FOOD STANDARDS
Australia New Zealand
Te Mana Kounga Kai – Ahitereiria me Aotearoa

6 May 2009
[7-09]

APPLICATION A1015
Ethyl LAUROYL ARGINATE AS A FOOD ADDITIVE ASSESSMENT REPORT

Executive Summary

Purpose

Food Standards Australia New Zealand (FSANZ) received an Application from Laboratarios Miret SA (LAMIRSA) on 28 August 2008. This Application seeks to amend Standard 1.3.1 – Food Additives of the *Australia New Zealand Food Standards Code* (the Code) to include a new food preservative, ethyl lauroyl arginate.

Ethyl lauroyl arginate is a synthetically produced cationic surfactant[1] that is intended to be used to protect food against microbial growth and thus spoilage. Cationic surfactants such as ethyl-N^{α}-lauroyl-L-arginate·HCl (active ingredient), can be used as food preservatives because they are able to disrupt the integrity of cell membranes in a broad spectrum of bacteria, yeasts and moulds. It is proposed to be used in a wide range of food groups.

Ethyl lauroyl arginate has been evaluated by other international agencies in recent years. In 2005, the US Food and Drug Administration (FDA) issued a Letter of No Objection regarding a submission that ethyl lauroyl arginate is Generally Recognised as Safe (GRAS, Notice No. GRN 000164) for use as an antimicrobial at levels up to 200 mg ethyl-N^{α}-lauroyl-L-arginate·HCl /kg in a specified range of foods. In April 2007, the European Food Safety Authority (EFSA) issued the opinion of the Scientific Committee on ethyl lauroyl arginate as a new food preservative for use in a range of food categories. An Acceptable Daily Intake (ADI) of 0-0.5 mg/kg body weight (bw) was established by EFSA. Most recently, in June 2008, JECFA (Joint FAO/WHO Expert Committee on Food Additives) considered ethyl lauroyl arginate as a food additive and allocated an ADI of 0-4 mg/kg bw for the active ingredient, ethyl-N^{α}-lauroyl-L-arginate·HCl. The large difference in the ADIs established by EFSA and JECFA is due to a difference in the interpretation of haematology data obtained in animal toxicity studies.

Based on the availability of an adequate range of suitable studies, FSANZ has been able to complete a safety assessment for ethyl lauroyl arginate and establish an ADI. The safety assessment reports that only minimal amounts of unchanged ethyl lauroyl arginate enter the bloodstream because the compound is rapidly metabolised by enzymes in the upper intestine before substantial absorption can occur. In the intestine ethyl lauroyl arginate is

[1] Surfactants are wetting agents that lower the surface tension of a liquid, allowing easier spreading, and lower the interfacial tension between two liquids.

rapidly degraded to compounds normally present in the diet such as the amino acid L-arginine and the fatty acid lauric acid.

In animal toxicity studies of up to one year duration, ethyl lauroyl arginate was well tolerated even at high concentrations in the diet. Ethyl lauroyl arginate and its major metabolites showed no evidence of genotoxic activity. In reproductive and developmental toxicity studies, the only notable and consistent finding was delayed onset of puberty in female rats. The ADI for ethyl lauroyl arginate established by FSANZ derived from this study was 0-5 mg/kg bw.

The ADI of 0-4 mg/kg bw published by JECFA was derived from this same study, however JECFA applied a correction factor for the content of active ingredient in the batch used in the study (88%) to arrive at an ADI expressed as the active ingredient, ethyl-N^{α}-lauroyl-L-arginate·HCl.

The dietary exposure assessment assumed the addition of ethyl lauroyl arginate at the proposed maximum use level for all food types proposed by the Applicant, i.e. assuming 100% uptake by food manufacturers. This scenario is highly protective of consumers as such complete uptake of ethyl lauroyl arginate is considered unlikely and actual use levels may be lower than maximum permitted levels. All estimated dietary exposures to ethyl lauroyl arginate for the population groups assessed were below the ADI of 5 mg/kg bw.

Estimated dietary exposure for high consumers of ethyl lauroyl arginate (90^{th} percentile) for Australian children aged 2-6 years approached 80% of the ADI, 90^{th} percentile dietary exposure for the whole population of Australians aged 2+ years was 30% of the ADI and for New Zealanders aged 15+ years 20% of the ADI. The major contributor to mean ethyl lauroyl arginate dietary exposure for Australians aged 2+ years and for New Zealanders aged 15+ years would be comminuted meat products and whole pieces of processed meat, assuming use in all requested food groups. For Australian children aged 2 to 6 years, the major contributor would be cordials.

The data provided by the Applicant supplemented with published peer reviewed information indicate that ethyl lauroyl arginate is an effective food preservative in the food categories proposed. This new antimicrobial agent is stable during storage in a range of food matrices and provides protection against microbial spoilage in these foods and extends their shelf life. Use of ethyl lauroyl arginate as a preservative in the specified food categories and at the maximum permitted level is technologically justified and it could be potentially a useful component of food preservation systems.

Based on the conservative assumptions in the dietary exposure calculations, FSANZ concludes that there are no public health and safety concerns for ethyl lauroyl arginate when used as a food additive at the maximum levels proposed by the Applicant.

The Application is being assessed under the General Procedure.

Assessing the Application

In assessing the Application and the subsequent development of a food regulatory measure, FSANZ has had regard to the following matters as prescribed in section 29 of the *Food Standards Australia New Zealand Act 1991* (FSANZ Act):

- Whether costs that would arise from the amendments of the Code to permit the use of the antimicrobial agent, ethyl lauroyl arginate, as a food additive would outweigh the direct and indirect benefits to the community, Government or industry.

- There are no other measures that would be more cost-effective than a variation to Standard 1.3.1 that could achieve the same end.
- There are no relevant New Zealand standards.
- There are no other relevant matters.

Preferred Approach after Assessment

FSANZ recommends the proposed draft variations to Standard 1.3.1, Schedule 1 – Food Additives, to include – permissions for ethyl lauroyl arginate in the food types at the specified maximum limits for the active ingredient, ethyl-N^{α}-lauroyl-L-arginate·HCl, as listed in Table 1.

Table 1: Intended uses of ethyl lauroyl arginate

Food types		Ethyl lauroyl arginate* (mg/kg; maximum)
0.1	Preparations of food additives	200
1.6	Cheese - soft/cream/processed and mozzarella	400 except for mozzarella at 200
1.6	Cheese – Hard/Semi-hard	1 mg/cm^2 of surface area of cheese (taken to a depth of 3 mm and not more than 5 mm)
4.1.3	Peeled and/or cut fruits and vegetables	200
4.3.8	Processed fruits and vegetables—rehydrated legumes only	200
6.3	Processed cereal and meal products- cooked rice only	200
6.4	Flour products (including noodles and pasta) – cooked pasta and noodles only	200
8.2	Processed meat, poultry and meat products in whole cuts or pieces	200
8.3	Processed comminuted meat and poultry products	315
9.3	Semi preserved fish and fish products	400
14.1.2	Fruit and vegetable juices and fruit and vegetable juice products (NOT apple juice)	50
14.1.3	Water based flavoured drinks	50
20.2	Savoury toppings or fillings - essentially sauces such as tomato paste used in ready to eat pizzas, etc.	200
20.2	Dairy and fat based desserts, dips and snacks	400

* Ethyl lauroyl arginate shall be calculated as ethyl-N^{α}-lauroyl-L-arginate·HCl.

Reasons for Preferred Approach

Amendments to the Code to include ethyl lauroyl arginate as a food preservative in Australia and New Zealand is proposed on the basis of the available scientific evidence for the following reasons:

- A detailed safety assessment has concluded the permission for the use of ethyl lauroyl arginate does not raise any public health and safety concerns, including considering development of antimicrobial resistance.

- Use of ethyl lauroyl arginate as a preservative in the specified food categories up to the maximum permitted level is technologically justified and it could be potentially a useful component of food preservation systems. Based on data provided by the Applicant, ethyl lauroyl arginate could possibly replace some approved food grade preservatives such as benzoates, sulphates and sorbates, which have some inherent limitations.

- The regulatory impact assessment concluded that the benefits of the potential use of ethyl lauroyl arginate in the specified food categories outweigh any costs associated with its use.

- The proposed variation to the Code is consistent with the section 18 objectives of the FSANZ Act.

Consultation

Public submissions are now invited on this Assessment Report. Comments are specifically requested on the scientific aspects of this Application, in particular, information relevant to the safety assessment of ethyl lauroyl arginate.

As this Application is being assessed a general procedure, there will be one round of public comment. Submissions to this Assessment Report will be used to develop the Approval Report for the Application.

Invitation for Submissions

FSANZ invites public comment on this Report and the draft variations to the Code based on regulation impact principles for the purpose of preparing an amendment to the Code for approval by the FSANZ Board.

Written submissions are invited from interested individuals and organisations to assist FSANZ in further considering this Application. Submissions should, where possible, address the objectives of FSANZ as set out in section 18 of the FSANZ Act. Information providing details of potential costs and benefits of the proposed change to the Code from stakeholders is highly desirable. Claims made in submissions should be supported wherever possible by referencing or including relevant studies, research findings, trials, surveys etc. Technical information should be in sufficient detail to allow independent scientific assessment.

The processes of FSANZ are open to public scrutiny, and any submissions received will ordinarily be placed on the public register of FSANZ and made available for inspection. If you wish any information contained in a submission to remain confidential to FSANZ, you should clearly identify the sensitive information, separate it from your submission and provide justification for treating it as confidential commercial material.

Section 114 of the FSANZ Act requires FSANZ to treat in-confidence, trade secrets relating to food and any other information relating to food, the commercial value of which would be, or could reasonably be expected to be, destroyed or diminished by disclosure.

Submissions must be made in writing and should clearly be marked with the word 'Submission' and quote the correct project number and name. While FSANZ accepts submissions in hard copy to our offices, it is more convenient and quicker to receive submissions electronically through the FSANZ website using the Standards Development tab and then through Documents for Public Comment. Alternatively, you may email your submission directly to the Standards Management Officer at submissions@foodstandards.gov.au. There is no need to send a hard copy of your submission if you have submitted it by email or the FSANZ website. FSANZ endeavours to formally acknowledge receipt of submissions within 3 business days.

DEADLINE FOR PUBLIC SUBMISSIONS: 6pm (Canberra time) 17 June 2009

SUBMISSIONS RECEIVED AFTER THIS DEADLINE WILL NOT BE CONSIDERED

Submissions received after this date will only be considered if agreement for an extension has been given prior to this closing date. Agreement to an extension of time will only be given if extraordinary circumstances warrant an extension to the submission period. Any agreed extension will be notified on the FSANZ website and will apply to all submitters.

Questions relating to making submissions or the application process can be directed to the Standards Management Officer at standards.management@foodstandards.gov.au.

If you are unable to submit your submission electronically, hard copy submissions may be sent to one of the following addresses:

Food Standards Australia New Zealand
PO Box 7186
Canberra BC ACT 2610
AUSTRALIA
Tel (02) 6271 2222

Food Standards Australia New Zealand
PO Box 10559
The Terrace WELLINGTON 6036
NEW ZEALAND
Tel (04) 473 9942

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INTRODUCTION

Food Standards Australia New Zealand (FSANZ) received an Application from Laboratarios Miret SA on 28 August 2008. The Application seeks to amend Standard 1.3.1 – Food Additives of the *Australia New Zealand Food Standards Code* (the Code) to include ethyl lauroyl arginate as a preservative for a wide range of food categories at specified maximum levels.

Ethyl lauroyl arginate is a new synthetically produced chemical preservative. The Applicant claims that because of the effectiveness of ethyl lauroyl arginate over a wide range of food matrices and a broad antimicrobial spectrum, some sectors of the food industry might prefer the use of ethyl lauroyl arginate over the other commonly used and approved antimicrobials. The Applicant has provided experimental data to demonstrate the relative effectiveness of ethyl lauroyl arginate.

In the original dossier submitted by the Applicant, their product is referred to as lauric arginate. However, FSANZ has referred to the product as ethyl lauroyl arginate throughout this assessment, in order to be consistent with international naming. Codex has proposed the name of the product as ethyl lauroyl arginate (INS 243). The abbreviation, ELA, will be used in Tables in this assessment report because of spacing limitation.

1. The Issue / Problem

Food additives, including preservatives, are required to undergo a pre-market safety assessment before they are included in Standard 1.3.1. Maximum limits for ethyl lauroyl arginate have to be established for all food types considered. The limits are established through consideration of:

- the safety assessment for ethyl lauroyl arginate; and
- the technological justification for and effectiveness of ethyl lauroyl arginate in the range of food groups requested.

There is currently no permission for ethyl lauroyl arginate in the Code.

2. Background

2.1 Current Standard

A food additive, as stated in the Purpose clause of Standard 1.3.1, 'is any substance not normally consumed as a food in itself and not normally used as an ingredient of food, but which is intentionally added to a food to achieve one or more of the technological functions as specified in Schedule 5. Preservation is one of the functions specified in Schedule 5 and a preservative is defined as an additive that 'retards or prevents the deterioration of a food by micro organisms'. Sub-classes of preservative are anti-microbial preservative, anti-mycotic agent, bacteriophage control agent, chemosterilant and disinfection agent.

This Standard regulates the use of food additives in the production and processing of food. A food additive may only be added to food where expressly permitted in this Standard. Additives may only be added to food in order to achieve an identified technological function according to Good Manufacturing Practice.

Currently, Standard 1.3.1, Schedule 1 permits one or more of the following preservatives for use in the food types, with the exception of cooked rice, in which the Applicant has proposed to use ethyl lauroyl arginate: sorbates, benzoates, parabens, sulphites, nisin, pimaricin, nitrates, nitrites, dimethyl dicarbonate and propionates.

2.2 Technological Purpose

The active component of ethyl lauroyl arginate, ethyl-N^{α}-lauroyl-L-arginate·HCl, is a cationic surfactant with a broad spectrum of activity against bacteria, yeasts and moulds. Ethyl lauroyl arginate is stable in relatively acidic product formulations (for example, pH 4). It is effective as an antimicrobial in a wide range of food categories at the proposed usage limits and thus provides the food industry with a flexible tool to control shelf life of foods. However, ethyl lauroyl arginate binds to proteins and therefore a higher limit of usage is proposed in protein-based foods.

The Applicant has provided information to demonstrate ethyl lauroyl arginate could be used as a potential alternative to the currently approved preservatives, which have some inherent limitations. For example, sulphite consumption exceeds the ADI for some high-level consumers in Australia[2] .

2.3 International Regulatory Status

The WHO Joint Expert Committee on Food Additives (JECFA) first considered ethyl lauroyl arginate at its 69th meeting in June 2008 (FAO/WHO 2008). The Committee established an ADI of 0–4 mg/kg bw for ethyl lauroyl arginate, expressed as the active ingredient ethyl-N^{α}-lauroyl-L-arginate·HCl.

The European Food Safety Authority (EFSA) published their opinion on ethyl lauroyl arginate in April 2007. EFSA established an ADI for ethyl lauroyl arginate of 0-0.5 mg/kg bw[3].

The US Food and Drug Administration has issued a Letter of No Objection regarding the submission that ethyl lauroyl arginate is Generally Recognised as Safe (GRAS) for use as an antimicrobial at levels up to 225 mg/kg of ethyl lauroyl arginate in the food categories specified (USFDA 2005).

3. Objectives

The objective of this assessment is to determine whether it is appropriate to amend the Code to include ethyl lauroyl arginate in the specified food categories and to establish maximum allowable limits. In developing or varying a food standard, 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; and
- the provision of adequate information relating to food to enable consumers to make informed choices; and
- the prevention of misleading or deceptive conduct.

[2] FSANZ 2005, 21st Australian Total Diet Study: a total diet study of sulphites, benzoates and sorbates.
[3] Reasons for discrepancy between JECFA and EFSA is given in Attachment 2.

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].

4. Questions to be answered

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

- What would the potential dietary exposure to ethyl lauroyl arginate be for mean and high consumers of foods containing the preservative?
- Are there any public health and safety issues as a consequence of approving the use of ethyl lauroyl arginate at the levels proposed in the range of food types listed in Table 1?
- Are the requested levels of ethyl lauroyl arginate technologically justified in the food categories applied for?

RISK ASSESSMENT

5. Risk Assessment Summary

5.1 Hazard Assessment

FSANZ has assessed the submitted evidence on the safety of ethyl lauroyl arginate including studies on absorption, metabolism, acute toxicity, repeat-dose toxicity, genotoxicity and reproductive toxicity. The submitted data were considered suitable for hazard assessment and assignment of an ADI for ethyl lauroyl arginate. For the full Hazard Assessment Report see **Attachment 2**.

JECFA first assessed the toxicity of ethyl lauroyl arginate in 2008 and arrived at an ADI of 0-4 mg/kg bodyweight expressed as the active ingredient. The ADI was based on the No Observed Adverse Effect Level (NOAEL) of 502 mg/kg bw/day (expressed as ethyl lauroyl arginate) established in a reproductive toxicity study. This NOAEL was corrected for the active ingredient content (88% w/w) to give a NOAEL for the active ingredient of 442 mg/kg bw/day. The ADI of 0-4 mg/kg bodyweight for the active ingredient was derived by applying a 100-fold safety factor (10-fold for inter-species differences and 10-fold to account for differences between individuals).

[4] In May 2008, the Australia and New Zealand Food Regulation Ministerial Council endorsed the Policy Guideline on Addition to Food of Substances other than Vitamins and Minerals. This includes policy principles in regard to substances added for technological purposes such as food additives and processing aids. FSANZ has given regard to each of these principles in assessing this Application.

After assessing all of the available data, FSANZ has used the same NOAEL of 502 mg/kg bw/day obtained in the reproductive toxicity study and applied a 100-fold safety factor to establish an ADI of 0-5 mg/kg bodyweight for ethyl lauroyl arginate. Thus, the only difference between the ADIs derived by JECFA and FSANZ was the correction for active ingredient content by JECFA. FSANZ did not correct for active ingredient content because the batch used in the relevant study conformed to the approved JECFA specifications for ethyl lauroyl arginate.

In the submitted studies, systemic exposure to orally administered ethyl lauroyl arginate was low because most of the compound is rapidly metabolised in the intestines before absorption occurs. Ethyl lauroyl arginate is rapidly degraded to endogenous compounds and compounds normally present in the diet such as the amino acid L-arginine and the fatty acid lauric acid. In animal toxicity studies of up to one year duration, ethyl lauroyl arginate was well tolerated even at relatively high doses. Ethyl lauroyl arginate had a minor local irritant effect on the rat forestomach probably due to its surfactant activity. However, the rodent forestomach is not protected by mucus and has no anatomical equivalent in humans. The forestomach findings were therefore not considered to be relevant for a risk assessment in humans.

Ethyl lauroyl arginate and its major metabolite showed no evidence of genotoxic activity. In reproductive and developmental toxicity studies the only notable and consistent finding was delayed onset of puberty in female rats. There was no information to indicate that this effect may not be relevant to humans. The finding was therefore considered suitable for deriving an ADI. Because of uncertainties regarding the mechanism of delayed puberty in female rats and the relevant exposure period for the effect, a conservative dose was chosen on which to base the ADI as discussed in the Hazard Assessment Report (Attachment 2). No other effects on reproduction or development attributable to ethyl lauroyl arginate were observed.

5.2 Dietary Exposure

FSANZ conducted a dietary exposure assessment for the food additive ethyl lauroyl arginate based on the information provided by the Applicant. For the full Dietary Exposure Assessment Report see **Attachment 3**.

Food consumption data from the 1995 Australian and 1997 New Zealand National Nutrition Surveys were used for the exposure assessments. The population groups assessed were the Australian population (2 years and above), the New Zealand population (15 years and above) and children (2 to 6 years for Australia only).

The Applicant provided FSANZ with information on proposed levels of use for ethyl lauroyl arginate for specific food groups and the expected foods within each food group that may contain it. Based on this information, dietary exposure was estimated assuming that ethyl lauroyl arginate was present in foods at the maximum permitted level suggested by the applicant correcting for the proportion of the active ingredient in ethyl lauroyl arginate. This scenario is highly protective of consumers.

Estimated mean exposures for consumers of ethyl lauroyl arginate for all population groups assessed were 38 mg/day (0.7 mg/kg bw/day) for the Australian population 2 years and above; 36 mg/day (2.0 mg/kg bw/day) for Australian children 2-6 years; and 32 mg/day (0.4 mg/kg bw/day) for the New Zealand population aged 15 years and above. Estimated 90th percentile exposures for consumers of ethyl lauroyl arginate were 82 mg/day (1.5 mg/kg bw/day) for the Australian population 2 years and above; 72 mg/day (3.9 mg/kg bw/day) for

Australian children 2-6 years; and 75 mg/day (1.0 mg/kg bw/day) for the New Zealand population aged 15 years and above.

Based on the food groups proposed by the Applicant, the major contributor to the estimated ethyl lauroyl arginate dietary exposure for Australians aged 2 years and above and for New Zealanders aged 15 years and above would be comminuted meat products and whole pieces of processed meat. For Australian children aged 2 to 6 years, the major contributor would be cordials.

5.3 Risk characterisation

Comparisons of the dietary exposure to ethyl lauroyl arginate with the ADI of 0-5 mg/kg bodyweight indicated that for all groups of Australian and New Zealand consumers assessed (including children), estimated dietary exposures were below this safe level of exposure. The estimated mean dietary exposures for consumers of ethyl lauroyl arginate correspond to 14% of the ADI for Australians aged 2 years and above, 39% of the ADI for Australian children aged 2-6 years, and 9% of the ADI for New Zealanders aged 15 years and above. The estimated 90th percentile dietary exposures for consumers of ethyl lauroyl arginate correspond to 31% of the ADI for Australians aged 2 years and above, 78% of the ADI for Australian children aged 2-6 years, and 21% of the ADI for New Zealanders aged 15 years and above. These comparisons raise no public health and safety concerns for the addition of ethyl lauroyl arginate at the proposed levels of use.

Ethyl lauroyl arginate showed no signs of intolerance even at very high dietary levels in animal studies of up to one year in duration. Ethyl lauroyl arginate has been approved for use and commercialised in the USA since 2005 with no reports of intolerance associated with consumption. Ethyl lauroyl arginate is rapidly metabolised to compounds which have not been associated with intolerance reactions.

5.4 Antimicrobial resistance

While there is a potential for resistance of microorganisms to antimicrobial agents, such as ethyl lauroyl arginate and other preservatives used in food production, this can be minimised through proper management and monitoring of their use. These measures include the setting of appropriate maximum limits and following the principles of GMP – i.e. the quantity of additive added to food shall be limited to the lowest possible level necessary to accomplish its desired effect.

While there is an absence of data in the peer-reviewed literature on the selection and/or development of microorganisms resistant to ethyl lauroyl arginate, resistance to other cationic surfactants, such as quaternary ammonium compounds, has been reported. Unpublished laboratory data provided by the Applicant showed that when test organisms were exposed to sub lethal concentrations of ethyl lauroyl arginate, an increased resistance to the antimicrobial was observed over time. This adaption was temporary, however, as resistant cultures quickly became susceptible following growth in ethyl lauroyl arginate-free media. See **Attachment 4** for the full review of antimicrobial resistance by FSANZ.

5.5 Food technology Assessment

FSANZ conducted a review of the technological justification of ethyl lauroyl arginate as a preservative based on the information provided by the Applicant and on published information. For the full Food technology Assessment Report see **Attachment 5**.

The Application requested ethyl lauroyl arginate as a preservative in a wide range of food groups as listed below:

- Food additive preparations
- Cheeses – soft, cream, processed, mozzarella, hard and semi hard
- Peeled and/or cut fruit and vegetables – rehydrated legumes
- Cereal products – cooked rice, noodles and pasta
- Semi processed fish and fish products – salted fish and roe
- Processed meat, poultry and meat products in whole or cut pieces or comminuted products
- Non-alcoholic beverages - fruit and vegetable juices and juice products (not including apple juice), water based flavoured drinks and high energy drinks and soft drinks
- Savoury toppings or fillings, dairy based desserts, dips and snacks

Within these foods, the Applicant proposed ethyl lauroyl arginate, expressed as the active ingredient, ethyl-N^{α}-lauroyl-L-arginate·HCl to be used in levels ranging between 50 mg/kg (e.g. beverages) and 400 mg/kg (in protein based foods, e.g. cheese and fish products).

The Applicant provided 36 experimental studies, 32 of which contain Confidential Commercial Information (CCI), to support their claims that ethyl lauroyl arginate effectively suppresses a broad spectrum of microorganisms in a wide range of food matrices. The Applicant provided information to demonstrate ethyl lauroyl arginate may be a potential alternative for some of the currently approved preservatives such as sulphites, benzoates and sorbates, which have some inherent limitations.

The data provided by the Applicant supplemented with published peer reviewed information indicate that ethyl lauroyl arginate is an effective food preservative to extend shelf life of foods in the food groups proposed above and that it also reduces the levels of certain pathogenic bacteria. This new antimicrobial agent is stable in storage and processing of a range of food groups.

Use of ethyl lauroyl arginate as a preservative in the specified food types up to the maximum requested level is technologically justified based on stability consideration and effectiveness. Along with good manufacturing practice, ethyl lauroyl arginate could be a useful component of food preservation systems.

RISK MANAGEMENT

6. Regulatory Options

There are no non-regulatory options for this Application. Two regulatory options have been identified for this Application:

Option 1 Maintain the *status quo* approach; do not permit the use of ethyl lauroyl arginate as a preservative.

Option 2 Amend Schedule 1 of Standard 1.3.1 to permit maximum limits for ethyl lauroyl arginate as a food additive in the range of food types specified in Table 1, and consequential amendments below:

Ethyl lauroyl arginate will be added to the list of food additive code numbers in Standard 1.2.4 – Labelling of Ingredients.

A specification for ethyl lauroyl arginate will be referenced in Standard 1.3.4 – Identity and Purity.[5]

7. Impact Analysis

FSANZ is required to consider the impact of various regulatory and non-regulatory options on all sectors of the community, especially relevant stakeholders who may be affected by this Application. The benefits and costs associated with the proposed amendment to the Code have been analysed using regulatory impact principles.

In accordance with the Best Practice Regulation Guidelines the preliminary assessment for this application indicated low or negligible impacts. The Office of Best Practice Regulation has advised that the analysis is adequate and approved the preliminary assessment (RIS ID 10222)

7.1 Affected Parties

The affected parties may include the following:

1. Those sectors of the food industry wishing to use this new food preservative.

2. Consumers who may be affected, either negatively or positively, as a result of a new preservative becoming available in processed foods.

3. Government agencies with responsibility for compliance and enforcement of the Code.

7.2 Benefit Cost Analysis

7.2.1 Option 1 – Do not permit the use of ethyl lauroyl arginate as a food preservative

- Food industries may be disadvantaged as they would be unable to capture the potential benefits of the new food preservative. Some sectors of the food industry are under pressure to reduce their levels of benzoates and sulphites. These sectors face increasing costs if alternatives are not permitted.

- There is no perceived impact on consumers.

- There is no perceived impact on government agencies.

7.2.1 Option 2 – Permit maximum limits for ethyl lauroyl arginate as a food additive in the range of foods specified in Table 1

- Food industries may benefit as they may be able to include ethyl lauroyl arginate in their products as part of their food preservation systems with consequent market advantages from reduced spoilage losses and extended shelf life. However, the food industries would incur the cost of labelling changes if they chose to use the new preservative.

[5] As ethyl lauroyl arginate complies with Monograph 5 published in the FAO Combined Compendium of Food Additive Specifications (Monograph 5) (JECFA, 2008), Monograph 5will be a primary source of specification, as required in Clause 2 of Standard 1.3.4. FSANZ is in the process of updating Clause 2 to include reference to Monograph 5 (in Proposal P1008).

- Consumers may benefit from foods containing ethyl lauroyl arginate through reduction in losses associated with food spoilage and potential for lowered consumption of some of the currently approved preservatives. However, some consumers may object to having a new chemical preservative added to foods.

- Government agencies may incur an increase in the cost of monitoring compliance, but this is expected to be minor as the method of analysis is published and uses typical laboratory apparatus.

7.3 Comparison of Options

Option 1 appears to provide no apparent benefits to industry, consumers or government. Option 1 denies industry access to a flexible preservative in a wide range of food products.

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 and to reduce the level of usage of some of the current approved preservatives.

An assessment of the costs and benefits of Option 1 and 2 indicates that there would be a net benefit in permitting the use of ethyl lauroyl arginate in the food categories listed in Table 1 at the specified maximum level of usage. Therefore Option 2 is the preferred option.

8. Other considerations

FSANZ notes that ethyl lauroyl arginate may also have applications in cosmetics and that NICNAS is considering an Application on ethyl lauroyl arginate in cosmetics currently. Prior to compiling the Approval Report, FSANZ will consider potential total exposure, including from non-food sources, if applicable.

COMMUNICATION AND CONSULTATION STRATEGY

9. Communication and consultation

FSANZ has developed a communication strategy to Application A1015 that involves advertising the availability of the assessment reports for public comment in the national press and placing the reports on the FSANZ website. In addition, FSANZ will issue a media release drawing journalists' attention to the matter.

The aim of the communication strategy is to inform the food industry and consumers about the issues raised in the Application and to communicate with health professionals about the proposed change to the standard and provide them with information for their clients if this should become necessary.

The process by which FSANZ considers standard matters is open, accountable, consultative and transparent. The purpose of inviting public submissions is to obtain the views of interested parties on the issues raised by the application and the impacts of regulatory options. The issues raised in the public submissions are evaluated and addressed in FSANZ assessment reports.

The Applicant, individuals and organisations that make 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.

9.1 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.

Amending the Code to include ethyl lauroyl arginate as a food additive is unlikely to have a significant effect on trade. The ethyl lauroyl arginate preparation is consistent with the international specifications for ethyl lauroyl arginate. For these reasons FSANZ has decided not to notify the WTO under either the Technical Barriers to Trade or Sanitary and Phytosanitary Measures Agreements.

CONCLUSION

10. Conclusion and Preferred Option

The Applicant has sought to amend Schedule 1 of Standard 1.3.1 – Food Additives, of the Code to permit maximum limits for the use of ethyl lauroyl arginate as a preservative in food types as listed in Table 1.

Preferred Approach

FSANZ recommends the proposed draft variations to Standard 1.3.1, Schedule 1 – Food Additives, to include ethyl lauroyl arginate in the food types at the specified maximum limits as listed in Table 1 with subsequent amendments to Standard 1.2.4 – Labelling of Ingredients and Standard 1.3.4 – Identity and Purity.

Table 1: Intended uses of ethyl lauroyl arginate

Food types		Ethyl lauroyl arginate* (mg/kg; maximum)
0.1	Preparations of food additives	200
1.6	Cheese - soft/cream/processed and mozzarella	400 except for mozzarella at 200
1.6	Cheese – Hard/Semi-hard	1 mg/cm^2 of surface area of cheese (taken to a depth of 3 mm and not more than 5 mm)
4.1.3	Peeled and/or cut fruits and vegetables	200
4.3.8	Processed fruits and vegetables—rehydrated legumes only	200
6.3	Processed cereal and meal products-cooked rice only	200
6.4	Flour products (including noodles and pasta) – cooked pasta and noodles only	200
8.2	Processed meat, poultry and meat	200

Food types		Ethyl lauroyl arginate* (mg/kg; maximum)
	products in whole cuts or pieces	
8.3	Processed comminuted meat and poultry products	315
9.3	Semi preserved fish and fish products	400
14.1.2	Fruit and vegetable juices and fruit and vegetable juice products (NOT apple juice)	50
14.1.3	Water based flavoured drinks	50
20.2	Savoury toppings or fillings - essentially sauces such as tomato paste used in ready to eat pizzas, etc.	200
20.2	Dairy and fat based desserts, dips and snacks	400

* Ethyl lauroyl arginate shall be calculated as ethyl-N^{α}-lauroyl-L-arginate·HCl.

10.1 Reasons for Preferred Approach

Amendments to the Code to include ethyl lauroyl arginate as a food preservative in Australia and New Zealand is proposed on the basis of the available scientific evidence for the following reasons:

A detailed safety assessment has concluded the permission for the use of ethyl lauroyl arginate does not raise any public health and safety concerns, including considering development of antimicrobial resistance. The relevant assessments are based on the best available scientific evidence.

Use of ethyl lauroyl arginate as a preservative in the specified food categories and at the maximum permitted level is technologically justified and it could be potentially a useful component of food preservation systems. Based on data provided by the Applicant, ethyl lauroyl arginate could potentially replace some approved food grade preservatives, such as benzoates, sulphates and sorbates.

The regulatory impact assessment concluded that the benefits of the potential use of ethyl lauroyl arginate in the specified food categories outweigh any costs associated with its use.

The proposed variation to the Code is consistent with the section 18 objectives of the FSANZ Act.

11. Implementation and Review

Following the consultation period for this document, an Approval Report will be completed and the draft variation will be considered for approval by the FSANZ Board. The FSANZ Board's decision will then be notified to the Ministerial Council. Following notification, the proposed draft variation to the Code is expected to come into effect on gazettal, subject to any request from the Ministerial Council for review of FSANZ's decision.

ATTACHMENTS

1. Draft variation to the *Australia New Zealand Food Standards Code*
2. Hazard Assessment Report

3. Dietary Exposure Assessment Report
4. Antimicrobial Resistance Report
5. Food Technology Report

Attachment 1

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

Section 87(8) of the FSANZ Act provides that standards or variations to standards are legislative instruments, but are not subject to disallowance or sunsetting

To commence on gazettal:

[1] ***Standard 1.2.4*** *of the Australia New Zealand Food Standards Code is varied by –*

[1.1] *inserting in* Part 1 *of* Schedule 2 –

Ethyl lauroyl arginate	243

[1.2] *inserting in* Part 2 *of* Schedule 2 –

Ethyl lauroyl arginate	243

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

[2.1] *inserting in subclause 5(2) –*

ethyl lauroyl arginate shall be calculated as ethyl-N^{α}-lauroyl-L-arginate·HCl

[2.2] *inserting in* Schedule 1, *under item* 0.1 Preparations of food additives –

243	Ethyl lauroyl arginate	200	mg/kg	

[2.3] *inserting in* Schedule 1*, under item* 1.6 Cheese and cheese products, *immediately following the last additive entry –*

1.6.1 Soft cheese, cream cheese and processed cheese

243	Ethyl lauroyl arginate	400	mg/kg	

Mozzarella cheese

243	Ethyl lauroyl arginate	200	mg/kg	

1.6.2 Hard cheese and semi-hard cheese

243	Ethyl lauroyl arginate	1	mg/cm^2	applied to the surface of food; maximum level determined in a surface sample taken to a depth of not less than 3 mm and not more than 5 mm.

[2.4] *inserting in* Schedule 1*, under item* 4.1.3 Peeled and/or cut fruits and vegetables –

243	Ethyl lauroyl arginate	200	mg/kg	

[2.5] *inserting in* Schedule 1*, under item* 4.3.8 Other fruit and vegetable based products* -

Rehydrated legumes

243	Ethyl lauroyl arginate	200	mg/kg	

[2.6] *inserting in* Schedule 1*, under item* 6.3 Processed cereal and meal products, *immediately following the last additive entry* –

6.3.1 Cooked rice

243	Ethyl lauroyl arginate	200	mg/kg	

[2.7] *inserting in* Schedule 1*, under item* 6.4 Flour products (including noodles and pasta)* –

243	Ethyl lauroyl arginate	200	mg/kg	cooked pasta and noodles only

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

243	Ethyl lauroyl arginate	200	mg/kg	

[2.9] *inserting in* Schedule 1*, under item* 8.3 Processed comminuted meat, poultry and game products –

243	Ethyl lauroyl arginate	315	mg/kg	

[2.10] *inserting in* Schedule 1*, under item* 9.3 Semi preserved fish and fish products –

243	Ethyl lauroyl arginate	400	mg/kg	

[2.11] *inserting in* Schedule 1*, under item* 14.1.2 Fruit and vegetable juices and fruit and vegetable juice products* –

243	Ethyl lauroyl arginate	50	mg/kg	not apple juice

[2.12] *inserting in* Schedule 1*, under item* 14.1.3 Water based flavoured drinks* –

243	Ethyl lauroyl arginate	50	mg/kg	

[2.13] *inserting in* Schedule 1*, under item* 20.2 Food other than beverages*, *sub-item* dairy and fat based desserts*,* dips and snacks –

243	Ethyl lauroyl arginate	400	mg/kg

[2.14] *inserting in* Schedule 1*, under item* 20.2 Food other than beverages*, *sub-item* sauces and toppings (including mayonnaises and salad dressings) –

243	Ethyl lauroyl arginate	200	mg/kg

[3] ***Standard 1.3.4*** *of the Australia New Zealand Food Standards Code is varied by omitting paragraph 2(a), substituting* –

(a) Combined Compendium of Food Additive Specifications, FAO JECFA Monograph 1 (2005) as superseded by specifications published in FAO JECFA Monographs 3 (2006) and FAO JECFA Monographs 4 (2007) and FAO JECFA Monographs 5 (2008), Food and Agriculture Organisation of the United Nations, Rome; or

Attachment 2

Hazard Assessment Report

Summary and Conclusions

FSANZ has assessed the submitted evidence on the safety of ethyl lauroyl arginate including studies on absorption, metabolism, acute toxicity, repeat-dose toxicity, genotoxicity and reproductive toxicity. The submitted data, comprising a suitable set of high quality studies, are considered suitable for hazard assessment and assignment of an acceptable daily intake (ADI) for ethyl lauroyl arginate.

Following oral ingestion, systemic exposure to ethyl lauroyl arginate was low in rats and negligible in humans. *In* vitro data suggest that the compound is rapidly metabolised in the intestines before substantial absorption can occur. Absorption from the stomach may be occurring in rats, particularly at higher doses. *In vivo*, significant systemic absorption of a metabolite does occur; however, this metabolite is rapidly degraded to endogenous compounds and compounds normally present in the diet.

In animal toxicity studies ethyl lauroyl arginate was well tolerated even at relatively high doses. Ethyl lauroyl arginate had a minor local irritant effect on the rat forestomach probably due to its surfactant activity. The rodent forestomach is not protected by mucus and has no anatomical equivalent in humans. The forestomach findings are therefore not considered to be relevant for a risk assessment in humans.

In genotoxicity assays, ethyl lauroyl arginate and its major metabolite showed no evidence of mutagenic or clastogenic activity. For ethyl lauroyl arginate, relatively low maximum concentrations were tested in the *in vitro* assays because of cytotoxicity at higher concentrations. A long term carcinogenicity study was not submitted which is considered acceptable because ethyl lauroyl arginate was not genotoxic and has no chemical structural alert and did not show evidence of pre-neoplasia or neoplasia in the repeat dose toxicity studies.

Reproductive and developmental toxicity studies were conducted in rats and rabbits. The only notable treatment related finding was delayed onset of puberty in female rats observed in two studies. A possible mechanism for this effect is not known; however, it may be related to reduced body weight gain in the pups in the week prior to weaning. The finding of delayed puberty onset was considered suitable by FSANZ for deriving an ADI. The No Observed Adverse Effect Level (NOAEL) for this effect was 502 mg/kg bw/day. Applying safety factors of 10 for inter-species differences and 10 for inter-individual differences to the NOAEL results in an ADI of 0-5 mg/kg bw for ethyl lauroyl arginate.

Background

Chemistry

Details of the physicochemical properties of ethyl lauroyl arginate (abbreviation: ELA)[6], including product specifications and the impurity profile, are included in the Food Technology Report. The compound is prepared as a hydrochloride salt (molecular weight 421.0) which is a white solid at room temperature (CAS number 60372-77-2). The active ingredient is ethyl-N^{α}-lauroyl-L-arginate·HCl. In solution, ethyl lauroyl arginate acts as a cationic surfactant and its preservative properties are reported to be due to disruption of bacterial cell membranes. The structural formula of ethyl lauroyl arginate is shown in Figure 1 on page 24.

Consideration of ethyl lauroyl arginate by various expert committees

The WHO Joint Expert Committee on Food Additives (JECFA) first considered ethyl lauroyl arginate at its 69th meeting in June 2008 (FAO/WHO 2008). The Committee established an ADI of 0–4 mg/kg bw for ethyl lauroyl arginate, expressed as the active ingredient ethyl-N^{α}-lauroyl-L-arginate·HCl, based on the NOAEL of 442 mg/kg bw per day identified in studies of reproductive toxicity and a safety factor of 100 (WHO 2009). The NOAEL was based on delayed vaginal opening observed in two reproductive toxicity studies in rats. The NOAEL for this effect was a dietary concentration of 6000 mg/kg, corresponding to an ethyl lauroyl arginate intake of 502 mg/kg bw/day (442 mg/kg bw per day expressed as ethyl-N^{α}-lauroyl-L-arginate·HCl).

The European Food Safety Authority (EFSA) published their opinion on ethyl lauroyl arginate in April 2007. EFSA established an ADI of 0-0.5 mg/kg bw for ethyl lauroyl arginate. The ADI was based on effects observed on white blood cell counts in repeat dose toxicity studies (EFSA 2007). The ADI derived by EFSA was based on an NOAEL of approximately 50 mg ELA/kg bw/day, which was the lowest dose tested in the 13 week study with Mirenat-N as test article. EFSA considered ELA prior to the availability of three expert reviews on the white blood cell findings. The expert reviews concluded that the white blood cell findings are unlikely to be of toxicological significance.

The EU Scientific Committee on Cosmetic Products and Non-Food Products Intended for Consumers (SCCP) issued an opinion on the safety of ethyl lauroyl arginate when used as a preservative in cosmetics, on 15th March 2005 (SCCP 2005). The SCCP considered that ethyl lauroyl arginate was safe for the consumers, when used:

- up to a maximum concentration of 0.4% as a preservative in cosmetic products, but excluding products for the lips, oral hygiene products and spray products
- up to a maximum concentration of 0.8% in soap, anti-dandruff shampoos, and non-spray deodorants.

The SCCP opinion was based on the use of ethyl lauroyl arginate in the specified cosmetic products only and took no account of other sources of exposure.

The US Food and Drug Administration has issued a Letter of No Objection regarding the submission that ethyl lauroyl arginate is Generally Recognised as Safe (GRAS) for use as

[6] Ethyl lauroyl arginate is the official proposed name for the compound according to Codex (December 2008). In many of the study titles quoted in this Attachment, lauric arginate and LAE are used as synonyms for ethyl lauroyl arginate.

an antimicrobial at levels up to 225 mg/kg of ethyl lauroyl arginate in the food categories specified (USFDA 2005).

Scope of the current hazard assessment

FSANZ has not previously assessed the safety of ethyl lauroyl arginate. Therefore, the aims of the current assessment were to:

- review all of the available data on the kinetics and toxicology of ethyl lauroyl arginate to determine its safety as a food additive; and
- establish an ADI for ethyl lauroyl arginate.

Evaluation of Submitted Data

FSANZ has assessed the submitted evidence on the safety of ethyl lauroyl arginate including studies on absorption, metabolism, acute toxicity, repeat-dose toxicity, genotoxicity and reproductive toxicity. The submitted data, comprising a relatively comprehensive set of high quality studies, were considered suitable for hazard assessment and assignment of an ADI for ethyl lauroyl arginate.

Absorption studies on ethyl lauroyl arginate were conducted in rats and humans. *In vitro* metabolism studies were conducted with S9 liver fractions from rats, simulated gastric fluid (with and without pepsin), simulated intestinal fluid (with and without pancreatin), and with human plasma and human hepatocytes. *In vivo* metabolism studies were conducted in rats. An excretion study was also conducted in rats.

Acute toxicity, repeat dose toxicity, genotoxicity studies were performed with: (i) ethyl lauroyl arginate (active ingredient content 88-90% w/w), and (ii) Mirenat-N (20-25% w/w ethyl lauroyl arginate dissolved in propylene glycol). An acute toxicity study and two genotoxicity studies were also performed with N^{α}-lauroyl-L-arginine (LAS)[7], the major metabolite of ethyl lauroyl arginate. Reproductive toxicity studies were performed with ethyl lauroyl arginate containing the active ingredient at 88% w/w. Developmental toxicity studies were performed with ethyl lauroyl arginate containing the active ingredient at 69% w/w. The relatively low content of active ingredient in this batch was due to high water content (23% w/w) because the synthesis product was not subject to a drying step. Because this batch does not meet the JECFA specifications for the content of the active ingredient (85 to 95% w/w), a correction factor was applied to the doses in the developmental toxicity studies to enable comparison with the studies that used batches conforming to the JECFA specifications.

Detailed descriptions of the absorption, metabolism and excretion studies, single and repeat dose toxicity, genotoxicity, reproductive and developmental toxicity studies considered in this assessment are given below.

Absorption, Metabolism and Excretion studies

In the studies below, the terms C_{max}, t_{max} and AUC refer to maximum plasma concentration, time of maximum plasma concentration, and area under the plasma concentration versus time curve, respectively.

[7] The reason for abbreviating N^{α}-lauroyl-L-arginine as LAS in the submitted study reports is not known but is retained for consistency throughout this report.

Absorption

Rats

HLS (2005c) **Study title**: Lauric arginate pharmacokinetics in rats. **Report no.**: LMA 057/053626 **Report date**: 21 Dec 2005 **Laboratory**: Huntingdon Life Sciences Ltd, Huntingdon, Cambridgeshire, England. **GLP**: Yes (OECD)

Sprague-Dawley rats in the fed state received single oral gavage doses of ethyl lauroyl arginate as shown in the table below.

Table 2.1: Treatment groups and dosing details

Group no.	ELA dose (mg/kg bw)	No. animals/group	Vehicle
1 (Pilot phase)	40	4/sex	Propylene glycol/water
2 (Main phase)	40	4 males	Propylene glycol/water
3 (Main phase)	120	4 males	Propylene glycol/water
4 (Main phase)	320	4 males	Propylene glycol/water
5 (Main phase)	120	4 males	Glycerol/water
6 (Main phase)	120	4 males	Water

Blood was sampled at 15, 30, 60, 90, 120 and 240 min post-dose for the pilot phase animals and at 30, 60, 90, 120, 240 min with a final sampling time at 8 h post-dose for animals in the main phase of the study. Plasma concentrations of ethyl lauroyl arginate and the metabolite LAS were measured by a partially validated LC-MS/MS method. Acceptable recovery, linearity, precision, accuracy and specificity were observed over the concentration range 1 – 200 ng/mL for ethyl lauroyl arginate and 5 – 1000 ng/mL for LAS. The limits in these ranges correspond to the lower and upper limits of quantification. The use of a dilution factor of x10 for ethyl lauroyl arginate was not validated primarily due to degradation during the procedure. The analytes were found to be unstable during one freeze-thaw cycle. Therefore this method is only applicable to rat plasma which is analysed immediately after sampling which was the case in this pharmacokinetics study.

Mean plasma C_{max}, t_{max} and $AUC_{0\text{-}8\ h}$ values for ethyl lauroyl arginate and LAS (propylene glycol/water vehicle, main phase) are shown in the table below.

Table 2.2: Mean plasma C_{max} and $AUC_{0\text{-}8\ h}$ values for ethyl lauroyl arginate and LAS

	ELA dose (mg/kg bw)					
	40		120		320	
Analyte	ELA	LAS	ELA	LAS	ELA	LAS
C_{max} (ng/mL)	2.02	24.2	1.23	23.2	2.60	96.9
t_{max} (h)	0.5 [a]	1.0 [a]	1.0 [a]	0.75 [a]	3.0 [a]	1.5 [a]
$AUC_{0\text{-}8\ h}$ (ng.h/mL)	-	52.5	-	103	7.50	315

[a] Median - Could not be calculated (insufficient data above the lower limit of quantification)

A comparison of the C_{max} and $AUC_{0\text{-}8\ h}$ values for ethyl lauroyl arginate in the presence of the three different vehicles at an ethyl lauroyl arginate dose of 120 mg/kg bw are shown below.

Table 2.3: C_{max} and $AUC_{0\text{-}8\ h}$ values for ethyl lauroyl arginate in the presence of the three different vehicles

	ELA dose = 120 mg/kg bw		
Vehicle	Propylene glycol/water	Glycerol/water	Water

Analyte	ELA	LAS	ELA	LAS	ELA	LAS
C_{max} (ng/mL)	1.23	23.2	9.42	28.8	10.6	31.2
t_{max} (h)	1.0 [a]	0.75 [a]	0.75 [a]	1.0 [a]	0.75 [a]	0.5 [a]
$AUC_{0\text{-}8\ h}$	-	103	12.6	115	8.78	109

[a] Median
- Could not be calculated (insufficient data above the lower limit of quantification)
Note that ethyl lauroyl arginate is freely soluble in water (greater than 247 g/kg at 20°C) and soluble up to 20% w/w in propylene glycol, glycerol and ethanol.

Conclusions: Ethyl lauroyl arginate was rapidly metabolised to LAS. The AUC for LAS was approximately proportional to the ethyl lauroyl arginate dose and was similar in the presence of all three vehicles at an ethyl lauroyl arginate dose of 120 mg/kg bw.

Humans

CentraLabS (2005a) **Study title**: LAE: An open label, single-dose study to determine the feasibility of measuring LAE and its breakdown products in plasma after oral administration of LAE to healthy male volunteers. **Report no.**: LMA 047/033421, **Report date**: 12 Jan 2005 **Laboratory**: CentraLabS Clinical Research Ltd., Alconbury, Cambridgeshire, England. (Clinical phase at PPD Development Clinic, Leicester, UK) **GLP**: Yes (OECD), for analytical phase of the study

Two healthy male volunteers each received an oral dose of 5 mg/kg bw ^{13}C-ELA dissolved in 15 mg/kg bw propylene glycol made up to a volume of 1 mL/kg bw with purified water. It was not stated whether the volunteers were in a fed or fasted state and whether the solution was consumed as a bolus or gradually ingested. Blood samples were taken pre-dose and at 5, 10, 15 and 30 min, and 1, 2, 4, 8, 12 and 24 h after dosing. Plasma concentrations of ^{13}C-ELA, ^{13}C-LAS and ^{13}C-arginine were measured by an LC-MS/MS method (lower limit of quantification 1 ng/mL for ethyl lauroyl arginate and LAS; 10 ng/mL for arginine). C_{max} and t_{max} values are shown in the table below. AUC values were not presented in the report.

Table 2.4: C_{max} and t_{max} values for ^{13}C-ELA, ^{13}C-LAS and ^{13}C-arginine

	^{13}C-ELA		^{13}C-LAS		^{13}C-arginine	
	Male 1	Male 2	Male 1	Male 2	Male 1	Male 2
C_{max} (ng/mL)	4.80	44.0	154	140	428	680
t_{max} (min)	30	15	120	120	60	60

The approximately 9-fold difference in C_{max} for ^{13}C-ELA observed in the two subjects may be due to a difference in the fed state of the subjects.

ELA appeared to be well tolerated except for a burning sensation on administration reported by both subjects and nausea in one subject. It was stated that the burning sensation, and possibly nausea, may have been due to the use of propylene glycol (15 mg/kg bw) as the solvent.

CentraLabS (2005b) **Study title**: LAE an open-label, single-dose study to determine the plasma levels of LAE and its breakdown products after a single oral dose to healthy male volunteers. **Report no.**: LMA 049/034017 **Report date**: 12 Jan 2005 **Laboratory**: CentraLabS Clinical Research Ltd., Alconbury, Cambridgeshire, England. (Clinical phase conducted at PPD Development Clinic, Leicester, UK) **GLP**: Yes (OECD), for analytical phase of study **GCP**: Yes

Approximately 15 min after the completion of a "standard" breakfast, six healthy male volunteers each received an oral dose of ^{13}C-ELA at dose levels of 2.5 mg/kg bw (subjects 1 and 2) or 1.5 mg/kg bw (subjects 3 to 6). Respective doses of propylene glycol vehicle were 7.5 and 4.5 mg/kg bw. Doses were made up to a volume of 1 mL/kg bw with purified water. Subjects swallowed the solution and the interior of the individual dosing vessel was rinsed twice with 50 mL purified water. Subjects swallowed each rinse. Blood samples were taken pre-dose and at 5, 10, 15 and 30 min, and 1, 2, 4, 8, 12 and 24 h after dosing. Standard meals were provided at 4 h and 10 h post dose and water was available *ad libitum* throughout the study. Plasma concentrations of ^{13}C-ELA, ^{13}C-LAS and ^{13}C-arginine were measured by a validated LC-MS/MS method with a lower limit of quantification of 1 ng/mL for ethyl lauroyl arginate and LAS and 20 ng/mL for arginine.

Due to rapid metabolism no meaningful pharmacokinetic data for ethyl lauroyl arginate were obtained. Plasma concentrations of ^{13}C-ELA were below the limit of quantification at all sampling times in all subjects, with the exception of subject 2 for whom quantifiable concentrations of ^{13}C-ELA were found in two samples. Mean pharmacokinetic parameters for the metabolites ^{13}C-LAS and ^{13}C-arginine are shown in the table below. AUC values for both LAS and arginine increased with dose.

Table 2.5: Mean pharmacokinetic parameters for ^{13}C-LAS and ^{13}C-arginine

	^{13}C-LAS		^{13}C-arginine	
Dose (mg/kg bw)	1.5	2.5	1.5	2.5
C_{max} (ng/mL)	18.2	23.9	124	240
t_{max} (h)	2 [a]	1.5 [a]	0.75 [a]	1.25 [a]
$AUC_{0-\tau}$ (ng.h/mL) [b]	90.6	118	383	764
τ (h) [b]	12	8	4	8
$AUC_{0-\infty}$ (ng.h/mL)	96.4	128	556	864
$t_{½}$ (h)	2.5	2.4	2.4	2.4

[a] Median
[b] Time intervals for these AUC values varied depending on the time of the last quantifiable sample.

No serious adverse events were reported during the study and no subject withdrew because of an adverse event. Three adverse events were reported by two subjects (one subject at each dose level): headache after the 2.5 mg/kg bw dose, and diarrhoea and flatulence 30 h after the 1.5 mg/kg bw dose. These adverse events were of mild severity and considered unlikely to be related to treatment. There were no clinically significant abnormalities in any of the laboratory data (clinical chemistry, haematology and urinalysis), no notable changes in vital signs during the study, and no clinically significant ECG findings.

Metabolism

In vitro

HLS (2003a) **Study title**: Nα-Lauroyl-L-arginine ethyl ester monohydrochloride *in vitro* stability. **Report no.**: LMA 043/032898 **Report date**: 29 July 2003 **Laboratory**: Huntingdon Life Sciences Ltd, Huntingdon, Cambridgeshire, England **GLP**: Yes (OECD)

This study investigated the stability of ethyl lauroyl arginate in simulated gastric fluid (pH 0.95), simulated intestinal fluids (at pH 6.8 and 7.5), human plasma, and in a preparation of human hepatocytes. Ethyl lauroyl arginate, radiolabelled with ^{14}C at the arginine carbons, was used at concentrations of 0.25 mg/mL (gastric and intestinal fluids) or 10 µg/mL (plasma and hepatocytes). Fluids were incubated at 37º C for 2 h (simulated gastric fluid with and without porcine pepsin) or 4 h (all other incubations). For incubations with simulated gastric

fluid, samples were taken for HPLC analysis at the following time-points (min): 0, 1, 5, 15, 30, 60, 120. An additional sample was taken at 4 h for incubations with simulated intestinal fluid. Sampling time-points for incubations with human plasma were 0, 1, 2 and 4 h, and with human hepatocytes were 0, 0.25, 0.5 and 3 h.

In simulated gastric fluid, with and without porcine pepsin, ethyl lauroyl arginate was stable over the 2 h period.

In simulated intestinal fluids (at both pH 6.8 and pH 7.5, and with porcine pancreatin), ethyl lauroyl arginate was immediately degraded to LAS (95 – 98% of total radioactivity immediately after mixing) and then to arginine (90 – 93% of total radioactivity at 60 min). This degradation was enzyme-mediated: in simulated intestinal fluids without porcine pancreatin, ethyl lauroyl arginate was stable at pH 7.5 (over 4 h), while at pH 6.8 degradation to LAS was not detectable until the 60 min sampling time and reached only 19% of total radioactivity by 4 h.

ELA was degraded to LAS (but not to arginine) by human plasma (40 – 50 % of total radioactivity at 4 h) and human hepatocytes (77 – 85 % of total radioactivity at 3 h).

HLS (2001d) **Study title**: N-α-Lauroyl-L-arginine ethyl ester monohydrochloride *in vivo* and *in vitro* metabolism in the rat. **Report no.**: LMA 033/012117 **Report date**: 8 May 2001 **Laboratory**: Huntingdon Life Sciences Ltd, Huntingdon, Cambridgeshire, England **GLP**: Yes (OECD)

In both the *in vitro* and *in vivo* parts of this study, ethyl lauroyl arginate was labelled with ^{14}C at the arginine carbon atoms.

In vitro: The S9 fraction from the liver of an untreated Sprague-Dawley rat was incubated with ^{14}C-ethyl lauroyl arginate for up to 24 h at 37° C. Unchanged ethyl lauroyl arginate, N^{α}-lauroyl-L-arginine (LAS), arginine ethyl ester, arginine, ornithine and urea were identified in the S9 treated samples as shown in the table below. Analysis was conducted by TLC, HPLC and LC-MS. Ornithine, an endogenous human amino acid, was the major metabolite. In a control incubation in the absence of S9 liver fraction no significant degradation of ethyl lauroyl arginate was observed.

Table 2.6: Radioactive components *in vitro* as a percentage of added radioactivity

Radioactive component	**Time (hours after start of incubation)**		
(% of administered radioactivity)	4	6	24
Ornithine / arginine	25.0 [a]	28.3	29.3
M3 [b]	1.8	2.1	2.9
M4 [b]	2.0	3.0	} 9.8
Arginine ethyl ester	2.7	1.5	
LAS	3.4	2.9	1.8
ELA	46.7	40.1	25.0
Urea	3.8	5.4	7.8
Others	2.0	2.3	4.1
Not extracted	9.2	14.4	12.6
Total	96.6	100	93.3

[a] By HPLC, ornithine and arginine were equivalent to 23.5% and 1.5%, respectively, of the added radioactivity at 4 h. The relative amounts of these amino acids were not quantified at 6 or 24 h.
[b] M3 co-chromatographed with a minor unknown impurity in the radiolabelled ^{14}C-arginine reference substance. M4 was also uncharacterised.

In vivo: Six male Sprague-Dawley rats (un-fasted) received ^{14}C-ELA (200 mg/kg bw) as a single oral (gavage) dose. The vehicle was 1% w/v methylcellulose in water. Pairs of rats were sacrificed and blood sampled at 0.5, 1 and 4 h after dosing. Concentrations of radioactivity in the plasma increased from 14.2 µg-equivalents ethyl lauroyl arginate/mL at 0.5 h to 118 µg-equivalents ethyl lauroyl arginate/mL at 4 h after dosing. Proportions of radioactive components in plasma for each rat are shown in the table below. Components were resolved using the same methods as in the above *in vitro* study.

Table 2.7: Radioactive components in plasma as a percentage of total radioactive residue[a]

Radioactive component (% of total radioactive residue[a])	Time (hours after administration)					
	0.5		1		4	
	Male 1	Male 2	Male 3	Male 4	Male 5	Male 6
Polar material (lowest retention time)[b]	17.2	13.0	13.2	21.5	7.4	7.3
Ornithine	8.5	6.8	6.9	2.6	1.5	1.6
Arginine	46.0	50.7	20.8	22.7	7.5	11.7
LAS	<1.9	<1.3	4.1	<1.2	<0.1	0.7
ELA	7.4	<1.3	11.1	4.0	0.5	<0.2
Others [c]	<1.9	<1.3	3.0	<1.2	0.6	0.7
Not extracted	20.3	22.8	37.9	48.9	73.1	72.8

[a] % of total radioactive residue was defined as the fraction of total radioactivity administered that was extracted from plasma. The increased with time in the fraction not extracted is consistent with the extensive degradation of arginine to smaller carbon-containing compounds units and incorporation into other biological components.
[b] Possibly urea.
[c] Arginine ethyl ester, which was observed *in vitro*, was not observed in rat plasma.

Excretion

HLS (1998g) **Study title**: N-α-lauroyl-L-arginine ethyl ester monohydrochloride metabolism in the rat. **Report no.**: LMA 017/983416 **Report date**: 26 August 1998 **Laboratory**: Huntingdon Life Sciences Ltd, Huntingdon, Cambridgeshire, England **GLP**: Yes (OECD)

Four male Sprague-Dawley rats (un-fasted) received ^{14}C-labelled ethyl lauroyl arginate (180 mg/kg bw) as a single oral (gavage) dose. Ethyl lauroyl arginate was labelled with ^{14}C at the arginine carbon atoms. The vehicle was 1% w/v methylcellulose in water. During the 5 days after dosing a mean of 36.6% of the dose was excreted as carbon dioxide in expired air, 11.8% in urine, 4.3% in faeces and 0.5% in the cage-wash. The HPLC radiochromatogram of urine showed a single peak which co-eluted with urea. Components in faeces were not analysed. A mean of 46.4% of the dose was retained in the carcass at sacrifice. The mean recovery of the administered radioactivity was 99.5%.

The proposed metabolic pathway, based on the results of the above metabolism and excretion studies, is shown below. The proposed degradation products lauric acid (a fatty acid found in various vegetable oils and in human milk) and ethanol were not identifiable in the submitted studies because they would not have contained a radiolabelled carbon. An unpublished review of the above metabolism and excretion studies was submitted by the applicant (Hawkins, 2005). The conclusions of this review are consistent with the results of the above studies and the proposed metabolic pathway.

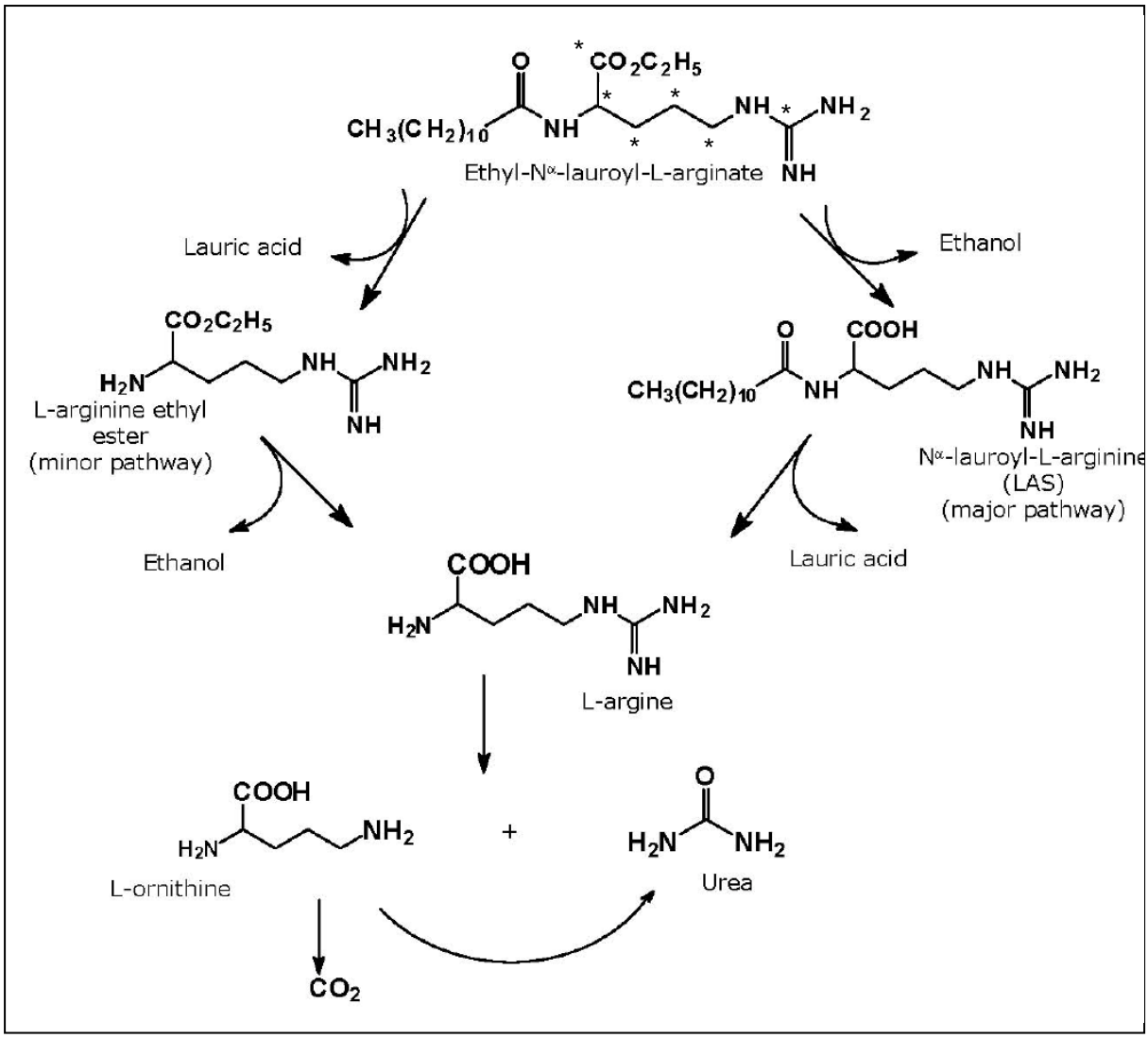

Figure 2.1: Proposed metabolic pathway for ethyl lauroyl arginate. The positions of the ^{13}C or ^{14}C labels in the studies with radiolabelled ethyl lauroyl arginate are indicated with asterisks.

Single-dose toxicity studies

Rats

Single dose toxicity studies in rats were conducted using Mirenat-N, ethyl lauroyl arginate and LAS as test articles.

(i) Ethyl lauroyl arginate

HLS (2000a) **Study title**: L.A.E. acute oral toxicity to the rat. **Report no.**: LMA 018/002881/AC **Report date**: 27 July 2000 **Laboratory**: Huntingdon Life Sciences Ltd, Huntingdon, Cambridgeshire, England **GLP**: Yes (OECD)

A group of six fasted Sprague-Dawley rats (3/sex) received a single oral gavage dose of ethyl lauroyl arginate (2000 mg/kg bw) formulated in 1% w/v aqueous methylcellulose.

Animals were observed for 15 days post-dose. There were no deaths. Treatment-related clinical signs consisted of piloerection and increased salivation which were both evident in all rats within 5 min of dosing. During the same period unsteady gait was observed in all females and hunched posture in all males. All clinical signs had resolved by day 3 or 4. All animals achieved satisfactory weight gain during the study period. No abnormalities were evident in any of the animals at necropsy on day 15.

(ii) LAS

Cidasal (2003a) **Study title**: Determination of acute toxicity in rats by oral route dose limit test. **Report no.**: CD02/8399T **Report date**: 31 January 2003 from **Laboratory**: Cidasal, Barcelona, Spain **GLP**: Yes (OECD)

The ethyl lauroyl arginate metabolite LAS was administered as a single dose to Sprague-Dawley rats (5/sex) by oral gavage at 2000 mg/kg bw. The observation period was 15 days followed by necropsy. There were no unscheduled deaths and no clinical signs of toxicity. Body weight gain was normal. No macroscopic alterations were observed at necropsy.

(iii) Mirenat-N

The formulation termed Mirenat-N is ethyl lauroyl arginate (20-25% w/w) dissolved in propylene glycol.

HRC (1995a) **Study title**: Mirenat-N acute oral toxicity to the rat. **Report no.**: LMA 4/951314/AC **Report date**: 17 July 1995 **Laboratory**: Huntingdon Research Centre Ltd, Huntingdon, Cambridgeshire, England. **GLP**: Yes (OECD)

A group of 10 fasted Sprague-Dawley rats (5/sex) received a single oral gavage dose of Mirenat-N (2000 mg/kg bw equivalent to 500 mg/kg bw ethyl lauroyl arginate). Animals were observed for 15 days post-dose. There were no deaths. Treatment-related clinical signs were confined to piloerection which was observed in all rats and resolved by day 2. All animals achieved satisfactory weight gain during the study period. No abnormalities were evident in any of the animals at necropsy on day 15.

Rabbits

RTC (2000) **Study title**: LAE Acute dermal irritation study in the rabbit **Report no.**: 7978/T/171/2000 **Report date**: 15 Dec 2000 **Laboratory**: Research Toxicology Centre, Rome, Italy **GLP**: Yes (OECD)

ELA (0.5 g + 0.5 mL water) was applied as a paste to the clipped dorsal skin of 3 female New Zealand White rabbits. The paste was spread out over a 2.5 x 2.5 cm^2 gauze square and applied to the skin as semi-occlusive barrier. After a period of 4 h, the patches were removed and the treated sites cleaned with cotton wool soaked in water. Reaction to treatment was assessed at approximately 1, 24, 48 and 72 h, and 7 and 14 days after the end of the exposure period. Slight to well-defined erythema was observed in all 3 treated animals approximately 1 h after the end of the 4 h exposure period. A slight erythema was still present 7 days after the end of the exposure period in 2 rabbits, with a slight oedema also present in one of these 2 animals. One of the 3 rabbits exhibited a slight erythema at the day 15 examination. Desquamation of the treated skin was also noted at 7 and 14 days after the end of the exposure. There was no indication of a systemic effect of treatment. No significant changes in body weight occurred during the course of the study.

Repeat dose toxicity studies

Repeat dose toxicity studies were conducted in two rat strains: Sprague-Dawley and Han Wistar. The test articles (i) ethyl lauroyl arginate and (ii) Mirenat-N (25% w/w solution of ethyl lauroyl arginate in propylene glycol) were administered via diet.
(i) ethyl lauroyl arginate.

HLS (2000b) **Study title**: LAE dose range finding/palatability study by dietary administration to Han Wistar rats for 4 weeks. **Report no.**: LMA 030/000063 **Report date**: 17 July 2000 **Laboratory**: Huntingdon Life Sciences Ltd, Huntingdon, Cambridgeshire, England **GLP**: No

Groups of Han Wistar rats (5/sex/group) received ethyl lauroyl arginate (90.1% w/w active ingredient) in the diet at concentrations of 0, 25000, 37500 or 50000 ppm for 4 weeks. The estimated intakes of ethyl lauroyl arginate were not presented in the report.

There were no deaths. Piloerection was observed in all females given 50000 ppm. Ungroomed coats were observed in two females receiving 37500 and all females receiving 50000 ppm. Salivation was observed in all treated females and in most males receiving 50000 ppm. Brown staining of the muzzle, probably dried saliva, was evident for most animals in each treated group. Body weight gain and food consumption were reduced in a dose-dependent manner in all treated animals during week 1. During weeks 2-4, body weight gain was similar to controls while food consumption remained low for treated animals. Reduced food consumption and body weight gain may be attributable to reduced palatability of the diet. Females receiving 50000 ppm had slightly elevated haemoglobin parameters. There were no other notable haematology findings. Clinical chemistry findings indicated slight effects on the liver as indicated by low total protein, albumin and calcium concentrations in males receiving 37500 and 50000 ppm. Females receiving 50000 ppm had high alkaline phosphatase, alanine aminotransferase, and aspartate aminotransferase. Females receiving 37500 ppm had slightly higher aspartate aminotransferase and alanine aminotransferase levels compared to controls.

There were no treatment-related findings for organ weights or gross pathology. Histopathology was not investigated. A maximum diet level of 50000 ppm ethyl lauroyl arginate was considered acceptable for a subsequent 13-week study.

HLS (2001c) **Study title**: LAE toxicity study by dietary administration to Han Wistar rats for 13 weeks. **Report no.**: LMA 031/004276 **Report date**: 28 March 2001 **Laboratory**: Huntingdon Life Sciences Ltd, Huntingdon, Cambridgeshire, England **GLP**: Yes (OECD)

Groups of Han Wistar rats (20/sex/group) received ethyl lauroyl arginate (batches contained 90.1-93.2% w/w active ingredient) in the diet at concentrations of 0, 5000, 15000 or 50000 ppm for 13 weeks. The control group received normal untreated diet. These diet levels resulted in ethyl lauroyl arginate intakes (calculated from weekly food consumption) of 0, 384, 1143 and 3715 mg/kg bw/day for males; and 0, 445, 1286 and 3915 mg/kg bw/day for females. Acceptable stability and homogeneity of ethyl lauroyl arginate in the 5000 ppm and 50000 ppm diets was confirmed by analysis.

There were no deaths. Evidence of mild toxicity was observed at 15000 and 50000 ppm with adverse effects on appearance (ungroomed coat, brown staining on the muzzle), body weight gain, food consumption, urinalysis, clinical chemistry and haematology parameters. Findings at 5000 ppm were restricted to slightly lower body weight gain and food consumption for males only during the first week of treatment. These changes were considered to be due to reduced palatability of the diet and not a toxic effect of treatment.

Functional observational battery tests gave no indication of neurotoxicity. There were no treatment-related ophthalmic findings. Urinalysis revealed a low pH for males receiving 15000 or 50000 ppm relative to the control group ($p < 0.01$ for both concentrations).

Clinical chemistry findings consisted of decreased total protein for animals receiving 50000 ppm, slightly decreased albumin for animals receiving 50000 ppm and females receiving 15000 ppm, and slightly decreased cholesterol for females receiving 50000 ppm (see Table below).

Table 2.8: Clinical chemistry findings

Parameter	Males				Females			
	Group 1	*Group 2*	*Group 3*	*Group 4*	*Group 1*	*Group 2*	*Group 3*	*Group 4*
Total protein (g/L)	65	65	64	63*	70	67	67	64**
Albumin (g/L)	37	37	37	36**	41	40	39*	38**
Cholesterol (mmol/L)	1.58	1.71	1.35	1.65	1.86	1.64	1.55	1.49*

Groups 1 to 4 refer to the 0, 5000, 15000 and 50000 mg/kg diet groups, respectively.
* $p \leq 0.05$; ** $p \leq 0.01$ (relative to control group).

Haematology findings consisted of slightly increased mean cell haemoglobin, mean cell haemoglobin concentration, and mean cell volume; and slightly decreased white blood cell and lymphocyte counts for males receiving 50000 ppm (females were unaffected). See table below.

Table 2.9: Haematology findings

Parameter	Males				Females			
	Group 1	*Group 2*	*Group 3*	*Group 4*	*Group 1*	*Group 2*	*Group 3*	*Group 4*
Mean cell haemoglobin (pg)	17.5	17.7	17.4	18.3**	18.8	18.7	18.5	18.7
Mean cell haemoglobin conc. (g/dL)	35.0	35.1	35.0	35.4*	35.2	35.4	35.6	35.3
Mean cell volume (fL)	50.1	50.3	49.7	51.7*	53.4	52.9	52.0	52.8
White blood cell counts (x 10^9/L)	6.97	6.87	7.15	5.15*	4.00	4.04	3.51	3.24
Lymphocyte counts (x 10^9/L)	5.31	5.24	5.22	3.82*	2.96	3.07	2.30	2.51

Groups 1 to 4 refer to the 0, 5000, 15000 and 50000 mg/kg diet groups, respectively.
* $p \leq 0.05$; ** $p \leq 0.01$ (relative to control group).

There were no adverse gross pathology or organ weight findings.

Histopathological findings considered to be related to treatment were restricted to the forestomach of rats receiving 15000 or 50000 ppm. These findings, tabulated below, comprised parakeratosis, ulceration, erosions, and epithelial hyperplasia.

Table 2.10: Histopathology findings for the forestomach (non-glandular region of the stomach)

Incidence of histopathology findings [a]	Males				Females			
	Group 1	*Group 2*	*Group 3*	*Group 4*	*Group 1*	*Group 2*	*Group 3*	*Group 4*
No. of animals examined	20	20	20	20	20	20	20	20
No. of animals with:								
Parakeratosis - *minimal*	0	0	0	2	0	0	1	2
- slight	0	0	0	9	0	0	0	7
- moderate	0	0	0	2	0	0	0	4
- total	0	0	0	13**	0	0	1	13**
Erosion, non-glandular region - *minimal*	0	0	0	0	0	0	0	3
Ulceration, non-glandular region - *minimal*	0	0	0	1	0	0	0	1
- slight	0	0	1	0	0	0	0	1
- total	0	0	1	1	0	0	0	2
Epithelial hyperplasia, non-glandular region - *slight*	0	0	0	0	0	0	0	1

Groups 1 to 4 refer to the 0, 5000, 15000 and 50000 mg/kg diet groups, respectively.
** $p \leq 0.01$ (relative to control group).
[a] Histopathology findings were graded in order of increasing severity as follows: minimal, slight, moderate, marked, severe.

Because of the known surfactant activity of ethyl lauroyl arginate, it is likely that these forestomach findings are due to a direct effect on epithelial cells and are not attributable to systemic toxicity.

Because of the clinical chemistry, haematology and forestomach findings at the high dietary level of 50000 ppm, the NOAEL was considered to be 15000 ppm which corresponds to an ethyl lauroyl arginate dose of 1143 mg/kg bw/day in males and 1286 mg/kg bw/day in females. However, despite the low incidence (1/20) of some forestomach findings at 15000 ppm, it is possible that these findings are treatment related and that this dietary concentration may approximate the threshold for the onset of adverse effects on the forestomach.

HLS (2005a) **Study title**: Lauric arginate toxicity study by dietary administration to CD rats for 52 weeks. **Report no.**: LMA 050/042556 **Report date**: 25 November 2005 **Laboratory**: Huntingdon Life Sciences Ltd, Huntingdon, Cambridgeshire, England. **GLP**: Yes (OECD)

Groups of Crl:CD(SD)IGS BR rats (20/sex/group) received diets containing ethyl lauroyl arginate (88.2% w/w active ingredient) at concentrations of 0, 2000, 6000 or 18000 mg/kg diet for 52 weeks (referred to as groups 1 to 4 in tables below). The control group received normal untreated diet. Acceptable stability of ethyl lauroyl arginate in samples from all treatment diets was confirmed by analysis. Homogeneity was analysed only for the 2000 mg/kg diet and shown to be acceptable. The diet levels resulted in ethyl lauroyl arginate doses of 0, 106, 307 and 907 mg/kg bw/day for males; and 0, 131, 393 and 1128 mg/kg bw/day for females. Clinical signs, body weight, and food and water consumption were recorded during the treatment period. During weeks 14, 26 and 52, haematological, clinical chemistry, urinalysis and ophthalmology were performed. During week 49, neurobehavioural screening (sensory reactivity, grip strength and motor activity) was performed on 10 males and 10 females from each group.

Gross pathology, organ weights, histopathology, bone marrow smears, and toxicokinetics were investigated at the end of the 52-week dosing period.

There were six unscheduled deaths during the study, one control male, 3 low dose animals and one high dose female. None of the deaths were considered to be attributable to treatment. There were no clinical signs of toxicity at 2000 ppm. At 6000 ppm, females exhibited increased incidences of brown fur staining in the period of week 1 to 13. At 18000 ppm, females exhibited increased incidences of brown fur staining in the period of weeks 1 to 13 and increased incidences of ungroomed coat during weeks 4 to 12.

Body weight gain was unaffected at 2000 ppm but was reduced in both sexes at 6000 and 18000 ppm, most notably in the early weeks of the study. Decreased food consumption was evident in the 6000 and 18000 ppm groups in the first week of the study. These effects are likely to be due to reduced palatability of the diet and not toxicologically relevant.

Haematology findings: there were treatment-related effects on white cell parameters for both sexes as shown in the table below. However, in the absence of any treatment-related effects on the bone marrow and the lack of any histopathology associated with the lymphoid tissues, the white cell changes were not considered to be of toxicological importance.

Table 2.11: White blood cell counts

Cell count (x 10^9/L)	Week	Males				Females			
		Group 1 [a]	*Group 2*	*Group 3*	*Group 4*	*Group 1*	*Group 2*	*Group 3*	*Group 4*
White blood cells	14	11.99	11.24	11.09	10.19	7.74	8.14	7.31	7.13
	26	12.24	9.49*	10.49*	9.09**	7.15	6.49	5.12*	4.67**
	52	11.48	9.10	10.39	8.72**	6.87	6.85	6.92	5.13
Neutrophils	14	1.45	1.45	1.28	1.46	0.93	0.90	0.82	0.59*
	26	1.68	1.56	1.71	1.39	0.96	0.92	1.16	0.59*
	52	2.38	2.19	1.97	1.23**	1.85	1.34	2.07	0.87*
Lymphocytes	14	9.80	9.11	9.15	8.15	6.24	6.82	6.03	6.17
	26	9.49	7.16*	7.94*	7.00**	5.59	5.17	3.60**	3.77**
	52	7.89	6.07	7.37	6.68	4.23	4.90	4.17	3.75
Basophils	14	0.04	0.03	0.04	0.04	0.02	0.02	0.02	0.02
	26	0.04	0.03	0.04	0.03	0.02	0.01	0.01	0.01
	52	0.04	0.02**	0.03**	0.02**	0.01	0.02	0.01	0.01
Monocytes	14	0.31	0.27	0.24*	0.23*	0.24	0.16*	0.18*	0.14**
	26	0.37	0.29	0.30	0.26*	0.24	0.15**	0.14**	0.12**
	52	0.58	0.41*	0.49*	0.38**	0.43	0.29	0.37	0.24**
Large unstained cells	14	0.29	0.26	0.27	0.19**	0.20	0.13*	0.15*	0.11**
	26	0.50	0.31**	0.37**	0.27**	0.23	0.13**	0.10**	0.11**
	52	0.43	0.28*	0.33*	0.25**	0.23	0.16	0.17	0.15*

[a] Groups 1 to 4 refer to the 0, 2000, 6000 and 18000 mg/kg diet groups, respectively.
* $p < 0.05$; ** $p < 0.01$ (relative to control group).

Clinical chemistry findings were limited to increased urea concentration (by 26% over control group, $p < 0.05$) observed at week 52 in females receiving 18000 ppm. Urinalysis findings were limited to decreased urine volume at week 12 in males receiving 18000 ppm (by 38%, $p < 0.01$) and at week 52 in females receiving 18000 ppm (by 49%, $p < 0.01$).

Gross pathology and histopathology findings were considered to be treatment-related only for the forestomach as shown in the table below. The severity of these findings was described as minimal or slight.

Table 2.12: Gross pathology and histopathology findings for the forestomach (non-glandular region of the stomach)

Incidence of gross pathology and histopathology findings	Males				Females			
	Group 1	*Group 2*	*Group 3*	*Group 4*	*Group 1*	*Group 2*	*Group 3*	*Group 4*
Gross pathology [a] *No. examined*	19	18	20	19	20	19	20	19
No. of animals with:								
Forestomach depression(s)	0	1	5	12	2	5	6	9
Histopathology [b] *No. examined*	20	20	20	20	20	20	20	20
No. of animals with:								
Epithelial hyperplasia, non-glandular- *minimal*	0	1	2	1	0	0	0	1
- *slight*	0	0	1	8	1	3	5	7
- *total*	0	1	3	9**	1	3	5	8*
Sub-epithelial/mucosal inflammation, non-glandular - *minimal*	0	1	5	5	1	4	3	7
- *slight*	0	0	0	1	1	1	2	1
- *total*	0	1	5	6	2	5	5	8
Sub-epithelial fibrosis, non-glandular - *minimal*	0	0	0	3	0	0	0	0
Muscle layer inflammation- *minimal*	0	0	1	1	1	3	3	4
Serosal inflammation - *minimal*	0	0	0	0	0	4	2	1
- *slight*	0	0	0	0	0	0	0	1
- total	0	0	0	0	0	4	2	2
Erosion, non-glandular epithelium - *slight*	0	0	0	0	0	0	0	1
Ulceration, non-glandular epithelium - *minimal*	0	0	1	0	0	1	0	3
- *slight*	0	0	2	0	1	2	3	3
- *total*	0	0	3	0	1	3	3	6
Re-epithelialisation, non-glandular - *minimal*	0	0	0	3	0	1	1	3
- *slight*	0	0	1	0	1	2	1	2
- *total*	0	0	1	3	1	3	2	5

Groups 1 to 4 refer to the 0, 2000, 6000 and 18000 mg/kg diet groups, respectively.
* $p < 0.05$; ** $p < 0.01$ (relative to control group).
[a] Statistical analysis was not conducted on the gross pathology findings.
[b] Histopathology findings were graded in order of increasing severity as follows: minimal, slight, moderate, marked, severe.

There was no correlation between the individual animals which showed lower body weight gain, poor grooming and/or brown fur staining and the presence of these forestomach findings. Nor was there any correlation between the animals which showed forestomach lesions and those which exhibited white blood cell and/or biochemical disturbances.

Because ethyl lauroyl arginate is a cationic surfactant which affects the integrity of cell membranes it is likely that these forestomach findings are due to a direct effect of ethyl lauroyl arginate on epithelial cells and are not attributable to systemic toxicity.

There were no treatment-related effects on neurobehavioural parameters, ophthalmology, bone marrow smears, and organ weights.

Blood samples were taken over a 24 h period during week 52 in order to assess systemic exposure to ethyl lauroyl arginate and the metabolite LAS. Blood samples were taken from 3 satellite rats per sex from each dose group at 6:00 pm, 10:00 pm, 2:00 am, 6:00 am, 10:00 am and 2:00 pm and analysed using a validated LC-MS/MS method. Toxicokinetics results shown in the table below indicate that exposure of males to ethyl lauroyl arginate, as indicated by maximum plasma concentrations, was approximately proportional to dietary concentration. Females exhibited greater exposure than males to ethyl lauroyl arginate at all concentrations. The increase in exposure with dietary concentration was less than proportional for both sexes. Exposure to the metabolite LAS was greater than exposure to ethyl lauroyl arginate for both sexes. Maximum plasma concentrations of ethyl lauroyl arginate and LAS usually occurred during the hours of darkness (corresponding to periods of nocturnal feeding activity).

Table 2.13: C_{max} and $AUC_{0\text{-}24\ h}$ values for ethyl lauroyl arginate and LAS

Dietary concentration (ppm)	ELA				LAS			
	C_{max} (ng/mL)		$AUC_{0\text{-}24\ h}$ (ng.h/mL)		C_{max} (ng/mL)		$AUC_{0\text{-}24\ h}$ (ng.h/mL)	
	males	*females*	*males*	*females*	*males*	*females*	*males*	*females*
2000	1.15	11.3	19.5	78.3	10.5	12.7	46.8	169
6000	6.92	22.9	66.8	130	18.7	26.4	286	368
18000	17.6	26.3	190	244	62.2	59.6	960	1130

In conclusion, the NOAEL was considered to be 6000 ppm corresponding to estimated ethyl lauroyl arginate intakes of 307 mg/kg bw/day for males and 393 mg/kg bw/day for females. This NOAEL was based on the local irritant changes in the forestomach at the high dietary concentration of 18000 ppm. The forestomach findings at 6000 ppm were not considered to be of sufficient severity or incidence to be regarded as toxicologically adverse.

(i) Mirenat-N

HLS (1995) **Study title**: Mirenat-N preliminary toxicity to rats by dietary administration for 4 weeks **Report no.**: LMA 2/952124 **Report date**: 14 December 1995 **Laboratory**: Huntingdon Life Sciences Ltd, Huntingdon, Cambridgeshire, England **GLP**: Yes (OECD)

Groups of Crl:CD(SD)BR rats (5/sex/group) received diets containing Mirenat-N at concentrations of 0, 3200, 12800 or 50000 mg Mirenat-N/kg diet for 4 weeks. These diet levels resulted in ethyl lauroyl arginate intakes (calculated from weekly food consumption) of 0, 84, 348 and 1317 mg/kg bw/day for males; and 0, 88, 350 and 1462 mg/kg bw/day for females. Control animals received normal untreated diet. Clinical signs, body weight, and food and water consumption were recorded during the treatment period. During week 4, haematological and clinical chemistry analyses were performed. Gross pathology and organ weights were also investigated.

There were no deaths or treatment-related findings at any dose level. It was concluded that a maximum level of 50000 mg Mirenat-N/kg diet was acceptable for a subsequent 13-week study.

HLS (1996) **Study title**: Mirenat-N toxicity to rats by dietary administration for 13 weeks. **Report no.**: LMA 3/961342. **Report date**: 4 November 1996 **Laboratory**: Huntingdon Life Sciences Ltd, Huntingdon, Cambridgeshire, England. **GLP**: Yes (OECD, FDA).

Groups of Crl:CD(SD)BR rats (10/sex/group) received diets containing Mirenat-N at concentrations of 0, 3200, 12800 or 50000 mg Mirenat-N/kg diet for 13 weeks. These diet levels resulted in ethyl lauroyl arginate intakes (calculated from weekly food consumption) of 0, 55, 226 and 831 mg/kg bw/day for males; and 0, 66, 267 and 982 mg/kg bw/day for females. Control animals received normal untreated diet. Clinical signs, body weight, and food and water consumption were recorded during the treatment period. During week 13, haematological, clinical chemistry, urinalysis and ophthalmology were performed. Gross pathology, organ weights and histopathology were also investigated.

One control male and one control female died during the treatment period. The male collapsed and died during week 1 on being returned to the cage following the daily clinical examination. No clinical signs were noted during its lifetime. Post mortem examination revealed a ruptured liver, probably incurred during handling, as the likely cause of death. The female died under anaesthesia during laboratory investigations. “Anaesthetic trauma” was listed as the possible cause of death.

There were no treatment-related clinical signs observed during the study. Body weight gain for treated females was lower than the controls (88, 79 and 86% that of the controls for the three doses, respectively). The lower body weight gain was statistically significant ($p \leq 0.01$) at the mid (12800 ppm) and high (50000 ppm) dietary levels. In the absence of a dose-response relationship and any effect in the males, the lower body weight gain in females is of uncertain relationship to treatment. Food intake was unaffected by treatment for males and females. Males receiving 50000 mg/kg diet had a slightly higher water consumption (117% of the control value); however this was not statistically significant. There were no treatment-related ophthalmology findings.

As shown in the table below, there was a slightly lower total white blood cell count in females receiving 12800 and 50000 mg/kg diet, predominantly due to reduced counts for lymphocytes, monocytes and large unstained cells. In males, there was a slightly lower neutrophil count at 12800 and 50000 mg/kg diet. Because there was no consistency in the type of white blood cell contributing to the lower total cell count these findings are unlikely to be toxicologically significant. There were no changes in the other haematological parameters investigated.

Table 2.14: White blood cell counts

Cell count (x 10^9/L)	Males				Females			
	Group 1	*Group 2*	*Group 3*	*Group 4*	*Group 1*	*Group 2*	*Group 3*	*Group 4*
White blood cells	14.58	14.03	13.54	12.65	8.65	8.01	6.71*	6.25**
Neutrophils	1.79	1.52	1.30**	1.29**	0.97	0.92	0.68	0.92
Lymphocytes	11.86	11.69	11.41	10.34	7.06	6.63	5.68*	4.98**
Eosinophils	0.17	0.13	0.16	0.18	0.16	0.12	0.09	0.12*
Basophils	0.04	0.03	0.03	0.03	0.01	0.02	0.01	0.01
Monocytes	0.22	0.20	0.22	0.34	0.16	0.13	0.09**	0.08**
Large unstained cells	0.50	0.46	0.42	0.47	0.28	0.20**	0.16**	0.13**

Groups 1 to 4 refer to the 0, 3200, 12800 and 50000 mg/kg diet groups, respectively.
* $p \leq 0.05$; ** $p \leq 0.01$ (relative to control group).

There were no treatment-related clinical chemistry changes. Slightly higher urine volume (121% of the control value, but not statistically significant) was observed in males receiving 50000 mg/kg diet. This is consistent with the increased water intake recorded for this group. In contrast, decreased urine volume was observed in a repeat-dose study in rats with ethyl lauroyl arginate as the test article [HLS (2005a), above]. The slightly higher water intake and urine volume observed in the present study may be related to the high concentration of propylene glycol in the Mirenat-N test article.

Females receiving 50000 mg/kg diet had a slightly higher group mean liver weight (body weight relative, 112% of control value, $p \leq 0.05$). However, no microscopic changes were detected in the liver and there were no associated clinical chemistry findings. The slightly increased mean relative liver weight is therefore not considered to be an adverse effect.

At necropsy, a high incidence (50 and 80%, respectively) of alopecia was observed in females at 12800 and 50000 mg/kg diet. In isolation this change is considered to be of uncertain biological significance. No other treatment-related changes were observed macroscopically or microscopically.

The NOAEL for Mirenat-N was concluded in the study report to be 12800 mg/kg diet (equal to 226 mg ethyl lauroyl arginate/kg bw/day) based on evidence of slight changes in males and females at 50000 mg/kg diet, when compared with controls (lower body weight gain of females, increased water consumption and urine volume of males, and higher group mean adjusted liver weights of females). However, as discussed above, all of these findings are unlikely to be toxicologically relevant, especially since animals in the control group received normal untreated diet. Comparisons between groups receiving Mirenat-N and the control group would be more robust if control animals had received propylene glycol vehicle in the diet.

In the absence of any adverse effects, a NOAEL of 50000 mg/kg diet (equivalent to 831 and 982 mg ethyl lauroyl arginate/kg bw/day for males and females, respectively) is considered appropriate.

Genotoxicity studies

Ethyl lauroyl arginate was tested in several *in vitro* genotoxicity assays and the major metabolite LAS was tested in an *in vitro* and an *in vivo* assay as summarised in the table below. These studies were conducted in compliance with GLP (OECD). The *in vitro* assays were performed both in the presence and absence of liver preparations from Aroclor 1254-induced rats (S9 mix, as indicated by ±S9 in the table). An appropriate high dose was tested in the *in vivo* study.

In the preliminary cytotoxicity tests for the *in vitro* assays, ethyl lauroyl arginate was cytotoxic at relatively low concentrations consistent with the cell membrane disrupting activity of the compound. The main tests in these assays used appropriate lower concentrations as shown in the table below. These *in vitro* concentrations of ethyl lauroyl arginate, while lower than those typically recommended for these assays, are far greater than the ethyl lauroyl arginate concentrations observed systemically in *in vivo* studies.

ELA and LAS showed no evidence of mutagenic or clastogenic activity in these assays. Some evidence of polyploidy was observed at cytotoxic concentrations in study HLS (2001b), but this is unlikely to be of biological significance. Negative and positive controls were used in all studies and gave expected results.

Table 2.15: Genotoxicity assays

Test type	Test system	Test article	Mirenat-N, ELA, LAS concentrations /dosages	Result	Reference
Bacterial reverse mutation	*S. typhimurium* TA 1535, TA 1537, TA 98, TA 100 *E. coli* WP2 uvrA pKM 101 (±S9)	ELA (93.2% w/w active ingredient) dissolved in DMSO	5 – 5000 µg/plate (preliminary) then 0.15 – 150 µg/plate (plate incorporation) and 1.5 – 150 µg/plate (pre-incubation)	Negative See footnote[a] regarding cytotoxicity	HLS (2001a)
Mammalian cell mutation	Mouse lymphoma L5178Y cells (±S9). 3 h (±S9) and 24 h (-S9) treatment.	ELA (88.2% w/w active ingredient) dissolved in DMSO	0.20 – 600 µg/mL (prelim. toxicity test) then 1 – 50 µg/mL	Negative. See footnote[b] regarding cytotoxicity	HLS (2004)
Chromosome aberration	Human lymphocytes *in vitro* (±S9). Test (i): 3 h treatment, 17 h recovery; (ii) 20 h treatment.	ELA (93.2% w/w active ingredient) dissolved in DMSO	50 – 200 µg/mL	Negative. Some evidence of polyploidy at cytotoxic concs (See footnote[c])	HLS (2001b)
Bacterial reverse mutation	*S. typhimurium* TA 1535, TA 1537, TA 98, TA 100 (±S9)	Mirenat-N (ELA 25% w/w in propylene glycol) dissolved in water	5 – 5000 µg/plate (preliminary) then 5 – 500 µg/plate (pre-incubation)	Negative See footnote[d] regarding cytotoxicity	HRC (1995b)

(cont.)

Test type	Test system	Test article	Mirenat-N, ELA, LAS concentrations /dosages	Result	Reference
Mammalian cell mutation	Mouse lymphoma L5178Y cells (±S9). 3 h (±S9) and 24 h (-S9) treatment.	Mirenat-N dissolved in water	15 – 2000 µg/mL (prelim. toxicity test) then 100 – 300 µg/mL (-S9) 100 – 500 µg/mL (+S9)	Negative. See footnote[e] regarding cytotoxicity	HRC (1995c)
Chromosome aberration	Human lymphocytes *in vitro* (±S9). Test (i): 3 h treatment, 15 h recovery; (ii) 3 h treatment, 29 h recovery	Mirenat-N dissolved in water	125 – 1000 µg/mL	Negative. See footnote[f] regarding cytotoxicity	HRC (1995d)
Bacterial reverse mutation	*S. typhimurium* TA 1535, TA 1537, TA 98, TA 100 *E. coli* WP2 uvrA pKM 101 (±S9)	LAS (N^{α}-lauroyl-L-arginine) dissolved in DMSO	156 – 5000 µg/plate	Negative See footnote[g] regarding cytotoxicity	Cidasal (2003b)
Micronucleus formation	Mouse (CD-1) bone marrow (sampled 24 h and 48 h post-dose). N = 5 males/group.	LAS dissolved in water	2000 mg/kg bw, single oral gavage dose	Negative	Cidasal (2003c)

[a] The maximum tested concentration for bacterial reverse mutation assays usually extends up to 5000 µg/plate, but testable concentrations were limited because of the antibacterial properties of the compound. Cytotoxicity, observed as an absence or thinning of the bacterial lawn or a reduction in the number of revertants, was observed in all strains at ≥ 150 µg/plate. Therefore, a maximum concentration of 150 µg/plate was therefore selected for the subsequent tests.

[b] Relative suspension growth was negligible at ELA concentrations ≥ 50 µg/mL.

[c] In the absence/presence of S9, ELA (200 µg/mL) caused a reduction in the mitotic index to 31%/32% of the solvent control value. Mitotic index reduction of > 50% is usually considered to represent an appropriate level of cytotoxicity at the maximum concentration for this assay.

[d] Cytotoxicity was observed in all strains at Mirenat-N concentrations ≥ 500 µg/plate. A maximum concentration of 500 µg/plate was therefore selected for the subsequent tests.

[e] Relative suspension growth was negligible at Mirenat-N concentrations ≥ 500 µg/mL.

[f] In the absence/presence of S9, Mirenat-N (1000 µg/mL) caused a reduction in the mitotic index to 7%/18% of the solvent control value.

[g] For LAS, cytotoxicity was observed in the strains TA-1535, TA-1537 and TA-100 only at the maximum tested concentration of 5000 µg/plate.

Carcinogenicity studies

No carcinogenicity studies were submitted. This is considered acceptable because the compound did not exhibit genotoxicity and there was no evidence of treatment-related pre-neoplasia or neoplasia in the 52-week repeat-dose toxicity study in rats.

Reproductive toxicity studies

HLS (2003b) **Study title**: LAE preliminary study of effects on reproductive performance in CD rats by dietary administration. **Report no.**: LMA 041/032575 **Report date**: 3 July 2003 **Laboratory**: Huntingdon Life Sciences Ltd, Huntingdon, Cambridgeshire, England. **GLP**: Yes (OECD)

Sprague-Dawley rats (n = 8/sex/group, age 9-10 weeks at commencement of treatment) were fed diets containing ethyl lauroyl arginate (88.2% w/w active ingredient) at concentrations of 0, 1500, 5000 or 15000 ppm for 4 weeks prior to mating. Animals were terminated after weaning of the litters. Pups selected to form the F1 generation (n = 12/sex/group) were continuously treated from the time of weaning until terminated at approximately 8 weeks of age.

The general condition of animals receiving diets containing ethyl lauroyl arginate was similar to that of controls and no unscheduled deaths occurred. Food consumption and body weight gain of F0 males and females were not adversely affected by treatment and there were no adverse effects on body weight gain for females during gestation and lactation. Pup body weight at day 1 of again, body weight gain to weaning, and body weight of selected F1 males and females to 8 weeks of age, were unaffected by treatment. Food consumption by selected F1 animals was similar to that of controls. Calculated intakes were proportional to the dietary concentrations as shown in the table below.

Table 2.16: Average intakes for F0 animals before pairing and F1 animals

Dietary concentration (ppm)	1500	5000	15000
Average F0 intake before pairing (mg/kg bw/day)			
Males	113	380	1151
Females	123	432	1295
Average intake for F1 animals (mg/kg bw/day)			
Males	173	589	1750
Females	169	586	1734

Mating performance, fertility, litter size and growth were unaffected by the presence of ethyl lauroyl arginate in the diet at 1500 and 5000 ppm. At 15000 ppm, the litters of 2 of the 8 females lost weight in the first 4 days after birth and when killed for humane reasons at day 4 of age were found to have no milk in their stomachs. Survival of pups within remaining litters at 15000 ppm was slightly below that of controls.

Sexual maturation in males was unaffected by treatment but vaginal opening was delayed by approximately 4 days in females treated at 15000 ppm as shown in the table below. Subsequent establishment of the normal oestrous cycle was demonstrated in all groups.

Table 2.17: Age at vaginal opening for pups selected as the F1 generation (days)[a]

Dietary concentration (ppm)	0	1500	5000	15000
Age at vaginal opening (days)[b]	35.6 (2.2)	36.8 (2.7)	36.3 (2.4)	39.5 (2.0)

[a] No analyses of statistical significance were performed in this preliminary study.
[b] Mean (Standard deviation)

Necropsy of F0 parental animals and pups (killed at approximately 8 weeks of age) did not reveal any effects related to treatment.

It was concluded that a dietary concentration of 15000 ppm could be used as the highest treatment level for a two-generation study.

HLS (2005b) **Study title**: LAE two generation reproductive performance study by dietary administration to CD rats. **Report no.**: LMA 042/032553 **Report date**: 6 April 2005 **Laboratory**: Huntingdon Life Sciences Ltd, Huntingdon, Cambridgeshire, England. **GLP**: Yes (OECD)

Sprague-Dawley rats (n = 28/sex/group, age 6 weeks at commencement of treatment) were fed diets containing ethyl lauroyl arginate (88.2% w/w active ingredient) at concentrations of 0, 2500, 6000 or 15000 ppm for 10 weeks prior to pairing, throughout pairing, gestation, lactation and until termination. All females were pregnant in the control group, 27 were pregnant in the 2500 and 6000 ppm groups and 26 in the 15000 ppm group. Animals selected for the F1 generation comprised 24 male and 24 female progeny from each group (typically one animal/sex/litter). After weaning, F1 animals received the relevant diet as per the F0 generation throughout the study until termination. The F1 generation were mated to produce the F2 generation which was raised to weaning and then the study was terminated. Mean achieved dosages (mg/kg bw/day) during the study were as follows:

Table 2.18: Mean achieved dosages (mg/kg bw/day)

	Males			Females		
Dietary concentration (ppm)	**2500**	**6000**	**15000**	**2500**	**6000**	**15000**
F0 generation						
Before pairing	181	434	1073	207	502	1226
During gestation	-	-	-	231	585	1518
During lactation	-	-	-	402	1018	2600
F1 generation						
Before pairing	224	537	1356	246	582	1489
During gestation	-	-	-	215	535	1430
During lactation	-	-	-	409	898	2353

The general condition of F0 and F1 animals receiving ethyl lauroyl arginate was similar to that of controls. Body weight and body weight gain of adult F0 and F1 animals were not affected by treatment. Food consumption was unaffected by treatment for both generations.

There were no adverse effects in either generation on pre-mating oestrous cycles, mating performance, fertility, litter size, pup survival and day 1 body weight. Pre-weaning reflex tests for F1 and F2 pups were not affected by treatment.

Pups were weaned on day 21 of age. Body weight gain was not affected by treatment during the periods 1-7 and 1-14 days of age. However, body weight gain over the full pre-weaning period (age 1-21 days) was reduced by approximately 10% for F1 males and females receiving 15000 ppm compared to controls as shown in the table below. The relative magnitude of this reduced body weight gain did not significantly change in the several days following weaning (*i.e.* the relative reduction in body weight gain was similar for the periods 1-21 days and 1-25 days). Thus, most of the reduction in body weight gain occurred in the week prior to weaning (days 14 to 21 of age).

Table 2.19: Body weight gain of F1 pups

Dietary concentration (ppm)		0	2500	6000	15000
Males					
Body weight gain (grams):	1-7 days of age	7.9 (1.3)	7.8 (2.8)	7.1 (2.6)	7.9 (1.6)
	1-14 days of age	24.2 (2.5)	25.0 (3.6)	22.4 (4.9)	24.4 (2.2)
	1-21 days of age	41.9 (5.5)	42.5 (5.7)	38.5 (6.0)	38.0 (2.8)**
	1-25 days of age	60.6 (6.7)	61.5 (7.7)	58.4 (6.4)	55.8 (3.7)**
Females					
Body weight gain (grams):	1-7 days of age	7.5 (1.2)	7.7 (2.3)	7.1 (2.3)	7.6 (1.5)
	1-14 days of age	23.6 (2.2)	24.1 (3.3)	23.0 (2.9)	23.1 (2.5)
	1-21 days of age	40.1 (4.5)	40.6 (4.4)	38.9 (3.3)	36.1 (2.4)**
	1-25 days of age	56.9 (5.2)	57.8 (7.3)	55.9 (5.9)	52.0 (3.7)**

[a] Mean (Standard deviation)
** Significant ($p < 0.01$) when compared with the control group.

Balano-preputial separation was unaffected at all dosage levels. A delay in vaginal opening of approximately 4 days was recorded at 15000 ppm as shown in the table below. Treatment had no impact on estrous cycles pre-pairing or pre-termination, fertility or primordial follicle counts. Anogenital distance in the F2 pups was also unaffected by treatment.

Table 2.20: Age at vaginal opening for pups selected as the F1 generation

Dietary concentration (ppm)	0	2500	6000	15000
Age at vaginal opening (days)	33.0 (2.3)	33.9 (2.6)	34.3 (2.7)	37.0 (2.0)**
No. aged ≥ 39 days at vaginal opening	0	0	1	5

[a] Mean (Standard deviation)
** Significant ($p < 0.01$) when compared with the control group.

Terminal investigations of F0 and F1 adult animals showed no effects on pre-termination oestrous cycles or on sperm assessments. Macroscopic examination of adult animals and pups revealed no changes attributable to treatment. In the 15000 ppm group, spleen weights (absolute and/or body weight relative) of F0 and F1 females at scheduled termination and of F1 male and F1 female weanlings and F2 female weanlings on day 30 of age were significantly lower than controls. The magnitude of the difference reduced with age and was not accompanied by any macroscopic or microscopic changes in F0 or F1 adult animals. This effect was therefore considered to be of no toxicological importance.

Because of the delay in vaginal opening observed at 15000 ppm in both the preliminary study and the main study, the NOAEL was considered to be 6000 ppm which corresponds to an ethyl lauroyl arginate intake of 502 mg/kg bw/day. This dose level corresponds to the calculated intake before pairing of males and females. Higher dietary intakes of ethyl lauroyl arginate were observed in females during gestation (585 mg/kg bw/day) and lactation (1018 mg/kg bw/day); however, because the relevant time of exposure for the delayed puberty effect is not known, the lower intake (before pairing) is considered to be the appropriate NOAEL.

Developmental toxicity studies

Developmental toxicity studies were conducted in rats and rabbits by the oral (gavage) route as summarised below. The active ingredient content in the ethyl lauroyl arginate batch used in these studies was 69.1% w/w. Therefore, this material does not meet the JECFA specifications for content of the active ingredient (85 to 95% w/w). However, the lower content of ethyl lauroyl arginate in this batch was due to a high water content (23% w/w) because the synthesis product was not subject to a drying step, not due to higher impurity levels. In the developmental toxicity studies below, the administered doses have been corrected for water content such that the active ingredient content corresponds to 90% w/w. This allows the doses used in these studies to be compared more readily to the doses in studies which used batches that conform to the JECFA specifications.

RATS

HLS (1998a) **Study title**: LAE study of tolerance in the rat by oral gavage administration. **Report no.**: LMA011/980114 **Report date**: 6 August 1998 **Laboratory**: Huntingdon Life Sciences Ltd, Huntingdon, Cambridgeshire, England. **GLP**: Yes (OECD)

ELA (69.1% w/w active ingredient) was administered by oral gavage to four non-pregnant Sprague-Dawley rats at an initial dosage of 250 mg/kg bw/day (corrected dose 192 mg/kg bw/day) for two days. The dosage was doubled every two days up to a maximum of 2000 mg/kg bw/day (corrected dose 1536 mg/kg bw/day). A separate group of presumed pregnant females (n = 4) received ethyl lauroyl arginate at 2000 mg/kg bw/day (corrected dose 1536 mg/kg bw/day) from days 6 to 13 of gestation.

There were no deaths during the treatment phase. Salivation was recorded on a number of occasions in both groups for a short period immediately after dosing. The frequency of salivation was increased at corrected doses of 768 and 1536 mg/kg bw/day. The general condition and body weights of the two groups of rats were not significantly affected by treatment and there were no treatment-related adverse gross pathology findings. All presumed-pregnant females were pregnant and embryo survival to gestation day 13 was unaffected by treatment.

HLS (1998b) **Study title**: LAE preliminary study of embryo-foetal toxicity in the CD rat by oral gavage administration. **Report no.**: LMA013/980140 **Report date**: 6 August 1998 **Laboratory**: Huntingdon Life Sciences Ltd, Huntingdon, Cambridgeshire, England. **GLP**: Yes (OECD)

ELA (69.1% w/w active ingredient) was administered by oral gavage at doses of 200, 600 or 2000 mg/kg bw/day to groups of presumed pregnant Sprague-Dawley rats (n = 6/group) from days 6 to 19 of gestation. The corrected doses are 154, 461 and 1536 mg/kg bw/day. Control animals received the vehicle, 1% w/v methylcellulose, throughout the same period.

All presumed-pregnant females were pregnant. One female in the lowest dose group (154 mg/kg bw/day) exhibited minimal food intake (2 g/day) and a body weight loss of 40 g on days 18-19 of gestation. This female was killed *in extremis* on day 19 of gestation after showing signs of pallor, piloerection, brown staining around one eye, red urine and perigenital discharge. Necropsy revealed a large amount of dark red fluid within the vagina and both uterine horns. The uterus contained 15 late resorptions. In the absence of similar findings in animals in the higher dosage groups it is considered that the findings in this animal were unrelated to treatment.

Salivation after dosing was observed occasionally at 461 mg/kg bw/day and frequently at 1536 mg/kg bw/day. Respiratory noises were noted for one animal in each of the treated groups. There were no other significant clinical signs observed in animals in the control group or the treatment groups.

ELA had no significant treatment-related effects on food consumption, body weight, gross pathology, fetal survival or fetal development.

HLS (1998c) **Study title**: LAE study of embryo-foetal toxicity in the CD rat by oral gavage administration. **Report no.**: LMA 014/984183 **Report date**: 24 November 1998 **Laboratory**: Huntingdon Life Sciences Ltd, Huntingdon, Cambridgeshire, England. **GLP**: Yes (OECD)

ELA (69.1% w/w active ingredient) was administered by oral gavage at doses of 200, 600 or 2000 mg/kg bw/day to groups of presumed pregnant Sprague-Dawley rats (n = 22/group) from days 6 to 19 of gestation. The corrected doses are 154, 461 and 1536 mg/kg bw/day. Control animals received the vehicle, 1% w/v methylcellulose, throughout the same period.

Three animals receiving 1536 mg/kg bw/day were humanely sacrificed on days 7 or 8 of gestation following severe signs of respiratory distress and salivation after dosing. Two of the animals had shown body weight loss prior to sacrifice. Necropsy revealed large amounts of gaseous material in the stomach of two females while in one female the entire GI tract was distended with gas. Two animals receiving 461 mg/kg bw/day were humanely sacrificed towards the end of gestation. These animals also exhibited respiratory distress, salivation, body weight loss and GI tract distention. Necropsy of the high and mid dose animals exhibiting respiratory distress did not indicate damage to the lungs. Accumulation of gas in the stomach and GI tract may be due to gasping respiration following possible aspiration of increased secretions and or traces of the dosing material following treatment with the more concentrated/viscous solutions at the higher doses. The observed respiratory distress is not considered to be a systemic toxic response to oral ingestion of ethyl lauroyl arginate but may suggest possible bronchial irritation if the test material is inhaled. Respiratory distress is also commonly observed in animals dosed using gavage due to intubation errors.

The general condition of the surviving animals was satisfactory and all the females were pregnant. Noisy respiration was observed in some animals from all ethyl lauroyl arginate treatment groups but not in controls. Salivation at the time of dosing was observed in all animals receiving 1536 mg/kg bw/day on approximately 50% of dosing occasions. At 461 mg/kg bw/day, salivation was observed in 14/22 animals on 1-3 dosing occasions, while at 154 mg/kg bw/day only one animal showed salivation on one dosing occasion. Salivation at the time of dosing was not observed in control animals.

There were no overall treatment-related effects on body weight or food consumption, although occasional animals in treatment groups showed periods of body weight loss and reduced food intake which were related to respiratory distress.

Apart from the gaseous distention of the GI tract in three high dose and two mid dose animals, there were no other maternal necropsy findings which were considered related to treatment.

There were no treatment-related effects on fetal survival, growth or development.

Although transient effects were observed on body weight and food consumption associated with animals exhibiting respiratory distress, they were not considered to be systemic toxic responses to oral ingestion of ethyl lauroyl arginate. Based on the absence of any compound related adverse effects a more appropriate NOAEL for dams and fetuses is considered to be 1536 mg/kg/day.

RABBITS

HLS (1998d) **Study title**: LAE study of tolerance in the rabbit by oral gavage administration. **Report no.**: LMA012/980115 **Report date**: 6 August 1998 **Laboratory**: Huntingdon Life Sciences Ltd, Huntingdon, Cambridgeshire, England. **GLP**: Yes (OECD)

ELA (69.1% w/w active ingredient) was administered by oral gavage to two non-pregnant NZW rabbits at an initial dosage of 60 mg/kg bw/day (corrected dose 46.1 mg/kg bw/day) for two days (group I). The dosage was doubled every two days up to a maximum of 1000 mg/kg bw/day (corrected dose 768 mg/kg bw/day). A separate group (group II) of two pregnant females received ethyl lauroyl arginate at 1000 mg/kg bw/day (corrected dose 768 mg/kg bw/day) from days 6 to 12 of gestation.

Group I: There were no deaths during the treatment phase. The general condition, clinical signs and body weights of the animals were not significantly affected by treatment and there were no treatment-related adverse gross pathology findings.

Group II: There were no deaths during the treatment phase. There was a transient reduction in water and food intake from about day 3 of treatment, associated with marked weight loss. One female showed abnormal stress reaction to the dosing procedure at the second dose and the second female showed marked respiratory distress after the third dose. One female exhibited continuous weight loss until termination but the other female showed some partial recovery in body weight towards the end of the dosing period. At necropsy, both animals showed some evidence of collapse of areas of the lung which was more extensive and accompanied by suggestions of infection in the lungs of the animal which had shown signs of respiratory distress during treatment. Both animals showed prominent dark vessels on the surface of the kidneys, but the significance of this observation was uncertain. Embryo survival was not affected by maternal treatment.

HLS (1998e) **Study title**: LAE preliminary study of embryo-foetal toxicity in the rabbit by oral gavage administration **Report no.**: LMA015/980169 **Report date**: 6 August 1998 **Laboratory**: Huntingdon Life Sciences Ltd, Huntingdon, Cambridgeshire, England **GLP**: Yes (OECD)

ELA (69.1% w/w active ingredient) was administered by oral gavage at doses of 250, 500 or 1000 mg/kg bw/day to presumed pregnant NZW rabbits (4/group) from day 6 to 19 after mating. The corrected doses are 192, 384 and 768 mg/kg bw/day. Six control presumed pregnant females received vehicle (1% w/v methylcellulose in water) for the same period. All females from each group were pregnant and were killed on day 29 after mating for examination of their uterine contents.

There were no pre-terminal deaths and no treatment-related clinical signs. Small losses in body weight were recorded during gestation days 6 to 12 for 3/4 animals at 768 mg/kg bw/day and for a lower proportion of control, low, and intermediate dose groups. There were no meaningful inter-group body weight differences by the end of pregnancy. Food consumption was lower in the 384 and 768 mg/kg bw/day groups.

There were no necropsy findings related to treatment and no effects on fetal survival or fetal anomalies.

HLS (1998f) **Study title**: LAE study of embryo-foetal toxicity in the rabbit by oral gavage administration. **Report no.**: LMA 016/992096 **Report date**: 26 March 1999
Laboratory: Huntingdon Life Sciences Ltd, Huntingdon, Cambridgeshire, England **GLP**: Yes (OECD)

ELA (69.1% w/w active ingredient) was administered by oral gavage at doses of 100, 300 or 1000 mg/kg bw/day to groups of presumed pregnant NZW rabbits (n = 22/group) from day 6 to 19 after mating. The corrected doses are 77, 230 and 768 mg/kg bw/day. Control females (n = 22) received the vehicle (1% w/v/ methylcellulose) throughout the same period. Surviving females were killed on day 29 after mating for examination of uterine contents followed by detailed fetal examination.

Signs of reaction to treatment, largely associated with dosing difficulty and respiratory signs (irregular, gasping, noisy), were observed in 5 animals per group at 230 and 768 mg/kg bw/day. These signs were largely alleviated by using a clean moist catheter (rather than a clean dry catheter) for dose administration. One animal at each of 230 and 768 mg/kg bw/day was killed for human reasons and necropsy revealed some lung congestion. One animal at 768 mg/kg bw/day aborted on day 24 of gestation.

Body weight gain and food consumption were unaffected at 77 and 230 mg/kg bw/day. Body weight gain of rabbits receiving 768 mg/kg bw/day was 40% that of controls during the treatment period ($p < 0.01$) while food consumption was low during the second week of treatment.

Necropsy revealed no treatment related effects on dams, litter parameters or fetuses. The number of females actually pregnant ranged from 19 to 22 per group.

Because of reduced body weight gain and the observation that respiratory signs were not completely alleviated at the high dose after modification of the gavage method, the NOAEL was considered to be 230 mg/kg bw/day. However, as for rats dosed with ethyl lauroyl arginate by gavage (see study LMA014/984183, above), the observed respiratory distress is not considered to be a systemic toxic response to oral ingestion of ethyl lauroyl arginate but may suggest possible bronchial irritation if the test material is inhaled.

For fetuses, the NOAEL was the high dose of 768 mg/kg bw/day.

Intolerance

Ethyl lauroyl arginate showed no signs of intolerance even at very high dietary levels in animal studies of up to one year in duration. Ethyl lauroyl arginate has been approved for use and commercialised in the USA since 2005 with no reports of intolerance associated with consumption. Ethyl lauroyl arginate is rapidly metabolised to compounds which have not been associated with intolerance reactions.

Discussion

The submitted data were considered suitable for hazard assessment and assignment of an ADI. The lack of a long term carcinogenicity study was not considered to be a deficiency as discussed below.

The overall quality of the submitted kinetics and toxicology studies was high. Adequate numbers of animals per group were evaluated in the main toxicity studies and appropriate investigations were conducted in these studies. All of the submitted kinetics and toxicology

studies, except one preliminary study, were conducted according to Good Laboratory Practice (GLP).

Absorption, metabolism and excretion

Absorption studies were conducted in rats and humans administered ethyl lauroyl arginate orally. In a study in rats receiving ethyl lauroyl arginate by gavage at single doses of 40, 120 or 320 mg/kg bw, both ethyl lauroyl arginate and the metabolite LAS were assayed in plasma. At doses of 40 and 120 mg/kg bw, the area under the plasma concentration versus time curve over 8 h ($AUC_{0\text{-}8\ h}$) for ethyl lauroyl arginate could not be calculated because of rapid metabolism to LAS. At 320 mg/kg bw, the highest dose tested, the $AUC_{0\text{-}8\ h}$ for ethyl lauroyl arginate was only 2.4% of the value for LAS (7.50 and 315 ng.h/mL, respectively). Thus, systemic exposure of rats to ethyl lauroyl arginate was very low at the doses used in this study. This study also investigated the absorption of ethyl lauroyl arginate (at a dose of 120 mg/kg bw) in three different vehicles: (i) propylene glycol/water, (ii) glycerol/water, and (iii) water. Ethyl lauroyl arginate was rapidly metabolised to LAS in the presence of each of the three vehicles. The AUC for LAS was approximately proportional to the ethyl lauroyl arginate dose and was similar for all three vehicles at an ethyl lauroyl arginate dose of 120 mg/kg bw.

Metabolism of ethyl lauroyl arginate to LAS was also rapid in humans. In the main human study, ethyl lauroyl arginate (radiolabelled with ^{13}C) was administered orally at single doses of 1.5 mg/kg bw (to 4 subjects) and 2.5 mg/kg bw (to 2 subjects). Ethyl lauroyl arginate, LAS and arginine were assayed in plasma over a 24 h period and ELA was shown to be rapidly metabolised to LAS and arginine. No meaningful AUC data could be obtained for ethyl lauroyl arginate because plasma concentrations of ^{13}C-ELA were below the limit of quantification at all sampling times in all subjects, with the exception of subject 2 (2.5 mg/kg bw) for whom quantifiable concentrations of ^{13}C-ELA were found in two samples. $AUC_{0\text{-}24\ h}$ values for LAS were 90.6 and 118 ng.h/mL at doses of 1.5 and 2.5 mg/kg bw, respectively. For arginine, the corresponding $AUC_{0\text{-}24\ h}$ values were 4- to 6-fold greater at 382 and 764 ng.h/mL, respectively.

A preliminary human absorption study using a single oral dose of 5 mg/kg bw also indicated rapid metabolism of ethyl lauroyl arginate to LAS and arginine; however, this study was limited by the use of only two subjects (fed state undocumented) and AUC values were not presented.

Studies specifically investigating metabolite formation were conducted *in vitro* and in rats *in vivo.* Rapid metabolism of ethyl lauroyl arginate to LAS and then to arginine was demonstrated to occur *in vitro* in simulated intestinal fluids but only in the presence of the pancreatic enzyme mixture pancreatin. In contrast, in simulated gastric fluid (with and without pepsin) ethyl lauroyl arginate was stable over the 2 h period investigated. Thus, negligible metabolism of ethyl lauroyl arginate is likely in the stomach, while rapid metabolism to LAS and subsequent slower metabolism to arginine likely occurs in the intestine.

Incubations of ethyl lauroyl arginate with human plasma and human hepatocytes also resulted in substantial conversion to LAS but not to arginine. Incubation of ^{14}C-ELA with the S9 fraction from rat liver resulted in the formation of LAS, arginine, ornithine, arginine ethyl ester, urea and several uncharacterised metabolites. However, substantial amounts of ^{14}C-ELA (25% of the added radioactivity) remained unchanged after 24 h.

An *in vivo* metabolism study in rats receiving ^{14}C-ELA as a single gavage dose also showed rapid metabolism of ethyl lauroyl arginate to LAS, arginine, ornithine and urea. At the first analysis time point (0.5 h), approximately 50% of the administered radioactivity was present

in arginine, 8% in ornithine, and 15% in small molecular weight polar material (probably urea based on retention time). It is not apparent why relatively larger concentrations of ethyl lauroyl arginate were observed in plasma in this study (greater than the LAS concentrations) compared to the other rat study.

As indicated above, a comparison of AUC values for ethyl lauroyl arginate in rats and humans is not possible because of the negligible systemic exposure to ethyl lauroyl arginate observed in the human studies. For the metabolite LAS, a comparison of the available rat and human AUC values is shown in the table below. This comparison shows that a human ethyl lauroyl arginate dose of approximately 2 mg/kg bw would result in an AUC value for LAS which is similar to that in rats receiving ethyl lauroyl arginate at approximately 120 mg/kg bw (*i.e.* a 60-fold lower dose in humans, on a body weight basis, results in a similar systemic exposure to LAS).
However, systemic exposure to arginine in humans (arising from the degradation of LAS) was 4- to 6-fold greater than exposure to LAS (based on plasma AUC values). Thus, in relative terms, systemic exposure to LAS is small because of rapid degradation to arginine. Data on systemic exposure to arginine arising from LAS were not available for rats.

Table 2.21: Plasma AUC data for the metabolite LAS

	Rat						Human	
	Single dose (mg/kg bw)			Repeat dose (mg/kg bw/day) [a]			Single dose (mg/kg bw)	
	40	120	320	119	350	1018	1.5	2.5
$AUC_{0-\tau}$ (ng.h/mL)	52.5	103	315	108	327	1045	90.6	118
τ (h)	8 [b]	8 [b]	8 [b]	24	24	24	12 [b]	8 [b]

[a] The dose levels of 119, 350, and 1018 are mean values calculated from food intake in the 52 week repeat dose toxicity study (dietary).
[b] The time intervals for these AUC values varied depending on the time of the last quantifiable sample.

An excretion study in rats administered an oral gavage dose of ^{14}C-ELA indicated that approximately 46% of the applied radioactivity was retained in the carcass at sacrifice, 5 days after administration. This finding is consistent with the formation of arginine as a metabolite which can be subsequently incorporated into endogenous proteins and undergo catabolism into smaller molecules. A large fraction of the dose (37%) was excreted as carbon dioxide in expired air which is also consistent with normal amino acid catabolism resulting in urea and carbon dioxide. Smaller fractions of the applied radioactivity were excreted in urine (12%) and faeces (4%).

The submitted absorption and metabolism studies on ethyl lauroyl arginate do not indicate any important differences between rats and humans with respect to these properties. The rat is therefore considered to be an appropriate animal species for toxicity studies.

Note that some reproductive toxicity studies were conducted in rabbits, a species for which the absorption, metabolism and excretion of ethyl lauroyl arginate has not been studied.

Single dose toxicity

Single dose toxicity studies in rats were conducted using Mirenat-N, ethyl lauroyl arginate and LAS as test articles. For Mirenat-N, administered by oral gavage at an effective ethyl lauroyl arginate dose of 500 mg/kg bw, there were no deaths and treatment-related clinical

signs were confined to transient piloerection. Body weight gain was normal and no abnormalities were evident in any of the animals at necropsy on day 15. For ethyl lauroyl arginate, administered by oral gavage at a dose of 2000 mg/kg bw, there were also no deaths. Treatment-related clinical signs consisted of piloerection, increased salivation, unsteady gait and hunched posture. All clinical signs of toxicity had resolved by day 3 or 4. All animals achieved satisfactory weight gain and no abnormalities were evident in any of the animals at necropsy on day 15. The metabolite LAS, also administered at a dose of 2000 mg/kg bw by oral gavage, exhibited lower acute toxicity than ethyl lauroyl arginate as indicated by an absence of clinical signs of toxicity. There were no deaths, body weight gain was normal and no macroscopic alterations were observed at necropsy 15 days after dosing.

In a dermal study in rabbits, ethyl lauroyl arginate applied as an aqueous paste caused slight to well-defined erythema followed by desquamation of the treated skin. There was no indication of a systemic effect of treatment.

Finally, in the preliminary absorption studies in humans (also discussed above), ethyl lauroyl arginate at a dose of 5 mg/kg bw appeared to be well tolerated except for a burning sensation and nausea reported on administration. These effects may have been due to the use of propylene glycol as the solvent in this study. These effects were not observed in the subsequent human study which employed lower ethyl lauroyl arginate and propylene glycol doses. In this study there were no clinically significant abnormalities in any of the laboratory investigations (clinical chemistry, haematology and urinalysis), no notable changes in vital signs during the study, and no clinically significant ECG findings.

Repeat dose toxicity

Three main repeat dose toxicity studies were conducted in rats. Two additional studies were preliminary studies of 4 weeks dosing duration. All of the studies were conducted according to GLP. The test articles, Mirenat-N (25% w/w solution of ethyl lauroyl arginate in propylene glycol) and ethyl lauroyl arginate, were administered via the diet.

ELA was generally well tolerated in these studies. There were no deaths in animals that received ethyl lauroyl arginate in the 4 and 13 week studies. There were 6 unscheduled deaths in the 52 week study; however, none of the deaths were attributable to treatment. Clinical signs of toxicity included piloerection, ungroomed coats and salivation in the 4 week study with ethyl lauroyl arginate at the high dietary concentration (50000 ppm), and ungroomed coat and brown staining of the muzzle in the 13 and 52 week studies with ethyl lauroyl arginate predominantly at the high concentration in each study (50000 and 18000 ppm, respectively). Neurobehavioural parameters (sensory reactivity, grip strength, motor activity) were investigated in the 52 week study with no significant findings.

Transient and relatively small reductions in body weight gain and food consumption were observed at the high dietary concentrations in 4 out of 5 studies. These findings may be attributable to reduced palatability of the diet at high ethyl lauroyl arginate concentrations.

Potentially treatment related effects on clinical chemistry parameters were observed in one 13 week study and in the 52 week study; however, these effects were not consistent across the studies and are unlikely to be toxicologically relevant. In the 13 week study with ethyl lauroyl arginate, decreased total protein was observed for animals receiving the high dietary concentration of 50000 ppm, slightly decreased albumin was observed for animals receiving 50000 ppm and females receiving 15000 ppm, and slightly decreased cholesterol was observed for females receiving 50000 ppm. In the 52 week study, clinical chemistry findings were limited to increased urea concentration in females receiving the high dietary concentration of 18000 ppm.

Potentially treatment related effects on white blood cell parameters were observed in both of the 13 week studies and in the 52 week study. The applicant provided expert opinions on the haematological findings from three scientists (Brown 2008; Escolar 2008; Maronpot 2008). Escolar (2008) and Maronpot (2008) considered that the haematological findings are unlikely to be toxicologically significant based on the following: (i) the absence of a clear dose-effect relationship; (ii) the findings were dependent on rat strains; (iii) the responses varied according to sex; (iv) there were no associated effects on bone marrow (investigated in the 52 week study); and (v) inconsistent effects both within and between studies. These arguments are consistent with the findings in the submitted studies and are considered to be valid. Brown (2008) considered that the changes may be treatment related, and if so, are likely to be a result of the local effect of ethyl lauroyl arginate on the forestomach (discussed below). Brown considered that the most likely reason for a reduction in mature white blood cells in the circulation was due to migration to the tissues.

However, there was no correlation between the animals which showed forestomach lesions and those which exhibited reduced white blood cell counts. Brown also stated that normal myeloid cell production was not disturbed and that there was no evidence of excessive cell destruction or damage. EFSA considered ELA before these expert reviews were available and concluded that the white blood findings may be related to treatment and that the ADI should therefore be based on these findings (EFSA 2007). The ADI derived by EFSA was based on the NOAEL of approximately 50 mg ELA/kg bw/day which was the lowest dose tested in the 13 week study with Mirenat-N as test article. Based on this NOAEL and a safety factor of 100, EFSA established an ADI of 0-0.5 mg ELA of the proposed specifications /kg bw.

Treatment related gross pathology and histopathology findings were limited to the forestomach and were observed in one of the 13 week studies and in the 52 week study. The findings, of generally minimal or slight severity even at the high dietary levels, were restricted to the non-glandular region of the stomach (the forestomach), and consisted of inflammation, erosion, parakeratosis, ulceration, epithelial hyperplasia and re-epithelialisation. The increase in the incidence of these findings was statistically significant only at the high dietary levels in each study. These findings are considered to arise from a direct effect on epithelial cells due to the surfactant action of ethyl lauroyl arginate. The effects are not considered to be indicative of systemic toxicity. Moreover, the rodent forestomach does not possess a protective mucus lining and has no counterpart in humans.

Genotoxicity

An appropriate set of genotoxicity studies was submitted comprising bacterial reverse mutation assays with Mirenat-N, ethyl lauroyl arginate and the metabolite LAS, mammalian cell mutation and chromosome aberration assays with Mirenat-N and ethyl lauroyl arginate, and a micronucleus formation study with the metabolite LAS. Relatively low maximum concentrations were tested in the *in vitro* assays because of cytotoxicity at higher ethyl lauroyl arginate concentrations. The cytotoxicity was particularly evident in the bacterial reverse mutation assay, this study is therefore of limited value for the evaluation of mutagenicity. None of the test articles showed evidence of mutagenic or clastogenic activity in the submitted assays while negative and positive controls gave expected results. There was some evidence of polyploidy induced by ethyl lauroyl arginate in a chromosome aberration assay; however, this was observed only at cytotoxic concentrations and is unlikely to be of biological significance.

Carcinogenicity

A long term carcinogenicity study was not submitted which is considered acceptable because ethyl lauroyl arginate was not genotoxic and has no chemical structural alert and did not show evidence of pre-neoplasia or neoplasia in the repeat dose toxicity studies. In addition, ethyl lauroyl arginate is rapidly metabolised to endogenous compounds or compounds naturally present in the diet and there were no significant systemic toxic effects observed in any of the studies.

Reproductive and developmental toxicity

A total of 5 main reproductive and developmental toxicity studies were conducted in rats and rabbits. The studies investigated fertility, reproductive performance, embryofetal development and postnatal development. Three additional studies were preliminary studies. All of the studies were conducted according to GLP.

The only notable finding potentially attributable to ethyl lauroyl arginate was the observation of delayed onset of puberty in female rats in two studies. The age at vaginal opening, which is an indicator of pubertal onset, was delayed by approximately 4 days at the high dietary ethyl lauroyl arginate level in a preliminary reproductive study in rats and also by 4 days in the main study. In the main study the difference was significant at the $p < 0.01$ level when compared to the control group (mean age at vaginal opening 33.0 days compared to 37.0 days in the high dietary level group). A possible mechanism for this effect is not known.

Body weight gain from birth to day 14 of age was not affected by treatment suggesting that palatability of milk from treated dams was not reduced. However, body weight gain during the week before weaning (days 14 to 21 of age) was reduced by approximately 10% in males and females receiving the high dietary concentration of 15000 ppm. It is possible that the observed delayed onset of puberty may be related to this reduced body weight gain; however, males also showed reduced body weight gain of similar magnitude but their development was not delayed.

Another potentially relevant consideration is that rat pups are coprophagic and begin to consume the maternal faeces in their second postnatal week. However, an excretion study showed that only approximately 4% of an administered dose of ^{14}C-labelled ethyl lauroyl arginate was excreted in faeces. The delay in vaginal opening was not associated with any deficit in other markers of development or subsequent reproductive parameters in these animals. The NOAEL for this effect was the mid dietary level of 6000 ppm ethyl lauroyl arginate which corresponds to 502 mg/kg bw/day. This dose level is the calculated intake before pairing of males and females. Higher dietary intakes of ethyl lauroyl arginate were observed in females during gestation (585 mg/kg bw/day) and lactation (1018 mg/kg bw/day); however, because the relevant time of exposure for the delayed puberty effect is not known, the lower intake (before pairing) is considered to be the appropriate NOAEL.

Toxicology studies relevant to hazard assessment

The repeat dose toxicity, reproductive toxicity and developmental toxicity studies relevant to the hazard assessment of ethyl lauroyl arginate are summarised in the table below.

Table 2.22: Levels relevant to hazard assessment

Species / strain	Dosing duration (weeks)	No. animals per group	Dose levels [a] (mg/kg bw/day)	NOAEL [b] (mg/kg bw/day)	LOAEL [c] (mg/kg bw/day)	Study no.
Rat, Han Wistar	13 weeks	20/sex	0, 384, 1143, 3715 (males) 0, 445, 1286, 3915 (females)	1143 (males) 1286 (females)	3715 (males) 3915 (females)	LMA 031/004276
Rat, SD	52 weeks	20/sex	0, 106, 307, 907 (males) 0, 131, 393, 1128 (females)	307 (males) 393 (females)	907 (males) 1128 (females)	LMA 050/042556
Rat, SD	See footnote[d]	28/sex	0, 181, 434, 1073 (males) 0, 207, 502, 1226 (females)	1073 (males) [e] 502 (females)	1226 (females)	LMA 042/032553
Rat, SD	Gestation days 6 to 19	22 females	0, 154, 461, 1536	1536 [e] (dams and fetuses)	-	LMA 014/984183
Rabbit, NZW	Gestation days 6 to 19	22 females	0, 77, 230, 768	230 (dams) 768 (fetuses) [e]	768 (dams)	LMA 016/992096

[a] Dose levels were calculated based on measured food consumption and dietary concentration of ethyl lauroyl arginate (corrected for active ingredient content only for study numbers LMA 014/984183 and LMA 016/992096 which used an ethyl lauroyl arginate batch containing 69.1% w/w of the active ingredient).
[b] No Observed Adverse Effect Level
[c] Lowest Observed Adverse Effect Level
[d] Two generation reproductive toxicity study. Animals were dosed for 10 weeks prior to pairing, throughout pairing, gestation, lactation and until termination. After weaning, F1 animals received the relevant diet as per the F0 generation throughout the study until termination. The F1 generation were mated to produce the F2 generation which was raised to weaning.
[e] Highest dose tested.

The forestomach effects observed in the 13 week and 52 week repeat dose toxicity studies are probably due to a local irritant effect arising from the cationic surfactant activity of ethyl lauroyl arginate. There were no adverse findings for the glandular region of the stomach. The rodent fore stomach has no protective mucus lining and has no anatomical equivalent in humans. It is therefore not considered appropriate to base the ADI on the forestomach findings. Effects on white blood cell counts observed in repeat dose toxicity studies were not consistent across studies and are not likely to be of biological significance. The white blood cell findings are also not considered to be appropriate for setting the ADI.

Because the delay in vaginal opening was observed in two reproductive toxicity studies, and the magnitude of the effect was similar in each case, it is considered that this finding may be due a systemic effect of ethyl lauroyl arginate and is thus suitable for assigning an ADI for ethyl lauroyl arginate. The NOAEL for this effect was 502 mg/kg bw/day. Applying safety factors of 10 for inter-species differences and 10 for inter-individual differences results in an ADI of 0-5 mg/kg bw for ethyl lauroyl arginate.

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Attachment 3

Dietary Exposure Assessment Report

EXECUTIVE SUMMARY

A dietary exposure assessment was undertaken by FSANZ for ethyl lauroyl arginate. Food consumption data from the 1995 Australian and 1997 New Zealand National Nutrition Surveys were used for the exposure assessments. The population groups assessed were the Australian population (2 years and above), the New Zealand population (15 years and above) and children (2 to 6 years for Australia only).

The Applicant provided FSANZ with information on proposed levels of use for ethyl lauroyl arginate for specific food groups and the foods within each food group that may be expected to contain it. Based on this information, dietary exposure was estimated assuming that ethyl lauroyl arginate is present in foods at the maximum permitted level suggested by the applicant. This scenario is highly protective of consumers.

Estimated dietary exposures were compared with the reference health standard, an Acceptable Daily Intake (ADI) of 0 - 5 mg/kg bw.

The dietary exposure assessment shows that if the requested permissions for ethyl lauroyl arginate are approved, consumers of foods containing ethyl lauroyl arginate including children are unlikely to exceed the ADI. All estimated dietary exposures for the population groups assessed were below the ADI, even when it was assumed that ethyl lauroyl arginate was in all foods for which permission is sought, at the maximum permitted level.

Based on the food groups proposed by the Applicant, the major contributor to ethyl lauroyl arginate dietary exposure for Australians aged 2 years and above and for New Zealanders aged 15 years and above was comminuted meat products and whole pieces of processed meat. For Australian children aged 2 to 6 years, the major contributor was cordials.

Introduction

FSANZ received an Application from Competitive Advantage on behalf of Laboratorios Miret, S.A. (LAMIRSA) on 18 August 2008 seeking to amend Standard 1.3.1 – Food Additives. The Applicant is seeking to add the use of ethyl lauroyl arginate to a food additive preparation that would be added to products such as beverages, cheeses, vegetables (including legumes), cooked rice, noodles and pasta, meats and meat products and mixed food items (e.g. savoury toppings and fillings, desserts, and dips). This dietary exposure assessment for ethyl lauroyl arginate for the Australian and New Zealand populations assumed use of ethyl lauroyl arginate was permitted as proposed.

Information provided by the Applicant for the dietary exposure assessment

The Applicant provided dietary exposure information considered at the 38th session of the Codex Committee on Food Additives and Contaminants and the 69th Session of the Joint FAO/WHO Expert Committee on Food Additives (FAO/WHO 2008). As an essential part of the Application the Applicant provided information (Table 1, page iii) on the foods and the concentrations of ethyl lauroyl arginate, as the active ingredient ethyl-N^{α}-lauroyl-L-arginate·HCl, that were proposed to be included in the Code.

Dietary modelling

The dietary exposure assessment used dietary modelling techniques that combine food consumption data with food chemical concentration data to estimate the exposure to the food chemical from the diet:

Dietary exposure = food chemical concentration x food consumption

Dietary exposure was estimated using FSANZ's dietary modelling computer program DIAMOND by combining usual patterns of food consumption derived from National Nutrition Survey (NNS) data with proposed levels of use of ethyl lauroyl arginate in foods.

Food consumption 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. Both of the NNSs used a 24-hour food recall methodology.

Conducting dietary modelling based on 1995 or 1997 NNS food consumption data provides the best available estimate of actual consumption of a food and the resulting estimated exposure to a food chemical. However, it should be noted that limitations exist within the NNS data. 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 which make up the majority of most people's diet is unlikely to have changed markedly.

However, there is an increasing level of uncertainty associated with the consumption of other foods where these may have changed in consumption since 1995 or 1997, or where new foods on the market were not available in 1995 in Australia or 1997 in New Zealand.

Dietary survey data from both New Zealand's 2002 National Children's Nutrition Survey and Australia's 2007 Children's Nutrition and Physical Activity Survey were not available at the time dietary modelling for Application 1015 – Ethyl lauroyl arginate was conducted.

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.

Population groups assessed

The dietary exposure assessment was conducted for both Australian and New Zealand populations. An assessment was conducted for the whole population, as well as for Australian children aged 2 to 6 years. Dietary exposure assessments for the whole population are a proxy for lifetime exposure. An exposure assessment was conducted on Australian children aged 2 to 6 years because children generally have higher exposures on a body weight basis as they consume more food per kilogram of body weight compared to adults. They also consume many of the foods and drinks proposed to contain ethyl lauroyl arginate, such as cordials, soft drinks and fruit and vegetable juice products. It is important to note that, while children aged 2 to 6 years have been assessed as a separate group, this group has also been assessed in the whole population's dietary exposure assessment.

Ethyl lauroyl arginate concentration levels

The levels of ethyl lauroyl arginate in foods that were used in the dietary exposure assessment were derived from data submitted by the Applicant, adjusting the levels submitted for the active ingredient ethyl-N^{α}-lauroyl-L-arginate·HCl to levels of ethyl lauroyl arginate to enable a direct comparison with the ADI for ethyl lauroyl arginate, established by FSANZ. For example, the applicant requested a level of 400 mg/kg of the active ingredient for soft, cream or processed cheese, that was corrected to 450 mg/kg of ethyl lauroyl arginate for modelling purposes. The foods and levels of use used in the dietary modelling are shown in Table 3.1.

Concentrations of ethyl lauroyl arginate were assigned to food groups using DIAMOND food classification codes. These codes are based on the Code. For example, Schedule 1 of Standard 1.3.1 contains a section *8.3 Comminuted meat products* with an entry for 'sausage', 'frankfurts' and 'saveloys'.

The foods proposed by the Applicant to contain ethyl lauroyl arginate were matched to the most appropriate processed foods in Schedule 1 for modelling purposes.

Scenarios for dietary modelling

Only one scenario was modelled for the purpose of this Application:

- assumed that ethyl lauroyl arginate was present in foods at the Maximum Permitted Level (MPL) currently suggested by the Applicant.

How were the estimated dietary exposures calculated?

Each individual's exposure to ethyl lauroyl arginate was calculated using his or her individual food records from the dietary survey. The DIAMOND program multiplies the specified concentration of ethyl lauroyl arginate by the amount of food that an individual consumed from that group in order to estimate the exposure to ethyl lauroyl arginate from each food. Once this has been completed for all of the foods specified to contain ethyl lauroyl arginate, the total amount of ethyl lauroyl arginate consumed from all foods is summed for each individual. Population statistics (mean for consumers and 90th percentile consumer exposures) are then derived from the individuals' ranked dietary 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 respondents did not provide a body weight. These respondents are not included in calculations of estimated dietary exposures 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 (in units per kilogram of body weight per day) is calculated as a percentage of the reference health standard, the results are then ranked, and population statistics derived.

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 ethyl lauroyl arginate and multiplying this by 100.

Table 3.1 Concentrations of ethyl lauroyl arginate used in the dietary modelling

ANZFCS	Food category used in model	Concentration (mg/kg)[#]	Notes
0.1	**Preparations of food additives**	225	
1.6	**Cheese – Soft /cream/processed**	450	
1.6	**Cheese – Mozzarella**	225	
1.6	**Cheese – Hard/semi hard**	100	Estimated 500 mg/kg for an average block of cheese based on surface area, then estimated 20% of cheese consumed is rind
4.1.3	**Peeled and/or cut fruits & vegetables**	225	Excludes potatoes products
4.3.8	**Re-hydrated legumes**	225	
6.3	**Cereal – Cooked rice**	225	
6.4	**Flour products – Cooked pasta/noodles**	225	
8.2	**Whole pieces of processed meat**	225	Ham, corned beef, pickled pork etc…
8.3	**Comminuted meat products**	350	Including poultry
9.3	**Fish products**	450	
14.1.2	**Fruit & vegetable juices and fruit & vegetable-based drinks**	55	Excludes apple juice and apple-based drinks
14.1.3	**Water-based flavoured drinks/high energy drinks/soft drinks**	55	Excludes regular cola products
20.2	**Cheese-based savoury topping and fillings**	450	E.g. pizza toppings
20.2	**Vegetable-based savoury topping and fillings**	225	E.g. sauces
20.2	**Dairy & fat-based desserts and dips**	450	

The concentration used for the dietary modelling is ethyl lauroyl arginate. This is different to the proposed levels in the draft standard in the Code which refer to the active ingredient, which is approximately 85-95% of ethyl lauroyl arginate and ethyl lauroyl arginate is the component reported on analysis of the food.

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 when only proposed concentration levels were available. However, where significant uncertainties in the data existed, conservative assumptions were generally used to ensure that the dietary exposure assessment did not underestimate exposure.

Assumptions made in the dietary exposure assessment include:

- all the foods within the group contain ethyl lauroyl arginate at the levels specified in Table 1. Unless otherwise specified, the maximum proposed concentration of ethyl lauroyl arginate in each food category has been used;
- consumption of foods as recorded in the NNS represent current food consumption patterns;
- consumers do not alter their food consumption habits besides substituting non-ethyl lauroyl arginate containing products with ethyl lauroyl arginate containing products;
- consumers do not increase their consumption of foods/food groups upon foods/food groups containing ethyl lauroyl arginate becoming available;
- where a food was not included in the exposure assessment, it was assumed to contain a zero concentration of ethyl lauroyl arginate;
- where a food has a specified ethyl lauroyl arginate concentration, this concentration is carried over to mixed foods where the food has been used as an ingredient; and
- there are no reductions in ethyl lauroyl arginate concentrations from food preparation or due to cooking.

These assumptions are likely to lead to a highly protective overestimate for ethyl lauroyl arginate dietary exposure.

Limitations of the dietary modelling

A limitation of estimating dietary exposure is that only 24-hour dietary survey data are 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 may specifically affect some of the food groups in this assessment, such as re-hydrated legumes.

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 exposure 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.

Results

Estimated dietary exposures to ethyl lauroyl arginate

The number of Australians and New Zealanders that reported consuming foods that may contain ethyl lauroyl arginate are listed in Table 3.2. In summary, approximately 86-95% of Australians and New Zealanders ate foods that might contain ethyl lauroyl arginate if permissions sought in the Application were added to the Code.

The estimated dietary exposures for each scenario for ethyl lauroyl arginate for Australia and New Zealand are shown in Table 3.3 and in Figure 3.1. Overall, Australian children 2 to 6 years had a higher exposure to ethyl lauroyl arginate on a bodyweight basis than the whole population.

The estimated mean respondent dietary exposures of ethyl lauroyl arginate ranged from 27.9 to 34.4 mg/day or 0.4 to 1.9 mg/kg bw/day. The 90th percentile dietary exposures for consumers of ethyl lauroyl arginate for Australia and New Zealand were between 72.0 and 82.1 mg/day (1.0 and 3.9 mg/kg bw/day) (Table 3.3).

Estimated mean and 90th percentile dietary exposures to ethyl lauroyl arginate for the Australian population (2 years and above) were higher than those for the New Zealand population (15 years and above). This may be due to different food consumption patterns (food types and/or amounts) between the two countries and/or differences in survey methodology.

Table 3.2: Population groups, number of consumers that reported consuming foods that may contain ethyl lauroyl arginate and consumers as a proportion of respondents to the surveys

Country	Population group	Number of consumers*	Consumers as a % of respondents
Australia	Whole population	12487	90.1%
	2 to 6 years	940	95.0%
New Zealand	Whole population	4001	86.3%

* Total number of respondents for Australia: whole population 2 years and above= 13 858, 2-6 years = 989; New Zealand: whole population 15 years and above = 4 636.

Table 3.3: Estimated mean and 90th percentile exposures to ethyl lauroyl arginate

Country	Population	Mean all respondents	Mean consumers	90th percentile consumers
		mg/day (mg/kg bw/day)	*mg/day (mg/kg bw/day)*	*mg/day (mg/kg bw/day)*
Australia	Whole population	33.9 (0.6)	37.7 (0.7)	82.1 (1.5)
	2 to 6 years	34.4 (1.9)	36.2 (2.0)	72.0 (3.9)
New Zealand	Whole population	27.9 (0.4)	32.3 (0.4)	75.2 (1.0)

Consumers include the people who have consumed a food that contains ethyl lauroyl arginate. *Respondents* include all members of the survey population whether or not they consumed a food that contains ethyl lauroyl arginate

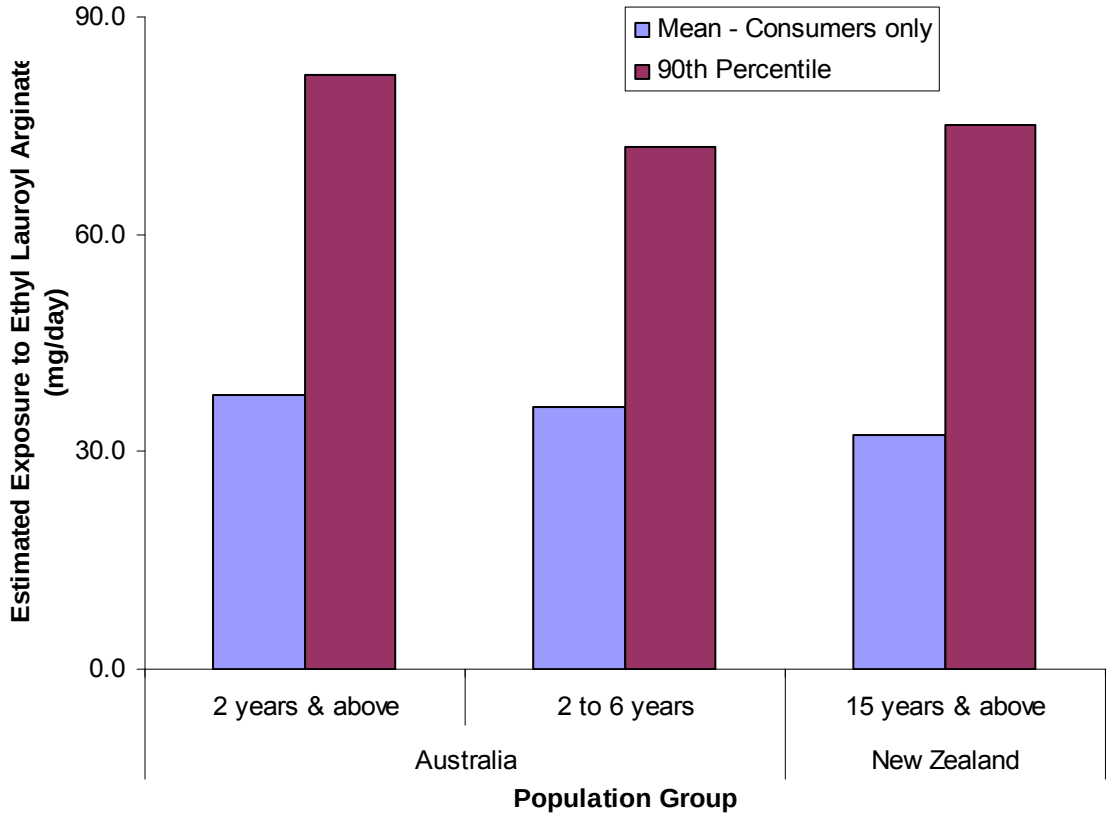

Figure 3.1: Estimated mean dietary exposures (mg/day) and 90th percentile dietary exposures (mg/day) for consumers of ethyl lauroyl arginate for the Australian and New Zealand population groups

Major contributing foods to total estimated dietary exposures

The major contributors (>5%) to total ethyl lauroyl arginate dietary exposures for the three population groups are shown in Figures 3.2 – 3.4 The main contributor for Australians aged 2 years and over and New Zealanders aged 15 and over was comminuted meat products and whole pieces of processed meat. For Australians aged between 2 and 6, cordial was the major contributor.

Other contributors to ethyl lauroyl arginate exposure in all population groups assessed were beverages (e.g. fruit and vegetable juices, fruit and vegetable-based drinks and soft drinks) and cheeses.

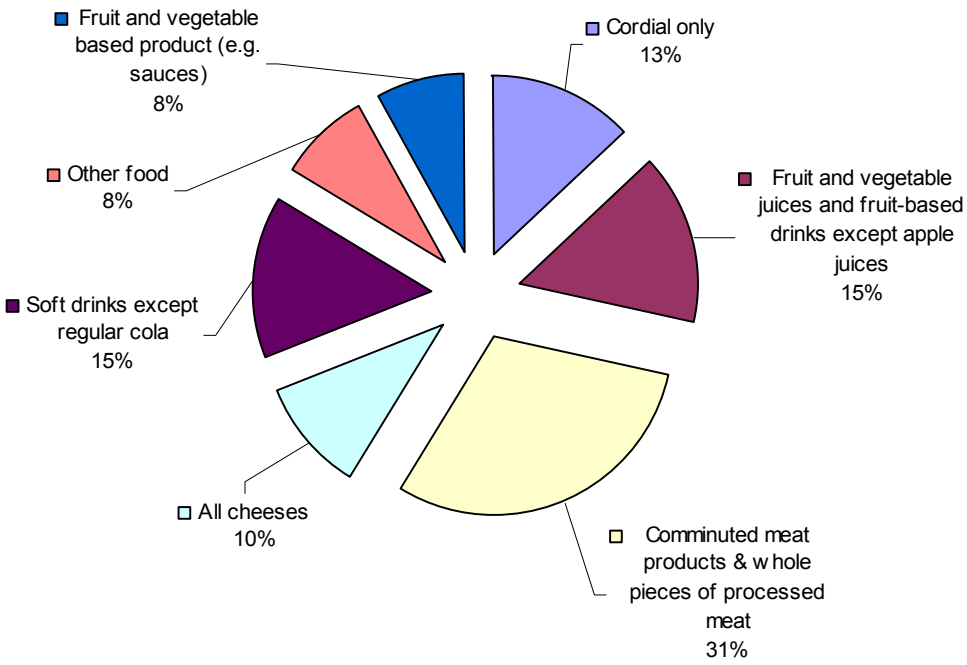

Figure 3.2: Major contributors to ethyl lauroyl arginate dietary exposures for Australians aged 2 years and above

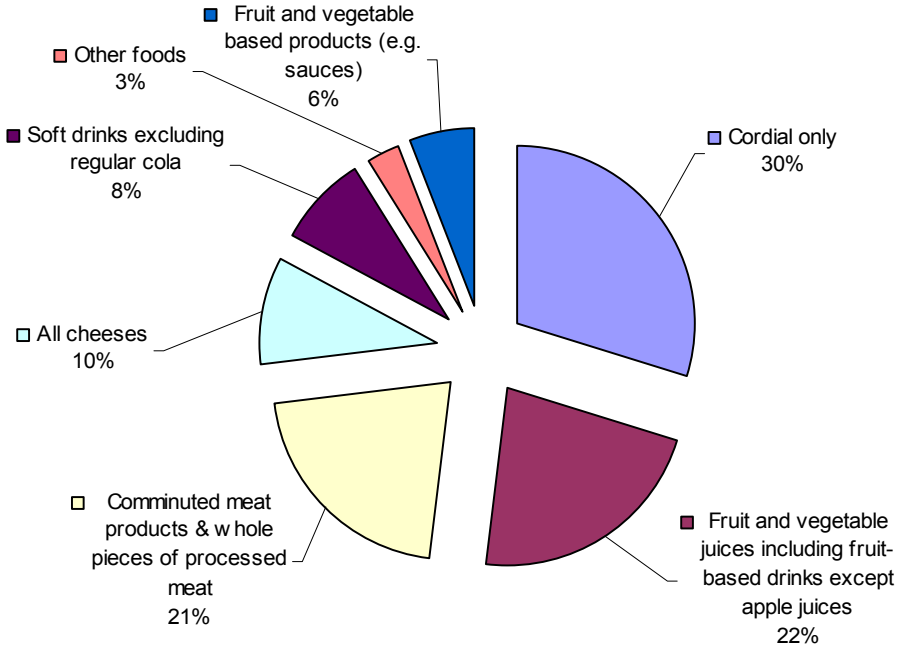

Figure 3.3: Major contributors to ethyl lauroyl arginate dietary exposures for Australians aged 2 to 6 years

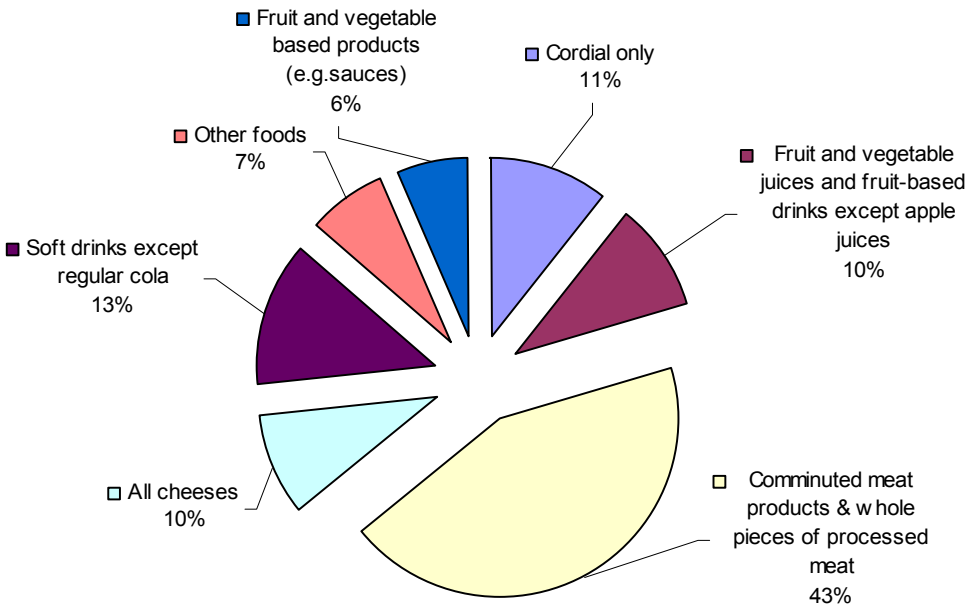

Figure 3.4: Major contributors to ethyl lauroyl arginate dietary exposures for New Zealanders aged 15 years and above

Comparison of the estimated dietary exposures with the reference health standard

In order to determine if the levels of dietary exposure to ethyl lauroyl arginate 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 – 5 mg/kg bw (Figure 3.5 and Table 3.4).

In summary, all estimated dietary exposures were below the ADI for the population groups assessed.

The estimated mean dietary exposures for consumers of ethyl lauroyl arginate were the lowest for New Zealanders aged 15 years and above (9% of the ADI) and Australians aged 2 years and above (14% of the ADI) and were the highest for Australian children aged 2 to 6 years at 39% ADI. The estimated 90th percentile dietary exposures for consumers were lowest at 21% of the ADI for New Zealanders aged 15 years and above followed by Australians aged 2 years and above at 31% of the ADI and highest for Australian children 2 to 6 years old at 78% of the ADI.

Conclusion

The dietary exposure assessment shows that if the permissions for ethyl lauroyl arginate are included in the Code, consumers of ethyl lauroyl arginate including children are unlikely to exceed the Acceptable Daily Intake (ADI) of 0 – 5 mg/kg bodyweight. All estimated dietary exposures for the population groups assessed were below the ADI, even when it was assumed that ethyl lauroyl arginate was in 100% of all permitted foods at the maximum permitted level.

Table 3. 4: Estimated mean and 90th percentile exposures to ethyl lauroyl arginate as a % of the ADI

Country	Population	Mean all respondents	Mean consumers	90th percentile consumers
		% ADI	*% ADI*	*% ADI*
Australia	Whole population	12	14	31
	2 to 6 years	37	39	78
New Zealand	Whole population	8	9	21

Consumers include the people who have consumed a food that contains ethyl lauroyl arginate
Respondents include all members of the survey population whether or not they consumed a food that contains ethyl lauroyl arginate
Acceptable Daily Intake (ADI) = 0 – 5 mg/kg bw

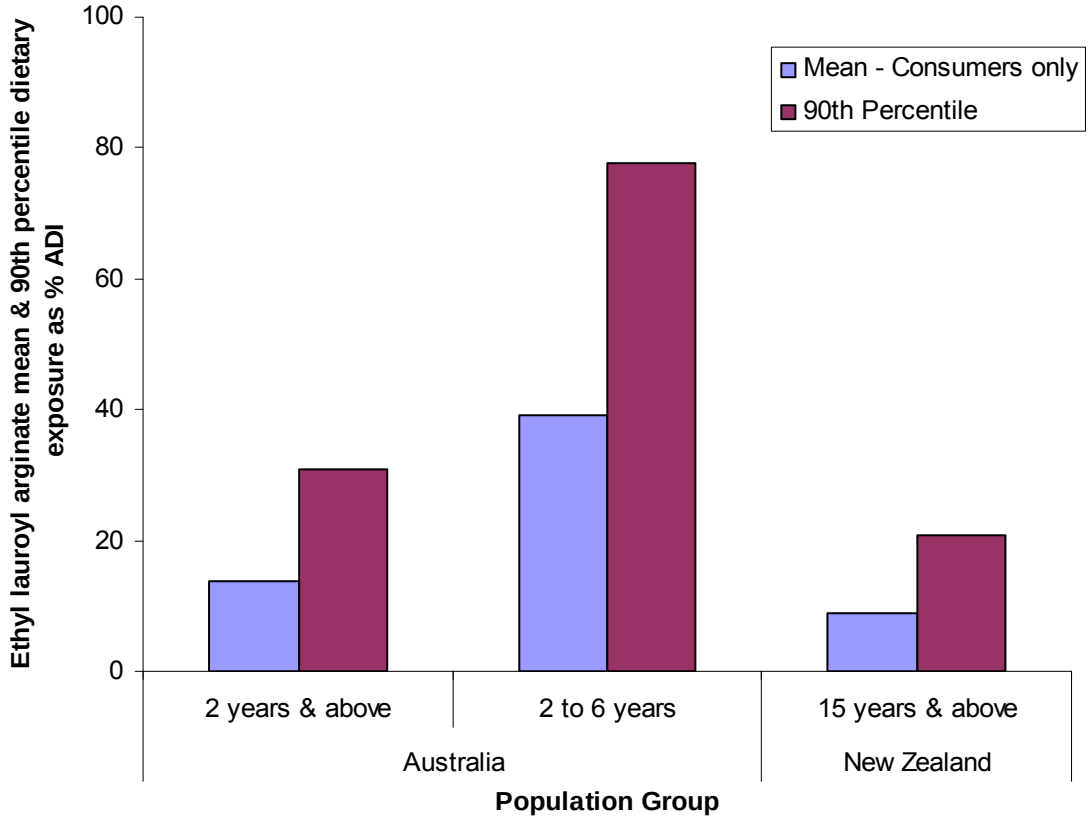

Figure 3.5: Estimated mean & 90th percentile dietary exposures to ethyl lauroyl arginate as a percentage of the ADI (consumers only)

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Attachment 4

Antimicrobial Resistance Assessment Report

Potential for microbial resistance

Microorganisms have the ability to adapt to a variety of physical and chemical environments. Tolerance, or resistance, of microorganisms to specific antimicrobial agents may be due to intrinsic factors, such as the nature and properties of cellular membranes, or be acquired through genetic mutation and/or acquisition of transferable genetic material (e.g. plasmids and transposons) (McDonnell and Russell, 1999). Variable levels of resistance of microorganisms to a wide range of antimicrobial agents, including disinfectants and preservatives, have been reported in the scientific literature (Potenski *et al*., 2003; Kramer *et al*., 2006; Capita *et al*., 2007; Plumridge *et al*., 2008).

Microorganisms that show a low-level resistance to an antimicrobial agent may be preferentially selected over sensitive populations, particularly when exposed to sub-lethal levels (i.e. below the minimum inhibitory concentration). If microorganisms were to develop resistance to an antimicrobial agent, their growth would no longer be inhibited in products where the antimicrobial had been added, and manufacturers would need to institute alternative procedures to mitigate microbiological growth.

While there is an absence of data in the peer-reviewed literature on the selection and/or development of microorganisms resistant to ethyl lauroyl arginate, resistance to other cationic surfactants, such as quaternary ammonium compounds, has been reported. The Applicant provided unpublished data from a laboratory study investigating the potential for *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Candida albicans* to develop resistance to ethyl lauroyl arginate. Microorganisms were cultured in a series of media containing increasing concentrations of ethyl lauroyl arginate, starting with levels below the minimum inhibitory concentration. The results showed that microorganisms increased their resistance to ethyl lauroyl arginate over time, however, this response was considered to be a physiological adaption of the microbial population to the stress from the presence of the antimicrobial. This adaption was temporary, as resistant cultures quickly became susceptible following growth in ethyl lauroyl arginate-free media.

It has been suggested that resistance of microorganisms to cationic surfactants and other biocides, may also confer resistance with certain antibiotics, although results from studies are inconclusive (McDonnell and Russell, 1999; Ishikawa *et al*., 2002; Joynson *et al*., 2002). For cross-resistance to occur, the organism must possess a common mechanism of resistance to both types of antimicrobial agents, for example up-regulation of efflux pumps or changes in membrane permeability (Poole, 2002). Evidence suggesting exposure of microorganisms to biocides at sub-lethal concentrations leads to increased antibiotic resistance is based primarily on results from in-vitro studies, with very few studies being undertaken in-situ. This raises questions around the complex interaction of biocides with microorganisms in various matrices, and the survival of resistant microorganisms in the environment compared with wild-type strains. There is also a lack of epidemiological data to indicate the public health significance of cross-resistance (Fraise, 2002).

The Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) – an independent committee that provides scientific advice to the European Commission – recently reviewed the literature in relation to the antibiotic resistance effects of biocides (SCENIHR, 2009). It concluded that current scientific evidence does indicate that the use of certain active substances in biocidal products in the health care, consumer, animal and food settings, may contribute to the increased occurrence of antibiotic resistant bacteria.

The committee also acknowledged that there was a lack of data and methodologies to clearly indentify the risks arising from the use, or misuse, of biocides.

In summary, while there is a potential for resistance of microorganisms to antimicrobial agents, such as ethyl lauroyl arginate and other preservatives used in food production, this can be minimised through proper management and monitoring of their use. These measures include the setting of appropriate maximum limits and following the principles of GMP – i.e. the quantity of additive added to food shall be limited to the lowest possible level necessary to accomplish its desired effect.

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Attachment 5

Food Technology Report

A1015 – Ethyl lauroyl arginate as a food preservative

Executive Summary

Ethyl lauroyl arginate is a synthetically produced chemical compound. Its active component is a cationic surfactant, ethyl-N^{α}-lauroyl-L-arginate·HCl, which has a broad spectrum of activity against bacteria, yeasts and moulds.

The Application requested ethyl lauroyl arginate as a preservative in a wide range of food groups as listed below:

- Food additive preparations
- Cheeses – soft, cream, processed, mozzarella, hard and semi hard
- Peeled and/or cut fruit and vegetables – rehydrated legumes
- Cereal products – cooked rice, noodles and pasta
- Semi processed fish and fish products – salted fish and roe
- Processed meat, poultry and meat products in whole or cut pieces or comminuted products
- Non-alcoholic beverages - fruit and vegetable juices and juice products (not including apple juice), water based flavoured drinks and high energy drinks and soft drinks
- Savoury toppings or fillings, dairy based desserts, dips and snacks

Within these foods, the Applicant proposed ethyl lauroyl arginate, expressed as the active ingredient, ethyl-N^{α}-lauroyl-L-arginate·HCl to be used in levels ranging between 50 mg/kg (e.g. beverages) and 400 mg/kg (in protein based foods, e.g. cheese and fish products).

The Applicant provided 36 experimental studies, 32 of which contain Confidential Commercial Information (CCI), to support their claims that ethyl lauroyl arginate effectively suppresses a broad spectrum of microorganisms in a wide range of food matrices. The Applicant provided information to demonstrate ethyl lauroyl arginate may be a potential alternative for some of the currently approved preservatives such as sulphites, benzoates and sorbates, which have some inherent limitations.

The data provided by the Applicant supplemented with published peer reviewed information indicate that ethyl lauroyl arginate is an effective food preservative to extend shelf life of foods in the food groups proposed above and that it also reduces the levels of certain pathogenic bacteria. This new antimicrobial agent is stable in storage and processing of a range of food groups.

Use of ethyl lauroyl arginate as a preservative in the specified food types up to the maximum requested level is technologically justified and along with good manufacturing practice could be a useful component of food preservation systems.

Contents of food technology report

1. Introduction

An Application was received from Laboratarios Miret SA on 28 August 2008. The Applicant seeks the listing of a new food additive, ethyl lauroyl arginate, in Schedule 1 of Standard 1.3.1 (Food Additives) of the *Australia New Zealand Food Standards Code* (the Code).

Ethyl lauroyl arginate is used as a chemical food preservative to protect food against growth of micro-organisms including food spoilage and to improve the storage capabilities of food products. Its active component, ethyl-N^{α}-lauroyl-L-arginate·HCl, is a cationic surfactant which has a wide spectrum of activity against Gram positive and negative bacteria, yeasts and moulds. It is therefore proposed to be used in a wide range of foods.

2. Ethyl lauroyl arginate

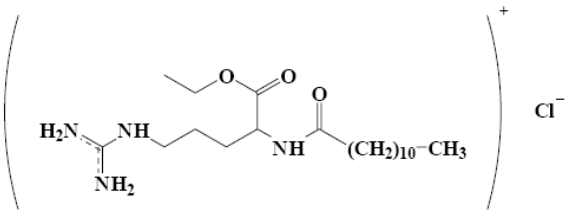
Chemistry

The active ingredient of ethyl lauroyl arginate is the hydrochloride salt of ethyl-N^{α}-lauroyl-L-arginate (ethyl-N^{α}-lauroyl-L-arginate·HCl, CAS number 60372-77-2). Ethyl lauroyl arginate contains between 85-95% of this active ingredient and it is a white powder.

The other names for ethyl lauroyl arginate are:

Lauric arginate ethyl ester
Lauramide ethyl ester
LAE
INS No. 243
Lauric arginate (Trade name)

The active ingredient is described as follows:

Chemical name:	ethyl-N^{α}-lauroyl-L-arginate·HCl
IUPAC name:	ethyl-N^{α}-dodecanoyl-L-arginate·HCl
C.A.S. number:	60372-77-2
Chemical formula:	$C_{20}H_{41}N_4O_3Cl$
Structural formula:	
Formula weight:	421.02 g/mol
Assay:	Not less than 85% and not more than 95%

Physical properties

Physical appearance:	White powder
Solubility:	Freely soluble in water (at 20°C, solubility greater than 247 g/kg) Soluble up to 20% in propylene glycol, glycerine and ethanol
pH (1% aqueous solution):	Between 3 and 5
Melting temperature:	50.5 – 58.0°C
Boiling temperature:	Decomposes from 107°C
Stability:	Over 2 years when the solid form is stored in a closed container

Methods of analysis in foods

The amount of ethyl-N^{α}-lauroyl-L-arginate·HCl (the active) in food matrices can be measured by Reverse Phase High Performance Liquid Chromatography (RP-HPLC). Different sample preparation techniques are required, which depend on the nature of the food matrix to be analysed (i.e. solid, semi-solid or liquid foods).

Stability

Ethyl lauroyl arginate as manufactured is a powdered substance and was shown to have a shelf life of more than two years when kept in a closed container at room temperature. However, the product is to be sold in a solution form with ethyl lauroyl arginate dissolved in appropriate carriers such as water, propylene glycol, glycerine and ethanol.

The stability of ethyl-N^{α}-lauroyl-L-arginate·HCl in aqueous solution has been evaluated under different pH conditions at 25°C. Results showed the active is most stable at pH 4, with a half life of greater than 1 year. Its half life decreased drastically at higher pH at that same temperature; that is, 57 days at pH 7 and 34 hours at pH 9.

The Applicant tested the stability of ethyl-N^{α}-lauroyl-L-arginate·HCl dissolved in propylene glycol at pH of 0.5, 1, 1.5, 2, 2.5, 3 and 3.5 and temperatures of 4°C, 25°C and 50°C. The tests included different food acids such as phosphoric, citric, tartaric, malic and fumaric acids.

Results showed that high temperature (i.e. 50°C) combined with very low pH (less than 3) causes the hydrolysis of ethyl-N^{α}-lauroyl-L-arginate·HCl to N^{α}-lauroyl-L-arginine (LAS) as the main product. Further hydrolysis of LAS produced arginine and lauric acid. However, the active compound was relatively stable at room temperature even at low pH.

It was concluded that ethyl lauroyl arginate should not be used in a food application that combines high temperatures (e.g. 50°C) and low pH (<3) for a period of time in excess of 10 days. The Applicant pointed out that the conditions studied above are unlikely conditions to be experienced in the proposed used of ethyl lauroyl arginate and therefore its stability would not be an issue under these storage conditions.

Stability of ethyl lauroyl arginate combined with other components

The Applicant evaluated the potential interaction of the active ingredient, ethyl-N^{α}-lauroyl-L-arginate·HCl, with other components in foods such as hydrocolloids, food preservatives and antioxidants, enzymes, colour additives and proteins or protein extracts. Out of a total of 33 samples, nine showed interaction between the active and the compounds that constituted the sample (EFSA 2007).

In four of these samples, ethyl-N^{α}-lauroyl-L-arginate·HCl was shown to decrease over time due to its hydrolysis to LAS (the main hydrolysis product). The remaining five samples showed interaction with other components including meat, soya proteins, ovo-albumin and lacto-albumin, resulting in degradation of ethyl-N^{α}-lauroyl-L-arginate·HCl to ethanol, arginine and lauric acid. Interaction between ethyl-N^{α}-lauroyl-L-arginate·HCl and nitrite was observed but the applicant indicated that no nitrosamines were detected.

Stability of ethyl lauroyl arginate within different food matrices

A further stability study was conducted on eight different food matrices, three uncooked foods and five processed foods. Ethyl-N^{α}-lauroyl-L-arginate·HCl was found to be stable through the duration of shelf life of all processed foods but a decrease was seen in the uncooked foods. This is because ethyl-N^{α}-lauroyl-L-arginate·HCl is subjected to enzymatic action and undergoes hydrolysis by the presence of inherent enzymes found in chickpeas, marinated meats, dried and salted cod and potentially other foods. As a result, a higher level of ethyl lauroyl arginate was used in these foods during the study to achieve the required shelf life.

3. Manufacturing

The manufacturing process

A Spanish patent application (ES-A-512643) (Beltran and Bonaventura 2001) and European Patent No. 1294678 describe how ethyl lauroyl arginate is produced (Kawamura and Whitehouse, 2008).

The manufacturing process involves esterification of the carboxyl group of L-arginate·HCl with ethyl alcohol, utilising thionyl chloride as the esterification agent, with ethyl arginate 2HCl as a resulting product in this step. The next step involves condensation of lauroyl chloride with the α-amino group of ethyl arginate·2HCl in an aqueous medium. The final production step of ethyl lauroyl arginate is the filtration of the reaction mixture through a press filter. After the filtration process, a white solid is obtained with the active ingredient content of between 71% and 81% and a water content of 12 to 19%. This final mixture can be further dried to produce a product with an active content of between 85 and 95%. The possible impurities are residual materials and by-products of the reactions and they are listed in the Product Specification below (Table 1).

Specifications

Table 5.1: Specification for ethyl lauroyl arginate

Compound	Purity
Ethyl-N^{α}-lauroyl-L-arginate·HCl	Between 85% and 95%
N^{α}-lauroyl-L-arginine	Not more than 3%
Lauric acid	Not more than 5%
Ethyl laurate	Not more than 3%
L-arginine·HCl	Not more than 1%
Ethyl arginate·2HCl	Not more than 1%
Ash	Not more than 2%
Water	Not more than 5%
Ethanol	Not more than 0.2%
pH of 1% solution	Not less than 3 and not more than 5
Arsenic	Not more than 3 mg/kg
Cadmium	Not more than 1 mg/kg
Lead	Not more than 1 mg/kg
Mercury	Not more than 1 mg/kg

The commercial product of this Application, ethyl lauroyl arginate complies with a relevant monograph published in the FAO Combined Compendium of Food Additive Specifications (Monograph 5) (JECFA, 2008). Monograph 5 is not yet a primary source of product specification, as required in Clause 2 of Standard 1.3.4 – Identity and Purity of the Code. FSANZ proposes to update Clause 2 to include reference to Monograph 5.

Allergenicity

No allergenic materials (as listed in the Code on Table to Clause 4, Standard 1.2.3) are likely to be present in the manufacture of this food additive.

Commercial preparations

Commercial products are formulations comprising of 20 – 25% solutions of ethyl lauroyl arginate in appropriate food grade solvents e.g. water, ethanol, propylene glycol, isopropyl alcohol, other glycols or mixtures of these. Examples of commercial product names are: Mirenat-N, Mirenat-NA, Mirenat-TT, Mirenat-LA and Mirenat-G.

4. Antimicrobial activity

Mode of Action

Ethyl lauroyl arginate is a cationic surfactant. The antimicrobial properties of ethyl lauroyl arginate include the reduction of surface tension and the formation of ionic aggregates leading to changes in the conductivity and solubility of cell membranes (Rodriguez *et al*., 2004). The disruption of proteins in the cellular membrane can lead to leaking of ions and other cellular constituents resulting in permanent alterations in cell permeability and subsequent inhibition of growth, or inactivation, of the microorganism. Ethyl lauroyl arginate is reported to have a broad spectrum of activity against Gram-negative and Gram-positive bacteria, yeasts and moulds (Bakal and Diaz, 2005).

The level of action of cationic surfactants against specific microorganisms is influenced by cell structure and physiology. Sakagami *et al*. (1989) reported that an increased quantity of phospholipids, fatty acids and neutral lipids in cell membranes inhibits the penetration of cationic surfactants. Another mechanism that has been associated with reduced sensitivity includes the increased activity of efflux pumps which act by reducing intracellular surfactant concentrations (Ishikawa *et al*., 2002).

Rodriguez *et al.* (2004) studied the structural alterations of cell membranes and subsequent changes in membrane potential following exposure of *Salmonella typhimurium* (ATCC 14028) and *Staphylococcus aureus* (ATCC 6538) to ethyl lauroyl arginate. Cell membrane damage was analysed by staining cells with fluorescent nucleic acid dyes: SYTO-13 which penetrates all cellular membranes and propidium iodide (PI) which only penetrates damaged membranes (non-viable cells).

Following exposure of *S*. *typhimurium* to ethyl lauroyl arginate at the minimum inhibitory concentration (MIC) of 32 μg ml^{-1} for 30 min, up to 94% of the population were stained with PI. For *S. aureus* treatment with 8 μg ml^{-1} LAE (MIC) for 30 min resulted in 43% of the population being stained with PI, however there was also a subpopulation of 21% that was double stained, indicating partially damaged membranes. Loss of viability was confirmed using conventional culture techniques.

Changes in membrane potential were determined by measuring the proton flux across the cell membrane and leakage of potassium ions. The flow of protons in cells treated with LAE was slightly less than that for untreated samples however this was not statistically significant. Leakage of potassium ions was rapid following exposure to LAE. Despite these observed

structural changes in membrane integrity, it was reported that cells remained intact when viewed by electron transmitting microscopy.

5. Technological Justification

The Applicant proposed that ethyl lauroyl arginate be permitted as a preservative in a range of foods and claimed that it may be used alone or in conjunction with other food preservatives such as sorbates, benzoates, sulphites and nitrates/nitrates.

There has been limited peer-reviewed published evidence describing the use of ethyl lauroyl arginate in food products because of the novelty and originality of the food preservative.

The Applicant has submitted the following studies to support their claims of the efficacy of ethyl lauroyl arginate in preserving cheese, meats, vegetables, beverages and other foods. Some of these studies contain confidential commercial information and hence full details cannot be disclosed in this report.

Milk and dairy products

The Applicant has requested that ethyl lauroyl arginate be permitted in meat and dairy products to be used at levels ranged from 225 to 450 mg/kg. The Applicant has provided data to demonstrate that ethyl lauroyl arginate was effective as an antimicrobial agent in the following studies conducted on different cheese:

- Studies conducted with hard cheese show the ethyl lauroyl arginate is an effective preservative in preventing microbial growth (especially the mould) on cheeses when used as a surface treatment. It was applied by dipping the cheese in an aqueous solution. Ethyl lauroyl arginate was found to migrate about 1 mm into hard cheeses (e.g. parmesan and Granda Padano). In these studies, no hydrolyses of ethyl lauroyl arginate was found to have taken place.

- Studies conducted with the soft cheese mascarpone showed that at the higher moisture content of soft cheese in the presence of milk proteins, it is more likely for the ethyl lauroyl arginate to interact with the milk protein and become inactivated within the cheese. However, its effectiveness in inhibiting growth of mesophilic aerobes was achieved by increasing the concentration of the preservative in the cheese to 500 mg/kg.

- Studies conducted with mozzarella (with ethyl lauroyl arginate added to the brine in which mozzarella is stored) showed that when ethyl lauroyl arginate was added at levels between 400 and 800 mg/kg to the brine, microbial counts (*Pseudomonas aeruginosa, Enterobacter earogenes and E. coli*) in both the brine and the solid cheese were reduced. The Applicant has requested a maximum concentration of 225 mg/kg of ethyl lauroyl arginate for mozzarella, which is resulted from soaking the cheese in brine with a concentration of 400 mg/kg of ethyl lauroyl arginate.

- Studies conducted with ricotta cheese inoculated with *Listeria monocytogenes* showed that treatment with ethyl lauroyl arginate resulted in a 2 logarithmic reduction over the period of a week.

- Studies were conducted on gorgonzola cheese by the Applicant to illustrate the need to maintain control of microbial populations throughout the production process and use of ethyl lauroyl arginate as a preservative to achieve this. The effect of using

preservative throughout the entire process and on the product was shown to inhibit growth of both mesophilic aerobes and *Listeria* spp.

The use of ethyl lauroyl arginate in dairy products may be limited by its reaction with the protein, casein, which can lead to the formation of a precipitate and consequent loss of activity. The Applicant has recommended that ethyl lauroyl arginate is not to be used in liquid milk products.

Vegetables

Studies have been conducted on various vegetables showing ethyl lauroyl arginate to be an effective food preservative including in rehydrated chickpeas, carrots and prepared salads.

- Chickpeas soaked in a bath of water containing 100 mg/kg ethyl lauroyl arginate showed inhibition of fermentation and reduction of the total viable microbiological counts in the chickpeas and the soaking bath water compared with an untreated control.

- Carrots dipped in a bath containing ethyl lauroyl arginate showed that the treated carrots exhibited a significant reduction in aerobic mesophiles count.

- A prepared ready-to-eat salad including washed, sliced, chopped or shredded vegetables combined with a dressing was treated with 200 mg/kg ethyl-N^{α}-lauroyl-L-arginate·HCl). The same salad was compared with an untreated control salad and a sodium benzoate plus potassium sorbate treated salad. The results indicated that ethyl lauroyl arginate provided a preservative effect in inhibiting growth of aerobic microorganism and enteric bacteria in the ready-to-eat salad at a level similar to that of sodium benzoate plus potassium sorbates for up to 30 days duration.

Meat and meat products

Ethyl lauroyl arginate has been shown to be an effective preservative in ham, stewed veal, marinated meat, smoked turkey slices, roast turkey slices and bratwurst sausages.

- Commercially-prepared hams treated with ethyl lauroyl arginate and utilising an innovative delivery method (the 'Sprayed Lethality In Container' – SLIC) when storing ham in shrink wrap showed that the application of the preservative appreciably reduced *L. monocytogenes* levels (Luchansky *et al.,* 2005).

- It has been reported that the preservative was effective in inhibiting the growth of *L. monocytogenes* on refrigerated cooked meats (Bakal and Diaz, 2005).

- Stewed veal vacuum sealed in a plastic bag inoculated with a mixture of bacteria (*E. coli, E. aerogenes, S. aureus, B cereus, C. albicans*) and yeasts (*Saccharomyces bailii*) was evaluated and showed that an added ethyl lauroyl arginate treatment at 100 mg/kg suppressed microbial growth more than nitrite treated or control veal. The shelf life of the stewed veal was increased from 14 days to 1 month by the treatment.

- Marinated non-cooked cured meat treated with 180 mg/kg ethyl lauroyl arginate was compared with marinated meat treated with an alternative preservative, sodium nitrite ($NaNO_2$), at 150 mg/kg. Results indicated that ethyl lauroyl arginate suppressed the growth of mesophilic aerobes in marinated meat more effectively than sodium nitrate over the two week trial.

- Sliced smoked turkey topically treated with ethyl lauroyl arginate showed a significant reduction of *L. monocytogenes* population over the 6 week study duration compared to untreated samples. Ethyl lauroyl arginate was also found to inhibit the growth of aerobic spoilage bacteria and thus has the potential to extend the shelf life of refrigerated sliced smoked turkey. Similar reductions in *L. monocytogenes* were also obtained from the same study design conducted on sliced roast turkey; however, the effect of ethyl lauroyl arginate on suppressing *L. monocytogenes* lasted only 2 weeks.

- Bratwurst sausages treated with 100 mg/kg of ethyl lauroyl arginate in the sausage mix significantly reduced the total aerobic bacteria count over the 90 day study. Aerobic bacteria counts remained the same inside both ethyl lauroyl arginate treated sausages and non-treated control sausages, but the growth of *Clostridium sp* was significantly reduced in the treated samples.

Fish

The Applicant has shown that ethyl lauroyl arginate is an effective preservative in dried and salted cod and fish roe in 9-day trial studies.

- Adding 80-160 mg/kg ethyl lauroyl arginate to a desalting bath during rehydration of salted cod has proven to be effective in reducing microbial spoilage and unpleasant odours compared to untreated controls.

- *S. aureus, E. coli* and total bacteria counts were significantly reduced by ethyl lauroyl arginate and benzoic acid (both at 200 mg/kg) treated roe compared to the untreated control over a 9 day study.

Processed foods

Ethyl lauroyl arginate has been shown to be effective as a preservative in a broad range of prepared foods, including:

- Refrigerated soups, in which ethyl lauroyl arginate added at 200 mg/kg, significantly reduced the level of aerobic mesophiles.

- Sauces with added ethyl lauroyl arginate showed suppressed microbial count: guacamole (300 mg/kg), fresh tomato sauce (200 mg/kg) and pizza topping (200 mg/kg).

- Pastries and bakery products with ethyl lauroyl arginate at 100-200 mg/kg added to their sugar coatings showed suppressed growth of fungi in the sugar coatings.

- Cooked pasta treated with 100 mg/kg ethyl lauroyl arginate exhibited 5 more days of shelf life.

- Cooked rice treated with 100-300 mg/kg ethyl lauroyl arginate had reduced viable counts compared to both potassium sorbate treated and untreated samples.

Beverages

The Applicant has provided studies on the use of ethyl lauroyl arginate as a preservative in the following beverages:

- A carbonated orange juice drink where ethyl lauroyl arginate was used at 50 mg/kg was found to have complete inhibition of *Saccharomyces bailii, Zygosaccharomyces bailii* (a resistant yeast) and mesophilic aerobes over 9 weeks storage period.

- Fruit based concentrates with 100 mg/kg ethyl lauroyl arginate demonstrated effective control against yeast growth

- Sport drinks (citrus flavour) and flavoured tea based drinks treated with 50 mg/kg ethyl lauroyl arginate is sufficient to inhibit yeast, resistant yeast, mould and mesophile bacteria. The Applicant claims that ethyl lauroyl arginate has the potential to replace benzoate and sorbate in these drink categories.

6. Regulatory status

JECFA

- In June 2008, during the 69th Session of JECFA, JECFA reviewed the proposal of LAMIRSA on ethyl lauroyl arginate as a food additive and allocated an ADI of 4 mg/kg bw/day for ethyl lauroyl arginate.

European Food Safety Authority

- In April 2007, EFSA issued the opinion of the Scientific Committee on ethyl lauroyl arginate as a new food preservative for use in a range of food categories. Based on NOAEL, EFSA established an ADI of 0.5 mg LAE/kg bw/day.

European List of Notified Chemical Substances (ELINCS)

- In 2000, European Directive 67/548/EEC notified ethyl lauroyl arginate as a new substance. The registration of the dossier was 00-11-0173 and L.A.E. was designated as the trade name of ethyl lauroyl arginate. The EC number assigned within ELINCS is 434-630-6.

Authorisation in the US

- In 2006, the Food Safety and Inspection Service (FSIS) Directive 7120.1 summarised the approval that ethyl lauroyl arginate is a safe and suitable ingredient for comminuted meat products, fresh cuts and ready-to-eat meat and poultry products.

- In September 2005, the US FDA issued a Letter of No Objection regarding a submission that LAE is Generally Recognised as Safe (GRAS, Notice No. GRN 000164) for use as an antimicrobial at levels up to 200 mg/kg ethyl-N$^{\alpha}$-lauroyl-L-arginate·HCl in meat and poultry products.

- In March 2005, the Applicant submitted a GRAS Notice to FDA and a complementary document establishing the efficacy of ethyl lauroyl arginate in meat and poultry products.

Authorisation in Mexico

- In July 2007, the Health Secretary of Mexico published in its Official Journal that ethyl lauroyl arginate is an allowed substance to be used as a food additive for human consumption.

7. Conclusion

Based on an assessment of the data provided to FSANZ, ethyl lauroyl arginate fulfils the technological purpose of a food preservative in a variety of foods.

The data provided by the Applicant supplemented with published peer reviewed information indicate that ethyl lauroyl arginate is an effective food preservative at the proposed usage levels in the following food categories cheeses, meats, semi preserved fish and fish products, vegetable products, salads, some prepared foods and beverages. This preservative is effective against a broad range of microorganisms and is relatively stable in a variety of food matrices. Table 5.2 (below) specifies the proposed food groups and proposed maximum allowable levels of ethyl lauroyl arginate.

Table 5.2: Intended uses of ethyl lauroyl arginate

Food types		Ethyl lauroyl arginate* (mg/kg; maximum)
0.1	Preparations of food additives	200
1.6	Cheese - soft/cream/processed and mozzarella	400 except for mozzarella at 200
1.6	Cheese – Hard/Semi-hard	1 mg/cm^2 of surface area of cheese (taken to a depth of 3 mm and not more than 5 mm)
4.1.3	Peeled and/or cut fruits and vegetables	200
4.3.8	Processed fruits and vegetables—rehydrated legumes only	200
6.3	Processed cereal and meal products-cooked rice only	200
6.4	Flour products (including noodles and pasta) – cooked pasta and noodles only	200
8.2	Processed meat, poultry and meat products in whole cuts or pieces	200
8.3	Processed comminuted meat and poultry products	315
9.3	Semi preserved fish and fish products	400
14.1.2	Fruit and vegetable juices and fruit and vegetable juice products (NOT apple juice)	50
14.1.3	Water based flavoured drinks	50
20.2	Savoury toppings or fillings - essentially sauces such as tomato paste used in ready to eat pizzas, etc.	200
20.2	Dairy and fat based desserts, dips and snacks	400

* Ethyl lauroyl arginate shall be calculated as ethyl-N^{α}-lauroyl-L-arginate·HCl.

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