European Food Safety Authority

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Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food on a request from the Commission related to an application

on the use of ethyl lauroyl arginate as a food additive

Question number EFSA-Q-2006-035

Adopted on 17 April 2007

SUMMARY

The Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food has been asked to evaluate the safety in use of ethyl lauroyl arginate as a food preservative for use in the food categories specified in the dossier.

The active ingredient of ethyl lauroyl arginate, ethyl-N^{α}-lauroyl–L-arginate HCl, is the hydrochloride salt of an N-fatty acylsubstituted amino acid ethyl ester.
Ethyl lauroyl arginate is intended to be used as a preservative. The anti-microbial activity of ethyl lauroyl arginate is due to the cationic surfactant properties of its active ingredient ethyl-N^{α}-lauroyl-L-arginate.

Ethyl lauroyl arginate has been shown by *in vivo* and *in vitro* studies in rats and humans to be rapidly metabolised by hydrolysis of the ethyl ester and lauroyl amide to the intermediate products, arginine ethyl ester and N^{α}-lauroyl-L-arginine, and then to ethanol, lauric acid and arginine. Arginine undergoes natural amino acid catabolism to urea and ornithine. Ornithine can then be further metabolised to CO_2 and urea. Lauric acid is a fatty acid that can enter normal fatty acid metabolism. Ethanol can be converted by alcohol dehydrogenase and aldehyde dehydrogenase to acetate, which can enter normal biochemical pathways. It is concluded that, on ingestion by humans, ethyl lauroyl arginate will be broken down to products of normal metabolism.

The Panel considered the bacterial reverse mutation assay study could not be used for evaluation of mutagenicity due to the high toxicity of ethyl-N^{α}-lauroyl-L-arginate towards the bacterial cells. The Panel noted that this toxicity was predictable since ethyl-N^{α}-lauroyl-L-arginate is a preservative with antimicrobial activity. Based on the results from the mouse lymphoma L5178Y cell mutation test and from the test for chromosomal aberrations in human lymphocytes it is concluded that ethyl-N^{α}-lauroyl-L-arginate is not genotoxic in mammalian cells. It showed no evidence of a genotoxic effect in the *in vivo* mouse micronucleus test.

According to the available evidence, ethyl lauroyl arginate is devoid of reproductive and developmental toxicity. Long-term carcinogenicity studies are lacking. However, the rapid metabolism of ethyl-N^{α}-lauroyl-L-arginate to compounds endogenously present in much higher levels, the absence of preneoplastic toxic effects in the *in vivo* studies performed, together with the absence of genotoxic activity in the mouse lymphoma assay, the human lymphocyte assay and the micronucleus test, does not suggest a carcinogenic potential. Therefore the Panel concludes that there is no need to perform carcinogenicity studies.

The Panel notes that effects on white blood cells were seen in different rat strains and in different sexes in two 90-day studies and in the 52-week study and concludes that these effects cannot be disregarded.
Therefore the Panel concludes, given the fact that the effects on white blood cell counts at 26 weeks are significant for all dose groups, that the NOAEL for this 52 week study is lower than the lowest dose levels tested, and thus lower than 106 mg/kg bw/day.
This is in line with the NOAEL of 47 and 56 mg ethyl lauroyl arginate /kg bw/day for males and females respectively from the 13 week study with the 19.5 % formulation of ethyl lauroyl arginate in propylene glycol.
Based on this NOAEL and a safety factor of 100, the Panel established an ADI of 0.5 mg ethyl lauroyl arginate of the proposed specifications /kg bw.
The safety factor of 100 is considered sufficient in spite of the fact that the ADI is based on a 90-day study because the effects on white blood cells do not become more severe upon prolonged exposure.

Potential dietary exposure to ethyl lauroyl arginate was estimated based on UK food consumption data and on the assumption that it would be present in all food categories for which use levels are proposed. Potential dietary exposure was found to be at or above the ADI in high consumers for both children aged 1.5 to 4.5 (580% of the ADI), children aged 4 to 18 (370% of the ADI) and adults (100% of the ADI). Potential mean dietary exposure to ethyl lauroyl arginate in consumers only was also at or above the ADI for both children aged 1.5 to 4.5 (170% of the ADI) and children aged 4 to 18 (106% of the ADI).

KEY WORDS

Ethyl lauroyl arginate, food additive, lauramide arginine ethyl ester, CAS Registry Number 60372-77-2.

BACKGROUND

Laboratorios Miret S.A. (LAMIRSA) has requested the authorisation of lauric arginate under Directive 95/2. This additive is to be used as a preservative in different food categories, e.g. non-alcoholic flavoured drinks containing fruit juices, energy and sports drinks, meat products, in use levels in the range of 115-225 ppm.

Lauric arginate has been notified as a new substance in accordance with the Council Directive 67/548/EEC of 27 June 1967 on the approximation of laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances, under the registration number 00-11-0173 and the EC number assigned within ELINCS is 434-630-6.

Lauric arginate has been previously evaluated for food safety as an antimicrobial in food by the US Food and Drug Administration (FDA) and for efficacy in meat and poultry products by the United States Department of Agriculture (USDA). Additionally, the Scientific Committee on Consumer Products (SCCP) issued an opinion on the safety of ethyl lauroyl arginate (lauric arginate) when used as a preservative in cosmetics, in 15th March 2005 (http://ec.europa.eu/health/ph_risk/committees/04_sccp/docs/sccp_o_017.pdf).

TERMS OF REFERENCE

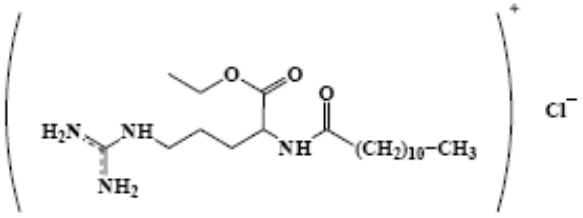
In accordance with Article 29 (1) (a) of Regulation EC No178/2002, the European Commission asks the European Food Safety Authority to provide a scientific opinion on the safety in use of lauric arginate as a food preservative for use in the food categories specified in the dossier.

ASSESSMENT

In the original dossier submitted by the applicant, the background and the terms of reference, the material is refered to as lauric arginate. However, the Panel notes that it would be preferable to name the product ethyl lauroyl arginate or lauramide arginine ethyl ester. Therefore the material is refered to as ethyl lauroyl arginate throughout the opinion.

Chemistry

The active ingredient of ethyl lauroyl arginate is the hydrochloride of the N-fatty acylsubstituted amino acid ethyl ester. Its chemical name is ethyl-N^{α}-lauroyl–L-arginate HCl (CAS number 60372-77-2). The molecular weight of ethyl-Nα-lauroyl–L-arginate HCl is 421.0.
Its structural formula is $C_{20}H_{41}N_4O_3Cl$:



Ethyl lauroyl arginate contains between 85-95% of ethyl-N^{α}-lauroyl–L-arginate HCl and it is a white powder with a solubility in water at 20ºC greater than 247 g/kg.
Commercial products are formulations comprising 20-25% solutions of ethyl lauroyl arginate in appropriate food-grade solvents.

Manufacturing Process

Ethyl-N^{α}-lauroyl–L-arginate, is synthesised from lauric acid, L-arginine HCl and ethyl alcohol. A detailed description of the manufacturing process has been provided by the applicant.
The manufacturing process of ethyl lauroyl arginate has been validated, according to GMP. The analysis of six lots of ethyl lauroyl arginate demonstrated that the production process yields a product of consistent quality.

Specifications

Specifications for ethyl lauroyl arginate were provided by the applicant and were as follows:
ethyl-N^{α}-lauroyl-L-arginate HCl not less than 85% and not more than 95%, containing the following levels of impurities: Nα-lauroyl–L-arginine (≤ 3%), lauric acid (≤ 5%), ethyl laurate (≤ 3%), L-arginine HCl (≤ 1%), ethyl arginate 2HCl (≤ 1%), water (≤ 5%), ethanol (≤ 0.2%), arsenic (≤ 3 mg/kg), cadmium (≤ 1 mg/kg), lead (≤ 1 mg/kg), mercury (≤ 1 mg/kg). The amount of ash upon drying should be no more than 2%. The compound is soluble in deionised water, propylene glycol, glycerol and ethanol, and the pH of a 1% solution should not be less than 3 and not more than 5

Determination of the active substance, ethyl-N^{α}-lauroyl–L-arginate, and its byproducts Nα-lauroyl–L-arginine (LAS), lauric acid (LOH) and ethyl laurate (LOEt), is carried out by high performance liquid chromatography (HPLC).
During the characterisation of ethyl lauroyl arginate, the applicant has identified three additional minor by-products, which share the structure of ethyl-N^{α}-lauroyl–L-arginate but have an additional lauroyl group. Quantification of these trace impurities is not included in the specification of ethyl lauroyl arginate because the applicant indicates that their presence (at $\leq$ 1%) was demonstrated to be consistently and reproducibly visualised by HPLC when historical data from analyses of ethyl lauroyl arginate batches are compared. The Panel concludes that these trace impurities should be included in the specifications.

Ethyl lauroyl arginate is a white powder proposed to be used for food preservation. The application of ethyl lauroyl arginate in its solid form can present technical difficulties as it has to be homogenously applied in low doses and, for the food industry, it is easier to work with liquids than with solids. For this reason, the applicant has developed some formulations of ethyl lauroyl arginate dissolved in appropriate food-grade solvents in order to provide ethyl lauroyl arginate in liquid form.
Propylene glycol was initially chosen as a solvent for ethyl lauroyl arginate in commercial products as it is widely used as a solvent for food additives and ethyl lauroyl arginate is very soluble in it. However, according to European Directive 95/2/EC, propylene glycol (E-1520) is a permitted carrier for colours, emulsifiers, antioxidants and enzymes but not for food preservatives.

As ethyl lauroyl arginate is intended to be used as a food preservative, the applicant has studied the use of other solvents as a basis for formulations of ethyl lauroyl arginate. Glycerol is one of these solvents. Studies of its stability and efficacy in different food matrices performed as

described below have demonstrated that this formulation was as effective as the propylene glycol formulation.

The applicant also described the results of a pharmacokinetic study performed in rats demonstrating that the bioavailability of ethyl lauroyl arginate in rats is similar when the test substance is formulated with propylene glycol, glycerol or water as the solvent.

Methods of analysis in foods

The content of ethyl-N^{α}-lauroyl–L-arginate in food matrices can be quantified by reversed-phase, high-performance liquid chromatography (RP-HPLC). The proposed analytical methods involve the use of different sample preparation techniques depending on the type of food matrix to be analysed. Two methods are described, one for solid and semi-solid food matrices, and another method for liquid food matrices.

Reaction and fate in foods, stability

Ethyl-N^{α}-lauroyl–L-arginate present in ethyl lauroyl arginate was found by the applicant to be stable for more than 2 years at room temperature when protected in a closed container.
The stability of ethyl-N^{α}-lauroyl–L-arginate in aqueous solution has also been evaluated. The results indicate that at 25 ^{o}C ethyl-N^{α}-lauroyl–L-arginate has a half-life greater than 1 year at pH 4, 57 days at pH 7, and 34 hours at pH 9.

The applicant also provided results of studies in which the stability of ethyl-N^{α}-lauroyl–L-arginate was assessed under different conditions of pH (i.e., 0.5,1, 1.5, 2, 2.5, 3 and 3.5) and temperature (i.e., 4, 25 and 50ºC) by exposing the test substance to citric, phosphoric, tartaric, malic or fumaric acid for time periods up to 50 days. The test substance employed was a formulation of ethyl lauroyl arginate consisting of a 19.3% solution of ethyl-N^{α}-lauroyl–L-arginate HCl in propylene glycol. The results of this study suggested that the effect of temperature combined with pH markedly influences the hydrolysis of ethyl-N^{α}-lauroyl–L-arginate to LAS and products resulting from further hydrolysis including arginine and lauric acid. At low pH and at room temperature (i.e. 25ºC), this molecule is quite stable. However, the combination of high temperature (i.e. 50ºC) and low pH (i.e. 2 or 3) can result in more extensive hydrolysis. The stability of ethyl-N^{α}-lauroyl–L-arginate and LAS decreases considerably when the temperature is increased and the pH is decreased resulting in full loss of ethyl-N^{α}-lauroyl–L-arginate after 10, 20, 30 and 40 days in respectively phosphoric acid pH 0.5 at 50^{o}C, citric acid pH 0.5 at 50^{o}C, tartaric acid pH 1.5 at 50 ^{o}C and malic acid pH 1.5 at 50^{o}C. In addition, the study demonstrated that the incubation with fumaric acid resulted in the lowest percentage of hydrolysis in comparison with the other acids studied, leading to 15% residual ethyl-N^{α}-lauroyl–L-arginate at pH 2 and 50ºC.
The results of this study suggest that ethyl lauroyl arginate should not be used in applications that combine very low pH (e.g. < 2 or 3) with high temperatures (e.g. 50ºC) for extended periods of time (e.g. more than 10 to 20 days). The applicant indicates that, given the proposed uses of this product it is difficult to envision these types of conditions being experienced in practice.

In order to establish the fate of ethyl-N^{α}-lauroyl–L-arginate in food, its possible incompatibilities with different hydrocolloids, food preservatives and antioxidants, enzymes, colour additives and proteins or protein extracts have been studied. The extent of hydrolysis of ethyl-N^{α}-lauroyl–L-arginate under various conditions was determined by measurement of the

percentage of ethyl-N^{α}-lauroyl–L-arginate recovered in samples of different constitution with respect to the parameters mentioned. In 24 out of 33 samples no hydrolysis took place. Only nine samples showed interaction between ethyl-N^{α}-lauroyl–L-arginate and the other compounds that constituted the sample.

In four of these nine samples, ethyl-N^{α}-lauroyl–L-arginate was hydrolysed to Nα-lauroyl-L-arginine (LAS) (the main metabolite of the active ingredient in ethyl lauroyl arginate). In the remainder of the samples, in which ethyl lauroyl arginate was combined with nitrite, meat or soya proteins or ovo-albumin or lacto-albumin, more extensive hydrolysis occurred.
The interaction of ovo-albumin, lacto-albumin, meat and soy proteins with ethyl-N^{α}-lauroyl-L-arginate resulted in the degradation of the active ingredient to ethanol, arginine and lauric acid. The interaction observed between nitrites and ethyl-N^{α}-lauroyl-L-arginate resulted in the degradation of the active ingredient. The applicant indicates that no formation of nitrosamines was observed. The Panel notes that this may be dependent on the detection limit. The Panel also notes that both secondary amine moieties in ethyl-N^{α}-lauroyl–L-arginate come from arginine which is a consituent normally present in biomacromolecules.

The applicant also evaluated the stability of the active ingredient of ethyl lauroyl arginate in eight different food matrices. Five of these matrices were examples of processed foods and the rest were examples of fresh foods. The duration of the study was up to the shelf-life of each food type varying from 18 hours (chickpeas) to 90 days (bratwurst sausage). The active ingredient was found to be stable throughout the duration of the study, in all processed food matrices with the exception of three samples tested. These were dried and salted cod, marinated meat and chickpeas. In these three cases a decrease in ethyl-N^{α}-lauroyl–L-arginate was observed, due to enzyme-mediated hydrolysis resulting in the formation of LAS as the main metabolite.

Case of need and proposed uses

Ethyl lauroyl arginate is intended to be used as a preservative. The active ingredient of ethyl lauroyl arginate, is a cationic surfactant, which has a wide spectrum of activity against Gram positive and Gram negative bacteria, yeasts and moulds. Specifically, ethyl lauroyl arginate affects negatively charged compounds such as microbial proteins present in cellular membranes or enzyme systems. One consequence of the interaction between ethyl lauroyl arginate and microorganisms is the denaturation of membrane proteins resulting in increased membrane permeability. This can result in the inhibition of growth or the death of the microorganism (Rodríguez *et al.*, 2004).

Ethyl lauroyl arginate is proposed to be used in the following food categories up to the maximum levels indicated in Table 1.

Table 1: Proposed uses and use levels of ethyl lauroyl arginate in foods and proposed use levels.

Food categories	**Ethyl lauroyl arginate (mg/kg)**
Non-alcoholic flavoured drinks containing fruit juices	115
Energy and sports drinks	115
Concentrates based on fruit juices	180*
Salted dried fish	225
Meat products: only heat treated, marinated and dried meat products	225
Savoury toppings or fillings for pizzas or similar products	225
Re-hydrated legumes	225
Fish roe products, Sturgeons' eggs	225
Prepared salads	225

* equivalent to 36 mg/kg of ethyl lauroyl arginate in finished drink.

The Panel notes that Table 1 includes proposed uses in meat products and salted dry fish despite stability experiments demonstrating limited stability of ethyl-N^{α}-lauroyl–L-arginate in these foods.

Exposure

The potential dietary exposure to ethyl lauroyl arginate has been estimated by the applicant in relation to the proposed levels of use (Table 1) on the basis of food consumption data from household budget/expenditure surveys and individual based surveys.

The DAFNE database comprises household budget or expenditure data from 13 European countries: Belgium, France, Germany, Greece, Hungary, Ireland, Italy, Luxemburg, Norway, Poland, Portugal, Spain and United Kingdom (Lagiou and Trichopoulou 2001; Trichopoulou and Naska 2003). Food consumption data relate to the amount of food purchased by the household, divided by the number of individuals in that household (per capita estimate). Based on the maximum use levels of ethyl lauroyl arginate and assuming an average body weight of 60 kg, the estimated potential exposure to ethyl lauroyl arginate per person per kg bw per day was calculated using the DAFNE database for each of the contributing European countries.
For the total population the estimated mean potential exposure to ethyl lauroyl arginate from all proposed food uses combined ranged from 0.14 mg/kg bw/day (France) to 0.50 mg/kg bw/day (Luxembourg), with an overall average of 0.32 mg/kg bw/day. The overall principal contributors to potential exposure were non-alcoholic flavoured drinks containing fruit juice (51%) and meat products (33%).

In contrast to aggregated data at the household level, data from individual based surveys reflect food consumption rather than food availability. The most refined estimates provided by the applicant were based on individual dietary records (4 and 7 days) from the UK National Diet and Nutrition Survey (NDNS). In this case, individual food consumption survey data were available for four UK populations covering pre-school children aged 1.5 to 4.5 years (Gregory *et al.*,1995), school children aged 4 to 18 years (Gregory *et al.*, 2000), adults 19 to 64 years (Henderson et al 2002),and the elderly aged 65 and over (Finch *et al.*, 1998). The NDNS data comprised records of the amounts of more than 2000 different food items. Individuals were considered to be consumers if they consumed one or more food products in which ethyl lauroyl arginate is proposed to be used in one of the survey days. NDNS food codes were matched to the food categories reported in Table 1 and then each individual potential exposure was

calculated based on the assumption that ethyl lauroyl arginate was present at the proposed use levels in all categories. Where treated foods (e.g. bacon, ham) occurred in other made-up foods a correction factor was applied based on the UK Food Standard Recipe Database. Individual body weights were available in all population groups to calculate individual's exposure per kg bw/day. The average and high percentile exposures were then calculated for the total population and for consumers only.

The results presented in table 2 confirmed the expectation that potential exposure is higher in children than in adults when expressed on a body weight basis.
The mean potential exposure to ethyl lauroyl arginate in consumers only ranged from 0.11 mg/kg bw/day in the elderly to 0.83 mg/kg bw/day in children aged 1.5 to 4.5 whereas high potential exposure (97.5th percentile in consumers only) ranged from 0.37 mg/kg bw/day in the elderly to 2.89 mg/kg bw/day in children aged 1.5 to 4.5.
Non-alcoholic fruit-based drinks and concentrates based on fruit juice were found to be the most important potential sources in the younger age groups. These beverages contributed approximately 80% (children aged 4 to 18) and 86% (children aged 1.5 to 4.5) of overall potential exposure in the total population. In adults and elderly this contribution was 42% and 28%, respectively. This contribution might be overestimated, since in some cases it was unclear whether the consumption figure provided related to the diluted or undiluted drink or if wastage of drinks (common in pre-school children) was taken into account. Percentage contribution from meat products ranged from 10-12% in children to 37% in adults and 63% in elderly. Dried salted fish, dehydrated legumes, fish roe products and prepared salads had little impact on potential exposure because of the limited numbers of consumers and relatively small amounts consumed.

Table 2. Potential* exposure to ethyl lauroyl arginate (mg/kg bw/day) in UK population groups

	Total population			Consumers only		
	N	mean	P97.5	N	Mean	P97.5
Pre-school children (1.5-4.5 years)	1335	0.74	2.77	1191	0.83	2.89
School children (4-18 years)	1686	0.52	1.83	1640	0.53	1.85
Adults (19-64 years)	1631	0.13	0.48	1490	0.14	0.50
Elderly (65 years and over)	1373	0.09	0.34	1076	0.11	0.37

* Based on the assumption that ethyl lauroyl arginate is present at the proposed use levels in all proposed food categories

These data represent conservative estimates of potential exposure because it was assumed that ethyl lauroyl arginate would be present in all foods for which it is proposed for use (i.e., that it would achieve a 100% share of the market). For individual food categories this might be realistic since consumer loyalty and individual preferences might cause a person to always choose particular brands containing the additive. However, when potential exposures from all foods are combined the scenario becomes less likely.
Based on these data, average exposure to ethyl lauroyl arginate across Europe is unlikely to exceed 1 mg/kg bw/day and high level exposure (at the 97.5th percentile) is unlikely to exceed 3 mg/kg bw/day.

Existing authorisations and evaluations

Ethyl lauroyl arginate was notified as a new substance in accordance with European Directive 67/548/EEC, under the registration number 00-11-0173 (Ministerio de Sanidad y Consumo 2005) and the EC number assigned within ELINCS is 434-630-6.

During March 2005, the applicant submitted a GRAS Notice for the use of ethyl lauroyl arginate as an antimicrobial in food to the US Food and Drug Administration (FDA), and a complementary document establishing the efficacy of ethyl lauroyl arginate in meat and poultry products was submitted to the United States Department of Agriculture (USDA). As a result of these submissions, on 1st September 2005, FDA 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 (FDA 2005). On 21st September and 13th October 2005, USDA issued two letters stating the efficacy and suitability of ethyl lauroyl arginate in meat and poultry products (USDA 2005a, 2005b). The Food Safety and Inspection Service (FSIS) Directive 7120.1 states that ethyl lauroyl arginate is a safe and suitable ingredient for fresh cuts of meat and poultry products and various non-standardized ready-to-eat meat and poultry products which permit ingredients of this type (FSIS 2006).

Ethyl lauroyl arginate has been previously evaluated for food safety as an antimicrobial in food by the US Food and Drug Administration (FDA) and for efficacy in meat and poultry products by the United States Department of Agriculture (USDA). Additionally, the Scientific Committee on Consumer Products (SCCP) issued an opinion on the safety of ethyl lauroyl arginate when used as a preservative in cosmetics, on 15th March 2005 (SCCP, 2005).

TOXICOLOGICAL DATA

The studies performed in order to demonstrate the safety of ethyl lauroyl arginate comprise *in vitro* and *in vivo* metabolism-toxicokinetics studies, *in vitro* and *in vivo* mutagenicity assays, acute, subchronic toxicity and chronic toxicity studies, reproduction and developmental toxicity studies (one generation and two generations) in animals, and human kinetic studies.
All studies were performed following OECD guidelines and GLP.
Some of the results of these studies have been published in the open literature (Ruckman *et al.*, 2004).

Absorption, Distribution, Metabolism and Excretion

A single oral dose of 180 mg/kg bw ^{14}C-ethyl-Nα-lauroyl-L-arginate (uniformly radio-labelled in the arginine portion of ethyl-N$^{\alpha}$-lauroyl-L-arginate) was administered to four male rats. Urine, faeces and exhaled carbon dioxide were collected and analysed at intervals throughout the 120 hours of the study, and gastrointestinal tract, including contents, liver, and the remaining carcass were analysed at termination of the study. Approximately 100% of the administered dose was recovered. The major route of excretion was as carbon dioxide by exhalation. A mean total of 23.9% of the dose was eliminated via this route in the 24 hours following dosing, increasing to a mean total of 36.6% of the dose over 5 days. Excretion in urine and faeces was low, a mean total of 11.8% and 4.3% of the dose respectively was excreted over the 5 day collection period. The low percentage in faeces indicated that a high

percentage of the test substance was absorbed. The largest proportion of radioactivity was recovered from the carcass, a mean total of 41.0% of the dose, of which 2.0% was measured in the gastrointestinal tract and 3.4% in the liver.
The high level of retention of radioactivity found in the carcass of the rats 5 days after dosing would be related to conversion of ethyl-N^{α}-lauroyl-L-arginate to metabolites entering normal metabolic pathways.

The results from this study together with additional *in vitro* and *in vivo* metabolism experiments reported by the applicant established a biotransformation pathway for ^{14}C- ethyl-N^{α}-lauroyl-L-arginate after ingestion (Figure 1) . Ethyl-N^{α}-lauroyl-L-arginate is rapidly hydrolysed either by loss of the lauroyl side chain to form arginine ethyl ester and/or cleavage of the ethyl ester to form N^{α}-lauroyl-L-arginine (LAS). Further hydrolysis of either intermediate results in the production of arginine, which is then further converted to ornithine and urea, following normal amino acid catabolism via the urea and citric acid cycles resulting in the formation of ^{14}C-carbon dioxide and ^{14}C-urea, which are excreted in the expired air and in urine respectively.
The ornithine could either be degraded further to citrulline and other amino acids in the urea cycle or to the metabolic intermediate α-ketoglutarate, which could then enter the citric acid cycle with subsequent metabolism to carbon dioxide (Voet and Voet 1990; Schlede *et al.*, 1994).

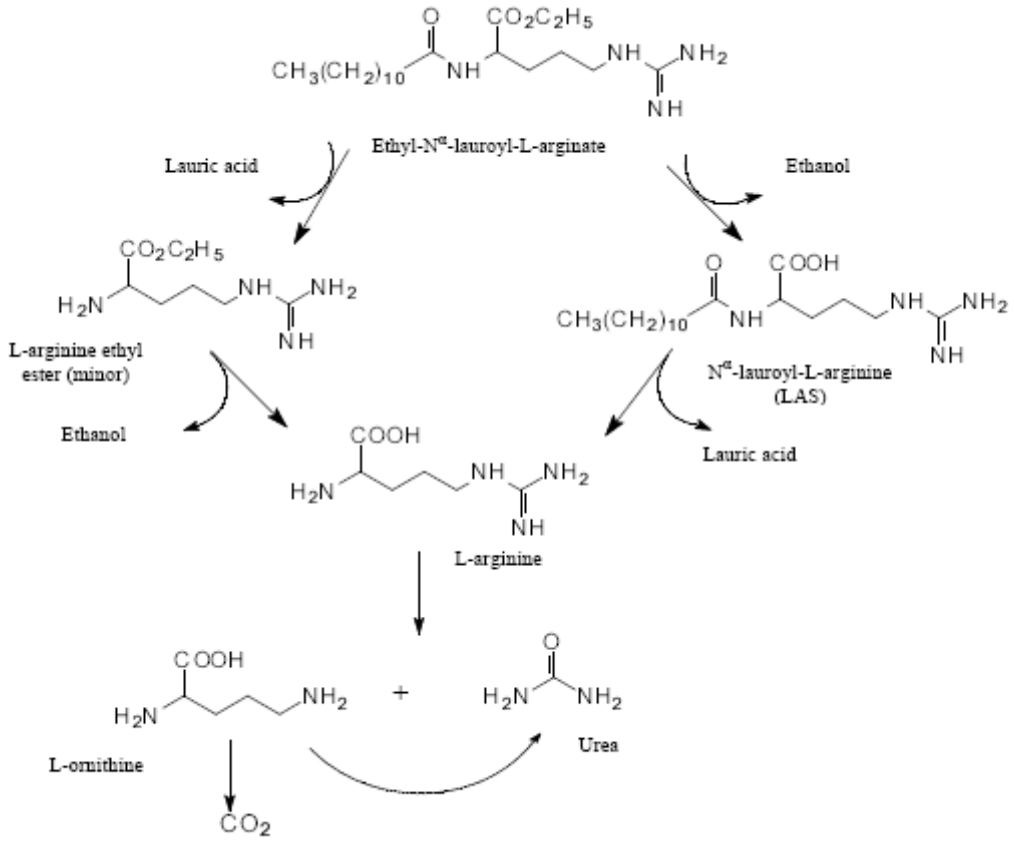


Figure 1: Biotransformation pathway of ethyl-N^{α}-lauroyl-L-arginate in rats based on *in vitro* and *in vivo* studies.

In another study reported by the applicant the pharmacokinetics of ethyl-Nα-lauroyl-L-arginate in rats was characterised. After oral administration of ethyl-Nα-lauroyl-L-arginate the rate and extent of systemic exposure of rats to intact ethyl-N^{α}-lauroyl-L-arginate was low and there did not appear to be a consistent relationship of plasma ethyl-N^{α}-lauroyl-L-arginate with dose following administration at 40, 120 and 320 mg/kg bw. The applicant ascribes this to the rapid hydrolysis of ethyl-N^{α}-lauroyl-L-arginate to N^{α}-lauroyl-L-arginine (LAS). The applicant indicates that plasma levels of LAS, and in particular the AUC for LAS, provide a better indication of the absorption of ethyl lauroyl arginate.

The applicant provided data on the stability of ethyl-N^{α}-lauroyl-L-arginate in rat and human plasma *in vitro* and *in vivo*, in simulated gastric fluid with or without pepsin, and in simulated gastrointestinal fluid in the presence or absence of pancreatin. The results obtained demonstrate that hydrolysis of ethyl-N^{α}-lauroyl-L-arginate is very rapid in the gastrointestinal tract and following very large doses to animals only very low transient levels of ethyl-N^{α}-lauroyl-L-arginate were found in plasma. In human studies, with low dose administration of ethyl lauroyl arginate, no, or only very low levels of ethyl-N^{α}-lauroyl-L-arginate could be detected in plasma. Together the studies support a half-life of ethyl-N^{α}-lauroyl-L-arginate of less than 1 hour in rat plasma and of around 4 hours in human plasma. The degradation in human plasma occurred more slowly than in simulated intestinal juice, where within 1 minute of incubation in the presence of pancreatin ethyl-N^{α}-lauroyl-L-arginate was almost fully hydrolysed to LAS.

The applicant reports a single oral dose human study performed in two volunteers who each received a solution of 5 mg ^{13}C-labelled ethyl-N^{α}-lauroyl-L-arginate (labelled in the arginine part) and 15 mg propylene glycol per kg bw made up to 1 ml/kg bw with purified water. Blood samples were taken for analysis from 5 minutes to 24 hours post dosing. No unchanged ethyl-N^{α}-lauroyl-L-arginate was detected in plasma samples from one subject (limit of quantification 1 ng/ml). In the other subject, quantifiable concentrations up to 44 ng/ml of ethyl-N^{α}-lauroyl-L-arginate were found at 10 and 15 minutes post dosing and of 13.6 ng/ml at 30 minutes post dosing. No ethyl-N^{α}-lauroyl-L-arginate was quantifiable at any time thereafter. For this study the applicant concluded that exact determination of a plasma half-life was not possible, but that the half-life would be much less than one hour. Ethyl-N^{α}-lauroyl-L-arginate was rapidly hydrolysed to LAS and then to arginine. The maximum concentration of ^{13}C-LAS in both subjects was observed 2 hours post dosing. The metabolites were essentially cleared from plasma within 24 hours after dosing.

The applicant also reports a further study carried out in six human volunteers. Single oral doses of 2.5 and 1.5 mg/kg bw ^{13}C-ethyl-N^{α}-lauroyl-L-arginate were administered to 2 and 4 subjects respectively. Although blood samples were taken 5, 10, 15 and 30 minutes after dosing, and thereafter up to 24 hours, the plasma concentrations of ^{13}C-ethyl-N^{α}-lauroyl-L-arginate were generally below the limit of quantification. The maximum plasma concentrations of ^{13}C-LAS generally occurred at 2 hours post dose and were generally quantifiable up to 12 hours post dose. The maximum concentration of ^{13}C-arginine occurred earlier or at the same time as for ^{13}C-LAS, indicating that the absorption of ^{13}C-arginine must have occurred more rapidly than the absorption of ^{13}C-LAS. The plasma levels of ^{13}C-arginine were quantifiable up to 4 or 8 hours post dosing. The plasma concentrations of ^{13}C-arginine were generally considerably higher than those of ^{13}C-LAS, indicating that there is an extensive breakdown of ethyl-N^{α}-lauroyl-L-arginate to arginine.
The applicant concludes that the half-life of both LAS and arginine were in the range of 2 to 4 hours and appeared to be independent of the dose.

Altogether the studies provided by the applicant show that ethyl-N^{α}-lauroyl-L-arginate is well absorbed and rapidly metabolised into arginine, lauric acid and ethanol. The arginine moiety is incorporated into naturally occurring products via the urea and citric acid cycles, distributed to the liver, and slowly excreted as carbon dioxide via the expired air, and to a low extent via the urine and faeces. Lauric acid is a fatty acid that can enter normal fatty acid metabolism. Ethanol can be converted by alcohol dehydrogenase and aldehyde dehydrogenase to acetate, which can enter normal biochemical pathways.

Acute Oral Toxicity

The oral (gavage) LD_{50} values of ethyl lauroyl arginate (purity 90.1%), a formulation of 19.5% ethyl-N^{α}-lauroyl-L-arginate HCl in propylene glycol and LAS (the principal metabolite) (purity 98.6%), were reported by the applicant to be all higher than 2,000 mg/kg bw in rats.

Short-term and sub-chronic toxicity

The applicant reported subchronic toxicity studies in rats performed with ethyl lauroyl arginate and a formulation of ethyl lauroyl arginate in propylene glycol.

Based on the findings in a 4 weeks range finding study, in a subsequent sub-chronic study ethyl lauroyl arginate (about 90% purity) was administered at dietary concentrations of 0 (control), 5000, 15000 and 50000 mg test substance/kg diet for 13 weeks, to groups of 20 male and 20 female Han Wistar rats. The overall group mean dosages for the 13 weeks of treatment were 0, 384, 1143, and 3714 mg/kg bw/day of ethyl lauroyl arginate for males and 0, 445, 1286 and 3915 mg/kg bw/day of ethyl lauroyl arginate for the equivalent female groups.
At 50000 mg/kg diet rats had ungroomed coats, associated with a high incidence of yellow staining of the coat and brown staining on the muzzle.

During week 1, rats that received a dietary concentration of 50000 mg/kg diet had a marked loss of body weight (-16 and –13% for males and females respectively). In animals given 15000 mg/kg diet and in males receiving 5000 mg/kg diet, the body weight gain was significantly lower than that of the control animals. Overall, the body weights of animals receiving 50000 and of males receiving 15000 mg/kg diet did not fully recover by the end of the study. During week 1, food consumption was markedly low (33% and 39%) for males and females respectively for animals that received 50000 mg/kg diet, compared with controls. Food consumption for animals receiving 15000 mg/kg diet and males receiving 5000 mg/kg diet was also slightly lower than that of the control group. In animals given 50000 mg/kg diet food consumption remained low during the subsequent weeks (overall, 79 and 78% of controls for males and females respectively).
There was no evidence of neurotoxicity in animals treated with ethyl lauroyl arginate for 13 weeks, at dietary levels of up to 50000 mg/kg diet as judged on the basis of gait, locomotion, activity and rearing scores, motor activity and ophthalmic measurements.

Haematology investigations during week 13 of treatment revealed slightly higher mean cell haemoglobin concentration and mean cell volume in males receiving 50000 mg/kg diet. In males the white blood cell counts in the 50000 mg/kg diet group amounted to 73.9 % of control values ($5.15 \pm 0.64 \times 10^9$/L versus $6.96 \pm 1.78 \times 10^9$/L in control)($p<0.01$). The lymphocyte counts amounted to 71.9% of control ($p<0.05$).
For animals receiving 50000 mg/kg diet and females that received 15000 mg/kg diet, low total protein concentration (50000 mg/kg diet) and slightly low albumin concentrations were observed. Slightly lower cholesterol levels were also apparent in females receiving 50000

mg/kg diet. The applicant indicates that in the absence of effects on liver weight or associated histopathology these changes are not considered to be of toxicological relevance.
Urinalysis in week 13 revealed a low pH in male rats only that received 15000 or 50000 mg/kg diet.
There were no organ weight changes attributable to treatment, and no treatment-related macroscopic findings.
The only histopathological changes were seen in the non-glandular region of the stomach, specifically in the area adjacent to the entry of the oesophagus. The predominant change was parakeratosis, which was present in the majority of male and female rats which received 50000 mg/kg diet, and in a single female that received 15000 mg/kg diet. Ulceration was seen in a single male and two females which received 50000 mg/kg diet and in a single male given 15000 mg/kg diet. In addition, erosions and epithelial hyperplasia were observed in female rats given the highest dietary level.
The no observed adverse effect level (NOAEL) identified in this study was 5000 mg/kg diet (384 and 445 mg/kg bw/day ethyl lauroyl arginate test substance for males and females respectively).

The effects of a formulation of ethyl lauroyl arginate in propylene glycol were investigated in additional 4- and 13-week studies.
Based on the findings in the 4-weeks range finding study, in the 13-week study a formulation of ethyl-N^{α}-lauroyl-L-arginate in propylene glycol (19.5 % solution of active ingredient ethyl-N^{α}-lauroyl-L-arginate in propylene glycol) was administered to groups of 10 male and 10 female Sprague-Dawley rats at dietary levels of 0, 3200, 12800 and 50000 mg formulation /kg diet resulting in overall mean intakes of the test formulation of 0, 220, 904 and 3324 mg/kg bw/day for males and 0, 262, 1067 and 3927 mg/kg bw/day for females in the treated groups. The controls did not receive ethyl-N^{α}-lauroyl-L-arginate and no propylene glycol. These dose levels correspond to intakes of propylene glycol of 0, 165, 678 and 2675 mg propylene glycol /kg bw/day for male rats and 0, 197, 800 and 2945 mg propylene glycol /kg bw/day for female rats. Correcting for the fact that the test formulation contained only 19.5% of the active ingredient ethyl-N^{α}-lauroyl-L-arginate in propylene glycol these intake levels would amount to ethyl-N^{α}-lauroyl-L-arginate intakes of 0, 43, 183, 648 mg ethyl-N^{α}-lauroyl-L-arginate/kg bw/day for male rats and 0, 51, 208 and 766 mg ethyl-N^{α}-lauroyl-L-arginate/kg bw/day for female rats. The applicant indicates that these dose levels amount to respectively 0, 47, 195 and 719 mg ethyl lauroyl arginate of the defined specifications/kg bw/day for male rats and 0, 56, 230 and 848 mg ethyl lauroyl arginate of the defined specifications /kg bw/day for female rats.

There were no treatment-related clinical signs noted during the study. The overall mean body weight gain for all treated groups of females was slightly lower than the concurrent controls. However, there was no relationship to the dietary levels. The mean cumulative food intake by the animals in the treated groups was unaffected by the treatment. Group mean water intake by males given 50000 mg/kg diet was slightly higher than that of controls.
When compared with control animals, slightly lower total white blood cell counts were noted amongst males and females receiving 12800 and 50000 mg of test formulation/kg diet. In males, the white blood cell counts amounted to 92.9% of control (change not significant) and 86.8% of control (change not significant) for the 12800 and 50000 mg/kg diet groups. For females, these values amounted to respectively 77.6% of control ($p \leq 0.05$) and 72.3 % of control ($p \leq 0.01$). There was no consistency in the cell type contributing to the lower total cell count.
Urine analysis revealed a slight increase in some individual and group mean urine volumes, which was associated with the slightly higher water intake of males given 50000 mg/kg diet.

There was a slightly higher mean adjusted (bodyweight as covariate) liver weight amongst females receiving 50000 mg/kg diet of the test formulation. No similar increase was apparent in the males at this dietary level and, since there were no histopathological changes detected in the liver and individual liver weights were within the same range as the controls, the applicant concludes that this finding is of uncertain toxicological significance.
The only change observed in macroscopic pathology was an increased incidence of alopecia amongst female rats receiving 12800 or 50000 mg/kg diet of the test substance. In microscopic pathology no treatment-related changes were detected in any of the tissues examined.
The applicant indicates that the no observed adverse effect level (NOAEL) for continuous administration of a 19.5% solution of ethyl lauroyl arginate in propylene glycol to rats for 13 weeks is considered to be 12800 mg/kg diet (904 and 1067 mg/kg bw/day for males and females respectively amounting to 183 and 208 mg/kg bw/day ethyl-N^{α}-lauroyl-L-arginate).
The Panel notes that the effects on white blood cell counts and alopecia indicate a NOAEL of 3200 mg formulation/kg diet amounting to 220 mg and 262 mg of test formulation/kg bw/day, to 43 and 51 mg ethyl-N^{α}-lauroyl-L-arginate /kg bw/day for males and females respectively and to 47 and 56 mg ethyl lauroyl arginate/kg bw/day for males and females respectively.

Since the controls in this study had untreated diet without any propylene glycol, and the propylene glycol intakes for the various groups were estimated by the applicant to amount to 0, 165, 678 and 2943 mg propylene glycol/kg bw/day for males and to 0, 197, 800 and 2945 mg/kg bw/day for females, the possibility that any effects observed in this study could have been caused by the the solvent propylene glycol used to prepare the testing formulation needs to be considered.
Propylene glycol has been evaluated by the Scientific Committee for Food (SCF 1996) and JECFA (JECFA 1974) and a (temporary) ADI of 25 mg/kg bw/day was allocated based on a NOEL of 2500 mg/kg bw/day in long-term studies in rats and dogs.
The Panel noted that for propylene glycol also haematological findings were reported in cats, and to a lesser degree in dogs. Cats were uniquely sensitive to propylene glycol responding with a significant increase in Heinz bodies in circulating erythrocytes. Since these effects are different from the heamatological effects observed upon exposure of rats to a formulation of ethyl lauroyl arginate in propylene glycol and since such effects of propylene glycol were not observed in rats, it is concluded that the effects observed in the present rat study cannot be ascribed to the solvent propylene glycol.

Reproductive and developmental toxicity

The applicant reports a series of reproductive and developmental toxicity studies with rats and rabbits.

Following a range finding study, three groups of 22 female Sprague-Dawley rats received a batch of ethyl lauroyl arginate containing 69.1% ethyl-N^{α}-lauroyl-L-arginate by oral gavage at dosages of 0 (control), 200, 600 or 2000 mg/kg bw/day for 14 days (days 6-19). The purity of this batch does not correspond to the specification for ethyl lauroyl arginate. The applicant indicates that this batch was obtained following the same synthesis process, and that the only difference from ethyl lauroyl arginate as specified is a difference in water content. Female rats were paired on one-to-one basis with stock males of the same strain.
Three females receiving 2000 mg/kg bw/day of ethyl-N^{α}-lauroyl-L-arginate were killed *in extremis* on the second or third day of treatment. All three animals had noisy and gasping respiration, and salivation after dosing. Necropsy revealed large amounts of gaseous material in the stomach of the three rats, the entire gastro-intestinal tract was distended with gas. In

addition one of the rats had enlarged and prominent lymph nodes, and another had haemorrhagic lungs, large amounts of pale yellow viscous material in the ileum, reduced and dehydrated faecal contents, dark and enlarged adrenals and a pronounced internal structure of the kidneys.
Two females at 600 mg/kg bw/day of ethyl-N^{α}-lauroyl-L-arginate were similarly affected towards the end of gestation, both had noisy respiration, salivation at the time of dosing and body weight losses. Necropsy of these animals revealed that the gastro-intestinal tract was distended with gaseous material. Both animals had grossly normal implantations.
The general condition of the surviving rats was satisfactory and all the females were pregnant. Noisy respiration occurred during the treatment period in three animals receiving 200 mg/kg bw/day of test substance, a total of 7 rats at 600, and in 9 rats at 2000 mg/kg bw/day of test substance (including animals which were killed prematurely).
Salivation at the time of dosing was seen in all animals receiving 2000 mg/kg bw/day of test substance on approximately 50% of dosing occasions, reaching peak daily incidence at about day 14 of gestation. Fourteen animals receiving 600 mg/kg bw/day of test substance occasionally salivated during the dosing period, and at 200 mg/kg bw/day of test substance salivation was seen in only one animal on one occasion. Neither noisy respiration nor salivation was seen in the control group.
A few rats of all treated groups showed transient body weight losses for periods following commencement of treatment on day 6 and some animals receiving 600 mg/kg bw/day of test substance lost weight towards the end of the treatment period. These weight losses coincided with episodes of respiratory distress. In the control groups, there were no similar bodyweight losses.
There were no overall treatment related effects upon food consumption. However, food intake, which appeared to be associated with episodes of respiratory distress, was reduced in occasional animals in the treatment groups.
Necropsy at 20 days of gestation revealed that there were no maternal necropsy findings that were considered to be related to treatment.
There were no effects on fetal survival as indicated by the extent of pre- and postimplantation loss and the numbers of live fetuses.
Fetal and placental weights were unaffected by treatment. The incidences and types of major fetal abnormalities were unaffected by treatment. The numbers of skeletal and visceral minor abnormalities and variants were unaffected by treatment.
Altogether it was concluded that respiratory distress was recorded among some animals receiving 600 or 2000 mg/kg bw/day of test substance. These effects were probably related to aspiration of the irritant dosing material, especially following treatment with the more concentrated/viscous suspensions at the higher doses.
With the exception of transient effects on bodyweight and food consumption associated with individual animals showing respiratory distress, at 600 and 2000 mg/kg bw/day, there were no adverse effects of treatment on the dams and there were no adverse effects on embryo fetal survival and development at dosages of up to 2000 mg/kg bw/day.
The applicant concluded that the no adverse effect level (NOAEL) for the dams was 200 mg/kg bw/day and for the fetuses was 2000 mg/kg bw/day amounting to respectively 138 and 1382 mg/kg bw/day when correcting for the 69.1 % purity of ethyl-N^{α}-lauroyl-L-arginate in the test material.
The Panel notes that the NOAEL in the dams is based on weight loss and/or the respiratory distress which could be an artefact of the dosing.

After a range finding study, embryo-fetal toxicity of a batch of ethyl lauroyl arginate containing 69.1% ethyl-N^{α}-lauroyl-L-arginate was also investigated in the rabbit. The purity of this batch

does not correspond to the specification for ethyl lauroyl arginate. The applicant indicates that this batch was obtained following the same synthesis process, and that the only difference from ethyl lauroyl arginate as specified is a difference in water content. Ethyl-N^{α}-lauroyl-L-arginate was administered by oral gavage to time mated (after pairing with proven males of the same stock), sexually mature female New Zealand White rabbits. Three groups of 22 female animals received ethyl-N^{α}-lauroyl-L-arginate by oral gavage at doses of 0 (control), 100, 300 and 1000 mg/kg bw/day, administered for 14 days (days 6-19 of gestation).

At 1000 mg/kg bw/day one animal was killed following periods of noisy respiration accompanied by reduced food consumption and faecal output and an aqueous discharge in the cage under tray on day 9 of gestation. Necropsy revealed a small amount of frothy liquid in the trachea, and congestion in the lungs. One animal at 300 mg/kg bw/day was also killed because of gasping respiration. Necropsy revealed incomplete collapse of the lungs, with occasional dark areas of change on the lung surfaces. One female at 1000 mg/kg bw/day aborted on day 24 of gestation: necropsy revealed three empty implantation sites in the left uterine horn but no implantations in the right horn of the uterus. Two dead fetuses, both of which appeared grossly normal, were found in the under tray of the cage.

Reactions to dosing were largely limited to changes in respiration pattern seen in 5 animals at 300 mg/kg bw/day and 5 animals at 1000 mg/kg bw/day (including the two animals, which were killed early in the study and replaced). Adverse respiratory reactions were believed to be associated with a higher risk of irritation being induced during the dosing procedure when high concentrations of test material were used; difficulties with dosing were much reduced when the surface of the catheter was washed clean rather than wiped dry before dose administration.

Bodyweight gain of animals receiving ethyl lauroyl arginate at 1000 mg/kg bw/day was slightly but significantly lower than that of the controls throughout most of the treatment period.

Food consumption by animals receiving the test substance at 1000 mg/kg bw/day fell slightly when treatment started and was significantly lower than that of the control group during the period days 13-19 of gestation but recovered to similar to control levels after completion of the dosing period.

The applicant states that there were no necropsy findings for females killed on day 29 after mating that were considered to be related to treatment with test substance.

There were no apparent treatment related effects on fetal survival. The numbers of corpora lutea implantations and live young in the control group were generally lower than in the treated groups but intergroup differences were not statistically significant. In the control, low, mid and high dose groups respectively, the pre-implantation losses were 8.9%, 13.4%, 12.3% and 11.3%, and the post-implantation losses were 9.3%, 11.9%, 10.3% and 6.9%. Litter sizes (live fetuses) in the controls to high dose groups respectively were 8.9, 9.1, 9.1 and 10.0.

There were no effects of treatment on fetal weight or placental weight. The incidences and types of major fetal abnormalities were unaffected by the treatment. The numbers of skeletal and visceral minor abnormalities and variants were unaffected by the treatment.

The applicant concludes that treatment of rabbits with the test substance by oral gavage at 300 or 1000 mg/kg bw/day was associated with difficulty in dosing and signs of respiratory distress in some animals. Similar respiratory signs were recorded during the preliminary study. This clinical sign was related to aspiration of traces of the test material. However, altering the standard dosing procedure, by using a clean moist catheter instead of a clean dry catheter, appeared to alleviate some of the dosing problems although there was still a residual incidence of respiratory noises.

Treatment at 1000 mg/kg bw/day was associated with reduced maternal bodyweight gain during treatment and also with reduced food intake. In contrast, these effects were not observed

at 100 and 300 mg/kg bw/day. There were no adverse effects upon fetal survival and development at any dosages up to the maximum level tested (1000 mg/kg bw/day).
Despite the slightly higher risk of irritation to the respiratory tract at dosages of 300 mg/kg bw/day and above, the applicant concluded that 300 mg/kg bw/day of the test substance was the no adverse effect level (NOAEL) for the dam and 1000 mg/kg bw/day of the test substance was the NOAEL for the fetus. These doses amount to 207 mg/kg bw/day and 691 mg/kg bw/day of ethyl-N^{α}-lauroyl-L-arginate. The Panel agrees with this NOAEL.

In addition to the developmental toxicity studies described above, the applicant has also submitted results of a two-generation study in rats.
In a range finding study in Sprague Dawley rats in the F1 generation, vaginal opening was delayed in the 15000 mg/kg diet ethyl lauroyl arginate (containing 88.2 % ethyl-N^{α}-lauroyl-L-arginate) treatment group. In the main two generation reproductive performance study groups of 28 adult male and 28 unrelated virgin female Sprague-Dawley received ethyl lauroyl arginate containing 88.2 % ethyl-N^{α}-lauroyl-L-arginate continuously in the diet at concentrations of 0 (control), 2500, 6000 or 15000 mg/kg diet for 10 weeks before pairing, throughout mating and until termination after weaning of the litters.
The lowest mean achieved dosages of the F0 generation amounted to 0, 181, 434 and 1073 mg/kg bw/dat for male and to 0, 207, 502 and 1226 mg/kg bw/day for females for the 0, 2500, 6000 and 15000 mg/kg diet groups respectively.
The lowest mean achieved dosages of the F1 generation amounted to 0, 224, 537 and 1356 mg/kg bw/day for males and to 0, 215, 535 and 1430 mg/kg bw/day for females for the 0, 2500, 6000 and 15000 mg/kg diet groups respectively.
No adverse effects on body weight performance, food consumption and food conversion efficiency were observed in adult animals. Measures of oestrous cycles, fertility and primordial follicle counts were also unaffected by dietary exposure to ethyl lauroyl arginate at concentrations of up to 15000 mg/kg diet.
In the F1 and F2 offspring there were no adverse effects on litter size, sex ratio, survival and day 1 bodyweight at dietary levels of up to 15000 mg/kg diet. Bodyweight gains up to day 14 of lactation were unaffected by exposure to ethyl lauroyl arginate, although in the latter stages of lactation/early stages of independent feeding animals at 15000 mg/kg diet showed a slight reduction in body weight gain.
There was a reduction in pass rate for startle response of 8.6% and 7.1% respectively for F2 offspring treated at 15000 and 6000 mg/kg diet. No similar effect had been seen in the F1 generation and no effect was seen on startle response in adult rats treated for 12 weeks at levels of up to 50000 mg/kg in the diet. Balano-preputial separation was unaffected at all dosage levels.
For the F1 females a delay of 4 days in vaginal opening at the dietary level of 15000 mg/kg diet was reported. Females were heavier than controls at completion of the development process.
The timing of sexual maturation occurs shortly after the time of highest intake (>1900 mg/kg bw/day) of ethyl lauroyl arginate, approximately 2 weeks after the initiation of the F1 generation.
The timing of the vaginal opening has no impact upon oestrous cycles pre-pairing or pre-termination on fertility or on primordial follicle counts.
The percentage of males in each litter and the measurement of anogenital distance in the F2 offspring were also unaffected by treatment.
The applicant concludes that these observations indicate that ethyl lauroyl arginate caused no changes in sexual differentiation and that the delay in vaginal opening was of no long-term toxicological importance.

In F0 and F1 adult males there were no effects on sperm assessment. Macroscopic examination of adult animals and offspring revealed no changes attributable to treatment.
In the 15000 mg/kg diet group absolute and/or body weight relative spleen weights of F0 and F1 females at scheduled termination and of male and female weanlings and F2 female weanlings on day 30 of age were significantly lower than in the controls. The magnitude of the difference reduced as age increased and was not accompanied by any macroscopic changes or microscopic changes in the adult animals and was therefore considered by the applicant to be of no toxicological importance.
Altogether, the applicant concludes that the NOAEL for reproductive performance in the Sprague Dawley rat is 15000 mg/kg diet ethyl lauroyl arginate which is equivalent to at least 1073 mg/kg bw/day based on the lowest average intake by adult rats before pairing and up to 2600 mg/kg bw/day for females during lactation. There was a slight reduction in offspring bodyweight gain just before weaning, a delay in vaginal opening of F1 females and reduced spleen weights among F1 and F2 offspring at 15000 mg/kg diet at a point when estimated achieved dosage would be in excess of 1900 mg/kg bw/day. However, these effects were transient and were regarded by the applicant as not toxicologically significant.

The Panel noted that the effect on the vaginal opening of F1 females at 15000 mg/kg diet is consistent among the studies and, although it is not accompanied by other functional changes, cannot be disregarded. The Panel further noted that at 15000 mg/kg diet there was also an effect on the offspring bodyweight gain just before weaning. Taking together all these effects the Panel derives a NOAEL from this study of 6000 mg/kg diet which amounted to an estimated intake of at least 434 mg/kg bw/day and 502 mg/kg bw/day of ethyl lauroyl arginate for males and females.

Mutagenicity

Several studies were performed to investigate the mutagenicity of ethyl lauroyl arginate, a formulation of ethyl-N^{α}-lauroyl-L-arginate in propylene glycol and the main metabolite of ethyl lauroyl arginate, N^{α}-lauroyl-L-arginine (LAS).

Ethyl lauroyl arginate containing 89.4% ethyl-N^{α}-lauroyl-L-arginate was tested in bacterial mutagenicity assays. For the bacterial assays the strains used were *S. typhimurium* TA1535, TA100, TA1537, TA98 and *E. coli* WP2uvrA/pKM101. No significant increases in revertant colony number were observed in any of the bacterial strains following exposure to ethyl lauroyl arginate at any concentration in either the presence or the absence of S-9 mix. Toxicity was seen in at exposures of 50 μg/plate and above in all *Salmonella typhimurium* strains and at 150 μg/plate and above in E.coli strains.
Likewise a formulation of ethyl-N^{α}-lauroyl-L-arginate (19.5 % in propylene glycol), was tested for potential bacterial mutagenic effects in strains of *Salmonella typhimurium* (TA1535, TA1537, TA98 and TA100). No substantial increases in revertant colony numbers of any of the tester strains were observed following treatment with the ethyl lauroyl arginate formulation at any dose level, in the presence or absence of S-9 mix, in any mutation test. In this test toxicity was observed towards all strains at 500 μg test material/plate and above amounting to 97.5 μg ethyl-N^{α}-lauroyl-L-arginate/plate and above.
The Panel considered the bacterial reverse mutation assay study could not be used for evaluation of mutagenicity due to the high toxicity of ethyl-N^{α}-lauroyl-L-arginate towards the bacterial cells. The Panel noted that this toxicity was predictable since ethyl-N^{α}-lauroyl-L-arginate is a preservative with antimicrobial activity.

Ethyl lauroyl arginate and a formulation of ethyl-N^{α}-lauroyl-L-arginate (19.5% in propylene glycol) were tested for potential mutagenicity in the mouse lymphoma L5178Y cell mutation test.

Ethyl lauroyl arginate was tested for 3 and 24 hours in the absence or presence of a metabolic activation system and in concentrations amounting to 1-50 μg/ml. These concentrations generally resulted in relative growth in suspension (RSG) values above 16% up to100%. There were no statistically significant increases in mutant frequency after exposure to ethyl lauroyl arginate.
A formulation of ethyl-N^{α}-lauroyl-L-arginate (19.5% in propylene glycol) was tested for 48 hours in the absence or presence of S9 in concentrations in the range of 100 to 500 μg/ml (amounting to 19.5 to 97.5 μg ethyl-N^{α}-lauroyl-L-arginate/ml). The resulting RSG values varied between 1 and 96%. There were no statistically significant increases in mutant frequency.

The ability of ethyl lauroyl arginate containing 89.4% ethyl-N^{α}-lauroyl-L-arginate to cause chromosomal aberrations in human lymphocytes cultured *in vitro* was assessed at dose levels of 12.5 to 1600 μg/ml. Based on toxicity data the dose levels selected for the metaphase analysis were 50, 100 and 200 μg/ml (with a reduction in mitotic index to about 30% at 200 μg/ml) in a first test, and 50,100 and 150 μg/ml (with a reduction in mitotic index to about 43-68% at 150 μg/ml) in a second test. There was no evidence that ethyl lauroyl arginate had clastogenic activity in this *in vitro* cytogenetic test system at the dose levels indicated. Some evidence of polyploidy-inducing activity was observed, which is however generally considered of uncertain significance in genotoxicity assessment. A formulation of ethyl-N^{α}-lauroyl-L-arginate (19.5% in propylene glycol) was tested at dose levels up to 2000 μg/ml of test formulation. In a first test this resulted in a reduction in mitotic index to 40% and 11% in the absence and presence of S9 respectively at 2000 μg/ml. The concentrations chosen for metaphase analysis were 125, 250 and 500 μg/ml in the absence of S9 and 125 and 250 μg/ml in the presence of S9. In a second test the levels analysed were 125 and 250 μg/ml (based on a mitotic index of 75% at 500 μg/ml) in the absence of S9 and 700 and 800 μg/ml (based on a mitotic index of 61% at 1000 μg/ml) in the presence of S9, all at 18 hours harvest, and 500 μg/ml (mitotic index 66%) in the absence of S9 and 1000 μg/ml (mitotic index 98%) in the presence of S9 at 32 hours harvest. Under these conditions ethyl-N^{α}-lauroyl-L-arginate did not show evidence of clastogenic activity in this *in vitro* cytogenetic test with human lymhocytes.

Based on the results from the mouse lymphoma L5178Y cell mutation test and from the test for chromosomal aberrations in human lymphocytes it is concluded that ethyl-N^{α}-lauroyl-L-arginate is not genotoxic in mammalian cells.

Another study assessed the possible mutagenic potential of the main metabolite of ethyl lauroyl arginate, N^{α}-lauroyl-L-arginine (LAS)(98.6 % purity), using four strains of *Salmonella typhimurium* and one strain of *Escherichia coli* in the presence and the absence of S9 at 6 concentrations from 156 to 5000 μg/plate. LAS at a concentration of 5000 μg/plate was toxic to the TA1535, TA1537 and TA100 strains of *S. typhimurium* without S9 and to TA1537 with S9. LAS produced no mutagenic activity in any of the five bacterial strains used.

The mutagenic potential of N^{α}-lauroyl-L-arginine (LAS) (98.6% purity), was also investigated by measuring its effects on the induction of micronuclei due to lagging chromosome fragments (clastogenicity) or whole chromosomes (aneugenicity) in polychromatic erythrocytes from

mouse bone marrow following a single oral administration by gavage of 2000 mg/kg bw. The induction of micronuclei ws analysed 24 and 48 hours after administration of the substance. When LAS was administered at a dose of 2000 mg/kg bw, there was no biologically significant increase in the frequency of micronucleated polychromatic erythrocytes in the bone marrow of the treated mice at any sampling time. Therefore, LAS was considered to be not genotoxic.

Carcinogenicity and long-term studies

Long-term carcinogenicity studies are lacking.

The applicant submitted a 52 week chronic study in which ethyl lauroyl arginate (88.2 % purity) was administered in the diet to Sprague Dawley rats (20 male and 20 female rats per group), at dose levels of ethyl lauroyl arginate of 0 (control), 2000, 6000 and 18000 mg/kg in the diet.
Overall group mean achieved intakes at 2000, 6000 and 18000 mg/kg diet for weeks 1 to 52 were 106, 307 and 907 mg/kg bodyweight/day for males and 131, 393 and 1128 mg/kg bodyweight/day for females.

The exposure of rats to ethyl lauroyl arginate at the different dose levels tested for up to 52 weeks was generally well tolerated. However, toxicologically significant effects were observed at the highest dietary level.
During the initial stages, dosage-related lower bodyweight gains were seen for both sexes receiving 6000 or 18000 mg/kg diet, with animals treated at 18000 mg/kg diet showing gains which were less than 50% of the concurrent controls. In week 1, decreased food intake was also clearly apparent for both sexes at 18000 mg/kg diet but the week 1 food efficiency data indicated that the initial lower gains were not solely due to lower food intake. After week 1, the efficiency of food utilisation for both sexes at 6000 or 18000 mg/kg diet was generally similar to controls, indicating a balance between lower food intake and reduced bodyweight gain. It is possible that the lower food intake noted after week 1 was at least partly due to unpalatability of the diet.

There were treatment-related effects on white blood cell parameters for both sexes at week 26 and 52 but not at week 14. In week 26, males at 2000, 6000 and 18000 mg/kg diet showed total white blood cell counts that amounted to respectively 77.5% ($p<0.05$), 85.7 ($p<0.05$) and 74.3% ($p<0.01$) of control values. For females these value amounted to 90.7 % (not significant), 71.6% ($p<0.05$) and 65.3% ($p<0.01$). In week 52 white blood cell counts in females were no longer statistically significantly different from those in control for all treatment groups. The values at 2000, 6000 and 18000 mg/kg diet amounted to 99.7%, 100.7% and 74.6% of the control respectively. In males a significant reduction in white blood cell counts to 76.0% ($p<0.01$) of control was only observed for the highest dose group and the values in the 2000 and 6000 mg/kg bw dose group were 79.3% and 90.5% of the control value. The applicant indicates that the differences were mainly due to lower neutrophils or lymphocytes and occasional effects on monocytes or large unstained cells though no consistent effects were observed. The effects on white blood cells were not accompanied by treatment related effects on bone marrow or histopathology associated with lymphoid tissue.

The only other toxicologically significant effect observed was in the forestomach in animals of the 18000 mg/kg diet group. The lesions were consistent with local irritation of the mucosa with hyperplasia, erosions and ulcerations, with evidence of healing and reepithelialisation.

The incidence of these lesions was only statistically significantly different from controls at this dietary level. The changes seen in the stomach were confined to a specific area of the forestomach, the oesophageal groove and are thought to represent a local response to irritation. Thus, they are considered not to be indicative of systemic toxicity. There was no correlation between the individual animals which showed lower bodyweight gain, poor grooming and/or brown fur staining and the presence of these stomach lesions. Nor was there any correlation between the animals, which showed stomach lesions and those, which exhibited white blood cell and/or biochemical disturbances. According to the applicant this further supports the hypothesis that the stomach lesions are not indicative of systemic toxicity.

Treatment related effects other than the forestomach findings were noted at the 6000 and 18000 mg/kg dietary levels. These consisted of low bodyweight gain and initial reduced food consumption in both sexes.
In animals receiving 18000 mg/kg diet there were more pronounced effects on bodyweight gain and irritant effects of treatment on the forestomach with limited ulceration and signs of healing. No significant toxicological effects were observed in the animals receiving 6000 mg/kg or 2000 mg/kg ethyl lauroyl arginate in the diet.

The applicant concludes that based on local irritant changes in the forestomach at 18000 mg/kg diet and the calculated intake data, the NOAEL in this study was 6000 mg/kg diet equivalent to 307 mg/kg bw/day in the males and 393 mg/kg bw/day in the females.

The Panel concludes, given the fact that the effects on white blood cell counts at 26 weeks are significant for all dose groups, that the NOAEL for this 52 week study is lower than the lowest dose levels tested, and thus lower than 106 mg/kg bw/day.

Human data

In a study submitted by the applicant, ethyl lauroyl arginate was administered to six healthy volunteers assigned to two dose groups. In this study two volunteers received an oral dose of ethyl lauroyl arginate of 2.5 mg/kg bw and four volunteers received an oral dose of 1.5 mg/kg bw. All of the subjects were clinically examined and samples of blood were analysed for haematology and clinical chemistry and urinalysis was also performed to identify possible adverse physiological effects of ethyl lauroyl arginate after a single oral dose. Blood was also taken to monitor plasma levels and to determine the pharmacokinetics of ethyl lauroyl arginate and its metabolites and the results of these analyses were described above under the section on absorption, distribution metabolism and excretion.

Three mild treatment-emergent adverse events were reported by two of the six subjects (33%). Headache, was observed in one subject after 2.5 mg/kg bw. Diarrhoea and flatulence, were observed in another subject who received 1.5 mg/kg bw. The applicant indicates that all of these adverse events were considered unlikely to be related to the treatment as there were no similar findings at 5 mg/kg bw of ^{13}C-LAE administered orally to 2 healthy male subjects in a study on the kinetics of ethyl laurolyl arginate described already in the section on absorption, distribution, metabolism and excretion.
There were no clinically significant abnormalities in any of the blood chemistry data for either of the two oral dose levels, and there were no notable changes in vital signs and no clinically significant findings in ECG interpretation.

DISCUSSION

Ethyl-N^{α}-lauroyl-L-arginate, the active ingredient of ethyl lauroyl arginate, has been shown by *in vivo* and *in vitro* studies in rats and humans to be rapidly metabolised by hydrolysis of the ethyl ester and lauroyl amide to the intermediate products, N^{α}-lauroyl-L-arginine and arginine ethyl ester and then to ethanol, lauric acid and arginine. Arginine undergoes natural amino acid catabolism to urea and ornithine. Ornithine can then be further metabolised to CO_2 and urea. Lauric acid is a fatty acid that can enter normal fatty acid metabolism. Ethanol can be converted by alcohol dehydrogenase and aldehyde dehydrogenase to acetate which can enter normal biochemical pathways.
It is concluded that, on ingestion by humans, ethyl lauroyl arginate will be broken down to products of normal metabolism. It does not accumulate in the body.

During the characterisation of ethyl lauroyl arginate, the applicant has identified three additional minor by-products, which share the structure of ethyl-N^{α}-lauroyl–L-arginate but have an additional lauroyl group. Quantification of these trace impurities is not included in the specification of ethyl lauroyl arginate because the applicant indicates that their presence (at less than 1%) was demonstrated to be consistently and reproducibly visualised by HPLC when historical data from analyses of ethyl lauroyl arginate batches are compared. The Panel concludes that these trace impurities should be included in the specifications.

The Panel notes that use levels for ethyl lauroyl arginate are proposed in meat products and salted dry fish despite stability experiments demonstrating limited stability of ethyl-N^{α}-lauroyl–L-arginate in these foods.

The toxicological studies performed on ethyl lauroyl arginate comprise *in vitro* and *in vivo* metabolism-toxicokinetics studies, *in vitro* and *in vivo* mutagenicity assays, acute, subchronic toxicity and chronic toxicity studies, reproduction and developmental toxicity studies (one generation and two generations) in animals, and human kinetic studies.

The Panel considered the bacterial reverse mutation assay study could not be used for evaluation of mutagenicity due to the high toxicity of ethyl lauroyl arginate towards the bacterial cells. The Panel noted that this toxicity was predictable since ethyl lauroyl arginate is a preservative with antimicrobial activity.

Based on the results from the mouse lymphoma L5178Y cell mutation test and from the test for chromosomal aberrations in human lymphocytes it is concluded that ethyl lauroyl arginate is not genotoxic in mammalian cells. It showed no evidence of a genotoxic effect in the *in vivo* mouse micronucleus test.

Based on the lack of pre-neoplastic leasions in the 52 week study the Panel concludes that the local gastric irritation observed in the 52 week study is unlikely to evolve into neoplasms.

Long-term carcinogenicity studies are lacking. However, the rapid metabolism of ethyl-N^{α}-lauroyl-L-arginate to compounds endogenously present in much higher levels, the absence of preneoplastic toxic effects in the *in vivo* studies performed, does not suggest carcinogenic potential. In combination with the absence of genotoxic activity in the mouse lymphoma assay, the human lymphocyte assay and the micronucleus assay, the Panel concludes that there is no need to perform carcinogenicity studies.

Two 13-week oral toxicity studies and a 52-week oral toxicity study were performed in rats. All these studies reported effects on white blood cell counts.Table 3 provides an overview of these three rat studies in which effects on white blood cell counts were reported.

Table 3: Overview of the rat studies reporting effects on white blood cell parameters and NOAELs derived from them by the Panel.

Study	Preparation tested	Diet	Doses	Effects	NOAEL
13 week Han Wistar rat study 20 males, 20 females	ethyl lauroyl arginate containing about 90% ethyl-N^{α}-lauroyl-L-arginate	0, 5000, 15000, 50000 mg test substance in diet	0, 384, 1143 and 3714 mg ethyl lauroyl arginate /kg bw/day males 0, 445, 1286 and 3915 mg/kg bw/day ethyl lauroyl arginate for females	Lower white blood cell counts (73.9% of control)($p<0.01$) in males at the highest dose group Lower lymphocyte counts (71.9% of control)($p<0.05$) in males at the highest dose group	1143 mg/kg bw/day
13 week Sprague-Dawley rat study 10 males 10 females	19.5% solution of ethyl lauroyl arginate in propylene glycol	0, 3200, 12800 and 50000 mg solution /kg diet	0, 47, 195 and 719 mg ethyl lauroyl arginate of defined specifications/kg bw/day for males and 0, 56, 230 and 848 mg ethyl lauroyl arginate of given specifications /kg bw/day for females	Lower total white blood cell counts (86.8% of control (ns) for males, 72.3% of control ($p<0.01$) for females in the 50000 mg/kg diet dose group The values were 92.9% of control (ns) in males and 77.6% of control ($p<0.05$) for females in the 12800 mg/kg diet group	47 and 56 mg ethyl lauroyl arginate /kg bw/day for males and females
52 week Sprague Dawley rat study 20 males, 20 females	ethyl lauroyl arginate (88.2% purity)	0, 2000, 6000 and 18000 mg/kg diet	0, 106, 307 and 907 mg ethyl lauroyl arginate /kg bw/day for males and 0, 131, 393, 1128 mg ethyl lauroyl arginate /kg bw/day for females	Treatment related effects on white blood cells for both sexes at week 26, with white blood cell counts amounting to 77.5% ($p<0.05$), 85.7% ($p< 0.05$) and 74.3% ($p<0.01$) of control for males in the 2000, 6000 and 18000 dose group respectively. For females at 26 weeks these values amounted to 90.7% (ns), 71.6% ($p<0.05$) and 65.3% (p,0.01) of control for the 2000, 6000 and 18000 dose groups. At week 52 significantly reduced white blood cells counts were observed only for males in the highest dose group amounting to 76.0% of control ($p<0.01$) whereas for females the value amounted to 74.6% of control (ns).	lower than 106 mg/kg bw/day

The applicant indicates that the effects on the white blood cells at the highest dose level in the 13-week study in Sprague Dawley rats was unlikely to be treatment related since there was no consistency in the type of cells contributing to the lower total counts (e.g. for males the

differences were due mainly to lower neutrophil counts, and in the females mainly due to lower lymphocyte counts). No effects on white blood cells were observed in the preliminary 4 week study at the same dose levels. Furthermore, in the 13-week Han Wistar rat study with much higher dose levels of 0 (control), 5000, 15000 and 50000 mg ethyl lauroyl arginate /kg diet, amounting to intakes of 0, 384, 1143, and 3714 mg/kg bw/day of ethyl lauroyl arginate for males and 445, 1286 and 3915 mg/kg bw/day of ethyl lauroyl arginate for the equivalent female groups, slightly lower total white blood cell and lymphocyte counts were only observed in males receiving 50000 mg/kg only. The applicant indicates that the changes in this 13 week Han Wistar rat study were marginal and that there was no significant effect on white blood cells at this much higher dose level of ethyl lauroyl arginate. Therefore the applicant concludes that the effects in the rat study with the 19.5% ethyl lauroyl arginate formulation were coincidental and not treatment related.

The Panel notes that two 13-week studies and the 52-week study in rats report consistent effects on white blood cell counts. In the 13 week rat study in which a formulation of ethyl-N^{α}-lauroyl-L-arginate in propylene glycol was tested significant effects on white blood cell counts and alopecia were observed at dose levels of 12800 and 50000 mg formulation (19.5% ethyl lauroyl arginate in propylene glycol)/kg diet, equivalent respectively to 195 and 719 and 230 and 848 mg of ethyl lauroyl arginate of the defined specifications/kg bw/day. The study indicates a NOAEL of 3200 mg test formulation/kg diet amounting to 47 and 56 mg ethyl lauroyl arginate of the defined specifications/kg bw/day for males and females respectively.
In the other 13 week study with much higher dose levels (384, 1143, 3714 mg ethyl lauroyl arginate /kg bw/day for males and 445, 1286 and 3915 mg ethyl lauroyl arginate /kg bw/day for females) slightly but significantly lower white blood cell and lymphocyte counts were observed only in males receiving 3714 mg ethyl lauroyl arginate/kg bw/day), pointing at a NOAEL of about 1143 mg/kg bw/day.

Furthermore, in the 52-week study with rats at dose levels of 2000, 6000 and 18000 mg ethyl lauroyl arginate/kg diet (amounting to dose levels of 0, 106, 307 and 907 mg ethyl lauroyl arginate/kg bw/day for males and 0, 131, 393, 1128 mg ethyl lauroyl arginate/kg bw/day for females) there was evidence of a treatment-related effects on white blood cell parameters for both sexes. In week 26 all treatment groups showed significantly lower total white blood cell counts compared with control.

At week 52 white blood cell counts in females were no longer statistically significantly different from those in control for all treatment groups. The values at 2000, 6000 and 18000 mg/kg diet amounted to 99.7%, 100.7% and 74.6% of the control respectively. In males a significant reduction in white blood cell counts to 76.0% ($p<0.01$) of control was still observed for the highest dose group and the values in the 2000 and 6000 mg/kg bw dose group were 79.3% and 90.5% of the control value.

The Panel notes that effects on white blood cells were seen in different rat strains and in different sexes in two 90-day studies and in the 52-week study and concludes that these effects cannot be disregarded.

Therefore the Panel concludes, given the fact that the effects on white blood cell counts at 26 weeks are significant for all dose groups, that the NOAEL for this 52- week study is lower than the lowest dose levels tested, and thus lower than 106 mg/kg bw/day.

This is in line with the NOAEL of 47 and 56 mg ethyl lauroyl arginate/kg bw/day for males and females respectively from the 13-week study with the 19.5 % formulation of ethyl lauroyl arginate in propylene glycol.
Based on this NOAEL and a safety factor of 100, the Panel established an ADI of 0.5 mg ethyl lauroyl arginate of the proposed specifations /kg bw.
The safety factor of 100 is considered sufficient in spite of the fact that the ADI is based on a 90-day study because the effects on white blood cells do not become more severe upon prolonged exposure.

Potential dietary exposure to ethyl lauroyl arginate was estimated based on UK food consumption data and on the assumption that it would be present in all food categories for which use levels are proposed. Potential dietary exposure was found to be at or above the ADI in high consumers for both children aged 1.5 to 4.5 (580% of the ADI), children aged 4 to 18 (370% of the ADI) and adults (100% of the ADI). Potential mean dietary exposure to ethyl lauroyl arginate in consumers only was also at or above the ADI for both children aged 1.5 to 4.5 (170% of the ADI) and children aged 4 to 18 (106% of the ADI).

CONCLUSIONS AND RECOMMENDATIONS

The Panel established an ADI of 0.5 mg ethyl lauroyl arginate of the proposed specifations /kg bw.

Potential dietary exposure resulting from the proposed uses and use levels was found to be at or above the ADI in high consumers for both children aged 1.5 to 4.5 (580% of the ADI), children aged 4 to 18 (370% of the ADI) and adults (100% of the ADI). Potential mean dietary exposure to ethyl lauroyl arginate in consumers only was also at or above the ADI for both children aged 1.5 to 4.5 (170% of the ADI) and children aged 4 to 18 (106% of the ADI).

DOCUMENTATION PROVIDED TO EFSA

Submission for the Safety Evaluation of ethyl lauroyl arginate as a food additive.
Dossier submitted by the applicant Laboratorios Miret S.A. (LAMIRSA).

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[*] Sue Barlow declared an indirect interest in this substance and therefore is not part of this opinion (Cf. minutes of the 22nd Plenary Meeting: http://www.efsa.europa.eu/en/science/afc/afc_meetings/afc_22nd_meeting.html).