity

Currently, a range of foods are permitted to contain nisin including cream products (up to 10 mg/kg) and flour products (up to 250 mg/kg).

In addition, other foods are permitted to contain nisin at GMP (Good Manufacturing Practice), including: cheese and cheese products; oil emulsions; tomato products; fruit and vegetable preparations; liquid egg products; tomato juices; beer and related products; dairy and fat-based desserts; dips and snacks; and sauces and toppings, mayonnaises and salad dressings. Nisin is typically added to foods as part of a food additive formulation which may contain allergens such as milk or soy constituents. The extension of use of nisin-containing formulations to processed meats therefore has the potential to increase the likelihood of exposure to these allergens. This potential increase in exposure and its implications for allergy sufferers will depend on the extent of use of nisin formulations in processed meats by industry and the existing presence of these allergens from other ingredients that may typically be used in processed meats. In accordance with clause 4 of Standard 1.2.3, the presence of potential allergens including milk, milk products and soy would need to be declared in the label of processed meat products that contain these potential allergens.

5.4 Antimicrobial Properties

Nisin is not used clinically but has been used extensively by the food industry as a preservative. It has a long history of safe use in a broad range of foods including fermented dairy products. Although sporadic occurrences of nisin-resistant bacterial mutants are reported, there is no evidence that these mutants can develop cross-resistance to clinically important antibiotics. This is because of the distinctly different mechanism of action of nisin and the glycopeptide antibiotics, specifically the different binding targets for the substances.

In assessing the proposed extended use of nisin in new food categories, FSANZ considered the potential of nisin to induce antimicrobial resistance. As part of these considerations FSANZ sought advice from the Expert Advisory Group on Antimicrobial Resistance (EAGAR) of the National Health and Medical Research Council (NHMRC). EAGAR concluded that nisin was unlikely to induce antimicrobial resistance under the proposed conditions of use.

5.5 Food Technology Assessment

The Applicant provided data indicating that the presence of nisin would inhibit the growth of Gram-positive bacteria in processed meat products, although its effects will be dependent upon the composition of the particular meat product. It is also considered that the effects of nisin are likely to be enhanced when used with other food preservation techniques.

The limit proposed by the Applicant of 12.5 mg/kg for nisin in meat products is considered to be consistent with the practical use of nisin in meat products, having regard to losses that could reasonably occur during processing and product shelf life. The use of nisin at levels proposed by the Applicant is also considered to be inhibitory to *L. monocytogenes*. In FSANZ's view, the data provided by the Applicant does not suggest that nisin eliminates *L. monocytogenes* i.e. that it is listericidal. If processed meat products were to contain *L. monocytogenes* then based on the data provided, it is questionable whether nisin will eliminate the pathogen, although it should inhibit its growth in the processed meat product over the product's shelf-life.

In accordance with Standard 1.6.1, packaged, cooked, cured/salted meat must not contain any *L. monocytogenes* (in 25g).

Good hygienic practices will therefore still need to be observed for these products to ensure the elimination of *L. monocytogenes* and to prevent subsequent contamination, even if nisin is added to these products.

On this basis and given the limited spectrum of activity of nisin, it is considered that there is technological justification for the use of nisin in processed meat products and that it will be a potentially useful component of food preservation systems for processed meat production.

Nisin is likely to be negative or inhibitory to starter cultures used in the production of fermented meat. In practice, nisin is not likely to be applied to meat prior to the completion of fermentation. Therefore the negative or inhibitory effect of nisin to starter cultures should not pose a problem to the performance of starter cultures in the production of fermented meat.

A full Food Technology Report is provided at **Attachment 4**.

RISK MANAGEMENT

Standard 1.3.1 – Food Additives includes limits for food additives in food and limits for nisin in a number of foods already exist in Schedule 1 of Standard 1.3.1. A specification for nisin is currently referenced in Standard 1.3.4 – Identity and Purity. As nisin is a food additive ingredient, the labelling requirements in the Code will also apply, including Standard 1.2.3 – Mandatory Warning and Advisory Statements and Declarations and Standard 1.2.4 – Labelling of Ingredients.

The Risk Assessment indicates that the extended use of nisin formulations in processed meats has the potential to increase the likelihood of exposure to certain allergens, including milk or soy. In accordance with clause 4 of Standard 1.2.3, the presence of potential allergens including milk, milk products and soy would need to be declared in the label of processed meat products that contain these potential allergens. For unpackaged foods, this declaration would need to be on or in connection with the display of a food or declared to the purchaser upon request. This declaration is required if potential allergens are added to a food as an ingredient or as part of a food additive formulation.

Processed meat products may currently contain constituents of milk (e.g. whey powder) or soy as ingredients. Information currently exists to indicate to consumers that these potential allergens are typically present in processed meats. For example, a Milk Allergen Card³ is available from Anaphylaxis Australia which indicates that deli meats are included in the list of foods that might contain milk.

Processed meats typically contain milk or soy ingredients and the presence of these allergens would already be declared. The extended use of nisin containing formulations may add milk or soy allergens to some processed meats that previously did not contain these allergens. For these products the presence of these allergens would be required to be declared or declared to the purchaser on request. On this basis, any additional presence of allergens through the extended use of nisin-containing formulations in processed meats is not considered to require additional risk management measures.

³ <http://www.foodstandards.gov.au/foodmatters/foodallergies/index.cfm>

6. Options

FSANZ is required to consider the impact of various regulatory (and non-regulatory) options on all sectors of the community, which includes consumers, food industries and government agencies in Australia and New Zealand.

There are no other options other than a variation to Standard 1.3.1 for this Application. Therefore the regulatory options available for this application are:

Option 1: not include a limit for nisin in processed meat products in Standard 1.3.1 and maintain the *status quo*;

Option 2: vary Standard 1.3.1 to include a limit for nisin in processed meat products and approve a broader use of nisin.

7. Impact Analysis

7.1 Affected Parties

Parties possibly affected by the regulatory options outlined above include:

1. Consumers who may be affected, either positively or negatively, if processed meat products contained nisin.
2. those sectors of the food industry wishing to use nisin in processed meat products to mitigate the costs associated with product spoilage from certain bacteria;
3. Government agencies where costs may increase as a result of the need to monitor compliance with the limits for nisin in processed meat products, including the development of methods to measure the nisin concentration in processed meats.

7.2 Benefit Cost Analysis

7.2.1 Option 1 – Not include a limit for nisin in processed meat products

Under Option 1, the affected parties and potential impacts are:

- Manufacturers and vendors of processed meat products may be disadvantaged as they would be unable to take advantage of the potential food preservation benefits and market opportunities for the development and sale of nisin containing processed meat products.
- Consumers may be disadvantaged as they would be denied any potential benefits resulting from nisin containing processed meat products, including potential shelf life extension.
- There is no perceived impact on government agencies, although lack of approval may be regarded as unnecessarily restrictive.

7.2.2 *Option 2 – Include a limit for nisin in processed meat products*

Under Option 2, the affected parties and potential impacts are:

- Manufacturers and vendors of processed meat products may benefit as they may be able to include nisin in their products as part of their food preservation systems with consequent market advantages from reduced spoilage losses.
- Consumers may benefit from foods containing nisin as there is a potential reduction in losses associated with food spoilage if nisin is used as part of a food preservation system, although the use of nisin-containing formulations may introduce a new source of allergens to some processed meats.
- There are potential impacts for government agencies as a result of ensuring that compliance with the proposed limits and labelling requirements (including allergen declarations) are observed by manufacturers.

7.3 **Comparison of Options**

Option 1 appears to provide no benefits to consumers, industry or government. Option 1 denies industry access to a preservative in processed meat products, which may potentially provide benefits to industry and consumers in reducing microbiological spoilage losses.

Option 2 does not appear to impose any significant costs on industry, consumers or government. Option 2 provides benefits to industry in terms of product innovation and potential benefits for industry and consumers in reducing the losses associated with food spoilage of processed meat products.

An assessment of the costs and benefits of Options 1 and 2 indicates that there would be a net benefit in including limits for nisin in processed meat products. Therefore, Option 2 is the preferred option.

COMMUNICATION

8. Communication and Consultation Strategy

This Application seeks to incorporate limits for nisin in processed meat products in Standard 1.3.1 to control food spoilage bacteria in these foods. Incorporating these limits would allow food manufacturers to add nisin formulations to processed meat products.

FSANZ has applied a basic communication strategy to Application A565. This involved advertising the availability of the Draft Assessment Report for public comment in the national press and making the reports available on the FSANZ website.

The Applicant, individuals and organisations that made submissions on this Application will be notified at each stage of the Application. If the FSANZ Board approves the draft variation to the Code, FSANZ will notify the Ministerial Council of its decision. The Applicant and stakeholders, including the public, will be notified on the gazettal of changes to the Code in the national press and on the website.

Nisin is typically added to foods as part of a food additive formulation which may contain allergens such as milk or soy constituents. The presence of these allergens in processed meats is either currently declared, or would be required to be declared if the addition of nisin-containing formulations resulted in allergens in processed meats that were not previously present. FSANZ will investigate whether the existing available information about the presence of allergens in food from food additive formulations is sufficient and will consider whether additional information may be required to improve awareness in the area of allergens in food from food additive formulations.

9. Consultation

9.1 Issues Raised in Public Consultation

The Draft Assessment Report was circulated for a round of public comment from 8 August 2007 until 19 September 2007 and eight submissions were received. One of these submitters opposed the Application, another submitter raised issues for further consideration by FSANZ and a third submitter did not object to further consideration of the Application. The remaining five submitters supported the incorporation of a limit for nisin in processed meat products, although one of these submitters raised an issue in relation to the draft variation. FSANZ has taken the submitters' comments into account in preparing the Final Assessment of this Application and the issues raised in submissions have been addressed.

9.1.1 Draft variation

The submission from the New Zealand Food Safety Authority (NZFSA) highlighted an issue in relation to the draft variation to the Code proposed at Draft Assessment and suggested deletion of the words 'commercially sterile canned cured meat' to apply the limit for nisin to all processed meat products categorised under item 8.2.

9.1.1.1 FSANZ Response

The Applicant sought limits for nisin for all processed meat products under item 8.2 and item 8.3 in Schedule 1 of Standard 1.3.1. It was also the intent of FSANZ for these limits to apply to all processed meat products under item 8.3 and item 8.2 in Schedule 1 of Standard 1.3.1. The draft variation circulated for public comment included the words 'commercially sterile canned cured meat' as part of the item 8.2 heading. If this draft variation had been approved, it would have had the unintended effect of restricting the nisin limit to commercially sterile canned cured meat, and other processed meat products standardised under item 8.2 would not have been permitted to contain nisin.

To address this FSANZ has amended the draft variation to delete the words 'commercially sterile canned cured meat' from the heading to item 8.2, thereby ensuring that the nisin limit applies to all processed meat products under item 8.2 and item 8.3 in Schedule 1 of Standard 1.3.1. **Attachment 1** includes the amended draft variation.

9.1.2 Antimicrobial resistance

The submission from Mr Paul Elwell-Sutton stated that Nisin is derived from *Streptococcus lactis* and states the European Parliament has determined that it causes antibiotic resistance and should not be used in foodstuffs.

9.1.2.1 FSANZ Response

In assessing the proposed use of nisin in new food categories, FSANZ has considered the potential of nisin to induce antimicrobial resistance (see Section 5.4). Since nisin is a polypeptide that is subject to proteolytic degradation, there is unlikely to be any biologically active nisin which could enter the colon and create conditions favourable for developing bacterial resistance in humans. In addition, nisin has a long history of safe use in a broad range of foods and although sporadic occurrences of nisin-resistant bacterial mutants are reported, there is no evidence that these mutants can develop cross-resistance to clinically important antibiotics.

FSANZ has also sought advice from the Expert Advisory Group on Antimicrobial Resistance (EAGAR) of the National Health and Medical Research Council (NHMRC). EAGAR concluded that nisin was unlikely to induce antimicrobial resistance under the proposed conditions of use.

FSANZ has not been able to locate any determination from the European Parliament that nisin not be used in food. FSANZ has located an ‘Opinion of the Scientific Panel on food additives, flavourings, processing aids and materials in contact with food (AFC) related to the use of nisin (E 234) as a food additive’ which stated that:

Nisin has a double mode of antimicrobial action; binding to lipid II and subsequent inhibition of cell wall synthesis as well as forming pores in the cytoplasmic membrane. Nisin is only used as a food preservative and has currently no therapeutic use. Sporadic nisin resistant mutants do not show any cross-resistance to therapeutic antibiotics. The Panel considered that this is probably due to the differences in the antimicrobial mode of action between therapeutic antibiotics and nisin and that development of antibiotic resistance is not of concern in relation to use of nisin in food.

Nisin is currently used in foods in Australia and New Zealand. Nisin is a food preservative produced by certain strains of the food-grade lactic acid bacterium *Lactococcus lactis* subsp. *lactis* during fermentation. This bacterium was formerly known as *Streptococcus lactis*. This naturally occurring bacterium commonly occurs in milk and fermented dairy and meat products. FSANZ does not consider that exposure to this naturally occurring organism has the potential to ‘cause’ or promote the development of antimicrobial resistance.

9.1.3 Nisin Specifications

Mr Paul Elwell-Sutton stated that China is a major producer of nisin and there may be undetected levels of contaminants in nisin from China.

9.1.3.1 FSANZ Response

Nisin is currently used in foods in Australia and New Zealand. The Food and Nutrition Paper 52 Compendium of Food Additive Specifications, addenda 1-12, published by the Food and Agriculture Organisation (FAO) of the United Nations in Rome (1992)⁴ contains a specification for nisin containing formulations.

⁴ <http://www.fao.org/ag/agn/jecfa-additives/specs/Monograph1/Additive-295.pdf>

This specification is currently referenced in Standard 1.3.4. Nisin currently used in foods in Australia and New Zealand must comply with this specification, which includes a specification for lead. In addition, Standard 1.3.4 applies further specifications for arsenic and heavy metals in nisin used in foods. FSANZ therefore considers that sufficient regulatory measures exist to ensure that contaminants in nisin from any source can be adequately managed.

FSANZ has noted that the FAO/World Health Organization Joint Expert Committee on Food Additives has recently published an amended specification for nisin, which includes nisin produced from non-dairy sources. The adoption of this specification will be considered by FSANZ as part of a separate proposal to regularly update the references in Standard 1.3.4. In this updated specification, the requirements for chemical contaminants remain unchanged.

9.1.4 Potential for Allergic reactions

The South Australian Department of Health raised the issue of allergic reactions to possible milk residues present on processed meat products and considered that appropriate labelling and information should be considered, including for unpackaged foods. This was on the basis that it is unlikely that consumers with milk allergies will expect meat products to contain milk residues.

9.1.4.1 FSANZ Response

Processed meat products may currently contain potential allergens such as the constituents of milk (e.g. whey powder). In accordance with clause 4 of Standard 1.2.3, the presence of potential allergens such as milk and milk products would need to be declared in the label of processed meat products. For unpackaged foods, this declaration would need to be on or in connection with the display of a food or declared to the purchaser upon request.

In relation to milk derived ingredients in processed meats, a Milk Allergen Card is available from Anaphylaxis Australia which already indicates that deli meats are included in the list of foods that might contain milk. Therefore some information currently exists to indicate to consumers that these allergens are typically present in processed meats.

The extended use of nisin-containing formulations may add allergens to some processed meats that previously did not contain these allergens. For these products the presence of these allergens would now be required to be declared or declared to the purchaser on request. Therefore, while the use of nisin containing formulations in processed meats is a potential new source of allergens, the requirements to declare the presence of these allergens in processed meats is considered sufficient to manage the risks associated with this potential new source of exposure.

Recognising the potential for exposure to certain allergens from food additive formulations, FSANZ will investigate whether the existing available information about the presence of allergens in food from food additive formulations is sufficient. If necessary, FSANZ will consider whether additional information may be required to improve awareness in the area of allergens in food from food additive formulations.

9.1.5 Use of nisin as a replacement or substitute for good hygienic practices

The South Australian Department of Health stated that nisin's effectiveness is yet to be proven sufficiently in meat products and it was therefore very important that good handling techniques are maintained and that nisin is not used as a replacement to good hygiene but as an additional measure to reduce contamination.

9.1.5.1 FSANZ Response

As stated above under Section 5.5, the limited activity of nisin to Gram-positive bacteria is such that good hygienic practices will still need to be observed in the production of processed meat products and that nisin would not mask the use of poor hygienic practices. Nisin is considered to be a potentially useful component of food preservation systems, not a replacement for good hygienic practices.

9.1.6 Control of *Listeria*

The South Australian Department of Health questioned whether the introduction of nisin use in the meat industry impacted on the product recall guideline for *Listeria* and specifically whether a nisin-treated meat product containing *Listeria* would still be required to be recalled.

9.1.6.1 FSANZ Response

While the Applicant refers to *L. monocytogenes* control as one of the advantages of the use of nisin in processed meat products, the Applicant also refers to the use of nisin inhibiting the growth of food spoilage bacteria in processed meat products. FSANZ has therefore considered the benefits of nisin in relation to the control of both food spoilage bacteria and *L. monocytogenes*. In accordance with Standard 1.6.1, packaged, cooked, cured/salted meat must not contain any *L. monocytogenes* (in 25 g). Good hygienic practices will therefore still need to be observed for these products to ensure the elimination of *L. monocytogenes* and to prevent subsequent contamination, even if nisin is added to these products.

As stated above under Section 5.5, FSANZ considers that adequate data were provided by the Applicant to indicate that nisin inhibits the growth of both Gram-positive spoilage bacteria and *L. monocytogenes*, although the effects will be dependent upon the composition of the meat product. Further details on this inhibition, including references are provided in the attached Food Technology Report (**Attachment 4**).

If processed meat products were to contain *L. monocytogenes*, then based on the data provided, it is questionable whether nisin would eliminate the pathogen, although it should inhibit its growth in the processed meat product over the product's shelf-life. The current 'Listeria Recall Guidelines for Packaged Ready-to-eat food' refer to the need to take inhibitors and documented evidence of this inhibition into account in determining the disposition of implicated product. If this inhibition can be adequately demonstrated by the manufacturer, then processed meat products would still need to comply with the <100 colony forming units requirement in the guidelines.

However, as stated in the *Listeria* recall guidelines, food processed correctly and protected from contamination after processing, should normally be able to comply with the 'not detected' in 25 g criterion.

The presence of *Listeria* in products, produced by a process which is capable of eliminating *Listeria*, may indicate that the process is not totally under control. The detection of *Listeria* in these products would be an indication of either inadequate processing or post-process contamination that should be further investigated.

In summary, FSANZ considers that the current *Listeria* recall guidelines remain current and appropriate for use in determining the disposition of nisin containing processed meats that have been found to contain *L. monocytogenes*.

9.2 Other Consultation Comments

9.2.1 Dietary Exposure, including Children

In their submission the NZFSA was satisfied that the estimated dietary exposure results do not raise any public health and safety concerns with nisin at the proposed levels of use. NZFSA accepts the rationale given by FSANZ for the assumption that the dietary exposures to nisin for New Zealand children are well below the ADI.

9.2.1.1 FSANZ Response

As stated at Draft Assessment, a separate dietary exposure assessment was not conducted for New Zealand children under 15 years old due to the absence of relevant consumption data in the correct format for dietary modelling. Based on the estimated 95th percentile dietary exposures for Australian children aged 2-6 years (10% of the ADI), it can be assumed that 95th percentile dietary exposures to nisin for New Zealand children are also well below the ADI.

Even if there was a difference in food consumption patterns between Australian and New Zealand children, it would need to be appreciably higher for New Zealand children in order to make dietary exposures approach the ADI. Such a large difference in consumption between Australian and New Zealand children is considered unlikely.

9.3 World Trade Organization (WTO)

As members of the World Trade Organization (WTO), Australia and New Zealand are obligated to notify WTO member nations where proposed mandatory regulatory measures are inconsistent with any existing or imminent international standards and the proposed measure may have a significant effect on trade.

The draft variation to the Code represents a reduction in regulation as it would permit nisin to be present in foods where it is currently prohibited.

The limit of 12.5 mg/kg, as proposed by the Applicant, is technologically justified and no information has been provided to justify a higher limit. While Codex has a limit of 500 mg/kg in processed meat products under consideration, this consideration has not been finalised. Therefore, the proposed limit is not considered more restrictive than international standards or to have a significant effect on international trade, although there is the potential for a more significant impact if the limit currently under consideration by Codex is progressed.

Recognising the potential for future impact, notification was made in accordance with Australia's and New Zealand's obligations under the WTO Technical Barriers to Trade Agreement. No comments were received.

CONCLUSION

10. Conclusion and Recommendation

The Applicant has sought to amend Schedule 1 of Standard 1.3.1 – Food Additives, of the Code to include limits for nisin of 12.5 mg/kg in processed meat products, specifically for:

- Processed meat, poultry and game products in whole cuts or pieces; and
- Processed comminuted meat, poultry and game products.

FSANZ has identified two regulatory options for this Application:

- Option 1: not include a limit for nisin in processed meat products in Standard 1.3.1 and maintain the *status quo* maintain the *status quo* approach;
- Option 2: vary Standard 1.3.1 to include a limit for nisin in processed meat products and approve a broader use of nisin.

Decision

Standard 1.3.1 – Food Additives be amended to include a limit of 12.5 mg/kg for nisin in processed meat products.

10.1 Reasons for Decision

This variation is proposed for the following reasons.

- The draft variation to the Code is consistent with the section 18 objectives of the FSANZ Act. In particular, it does not raise any public health and safety concerns, including in relation to antimicrobial resistance. The relevant assessments are based on the best available scientific evidence and the use of nisin in processed meat products may assist in promoting an efficient and internationally competitive food industry.
- Use of nisin in processed meats is technologically justified and it will be a potentially useful component of food preservation systems for processed meat production. Based upon data provided by the Applicant, the presence of nisin would inhibit the growth of Gram-positive bacteria, and its effects are likely to be enhanced when used with other food preservation techniques.
- The regulation impact assessment concluded that the benefits of the potential use of nisin in processed meats outweigh any costs associated with its use.
- To achieve what the Application seeks, there are no alternatives that are more cost-effective than a variation to Standard 1.3.1 – Food Additives.

11. Implementation and Review

The variation will come into effect on the date of gazettal.

FSANZ will investigate whether the existing available information about the presence of allergens in food from food additive formulations is sufficient and will consider whether additional information may be required to improve awareness in the area of allergens in food from food additive formulations.

ATTACHMENTS

1. Draft variation to the *Australia New Zealand Food Standards Code*
2. Safety Assessment Report
3. Dietary Exposure Assessment Report
4. Food Technology Report
5. Summary of issues raised in public submissions to the Draft Assessment Report

Draft variation to the *Australia New Zealand Food Standards Code*

Standards or variations to standards are considered to be legislative instruments for the purposes of the Legislative Instruments Act (2003) and are not subject to disallowance or sunseting.

To commence: on gazettal

[1] **Standard 1.3.1** of the *Australia New Zealand Food Standards Code* is varied by –

[1.1] *inserting in* Schedule 1, *under item* 8.2 Processed meat, poultry and game products in whole cuts or pieces* –

234	Nisin	12.5	mg/kg
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[1.2] *inserting in* Schedule 1, *under item* 8.3 Processed comminuted meat, poultry and game products* –

234	Nisin	12.5	mg/kg
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Safety Assessment Report

APPLICATION A565 – NISIN – USE IN PROCESSED MEAT PRODUCTS

This safety assessment was conducted to identify potential public health and safety risks associated with the addition of nisin to two new food categories: processed meat, poultry and game products in whole cuts or pieces; processed comminuted meat, poultry and game products. The assessment was based on data on the chemistry, metabolism and toxicity of nisin provided by the applicant and obtained from the scientific literature. In addition, safety assessments conducted by the Joint FAO/WHO Expert Committee on Food Additives (JECFA), EU Scientific Committee for Food, and European Food Safety Authority (EFSA) were also considered.

Summary and Conclusions

Nisin (INS 234) is a naturally occurring antimicrobial agent, also known as a bacteriocin, which is produced by the bacterium *Lactococcus (Streptococcus) lactis* ssp. *lactis*. Bacteriocins are proteins or polypeptides produced by bacteria that kill or inhibit the growth of other bacteria. Many lactic acid bacteria, such as *Lactococcus lactis*, found in various fermented dairy and meat products, produce a diverse range of bacteriocins. Nisin is a polypeptide composed of 34 amino acids produced by the bacterium *L. lactis* ssp. *lactis*. It has a narrow spectrum of activity affecting primarily vegetative cells and spores of Gram-positive bacteria. Bacteria susceptible to nisin include other lactic acid bacteria, *Bacillus*, *Clostridium*, *Listeria*, and *Streptococcus* genera. In the absence of other preservation methods, nisin does not inhibit Gram-negative bacteria, yeasts, or moulds. Nisin may be present in food due to the presence of the lactic acid bacteria used as starter cultures, or may be added directly. Therefore it is likely that human exposure to nisin has been occurring naturally for many years.

Ingested nisin is inactivated by digestive enzymes in a similar way to other dietary proteins or peptides and has therefore no effect on the colonic microflora. The Expert Advisory Group on Antimicrobial Resistance (EAGAR) of the National Health and Medical Research Council (NHMRC) has considered the potential of nisin for developing antimicrobial resistance through the extended use of nisin as a food preservative. EAGAR concluded that nisin was unlikely to induce antimicrobial resistance when used as proposed.

Based on the absence of any appreciable systemic exposure there are unlikely to be any safety issues associated with the presence of nisin in food. A range of hazard testing studies in laboratory animals confirmed the absence of any adverse effects. The dietary modelling of nisin-treated foods, including the proposed new range of treated foods, indicated that consumers would not exceed the ADI of 0-0.625 mg/kg bw even for the high intake consumers (i.e. 95th percentile intake levels).

Existing Permissions in the Code

Nisin is currently permitted in the Code in a wide range of foods including, cream products, cheese and cheese products, oil emulsions, tomato products, fruit and vegetable preparations, flour products, liquid egg products, tomato juices, beer and related products, dairy and fat-based desserts, dips and snacks, and sauces and toppings including mayonnaises and salad dressings, at levels ranging from 10 mg/kg to GMP.

Existing Safety Standards

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) allocated an ADI of 33,000 units per kg body weight (bw) in 1969 based on a long term toxicity study (JECFA, 1969). The WHO Committee on Biological Standardization defined the International Unit (IU) as 0.001 mg of a reference preparation containing 25 ng of nisin. Subsequently, an error in calculating the administered dose in the JECFA report was identified by the US Food and Drug Administration (FDA). The amended value was calculated to be 4.9 mg /kg bw/day. Using a safety factor of 100, an Acceptable Daily Intake (ADI) of 0-0.049 mg/kg bw was established (FDA, 1988).

In 1992, the EU Scientific Committee for Food (SCF) allocated an ADI of 0-0.13 mg nisin/kg bw for a product with a potency of 40 000 units/mg based on a multi-generational reproduction study (SCF, 1992). In 2006 the European Food Safety Authority (EFSA) reviewed the same study and concluded that it indicated a NOEL of 62.5 mg/kg bw/day for nisin but confirmed the ADI of 0-0.13 mg/kg bw previously established by SCF (EFSA, 2006).

FSANZ has assessed the original data of this study and found no consistent evidence to indicate adverse effects associated with the presence of nisin even at the highest dose (62.5 mg/kg bw). After reviewing all the available data, FSANZ has used the NOEL of 62.5 mg/kg bw/day and applied a 100-fold safety factor to establish an ADI of 0-0.625 mg/kg bw for nisin.

1. INTRODUCTION

Danisco Australia Pty Ltd is seeking approval for the broader use of nisin as a preservative, to a maximum level of 12.5 mg/kg, in two food categories:

- processed meat, poultry and game products in whole cuts or pieces;
- processed comminuted meat, poultry and game products;

Nisin (INS 234) is a naturally occurring antimicrobial peptide produced by the bacterium *Lactococcus lactis* ssp. *lactis*. Nisin is active against a wide range of Gram-positive vegetative bacteria, including *Bacillus*, *Clostridium* and *Lactobacillus* as well as the highly significant gram-positive pathogen *Listeria monocytogenes*. Nisin can also inhibit spore outgrowth through double bond interactions between its didehydroalanine residue (in position 5) and a spore-associated factor essential in sporulation (reviewed by Bauer and Dicks, 2005). Nisin is ineffective against yeasts, moulds and Gram-negative bacteria.

Nisin is produced commercially by fermentation of skim milk using nisin-producing strains of *L. lactis* ssp. *Lactis*.

2. HISTORY OF USE

Many lactic acid bacteria, such as *L. lactis*, are found in various fermented dairy and meat products and produce a diverse range of bacteriocins, including nisin. Nisin may be present in some foods due to the presence of lactic acid bacteria used as starter cultures, or it may be added directly as a preservative. A level of natural human exposure to nisin may have occurred for many centuries.

Nisin preparations have been used for more than 50 years as a preservative in food. It is currently approved in more than 70 countries and permitted in a wide range of foods. In Australia and New Zealand, nisin is permitted in a wide range of foods (cream products, cheese and cheese products, oil emulsions, tomato products, fruit and vegetable preparations, flour products, liquid egg products, tomato juices, beer and related products, dairy and fat based desserts, dips and snacks, and sauces and toppings including mayonnaises and salad dressings) at levels ranging from 10 mg/kg to GMP.

3. EXISTING SAFETY STANDARDS

Nisin was evaluated for safety by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 1969. JECFA concluded that:

The available evidence indicates that a level of 3,300,000 units of nisin/kg body weight has no adverse effect. This finding permits an unconditional ADI to be set at 33,000 units per kg body weight. At the level noted above, nisin has no microbiological, toxic or allergic effects.

The WHO Committee on Biological Standardization defined the International Unit (IU) as 0.001 mg of a reference preparation containing 25 ng of nisin.

However, there was a mistake in the units used by JECFA (3,300,000 units/kg was the concentration in the diet, not a daily intake dose) and the US Food and Drug Administration (FDA) subsequently calculated the equivalent dose of 4.9 mg /kg bw. Using a safety factor of 100, an Acceptable Daily Intake (ADI) of 0.049 mg/kg bw was established. This corresponds to 2.9 mg/person/day (FDA, 1988).

In 1992, the EU Scientific Committee for Food (SCF) reassessed the safety information available to JECFA and considered a further reproduction and teratogenicity study, as well as mutagenicity studies. Based on the available data, this Committee allocated an ADI of 0.13 mg/kg bw for a product with a potency of 40 000 units/mg based on the newer reproduction study (SCF, 1992).

In 2001, based on a report prepared by the applicant, FDA accepted nisin as Generally Recognized As Safe (GRAS) for use as an anti-microbial on cooked meat and poultry products when used at a level that delivers a maximum of 0.025% nisin in the finished product (FDA, 2001).

In 2006, the European Food Safety Authority (EFSA) re-evaluated the safety of nisin and considered the issue of antimicrobial resistance. When ingested, nisin is inactivated by digestive enzymes such as trypsin and pancreatin and has no effect on intestinal microflora. Based on the available toxicity studies and its long history of safe use, EFSA confirmed the ADI previously established by the SCF and concluded that the development of resistance to nisin is not a concern in relation to the use in food (EFSA, 2006).

4. STRUCTURE AND PROPERTIES

4.1 Chemistry

Nisin is a peptide antibiotic, or bacteriocin, that contains the unusual amino acid lanthionine, and thus is classed as a lantibiotic. Nisin is composed of 34 amino acids of which 13 amino acids are post-translationally modified to create five lanthionine-based rings in the peptide core.

There are two naturally occurring nisin variants, nisin A and nisin Z, which are produced by different *Lactococcus lactis* stains. The variants only differ by an amino acid substitution at position 27 in the polypeptide. Nisin A has a histidine residue at position 27, while nisin Z has asparagine (Mulders et al. 1991). Nisin A and nisin Z have identical MICs against indicator strains of 6 different species of gram-positive bacteria. The genes encoding nisin A and nisin Z among 16 *Lactococcus lactis* stains were investigated and the results indicated that there were 14 strains producing nisin Z and 12 nisin A (de Vos et al. 1993). Nisin A has the formula of C₁₄₃H₂₃₀N₄₂O₃₇S₇ and a molecular weight of 3354 Daltons (Bauer and Dicks, 2005).

4.2 Solubility and stability

Nisin is an amphiphilic molecule. It is soluble in water and other polar solvents and virtually insoluble in non-polar solvents.

Nisin is most soluble and stable in acid conditions. The solubility is reduced with an increase in pH. It was reported that 12% solubility was achieved at pH 2.5, but only 4% at pH 5.0. It is also heat-stable at low pH. At pH 2.0 it was found to be stable on heating to 115.6°C, but lost 40% of its activity at pH 5.0 and more than 90% at pH 6.8 at the same temperature (Liu and Hansen, 1990).

5. TOXICOLOGY DATA

EFSA (2006) reviewed existing toxicity studies on nisin and their summaries are presented here.

5.1 Metabolism

Based on the EFSA's review, nisin was not detected in human saliva 10 min after the consumption of chocolate milk containing 0.005 mg nisin/kg. Daily consumption of a milk-based beverage containing 0.625 mg/kg nisin, for 14 days, did not adversely affect the oral microflora, or lead to the emergence of nisin-resistant strains.

Nisin was found to be readily inactivated and by pancreatin at pH 8.0 *in vitro* when held at a temperature of 37°C for 15-30 min. Inactivation through exposure to alpha-chymotrypsin has also been reported.

Based on *in vitro* studies, proteolytic enzymes in the small intestine are anticipated to hydrolyze the 34 amino acid-nisin into smaller peptides that can be absorbed and further metabolized to their constituent amino acids.

5.2 Toxicity Studies

Acute toxicity

The oral LD₅₀ of nisin in rats was reported to be >25 mg/kg bw by Frazer *et al* (1962), whereas in the mouse Hara *et al.* (1962) determined it to be 174 mg/kg bw.

Short term studies

Several subchronic studies in rats with a duration ranging from 84 to 90 days were conducted. The highest tested dose tested varied from 0.125 mg/kg bw/day to 60 mg/kg bw/day. The results in all measured parameters were indistinguishable from controls.

Long-term studies

Three groups of 30 females and 15 male rats (generation 0) were fed a control diet and diet containing nisin at levels of 3.33×10^4 or 3.33×10^6 units/kg diet (Frazer *et al.* 1962, in EFSA 2006). All animals were weighed weekly for the first 16 weeks and food consumption measured. After 16 weeks, males and females from the same group were mated and reproductive performance recorded.

Thirty females and ten males from the offspring in each group (generation 1) were fed on the same diet as their parents. After 35 weeks of feeding, 5 male and 5 female from generation 1 were tested for haematological parameters (erythrocyte, lymphocyte, monocyte, granulocytes and haemoglobin contents). At 37 and 38 weeks of age, kidney and gastrointestinal functions were determined respectively. Organ weights (heart, spleen, liver, kidneys, stomach, small intestines, caecum, adrenals, ovaries and uterus) were recorded for 10 females and testes weighed in 10 males from each group. At approximately 40 weeks of age, generation 1 animals were autopsied and histopathological examinations carried out on organs of 10 animals from each group. For generation 0 animals, the date an animal died was recorded and post mortem carried out wherever appropriate. After 2 years, the remaining animals were euthanized and autopsied.

On the basis that the mean body weight of the rats was 250 g and that they ate 15 g per day, the daily doses were equivalent to 0.049 or 4.9 mg/kg bw. No differences were found in the survival and reproductive performance between the control and test groups or in the autopsy and histological findings. Also, no differences in hepatic, renal or gastrointestinal function between the controls and experimental groups were observed. Similarly, the study revealed no increase in the incidence of tumours compared to the control.

Genotoxicity

Nisin did not show any mutagenic activity in the *Salmonella typhimurium* test system at doses up to 300,000 units/plate. Similarly, at oral doses of up to 8000 mg/kg bw nisin did not increase the incidence of micronucleated polychromatic erythrocytes in mice.

Reproductive toxicity

Nisin has an antibacterial activity of about 40,000 iu/mg. Nisaplin is a commercial formulation containing about 2.5% nisin, 76% NaCl and 20% non-fat milk solid and a antibacterial activity of 1,000 iu/mg.

In a multigenerational study, 12 male and 24 female rats per test group were fed diets containing Nisaplin at three different concentrations throughout three generations (Report APL1/801028, 1981). The Nisaplin concentrations used were 0%, 0.2%, 1%, or 5 % of the diet and these levels correspond to approximately 2.5, 12.5 and 62.5 mg nisin/kg bw/day for rats having a bodyweight of 300 g. Two control groups were used: a negative control group used diet containing no Nisaplin and a salt control group a diet containing 3.8% micronised NaCl which was equivalent to the salt content of 5% Nisaplin diet.

Rats of the F0 generation were maintained on their respective diet for at least 60 days prior to mating. The rats were mated on a one male to two female basis for a period of 20 days. The resulting litters (F1a) were reared to 21 days postpartum. The F1a pups were sacrificed and subjected to post mortem examination for the detection of macroscopic changes. Shortly after (approximately 10 days) the weaning of the F1a litters, the F0 females were re-mated for a period of 20 days. Offspring of this pairing were reared to 21 days post partum, when 12 males and 24 females were selected from each group to form the basis of the second generation (F1b).

The F1b generation was reared on their respective diet until they were at least 90 days old. Rats were mated on a one male to two female basis for a period of 20 days (avoiding brother and sister pairings) to produce an F2a generation, which was reared to 21 days post partum. The treatment and rearing of F2a and F2b pups were the same as for F1a and F1b.

The F2b generation proceeded as outlined above except that - at around day 21 post partum 10 male and 10 female F3b pups were selected for detailed macroscopic examination which also included organ weight analysis. Subsequent histopathological examination of preserved tissues was restricted to the control and the high dose group.

Over the three generations, there was one death among males of the F1b generation fed on 5% Nisaplin diet. Among females, occasional deaths did not reflect any treatment-related trend. Overall, no treatment-related mortality increase was observed.

There were no treatment-related effects on food or water intake although rats on high salt diets i.e. 5% Nisaplin and the salt control, had increased water intake and urination. Similarly, there were no treatment-related effects on bodyweight gain, reproductive performance, litter loss, litter size or pup mortality over the three generations. Macro- and microscopic analyses of the collected tissues also did not reveal any treatment related effects.

Based on the absence of any observed effects at the highest tested dose the NOEL is 62.5 mg/kg bw/day.

Special studies

Reddy *et al.* (2004) conducted a study on the effects of purified nisin on reproductive capacity of male rats upon oral administration of nisin for 13 consecutive weeks.

Nisin was administered by gavage, at a dose level of 5 mg/kg bw/day for 13 weeks. No abnormal weight changes in reproductive organs (testes, epididymes, prostate and seminal vesicle) were observed. No change was observed in the total sperm count. The reproductive performance of the treated rats remained unaffected. Furthermore, nisin treatment did not reduce the number of pups born (6 - 10 in both groups), weight (3 - 4g) and general health of the pups with no perinatal or postnatal repercussions. Growth of the pups was observed until the end of lactation and was found to be normal and similar to the control group.

A study using crude nisin demonstrated no effect on the mobility of leucocytes or the stability of erythrocytes at a concentration of 1:250 (4 mg/mL) (Mattick and Hirsch, 1947 in EFSA 2006).

Using a trypan blue exclusion test, nisin was shown to cause some cytotoxicity after 48 hours of incubation with SV40-HC cells and Vero cell lines (3.4 mM). The cytotoxicity was more pronounced for the SV40-HC cells (0.85 mM) (Murinda *et al.*, 2003). Nisin was found to be spermicidal *in vitro* at a concentration of 400µg/mL in a study of evaluating nisin as a vaginal contraceptive agent in rabbits (Reddy *et al.*, 2004).

A study was conducted to compare antibacterial activity and cytotoxicity of various antimicrobial peptides by Maher and McClean (2006). Nisin was shown to have an IC₉₀ of 0.13 µM against a Gram-positive indicator bacterial *Micrococcus luteus*, about one third of the IC₉₀ of vancomycin (0.45 µM). In cytotoxicity assays using human intestinal epithelial cell lines HT29 and Caco-2, both MTT (methylthiazolyldiphenyl-tetrazolium bromide) and neutral red dye uptake assays indicated an IC₅₀ of around 100 µM for nisin and 6000 µM for vancomycin, indicating a much higher cytotoxicity for nisin. Similarly, nisin caused significant haemolysis (12%) on live sheep erythrocytes compared to vancomycin (0.6%) at a concentration of 2 times IC₅₀. Although nisin exhibited much higher cytotoxicity than vancomycin, the concentrations at which nisin caused cytotoxicity were 1000-fold higher than those required for antimicrobial activity.

5.3 Risk Characterisation

SCF allocated an ADI of 0-0.13 mg/kg bw for nisin in 1992. This ADI was based on the second highest dose used in a multigenerational reproduction study (Report APL1/801028, 1981). EFSA (2006) reviewed the same study and concluded that it indicated a NOEL of 62.5 mg/kg bw/day for nisin. FSANZ has assessed the original data of this study and found no evidence to indicate adverse effects associated with the presence of nisin even at the highest tested dose of 62.5 mg/kg bw/day. After reviewing all the available data, FSANZ has used the NOEL of 62.5 mg/kg bw/day and applied a 100-fold safety factor to establish an ADI of 0-0.625 mg/kg bw for nisin.

The mean and 95th percentile estimated dietary exposures with the extension of use were well below the ADI (0.625 mg/kg bw) for all population groups examined. These results do not raise any public health and safety concerns associated with dietary exposure to nisin at the proposed levels of use.

6. ANTIMICROBIAL RESISTANCE

Emergence of resistance to antimicrobial compounds, especially those used in clinical medicine, is a potential public health concern.

The World Health Organization developed a 'WHO Global Strategy for Containment of Antimicrobial Resistance' to address this issue (WHO 2001). In assessing the proposed extended use of this antimicrobial agent in new food categories, FSANZ considered the potential of nisin to induce antimicrobial resistance. As part of these considerations FSANZ sought advice from EAGAR. EAGAR concluded that nisin was unlikely to induce antimicrobial resistance under the proposed conditions of use.

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Dietary Exposure Assessment Report

APPLICATION A565 – NISIN USE IN PROCESSED MEAT PRODUCTS

1. Executive Summary

An Application was received by FSANZ from Danisco Australia Pty Ltd requesting a variation to Standard 1.3.1 – Food Additives to extend the current permissions for nisin to include use in processed meat, poultry and game products, in whole cuts or pieces, and processed comminuted meat, poultry and game products to a maximum level of 12.5 mg/kg.

Dietary exposures to nisin were calculated for the Australian and New Zealand populations (Australians aged 2 years and above and New Zealanders aged 15 years and above), based on existing permissions and use levels ('**Baseline**') and including proposed permissions from the Application (**Scenario 1 'Baseline plus A565'**). The population groups examined were Australians 2 years and above and 2-6 years and New Zealanders aged 15 years and above.

The food consumption data used were from the 1995 Australian National Nutrition Survey (NNS) and the 1997 New Zealand NNS. The NNSs used a 24-hour food recall methodology. The concentration data used for both Baseline and Scenario 1 assessments were manufacturer use levels provided by the Applicant or Maximum Permitted Levels (MPLs) under the Code.

Estimated mean dietary exposures for consumers of nisin at Baseline using current MPLs and manufacturer use levels were up to 0.2 mg/day or up to 0.002 mg/kg bw/day, depending on the population group assessed. Estimated 95th percentile exposures for consumers of nisin were up to 1.0 mg/day or up to 0.01 mg/kg bw/day, depending on the population groups assessed.

Under Scenario 1, estimated mean dietary exposures for consumers of nisin were up to 0.7 mg/day or up to 0.02 mg/kg bw/day, depending on the population groups assessed. Estimated 95th percentile exposures for consumers of nisin were up to 2.6 mg/day or up to 0.07 mg/kg bw/day, depending on the population groups assessed.

Of the population groups assessed, New Zealanders aged 15 years and above had the highest estimated total dietary exposures for consumers of nisin (mg/day) for the Baseline assessment and the extension of use assessment (Scenario 1). When estimated mean dietary exposures were expressed on a body weight basis (mg/kg bw/day), the Australian and New Zealand populations had the same dietary exposures to nisin for the Baseline assessment. The sub-population group, Australians aged 2-6 years, had lower dietary exposures to nisin on a body basis (mg/kg bw/day), compared to the whole Australian population, for the Baseline assessment. However, based on the proposed extensions of use (Scenario 1), Australian children aged 2-6 years would have the highest dietary exposures to nisin on a body weight basis (mg/kg bw/day).

Should the requested permissions be approved, processed meat products (including processed poultry and game products) would be the highest contributor to nisin exposures for all population groups.

Other major contributors to nisin exposures would be beer (Australians aged 2 and above and New Zealanders aged 15 and above) and cream products (all population groups including Australians aged 2-6 years).

Estimated dietary exposures to nisin were compared to the Acceptable Daily Intake (ADI) of 0.625 mg/kg bw/day for risk characterisation purposes. Estimated dietary exposures for all population groups assessed were well below the ADI for both the Baseline and Scenario 1 assessments for both mean and 95th percentile consumers of nisin, at 10% of the ADI or less.

2. Background

An Application was received by FSANZ from Danisco Australia Pty Ltd requesting a variation to Standard 1.3.1 – Food Additives of the *Australia New Zealand Food Standards Code* (the Code) to extend the current permissions for nisin to include use in processed meat, poultry and game products, in whole cuts or pieces, and processed comminuted meat, poultry and game products to a maximum level of 12.5 mg/kg.

Nisin is used as an antimicrobial preservative in foods. It has been used as a food additive for over 50 years and is currently approved in more than 70 countries, including Australia and New Zealand. Nisin is a naturally occurring antibacterial agent, produced by a strain of the dairy starter culture *Lactococcus lactis* spp. *lactis*. Nisin is active against a wide range of Gram-positive bacteria but is generally not effective against Gram-negative bacteria, yeasts or moulds. The mechanism of action of nisin is thought to involve the incorporation of nisin into the bacterial cell membrane and the formation of pores, resulting in the efflux of various cellular constituents and the depletion of proton motive force (Davidson et al 2002). Nisin also inhibits the outgrowth of bacterial spores. The main purpose of nisin as a food additive is to inhibit the growth of food spoilage bacteria.

2.1 Existing permissions for use of nisin

The current maximum permitted levels (MPLs) for nisin in the Australia New Zealand Food Standards Code (the Code) in Standard 1.3.1 – Food Additives are listed in Table 1. Many foods are permitted to contain nisin at ‘GMP’ or Good Manufacturing Practice. This means that manufacturers should not add more than is required to achieve a technological function.

Table 1: Current Maximum Permitted Levels (MPLs) for nisin in the Code

Food Name	Nisin Concentration (mg/kg)
Cream products (flavoured, whipped, thickened, sour cream etc.)	10
Cheese and cheese products	GMP
Oil emulsions (<80% fat)	GMP
Tomato products pH < 4.5	GMP
Fruit and vegetable preparations including pulp	GMP
Flour products cooked on hot plates	250
Liquid egg products	GMP
Tomato juices pH < 4.5	GMP
Beer and related products	GMP
Dairy and fat based desserts, dips and snacks	GMP
Sauces and toppings (including mayonnaises and salad dressings)	GMP

2.2 Extension of permissions requested by Applicant

The foods, and their nisin concentrations, that the Applicant is requesting extensions of use for are listed in Table 2.

Table 2: Proposed uses of nisin in foods, as provided by the applicant

Food Name	Nisin Concentration (mg/kg)
Processed meat, poultry and game products in whole cuts or pieces	12.5
Processed comminuted meat, poultry and game products	12.5

2.3 Dietary exposure assessment provided by the Applicant

The Applicant provided dietary exposure information, stating that the use of nisin in the food categories and at the MPLs requested in the Application, would not result in any adverse dietary implications.

The food consumption data in the Application were taken from the publication *National Nutrition Survey Foods Eaten in Australia 1995*. The Applicant states that exposure estimates are only provided for mean exposure. Ninety-fifth percentile exposures were not calculated as the food consumption data were not reported at this level in the publication.

The Applicant stated that, based on the consumption amounts reported for ‘Sausages, frankfurts and saveloys’ and ‘Processed meat’ at the maximum level requested for these foods of 12.5 mg/kg, Australian children and adults have estimated exposures to nisin of 700 and 120 units/kg bw/day, respectively. One unit is defined by the WHO Committee on Biological Standardisation as 0.001 mg of a reference preparation containing 25 ng of nisin (SCF 1992). The Applicant compared these exposures to an ADI of 33,000 units assigned by JECFA in 1969 (JECFA 1969) and commented that the estimated exposures were well below this ADI.

The dietary exposure assessment provided by the Applicant was not detailed enough to allow FSANZ to determine a conclusion about the likely exposure to nisin for the following reasons:

- the Applicant provided dietary exposure information for Australia only;
- the Applicant only provided estimated mean exposures;
- the Applicant did not include existing permissions and uses of nisin in the exposure assessment; and
- the ADI was revised by FSANZ.

In addition, the ADI used by the Applicant is not a valid reference health standard for nisin as there was a mistake in the units used in the JECFA evaluation (SCF 1992). The ADI for nisin has been re-evaluated since 1969 as more recent toxicological data on nisin have become available. Based on an evaluation of recent toxicological studies FSANZ has assigned an ADI of 0.625 mg/kg bw/day for nisin.

For the estimated dietary exposure assessment to be comprehensive, a dietary exposure assessment was required for the Australian and New Zealand populations and for vulnerable sub-groups (children 2-6 years), which includes estimated dietary exposures at the 95th percentile.

3. Dietary exposure assessment

3.1 What are dietary exposure assessments?

Dietary modelling is a tool used to estimate exposures to food chemicals from the diet as part of the risk assessment process. To estimate dietary exposure to food chemicals, records of what foods people have eaten along with information on how much of the food chemical is in each food are required. The accuracy of these exposure estimates depends on the quality of the data used in the dietary models. Sometimes not all of the data required are available or there is uncertainty about the accuracy. Therefore, assumptions are made, either about the foods eaten or about chemical levels, based on previous knowledge and experience. The models are generally set up according to international conventions for food chemical exposure estimates, however, each modelling process requires decisions to be made about how to set the model up and what assumptions to make; a different decision may result in a different answer. Therefore, FSANZ documents clearly all such decisions and model assumptions to enable the results to be understood in the context of the data available and so that risk managers can make informed decisions.

3.2 Dietary Modelling Approach for consideration of A565

The dietary exposure assessment was conducted using dietary modelling techniques that combine food consumption data with nisin concentration data to estimate the exposure to the nisin from the diet. The dietary exposure assessment was conducted using FSANZ's dietary modelling computer program, DIAMOND.

$$\boxed{\text{Dietary exposure} = \text{nisin concentration} \times \text{food consumption}}$$

The exposure was estimated by combining usual patterns of food consumption, as derived from national nutrition survey (NNS) data, with current concentrations of nisin in foods, in addition to the proposed levels of nisin in foods.

A detailed explanation of how the estimated dietary exposures are calculated can be found in Appendix 1.

3.3 Dietary survey data

DIAMOND contains dietary survey data for both Australia and New Zealand; the 1995 NNS from Australia that surveyed 13 858 people aged 2 years and above, and the 1997 New Zealand NNS that surveyed 4 636 people aged 15 years and above. The NNS used a 24-hour food recall methodology.

It is recognised that these survey data have several limitations. For a complete list of limitations see section 6 on *Limitations*.

3.4 Additional food consumption data or other relevant data

No further information was required or identified for the purpose of refining the dietary exposure estimates for this Application.

3.5 Nisin concentration levels

Many of the foods permitted to contain nisin have a ‘GMP’ permission. However, a numerical figure is required for running the dietary exposure assessment in DIAMOND, otherwise a zero concentration is imputed as a default. Therefore, likely use values for foods with ‘GMP’ permissions had to be found.

The levels of nisin in foods used in the dietary exposure assessment were supplied by the Applicant. These were the manufacturer use levels in foods available in Australia and New Zealand. Levels of zero were used for some food categories where nisin is permitted but is not actually added to foods available in Australia or New Zealand. Actual use levels for cream and cream products were not available and so for this category, the MPL was used.

Concentrations of nisin were assigned to food groups using DIAMOND food classification codes (as shown in Table 3). These codes are based on the Australian New Zealand Food Classification System (ANZFCS) used in Standard 1.3.1 Food Additives (for example, 1.6.4 represents processed cheese products). The foods proposed by the Applicant to contain nisin (as shown in Table 3) were matched to the most appropriate ANZFSC codes for dietary exposure assessment purposes. Manufacturer use levels cannot be disclosed due to Commercial in Confidence reasons. These are indicated as ‘*’ in Table 3.

3.6 Scenarios for the Dietary Exposure Assessment

Two scenarios were modelled for the purpose of this Application:

- ‘Baseline’ estimates current dietary exposures to nisin based on current permissions and assumes that nisin is present at manufacturer use levels, except for cream and cream products, where it is assumed nisin is present at the MPL.
- Scenario 1 (‘Baseline plus A565’) estimates the dietary exposures to nisin should the extension of use be granted and assumes that nisin is present at the levels used in modelling for Baseline and at the MPL for the foods proposed by the Applicant.

Table 3: Nisin concentrations used for estimating dietary exposures to nisin

DIAMOND Food Code	Food Name	Concentration Level (mg/kg)	
		‘Baseline’	Scenario 1 ‘Baseline plus A565’
1.4.2	Cream products (flavoured, whipped, thickened, sour cream etc)	10	10
1.6	Cheese and cheese products	*	*
2.2.2	Oil emulsions (< 80 % oil)	*	*
4.3.0.3	Tomato products pH < 4.5	*	*
4.3.6	Fruit and vegetable preparations including pulp	*	*
6.4.1	Hotplate products	*	*
8.2	Processed meat, poultry and game products, whole or pieces/cuts	0	12.5
8.3	Processed comminuted meat, poultry & game products	0	12.5
10.2	Liquid egg products	*	*
14.1.2.4	Tomato juices pH < 4.5	*	*
14.2.1	Beer & related products	*	*
20.2.4	Sauces, toppings, mayonnaises and salad dressings	*	*
20.2.8	Fat based dips and other fat based products	*	*

3.6 Population groups assessed

The dietary exposure assessment was conducted for both Australian and New Zealand populations. Dietary exposure assessments were conducted for Australians aged 2 years and above and New Zealanders aged 15 years and above as a proxy for lifetime exposure.

A dietary exposure assessment was conducted for children aged 2-6 years (Australia only) because children generally have higher exposures on a body weight basis due to their smaller body weight, and they consume more food per kilogram of body weight compared to adults. It is important to note that, while Australian children aged 2-6 have been assessed as a separate group, this group has also been assessed in the 2 years and above dietary exposure assessment.

A dietary exposure assessment was not conducted for New Zealand children as food consumption data for New Zealand children were not available to FSANZ in the correct format for the DIAMOND program at the time the dietary exposure assessment was undertaken. However, it is assumed that the diets of New Zealand children are similar to those of Australian children. This issue is discussed further in the risk characterisation section of this report (Section 7).

4. Assumptions in the dietary exposure assessment

The aim of the dietary exposure assessment was to make as realistic an estimate of dietary exposure as possible. However, where significant uncertainties in the data existed, conservative assumptions were generally used to ensure that the dietary exposure assessment did not underestimate exposure.

The assumptions made in the dietary exposure assessment are listed below, in several categories.

Concentration data

- all the foods within the group contain nisin at the levels specified in Table 3;
- where there were no Australian or New Zealand manufacturer use data on nisin concentrations for food groups, it was assumed that the concentration was equal to the MPLs for these food groups;
- where a food was not included in the exposure assessment, it was assumed to contain a zero concentration of nisin; and
- where a food has a specified nisin concentration, this concentration is carried over to mixed foods where the food has been used as an ingredient e.g. processed meats in pizza.

Consumption data

- consumption of foods as recorded in the NNS represents current food consumption patterns.

Consumer behaviour

- consumers always select the products containing nisin;

- consumers do not alter their food consumption habits besides to substitute non-nisin containing products with nisin containing products; and
- consumers do not increase their consumption of foods/food groups upon foods/food groups containing nisin becoming available.

General

- all nisin present in food is absorbed by the body;
- endogenous production of nisin has not been included in the dietary exposure assessment;
- naturally occurring sources of nisin have not been included in the dietary exposure assessment;
- there are no reductions in nisin concentrations from food preparation or due to cooking;
- for the purpose of this assessment, it is assumed that 1 millilitre is equal to 1 gram for all liquid and semi-liquid foods (e.g. tomato juice and beer); and
- there is no contribution to nisin exposure through the use of complementary medicines (Australia) or dietary supplements (New Zealand).

These assumptions are likely to lead to a conservative estimate for nisin dietary exposure.

5. Dietary Exposure Assessment Results

5.1 Estimated dietary exposures to nisin

The estimated dietary exposures to nisin for each scenario for Australia and New Zealand are shown in Figures 1 and 2 (full results in Tables A2.1 and A2.2 in Appendix 2). Estimated nisin dietary exposures are presented for consumers of nisin only and not for all respondents (every person in the population group). Consumer numbers are also shown in Tables A2.1 and A2.2.

5.1.1 Estimated mean dietary exposures to nisin

At Baseline, the estimated mean dietary exposures for consumers of nisin were:

- 0.16 mg/day (0.002 mg/kg bw/day) for Australians aged 2 years and above;
- 0.02 mg/day (0.001 mg/kg bw/day) for Australians aged 2-6 years; and
- 0.16 mg/day (0.002 mg/kg bw/day) for New Zealanders aged 15 years and above.

Under Scenario 1, the estimated mean dietary exposures for consumers of nisin were:

- 0.57 mg/day (0.009 mg/kg bw/day) for Australians aged 2 years and above;
- 0.32 mg/day (0.02 mg/kg bw/day) for Australians aged 2-6 years; and
- 0.66 mg/day (0.009 mg/kg bw/day) for New Zealanders aged 15 years and above.

5.1.2 Estimated 95th percentile dietary exposures to nisin

At Baseline, the estimated 95th percentile dietary exposures for consumers of nisin were:

- 0.91 mg/day (0.01 mg/kg bw/day) for Australians aged 2 years and above;

- 0.13 mg/day (0.007 mg/kg bw/day) for Australians aged 2-6 years; and
- 0.97 mg/day (0.01 mg/kg bw/day) for New Zealanders aged 15 years and above.

Under Scenario 1, the estimated 95th percentile dietary exposures for consumers of nisin were:

- 2.26 mg/day (0.04 mg/kg bw/day) for Australians aged 2 years and above;
- 1.42 mg/day (0.07 mg/kg bw/day) for Australians aged 2-6 years; and
- 2.58 mg/day (0.03 mg/kg bw/day) for New Zealanders aged 15 years and above.

For all population groups assessed, estimated mean and 95th percentile dietary exposures to nisin increased from Baseline to Scenario 1. There was little difference between estimated dietary exposures for Australians aged 2 years and above and New Zealanders aged 15 years and above at Baseline and under Scenario 1. The Australian and New Zealand populations had the same exposures to nisin based on mg/kg bw/day at Baseline. The sub-population group, Australians aged 2-6 years, had lower dietary exposures to nisin on a body basis (mg/kg bw/day), compared to the whole Australian population, for the Baseline assessment. Under Scenario 1, Australians aged 2-6 years had the highest exposure to nisin based on mg/kg bw/day.

Figure 1: Estimated mean dietary exposures, in mg/day, for consumers of nisin for different scenarios for Australia and New Zealand population groups.

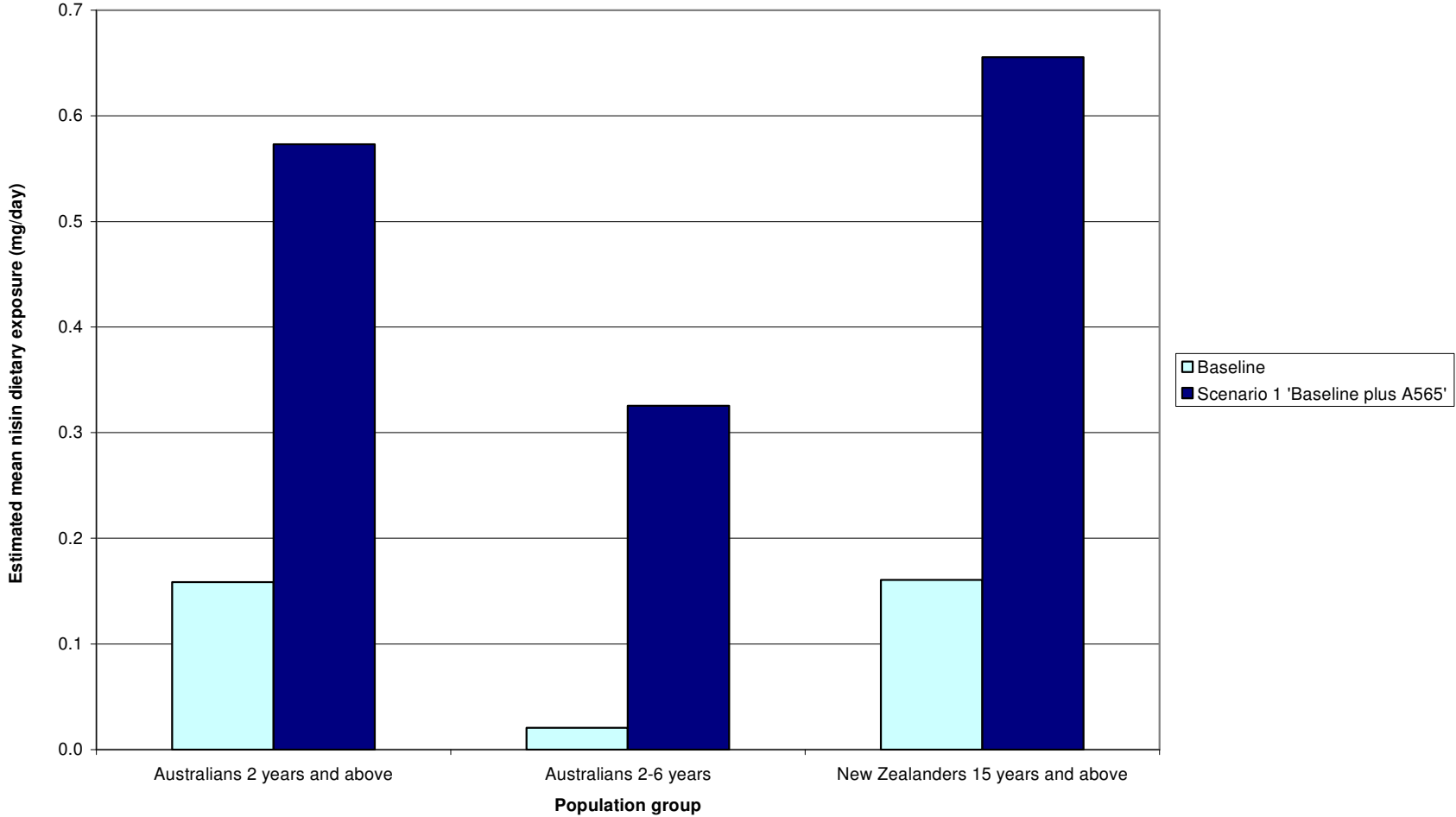
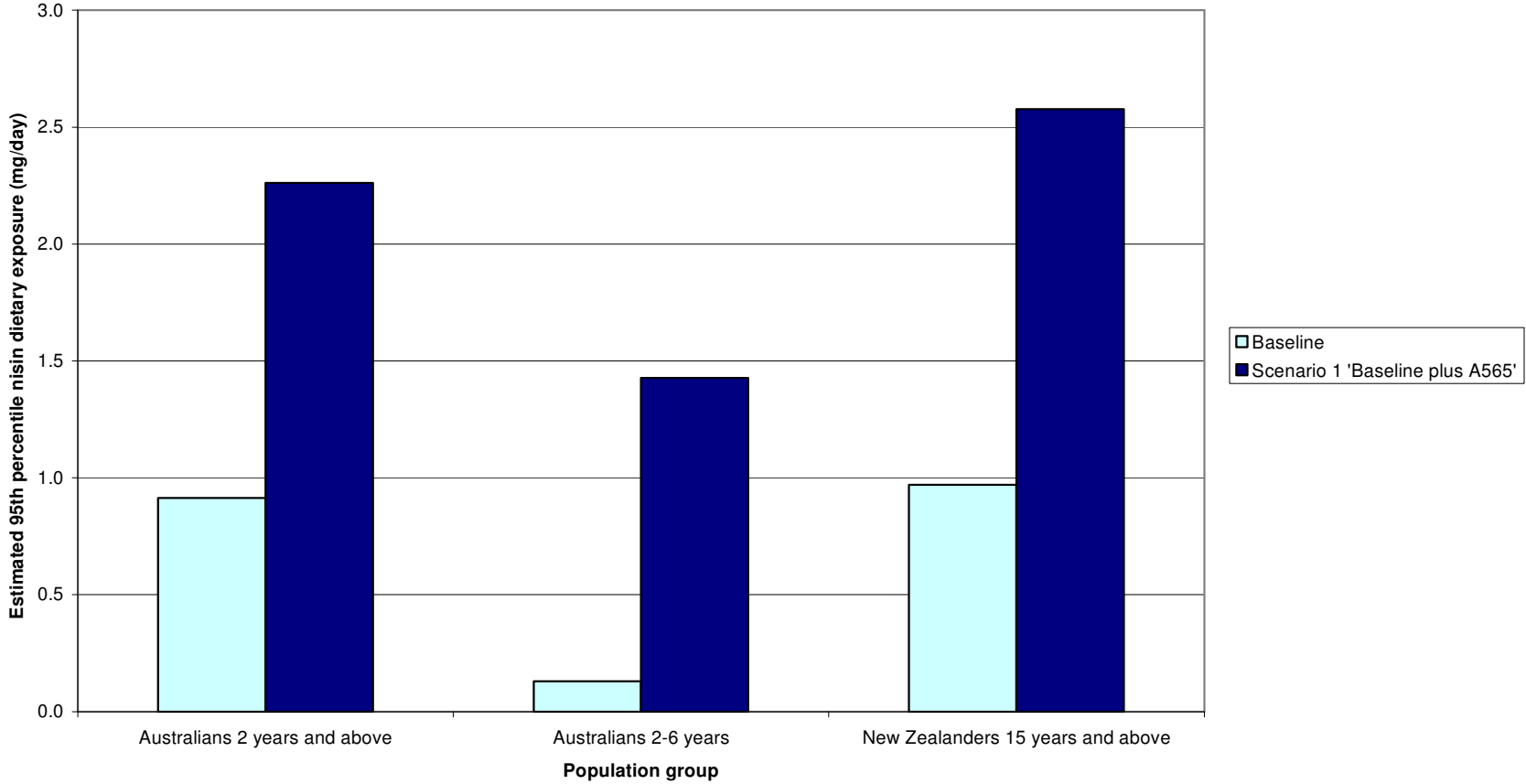


Figure 2: Estimated 95th percentile dietary exposures, in mg/day, for consumers of nisin for different scenarios for Australia and New Zealand population groups.



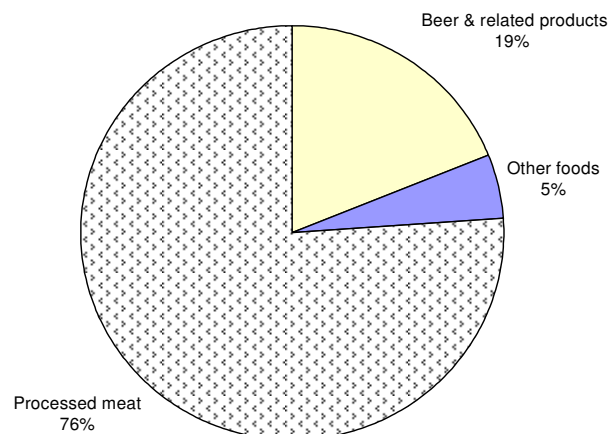
5.2 Major contributing foods to estimated nisin dietary exposures

At Baseline, beer was the main contributor to estimated nisin dietary exposures for Australians aged 2 years and above and New Zealanders aged 15 years and above (76% and 83% respectively). For all population groups assessed cream and cream products was a major contributor to estimated nisin dietary exposures (12%-61%, depending on the population group). This food group was the major contributor for Australians aged 2 to 6. Sauces, toppings, mayonnaises and salad dressings were major contributors for Australians aged 2-6 years and New Zealanders aged 15 years and above (5% and 14% respectively). For Australians aged 2 to 6, processed cheese was also a major contributor (17%).

The major contributors (>5%) to estimated nisin dietary exposures under Scenario 1 (baseline plus A565) are shown in Figure 3 for Australians aged 2 years and above, Figure 4 for Australians aged 2-6 years and Figure 5 for New Zealanders aged 15 years and above. For all population groups assessed in Scenario 1 the main contributor was processed meat products (including processed poultry and game products) (76%-94%, depending on the population group). For Australians aged 2 years and above and New Zealanders aged 15 years and above, beer was also a major contributor (18% and 19% respectively). For Australians aged 2-6 years, no other food category was a major contributor to estimated nisin dietary exposure.

A full list of all the food groups and their contributions to total dietary exposure to nisin can be found in Tables A2.3 and A2.4 in Appendix 2.

Figure 3: Major contributors to estimated nisin dietary exposures for scenario 1 for Australians aged 2 years and above⁵



⁵ Note: The per cent contribution of each food group is based on total nisin intakes for all consumers in the population groups assessed. Therefore the total nisin intakes differ for each population group and each scenario.

Figure 4: Major contributors to estimated nisin dietary exposures for scenario 1 for Australians aged 2-6 years⁶

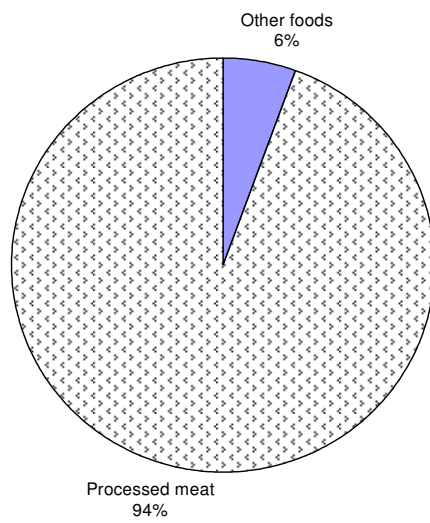
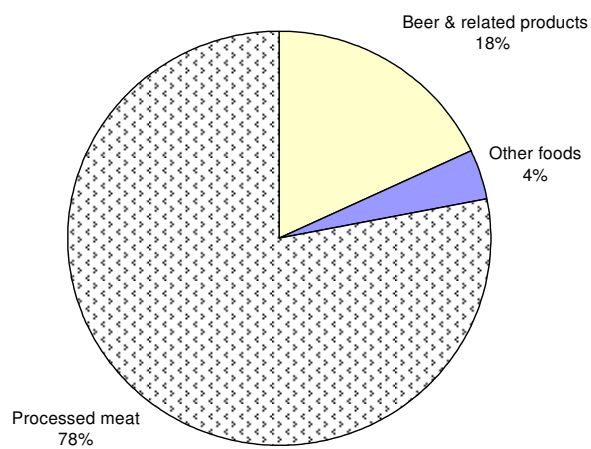


Figure 5: Major contributors to estimated nisin dietary exposures for scenario 1 for New Zealanders aged 15 years and above²



⁶ Note: The percent contribution of each food group is based on total nisin intakes for all consumers in the population groups assessed. Therefore the total nisin intakes differ for each population group and each scenario.

6. Limitations of the dietary exposure assessment

Dietary exposure assessment based on 1995 or 1997 NNS food consumption data provides the best available estimate of actual consumption of a food and the resulting estimated dietary intake of a nutrient for the population. However, it should be noted that the NNS data do have these limitations. These limitations relate to the age of the data and the changes in eating patterns that may have occurred since the data were collected. Generally, consumption of staple foods such as fruit, vegetables, meat, dairy products and cereal products, which make up the majority of most people's diet, is unlikely to have changed markedly since 1995/1997 (Cook *et al.*, 2001a; Cook *et al.*, 2001b).

A limitation of estimating dietary exposure over a period of time associated with the dietary exposure assessment is that only 24-hour dietary survey data were available, and these tend to over-estimate habitual food consumption amounts for high consumers. Therefore, predicted high percentile exposures are likely to be higher than actual high percentile exposures over a lifetime.

Daily food consumption amounts for occasionally consumed foods based on 24-hour food consumption data would be higher than daily food consumption amounts for those foods based on a longer period of time. This specifically affects the food groups in this assessment such as cream products or fat based dips.

Over time, there may be changes to the ways in which manufacturers and retailers make and present foods for sale. Since the data were collected for the Australian and New Zealand NNSs, there have been significant changes to the Food Standards Code to allow more innovation in the food industry. As a consequence, another limitation of the dietary exposure assessment is that some of the foods that are currently available in the food supply were either not available or were not as commonly available in 1995/1997.

While the results of NNSs can be used to describe the usual intake of groups of people, they cannot be used to describe the usual intake of an individual (Rutishauser, 2000). In particular, they cannot be used to predict how consumers will change their eating patterns as a result of an external influence such as the availability of a new type of food.

FSANZ does not apply statistical population weights to each individual in the NNSs in order to make the data representative of the population. This prevents distortion of actual food consumption amounts that may result in an unrealistic intake estimate. Maori and Pacific Islanders were over-sampled in the 1997 New Zealand NNS so that statistically valid assessments could be made for these population groups. As a result, there may be bias towards these population groups in the dietary exposure assessment because population weights were not used.

7. Risk Characterisation

7.1 Current Acceptable Daily Intake (ADI) for nisin

In order to determine if the levels of dietary exposure to nisin are likely to be of a public health and safety concern, the estimated dietary exposures were compared to an Acceptable Daily Intake (ADI) of 0.625 mg/kg bw/day that was determined by FSANZ in 2007, following an evaluation of recent toxicological studies on nisin.

The ADI is defined as an estimate of the amount of a chemical that can be ingested daily over a lifetime without appreciable risk to health (WHO 2001).

7.2 Characterisation of Estimated Mean Dietary Exposures

The estimated mean dietary exposures to nisin, as a percent of the ADI, are shown in Figure 6 for Australia and New Zealand at Baseline and under Scenario 1 (full results in Table A3.1 in Appendix 3).

Estimated mean dietary exposures to nisin were below the ADI for all scenarios and population groups assessed.

Baseline estimated mean dietary exposures to nisin were the less than 1% of the ADI for all population groups assessed.

Scenario 1 estimated mean dietary exposures to nisin were 1% of the ADI for Australians aged 2 years and above and New Zealanders aged 15 years and above and 3% of the ADI for Australians aged 2-6 years.

7.3 Characterisation of 95th Percentile Dietary Exposures

The estimated 95th percentile dietary exposures to nisin, as a percent of the ADI, are shown in Figure 7 for Australia and New Zealand at Baseline and under Scenario 1 (full results in Table A3.2 in Appendix 3).

Estimated 95th percentile dietary exposures to nisin were below the ADI for all scenarios and population groups assessed.

At Baseline, the estimated 95th percentile dietary exposures were 1% of the ADI for Australians aged 2-6 years and 2% of the ADI for Australians aged 2 years and above and New Zealanders aged 15 years and above.

Estimated 95th percentile dietary exposures for consumers of nisin under Scenario 1 were lowest for New Zealanders aged 15 years and above at 5% of the ADI and highest for Australian children aged 2-6 years at 10% of the ADI.

A separate dietary exposure assessment was not conducted for New Zealand children. Based on the estimated 95th percentile dietary exposures under Scenario 1 for Australian children aged 2-6 years (10% of the ADI), it can be assumed that 95th percentile dietary exposures to nisin for New Zealand children are also well below the ADI. Even if there was a difference in food consumption patterns between Australia and New Zealand children, it would need to be significantly higher in order to make dietary exposures approach the ADI, which is highly unlikely.

Figure 6: Estimated mean dietary exposures, as a %ADI, for consumers of nisin for different scenarios for Australia and New Zealand population groups.

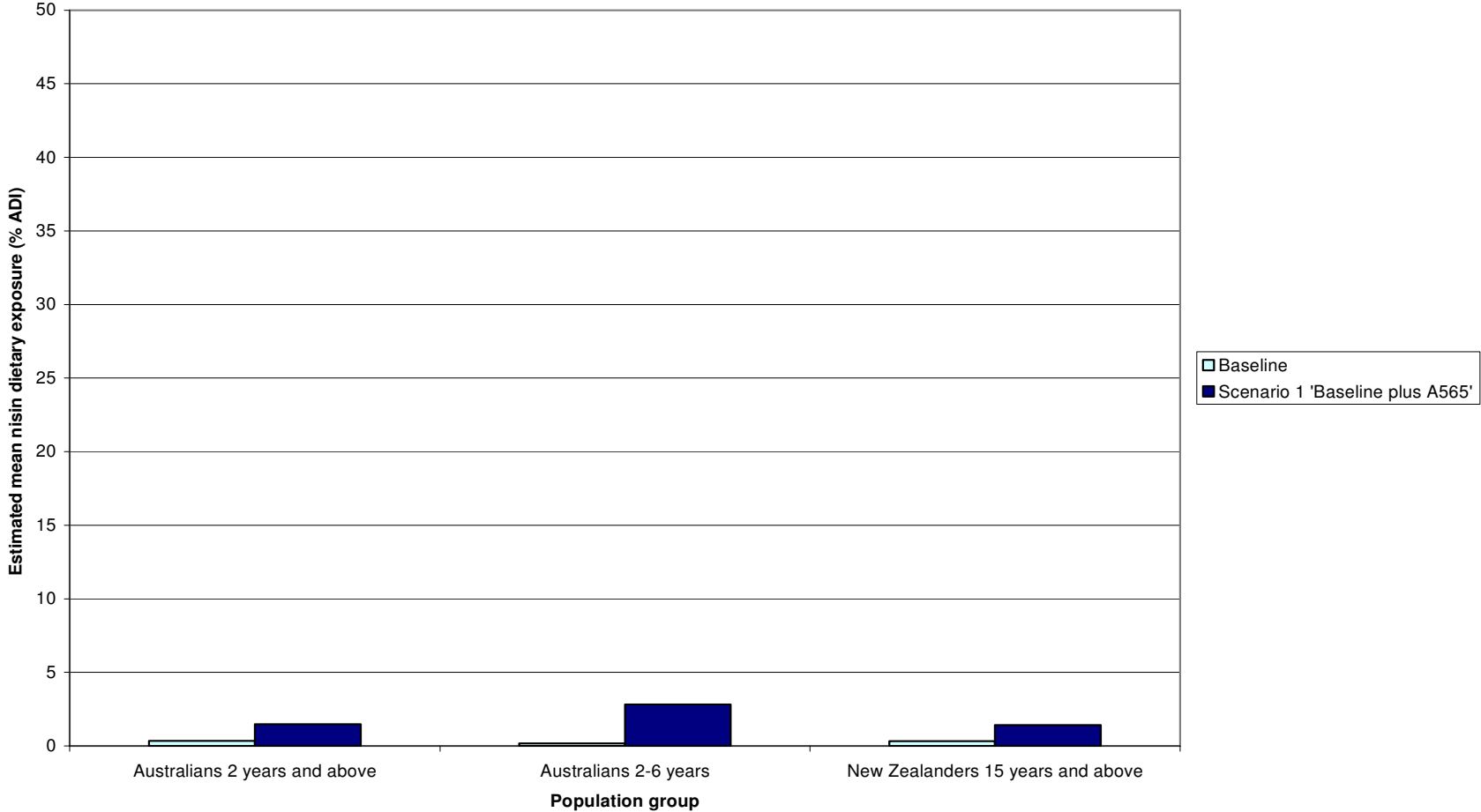
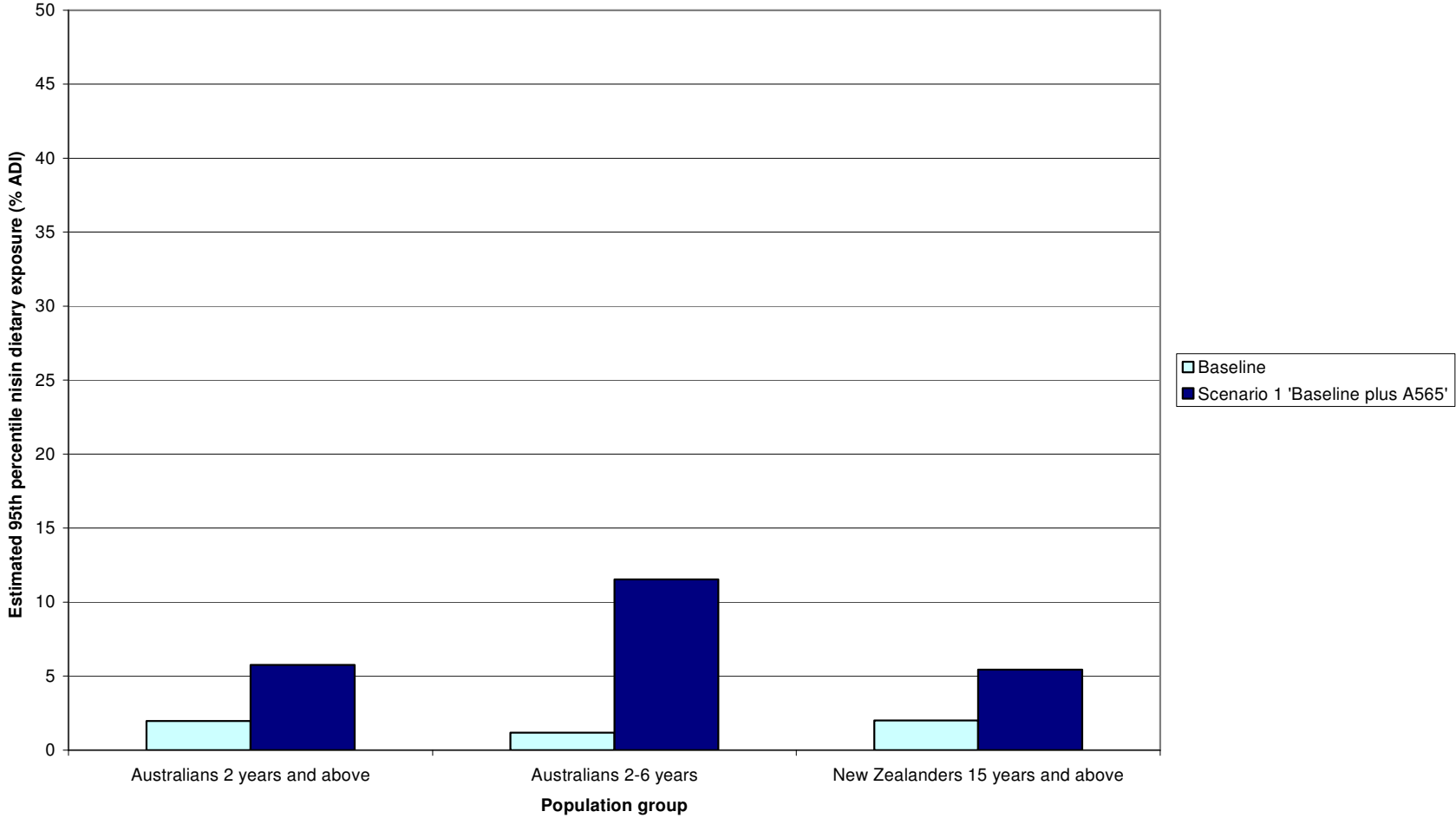


Figure 7: Estimated 95th percentile dietary exposures, as a %ADI, for consumers of nisin for different scenarios for Australia and New Zealand population groups.



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How were the estimated dietary exposures calculated?

How were estimated dietary exposures calculated?

The DIAMOND program allows nisin concentrations to be assigned to food groups and sub-categories within food groups. For instance, nisin is permitted to be added to 1.6 Cheese and cheese products. However, nisin is currently used only in 1.6.4 Processed cheese. Therefore, for the purposes of dietary modelling, a nisin concentration was assigned only to the sub-category 1.6.4 Processed cheese. In other cases, nisin permissions are restricted to specific food sub-categories. For example, nisin is permitted to be added to 6.4.1 Hot plate products, which is a sub-category of the higher level classification code 6.4 Flour products, but is not permitted to be added to any other Flour products. In this case, a nisin concentration was assigned to the sub-category 6.4.1 Hot plate products only.

Each individual's exposure to nisin was calculated using his or her individual food records from the dietary survey. The DIAMOND program multiplies the specified concentration of nisin by the amount of food that an individual consumed from that group in order to estimate the exposure to nisin from each food. Once this has been completed for all of the foods specified to contain nisin, the total amount of nisin consumed from all foods is summed for each individual. Population statistics (mean and 95th percentile exposures) are then derived from the individuals' ranked exposures.

Where estimated dietary exposures are expressed per kilogram of body weight, each individual's total dietary exposure is divided by their own body weight, the results ranked, and population statistics derived. A small number of NNS respondents did not provide a body weight. These respondents are not included in calculations of estimated dietary intakes that are expressed per kilogram of body weight.

Where estimated exposures are expressed as a percentage of the reference health standard, each individual's total exposure on a body weight basis is calculated as a percentage of the reference health standard, the results are then ranked, and population statistics derived.

Food consumption amounts for each individual take into account where each food in a classification code is consumed alone and as an ingredient in mixed foods. For example, ham eaten as a slice of ham, ham eaten in a ham sandwich and ham on a pizza, are all included in the consumption of ham. Where a higher level food classification code, for example, 14.2 Alcoholic beverages is given a nisin concentration, as well as a sub-category, for example 14.2.1 Beer and related products, the consumption of the foods in the sub-classification is not included in the higher level classification code.

In DIAMOND, all mixed foods in classification codes 20 and 21 have a recipe. Recipes are used to break down mixed foods into component ingredients which are in classification codes 1-14. The data for consumption of the ingredients from the recipe are added to consumption of these foods when not used in recipes then used in the exposure assessment and multiplied by nisin concentrations for each of the raw ingredients. This only occurs if the *Mixed food* classification code (classification code 20) is not assigned its own nisin permission. If the *Mixed foods* classification is assigned a nisin concentration, the total consumption of the mixed food is multiplied by the proposed level, and the recipes are not used for that food group.

When a food that does not have a recipe is classified in two food groups in classification codes 1-14, and these food groups are assigned different permissions, DIAMOND will assume the food is in the food group with the highest assigned nisin level to assume a worst-case scenario. If the food groups have the same permitted nisin level, DIAMOND will assume the food is in the food group that appears first, based numerically on the ANZFCS.

When a food is classified in two food groups (for example, mixed fruit juice may be entered in the apple and pear groups), and these food groups are assigned different nisin permissions, DIAMOND will assume the food is in the food group with the highest assigned nisin level to assume a worst case scenario. If the food groups have the same permitted nisin level, DIAMOND will assume the food is in the food group that appears first, based numerically on the DIAMOND food classification code.

In DIAMOND, hydration factors are applied to some foods to convert the amount of food consumed in the dietary survey to the equivalent amount of the food in the form to which a food chemical concentration is assigned. Factors are only applied to individual foods, and not major food group codes. For example, consumption figures for tomato juice concentrate would be converted to the equivalent amount of fluid tomato juice consumed.

How were percentage contributions calculated?

Percentage contributions of each food group to total estimated exposures are calculated by summing the exposures for a food group from each individual in the population group who consumed a food from that group and dividing this by the sum of the exposures of all individuals from all food groups containing nisin, and multiplying this by 100.

Appendix 2

Complete information on dietary exposure assessment results

Table A2.1: Estimated dietary exposures to nisin for Australian and New Zealand population groups at Baseline

Country	Population group	Number of consumers of nisin	Consumers [♦] as a % of total respondents [#]	Mean all respondents mg/day (mg/kg bw/day)*	Mean consumers mg/day (mg/kg bw/day)	95 th percentile consumers mg/day (mg/kg bw/day)
<u>Australia</u>	2 years and above	10,400	75	0.1 (0.002)	0.2 (0.002)	0.9 (0.01)
	2-6 years	753	76	0.02 (0.0008)	0.02 (0.001)	0.1 (0.007)
New Zealand	15 years and above	3,492	75	0.1 (0.002)	0.2 (0.002)	1.0 (0.01)

Total number of respondents for Australia: 2 years and above = 13 858, 2-6 years = 989; New Zealand: 15 years and above = 4 636. Respondents include all members of the survey population whether or not they consumed a food that contains nisin.

♦ Consumers only – This only includes the people who have consumed a food that contains nisin.

*Mean body weights: Australia 2 years and above = 67 kg, 2-6 years = 19 kg, New Zealand 15 years and above = 71 kg.

Table A2.2: Estimated dietary exposures to nisin for Australian and New Zealand population groups under Scenario 1 ('Baseline plus A565')

Country	Population group	Number of consumers of nisin	Consumers [♦] as a % of total respondents [#]	Mean all respondents mg/day (mg/kg bw/day)*	Mean consumers mg/day (mg/kg bw/day)	95 th percentile consumers mg/day (mg/kg bw/day)
<u>Australia</u>	2 years and above	11626	84	0.5 (0.008)	0.6 (0.009)	2.3 (0.04)
	2-6 years	841	85	0.3 (0.01)	0.3 (0.02)	1.4 (0.07)
New Zealand	15 years and above	3887	84	0.5 (0.007)	0.7 (0.009)	2.6 (0.03)

Total number of respondents for Australia: 2 years and above = 13 858, 2-6 years = 989; New Zealand: 15 years and above = 4 636. Respondents include all members of the survey population whether or not they consumed a food that contains nisin.

♦ Consumers only – This only includes the people who have consumed a food that contains nisin.

*Mean body weights: Australia 2 years and above = 67 kg, 2-6 years = 19 kg, New Zealand 15 years and above = 71 kg.

Table A2.3: Contributors to Total Estimated Nisin Dietary Exposures for Australian and New Zealand Population Groups at Baseline

DIAMOND Food Code	Food Name	% Contribution to nisin dietary exposure*		
		Australians 2 years and above	Australians 2-6 years	New Zealanders 15 years and above
1.4.2	Cream products (flavoured, whipped, thickened, sour cream etc)	18	61	12
1.6	Cheese and cheese products	<1	17	<1
2.2.2	Oil emulsions (< 80 % oil)	0	0	0
4.3.0.3	Tomato products pH < 4.5	0	0	0
4.3.6	Fruit and vegetable preparations including pulp	<1	4	<1
6.4.1	Hotplate products	<1	3	<1
10.2	Liquid egg products	0	0	0
14.1.2.4	Tomato juices pH < 4.5	0	0	0
14.2.1	Beer & related products	76	<1	83
20.2.4	Sauces, toppings, mayonnaises and salad dressings	4	14	5
20.2.8	Fat based dips and other fat based products	<1	<1	<1

* Zero values for % contribution to nisin dietary exposure from food categories could be either because nisin is not used in those foods or those foods are not consumed.

Table A2.4: Contributors to Total Estimated Nisin Dietary Exposures for Australian and New Zealand Population Groups under Scenario 1

DIAMOND Food Code	Food Name	% Contribution to nisin dietary exposure*		
		Australians 2 years and above	Australians 2-6 years	New Zealanders 15 years and above
1.4.2	Cream products (flavoured, whipped, thickened, sour cream etc)	4	3	3
1.6	Cheese and cheese products	<1	<1	<1
2.2.2	Oil emulsions (< 80 % oil)	0	0	0
4.3.0.3	Tomato products pH < 4.5	0	0	0
4.3.6	Fruit and vegetable preparations including pulp	<1	<1	<1
6.4.1	Hotplate products	<1	<1	<1
8.2	Processed meat, poultry and game products, whole or pieces/cuts	31	23	31
8.3	Processed comminuted meat, poultry & game products	45	71	47
14.1.2.4	Tomato juices pH < 4.5	0	0	0
14.2.1	Beer & related products	19	<1	18
20.2.4	Sauces, toppings, mayonnaises and salad dressings	<1	<1	1
20.2.8	Fat based dips and other fat based products	<1	<1	<1

* Zero values for % contribution to nisin dietary exposure from food categories could be either because nisin is not used in those foods or those foods are not consumed.

Complete information on risk characterisation

Table A3.1: Estimated dietary exposures to nisin for Australian and New Zealand population groups, as a percentage of ADI at Baseline

Country	Population group	Number of consumers of nisin	Consumers [♦] as a % of total respondents [#]	Mean all respondents (% ADI*)	Mean consumers (% ADI*)	95 th percentile consumers (% ADI*)
Australia	2 years and above	10400	75	<1	<1	2
	2-6 years	753	76	<1	<1	1
New Zealand	15 years and above	3492	75	<1	<1	2

Total number of respondents for Australia: 2 years and above = 13 858, 2-6 years = 989; New Zealand: 15 years and above = 4 636. Respondents include all members of the survey population whether or not they consumed a food that contains nisin.

♦ Consumers only – This only includes the people who have consumed a food that contains nisin.

* ADI = 0.625 mg/kg bw/day

Table A3.1: Estimated dietary exposures to nisin, as a percentage of ADI Scenario 1 ('baseline plus A565')

Country	Population group	Number of consumers of nisin	Consumers [♦] as a % of total respondents [#]	Mean all respondents (% ADI*)	Mean consumers (% ADI*)	95 th percentile consumers (% ADI*)
Australia	2 years and above	11626	84	1	1	6
	2-6 years	841	85	2	3	10
New Zealand	15 years and above	3887	84	1	1	5

Total number of respondents for Australia: 2 years and above = 13 858, 2-6 years = 989; New Zealand: 15 years and above = 4 636. Respondents include all members of the survey population whether or not they consumed a food that contains nisin.

♦ Consumers only – This only includes the people who have consumed a food that contains nisin.

* ADI = 0.625 mg/kg bw/day

Food Technology Report

APPLICATION A565 – NISIN USE IN PROCESSED MEAT PRODUCTS

Summary and Conclusions

An Application (A565) was received on 23 June 2005 from Danisco Australia Pty Ltd, submitted by Axiome Pty Ltd, seeking to amend Schedule 1 of Standard 1.3.1 to include limits for nisin of 12.5 mg/kg in processed meat products, specifically for:

- processed meat, poultry and game products in whole cuts or pieces; and
- processed comminuted meat, poultry and game products.

The Applicant provided data demonstrating that the presence of nisin would inhibit the growth of certain spoilage bacteria and *Listeria monocytogenes*. While limited data were provided on its use with other food preservation techniques, it is considered that the effects of nisin are likely to be enhanced when used with these other techniques, as with other approved preservatives. On this basis and given the limited spectrum of activity of nisin, it is considered that there is technological justification for the use of nisin in processed meat products and that it will be a potentially useful component of food preservation systems for processed meat production.

Taking into account the losses of nisin that are likely to occur during processing and during the shelf life of processed meat products, the limit proposed by the Applicant of 12.5 mg/kg for nisin in meat products is considered to be in the range for inhibiting the growth of spoilage bacteria and *L. monocytogenes*. While the use of nisin at levels proposed by the Applicant is considered to inhibit the growth of *L. monocytogenes*, the data did not suggest that nisin would eliminate *L. monocytogenes*.

Good hygienic practices should ensure that the potential for nisin-resistant bacteria to contaminate meat products in food processing establishments is mitigated. If resistance were to develop and nisin was no longer able to inhibit microbiological growth then manufacturers would have to use other techniques to inhibit this growth. Taking this into account as well as the lack of data indicating increasing resistance and the long history of use, it is considered that use of nisin in meat products is unlikely to increase the prevalence of nisin resistant bacteria in food processing establishments. If it were to develop then manufacturers would need to institute alternative procedures to mitigate microbiological growth.

Nisin is unlikely to affect starter cultures used in the production of fermented meat. In practice, nisin is applied to meat product after completion of fermentation. Therefore nisin should not pose a problem to the performance of starter cultures in the production of fermented meat.

Introduction

Danisco Australia Pty Ltd submitted an Application on 23 June 2005 to FSANZ seeking to amend Schedule 1 of Standard 1.3.1, to include a limit of 12.5 mg/kg for nisin in the following foods, which would include ready to eat meat products:

- processed meat, poultry and game products in whole cuts or pieces.
- processed comminuted meat, poultry and game products.

During the development of the Code, the current limits for nisin were included in Schedule 1 of Standard 1.3.1 for the following foods:

- cream products (flavoured, whipped, thickened, and sour cream) to a maximum level of 10 mg/kg;
- flour products (including noodles and pasta) e.g. crumpets, flapjacks and pikelets (hot plate flour products) to a maximum level of 250 mg/kg;

and at levels compliant with good manufacturing practice (GMP) in:

- cheese and cheese products;
- oil emulsions (<80% oil);
- tomato products pH <4.5;
- fruit and vegetables preparations including pulp;
- liquid egg products;
- fruit and vegetable juices (e.g. tomato juice pH <4.5);
- beer and related products; and
- dairy and fat-based desserts, dips and snacks, sauces, toppings, mayonnaises and salad dressings.

Nisin is a food preservative. It is a polypeptide produced by certain strains of the food-grade lactic acid bacterium *Lactococcus lactis* subsp. *lactis* during fermentation. It is a small heat stable peptide belonging to a group of bacteriocins known as lantibiotics, present in milk. Nisin has been used to control bacterial spoilage in both heat-processed and low-pH foods (Davies et al, 1999).

A formulation containing nisin has been sold under the trade name of *Nisaplin*®. *Nisaplin*® contains approximately 2.5% nisin and includes milk and milk solids derived from the fermentation of a modified milk medium by nisin producing strains of *L. lactis*. The product is standardised to an activity of one million international units per gram, which equates to 2.5% nisin by weight. The Applicant indicates that the nisin containing formulation could be added to meat mix, or meat products could be dipped in the formulation solution or casings pre-treated by dipping in a solution of the nisin containing formulation.

Nisin is reported to be effective against a wide range of Gram-positive bacteria as vegetative cells (Davies *et al.* 1999). Nisin does not normally kill spores and the effect on spores is predominantly sporostatic. Its preservative action is dependent on an effective level of nisin being sustained throughout the shelf-life of the food. It shows little or no activity against Gram-negative bacteria, yeast, and moulds.

Regulatory Status

Codex Standards

Nisin is currently under consideration for inclusion in the Codex Alimentarius General Standard for Food Additives for use in a wide range of foods including meat and meat products including poultry and game at a maximum level of 500 mg/kg⁷.

Nisin is currently included as a preservative in the following Codex Standards:

- Codex General Standard for Named Variety Processed Cheese and Spreadable Processed Cheese (Ref: Codex Standard A-8(a))⁸.
- Codex General Standard for Processed Cheese and Spreadable Processed Cheese (Ref: Codex Standard A-8(b))⁹.
- Codex General Standard for Processed Cheese Preparations, Processed Cheese Food and Processed Cheese Spread (Ref: Codex Standard A-8(c))¹⁰.
- Codex General Standard for Cheese (Ref: Codex Standard A-6)¹¹.

The maximum level of nisin permitted in all of these standards is 12.5 mg/kg.

International Legislation

Nisin is approved as a preservative in specific foods in a number of countries and jurisdictions including the European Union, USA, China and MERCOSUR countries (Argentina, Brazil, Paraguay, Venezuela and Uruguay).

For meat products, in the USA, a GRAS (generally recognized as safe) notice response was issued on April 20, 2001 (GRN 000065) about nisin as an antimicrobial agent for use on casings for frankfurters and on cooked meat and poultry products¹². The notice states that:

- nisin is considered to be GRAS by Rhodia Inc. (a manufacturer of nisin containing formulations) for use on casings for frankfurters and on cooked meat and poultry products as an antimicrobial agent;
- nisin would be used in casings for frankfurters at a level of 3.15 milligrams (mg) per pound (lb) (equivalent to approximately 6.9 mg per kilogram (kg)) in the finished frankfurter and in cooked meats and poultry products sold ready-to-eat at a level of 2.5 mg/ lb (equivalent to 5.5 mg/kg);
- the FDA has not published its determination regarding the GRAS status of the subject use of nisin; and
- based on the information submitted, the Food Safety Inspection Service (FSIS) concluded that nisin-containing antimicrobial formulations, when used under the conditions specified in the notice, would be acceptable to control *L. monocytogenes* in various non-standardized meat and poultry products.

⁷ <ftp://ftp.fao.org/codex/ccfac38/fa3808ae.pdf>

⁸ <http://www.codexalimentarius.net/download/standards/177/CXSA08ae.pdf> *

⁹ <http://www.codexalimentarius.net/download/standards/178/CXSA08be.pdf> *

¹⁰ <http://www.codexalimentarius.net/download/standards/179/CXSA08ce.pdf> *

¹¹ http://www.codexalimentarius.net/download/standards/175/CXS_A06e.pdf *

* accessed on 28 February 2007.

¹² <http://www.cfsan.fda.gov/%7Erdb/opa-g065.html>

For cheese products, nisin was given (GRAS) designation in the U.S. Federal Register of April, 1988 as a food preservative. In the U.S., nisin is used to inhibit outgrowth of *Clostridium botulinum* spores (the cause of botulism) and toxin formation in pasteurized processed cheese spreads containing fruits, vegetables or meats at levels not exceeding good manufacturing practice (GMP). Current GMP in this case is the quantity of the ingredient that delivers a maximum of 250 ppm (or mg/kg) of nisin in the finished product. Nisin is also approved in the USA for liquid egg products, dressings, and sauces. In other countries it is also used in milk and milk products, fermented beverages such as beer, canned foods, frozen desserts, and high moisture/reduced fat foods.

Chemical Structure of Nisin

Nisin is composed of 34 amino acids and has a molecular weight of 3353 (Hurst, 1981). It contains some unusual amino acids, including didehydroalanine (Dha), β -methyldehydroalanine (β -MeDha) or didehydrobutyrine (Dhb) and the thioether amino acids lanthionine (Lan, Ala-S-Ala) and β -methylanthionine (β -MeLan, Ala-S-Abu, amino butyric).

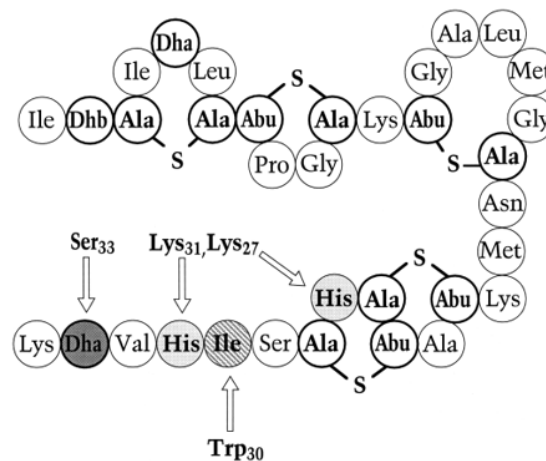


Figure 1: Molecular structure of nisin A

The modified residues, indicated in bold, are Dha, dehydroalanine; Dhb, dehydrobutyrine; Abu, aminobutyrate; Ala-S-Ala, Lanthionine; Abu-S-Ala, β -methylanthionine.

Specification

The Food and Nutrition Paper 52 Compendium of Food Additive Specifications, addenda 1-12, published by the Food and Agriculture Organisation of the United Nations in Rome (1992) contains a specification for nisin containing formulations (FAO, 1992). This specification is currently referenced in Standard 1.3.4 – Identity and Purity.

Technical specifications for nisin formulations indicate that the process begins by fermenting milk bacteria. The resulting nisin containing material is concentrated, separated, and spray-dried before milling into fine particles and standardised by the addition of sodium chloride (salt). The resulting typical composition is:

- nisin - 2.5%
- sodium chloride - 77.5%

- protein - 12%
- carbohydrate - 6%
- moisture - 2%

Nisin	
INS No.	234
CAS Registry No.	1414-45-5
Trade Names (formulation)	Nisaplin®
Description	Antimicrobial preservative agent
Appearance	Off-white powder
Chemical Formula	C ₁₄₃ H ₂₂₈ N ₄₂ O ₃₇ S ₇
Molecular Structure	Nisin, a 34-residue peptide bacteriocin, contains the less common amino acids lanthionine, β-methyl-lanthionine, dehydroalanine (Dha), and dehydrobutyrine (Dhb).
Molecular Weight	3,353
Melting Point	270°C
Stability	It is heat stable (100°C, 10 min) and very stable in the dry state.
Sensitivities	May cause skin and eye irritation.
Solubility	At pH 2.5 the solubility of nisin is 12%, at pH 5.0 it is 4%, and it is insoluble in neutral or alkaline pH.

Stability

Nisin formulations are stable, showing no loss of activity over two years when stored under dry conditions in the dark, below 25°C. Nisin is most stable in acid conditions and it is soluble in aqueous environments. A nisin solution in dilute HCl at pH 2.5 is stable upon boiling with no marked loss of activity. At pH 7.0, inactivation occurs even at room temperature (Davies and Delves-Broughton, 1999).

Some loss of activity is expected when nisin is used in heat-processed foods. Nisin solutions are most stable to autoclaving (121°C for 15 min) in the pH range 3.0–3.5 (<10% activity loss). At pH values below and above this range, there is a marked decrease in activity (>90% loss at pH 1 or 7). Losses of activity at pasteurisation temperatures are significantly less (approximately 20% during standard processed cheese manufacture at pH 5.6–5.8) (Davies and Delves-Broughton, 1999).

The stability of nisin in a food during storage is dependent upon the storage temperature, length of storage, and pH. Greater nisin retention occurs at lower temperatures and the Applicant reported that the manufacture of a pasteurised processed cheese spread (85–105°C for 5–10 min at pH 5.6–5.8) results in an initial 20–30% loss, after which the nisin retention after 30 weeks storage is approximately 80% at 20°C, 60% at 25°C and 40% at 30°C. A higher level of nisin addition will be required if storage at unusually high ambient temperature is intended.

In cold processed foods, proteolytic enzymes can affect nisin stability. The food additives, titanium dioxide and sodium metabisulphite can also adversely affect nisin stability.

Mode of Action

Nisin is reported to bind to the anionic phospholipids of the cell membrane which then form part of the cell membrane. This incorporation in the cell membrane results in pores in the membrane through which intracellular components pass. The loss of these components depletes the energy forces in the cell resulting in cell death. (European Food Safety Agency, Scientific Panel on Food Additives, 2006).

Nisin is reported to be effective at controlling a wide range of Gram-positive organisms including: *Listeria* sp., *Bacillus sporothermodurans*, and *Clostridium* sp. Used alone, it is not effective on Gram-negative bacteria (such as *E. coli*), yeasts, and moulds. Gram-negative bacteria are reported to be protected from nisin by the presence of an outer wall membrane but may show sensitivity to nisin if this membrane is weakened (e.g. osmotic shock or freezing). Nisin's action against spores is considered to be predominantly sporostatic rather than sporicidal and this effect is reported to be dependent upon a sufficient level of nisin being available throughout the shelf life of the product. The main use for nisin is reported to be as an antimicrobial in heat treated but not sterilised foods to prevent outgrowth of heat-resistant spores (Thomas and Delves-Broughton, 2001).

Nisin sensitivity of Gram-positive bacteria is reported to vary considerably with its efficacy being dependent on the concentration of the nisin and the number of spores or bacteria present. Effects are reported to be enhanced when the bacteria are growing and when nisin is used as part of a multi-preservation system (Davies *et al*, 1999).

Technological Justification

In assessing the technological justification for nisin addition to processed meat products (including processed comminuted meat products), FSANZ has considered:

- the addition of nisin to these foods and whether it will serve any purpose that is not already achieved through existing measures, or would provide an appropriate alternative to existing measures; and
- the amount of nisin proposed to be added to processed meats and whether this is necessary to achieve the technological function requested.

In addition to these issues, FSANZ has also considered the issues of

- control of *Listeria monocytogenes*, including potential development of resistance in food processing establishments; and
- potential impacts of nisin on starter cultures used for fermented meat products.

Addition of Nisin to Processed Meats

The Applicant proposes that nisin be used in processed meat products. Whilst several food additives are currently permitted (e.g. nitrites/nitrates, sorbates), the Applicant has stated that they are not completely effective and spoilage in processed meat products is not uncommon. Preservatives inhibit the growth of micro-organisms under specific conditions but typically do not ensure sterility. The Applicant has stated that:

- Gram-positive bacteria associated with processed meat products (unlike many Gram-negative bacteria) have a relatively high tolerance to reduced water activity, refrigeration temperatures, low pH and the presence of nitrate and phosphate emulsifying salts; and
- the temperatures used in processing are sufficient to kill most bacteria, however, they are not effective against heat resistant spores;
- common spoilage bacteria for processed meat products are Gram-positive lactic acid bacteria (*Lactobacillus* species) and *Brochothrix thermosphacta*.

Davies *et al* reported that nisin is only effective in meat products at levels of 12.5 mg/kg and above, although more promising control has been reported in modified atmosphere and vacuum packaged products. Davies *et al* also reported that nisin has lower efficacy in meat systems because of binding onto fats and proteins making the nisin unavailable for antimicrobial activity (Davies *et al*, 1999).

Thomas and Delves-Broughton reported that nisin has been shown to be effective in meat situations although a number of aspects of the potential products need to be considered to ensure the appropriate preservation outcome (Thomas and Delves-Broughton, 2001). This publication also reported that:

- 6.25 mg/kg nisin inhibited lactic acid spoilage bacteria in vacuum packed refrigerated bologna-type sausages for over 4 weeks;
- improved nisin activity was observed when combined with other additives and preservation technologies, such as temperature control and vacuum packaging; and
- the Gram-positive organism *Brochothrix thermosphacta* has been associated with off odours in refrigerated meat products and this organism has been found to be sensitive to nisin.

In a study by Davies *et al* in 1999, it was reported that:

- 25 mg/kg nisin was found to inhibit the growth of lactic acid bacteria in cooked vacuum packed bologna type sausages for over five weeks and a level of 6.25 mg/kg inhibited growth for 4 weeks;
- there were variations in the effect of nisin depending upon fat content, although it was reported that a concentration of 12.5 mg/kg nisin prevented lactic acid bacteria growth in a sausage with 25% fat content for five weeks;
- nisin losses of between 13% and 29% were observed as a result of processing (pasteurisation);
- nisin losses of as much as 50% were reported over the study period (approx. 80 days);
- the results were based upon bologna type sausages stored at 8 degrees Celsius;
- nisin levels between 6.25 mg/kg and 25 mg/kg were able to inhibit the growth of lactic acid bacteria and extend the shelf life of bologna type sausages; and
- nisin may be poorly recovered from meat and meat products resulting in misleading and variable bioassay results.

Collins-Thompson *et al*, in 1985, reported that lactic acid bacteria isolated from commercial cured meat products had sensitivity to nisin and that nisinase activity was not a common characteristic among the isolates studied.

Gill *et al*, in 2000, reported that nisin application as a surface gel treatment inhibited the growth of the Gram-positive organisms, *Brochothrix thermospacta*, *Lactobacillus sakei*, *Leuconostoc mesenteroides* and *Listeria monocytogenes*. This study also reported that the antimicrobial gel had a bactericidal effect that reduced the number of the Gram-positive organisms by at least 4 log CFU/cm². The study also reported the appearance of detectable numbers of *Listeria monocytogenes* at or after three weeks of storage, and stated that this indicates that the bactericidal effect may not eliminate these organisms from treated samples.

Beuchat *et al*, in 1997 reported that psychrotrophic enterotoxigenic *Bacillus cereus* in beef gravy at 8 degrees Celsius was inhibited with a concentration of between 5 and 50 microgram/ml (5-50 mg/kg) of nisin. Fang and Lin in 1994 reported that the growth of *Listeria monocytogenes* was prevented when cooked pork samples in a modified atmosphere were treated with 25 mg/kg nisin (Fang and Lin, 1994).

The Applicant also provided a number of other studies that reported the growth and incidence of spoilage and pathogenic bacteria in meat products. These were not assessed in great detail as the association of spoilage and pathogenic bacteria in some meat products has been documented. It was noted that some of these studies indicated that the contamination of meat products occurred as a result of manufacturing and handling processes. One study indicated that while in-package pasteurisation increased the shelf life of vacuum packaged meat products, it did not eliminate bacterial spoilage (Von Holy *et al*, 1989).

Discussion

Nisin has a limited spectrum of activity and by itself only inhibits the growth of certain bacteria, namely Gram-positive bacteria. The extent of this inhibition in meat products is highly dependent upon the nature of the product and the storage temperature. Given this limited activity, nisin could not be used as a replacement for poor hygienic practices because such practices would introduce other micro-organisms that are not inhibited by nisin. Manufacturers would therefore still need to use good hygienic practices to prevent the growth or introduction of these other micro-organisms. Furthermore, there is the potential for the activity of nisin to be extended by combining it with other substances.

Most of the data provided by the Applicant indicated that spoilage was related to post processing contamination. While good hygienic practices should ensure that processed meat products should not become contaminated after processing, and are stored under appropriate temperature control, there is still the potential for some bacteria to survive this processing and grow under refrigeration.

The Applicant provided data demonstrating that the presence of nisin would inhibit the growth of certain spoilage bacteria. While limited data were provided on its uses in conjunction with other preservation techniques, it is considered that the effects of nisin are likely to be enhanced when used with these other techniques. On this basis and given the limited spectrum of activity of nisin, it is considered that there is adequate technological justification for the use of nisin in processed meat products and that it will be a potentially useful component of food preservation systems for processed meat production.

Proposed Amount of Nisin Added to Processed Meats

The Applicant proposed a limit of 12.5 mg/kg for nisin in processed meat products. This limit is consistent with the limit for nisin in Codex Standards for cheese products.

The Applicant provided data indicating that nisin was effective in inhibiting spoilage bacteria in the concentration between 6.25 mg/kg and 25 mg/kg in sausage products. A concentration of 12.5 mg/kg nisin prevented lactic acid bacteria growth in a sausage with 25% fat content for five weeks (Davies et al in 1999).

The effect of nisin is dependent upon a number of factors and its concentration declines throughout the shelf life of the product, as is the case for other currently permitted food additives. Some of the nisin added to a product may also be lost during processing. Based on the data reported by Davies *et al*, losses of nisin of between 10% and 50% may occur during processing and over the shelf life of the product.

One of the studies provided by the Applicant indicated problems with the recovery of nisin from meat products (Davies et al, 1999). The Applicant provided a method for the determination of nisin content of food samples using the *Micrococcus luteus* plate diffusion assay. This method was represented as being appropriate for food samples generally.

Discussion

It is necessary for any maximum limit in the Code to be high enough to account for losses during processing and shelf life of processed meat products. Based upon the data provided by the Applicant, the Applicant's proposed limit of 12.5 mg/kg is considered to be:

- a suitable maximum level to account for losses that could occur during processing and shelf life (between 10% and 50%);
- adequate for inhibiting the growth of certain spoilage bacteria throughout the shelf life of processed meat products; and
- sufficiently low to minimise dietary exposure to unnecessary amounts of nisin.

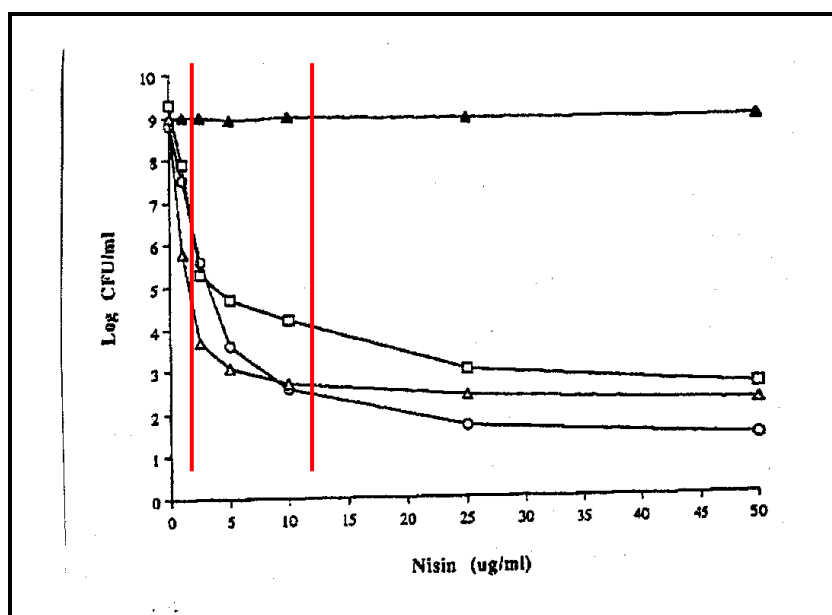
The maximum level of 12.5 mg/kg for nisin in processed meat products, as proposed by the Applicant, is considered to be technologically justified.

Control of Listeria monocytogenes

The Applicant has drawn specific attention to the ability of nisin to control *Listeria monocytogenes*. On this basis, the effect of nisin on this organism has been specifically assessed.

Since *Listeria monocytogenes* can grow on a variety of processed meat products at refrigeration temperatures (Glass *et al.*, 1989) the use of nisin to control it could be of particular interest for the food industry. Nisin can be directly incorporated into processed meat products, or can be applied as a dip, spray, or incorporated in a brine or cure. According to the Applicant, nisin is effective in inhibiting the growth of *Listeria monocytogenes* in the food following processing.

According to Harris *et al.* (1991), nisin inhibition of the growth of *L. monocytogenes* exhibits a biphasal pattern. The first phase is a linear inhibition where concentration of nisin increases arithmetically, and the inhibition to the growth of *L. monocytogenes* responds almost logarithmically. The second phase exhibits an increased deceleration of the growth inhibition where significant further increase of nisin concentration has little or no additional effect in inhibiting the growth of *L. monocytogenes*. The turning point of the two phases appears to be between 5 and 10 $\mu\text{g/ml}$ (Figure 2). Because of insignificant changes of *L. monocytogenes* inhibition at the second phase, it is suggested that nisin concentration at the turning point of the two inhibition phases would be the most effective dose in inhibiting *L. monocytogenes* growth, i.e. nisin concentration at 5 to 10 $\mu\text{g/ml}$ (5 to 10 mg per litre or per kg).



Note: ▲: Nisin producing *Lactococcus lactis* subspecies *lactis* ATCC 11454 is a reference organism of nisin resistant; □: *L. monocytogenes* strain Scott A; ○: *L. monocytogenes* strain UAL500; △: *L. monocytogenes* strain ATCC 19115.

Figure 2: Inhibition of *L. monocytogenes* growth under different concentration of nisin

An increase of nisin concentration from 25 $\mu\text{g/ml}$ (25 mg/kg) to 50 $\mu\text{g/ml}$ (50 mg/kg) results in no appreciable change in the growth inhibition of *L. monocytogenes* as shown in Figure 2.

According to a FDA GRAS notice letter (20 April, 2001)¹³, a nisin-containing antimicrobial formulation prepared by Rhodia Inc., when used under the specified conditions, is acceptable to control *L. monocytogenes* in various non-standardised meat and poultry products. The extent of *L. monocytogenes* control by nisin however, is not stated.

Potential resistance development in food processing establishments

According to Chi-Zhang *et al.* (2004), the effectiveness of nisin in inhibiting the growth of *L. monocytogenes* varies by the way the organism is exposed to nisin. If *L. monocytogenes* is exposed to nisin instantaneously, the organism tends to develop resistance to nisin over a period of time. However, there appears to be a lack of surveillance data on the prevalence of spontaneously developed nisin-resistant *L. monocytogenes* in food.

¹³ <http://www.cfsan.fda.gov/%7Erd/b/opa-g065.html>

Upon exposure to nisin (concentration range from 1 to 50 µg/ml for 4 days) in a laboratory study, Harris *et al.* (1991) observed that the occurrence of mutant *L. monocytogenes* that are resistant to 50 µg/ml (or 50 mg per litre) varied from 10^{-8} to 10^{-6} . Harris *et al.* also reported that despite its bactericidal effect, nisin cannot be solely relied upon to control *Listeria monocytogenes*. Martinez *et al.* (2005) studied the frequency of development of nisin resistance in 4 strains of *L. monocytogenes* and found this to be in the range of 10^{-6} to 10^{-3} . *L. monocytogenes* strains were exposed to approximately 5.3 µg/ml of nisin for 24 hours.

Gravesen *et al.* (2002) reported the frequency of developing resistance to nisin at 10^{-7} to 10^{-2} when 14 strains of *L. monocytogenes* were exposed to 12.5 µg/ml nisin. Both Gravesen *et al.* (2002) and Martinez *et al.* (2005) found that development of nisin resistance varies among strains of *L. monocytogenes*, and the resistant phenotype is apparently stable. Nisin resistant *L. monocytogenes* have been selected under a wide range of nisin concentration, for example, at 2.3 µg/ml (Martinez *et al.*, 2005), at 1 to 50 µg/ml (Harris *et al.*, 1991), and at 12.5 µg/ml (Gravesen *et al.*, 2002).

Discussion

Nisin has been used extensively by the food industry as a preservative. It has a long history of safe use in a broad range of foods including fermented dairy products. Although sporadic occurrences of nisin-resistant bacterial mutants are reported, there is no evidence that these mutants can develop cross-resistance to clinically important antibiotics (Davidson and Harrison, 2002; Martinez and Rodriguez, 2005). This is because of the distinctly different mechanism of action of nisin and the glycopeptide antibiotics, including the different binding targets for the substances.

Despite the prolonged use of nisin by the food industry there are no published reports of increasing resistance. The dual mode of action of nisin, blocking cell wall synthesis as well as forming membrane pores, may limit the prevalence of nisin resistance (Kramer *et al.*, 2004). In addition, Bonev *et al.* (2004) have postulated that, as the pyrophosphate moiety that is the target for nisin binding has no biochemical analogs with comparable properties, it is not so amenable to bacterial adaptations that may confer resistance.

Good hygienic practices in manufacturing facilities should include appropriate cleaning and sanitation procedures to control hazards associated with the microbiological contamination of meat products. These procedures would also ensure that the potential for nisin-resistant bacteria to contaminate meat products in food processing establishments is mitigated. If resistance were to develop and nisin was no longer able to inhibit microbiological growth then manufacturers would have to use other techniques to inhibit this growth. Taking this into account as well as the lack of data indicating increasing resistance and the long history of use in other foods, it is considered that use of nisin in meat products is unlikely to increase the prevalence of nisin resistant bacteria in food processing establishments. If this resistance were to develop then manufacturers would have to institute alternative procedures to mitigate microbiological growth.

Fermented products that rely on starter cultures for hurdle technology

Nisin produced by *Lactococcus lactis*, is an inhibitor for Gram-positive bacteria that are often closely related, for example *L. monocytogenes* and *L. innocua*. In practice, it is inhibitory also to some of the lactic acid bacteria used in food fermentation. This was demonstrated by the work of Ho and Park (1998).

There is little published information on the inhibitory effect of nisin on starter cultures used in manufacturing fermented meat. Starter cultures used in meat fermentation are largely selected strains of lactic acid bacteria including strains under the genus of *Lactobacillus*, *Pediococcus* and *Leuconostoc*. This lack of published information is presumably because nisin would not be applied to fermented meat products until the fermentation by starter culture is completed. Another reason for this lack of published information is that starter cultures used in meat fermentation produce their own antimicrobials although not nisin (Verluyten et al. (2004).

In summary, nisin is unlikely to affect starter cultures used in the production of fermented meat. In practice, nisin is applied to meat product after completion of fermentation. Therefore nisin should not pose a problem to the performance of starter cultures in the production of fermented meat.

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Business Cost Calculator Report

A 565 - Use of nisin in processed meat products

Problem: Limits for nisin currently exist in Standard 1.3.1 for a number of foods. This application seeks to include limits for nisin in processed meat products so that it may be present in these foods. The issue here is:

The safety of increased dietary exposure to nisin; and

The technological justification for nisin in processed meat products.

Objective: The objective of this assessment is to determine whether it is appropriate to amend the code to include limits for nisin in processed meat products.

Policy Options

Option Name	Quickscan Result
No change to Standard 1.3.1	FALSE
Amend Standard 1.3.1 to approve a broader use of nisin	FALSE

Compliance Cost Summary

Option Name:	No change to Standard 1.3.1		
Businesses Affected:	N/A		
Type	Cost per Business	Total Cost of Regulation	
N/A	N/A	N/A	
Option Name:	Amend Standard 1.3.1 to approve a broader use of nisin		
Businesses Affected:	N/A		
Type	Cost per Business	Total Cost of Regulation	
N/A	N/A	N/A	

Caution should be used comparing options and interpreting results over time. The Business Cost Calculator does not estimate the future values of ongoing costs. Refer to the User Guidelines for further information.

This report contains summaries of compliance costs only. An assessment on the compliance cost in itself does not provide an answer to which policy option is the most effective and efficient one. Rather, it provides information which needs to be considered alongside other relevant factors and issues when deciding between alternative policy options.

Summary of Submissions

The Draft Assessment Report identified the following regulatory options in relation to Application A565:

Option 1: no change to Standard 1.3.1, do not include a limit for nisin in Standard 1.3.1, maintain the *status quo* approach;

Option 2: vary Standard 1.3.1 to include limits for nisin in processed meat products.

The following submissions were received by FSANZ:

Submitter Organisation

Queensland Health
 Food Technology Association of Australia
 Private Citizen
 New Zealand Food Safety Authority
 Australian Food and Grocery Council
 Department of Health – South Australia
 George Weston Foods Limited
 New South Wales Food Authority

Name

Mr Gary Bielby
 Mr David Gill
 Mr Paul Elwell-Sutton
 Ms Carole Inkster
 Mr Kim Leighton
 Ms Joanne Cammans
 Ms Fiona Fleming
 Mr Craig Sahlin

Submitter	Position	Comments
Food Technology Association Vic	Supports option 2	
Queensland Health	Supports option 2	
Paul Elwell-Sutton	Opposes the Application – supports Option 1	<ul style="list-style-type: none"> Nisin is derived from <i>Streptococcus lactis</i> and states the European Parliament has determined that it causes antibiotic resistance and should not be used in foodstuffs. China is a major producer of nisin and there may be undetected levels of contaminants in it.
New Zealand Food Safety Authority	Supports Option 2	<ul style="list-style-type: none"> satisfied that the estimated dietary exposures results do not raise any public health and safety concerns with nisin at the proposed levels of use; accepts the rationale given by FSANZ for the assumption that the dietary exposures to nisin for New Zealand children are well below the ADI. highlighted an issue in relation to the draft variation to the Code proposed at Draft Assessment and suggested deletion of the words ‘commercially sterile canned cured meat’ to apply the limit for nisin to all processed meat products categorised under item 8.2.
Australian Food and Grocery Council	Supports option 2	

Submitter	Position	Comments
Department of Health – South Australia	Raised Issues	<ul style="list-style-type: none"> • noted the issue of allergic reactions to milk residues if residues of Nisaplin are present on processed meat products and that appropriate labelling and information should be considered, including for unpackaged foods as it is unlikely that consumers with milk allergies will expect meat products to contain milk residues; • it is very important that good handling techniques are maintained and that nisin is not used as a replacement to good hygiene but as an additional measure to reduce contamination. Nisin’s effectiveness is yet to be proven sufficiently in meat products; • questions whether the introduction of nisin use in the meat industry will impact on the product recall guideline for Listeria? This is a possibility if nisin is a reliable bacteriostatic product. For example, if a nisin treated meat product is found to contain a very low number of Listeria organisms, but the product cannot support growth of the bacterium, does the meat product still require removal from sale?.
George Weston Foods Ltd	Supports option 2	
New South Wales Food Authority	Does not object to further consideration of this application.	<ul style="list-style-type: none"> • notes that the Draft Assessment Report (DAR) provides adequate information regarding dietary intake estimates and the potential for increasing bacterial resistance to nisin. • notes that the DAR provides adequate information that justifies the proposed maximum level of 12.5 mg/kg. • notes that the DAR includes sufficient information demonstrating that the nisin-containing antimicrobial formulations are capable of inhibiting <i>L. monocytogenes</i> in various processed whole and comminuted meat and poultry products. • acknowledges this Application will result in potential benefits from possible reductions in food-borne illness. • There is no potential cost from the Food Authority perspective.