



EUROPEAN COMMISSION



## Scientific Committee on Consumer Products

SCCP

### **OPINION ON Ethyl lauroyl arginate HCl**

COLIPA n° P95



The SCCP adopted this opinion at its 15<sup>th</sup> plenary of 15 April 2008

### About the Scientific Committees

Three independent non-food Scientific Committees provide the Commission with the scientific advice it needs when preparing policy and proposals relating to consumer safety, public health and the environment. The Committees also draw the Commission's attention to the new or emerging problems which may pose an actual or potential threat.

They are: the Scientific Committee on Consumer Products (SCCP), the Scientific Committee on Health and Environmental Risks (SCHER) and the Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) and are made up of external experts.

In addition, the Commission relies upon the work of the European Food Safety Authority (EFSA), the European Medicines Evaluation Agency (EMEA), the European Centre for Disease prevention and Control (ECDC) and the European Chemicals Agency (ECHA).

### SCCP

Questions concerning the safety of consumer products (non-food products intended for the consumer).

In particular, the Committee addresses questions related to the safety and allergenic properties of cosmetic products and ingredients with respect to their impact on consumer health, toys, textiles, clothing, personal care products, domestic products such as detergents and consumer services such as tattooing.

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[http://ec.europa.eu/health/ph\\_risk/risk\\_en.htm](http://ec.europa.eu/health/ph_risk/risk_en.htm)

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## 1. BACKGROUND

Submission I for Ethyl lauroyl arginate HCl was submitted in February 2003 by COLIPA<sup>1</sup>.

The Scientific Committee on Consumer Products (SCCP) adopted its opinion SCCP/0837/04 at the 3<sup>rd</sup> plenary meeting of 15 March 2005 with the conclusion, that:

*"The SCCP is of the opinion that the information submitted suggests that ethyl lauroyl arginate causes mucosal irritation. Before any further consideration, the following additional information is required by the end of 2005:*

- \* clarification on purity, composition and impurities;
- \* an acute inhalation toxicity study."

In the current submission II, the company apply for inclusion of Ethyl lauroyl arginate HCl in Annex VI as a preservative with a new maximum concentration of 0.4% in all cosmetic products, and for inclusion in Annex III as an antimicrobial in soap, as oral care and antiplaque in oral care products, as deodorant in deodorant products and antidandruff agent in shampoos with a new maximum concentration of 0.8%.

## 2. TERMS OF REFERENCE

1. Does the SCCP consider, with the data provided in the attached submission that Ethyl lauroyl arginate HCl is safe for the consumers, when used as a preservative up to a maximum authorised concentration of 0.4 % in cosmetic products?
2. Does the SCCP consider, with the data provided in the attached submission that Ethyl lauroyl arginate HCl is safe for the consumers, when used for specific use up to a maximum authorised concentration of 0.8 % in the following cosmetic products: soap, anti-dandruff shampoos, deodorants and oral hygiene products?
3. Does the SCCP recommend any limitations and requirements or conditions of use for Ethyl lauroyl arginate HCl in cosmetic products based on the toxicological profile and risk assessment presented in the attached submission?

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<sup>1</sup> COLIPA - European Cosmetics Toiletry and Perfumery Association

### 3. OPINION

#### 3.1. Chemical and Physical Specifications

##### 3.1.1. Chemical identity

###### 3.1.1.1. Primary name and/or INCI name

Ethyl lauroyl arginate HCl (INCI name)

###### 3.1.1.2. Chemical names

Ethyl-N<sup>a</sup>-dodecanoyl-L-arginate hydrochloride (IUPAC)  
Monohydrochloride of L-arginine, N<sup>a</sup>-lauroyl-ethylester

###### 3.1.1.3. Trade names and abbreviations

LAE-P abbreviation for pure compound

LAE

Lauric arginate

Mirenat-N

Aminat

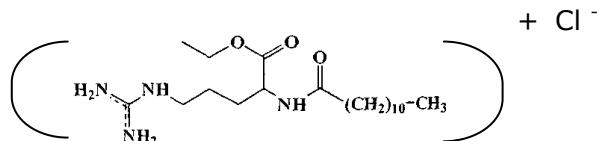
Lauramide arginine ethyl ester

###### 3.1.1.4. CAS / EINECS / ELINCS number

CAS: 60372-77-2

ELINCS: 434-630-6

###### 3.1.1.5. Structural formula



###### 3.1.1.6. Empirical formula

Formula: C<sub>20</sub>H<sub>41</sub>N<sub>4</sub>O<sub>3</sub>Cl

##### 3.1.2. Physical form

White solid

##### 3.1.3. Molecular weight

Molecular weight: 421.02

**Opinion on ethyl lauroyl arginate HCl****3.1.4. Purity, composition and substance codes**

Ethyl lauroyl arginate HCl is the active ingredient in the commercial product, LAE. In the crude technical product the aqueous paste contains 74-84% Ethyl lauroyl arginate HCl. LAE is the dehydrated crude product containing 84-95% Ethyl lauroyl arginate HCl.

Table 1: Specifications from submission II

<b>Product</b>	<b>Ethyl lauroyl arginate HCl Content</b>	<b>Physical form</b>	<b>Comments</b>
Crude Technical product	74-84%	White solid. H <sub>2</sub> O Content: 14-22%	Obtained at the end of the synthesis of Ethyl-Na-dodecanoyl-L-arginate HCl
LAE (Dehydrated commercial product)	85-95%	White solid. H <sub>2</sub> O Content: 0-1.5%	Obtained after drying the crude technical product
Ethyl lauroyl arginate HCl formulated			
MIRENAT-N AMINAT	20-20.4%	Liquid form Formulation of Ethyl lauroyl arginate HCl in propylene glycol	Both can be formulated from the Crude Technical or from LAE

'Impurities in the commercially available products have no toxicological relevance. Ethyl lauroyl arginate HCl is rapidly hydrolysed to the naturally occurring amino acid (arginine) and to the corresponding carboxylic acid (lauric acid) in plasma. The impurities correspond to these metabolites or are esters thereof, which are rapidly hydrolysed. Arginine is further metabolised to ornithine and urea. Moreover, the impurities of Ethyl lauroyl arginate HCl are also implicitly assessed in the toxicological studies performed with Ethyl lauroyl arginate HCl as they form part of the test substance.'

Table 2 lists the Ethyl lauroyl arginate HCl content and accompanying contaminants of the batches used in the provided studies. The main impurities are N<sup>ω</sup>-lauroyl-L-arginine, lauric acid and ethyl laurate. It should be noted Batch 5159 had a higher water content. It was stated in the submission that it was used in some of the older tests. However, it was only used in the embryo-foetal toxicity studies between 1998 and 1999. The batches used in the new studies provided in submission II are included.

Table 2: Ethyl lauroyl arginate HCl content and accompanying contaminants in LAE

<b>Batch name/number</b>	<b>LAE-P</b>	<b>3036</b>	<b>5733</b>	<b>2625</b>	<b>5159</b>	<b>7446</b>	<b>10234</b>	<b>12547</b>
	%	%	%	%	%	%	%	%
Ethyl lauroyl arginate HCl	99.0	93.2	90.3	90.1	69.1	88.2	88.2	91.87
Water		4.1	0.9	0.4	23.1	3.7	2.8	1.72
Ethyl laurate		1.5	2.0	0.7	1.0	1.0	1.4	0.83
Lauric acid		2.7	3.0	4.2	1.7	2.7	2.5	2.11
N <sup>ω</sup> -lauroyl-L-arginine (LAS)		1.5	2.1	3.3	1.0	1.9	1.6	1.83
L-arginine ethyl ester		0.3	0.4	0.3	0.2			
L-arginine		0.3	0.3	0.2	0.2			
Arginate HCl						0.1	0.4	0.18
Ethyl arginate 2HCl						<0.1	<0.1	
Salts (mostly NaCl)		0.7	0.9	0.8	1.6	1.5	0.8	
Ethanol					1.9			

In the acute inhalation toxicity study dossier, the test substance was RGR 6895, LAE in ethanol, batch no LI-531 (October 19, 2005); stated as "purity" of 0.63% LAE is the concentration. There was no further information. In the study dossier, Ethyl lauroyl arginate HCl and LAE seem to be considered equivalent.

**Opinion on ethyl lauroyl arginate HCl**

**Mirenat-N** is reported to be a formulation of 21.6 – 22% (w/w) LAE. Details of the Ethyl lauroyl arginate HCl content and impurities of the batches used the studies are in the Table 3.

Table 3: Ethyl lauroyl arginate HCl content (%) and accompanying contaminants in Mirenat

Batch	0000001 4-12-95	0000003	12 June 1995	13 Dec 1995	3128
	% (w/w)	% (w/w)	% (w/w)	% (w/w)	% (w/w)
Ethyl lauroyl arginate HCl	20.2	20.3	20.4	20.4	20.0
N <sup>ω</sup> -lauroyl-L-arginine	0.4	0.4	0.3	0.2	0.3
Lauric acid	0.7	0.6	0.7	0.7	0.6
Ethyl laurate	0.3	0.3	0.2	0.3	0.3
Water	3.8	3.4	3.5	76.9	3.8
Ethanol	0.3	0.2	0.2	0.2	0.2
Citric acid	1.2	1.2	1.2	0.2	1.2
Propylene glycol	73.0	73.5	73.3	1.2	73.7
LAE in formulation	21.6	21.6	21.6	21.6	21.2

There are some inconsistencies between the submission and the study reports. Batch 0000003 was given as 25% N-Lauroyl ethyl arginate monochlorohydrate. Batch 13 Dec 1995 differs from the other batches of Mirenat since the propylene glycol content is only 1.2% compared with 73% in the other batches of Mirenat.

**Aminat**, in these studies, is referred to only as a dilution of Mirenat. However, in the submission, it was indicated that Mirenat-N and Aminat were 20.0 –20.4% Ethyl lauroyl arginate HCl.

Submission II states that 'Mirenat-N and Aminat are trade names for a formulation of 21.2 – 21.6% LAE (which means 20-20.4% ethyl lauroyl arginate HCl) in propylene glycol.' These are marketing strategies. Mirenat is used for to preserve food products, while Aminat is the same formulation but proposed for cosmetics.

**Table 4: Mirenat-N and Aminat (20.0-20.4% Ethyl lauroyl arginate HCl)**

Composition	Range w/w (%)
LAE	21.2-21.6
Propylene glycol	73-74
Water	3-4
Citric acid	1.1-1.3
Ethanol	0.1-0.3

Aminat 4%, in the new mucous membrane irritation test, was prepared from Aminat, batch JMR-672. This was described as 20% LAE, Ethyl-N<sup>ω</sup>-dodecanoyl-L-arginate HCl on the certificate of analysis. No other information on the formulation of Aminat was provided. It is not stated if it was formulated in water or propylene glycol.

### 3.1.5. Impurities / accompanying contaminants

The accompanying contaminants are listed in 3.1.4

### 3.1.6. Solubility

In water, the solubility is greater than 247 g/l at 20°C.

In dimethyl sulphoxide (DMSO), LAE solubility is approximately 236 mg/ml. However precipitation occurred in cell culture medium, when dosed at 1% in media, to as low as 118 mg/ml. Solutions of LAE from 15 mg/ml, 30 mg/ml and 59 mg/ml formed cloudy/milky suspensions in medium, whereas 7 mg/ml solutions and lower did not form visible precipitate in medium. No colour change was observed at any of the concentrations.

**Opinion on ethyl lauroyl arginate HCl**

Solubility in ethanol is not provided, but in the acute inhalation toxicity study, the test substance was described as LAE in ethanol.

<b>3.1.7. Partition coefficient (Log P<sub>ow</sub>)</b>
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Log P<sub>ow</sub>: 1.43 at 20 °C

<b>3.1.8. Additional physical and chemical specifications</b>
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No specific characteristics were given for Ethyl lauroyl arginate HCl, only for LAE

**Organoleptic properties:**

Melting point:	50.5 to 58.0 °C
Boiling point:	decomposition from 107 °C
Flash point:	/
Vapour pressure:	5.45 × 10 <sup>-4</sup> Pa at 25 °C
Density:	1.11
Viscosity:	/
pKa:	/
Refractive index:	/
Stability:	not specified but assumed to be 6 months at 4°C in the dark by study authors

**Mirenat**

Stability: 6 months at 4°C in the dark

### **3.2. Function and uses**

Ethyl lauroyl arginate HCl is a cationic surfactant, active against bacteria, algae and fungi by modifying the permeability of membranes.

It is envisaged that it would be used as a multi-functional component in the formulation of cosmetic products. "It has applications as an anti-static agent and a surfactant with antimicrobial properties in cosmetics and toiletry formulations. The concentration used in any product depends on the susceptibility of each to microbial contamination."

In Submission I, the use concentration of Ethyl lauroyl arginate HCl was foreseen as 0.04 - 0.2% as a preservative in cosmetic formulations and up to 0.4%, as an antistatic agent and surfactant in soaps, oral care products, deodorants and anti-dandruff shampoos.

In Submission II, the application is for inclusion of Ethyl lauroyl arginate HCl in annex VI as a preservative with a new maximum concentration of 0.4% in all cosmetic products, and for inclusion in annex III as an antimicrobial in soap, as oral care and anti-plaque in oral care products, as deodorant in deodorant products and antidandruff agent in shampoos up to a maximum concentration of 0.8%.

EFSA (2007) approved ethyl lauroyl arginate as a food additive with an ADI of 0.5 mg/kg bw ethyl lauroyl arginate. The proposed uses include non-alcoholic drinks and fruit juices (115 mg/kg), salted fish, specified meat products, toppings and prepared salads (225 mg/kg).

### 3.3. Toxicological Evaluation

#### 3.3.1. Acute toxicity

##### 3.3.1.1. Acute oral toxicity

**Taken from SCCP/0837/04**

#### LAE

Guideline:	OECD 423 (1987)
Species/strain:	Rat, Sprague-Dawley Hsd, (CD)
Group size:	3 males + 3 females
Active ingredient:	90.1% Ethyl lauroyl arginate HCl
Test substance:	LAE
Batch:	2625
Dose:	1800 mg/kg bw Ethyl lauroyl arginate HCl in 1% aqueous methylcellulose by gavage (10 ml/kg bw)
Controls:	None
Observation period:	14 days
GLP:	in compliance

No control animals were included. Animals were observed for 14 days after treatment. There were no deaths.

Clinical reactions to treatment were piloerection and increased salivation in all rats within five minutes of dosing. Simultaneously, a waddling/unsteady gait was noted in females, whereas the males adopted a hunched posture. These signs persisted. Later during Day 1, females also adopted hunched posture and soiled fur (associated with the increased salivation). Piloerection (all rats) and hunched posture (females) were resolved by Day 2. All other signs had resolved by Day 3 (males) or Day 4 (females). All animals were considered to have achieved satisfactory bodyweight gains throughout the study. No macroscopic abnormalities were seen in any animals on Day 15. This macroscopic examination consisted of opening the cranial, thoracic and abdominal cavities.

The acute lethal oral dose to rats of Ethyl lauroyl arginate HCl was shown to be greater than 1800 mg of Ethyl lauroyl arginate HCl/kg bw in this study.

Ref.: 1

#### Mirenat

Guideline:	/
Species/strain:	Rat, Sprague-Dawley Hsd, (CD)
Group size:	5 males + 5 females
Active ingredient:	20.3% Ethyl lauroyl arginate HCl
Test substance:	Mirenat-N (25% N-Lauroyl ethyl arginate monochlorohydrate, 75% propylene glycol)
Batch:	000003
Stability:	6 months
Dose:	2000 mg/kg bw by gavage
Controls:	None
Observation period:	14 days
GLP:	in compliance

There were no deaths. Piloerection was the only clinical sign noted during this study.

Ref.: 3

**3.3.1.2. Acute dermal toxicity**

**Taken from SCCP/0837/04**

Guideline: OECD 402 (1987)  
 Species/strain: Rat, Sprague-Dawley Hsd, (CD)  
 Group size: 5 males + 5 females  
 Active ingredient: 90.1% Ethyl lauroyl arginate HCl  
 Test substance: LAE  
 Batch: 2625  
 Formulation: 601 mg/kg bw Ethyl lauroyl arginate HCl) in 1% aqueous methylcellulose  
 Dose: 2000 mg  
 Controls: None  
 Observation period: 14 days  
 GLP: in compliance

One day prior to treatment, hair was removed from the dorso-lumbar region (approx 10% total body surface) with electric clippers. A single topical dose of 667 mg/kg bw LAE (601 mg/kg bw Ethyl lauroyl arginate HCl) in 1% aqueous methylcellulose was applied at 3.0 ml/kg bw evenly to the prepared skin. The treated area (50mm<sup>2</sup>) was covered with gauze, held by a non-irritating dressing and further covered with a waterproof dressing for 24 h. These were removed and the treated area washed to remove any residual test substance. Animals were observed for 14 days after treatment. There were no deaths and no evidence of a systemic response in any animal throughout the study. Bodyweight gain throughout the study was satisfactory.

Well-defined irritation (erythema and oedema) was noted in all rats following removal of the dressings on Day 2. This was resolved by Day 9 in 8/10 animals, but in the other two rats, this dermal response persisted to Day 12 or 14. Associated with the dermal irritation, were reactions characterised by skin blanching, localised spots and/or scabbing and/or thickening of the skin and desquamation. These responses had resolved in all but three rats by Day 15. They continued to show scabbing at the dose sites, plus skin thickening in two rats. No macroscopic abnormalities were seen in the cranial, thoracic and abdominal cavities.

The acute lethal dermal dose to rats of LAE was shown to be greater than 1802 mg/kg bw Ethyl lauroyl arginate HCl.

Ref.: 2

**3.3.1.3. Acute inhalation toxicity**

**Taken from submission II**

Guideline: OECD 403 (1987)  
 Species/strain: Rat, Wistar Hsd, CPB: WU (SPF)  
 Group size: 5 males + 5 females  
 Active ingredient: 0.63% LAE  
 Purity: /  
 Test substance: RGR 6895, LAE in ethanol  
 Batch: LI-531 (October 19, 2005)  
 Route: Nose only 4h test period  
 Dose: 5800 mg/m<sup>3</sup> aerosolized LAE  
 Controls: Dry air atmosphere  
 Recovery period: 14 days  
 GLP: in compliance

LAE in ethanol was sprayed from aerosol cans in bursts of approximately 0.5 seconds into the inhalation chamber intermittently. The particle size had a Mass Median Aerodynamic

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Diameter of 4.67 $\mu\text{m}$  (geometric standard deviation 1.92). The particle size and distribution seemed constant over the dosing period. Air was supplied at a constant flow rate of 20 litres/minute. The concentrations present in the rat breathing zone were determined by back calculation from the propellants. The mean recovery of the propellants (vapour) was 68% and the active ingredient (LAE in ethanol as an aerosol) was 14%.

The test conditions fulfilled current test guidelines of steady-state concentration, respirability of particles and the 'limit' concentration tested. Due to the higher recovery of the latter, this was used to back-calculate the actual concentration, since this represented the amount of sprayed formulation best. The lower recovery of the aerosolized LAE at this high concentration appears to be related with the settling velocity of larger particles still containing ethanol and isobutane in addition to the non-volatile LAE. The results are the actual concentration in the breathing zone for the rat.

The animals were treated by nose only exposure for 4h and observed for 14 days after treatment. There were no deaths.

Clinical reactions to treatment were piloerection and irregular breathing patterns in all rats after dosing and lasted up to Day 4. In males, bradypnea, laboured breathing patterns, irregular breathing patterns, high-legged gait were seen following dosing but were mainly resolved by Day 1. Bodyweight gains throughout the study were reduced compared with the control in males but were not significant. During dosing, rectal temperatures were statistically significant lower, indicative of hypothermia, in the exposure group compared with the controls. No macroscopic abnormalities were seen in any animals.

The 4 h aerosol LC<sub>50</sub> is greater than 28150 mg/m<sup>3</sup> for the volatile fraction and greater than 5883 mg/m<sup>3</sup> for the aerosol fraction of LAE.

The study authors suggested that the test substance has a mild respiratory tract irritation if exposure to the aerosol is sufficiently high.

Ref.: 32

#### Comment

The purity of LAE was not supplied. Solubility of LAE in ethanol was not provided. The exposure to the non-volatile LAE is difficult to assess since much appears to have been lost before reaching the breathing zone.

### **3.3.2 Irritation and corrosivity**

#### 3.3.2.1. Skin irritation

##### **Taken from SCCP/0837/04**

Guideline:	OECD 404 (1992)
Species/strain:	New Zealand albino rabbit
Group size:	3 females
Active ingredient:	90.1% Ethyl lauroyl arginate HCl
Test substance:	0.5 g LAE, moistened with 0.5 ml sterile water
Batch:	2625
Dose:	0.07 mg Ethyl lauroyl arginate HCl/cm <sup>2</sup>
GLP:	in compliance

A paste, (0.5 g LAE with 0.5 ml water), was applied evenly to 6.25 cm<sup>2</sup> gauze square. This was applied to the dorsum of the rabbit. Semi-occlusive patches were applied and left in place for 4 hours. The test site was cleaned by gently swabbing with cotton wool. The skin was examined for erythema, eschar formation and oedema at 1, 24, 48 and 72 hours, 7 and 14 days after removal of the patches.

#### Results

All 3 animals showed slight erythema and 1 animal also showed slight oedema at the end of the exposure period. This continued up to 48 h. After 7 days, 2 animals still exhibited erythema (with the same erythema scores) and one also oedema. In addition, desquamation

**Opinion on ethyl lauroyl arginate HCl**

of the treated skin was noted in all 3 animals. By Day 15, only 1 of 3 animals had erythema, but the desquamation was still evident in 2 animals.

There was no indication of a systemic effect of treatment. No changes in body weight occurred during the course of the study.

The results of this study indicate that the test item, 90.1% of Ethyl lauroyl arginate HCl, has some irritant effect on the skin of the rabbit. The study authors concluded that incidence and severity of this reaction were not sufficient to require classification of the test item.

Ref.: 5

### 3.3.2.2. Mucous membrane irritation

#### **Taken from SCCP/0837/04**

Guideline:	OECD 405 (1987)
Species/strain:	New Zealand albino rabbit
Group size:	3 males
Active ingredient:	99% Ethyl lauroyl arginate HCl
Test substance:	LAE-P
Batch:	LAE-P
Purity:	99% Ethyl lauroyl arginate HCl
Dose:	100 mg LAE-P
GLP:	in compliance

The test substance was instilled in a single application. The product was poured into the right conjunctival sac. After application, the lids of the treated eye were held closed for approximately one second. The quantity of test substance administered was 100 mg (99 mg of Ethyl lauroyl arginate HCl). The untreated left eye was used as a control. The degree of eye irritation was evaluated at 1, 24, 48 and 72 hours and 7, 14 and 21 days after treatment. The behaviour and physical condition of the rabbits were normal throughout the study.

One hour post-administration, redness of the conjunctiva with some hyperaemic blood vessels was observed in all animals. All animals showed swelling with the eyelids closed and scattered or diffuse corneal opacity, obscuring the iris.

Seventy-two hours after treatment, all animals continued to show redness of conjunctiva, corneal opacity, with no discernible iris through opacity, swelling with lids closed and lacrimation, moistening of the eye lids and the fur.

21 days post-administration, all animals still had a diffuse, crimson redness of the conjunctiva with individual vessels not easily discernible, swelling with lids half closed. Two animals continued to display lacrimation with moistening of lids and the fur. All animals had tissue growth in the cornea. Cornea opacity was noted in one animal, whilst the other two showed areas of corneal opacity with no visible iris.

The mean values for each type of lesion at 24, 48 and 72 hours post-administration, for the 3 animals were:

Corneal opacity	4.0
Iridial lesions	no quantification possible
Hyperaemia	3.0
Oedema	4.0

The test substance, 99% Ethyl lauroyl arginate HCl, was considered to cause serious damage to eyes under the test conditions of the study.

Ref.: 8

**Taken from SCCP/0837/04****Mirenat-N, study 1**

Guideline: OECD 405 (1987)  
 Species/strain: New Zealand albino rabbit  
 Group size: 1  
 Active ingredient: 20.4% Ethyl lauroyl arginate HCl  
 Test substance: Mirenat -N  
 Batch: 12 June 1995  
 Purity: 21.6% LAE  
 Dose: 0.1 ml equivalent to 20.4 mg Ethyl lauroyl arginate HCl  
 GLP: in compliance

The test substance was instilled in a single application. The product was poured into the right conjunctival sac. After application, the lids of the treated eye were held closed for approximately one second. The untreated left eye was used as a control. The degree of eye irritation was evaluated at 1, 24, 48 and 72 hours and 7 days after treatment. The behaviour and physical condition of the rabbit was normal throughout the study.

One hour post-administration, diffuse corneal opacity was noted, with translucent corneal opacity at 24-h and opalescent corneal opacity at the 48-h. Sloughing of the cornea was noted both at the 24 and 48-h. Iridial irritation was noted at 1, 24 and 48-h.

Severe conjunctival irritation was noted at 1-h, with moderate conjunctival irritation at 24 and 48-h. Petechial haemorrhage of the upper conjunctival membrane was noted at 1, 24 and 48-h with sloughing of the conjunctivae at the 48-h. Due to sloughing of the nictitating and conjunctival membranes, the animal was killed after 48 hours in accordance with Company policy and Home Office Guidelines. No further animals were treated.

20.4% Ethyl lauroyl arginate HCl produced a maximum total score of 77.0 in the Kay and Calendra classification for the rabbit eye; as class 6, 'at least a severe irritant' based on a 1 to 8 scale. Under EU labelling regulations, it would be 'an irritant'.

Ref.: 9

**Taken from SCCP/0837/04,****Mirenat-N, study 2**

Guideline: OECD 405 (1987)  
 Species/strain: New Zealand albino rabbit  
 Group size: 1  
 Active ingredient: 20.4% Ethyl lauroyl arginate HCl  
 Test substance: Mirenat -N  
 Batch: 13 December 1995  
 Purity: 21.6% LAE  
 Dose: 0.1 ml equivalent to 20.4 mg Ethyl lauroyl arginate HCl  
 GLP: in compliance

The test substance was instilled in a single application. The product was poured into the right conjunctival sac. After application, the lids of the treated eye were held closed for approximately one second. The untreated left eye was used as a control. The degree of eye irritation was evaluated at 1, 24, 48 and 72 hours and 7 days after treatment.

One hour post-administration, diffuse corneal opacity was noted, with translucent corneal opacity at 24-h. Sloughing of the cornea was noted both 1 and 24-h. Iridial irritation and moderate conjunctival irritation was noted at 1 and 24h.

**Opinion on ethyl lauroyl arginate HCl**

Due to sloughing of the conjunctival membranes, the animal was killed after 24 hours in accordance with Company policy and Home Office Guidelines. No further animals were treated.

20.4% Ethyl lauroyl arginate HCl produced a maximum total score of 57.0 in the Kay and Calendra classification for the rabbit eye, as class 6; 'at least a severe irritant' based on a 1 to 8 scale. Under EU labelling regulations, it would be 'an irritant'.

Ref.: 10

**Comment**

20.4% Ethyl lauroyl arginate HCl would be classified as an irritant in both these Mirenat studies, independent of the vehicle (Study 1: vehicle propylene glycol; Study 2: vehicle water).

***Taken from SCCP/0837/04*****Aminat, study 1**

Guideline:	OECD 405 (1987)
Species/strain:	New Zealand albino rabbit
Group size:	3 male
Active ingredient:	0.02%. Ethyl lauroyl arginate HCl
Test substance:	Aminat 0.1%: [Mirenat-N & deionised water]
Batch:	Mirenat 3128
Purity:	21.2% LAE
Dose:	0.1 ml equivalent to 20 µg Ethyl lauroyl arginate HCl
GLP:	in compliance

The test substance was instilled in a single application. The product was poured into the right conjunctival sac. After application, the lids of the treated eye were held closed for approximately one second. The untreated left eye was used as a control. The degree of eye irritation was evaluated at 1, 24, 48 and 72 hours and 7 days after treatment. Animals were observed for 7 days after treatment. The behaviour and physical condition of the rabbits were normal throughout the study.

One hour post-administration, redness of the conjunctiva with some hyperaemic blood vessels were observed in all animals. In addition, one animal also presented oedema with slight swelling.

At 24 hours, the hyperaemia persisted in one animal and oedema in another. One animal presented scattered or diffuse areas of opacity covering one fourth or less of the corneal area.

At 48 h, no ocular lesions were observed in any animal. At 72 h post-administration, redness of the conjunctiva with some hyperaemic blood vessels was seen in one animal, which had disappeared by day 7.

The mean values at 24, 48 and 72 hours post-administration, for each type of lesion, of the 3 animals were:

Corneal opacity	0.11
Iridial lesions	0.00
Hyperaemia	0.22
Oedema	0.11

0.02% of Ethyl lauroyl arginate HCl was considered to cause no ocular irritation under the test conditions. However the concentration of Ethyl lauroyl arginate HCl (0.02%) used is well below the concentrations that are being requested for use.

Ref.: 11

**Taken from SCCP/0837/04****Aminat, study 2**

Guideline: OECD 405 (1987)  
 Species/strain: New Zealand albino rabbit  
 Group size: 3 male  
 Active ingredient: 0.03%. Ethyl lauroyl arginate HCl  
 Test substance: Aminat 0.15%: [Mirenat-N & deionised water]  
 Batch: Mirenat 3128  
 Purity: 21.2% LAE  
 Dose: 0.1 ml equivalent to 30 µg Ethyl lauroyl arginate HCl  
 GLP: in compliance

One hour post-administration, all animals showed conjunctival redness with some hyperaemic blood vessels. In addition, one animal also had oedema with slight swelling. Slight lacrimation was observed in another animal. At 24 hours, two animals showed symptoms, one had persistent redness and the other had slight swelling. At 48 hours no ocular lesions were observed in any of the animals. At 72 h post-administration, conjunctival redness with some hyperaemic blood vessels was seen in one animal, which had disappeared by day 7. The mean values at 24, 48 and 72 hours post-administration, for each type of lesion, of the 3 animals were:

Corneal opacity	0.00
Iridial lesions	0.00
Hyperaemia	0.22
Oedema	0.11

0.15% (0.03% of Ethyl lauroyl arginate HCl) was considered to cause no ocular irritation under the test conditions. However the concentration of Ethyl lauroyl arginate HCl (0.03%) used is well below the concentrations that are being requested for use.

Ref.: 12

**Taken from SCCP/0837/04****Aminat, study 3**

Guideline: OECD 405 (1987)  
 Species/strain: New Zealand albino rabbit  
 Group size: 3 male  
 Active ingredient: 0.04%. Ethyl lauroyl arginate HCl  
 Test substance: Aminat 0.2%: [Mirenat-N & deionised water]  
 Batch: Mirenat 3128  
 Purity: 21.2% LAE  
 Dose: 0.1 ml equivalent to 40 µg Ethyl lauroyl arginate HCl  
 GLP: in compliance

One hour post-administration, redness of the conjunctiva with some hyperaemic blood vessels were observed in 2 animals, whilst in the third animal the redness was diffuse, crimson coloured with individual vessels not easily discernible. In addition, 2 animals also presented oedema with slight swelling and lacrimation. At 24 hours, the redness of the conjunctiva with some blood vessels definitely hyperaemic persisted in two animals. In addition, one animal had oedema with slight swelling. The other animal presented scattered or diffuse areas of opacity, covering a fourth or less of the corneal area. At 48 hours, two animals showed redness of the conjunctiva with some hyperaemic blood vessels. At 72 h post-administration, redness of the conjunctiva with some hyperaemic blood vessels was seen in one animal that disappeared by day 7.

The mean values at 24, 48 and 72 hours post-administration, for each type of lesion, of the 3 animals were:

Corneal opacity	0.11
Iridial lesions	0.00
Hyperaemia	0.56
Oedema	0.11

0.04% of Ethyl lauroyl arginate HCl was considered to cause no ocular irritation under the test conditions. However the concentration of Ethyl lauroyl arginate HCl (0.04%) used is well below the concentrations that are being requested for use.

Ref.: 13

**Taken from SCCP/0837/04**

**Aminat, study 4**

Guideline:	OECD 405 (1987)
Species/strain:	New Zealand albino rabbit
Group size:	3 male
Active ingredient:	0.4% Ethyl lauroyl arginate HCl
Test substance:	Aminat 2.0%: [Mirenat-N & deionised water]
Batch:	Mirenat 3128
Purity:	21.2% LAE
Dose:	0.1 ml equivalent to 400 µg Ethyl lauroyl arginate HCl
GLP:	in compliance

One hour post-administration a diffuse, crimson redness of the conjunctiva with individual vessels not easily discernible (grade 2) was observed in all animals. In addition, one animal presented oedema of the conjunctiva with slight swelling (grade 1) whereas the other two presented swelling with lids about half closed (grade 3). Two animals presented lesion in the iris (grade 1). Similarly, two animals were lacrimating, moistening the lids and fur, and the third animal presented increased lacrimation, moistening lids, fur and affecting a considerable area around the eye.

At 24 hours, the diffuse, crimson coloured redness of the conjunctiva with individual vessels not easily discernible (grade 2) persisted in two animals while in third, redness with vessels clearly hyperaemic (grade 1) was observed. In addition, two animals also presented oedema with slight swelling (grade 1) and one of them slight lacrimation.

At 48 and 72 hours, redness with hyperaemic vessels (grade 1) was recorded in two animals and this lesion was accompanied by oedema with slight swelling (grade 1) in the two animals at 48 hours, and in one animal at 72 hours. On day 7, redness with hyperaemic vessels (grade 1) was observed in the conjunctivas of one animal that disappeared by day 14.

The mean values at 24, 48 and 72 hours post-administration, for each type of lesion, of the 3 animals were:

Corneal opacity	0.00
Iridial lesions	0.00
Hyperaemia	1.00
Oedema	0.56

0.4% of Ethyl lauroyl arginate HCl was considered to cause no ocular irritation under the test conditions.

Ref.: 14

**From submission II**

Guideline: OECD 405 (2002)  
 Species/strain: New Zealand albino rabbit  
 Group size: 3 males  
 Active ingredient: Ethyl-N<sup>α</sup>-dodecanoyl-L-arginate HCl  
 Test substance: Aminat 4% in water  
 Batch: JMR-684  
 Purity: /  
 Dose: 0.1 ml  
 GLP: in compliance

The test substance was instilled in a single application. The product was poured into the right conjunctival sac. After application, the lids of the treated eye were held closed for approximately one second. The untreated left eye was used as a control. The degree of eye irritation was evaluated at 1, 24, 48 and 72 hours and 7 days in all 3 animals and one up to day 14 after treatment.

One animal was used initially to determine if there were any ocular reactions. Since these occurred, two further animals were dosed.

The one animal showed a 20g weight loss in the first 24h post-dosing. Bodyweight gain after that was normal. The behaviour and physical condition of the rabbits were normal throughout the study.

The mean values for each type of lesion at 24, 48 and 72 hours post-administration, for the 3 animals were:

Corneal opacity	0.11
Iridial lesions	0
Hyperaemia	1.22
Oedema	0.33

The test substance, Aminat 4%, equivalent to 0.8% Ethyl-N<sup>α</sup>-dodecanoyl-L-arginate HCl, was considered as non-irritant to the eyes under the test conditions of the study.

Ref.: 41

Comment: The certificate of analysis showed that Aminat 4% was prepared from Aminat, batch JMR-672. This was described as 20% LAE, Ethyl-N<sup>α</sup>-dodecanoyl-L-arginate HCl. No information on the composition of Aminat, batch JMR-672 was provided, but the study text implies the solvent was water rather than propylene glycol.

### 3.3.3. Skin sensitisation

**Taken from SCCP/0837/04**

#### Magnusson and Kligman study

##### LAE

Guideline: OECD 406 (1992)  
 Species/strain: Dunkin Hartley female guinea pig  
 Group size: Preliminary tolerance test: intradermal 2, topical 5  
 Main study: test group 10, control 5  
 Active ingredient: 90.1% Ethyl lauroyl arginate HCl  
 Test substance: LAE  
 Batch: 2625

**Opinion on ethyl lauroyl arginate HCl**

Purity:	90.1%
Concentrations	
Preliminary tolerance test: intradermal:	45%, 18%, 9%, 4.5%, 0.9% and 0.45% Ethyl lauroyl arginate HCl
topical application:	45%, 18%, 9%, 4.5% and 0.9% Ethyl lauroyl arginate HCl
Main study-	Intradermal injection: 0.09% Ethyl lauroyl arginate HCl
Main study-	Topical application: 18% Ethyl lauroyl arginate HCl
Vehicle:	Sterile water
Resting phase:	21 days
Challenge:	4.5% of Ethyl lauroyl arginate HCl
GLP:	in compliance

**Preliminary tolerance tests** to establish suitable concentrations for the main study.

Intradermal injection: The scapulae were shaved. Six sites per animal were injected intradermally with 0.1 ml of the test formulation (one per site). These sites were examined 5 days later for any signs of treatment reaction.

The study authors suggested that 0.9% Ethyl lauroyl arginate HCl seemed reasonably tolerated.

Topical application: Each animal was injected intradermally twice at the prepared sites with 0.1 ml emulsified Freund's complete adjuvant. Seven days later, the flanks of each animal were clipped free of hair. Each animal was dosed with 2 concentrations of the test item, 1 on either flank. A gauze patch, 20 x 20 mm, soaked with 0.2 ml of the selected concentration was placed on the treatment site. When both sites had been treated, they were covered with a strip of aluminium foil to act as an occlusive barrier and the trunk of the animal was wrapped with an elastic adhesive bandage to maintain the test item in contact with the skin. Within the group, each concentration was duplicated. The adhesive dressings and gauze patches were removed after 24 h contact. The sites were examined for signs of treatment reactions 24 and 48 h after removal of the dressings, 18% Ethyl lauroyl arginate HCl produced mild erythema after 24 h that had cleared by 48h.

4.5% Ethyl lauroyl arginate HCl was selected for use at challenge, being judged non-irritant.

### **Main study**

**Intradermal Induction:** Intradermal injections (0.1 ml) were made at the prepared skin site of each animal. Test animals were treated as follows:

<u>Injection site (paired)</u>	<u>Treatment</u>
Anterior	Emulsified Freund's complete adjuvant
Median	0.1% test item in sterile water
Posterior	0.1% test item in emulsified Freund's complete adjuvant

Skin reaction at the injection sites was assessed approximately 24 hours after injection. Well defined erythema was apparent at the FCA and FCA+ test substance injection sites at 24h but not at 48h.

**Topical Induction:** Day 8 of the study, 0.4 ml of 18% Ethyl lauroyl arginate HCl on a gauze patch was placed over the injection sites. After 48 h, the dressings were removed and the treated sites gently cleaned with warm water. The control group were treated the vehicle. There were no reactions to the treatment 24 h after removal of the dressings.

**Challenge:** All animals were prepared for challenge by clipping 50 mm x 50 mm, on each flank. The right flank of each animal had gauze patches, 20 x 20 mm, with 0.2 ml aliquots of the test item 4.5% Ethyl lauroyl arginate HCl placed in the centre of the prepared skin site. The left flank was treated with similar patches of 0.2 ml of the vehicle. The treated sites were covered with a strip of aluminium foil to act as an occlusive barrier and each animal then wrapped with a length of elastic adhesive bandage to keep the test item and

**Opinion on ethyl lauroyl arginate HCl**

vehicle in contact with the skin. After a contact period of 24 hours the dressings and patches were removed. Approximately 21 hours after removal of the dressings and patches, the treated sites were closely clipped to remove any hair that may have grown.

24 h after removal of the dressings, no response was observed in any animals from the test or control group.

Body weight during the period of the study were similar in both test and control groups.

**Conclusion**

The results indicate that 18% Ethyl lauroyl arginate HCl does not induce sensitisation in the guinea pig.

Ref.: 6

**Taken from SCCP/0837/04****Mirenat-N**

Guideline:	OECD 406 (1992)
Species/strain:	Dunkin Hartley male guinea pig albino
Group size:	Preliminary test: intradermal 2, topical induction, 2 topical challenge Main study: test group 10, control 5
Active ingredient:	20.4% Ethyl lauroyl arginate HCl
Test substance:	Mirenat-N
Batch:	12 June 1995, 21.6% of LAE
Purity:	20.4% Ethyl lauroyl arginate HCl
Concentrations	
Preliminary tolerance test:	Intradermal: 0.2 % and 1.0% Ethyl lauroyl arginate HCl topical application: 15.3%, 10.2%, 5.1% Ethyl lauroyl arginate HCl Challenge: 10.2%, 5.1%, 2.4%, 1.2% Ethyl lauroyl arginate HCl
Main study:	Intradermal induction: 0.2% Ethyl lauroyl arginate HCl Topical induction: 50% v/v 10.2% Ethyl lauroyl arginate HCl
Resting phase:	21 days
Challenge:	50% v/v 10.2% Ethyl lauroyl arginate HCl
Vehicle:	Sterile water
GLP:	in compliance

**Preliminary tolerance tests** to establish suitable concentrations for the main study.

**Intradermal induction:** each animal received 0.1 ml injections of any one concentration of test material at 4 sites. These sites were examined 24, 48, 72h and 7 days later for any signs of treatment reaction. The degree of oedema was not evaluated. The highest concentration that caused only mild to moderate skin irritation and well tolerated systemically was 0.2% Ethyl lauroyl arginate HCl. This was selected for this phase of the main study.

**Topical induction:** applications were made to the clipped flanks under occlusive dressings for a 48 h exposure period to animals that had been intradermally injected with Freund's Complete Adjuvant eleven days earlier.

The degree of erythema and oedema was evaluated approximately 1, 24 and 48 hours after dressing removal. The highest concentration, 10.2% Ethyl lauroyl arginate HCl that caused only mild to moderate skin irritation and well tolerated systemically was selected for this phase of the main study.

**Topical challenge:** 10.2%, 5.1%, 2.0% and 1 .0% of Ethyl lauroyl arginate HCl.

The four test concentrations were applied to the clipped flanks of the animals under occlusive dressings for an exposure period of 24 hours to animals that had been treated

**Opinion on ethyl lauroyl arginate HCl**

identically to the control animals of the main study, up to Day 14. The degree of erythema and oedema was evaluated approximately 1, 24 and 48 hours after dressing removal. 10.2% and 5.1% Ethyl lauroyl arginate HCl, as the highest non-irritant challenge concentration and one lower concentration were selected for this phase of the main study.

**Main study**

Intradermal induction: Intradermal injections (0.1 ml) were made at the prepared skin site of each animal. Test animals were treated as follows:

Injection site (paired)	Treatment
Anterior	Emulsified Freund's complete adjuvant. Water (1:1)
Median	0.1% test item in sterile water.
Posterior	0.1% test item in emulsified Freund's complete adjuvant (1:1)

Well-defined to moderate to severe erythema was noted at all induction sites at 24 h and very slight to moderate to severe erythema at 48 h in the test group animals. In the control group, very slight erythema was noted at 3 treatment sites at 24-h but no dermal reactions at 48 h.

**Topical induction:** On Day 7, the same area on the shoulder region used previously for intradermal injections was clipped again and treated with a topical application of the test material formulation. A filter paper patch (approximate size 40 mm x 20 mm), saturated with the test material formulation 10.2% Ethyl lauroyl arginate HCl was applied to the prepared skin and held in place with a strip of surgical adhesive tape (50 mm x 30 mm) covered with an overlapping length of aluminium foil. The patch and foil were further secured with a strip of elastic adhesive bandage (approximate size 250 mm x 350 mm) wound in a double layer around the torso of each animal. This occlusive dressing was kept in place for 48 hours.

Slight erythema was noted at all induction sites at the 1 h and two induction sites at the 24 h observation in the test group animals. No dermal reactions were noted at the treatment sites of the control group animals at the 1 or 24-h observation.

**Topical Challenge Phase:** shortly before treatment on Day 21, an area of approximately 50 mm x 70 mm on both flanks of each animal, was clipped free of hair. A square filter paper patch (20 mm x 20 mm), saturated with the test material formulation at the maximum non-irritant concentration 10.2% Ethyl lauroyl arginate HCl was applied to the right flank. To ensure that the maximum non-irritant concentration was used at challenge, the test material at a concentration of 5.1% Ethyl lauroyl arginate HCl was applied to the left shorn flank. The patches were occluded. After 24 hours, the dressings were removed. The challenge sites were swabbed with distilled water to remove residual material.

No skin reactions were noted at the challenge sites of the test or control group animals at the 24 or 48 h observations at 10.2% and 5.1% Ethyl lauroyl arginate HCl.

Bodyweight: Bodyweight gains of guinea pigs in the test group, between Day 0 and Day 24, were comparable with the control group animals over the same period.

20.4% Ethyl lauroyl arginate HCl produced 0% sensitisation (0/10) at 50% dilution. The results indicate that 20.4% Ethyl lauroyl arginate HCl does not induce a sensitisation response in the guinea pig.

Ref.: 7

### 3.3.4. Dermal / percutaneous absorption

#### **From SCCP/0837/04 and reassessed in this opinion**

Guideline: According to the 'Guidelines for *in vitro* methods to assess percutaneous absorption of cosmetic ingredients' adopted by SCCNFP  
 Species/strain: Female pig skin, unboiled back. Animal weight: about 80 kg

Test Substance: LAE  
 Active ingredient: 90.3% Ethyl lauroyl arginate HCl  
 Batch: 5733  
 Purity: 90.3% Ethyl lauroyl arginate HCl  
 Test solutions: 0.39% and 1.96% Ethyl lauroyl arginate HCl in propylene glycol/water 30/70 solution  
 Dose application: 4.9 µl/cm<sup>2</sup> of test solution, 7 replicates  
 GLP: in compliance

### **Skin Preparation**

Subcutaneous fat was removed with a scalpel and the skin was rinsed with tap water. The bristles were cut with a special electric clipper for animals. The skin was then dermatomed to a thickness of about 700 µm. A punch with 2.6 cm inner diameter was used to obtain skin discs that fit the penetration cells. Only intact skin discs were used for the experiments. The integrity of the skin membranes was checked for each diffusion cell by measuring the Transepidermal Water Loss (TEWL). The diffusion cells were stabilised for one hour in the bath, TEWL was registered over one minute, after an initial 2 min stabilisation of the probe on the skin Cells that gave a TEWL higher than 15 g/m<sup>2</sup>.h were replaced.

### **Application**

The test solution (9 µl) was applied by micro-pipette to the entire epidermal surface delimited by the upper cell (1.86 cm<sup>2</sup> of exposed area; 4.8 µl formulation/cm<sup>2</sup> of skin). The solutions were in contact with the skin for 24 h. At the end of the contact period, the receptor fluid was recovered into a 5 ml volumetric flask. Then, both the skin bottom and the lower section of the diffusion cell were washed with distilled water, which was added to the receptor fluid taken to a final volume of 5 ml. The test solution remaining on the skin surface treated was washed off with water. Water aliquots, all tips, all cotton swabs as well as the top of the cell were collected together constituting the fraction of the active compound remaining in the surface.

### **Skin Stripping**

Eight strippings were carried out on the stratum corneum uniformly. The epidermis was separated from the dermis after heating the skin at 80°C for a few seconds.

### **Dose levels**

In the 0.39% Ethyl lauroyl arginate HCl solution, this is 4.8 mg solution/cm<sup>2</sup> and 18.7 µg/cm<sup>2</sup> of active substance.

In the 1.96% Ethyl lauroyl arginate HCl solution, it means 4.9 mg solution/cm<sup>2</sup> and 96.5 µg/cm<sup>2</sup> of active substance.

### **Recovery of test substance**

In the experiment using 0.39% Ethyl lauroyl arginate HCl solution, the quantities of active ingredient were below the Limit of Quantification (LOQ) in all the compartments analysed. The LOQ was given as 4.8383 mg/L.

Under the experimental conditions of this study, the percutaneous absorption of 1.96% Ethyl lauroyl arginate HCl in propylene glycol/water 30/70 after an exposure time of 24 h may be considered to be 5.24 -± 2.29 µg/cm<sup>2</sup>. The quantities of active ingredient found in all the compartments analysed were as follows:

<u>Compartments</u>	<u>µg/cm<sup>2</sup></u>
Surface	56.09 ±10.79
Stratum Corneum	28.80 ± 9.04
Epidermis	3.78 ± 1.84
Dermis	1.46 ± 1.65
Receptor Fluid	not detected

**Opinion on ethyl lauroyl arginate HCl**

<i>Total Recovery</i>	<i>90.13 ± 7.21</i>
Total Absorbed	5.24 ± 2.29

Ref.: 4

**Comment**

The 1.96% Ethyl lauroyl arginate HCl solution is 5 times higher than the maximum dose for the proposed use as preservative in cosmetics. Back extrapolation of the percutaneous absorption from 1.96% Ethyl lauroyl arginate HCl to 0.4% Ethyl lauroyl arginate HCl was not considered to be appropriate.

100% absorption of requested concentration 0.4% Ethyl lauroyl arginate HCl was therefore used to calculate the MOS.

### **3.3.5. Repeated dose toxicity**

#### **3.3.5.1. Repeated Dose (28 days) oral / dermal / inhalation toxicity**

**Taken from SCCP/0837/04 and reassessed in this opinion**

##### **LAE, study 1**

Guideline:	/
Species/strain:	Rat, Han Wistar
Group size:	5 males + 5 females per dose
Active ingredient:	90.1% Ethyl lauroyl arginate HCl
Test substance:	LAE
Batch:	2625
Purity:	90.1% Ethyl lauroyl arginate HCl
Dose:	0, 25000, 37500 or 50000 ppm LAE in diet (0, 22528, 33793 and 45057 ppm Ethyl lauroyl arginate HCl)
Observ. Period:	28 days
GLP:	in compliance

This was a pilot study for the 90 day study.

The LAE (final doses of 25000, 37500 or 50000 ppm) was incorporated in the diet to provide the required concentrations by initial preparation of a premix. The diet was prepared and issued weekly to the animal unit.

Group mean dosages over the 4 weeks of treatment were 2353, 3438 and 4273 mg LAE/kg bw/day (equivalent to 2120, 3098 and 3850 mg Ethyl lauroyl arginate HCl/kg bw/day) for males and 2379, 3329 and 4641 mg/kg bw/day (equivalent to 2143, 2999 and 4182 mg Ethyl lauroyl arginate HCl/kg bw/day) for the females. Control animals received the basal diet only.

Throughout the study, animals were inspected at least twice daily. The bodyweight of each animal was recorded prior to dosing, at the end of Week 1, twice weekly for Weeks 2-4, and before necropsy.

##### **Results**

There were no deaths. Piloerection was seen in all high dose females. The high and mid dose females had ungroomed coats. Excess salivation was evident in all treated females and most high dose males. Some animals in each treated group had brown staining of the muzzle.

The high dose animals lost weight or did not gain weight during Week 1. Low and mid dose animals had markedly low weight gain during this period. From week 2, the weight gain of treated animals was similar or greater than the controls. However, the overall gain (Days 0 - 27) for treated males was still low, particularly noticeable in the high dose group. Food consumption during Week 1 was low for all treated animals. A similar but less marked affect was evident during the remaining three weeks of treatment. Overall food intake was low and a dosage relationship was evident for the males.

**Haematology:** Minor changes and longer prothrombin and partial thromboplastin times for mid and high dose males. Females, at high dose, showed increased high mean cell haemoglobin concentration, mean cell volume and longer activated partial thromboplastin times.

**Blood chemistry:** Males had lower protein concentrations at all doses and low albumin and calcium levels in the high and mid doses.

Females had higher alanine amino-transferase and aspartate amino-transferase activities at the high and mid dose and increased alkaline phosphatase at the high dose.

**Organ weights analysis and macroscopic examination** did not reveal any significant findings. The top dose, 45057 ppm Ethyl lauroyl arginate HCl in diet, was tolerated and was selected for the 13 week study. This was a mean dosage of 3850 mg (male) and 4182 mg (female) Ethyl lauroyl arginate HCl/kg bw/day).

Ref.: 15

**Taken from SCCP/0837/04 and reassessed in this opinion**

**Mirenat-N**

Guideline:	/
Species/strain:	Rat, Crl: CD BR
Group size:	5 males + 5 females per dose
Active ingredient:	20.3% Ethyl lauroyl arginate HCl
Test substance:	Mirenat-N (21.6% of LAE)
Batch:	0000003
Purity:	20.3 Ethyl lauroyl arginate HCl % w/w
Dose:	0, 3200, 12800 and 50000 ppm of Mirenat-N in diet (0, 650, 2598 and 10150 ppm of Ethyl lauroyl arginate HCl)
Observ. Period:	28 days
GLP:	in compliance

This was a pilot study for the 90 day study.

The test substance, Mirenat-N (3200, 12800 and 50000 ppm of Mirenat-N) was incorporated in the diet to provide the required concentrations by initial preparation of a premix. The diet was prepared and issued weekly to the animal unit.

The mean Mirenat-N intakes over the 4 weeks of treatment were 336, 1393 and 5269 mg/kg bw/day (equivalent to Ethyl lauroyl arginate HCl of 68, 283 and 1070 mg/kg bw/day) for males and 352, 1400 and 5846 mg/kg bw/day (equivalent to Ethyl lauroyl arginate HCl of 71, 284 and 1187 mg/kg bw/day) for females.

Throughout the study, animals were inspected at least twice daily. The bodyweight of each rat was recorded at the time of allocation of animals to groups, on the day of commencement of treatment and once a week thereafter

**Results**

There were no treatment-related findings at any dose level.

The no-observed effect level in this 4 week dietary study was 1070 mg in males and 1187 mg in females Ethyl lauroyl arginate HCl/kg bw/day.

Ref.: 17

**3.3.5.2. Sub-chronic (90 days) oral / dermal / inhalation toxicity**

**Taken from SCCP/0837/04 and reassessed in this opinion**

**LAE study**

Guideline:	OECD 408 (revised 1998)
Species/strain:	Rat, Han Wistar

**Opinion on ethyl lauroyl arginate HCl**


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Group size:	20 males + 20 females per dose
Active ingredient:	90.1% and 90.3% Ethyl lauroyl arginate HCl
Test substance:	LAE
Batch:	2625 and 3036
Purity:	90.1% and 93.3% Ethyl lauroyl arginate HCl
Dose:	0, 5000, 15000 or 50000 ppm in diet (0, 4506, 13517 and 45057 ppm Ethyl lauroyl arginate HCl)
Test substance:	LAE
Observ. Period:	13 weeks
GLP:	in compliance

The experiment was conducted in accordance with the requirements of the OECD guideline No.408 (revised 1998) and the Toxicological Principles for the Safety Assessment of Direct Food Additives and Colour Additives used in Food-Red Book 1 (1982)

The LAE (final doses of 5000, 15000 or 50000 ppm) was incorporated in the diet to provide the required concentrations by initial preparation of a premix. The diet was prepared and issued weekly to the animal unit.

Group mean dosages over the 4 weeks of treatment were 384, 1143, 3714 mg LAE/kg bw/day (equivalent 346, 1030 and 3346 mg Ethyl lauroyl arginate HCl/kg bw/day) for males and 445, 1286, 3915 mg/kg bw/day (equivalent to 401, 1159 and 3527 mg Ethyl lauroyl arginate HCl/kg bw/day) for females. Control animals received the basal diet only.

A conversion factor was applied for batch 3036 LAE to take into account the water content. It is unclear if only LAE batch 3036 was used in this experiment. The study report indicates it was used from week 1, but does not indicate if this was at all doses.

During the study, the animals were observed daily for clinical signs and mortality, weekly for bodyweight and food consumption. During week 13, blood was sampled from the lateral tail vein for haematology and blood biochemistry and overnight urine was collected for urinalysis. At the end of the treatment periods, a full autopsy was conducted with recording of weights and macroscopic and microscopic examination of major organs. Ophthalmoscopy was conducted before the start of the study in all animals and at the end of the treatment period in 10 of each dose group. A battery of functional observations was assessed weekly after removal in 10 males and 10 females from each group. Animals were killed in the first four days of Week 13. There was no recovery period.

### Results

No deaths occurred during the study. Ungroomed coat was observed for the most high dose males and females and two mid dose females. This was associated with high incidences of yellow staining of the coat in the high dose animals. Brown staining on the muzzle was also observed in most high and mid dose animals. There was evidence of neurotoxicity during the weekly functional observational battery tests.

Marked bodyweight losses were observed during the first week of treatment in the high dose animals and significantly low bodyweight gains were observed in the mid dose group. From Week 2 onwards, bodyweight gains for treated animals were similar to, or higher than those of the controls. Despite this, all high dose animals or mid dose males after 13 weeks had lower overall bodyweights.

Food consumption was markedly lower in the high dose group and slightly lower in the mid dose group and low dose males during the first week. Food consumption remained low during subsequent weeks for the high dose animals.

It was not possible to calculate food conversion efficiency during Week 1 for the high dose animals due to bodyweight losses recorded. During the remainder of the treatment period it was similar, or slightly higher than the controls.

There were no treatment-related ophthalmic findings.

Only minor haematological changes were seen in the high dose males. Significant increases in mean cell haemoglobin, mean cell haemoglobin concentration, mean cell volume and reduced total white blood cell and lymphocyte counts with the concurrent controls were noted. As these differences were within the normal range, the study authors considered

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these were not of toxicological importance. They suggested the lower white blood count 'may be a reflection of the antibacterial nature of the substance'. Females were unaffected. Blood chemistry investigations revealed low total protein concentrations for the high dose animals and a lower albumin concentration for the high dose animals and the mid dose females. Slightly low cholesterol concentrations were also noted in the high dose females. Urinalysis investigations revealed a low pH for high and mid dose males. This was not seen in females.

No organ weight changes or macroscopic findings were attributable to treatment with LAE. The main treatment-related pathological changes with LAE 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, present in the majority of the high dose males and females and in one mid dose female. Ulceration was seen in 1 high dose and 1 mid dose male and two high dose females. In addition, erosions and epithelial hyperplasia were seen in all the high dose females. This was considered to indicate that the test substance had an irritant action on mucosal tissue, but that it was unusual for the changes to be restricted to such a specific area, e.g. adjacent to the entry of the oesophagus.

**Conclusion**

The No Observed Adverse Effect Level (NOAEL) was considered to be 346 mg/kg bw/day for males and 401 mg/kg bw/day for females of Ethyl lauroyl arginate HCl.

Ref.: 16

***Taken from SCCP/0837/04 and reassessed in this opinion*****Mirenat-N**

Guideline:	OECD No.408 (1981)
Species/strain:	Rat, Sprague Dawley Crl: CD BR
Group size:	10 males + 10 females per dose
Active ingredient:	20.2% Ethyl lauroyl arginate HCl
Test substance:	Mirenat-N (21.6% of LAE in 73% propylene glycol)
Batch:	0000001, 4-12-95
Purity:	20.2% Ethyl lauroyl arginate HCl
Dose:	0, 3200, 12800 and 50000 ppm Mirenat-N (equivalent to 646, 2586 and 10100 ppm Ethyl lauroyl arginate HCl)
Observ. Period:	13 weeks
GLP:	in compliance

The test substance, Mirenat-N (3200, 12800 and 50000 ppm of Mirenat-N) was incorporated in the diet to provide the required concentrations by initial preparation of a premix. The diet was prepared and issued weekly to the animal unit.

The mean Mirenat-N intakes over the 13 weeks of treatment were 220, 904 and 3324 mg/kg bw/day (equivalent to 44, 183 and 671 Ethyl lauroyl arginate HCl mg/kg/day) for males and 262, 1067 and 3927 mg/kg bw/day (equivalent to 53, 216 and 793 Ethyl lauroyl arginate HCl mg/kg bw/day) for females.

Throughout the study, animals were inspected at least twice daily. The bodyweight of each rat was recorded when they were allocated to groups, at the start of treatment and once a week thereafter.

**Results**

There was one unscheduled death amongst control males during Week 1 of the study. This was not considered related to treatment.

There were no adverse treatment-related clinical signs noted during the study. A slightly higher incidence of hairloss amongst the mid and high dose females was considered coincidental.

The overall mean bodyweight gain of females in all treatment groups was lower than the controls but there was no dose-relationship. Since bodyweight gain of males was unaffected

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by treatment, it is uncertain if the effect on females is treatment related. Marginally lower mean efficiencies of food utilisation were apparent for treated females but not males. Mean food intake of males and females was unaffected by treatment. The mean water intake of the high dose males was slightly higher than controls in Week 12.

There were no ocular lesions considered to be attributable to treatment.

Slightly lower total white blood cell counts were noted amongst mid and high dose male and females, though there was no consistency in the individual cell types affected.

No treatment-related changes in biochemical parameters were observed. Slightly higher urine volume amongst the high dose males was noted.

In the high dose females, higher mean adjusted liver weights were seen compared with concurrent controls.

No treatment-related changes were detected in any of the tissues examined.

**Conclusion**

The study authors concluded that treatment with Miranat at 50000 ppm (10100 ppm Ethyl lauroyl arginate HCl, equivalent to mean doses of 671 and 793 mg Ethyl lauroyl arginate HCl/kg bw/day males and females respectively) was associated with slight changes, evident as an equivocal lower body weight gain in females and an increase in water consumption and urine volume of males. They considered the no-effect level for continuous administration of Mirenat-N to rats for 13 weeks was likely to be 12800 ppm, (2586 ppm Ethyl lauroyl arginate HCl equivalent to mean doses of 183 mg Ethyl lauroyl arginate HCl/kg bw/day for males and 216 mg Ethyl lauroyl arginate HCl /kg bw/day for females).

Ref.: 18

<b>3.3.5.3. Chronic (&gt; 12 months) toxicity</b>
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**From submission II**

Guideline:	OECD No.452 (1981)
Species/strain:	Rat, Sprague Dawley Crl: CD (SD)IGS BR
Group size:	20 males + 20 females per dose
Active ingredient:	88.2% Ethyl lauroyl arginate HCl
Test substance:	Lauric arginate
Batch:	7446
Dose:	0, 2000, 6000 and 18000 ppm LAE
Observ. Period:	52 weeks
GLP:	in compliance

Overall group mean achieved intakes at 2000, 6000 and 18000 ppm for the period Week 1 to 52 were 106, 307 and 907 mg/kg bw/day (93.5, 271 and 800 mg/kg bw/day Ethyl lauroyl arginate HCl) for males and 131, 393 and 1128 mg/kg bw/day for females.

During the study, clinical condition, bodyweight and food consumption were recorded for all animals. Ophthalmic examination, haematology (peripheral blood), blood chemistry and urinalysis were carried out at scheduled intervals. In addition, detailed physical examination and arena observations were also performed at regular intervals and sensory reactivity, grip strength and motor activity were tested towards the end of the study. Blood samples were also withdrawn from animals towards the end of the study for the toxicokinetic and bioanalytical investigations. Following completion of the 52 weeks of treatment, all animals were killed. Bone marrow smears were prepared and a full myelogram completed. A full macroscopic examination was performed, organ weights were recorded, and tissues were retained for subsequent histopathological examination.

**Results**

Toxicokinetics: At the low dose, plasma LAE and LAS concentrations were below the LOQ. The rate and extent of systemic exposure of rats to LAE and its metabolite LAS appeared to be characterised by dose-independent (linear) kinetics over the dietary concentration 2000 to 18000 ppm during Week 52 of the 52-week toxicity study. High inter-individual variation

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in plasma LAE concentrations was noted, but this was less marked in plasma LAS concentrations in both sexes. There was some evidence that the kinetics of LAE in female rats may have been non-linear (dose-dependent), however, this did not reach statistical significance. The study also provided evidence that in general there were no differences in the systemic exposure of male and female rats to LAE or LAS.

Clinical observation in the period up to Week 13 females only showed higher weekly incidences of generalised brown fur staining and ungroomed coats in the 18000 ppm dose group and to a lesser extent in the 6000 ppm dose group than in the controls. Thereafter, no clear difference between groups was observed.

At 6000 and 18000 ppm both sexes showed low bodyweight gain and initial reduced food conversion efficiency in both sexes. The reduced food conversion efficiency was confined to the first week of treatment, and for males at both dose levels and for females at 18000 ppm, this coincided with lower than control food intake. However females receiving 6000 ppm also showed reduced food conversion efficiency in Week 1 without a concomitant effect on food intake. From approximately Week 26 of treatment, lower bodyweight gains were again apparent for these females at 6000 or 18000 ppm. The group treated with 2000 ppm LAE in the diet was not affected.

Higher high and low beam motor activity scores were noted for males only in Week 49 in the 18000 ppm dose group.

Compared with concurrent controls, there were statistically significant, but inconsistent effects on peripheral blood cell parameters at all doses, particularly the white blood cell parameters.'

At Week 14, at both mid and high dose, there were statistically significant decreases in monocytes and LUC in males. In females, at all doses, there were statistically significant decreases in monocytes and LUC. At the high dose there were also statistically significant decreases in neutrophils and reticulocytes

By Week 26, in males, there were statistically significant increases in MCHC and decreases in the total WBC counts at all doses, (decreases in differential cell counts mainly of lymphocytes and LUC at all doses and monocytes at the high dose). In females, there were statistically significant decreases in the total WBC counts at the mid and high doses, (statistically significant decreases in differential cell counts mainly of lymphocytes, monocytes and LUC at all doses).

By Week 52, in males, a statistically significant decrease in the total WBC count was only seen at the high dose. Similar statistically significant decreases in differential cell counts of lymphocytes, monocytes and LUC were seen in all doses but at the high dose there was also a decrease in neutrophils. In females, there were only statistically significant decreases in differential cell counts of neutrophils, monocytes and LUC and an increase in the MCHC at the top dose. There were no clear effects of treatment on the bone marrow. All differences from control were minor, not dosage-related and/or inconsistent across both sex groups, and as such considered not to be associated with treatment.

No significant changes were observed in the blood chemistry in any groups compared with controls.

Macroscopically evident depressions on the epithelia of the forestomach (in the oesophageal groove) were observed in 12/19 male and 9/19 female rats in the high dose group (18000 ppm), 5/20 male and 6/20 female rats in the mid dose and 1/18 male and 5/19 female rats in the low dose group compared with 0/19 male control rats and 2/20 female control rats.

These were reflected microscopically. In the high dose group (9/19 male and 8/19 female) showed erosion, an increased incidence/severity of ulceration, re-epithelialisation and hyperplasia of the non-glandular epithelium of the forestomach in the oesophageal groove area.. These lesions in association with the accompanying sub-epithelial submucosal inflammation, subepithelial fibrosis and inflammation of the muscle and serosal layers of the same area, broadly reflected the depressions in the forestomach recorded at necropsy. These changes were statistically significant compared with the controls. Similar forestomach lesions was observed in the mid (4/20 male and 5/20 female) and low dose (1/18 male and 5/19 female) groups but were not statistically significant compared with the controls (0/19 male and 2/20 female).

### Conclusion

Low bodyweight gain and initial reduced food consumption in both sexes were noted at the 6000 and 18000 ppm dosage levels. These bodyweight changes at both 6000 and 18000 were considered to be treatment-related, but at 6000 ppm, based on a combination of the short duration, the magnitude of difference from controls, lack of adverse effects on survival or general condition of the animals, they were not considered to be of toxicological importance. Animals receiving 18000 ppm showed a more pronounced effect on bodyweight gain and irritant effects of treatment on the forestomach with limited ulceration and signs of healing.

The study authors considered the observed white cell disturbances were not of toxicological importance, since there was no clear dose-relationship. In support of this, there were no treatment related effects on the bone marrow and a lack of any histopathology findings associated with the lymphoid tissues.

The study authors concluded that no significant toxicological effects were observed in the animals receiving 6000 ppm or 2000 ppm LAE in the diet.

Based on the calculated intake data, the NOAEL in this study was 6000 ppm equivalent to 307 mg/kg bw/day (271 mg/kg bw/day Ethyl lauroyl arginate HCl in the males and 393 mg/kg bw/day in the females). The corresponding LOAEL was 18000 ppm equivalent to 907 mg/kg bw/day and 1128 mg/kg bw/day for the males and females respectively, based on local irritant changes in the forestomach.

Ref.: 33

### Comments and reassessment of the oral toxicity studies (28 day, subchronic and chronic)

Hairloss was not seen in the subchronic and chronic LAE, only in the subchronic Mirenat study. This hairloss was seen in controls (4/9 male, 4/10 female) and at all dose levels (low dose: 3/10 male, 3/10 female; mid dose: 7/10 male, 6/10 female; high dose: 6/10 male, 8/10 female). However these included the animals where hairloss was also categorised alopecia (low dose: 0/10 male, 1/10 female; mid dose: 2/10 male, 5/10 female; high dose: 1/10 male, 7/10 female). The rationale for this dual categorisation was not provided as there was no indication of increased severity. The SCCP agreed that this finding was considered coincidental in the subchronic Mirenat study.

### Haematology

In all subchronic and chronic studies with Ethyl lauroyl arginate HCl, alterations of peripheral haematological parameters were observed. This concerned mainly decreases in white blood cells counts.

The initial response in the 28-day studies was an increase in corpuscular haemoglobin. This occurred only at the high dose in both studies. In males, similar statistically significant increases were seen (LAE study: MCHC; Mirenat study: MCH) and in females in the LAE study (Hb, MCH and MCV).

In the sub-chronic LAE study, at the high dose, in males only, statistically significant increases were seen in the MCH, MCHC and MCV and a decrease in the total WBC counts (due to statistically significant decrease in the neutrophils).

In the sub-chronic Mirenat study, statistically significant decreases were seen in the total WBC counts in females only at the mid and high dose, (due to statistically significant decreases in differential cell counts mainly of lymphocytes, monocytes and LUC). In males, statistically significant decreases in the neutrophils only were seen at these doses. In the LAE chronic study, at week 14, there seemed to be a similar more muted response in both sexes at both mid and high dose, (statistically significant decreases in differential cell counts mainly of monocytes and LUC) but not sufficient to decrease the total WBC significantly. The doses were within the same range (Mirenat 44, 183, 793 mg/kg body wt; chronic LAE 93.5, 271, 800 mg/kg body wt).

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In the chronic study, at week 26, males had statistically a significant increase in MCHC and decreases in the total WBC counts at all doses, (decreases in differential cell counts of lymphocytes and LUC at all doses and also monocytes at the high dose). By week 52, a statistically significant decrease in the total WBC count was only seen at the high dose. However, there were statistically significant decreases in differential cell counts of monocytes and LUC at all doses and also in neutrophils at the high dose.

In females, at week 26, there were statistically significant decreases in the total WBC counts at the mid and high doses, (statistically significant decreases in differential cell counts of monocytes and LUC at all doses and lymphocytes at the mid and high doses). By week 52, there was a change. The total WBC counts were similar to the controls at all doses. At the top dose only, there were statistically significant decreases in differential cell counts of neutrophils, monocytes and LUC and an increase in the MCHC.

These results are difficult to interpret since there were no individual pre-dosing haematology data available in the chronic study. The ranges of haematological parameters in the control group were wider than in the treated animals at most doses and there were also no consistent outliers within the control animals. Haematological data for replacement animals was not provided until after inclusion in the study. In addition, the percentage of the different white blood cells in the total WBC counts were within historical range (Charles River, 2006) at all doses and all studies, though the concurrent control monocytes and LUC were significantly higher in the chronic study.

Thus the effect of Ethyl lauroyl arginate HCl on peripheral haematological parameters is equivocal. No consistent pattern for the decrease of white blood cells was seen when different doses, time points and rat strains were compared.

Overall, when the effects from the different studies available are compared, there was little consistency in the cell types affected by these alterations. In the chronic studies, where data on haematological parameters were collected at several time points, the observed effects were also not consistent over time. Moreover, the decreases showed little dose-dependency.

No abnormal morphological changes in the blood cells were noted in the studies. In addition, no treatment related effects on the bone marrow and no histopathology findings associated with the lymphoid tissues have been reported.

#### **Histopathology**

In the chronic toxicity Sprague Dawley rat study, the post mortem effects in the stomach seemed to be dose-related: ulceration and epithelial hyperplasia were seen in animals in all dose groups and accompanied by subepithelial/submucosal inflammation and subepithelial fibrosis of the nonglandular epithelium, along with some inflammation of the underlying muscle and serosal layer, especially at the high dose. The study authors considered these minimal to slight treatment related findings in the non-glandular forestomach epithelium not to be a significant toxic effect as they were so localised. These effects on gastric mucosa appear to be local irritation effects induced by Ethyl lauroyl arginate HCl and are unrelated to a systemic toxicity. These effects were similar to the statistically significant gastric mucosal effects seen in the high dose in the sub-chronic LAE study in the Wistar rat. No gastric mucosal effects were seen after 13 weeks in the Mirenat Sprague Dawley rat study at comparable doses to the chronic study.

#### **NOAEL**

Despite the different rat strains, the NOAEL/NOEL for the subchronic and chronic LAE and Mirenat studies are within the same range. Dosing in the subchronic LAE study was higher, but the histopathology seen in both the subchronic and chronic studies was similar. This could explain the lower total WBCs at week 26, as the lesions developing in the gastric mucosa would cause concomitant migration of WBC to the damaged tissue. The normal morphology of the blood cells throughout this study supports this.

Individual pre-dosing haematology values in the chronic study would have assisted in assessing whether the variations seen in WBC parameters were normal or treatment

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induced. The high inter-individual variation seen in plasma LAE concentrations in the toxicokinetic data would suggest that similar variation could be feasible for the blood parameters.

The table indicates the achieved doses of Ethyl lauroyl arginate HCl dose mg/kg bw /day in diet and the NOAEL/NOEL derived by the study authors.

<b>Study</b>	<b>Test substance</b>	<b>Strain</b>	<b>Sex</b>	<b>Low dose mg/kg bw/day</b>	<b>Mid dose mg/kg bw/day</b>	<b>High dose mg/kg bw/day</b>	<b>NOAEL mg/kg bw/day</b>
28 day	LAE	Han, Wistar	M	2120	3098	3850	/
			F	2143	2999	4182	
	Mirenat	Sprague Dawley	M	68	283	1070	
			F	71	284	1187	
Sub-chronic	LAE	Han, Wistar	M	346	1030	3346	346
			F	401	1159	3527	
	Mirenat	Sprague Dawley	M	44	183	671	183 (NOEL)
			F	53	216	793	
Chronic	LAE	Sprague Dawley	M	93.5	271	800	271
			F	131	393	1128	

The NOAEL of 271 mg/kg bw/day from the chronic study will be used for the calculation of the Margin of Safety.

### 3.3.6. Mutagenicity / Genotoxicity

#### **Taken from SCCP/0837/04**

#### **Bacterial Reverse Mutation Test**

##### Study 1

Guideline:	OECD 471 (1997)
Species/strain:	<i>S. typhimurium</i> , TA98, TA100, TA1535, TA1537, TA 1538 and <i>E. coli</i> WP2 uvrA/pKM101
Active ingredient:	90.1% and 90.3% Ethyl lauroyl arginate HCl
Substance:	LAE
Batch:	3036
Purity:	93.3% Ethyl lauroyl arginate HCl
Vehicle:	DMSO
Concentration	
Test 1 range finding:	15, 50, 150 500, 1500, 5000 µg/ Ethyl lauroyl arginate HCl/plate
Test 1 repeat:	1.5, 5, 15, 50, 150 µg/ Ethyl lauroyl arginate HCl/plate
Test 2:	1.5, 5, 15, 50, 150 µg/ Ethyl lauroyl arginate HCl/plate
Positive controls:	without S9-mix: sodium azide TA1535 and TA100 strains 0.5 µg/plate 9-aminoacridine TA1537 strain 30 ng/plate 2-nitrofluorene TA98 strain 1 µg /plate and 2-(2-furyl)-3-(5-nitro-2-furyl)-acrylamide <i>E. coli</i> 0.05 µg/plate with S9-mix: 2-aminoanthracene: TA1535 strain 2µg /plate, <i>E. coli</i> 10 µg /plate benzo[a]pyrene: TA1537, TA98 and TA100 strains 5 µg/plate
Negative controls:	Vehicle
GLP:	in compliance

The first range-finding tests were standard plate incorporation assay. The second test involved a pre-incubation. The tests were performed in the presence and absence of S9 liver preparations from Aroclor 1254-induced rats.

## Results

**First tests (range-finding):** No substantial increases in revertant colony numbers over control counts were obtained with any of the tester strains following exposure to Ethyl lauroyl arginate HCl at any concentration with or without S9-mix.

Toxicity (thinning of the background lawn of non-revertant cells, combined with a reduction in revertant colony numbers) was seen in all *Salmonella* strains at 50 µg/plate and in the *E. coli* strain at 150 µg/plate. A maximum exposure concentration of 150 µg/plate was selected for use in the second test

**Second test:** No substantial increases in revertant colony numbers over control counts were obtained with any of the tester strains at any concentration with or without S9-mix. Toxicity was seen in all strains following exposure at 150 µg/plate.

## Conclusion

Under the test conditions employed, LAE (93.2% of Ethyl lauroyl arginate HCl) showed no evidence of mutagenic activity in this bacterial system.

Ref.: 19

## Taken from SCCP/0837/04

### Study 2

Guideline:	OECD 471 (1983)
Species/strain:	<i>S. typhimurium</i> , TA98, TA100, TA1535, TA1537, TA 1538
Active ingredient:	20.3% Ethyl lauroyl arginate HCl
Test Substance:	Mirenat-N
Batch:	0000003, 21.6% of LAE
Purity:	20.3%Ethyl lauroyl arginate HCl
Vehicle:	Water
Concentrations	
Test 1(range finding):	5, 50, 500, 5000 µg/ Mirenat-N (1, 10.2, 101.5 and 1015 µg/Ethyl lauroyl arginate HCl/plate)
Test 2:	15, 50, 150, 500, 1500, 5000 µg/ Mirenat-N (3, 10.2, 30.5, 101.5 304.5 and 1015 µg/Ethyl lauroyl arginate HCl/plate)
Positive controls:	without S9-mix: N-Ethyl-N'-nitro-N-nitrosoguanidine TA1535 strain, 5 µg /plate, TA100 strain 3 µg /plate 9-aminoacridine TA1537 strain 80 µg /plate 2-nitrofluorene TA98 strain 1 µg /plate with S9-mix: 2-aminoanthracene: TA1535 and TA1537strains 2µg/plate, TA98 0.5µg/plate and TA100 strains 1µg/plate
Negative controls:	Vehicle
GLP:	in compliance

The tests were performed in the presence and absence of S9 liver preparations from Aroclor 1254-induced rats.

## Results

Test 1 : Toxicity was seen at 5000 µg /plate (1015 µg of Ethyl lauroyl arginate HCl/plate) in tester strains TA 98 and TA 100; TA 98 at 500 µg /plate (101.5 µg of Ethyl lauroyl arginate HCl/plate) in the absence of S9-mix and TA 1537 only in the presence of S9-mix.

Test 2: No substantial increases in revertant colony numbers in any tester strains were observed following treatment with Mirenat-N at any dose level, in the presence or absence of S9-mix.

Toxicity was seen in all strains at 500 µg/plate (101.5 µg of Ethyl lauroyl arginate HCl/plate), except TA 1535 in the presence of S9-mix.

## Conclusion

20.3% Ethyl lauroyl arginate HCl in water shows no mutagenic activity in these bacterial systems

Ref.: 20

**Taken from SCCP/0837/04**

**In vitro Mammalian cell mutation assay**

Guideline:	OECD 476 (1984)
Species/strain:	Mouse lymphoma cells L5178Y
Active ingredient:	20.3% Ethyl lauroyl arginate HCl
Test Substance:	Mirenat-N
Batch:	0000003, 21.6% of LAE
Purity:	20.3%Ethyl lauroyl arginate HCl
Vehicle:	Water
Concentrations	
Preliminary Test:	15, 31.25, 62.5, 125, 250, 500, 1000, 1500, 2000 µg Mirenat-N/ml (3.0, 6.3, 12.7, 25, 51, 102, 203, 305, 406 µg Ethyl lauroyl arginate HCl/ml).
Test without S9-mix:	100, 150, 200, 220, 240, 260, 280, 300, µg/ml Mirenat-N (20, 30, 41, 45, 49, 53, 57, 61 µg Ethyl lauroyl arginate HCl/ml)
Test with S9-mix:	100, 200, 300, 375, 400, 425, 450, 500 µg/ml Mirenat-N (20, 41, 61, 76, 81, 86, 91, 102 µg Ethyl lauroyl arginate HCl/ml)
Positive controls:	without S9-mix: Ethyl methanesulphonate at 500 µg/ml with S9-mix: 20-Methyl cholanthrene at 2.5 µg/ml.
Negative controls:	Vehicle
GLP:	in compliance

Preliminary test: Relative growth, with and without S9-mix, resulted in suspension of 120 - 1% and 109 - 0% respectively compared with the controls. Concentrations in the main test were based upon this data.

Without S9-mix: Mean cell growths in suspension in Test 1 and Test 2 were 96 - 21% and 94 - 1% respectively. Relative Total Growth (RTG) in soft agar cultures in Test 1 (100, 200, 280 and 300 µg/ml Mirenat) and Test 2 (150, 220, 240 and 280 µg/ml Mirenat) were 86 - 24% and 77 - 35% respectively relative to the controls.

No significant increases in mutation frequency were observed after treatment with Mirenat-N in either test. The positive control induced significant increases in both tests.

With S9-mix: Mean cell growths in suspension in Test 1 and Test 2 were 82 - 2% and 90 - 2% respectively. RTG in Test 1 (200, 300, 400, 425 and 450 µg/ml Mirenat) and Test 2 (200, 300, 400 and 450 µg/ml (41, 61, 81 and 91 µg/ml Mirenat) were 70 - 8% in Test 1 and 83 - 32% in Test 2 relative to the controls.

Mirenat-N did not significantly increase mutant frequency after treatment in either test. In the second test a higher than normal control mutant frequency was observed but this was not considered to adversely affect the results obtained. The positive control induced significant increases in mutant frequency in both test.

**Conclusion**

It was concluded that 20.3% Ethyl lauroyl arginate HCl did not demonstrate mutagenic potential in this *in vitro* gene mutation assay.

Ref.: 21

**From submission II*****In vitro Mammalian cell mutation assay***

Guideline:	OECD 476 (1997)
Species/strain:	Mouse lymphoma cells L5178Y
Active ingredient:	Ethyl lauroyl arginate HCl
Test Substance:	LAE
Batch:	7446
Purity:	88.2% Ethyl lauroyl arginate HCl
Vehicle:	DMSO
Concentrations	
Preliminary Test:	0.29, 0.59, 1.17, 2.34, 4.68, 9.38, 18.75, 37.5, 75, 150, 300 600 µg/ml LAE (0.26, 0.52, 1.03, 2.06, 4.13, 8.27, 16.54, 33.1, 66, 132, 265, 529 µg/ml Ethyl lauroyl arginate HCl)
without S9-mix:	Test 1: 10, 24, 28, 30, 34, 38, 40, 45, 50µg/ml LAE (8.8, 21.2, 25, 26, 30, 34, 35, 40, 44 µg/ml Ethyl lauroyl arginate HCl). Test 2: 10, 24, 26, 28, 30, 31, 32, 33, 34 µg/ml of LAE (8.8, 21.2, 23, 25, 26, 27, 28, 29, 30 µg/ml Ethyl lauroyl arginate HCl) Test 3: 1, 10, 20, 30, 32, 34, 36, 38, 40, 45 µg/ml of LAE (0.88, 8.8, 18, 27, 28, 30, 32, 34, 35, 40 µg/ml of Ethyl lauroyl arginate HCl) Test 4: 1, 20, 30, 40, 42.5, 45, 47.5, 50 µg/ml of LAE (0.88, 18, 27, 35, 38, 40, 42, 44 µg/ml of Ethyl lauroyl arginate HCl)
with S9-mix:	Test 1 10, 40, 42, 43, 45, 46, 47, 48, 50 µg/ml LAE (8.8, 35, 37, 38, 40, 41, 41.5, 42, 44 µg/ml of Ethyl lauroyl arginate HCl) Test 2: 15, 30, 42, 43, 43.5, 44, 44.5, 45, 45.5, 46 µg/ml LAE (13, 27, 37, 38, 38.4, 39, 39.2, 40, 40.1, 41µg Ethyl lauroyl arginate HCl)
Positive controls:	without S9-mix: Methyl methanesulphonate at 10 µg/ml/3h; 5µg/ml/24h with S9-mix: 3-Methyl cholanthrene at 2.5 µg/ml.
Negative controls:	Vehicle
GLP:	in compliance

LAE was found to be soluble at approximately 236 mg/ml in dimethyl sulphoxide (DMSO). Solutions at this concentration and at approximately 118 mg/ml, when dosed at 1 % in the cell culture medium formed precipitate that settled out. Solutions of LAE at approximately 15 mg/ml, 30 mg/ml and 59 mg/ml formed cloudy/milky suspensions in medium. Solutions of approximately 7 mg/ml and lower did not form visible precipitate in medium. No colour change was observed at any of the concentrations. The maximum concentration tested in the preliminary toxicity test was 60 mg/ml (final concentration in medium of 600 µg/ml the objective being to test up to and beyond the limit of solubility).

**Preliminary test:** Relative suspension growth (RSG), with and without S9-mix, resulted in suspension of 105 - 0% and 114 - 0% respectively compared with the controls over 3h exposure. The RSG over 24h exposure without S9-mix was 98 - 0%. Concentrations in the main test were based upon this data.

**Without S9-mix, 3h exposure:**

**Test 1:** 10-50 µg/ml of LAE (8.8-44 µg/ml Ethyl lauroyl arginate HCl) resulted in RSG values of 96-0%. Cultures exposed at concentrations of 10-30 µg/ml (8.8-27 µg/ml Ethyl lauroyl arginate HCl) were assessed to determine mutant frequency. Relative total growth (RTG) values of 90-29% and cloning efficiencies of 118-93% relative to the solvent control were obtained.

**Test 2:** 10-34 µg/ml of LAE (8.8-30 µg/ml Ethyl lauroyl arginate HCl) resulted in RSG values of 89-16%. Cultures exposed at concentrations of 10-30 µg/ml (8.8-27 µg/ml Ethyl lauroyl arginate HCl) were assessed to determine mutant frequency. RTG values of 79-20% and cloning efficiencies of 120-81% relative to the solvent control were obtained.

With a 3-hour exposure, the maximum concentration assessed for determination of mutant frequency was 34 µg/ml LAE (30 µg/ml Ethyl lauroyl arginate HCl). There were no statistically significant increases in mutant frequency at any of the concentrations tested at acceptable levels of toxicity.

#### 24 h exposure

**Test 3:** 1-45 µg/ml LAE (0.88-40 µg/ml Ethyl lauroyl arginate HCl) resulted in RSG values of 110-34%. Cultures exposed at concentration LAE of 1-45 µg/ml (0.88-40 µg/ml Ethyl lauroyl arginate HCl) were assessed for determination of mutant frequency. RTG values of 142-32% and cloning efficiencies of 129- 83%, relative to the solvent control, were obtained.

**Test 4** treatment cells at 1-50 µg/ml of LAE (0.88-44 µg/ml Ethyl lauroyl arginate HCl) resulted in RSG values of 107-20%. Cultures exposed at concentration of LAE of 1-50 µg/ml (0.88-44 µg/ml Ethyl lauroyl arginate HCl) were assessed for determination of mutant frequency. RTG values of 115-22% and cloning efficiencies of 130- 92%, relative to the solvent control, were obtained.

With a 24-hour exposure, the maximum concentration assessed for determination of mutant frequency was 40 µg/ml LAE. There were no statistically significant increases in mutant frequency at any of the concentrations tested at acceptable levels of toxicity.

#### With S9-mix, 3h exposure

**Test 1** 10-50 µg/ml of LAE (8.8-44 µg/ml Ethyl lauroyl arginate HCl) resulted in RSG values of 101-0%. Cultures exposed at concentrations of 10-46 µg/ml (8.8-41 µg/ml Ethyl lauroyl arginate HCl) were assessed to determine mutant frequency. Relative total growth (RTG) values of 101-5% and cloning efficiencies of 116-87% relative to the solvent control were obtained.

**Test 2:** 15-46 µg/ml of LAE (13-41 µg/ml Ethyl lauroyl arginate HCl) resulted in RSG values of 93-33%. Cultures exposed at concentrations of 15-46 µg/ml of LAE (13-41 µg/ml Ethyl lauroyl arginate HCl) were assessed to determine mutant frequency. RTG values of 94-32% and cloning efficiencies of 114-92 % relative to the solvent control were obtained.

In all tests the concurrent solvent and positive control were within the ranges found from the historical solvent and positive control data.

#### Conclusion

It was concluded that 88.2% Ethyl lauroyl arginate HCl did not demonstrate mutagenic potential in this *in vitro* gene mutation assay.

Ref.: 34

#### Taken from SCCP/0837/04

#### *In vitro* mammalian chromosome aberration test in human lymphocytes

Guideline:	OECD 473 (1997)
Species/strain:	Human lymphocytes
Active ingredient:	93.3% Ethyl lauroyl arginate HCl
Test substance:	LAE
Batch:	3036
Purity:	93.3% Ethyl lauroyl arginate HCl
Vehicle:	DMSO
Culture medium:	RPMI 1640 tissue culture medium (Sigma) supplemented with 10% foetal calf serum (Globepharm), 1 unit/ml Heparin (CP Pharmaceuticals), 20 I.U./ml penicillin/20 µg /ml streptomycin and 2.0 mM glutamine (Imperial)

Concentrations

**Opinion on ethyl lauroyl arginate HCl**


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First test:	12.5, 25, 50, 100, 200, 400, 800 and 1600 µg LAE/ml (11.7, 23.3, 4-6.6, 93.2, 186.4, 372.8, 745.6 and 1491.2 µg Ethyl lauroyl arginate HCl/ml)
Second Test:	without S9-mix: 12.5, 25, 50, 75, 100, 150, 200 and 300 µg LAE/ml (23.3, 46.6, 93.2, 139.8, 186.4 and 279.6 µg Ethyl lauroyl arginate HCl/ml) with S9-mix: 25, 50, 100, 150, 200 and 300 µg LAE/ml (20, 41, 61, 76, 81, 86, 91, 102 µg Ethyl lauroyl arginate HCl/ml)
Metaphase analysis	50, 100 and 200 µg LAE/ml (46.6, 93.2 and 186.4 µg Ethyl lauroyl arginate HCl/ml)
Positive controls:	without S9-mix: Mitomycin C 0.1µg/ml with S9-mix: Cyclophosphamide 6 µg/ml
Negative controls:	Vehicle
GLP:	in compliance

**Results**

**First test - Toxicity data:** With and without S9-mix, 200 µg LAE/ml (186.4 µg Ethyl lauroyl arginate HCl/ml) reduced the relative mitotic index to 31% and 32% respectively of the control value.

LAE (200 µg/ml) caused statistically significant increases ( $P<0.01$ ) in the proportion of polyploid cells, with and without S9-mix. These increases, to 0.7% and 0.6%, respectively, lay outside the upper limit of the historical control range (0.3% and 0.4%, respectively).

**Metaphase analysis:** With and without S9-mix, LAE did not cause statistically significant increases in the cell numbers with chromosomal aberrations at any dose level compared with the control. Positive control compounds, mitomycin C and cyclophosphamide, caused significant increases ( $P<0.001$ ) in aberrant cells.

**Second Test - Toxicity data:** Without S9-mix, LAE (150 µg/ml; 139.8 µg Ethyl lauroyl arginate HCl/ml) caused a reduction in the relative mitotic index to 32% of the control.

With S9-mix, LAE (150 µg/ml; 139.8 µg Ethyl lauroyl arginate HCl/ml) caused a reduction in the relative mitotic index to 57% of the control. Statistically significant increases ( $P<0.001$ ) of polyploid cells were seen with LAE (93.2 and 139.8 µg Ethyl lauroyl arginate HCl/ml). These increases, to 0.6%, lay outside the upper limit of the historical control range.

**Metaphase analysis:** With and without S9-mix. LAE caused no statistically significant increases in cells with chromosomal aberrations at any dose level compared with the controls. Both positive control compounds, mitomycin C and cyclophosphamide, caused large, statistically significant increases ( $P<0.001$ ) in the proportion of aberrant cells.

The study authors concluded that LAE (93.2 % of Ethyl lauroyl arginate HCl) showed no evidence of clastogenic activity in this test system, under the experimental conditions described. The authors considered the increases in polyploidy in both tests were seen mainly at cytotoxic dose levels and possibly are related to the toxicity and not of biological significance. This increased frequency of polyploid metaphases could indicate the possibility of aneugenic activity.

Ref.: 22

**Take from SCCP/0837/04****Metaphase chromosome analysis of human lymphocytes cultured *in vitro***

Guideline:	OECD 473 (1983)
Species/strain:	Human lymphocytes
Active ingredient:	20.3% Ethyl lauroyl arginate HCl
Test substance:	Mirenat-N
Batch:	0000003
Purity:	21.6% LAE
Vehicle:	Water
Culture medium:	RPMI 1640 tissue culture medium (Sigma) 20% foetal calf serum (PAA), phytohaemagglutinin (Wellcome)

**Concentrations**

Test 1:	15.6, 31.3, 62.5, 125, 250, 500, 1000 and 2000 µg Mirenat/ml (3.2, 6.4, 12.7, 25.4, 50.8, 101.5, 203 and 406 µg Ethyl lauroyl arginate HCl/ml)
Test 2:	without S9-mix 32 h: 125, 250, 500, 1000 and 2000 µg Mirenat/ml (25.4, 50.8, 101.5, 203 and 406 µg Ethyl lauroyl arginate HCl/ml). with S9-mix 32 h: 250, 500 and 1000 µg Mirenat/ml (50.8, 101.5 and 203 µg Ethyl lauroyl arginate HCl/ml) without S9-mix 18 h: 125, 250, 500, 750, 1000, 1500 and 2000 µg Mirenat/ml (25.4, 50.8, 101.5, 152.3, 203, 304.5 and 406 µg Ethyl lauroyl arginate HCl/ml) with S9-mix 18 h : 125, 250, 500, 600, 700, 800 and 1000 µg Mirenat/ml (25.4, 50.8, 101.5, 121.8, 142.1, 162.4 and 203 µg Ethyl lauroyl arginate HCl/ml)
Metaphase analysis	Test 1 125, 250 and 500 µg Mirenat/ml Test 2 without S9-mix 18 h 125, 250 and 500 µg Mirenat/ml Test 2 with S9-mix 18 h 700, 800 and 1000 µg Mirenat/ml Test 2 without S9-mix 32 h 500 µg Mirenat/ml Test 2 with S9-mix 32 h 1000 µg Mirenat/ml
Positive controls:	without S9-mix: Ethyl methanesulphonate 250, 500 and 750 µg/ml with S9-mix: Cyclophosphamide 2.5, 5, 10, and 15 µg/ml.
Negative controls:	Vehicle
GLP:	in compliance

**Results****Test 1**

Toxicity data: The relative mitotic index fell to 40% without S9-mix and to 11% with S9-mix of the control at the high concentration, 2000 µg/ml (406 µg Ethyl lauroyl arginate HCl/ml). 500 µg/ml (relative mitotic index 75%) was selected the highest dose level for metaphase analysis, as higher concentrations were toxic.

Metaphase analysis: There were no statistically significant increases in metaphase figures with chromosomal aberrations. The positive control compounds caused statistically significant increases ( $P<0.001$ ) in aberrant cells, demonstrating the efficacy of the S9-mix and the sensitivity of the test system.

**Test 2 - 18 hour harvest**

Toxicity data: Without S9-mix, the relative mitotic index fell to 39% of the control at the high concentration, 2000 µg/ml (406 µg Ethyl lauroyl arginate HCl/ml). 500 µg/ml (relative mitotic index 146%) was selected the highest dose level for metaphase analysis, as higher concentrations were toxic.

With S9-mix, the relative mitotic index was 61% of the control at the high concentration, 1000 µg/ml (203 µg Ethyl lauroyl arginate HCl/ml). This was selected the highest dose level for metaphase analysis.

Metaphase analysis: There were no statistically significant increases in metaphase figures with chromosomal aberrations.

**Test 2 - 32 hour harvest**

Toxicity data: Without S9-mix, the relative mitotic index fell to 6% of the control at the high concentration, 2000 µg/ml (406 µg Ethyl lauroyl arginate HCl/ml). 500 µg/ml (relative mitotic index 66%) was selected the highest dose level for metaphase analysis, as higher concentrations were toxic.

With S9-mix, the relative mitotic index was 98% of the control at the high concentration, 1000 µg/ml (203 µg Ethyl lauroyl arginate HCl/ml) and was selected for metaphase analysis.

Metaphase analysis: There were no statistically significant increases in metaphase figures with chromosomal aberrations.

Conclusion

20.3% of Ethyl lauroyl arginate HCl has shown no evidence of clastogenic activity in this in vitro cytogenetic test system. There was a high variability in the mitotic index. Polyploidy was not determined.

Ref.: 23

### **3.3.7. Carcinogenicity**

No data submitted

### **3.3.8. Reproductive toxicity**

*Taken from SCCP/0837/04*

#### **Rat, preliminary range finding study**

Guideline:	/
Species/strain:	Sprague-Dawley rat,
Group size:	4 non pregnant and 4 pregnant females, 1 male
Active ingredient:	69.1% Ethyl lauroyl arginate HCl
Test substance:	LAE
Batch:	5159
Purity:	69.1% ethyl lauroyl arginate HCl
Vehicle:	1% w/v aqueous methyl cellulose
Staircase dose:	Day 1 and 2: 173 mg/kg/day ethyl lauroyl arginate HCl Day 3 and 4: 346 mg/kg/day ethyl lauroyl arginate HCl Day 5 and 6: 691 mg/kg/day ethyl lauroyl arginate HCl Day 7 and 8: 1382 mg/kg/day ethyl lauroyl arginate HCl
Constant dose:	1382 mg/kg/day ethyl lauroyl arginate HCl
Volume:	1 ml/ kg bw by gavage
Treatment period:	Gestation Days 6 to 13 gestation
GLP:	in compliance

This is a preliminary study and not need to follow an official guideline. It was conducted in compliance with GLP. The animals were dosed by gavage.

**Staircase phase (4 non pregnant females):** The general condition was unaffected by treatment. No deaths occurred. Increased salivation was noted on occasions for a short period immediately after dosing. The frequency of recorded salivation was increased at dosages of 173 and 1382 mg of Ethyl lauroyl arginate HCl/kg/day. Bodyweight and bodyweight gain in the non-pregnant female was essentially unaffected with LAE at dosages up to 1382 mg of Ethyl lauroyl arginate HCl/kg/day. At the highest dose (1382 mg of Ethyl lauroyl arginate HCl/kg/day) 3/4 animals showed minor bodyweight loss after the first dose, but recovered the following day. No adverse pathological findings were recorded after the 8 doses of LAE, stepped from 173 to 1382 mg of Ethyl lauroyl arginate HCl/kg/day.

**Constant dosage phase (4 pregnant females):** The general condition was unaffected by treatment. No deaths occurred.

Increased salivation in all animals was noted on occasions for a short period immediately after dosing. The frequency of recorded salivation was increased at dosages of 173 and 1382 mg of Ethyl lauroyl arginate HCl/kg/day. Bodyweight and cumulative bodyweight gain in the pregnant female appeared to be unaffected by treatment with LAE at a dosage of 1382 mg of Ethyl lauroyl arginate HCl/kg/day.

All females were pregnant at termination and no adverse findings were recorded at *post mortem* of the pregnant females after 7 doses of LAE at 1382 mg of Ethyl lauroyl arginate HCl/kg/day.

Maternal bodyweight and embryo survival appeared normal.

It was concluded from this investigation that 1382 mg of Ethyl lauroyl arginate HCl/kg/day should be used in a preliminary embryo-foetal study in the rat. This was the highest dosage that could be used without exceeding guideline figures for volume dosage.

Ref.: 26

### Preliminary embryo-foetal toxicity study

Guideline:	/
Species/strain:	Sprague-Dawley CD rat
Group size:	6 pregnant females
Active ingredient:	69.1% Ethyl lauroyl arginate HCl
Test substance:	LAE
Batch:	5159
Purity:	69.1% ethyl lauroyl arginate HCl
Vehicle:	1% w/v aqueous methyl cellulose
Dose:	0, 200, 600 and 2000 mg LAE/kg/day (0, 138, 415, 1382 mg ethyl lauroyl arginate HCl /kg/day)
Volume:	1 ml/ kg bw by gavage
Treatment period:	Gestation Days 6 to 19
GLP:	in compliance

This is a preliminary study and not need to follow an official guideline. It was conducted in compliance with GLP. The animals were dosed by gavage.

21 of the 23 females surviving to the end of the study were pregnant. One control animal was considered "not pregnant" and was excluded, although staining the uterus revealed a single implantation site. One low dose female was killed *in extremis* on Gestation Day 19 of after showing reduced food intake bodyweight loss (40g) on Days 18-19 of gestation. This female had signs of pallor, piloerection, brown staining around left orbital, red urine and a perigenital discharge. Necropsy revealed a large amount of dark red fluid within the vagina and both uterine horns. The uterus contained 15 late resorptions.

Increased salivation after dosing was seen occasionally in 3/6 mid-dose animals and about 50% of occasions in all high-dose animals. One animal from each treated group had periods when respiration sounded noisy. There were no other significant clinical signs recorded in either the control group or any of the treatment groups.

Bodyweight: there were no intergroup differences in bodyweight or bodyweight gain that were considered to be treatment-related. During the first two days of treatment, occasional animals in all groups showed bodyweight loss, but this was considered to be related to animals adapting to the dosing process rather than to the test material itself.

Food consumption: food consumption was similar for all groups of animals throughout the treatment period, apart from the one low-dose female that was killed.

Necropsy findings: there were no necropsy findings which were considered to be related to treatment.

Litter responses: one control female had only one single implantation site, revealed by staining the uterus, and has been excluded from group mean values. Rats with very low implantation rates frequently show spontaneous resorption at an early stage of pregnancy. Another two animals, low-dose and one high-dose, showed high pre-implantation losses, but these losses almost certainly occurred before the start of treatment, these were not considered to be treatment related. The group mean value for post-implantation loss was slightly higher in the mid-dose animals, but since it was not seen in the high-dose group, it was not considered treatment related.

Maternal parameters: (group mean values)

**Opinion on ethyl lauroyl arginate HCl**

Dose (mg/kg/day)	0	138	415	1382
Corpora lutea	14.8	14.4	15.0	14.7
Implantations	14.5	12.5	14.5	13.8
Resorptions	0.5	0.2	1.2	0.3

**Foetal parameters** There were no obvious treatment related effects upon foetal development assessed by foetal weight and macroscopic examination at necropsy.

(group mean values)

Dose (mg/kg/day)	0	138	415	1382
Live foetuses	14.0	12.2	13.3	13.5
Dead foetuses	0	0	0	0
Abnormalities	0/56	0/61	0/80	0/81

### Conclusion

The high dose, 1382 mg of Ethyl lauroyl arginate HCl/kg/day, was considered as the highest dose for a main embryo-foetal study in the rat.

Ref.: 27

**Taken from SCCP/0837/04**

### Embryo-foetal toxicity

Guideline:	OECD 414
Species/strain:	Sprague-Dawley CD rat,
Group size:	22 pregnant females
Active ingredient:	69.1% Ethyl lauroyl arginate HCl
Test substance:	LAE
Batch:	5159
Purity:	69.1% ethyl lauroyl arginate HCl
Vehicle:	1% w/v aqueous methyl cellulose
Dose:	0, 200, 600 and 2000 mg LAE/kg/day (0, 138, 415, 1382 mg ethyl lauroyl arginate HCl /kg/day)
Volume:	1 ml/ kg bw by gavage
Treatment period:	Gestation Days 6 to 19
GLP:	in compliance

The animals were dosed by gavage.

### Maternal responses

**Mortality:** three high-dose animals were killed *in extremis* on Gestation Days 7 or 8 (the second or third day of treatment). They showed signs of noisy and gasping respiration, and excess salivation after dosing. Two showed bodyweight loss and the third showed decreased activity and piloerection. Necropsy revealed amounts of gaseous material in the stomach and in third the entire gastro-intestinal tract was distended with gas. In addition one had enlarged and prominent lymph nodes, and the other had haemorrhagic lungs, large amounts of pale yellow viscous material in the ileum, reduced and dehydrated caecal contents, dark and enlarged adrenals and a pronounced internal structure of the kidneys. All animals were pregnant.

Two mid-dose animals showed similar effects at the end of gestation, both showing signs of noisy respiration, salivation at the time of dosing and bodyweight losses. Both were killed on Gestation Day 17 for ethical reasons. Necropsy of these animals revealed that gastro-intestinal tract was distended with gaseous material. Both animals had normal implantations.

**General condition** of the surviving animals was satisfactory and all were pregnant. Noisy

**Opinion on ethyl lauroyl arginate HCl**

respiration was seen during the treatment period in 3 low-dose animals, 7 mid-dose animals and 9 high-dose animals (including those killed prematurely).

Excess salivation at the time of dosing was seen in all high-dose animals on approximately 50% of dosing occasions reaching peak daily incidence at about Day 14 of gestation. It was occasionally noted in 14 mid-dose animals and 1 low-dose animal on one occasion. Neither noisy respiration nor salivation were seen in the Control group.

**Bodyweight:** there were no overall treatment related effects upon bodyweight. Occasional animals in all groups receiving LAE showed Transient bodyweight losses at the start of treatment on Day 6 and in some mid-dose animals towards the end of treatment were noted. Weight loss and reduced food consumption coincided with episodes of respiratory distress. There were no treatment-related effects at post-mortem on Gestation Day 20.

**Maternal parameters: (group mean values)**

Dose (mg/kg/day)	0	138	415	1382
Corpora lutea	15.6	15.5	15.7	16.0
Implantations	15.0	14.5	14.7	15.4
Resorptions	1.0	0.5	0.6	0.6

**Litter responses:**

There were no treatment-related effects on foetal survival as indicated by the extent of pre- and post-implantation loss and the numbers of live foetuses.

**Foetal parameters: (group mean values)**

Dose (mg/kg/day)	0	138	415	1382
Live foetuses	14.0	14.0	14.1	14.7
Dead foetuses	0.1	0.0	0.0	0.1
Major foetal abnormalities	2/307	2/309	0/282	4/280

The no-adverse-effect level (NOAEL) for the dam was taken to be 138 mg of Ethyl lauroyl arginate HCl/kg/day concluded, because of the maternal deaths at the higher doses. The NOAEL for the foetuses was taken to be 1382 mg of Ethyl lauroyl arginate HCl/kg/day.

Ref.: 28

**Taken from SCCP/0837/04****Rabbit, preliminary range finding study**

Guideline:	/
Species/strain:	New Zealand White rabbits female
Group size:	2 non pregnant and 2 pregnant females
Active ingredient:	69.1% Ethyl lauroyl arginate HCl
Test substance:	LAE
Batch:	5159
Purity:	69.1% ethyl lauroyl arginate HCl
Vehicle :	1% w/v aqueous methyl cellulose
Staircase dose:	Day 1 and 2: 41 mg/kg/day ethyl lauroyl arginate HCl Day 3 and 4: 83 mg/kg/day ethyl lauroyl arginate HCl Day 5 and 6: 173 mg/kg/day ethyl lauroyl arginate HCl Day 7 and 8: 346 mg/kg/day ethyl lauroyl arginate HCl Day 9 and 10: 691 mg/kg/day ethyl lauroyl arginate HCl
Constant treatment period:	Gestation Days 6 to 12
Constant dose:	691 mg/kg/day ethyl lauroyl arginate HCl
Volume:	5 ml/ kg bw by gavage
GLP:	in compliance

This is a preliminary study and not need to follow an official guideline. It was conducted in compliance with GLP. The animals were dosed by gavage.

**Staircase phase (2 non pregnant females):** The general condition was unaffected by treatment. No deaths occurred. Bodyweight and bodyweight gain in the non-pregnant female was essentially unaffected with LAE at dosages up to 691 mg Ethyl lauroyl arginate HCl/kg/day. Marginal losses in bodyweight were recorded for one of the females on one day at 346 mg Ethyl lauroyl arginate HCl/kg/day and for both females at 691 mg Ethyl lauroyl arginate HCl/kg/day on one day only. No adverse findings were recorded at *post mortem* of the non-pregnant females after a total of 10 doses of LAE progressively increased from 41 to 691 mg Ethyl lauroyl arginate HCl/kg/day.

**Constant dosage phase (2 pregnant females):** No deaths occurred. Both females showed reduced food and water intake during the treatment period (Gestation Days 8-9). The effect on water intake was transient but reduced food consumption and reduced faecal output were observed daily until termination on Gestation Day 13. On Gestation Day 7 (treatment Day 2), one animal became stressed during dosing and dosing had to be delayed by 30 minutes. On Gestation Day 8 (treatment Day 3), the second animal showed marked respiratory noises leading to irregular respiration, blue extremities, inactivity and hunched posture within an hour of dosing. The more severe signs lasted until the end of the day and noisy respiration was recorded daily until termination. Weight loss, in excess of 400 g, was recorded for both animals by Gestation Day 10. One animal continued to lose weight to termination on Gestation Day 13. The other, that had shown the respiratory problems, had small weight gains between Day 10 and Day 13 gestation.

*Post mortem:* Both animals were pregnant and there were no apparent adverse foetal effects. Both animals showed evidence of collapse of the areas of the lung. This was more extensive in the animal that had respiratory distress, with a hint of infection in the lungs. Both animals showed prominent dark vessels on the surface of the kidneys, but the significance of this observation was uncertain. Both animals had 12 implantations and 1 resorption in one animal.

#### Conclusion

Despite maternal findings, treatment at 691 mg of Ethyl lauroyl arginate HCl/kg/day for 7 consecutive days did not result in any significant effect on embryo survival.

The study authors recommended that the highest dosage for the preliminary teratology study should be 691 mg Ethyl lauroyl arginate HCl/kg/day, despite maternal effects on the lungs. These were considered possible non-treatment related changes. This would ensure that the effects of LAE on the pregnant rabbit were investigated at a dosage which is commonly accepted as a maximum limit dosage in studies of this type. There was no evidence of the expected loose faeces, often a reaction in rabbits to antibiotics that kill the gut flora and hence disturb the normal nutritional pattern of the rabbit.

Ref.: 29

**Taken from SCCP/0837/04**

#### Preliminary embryo-foetal toxicity study

Guideline:	/
Species/strain:	New Zealand White rabbits female
Group size:	18 pregnant females, 4 per dose, 6 controls
Active ingredient:	69.1% Ethyl lauroyl arginate HCl
Test substance:	LAE
Batch:	5159
Purity:	69.1% ethyl lauroyl arginate HCl

**Opinion on ethyl lauroyl arginate HCl**


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Vehicle:	1% w/v aqueous methyl cellulose
Dose:	0, 250, 500 and 1000 mg LAE/kg/day (0, 173, 346, 691 mg ethyl lauroyl arginate HCl /kg/day)
Volume:	5 ml/kg bw by gavage
Treatment period:	Gestation Days 6 to 19
Post mortem:	Gestation Day 29
GLP:	in compliance

This is a preliminary study and not need to follow an official guideline. It was conducted in compliance with GLP. The animals were dosed by gavage.

**Maternal responses:**

All animals were pregnant and had live foetuses on Gestation Day 29. One low-dose animal and one mid-dose animal had periods when the respiration was noisy and/or slow but this did not appear to be dose related. No other remarkable clinical signs were recorded.

Bodyweight: Small losses in bodyweight were recorded between Gestation Days 6 and Day 12 (the first week of treatment) for 2/6 controls, 1/4 low-dose animal, 2/4 mid-dose animals, 3/4 high-dose animals. Other animals showed brief periods of weight loss. By Gestation Day 28, most animals gained weight and group mean bodyweight gains were similar in the control and low-dose group and only marginally low in the mid and high dose groups.

Food consumption was lower in the high-dose group and was slightly depressed in the mid-dose compared with the controls during Gestation Days 6 - 12. It was slightly low for the rest of the treatment period. In the first four days post-treatment, mid and high dose animals had increased food consumption relative to consumption during the treatment period.

Post mortem findings: there were no treatment-related findings at the end of pregnancy.

Maternal parameters: (group mean values)

Dose (mg/kg/day)	0	173	346	691
Corpora lutea	11.2	11.0	11.3	13.0
Implantations	8.7	11.0	9.5	12.0
Resorptions	0.7	1.0	2.8	0.8

**Litter responses:**

Dose (mg/kg/day)	0	173	346	691
Life foetuses	14.0	14.0	6.8	11.3
Dead foetuses	0.1	0.0	1.5	0.3
Major foetal abnormalities	2/307	2/309	2/27	4/45

There were no apparent treatment-related effects on embryo-foetal survival. Corpora lutea numbers were essentially similar in all groups but intergroup variation in pre-implantation loss (occurring before treatment started) and slightly high levels of post-implantation loss in mid-dose animals resulted in considerable differences in mean live litter size. Overall foetal weight was lowest in the high-dose group, but this was attributable to the effect of larger litter size. There was no indication that the ability of the dam to support a litter was impaired by treatment. There was a low incidence of foetal anomalies but no indication of any adverse effect of treatment.

The low-dose, 173 mg Ethyl lauroyl arginate HCl/kg/day, was considered to be the no-effect-level (NOEL) for the mother, since both 346 and 691 mg Ethyl lauroyl arginate HCl/kg/day were associated with reduced food consumption and bodyweight gain. The high dose was considered to be the NOEL for the foetus. A dosage of up to 691 mg Ethyl lauroyl arginate HCl/kg/day would be suitable as the highest dosage level for a main embryo-foetal study in the rabbit.

**Taken from SCCP/0837/04**

### **Embryo-foetal toxicity**

Guideline:	OECD Guideline 414
Species/strain:	New Zealand White rabbits female
Group size:	88 pregnant females, 22 per dose,
Active ingredient:	69.1% Ethyl lauroyl arginate HCl
Test substance:	LAE
Batch:	5159
Purity:	69.1% ethyl lauroyl arginate HCl
Vehicle:	1% w/v aqueous methyl cellulose
Dose:	0, 100, 300 and 1000 mg LAE/kg/day (0, 69, 207, 691 mg ethyl lauroyl arginate HCl /kg/day)
Volume:	5 ml/kg bw by gavage
Treatment period:	Gestation Days 6 to 19
Post mortem:	Day 29 gestation
GLP:	in compliance

The animals were dosed by gavage.

#### **Maternal responses:**

Mortality: Two animals, high dose, (Day 9 gestation) and mid dose (Day 14 gestation) were killed for humane reasons. The high dose animal had periods of noisy respiration accompanied by reduced food consumption and faecal output with an aqueous discharge in the cage undertray on Gestation Day 9. Post-mortem revealed a frothy liquid in the trachea and congestion in the lungs. The mid dose animal was killed because of gasping respiration following dosing. Post-mortem revealed incomplete collapse of the lungs, with occasional dark areas of change on the lung surfaces

One high dose animal aborted on Gestation Day 24. There were three empty implantation sites in the left uterine horn, but no implantations in the right horn of the uterus. Two dead foetuses, that appeared normal, were found in the undertray of the cage.

In addition two high dose animals were culled from the study on Gestation Day 7 because of poor acclimatisation and difficulties with dosing but replaced. Both had congested lungs, frothy fluid in the trachea and yellow stained fur.

Reactions to dosing were largely limited to changes in the respiratory pattern seen in 5/22 mid-dose animals and 5/22 high dose animals, including the two animals that 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. The study authors reported that the difficulties with dosing were much reduced when the surface of the catheter was washed clean rather than wiped dry before dose administration. There were no other signs of adverse reaction to treatment.

Bodyweight gain in the high dose group was slightly but significantly lower than that the controls throughout most of the treatment period. This was considered to be treatment related, since they showed recovery of weight gain once treatment ceased. The interpretation was complicated by the fact that animals allocated to the high dose group gained more bodyweight than controls between mating and start of treatment. Bodyweight gain of in the low and mid dose animals was similar to the controls throughout

gestation.

Food consumption in the high dose group fell slightly when treatment started and was significantly lower than the control group from Gestation Day 13 to 19 but recovered to control levels after the dosing period. Food consumption in the low and mid dose group was unaffected by treatment.

Post mortem: there were no treatment-related effects for females killed on Gestation Day 29.

Maternal parameters: (group mean values)

Dose mg/kg/day	0	69	207	691
Corpora lutea	10.7	11.8	11.6	12.2
Implantations	9.8	10.4	10.1	10.8
Resorptions	0.9	1.3	1.0	0.8

**Litter responses:**

There were no apparent treatment-related effects on foetal 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.

Foetal evaluation: there were no effects of treatment on foetal weight or placental weight. There was a low incidence of foetal anomalies seen at post mortem or at subsequent detailed examinations but no indication of any adverse effect of treatment.

Foetal parameters: (group mean values)

Dose mg/kg/day	0	69	207	691 (mg/kg/day)
Life foetuses	8.9	9.1	9.1	10.0
Dead foetuses	0.3	0.9	0.2	0.6
Major foetal abnormalities	3/169	4/200	3/163	2/170

Despite the slightly higher risk of irritation to the respiratory tract at concentrations of 60 mg Ethyl lauroyl arginate HCl/ml and above (dosages of 207 and 691 mg of Ethyl lauroyl arginate HCl/kg/day), it was concluded that 207 mg Ethyl lauroyl arginate HCl/kg/day was the no adverse-effect level (NOAEL) for the dam and 691 mg of Ethyl lauroyl arginate HCl/kg/day was the NOAEL for the foetus.

Ref.: 31

**Comment**

Attached to this submission was a letter from the head of Reproductive Studies Group, commenting on the effect seen when Ethyl lauroyl arginate HCl was administered by gavage. He considered that the respiratory effects following gavage were due to the route of administration and not a systemic toxic response but said "to base the NOAEL on results of dietary studies should be considered in the light of the proposed route of administration for use in the material in cosmetics. It should be acceptable if the material is applied as a lotion to the body skin, but might be less acceptable if applied as a spray or to the lip/face."

**From submission II**

**Preliminary study of effects on reproductive performance**

Guideline:	/
Species/strain:	Rats, Sprague-Dawley (Crl:CD®(SD)IGSBR(IGS))
Group size:	F 0: 8 male, 8 female F1: 12 male, 12 female
Test substance:	LAE
Batch no:	10234

**Opinion on ethyl lauroyl arginate HCl**

Purity: 88.2% ethyl lauroyl arginate HCl  
 Dose: 0, 1500, 5000 and 15000 ppm.  
 GLP: in compliance

Diets were prepared fortnightly. For each concentration, the required amount of LAE was stirred together with an approximately equal amount of basal diet. The treated groups received LAE continuously for 4 weeks before pairing, through mating and until termination after weaning the litters.

Clinical signs: all animals were inspected at least twice daily throughout the study and reactions recorded. Once each week all parental and selected F1 animals were given a thorough physical examination.

Bodyweight: males were weighted on the first day of treatment and then twice weekly until termination. Females were weighed on the first day of treatment and then twice weekly until mating was detected. Subsequently, females were weighted on Days 0, 6, 13 and 20 after mating and on Days 1, 4, 7, 14 and 21 of lactation. Selected F1 animals were weighed twice weekly from a nominal 4 weeks of age until termination.

Food consumption recorded twice weekly for the F0 animals until they were paired for mating. After mating, food consumption for the females was recorded for the periods 0-5, 6-12, 13-19 Days and Days 1-3, 4-6, 7-13, 14-20 during lactation.

Food consumption for the F1 selected animals was recorded twice weekly from nominal week 4 of age until termination.

Mating procedure: F0 females were paired on a one-to one basis with males from the same treatment group for a maximum of two weeks. After pairing, vaginal smears were taken daily from all F0 females until evidence of mating was observed. The day on which mating was detected was designated gestation day (GD) 0.

Parturition and duration of gestation: from day 20 after mating, F0 females were inspected three times daily for the onset, progress and completion of parturition. All F0 females were permitted to deliver their young naturally and rear their own offspring until day 21 of lactation where possible.

For litters:

All offspring were examined 24 hours after birth. For each litter, the number of offspring (live and dead); individual offspring bodyweight; sex ratio; observations on individual offspring were recorded up to Day 21 of age.

Sex ratio: the sex of the offspring was determined on days 1 and 4 of age and at weaning.

Bodyweight: the live offspring in each litter were weighed individually on Days 1, 4, 7, 14, 21 and 25 of age and then twice weekly for selected F1 males and females.

## Results

The general condition of animals receiving diets containing LAE was similar the controls.

Bodyweight and bodyweight gain of F0 males and females were not adversely affected by treatment and there were no adverse effects on bodyweight gain for females during gestation and lactation. Offspring bodyweight at Day 1 of age, gain to weaning, and bodyweight of selected F1 males and females to eight weeks of age, were unaffected by treatment.

Food consumption was similar in all groups of F0 animals, both before mating and for females during gestation and lactation. Food consumption by selected F1 animals was similar to that of controls. There were no consistent clear effects on food conversion efficiency in the females or males before pairing or in the F1 generation to 8 weeks of age.

At 15000 ppm achieved intakes of LAE at the start of F0 treatment were approximately 1270/1330 mg/kg/day and the overall average before pairing was 1150/1295 mg/kg/day for males/females respectively. The peak intake (approximately 3070 mg/kg/day) by females occurred during the second week of lactation. Selected F1 animals had higher achieved intakes than their F0 parents, averaging approximately 1750/1735 mg/kg/day for males/females respectively between ages of 4-8 weeks. Calculated intakes at lower treatment levels were in proportion to the dietary concentrations.

Mating performance, fertility, litter size, and growth were unaffected by LAE in the diet at levels of up to 15000 ppm. The two litters in the 15000 ppm females lost weight in the first

**Opinion on ethyl lauroyl arginate HCl**

four days after birth and when killed for humane reasons. They were found to have no milk in their stomachs. Survival of offspring within remaining litters at this dietary level was slightly below that of the controls.

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

Necropsy of F0 parental animals, weanling offspring and selected offspring killed at approximately 8 weeks of age did not detect any effects of treatment.

It was concluded that a dietary concentration of 15000 ppm could be used as the highest treatment level for the two-generation study in the CD rat. The general condition of rats receiving a diet containing LAE was similar to that of the control. No deaths occurred.

Ref.: 35

### **From submission II**

#### **Study of effects on reproductive performance**

Guideline:	OECD Guideline 416, 2001
Species/strain:	Rats, Sprague-Dawley (Crl:CD®(SD) IGS BR)
Group size:	F 0: 28 male, 28 female F1: 24 male, 24 female F2: 5 male, 5 female
Test substance:	LAE
Batch:	10234
Purity:	88.2% ethyl lauroyl arginate HCl
Dose:	0, 2500, 6000 and 15000 ppm
GLP:	in compliance

Diets were prepared fortnightly. For each concentration, the required amount of LAE was stirred together with an approximately equal amount of basal diet. The treated groups received LAE continuously for 10 weeks before pairing, through mating and until termination after weaning the litters.

Clinical signs: all animals were inspected at least twice daily throughout the study and reactions recorded. Once each week all parental and selected F1 animals were given a thorough physical examination.

Bodyweight: males were weighted on the first day of treatment and then weekly until termination. Females were weighed on the first day of treatment and then weekly until mating was detected. Subsequently, females were weighted on Days 0, 6, 13 and 20 after mating and on Days 1, 4, 7, 14 and 21 of lactation. Selected F1 animals were weighed twice weekly from a nominal 4 weeks of age until termination.

Food consumption was recorded weekly for the F0 animals until they were paired for mating. After mating, food consumption for the females was recorded for the periods 0-5, 6-12, 13-19 Days and Days 1-3, 4-6, 7-13, 14-20 during lactation.

Food consumption for the F1 selected animals was recorded twice weekly from nominal week 4 of age until termination.

Mating procedure: F0 females were paired on a one-to one basis with males from the same treatment group for a maximum of two weeks. After pairing, vaginal smears were taken daily from all F0 females until evidence of mating was observed. The day on which mating was detected was designated gestation day (GD) 0

Parturition and duration of gestation: from day 20 after mating, F0 females were inspected three times daily for the onset, progress and completion of parturition. All F0 females were permitted to deliver their young naturally and rear their own offspring until day 21 of lactation where possible.

For litters:

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All offspring were examined 24 hours after birth. For each litter number of offspring (live and dead); individual offspring bodyweights; sex ratio; observations on individual offspring were recorded up to Day 21 of age.

Sex ratio: the sex of the offspring was determined on days 1 and 4 of age and at weaning.

Bodyweight: the live offspring in each litter were weighed individually on Days 1, 4, 7, 14, 21 and 25 of age and then twice weekly for selected F1 males and females.

### Results

Mean achieved dosages (mg/kg bw/day) throughout study were as follows:

<b>F0 Generation</b>	<b>Males</b>			<b>Females</b>		
Dietary Conc. (ppm)	2500	6000	15000	2500	6000	15000
Before pairing	181	434	1073	207	502	1226
During gestation	-	-	-	231	585	1518
During lactation	-	-	-	402	1018	2600
<b>F1 Generation</b>						
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 generation animals receiving diets containing LAE was similar to that of the controls.

Bodyweight and bodyweight gain of adult F0 and F1 males and females was not adversely affected by treatment. At 15000 ppm, bodyweight gains as F1 and F2 offspring began to eat the diet were slightly lower than control offspring. Food consumption and food conversion efficiency was unaffected by the level of LAE in the diet in both generations.

There were no adverse effects in either generation on pre-mating oestrous cycles, mating performance, fertility, litter size, offspring survival and Day 1 bodyweight at levels of up to 15000 ppm of LAE in the diet. Pre-weaning examinations of surface, air righting startle response and pupil response for F1 and F2 offspring were not significantly affected by treatment.

Balano-preputial separation was unaffected at all dosage levels. A delay in vaginal opening of 4 days was recorded with treatment at 15000 ppm. The timing of vaginal opening occurs approximately within 2 weeks after the initiation of the selected F1 generation. At this time, achieved dosages in the 15000 ppm group were 2269 and 1957 mg/kg bw/day. Treatment had no impact upon oestrous cycles pre-pairing or pre-termination, fertility or primordial follicle counts. The effect on vaginal opening appears to be a transient effect at the time of highest exposure. The measurement of anogenital distance in the F2 offspring was also unaffected by treatment, confirming that LAE caused no changes in sexual differentiation. Terminal investigations from F0 and F1 adult animals showed no effects on pre-termination oestrous cycles or on sperm assessments. Macroscopic examination of adult animals and offspring revealed no changes attributable to treatment. In the 15000 ppm group absolute and/or bodyweight relative spleen weights 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 in controls. The magnitude of the difference reduced as age increased and was not accompanied by any macroscopic changes or microscopic changes in F0 and F1 adult animals so that the effect was therefore considered to be of no toxicological importance.

### Conclusion

The study authors considered the NOAEL for reproductive performance in the Sprague Dawley CD rat to be 15000 ppm LAE, which was equivalent to at least 1073 mg/kg bw/day of LAE (946 mg/kg bw/day of Ethyl lauroyl arginate HCL) based on the lowest average intake by adult rats before pairing and up to 2600 mg/kg bw/day of LAE (92293 mg/kg bw/day of Ethyl lauroyl arginate HCL) for females during lactation.

Ref.: 36

### 3.3.8.2. Teratogenicity

Data in the previous section

### 3.3.9. Toxicokinetics

#### **Taken from SCCP/0837/04**

##### **In vivo and in vitro metabolism in the rat**

Guideline:	/
Species/strain:	Rat, Sprague DawleyCrl:CD.BR male
Group size:	6 males, in vitro 1 male liver
Active ingredient:	93.2% Ethyl lauroyl arginate HCl
Test substance:	LAE and radio-labelled arginine LAE in all carbons of arginine
Batch:	3036 Radio-labelled NPE/LMA001/65
Purity:	93.2% ethyl lauroyl arginate HCl 99.8% radiochemical purity
Vehicle:	80 mg/ml in 1% w/v aqueous methyl cellulose
Dose:	<i>in vitro</i> 10 µg <sup>14</sup> C-LAE/ml (9.3 µg Ethyl lauroyl arginate HCl) 200 mg LAE/kg bw (186 mg ethyl lauroyl arginate HCl /kg bw)
Volume:	200 ml/kg bw by gavage
Sample collection:	<i>in vitro</i> S9 fraction: 4, 6 and 24 h after treatment <i>in vitro</i> control plasma: 1 and 4 h after dosing <i>in vivo</i> blood: 0.5, 1 and 4 h post treatment (2 rats each sample).
GLP:	in compliance

The study was a bespoke design to answer specific questions about the metabolic fate and not designed to fulfil any particular regulatory guideline. It was conducted in compliance with GLP.

**In vitro incubation at 37 °C**, using liver S9 fractions or plasma, with <sup>14</sup>C-LAE for up to 24h showed the metabolism of LAE. In the extracted S9 samples, unchanged LAE, N<sup>a</sup>-lauroyl-L-arginine ethyl ester, arginine, ornithine and urea were identified. Ornithine was the major metabolite. In a control incubation with no S9 fraction, there was no significant degradation of <sup>14</sup>C-LAE.

Incubation of plasma with <sup>14</sup>C-LAE for up to 4 hours showed that esterase activity in the plasma rapidly hydrolyses Ethyl lauroyl arginate HCl to LAS (N<sup>a</sup>-lauroyl-L-arginine) and arginine. Arginine was further metabolised to ornithine. The plasma extract showed qualitatively similar profiles to those seen in the S9 liver fractions. Extraction of radioactivity declined from 99.5% at zero-time to 86.4% at 4 h. This suggests that some binding of radioactivity to plasma proteins occurred.

**In vivo**, concentrations in plasma rose rapidly to a mean of 118 µg equivalents Ethyl lauroyl arginate HCl/ml, 4 h after dosing with 200 mg <sup>14</sup>C-LAE /kg bw by gavage. Extraction of radioactivity from plasma declined from a mean of 74.8% total radioactive residue (TRR) at 0.5 h to a mean of 19.7% TRR at 4 h. In the 0.5 h samples, Ethyl lauroyl arginate HCl accounted for less than 10% TRR. Arginine was the major metabolite (mean maximum of 48% TRR) with ornithine (7.7% TRR) An unretained polar fraction accounted for a mean maximum of 17% TRR but was unidentified.

#### Conclusion

*In vitro* experiments with <sup>14</sup>C-LAE in plasma demonstrated that Ethyl lauroyl arginate HCl and N<sup>a</sup>-lauroyl-L-arginine could be quantitatively recovered from plasma by extraction with methanol. This suggests that the increase in the non-extractable radioactivity observed in

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*vitro* and to a lesser extent *in vivo* is probably due to binding of the Ethyl lauroyl arginate HCl and/or its metabolites to plasma proteins and/or natural incorporation.

This study has helped to elucidate the *in vitro* and *in vivo* metabolic pathway of Ethyl lauroyl arginate HCl. It is rapidly metabolised by hydrolysis of the ethyl ester and lauroyl amide to arginine and then further catabolism to ornithine and urea. The study authors conclude that due to the rapid hydrolytic degradation in liver and plasma, exposure to Ethyl lauroyl arginate HCl and N<sup>α</sup>-lauroyl-L-arginine *in vivo* is likely to be very short.

Ref.: 25

**From submission II****In vivo metabolism in the rat**

Guideline:	/
Species/strain:	Rat, Sprague Dawley Crl:CD.BR, male
Group size:	4
Active ingredient:	Ethyl lauroyl arginate HCl
Test substance:	LAE
Batch:	12547
Purity:	91.8% Ethyl lauroyl arginate HCl
Dose:	Single oral doses (gavage)
	In propylene glycol/water: 40, 120 and 320mg/kg bw LAE (36.7, 110 and 294 mg/kg bw Ethyl lauroyl arginate HCl)
	In glycerol/water or water: 120 mg/kg bw LAE (110 mg/kg bw Ethyl lauroyl arginate HCl)
Volume:	5 ml/kg bw
Sample collection:	Blood samples 30, 60, 90, 120, 240 minutes and 8 hours post dose
GLP:	in compliance

All dose solutions were prepared on the day of dosing. Doses were mixed at a ratio of 1:3.5 LAE: solvent and then to volume with distilled water. In water alone, LAE was dissolved by sonication in 10 ml

Blood was collected 30, 60, 90, 120, 240 minutes and 8 hours post dose. Plasma was separated immediately after sampling and then processed.

**Results**

For LAE in propylene glycol/water, the mean maximum concentration (Cmax) 2.02, 1.23 and 2.60 ng/ml respectively for the dose levels of 36.7, 110 and 294 mg/kg bw/day Ethyl lauroyl arginate HCl. The area under the curve (AUC<sub>8</sub>) was 7.50 ng·h/ml at the high dose, but could not be calculated for the other doses. Absorption of LAE was between 30 min or 1 hour post-dose at 40 and 120 mg/kg bw and between 0.5 to 4 hours for 320 mg/kg bw. This suggests it is dose dependent.

For the main metabolite of Ethyl lauroyl arginate HCl, N- $\alpha$ -lauroyl-L-arginine (LAS) the Cmax: 24.2, 23.2 and 96.9 ng/ml and the AUC<sub>8</sub>: 52.5, 103, 315 ng·h/ml respectively.

Plasma concentrations of LAS at 8 hours post-dose were quantifiable in all animals at 120 and 320 mg/kg bw dose levels.

Plasma concentrations of LAS were higher than those of Ethyl lauroyl arginate HCl, indicating that there was extensive conversion of Ethyl lauroyl arginate HCl to LAS.

For 120 mg/kg bw LAE in glycerol/water or in water the mean Cmax were 9.42 and 10.6 ng/ml and the AUC<sub>8</sub>: 12.6 and 8.78 ng·h/ml respectively. For LAS, these were Cmax: 28.8 and 31.2 ng/ml and AUC<sub>8</sub>: 103, 115 and 109 ng·h/ml respectively.

Absorption was again rapid, between 30 min or 1 hour post-dose and was not greatly influenced by whether propylene glycol or glycerol was added to aid solubility in water.

**Conclusion**

LAE is very rapidly hydrolysed to LAS. The low levels of LAE, and consequently Ethyl lauroyl arginate HCl, detected in this study are due to rapid metabolism. The levels of LAS give a

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better indication of the absorption of Ethyl lauroyl arginate HCl. The present study gives no information about the absolute bioavailability of Ethyl lauroyl arginate HCl, but suggests that similar absorption is observed whether propylene glycol or glycerol was added to aid solubility in water.

Ref.: 37

***From submission II******In vitro stability in simulated gastric & intestinal fluid, human plasma and hepatocytes***

Guideline: /  
 Active ingredient: Ethyl lauroyl arginate HCl  
 Test substance: LAE and radio-labelled arginine LAE in all carbons of arginine  
 Batch no: Q.98.250.6  
                   Radio-labelled NPE/LMA054/8  
 Purity:        95.62% ethyl lauroyl arginate HCl  
                   95% radiochemical purity  
 GLP:            in compliance

The study investigated the stability of LAE in simulated gastric and intestinal fluids (at pH 6.8 and pH 7.5), in human plasma and in a preparation of human hepatocytes. LAE, radiolabelled uniformly with  $^{14}\text{C}$  in the arginine carbons, was used at nominal initial concentrations of 0.25 mg/ml (gastric and intestinal fluids) or 10  $\mu\text{g}/\text{ml}$  (plasma and hepatocytes).

**Incubation solutions**

Non-radiolabelled LAE was dissolved in water. Ethanolic solution of radiolabelled LAE stock was evaporated to dryness under  $\text{N}_2$ . The radioactive residue, re-dissolved in water was added to the non-radiolabelled LAE and made up to volume.

**Simulated gastric fluid with or without pepsin:** sodium chloride with or without pepsin as appropriate was dissolved in diluted hydrochloric acid. The pH was 0.91 and 0.95 respectively.

**Simulated intestinal fluid with or without pancreatin (pH 7.5 and 6.8):** monobasic potassium phosphate was dissolved in water. 0.2 M Sodium hydroxide solution was added to adjust the pH to 7.5 or 6.8 and then pancreatin was added as appropriate.

**Human plasma:** blood was taken from four volunteers, centrifuged and the plasma from each volunteer separated. The radiolabelled ethanolic LAE stock solution was evaporated to dryness under  $\text{N}_2$ . The residue was re-dissolved in 0.9% w/v sodium chloride solution.

**Human hepatocytes:** male human cryopreserved hepatocytes were thawed and pooled before use. An ethanolic solution of radiolabelled LAE stock was evaporated to dryness under  $\text{N}_2$  and the residue dissolved in methanol.

Incubation was at 37°C and samples taken at intervals up to 2 - 4 hours. All samples were analysed by HPLC to determine the proportions of LAE and any degradation products.

**Results**

The initial concentrations of LAE were approximately 0.25 mg/ml in the simulated gastric and intestinal fluids and approximately 10  $\mu\text{g}/\text{ml}$  in plasma and the hepatocytes samples.

**Gastric fluid with or without pepsin and intestinal fluid**

LAE was stable in simulated gastric fluid with or without pepsin for 120 minutes. LAE represented at least 96.1 % of the sample radioactivity in all samples. Small amounts of LAS (up to 3.6% sample radioactivity) were present in some samples.

In simulated intestinal fluid at pH 7.5 containing pancreatin, LAE was immediately degraded to N- $\alpha$ -lauroyl-L-arginine (LAS), which was subsequently degraded to arginine. At zero-time, LAS and arginine represented 95.2 and 4.8% sample radioactivity, respectively. After 240 minutes the proportions were reversed: LAS and arginine represented 4.0 and 96.0% sample radioactivity, respectively. LAE was not detected in any sample. LAE was stable in

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simulated intestinal fluid at pH 7.5 in the absence of pancreatin. LAE represented at least 99.6% of the sample radioactivity in all samples.

At pH 6.8, LAE was also immediately degraded to LAS and then to arginine. LAS and arginine represented 98.2 and 1.8% sample radioactivity, respectively at zero-time, and 5.5% and 94.5% sample radioactivity, respectively, after 240 minutes. No LAE was detected.

In the absence of pancreatin, LAE was stable up to 30 minutes (98.1 % sample radioactivity) but then degraded to LAS. After 240 minutes, LAE represented 80.6% sample radioactivity and LAS represented 19.4% sample radioactivity.

**Human Plasma**

LAE degraded over the course of the incubation period, from 98.4% sample radioactivity at zero-time, to 86.8% after one hour, 64.3% after two hours and 51.3% after 4 hours. LAS was the only degradation product and represented 46.7% sample radioactivity after four hours. Arginine was not detected in these samples.

**Human hepatocytes**

LAE was degraded in the presence of human hepatocytes. At zero-time, LAE represented 75.7% of the sample radioactivity and LAS, 24.4%. After 3 hours LAE had declined to 6.1 % sample radioactivity, while LAS had increased to 81.2%. The other radioactivity present in the samples after 3 hours (12.7%) was of a number of minor components, but not including arginine.

In the absence of hepatocytes, however, LAE also similarly degraded to LAS. After 3 hours LAE represented 12.5% sample radioactivity and LAS, 84.2%.

**Conclusion**

Ethyl lauroyl arginate HCl, the active ingredient of LAE, was stable for 2 hours in simulated gastric fluid, with and without pepsin. Ethyl lauroyl arginate HCl was immediately hydrolysed to LAS and thence to arginine in simulated intestinal fluids with pancreatin at pH 6.8 and at pH 7.5. This hydrolysis was enzyme-mediated. In simulated intestinal fluids without pancreatin, Ethyl lauroyl arginate HCl was stable at pH 7.5 while hydrolysis to LAS was considerably slower at pH 6.8. Ethyl lauroyl arginate HCl was hydrolysed to LAS (but not arginine) by human plasma and human hepatocytes. In human plasma and hepatocytes over 4 h, the only hydrolysis product was LAS. No arginine was detected.

Ref.: 38

**3.3.10. Photo-induced toxicity**

No data submitted

**3.3.11. Human data*****From submission II*****Single dose oral study with radiolabelled LAE to male volunteers**

Guideline:	/
Subjects:	2 healthy Caucasian male
Test substance:	Radiolabelled LAE; arginine portion uniformly <sup>13</sup> C labelled (purity 97.6%).
Batch:	2625 Radio-labelled NPE/LMA0044/41
Purity:	86.7% ethyl lauroyl arginate HCl 96% radiochemical purity
Vehicle:	15 mg propylene glycol mg/kg
Dose:	5 mg/kg/bw <sup>13</sup> C-LAE
Volume:	made up to 1 ml/kg bw in purified water
Sample collection:	0, 5, 10, 15, 30 min and 1, 2, 4, 8, 12 and 24 h post-dose

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GLP: in compliance

2 healthy Caucasian male subjects between 20-30 years, body mass index (BMI) of 19 and 29 kg/m<sup>2</sup> and weighing between 72.3 and 75.8 kg.

<sup>13</sup>C-LAE (N- $\alpha$ -lauroyl-L-arginine ethyl ester monohydrochloride) was given orally to the healthy male volunteers.. The study design included a 2-week pre-study screening interval, a 2-day study period and a post-study assessment for 7 days after dose administration. Blood samples were taken at 0 hours (pre-dose), 5, 10, 15, 30 minutes, 1, 2, 4, 8, 12 and 24 hours post-dose. The resultant plasma samples were stored frozen and a total of 22 plasma samples were for analysis. Plasma concentrations of <sup>13</sup>C -LAE, <sup>13</sup>C -LAS and <sup>13</sup>C -arginine in these samples were measured using a liquid chromatographic-tandem mass spectrometric (LC-MS/MS) method (Huntingdon Life Sciences letter report LMAI045). The performance of the bio-analytical method was monitored by measuring concentrations of each analyte in quality control samples that were analysed concurrently with the test samples.

Plasma levels of <sup>13</sup>C -LAE (concentrations as the hydrochloride salt) and <sup>13</sup>C -LAS ranged from below the low limit of quantification (1 ng/ml) to 44.0 ng/ml and 154 ng/ml respectively, and <sup>13</sup>C-arginine ranged from below the low limit of quantification (10 ng/ml) to 680 ng/ml.

Doses of <sup>13</sup>C-LAE at a level of 5 mg/kg bodyweight appeared to be well tolerated by the two healthy male subjects except for the burning sensation reported by both and nausea in one. However, it was assumed that the burning sensation (and possibly the nausea) were not due to LAE but rather due to the solvent, propylene glycol, (15 mg/kg bw).

Ref.: 39

#### Comment

The burning sensation felt by both subjects and nausea in one was dismissed by the study authors as the effect of solvent, propylene glycol. There was no justification for this statement. [WHO ADI for propylene glycol is up to 25 mg/kg]

Guideline: /  
 Subjects: 6 healthy Caucasian male  
 Test substance: Radiolabelled LAE; 2arginine portion uniformly <sup>13</sup>C labelled (purity 97.6%).  
 Batch: 2625  
 Purity: Radio-labelled NPE/LMA0044/41  
 86.7% ethyl lauroyl arginate HCl  
 96% radiochemical purity  
 Dose: 2 subjects: 2.5 mg/kg/bw <sup>13</sup>C-LAE and 7.5 mg propylene glycol /kg  
 4 subjects: 1.5 mg/kg/bw <sup>13</sup>C-LAE and 4.5 mg propylene glycol /kg  
 Volume:  
 Sample collection: 0, 5, 10, 15, 30 min and 1, 2, 4, 8, 12 and 24 h post-dose  
 GLP: in compliance

Six healthy Caucasian male subjects with a mean age of 33.7 years, a mean body mass index (BMI) of 24.87 kg/m<sup>2</sup> and with a mean weight of 79.77 kg.

<sup>13</sup>C-LAE (N-a-Iauroyl-L-arginine ethyl ester monohydrochloride) was administered orally to healthy male volunteers after an overnight fast followed by a standard breakfast, at dose levels of 2.5 mg/kg bw or 1.5 mg/kg bw. The study design included a 2-week pre-study screening interval, a 2-day study period and a post-study assessment for 7 days after dose administration. Blood samples were taken at 0 hours (pre-dose), 5, 10, 15, 30 minutes, 1, 2, 4, 8, 12 and 24 hours post-dose. The separated plasma was transferred to tubes containing sodium metabisulphite (acting as a stabilising agent), "snap"-frozen in solid carbon dioxide and stored at ca -20°C or below until analysed.

### **Pharmacokinetic results**

Plasma concentrations of  $^{13}\text{C}$ -LAE were below the limit of quantification at all sampling times in 5 subjects. Since there is only data from one subject and at only two sampling times, there were insufficient quantifiable data to assess the pharmacokinetics of  $^{13}\text{C}$ -LAE.

The time at which the maximum plasma concentration ( $T_{\max}$ ) for  $^{13}\text{C}$ -arginine occurred either earlier or at the same time as  $T_{\max}$  for  $^{13}\text{C}$ -LAS, indicating that the absorption of  $^{13}\text{C}$ -arginine occurred more rapidly than absorption of  $^{13}\text{C}$ -LAS (from which it was released).

Plasma concentrations of  $^{13}\text{C}$ -arginine were generally considerably higher than those of  $^{13}\text{C}$ -LAS (this difference exists without factoring in the two-fold difference in molecular weight), indicating that there was relatively extensive breakdown of LAE to arginine.

The terminal half-life ( $t^{1/2}$ ) of  $^{13}\text{C}$ -LAS was in the range 2.2 to 3.3 hours, and generally appeared to be similar to that of  $^{13}\text{C}$ -arginine (range 1.6 to 4.0 hours). However, it should be noted that  $t^{1/2}$  of  $^{13}\text{C}$ -arginine could not be determined in accordance with the acceptance criteria for all subjects and so these values should be interpreted with caution.

### **Clinical results**

No serious adverse events were reported during the study and no subject withdrew because of an adverse event. Three treatment-emergent adverse events were reported in the study by two of the six subjects (33%), one subject at each dose level: headache after the 2.5 mg/kg bodyweight dose, diarrhoea and flatulence after the 1.5 mg/kg bodyweight dose - all were of mild severity.

The headache was considered unlikely to be related to treatment.

The findings of diarrhoea and flatulence occurred approximately 30 hours after receiving a dose of 1.5 mg/kg bodyweight LAE with 4.5 mg/kg bodyweight propylene glycol and were recorded at the time by the investigator as "possibly related" to the study treatment. The study authors re-assessed these adverse events. They considered them unlikely to be related to treatment since no similar findings at higher dose levels in a preliminary study at 5 mg/kg bw of LAE (Ref 39) nor in this study at 2.5 mg/kg bodyweight of LAE. In addition, LAE in preclinical testing has not been reported to induce these effects (no data provided).

There were no clinical statistically significant variations in the haematological, blood chemistry and urinalysis comparing pre-study (within 14 days of dosing) and post study (within 7 days of discharge) No notable changes were noted in vital signs and no clinically significant ECG findings during the study at the two oral dose levels.

Ref.: 40

#### **Comment**

The burning sensation and nausea reported in the first study and the diarrhoea noted in the second study could be due to mucosal irritation as seen in the animal studies.

#### **3.3.12. Special investigations**

No data submitted

#### **3.3.13. Safety evaluation (including calculation of the MoS)**

The Margin of Safety is based on dermal application only in cosmetics (see 3.3.14).

#### **CALCULATION OF THE MARGIN OF SAFETY**

Dermal absorption of 0.4% ethyl lauroyl arginate, the requested concentration for general use as a preservative in cosmetics, was below the Limit of Quantification. Back extrapolation of the percutaneous absorption from 1.96% Ethyl lauroyl arginate HCl to 0.4% Ethyl lauroyl arginate HCl was not considered to be appropriate. Therefore, 100% absorption of 0.4% Ethyl lauroyl arginate HCl is used to calculate the MOS.

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The NOAEL derived from the chronic toxicity study (52 week, oral, rat) for ethyl lauroyl arginate of 271 mg/kg bw/day was used.

**0.4%  
preservative use only**

$$\boxed{\text{SED} = \frac{\mathbf{A} (\text{g/day}) \times 1000\text{mg/g} \times \mathbf{C} (\%) / 100 \times \mathbf{DA}_p (\%) / 100}{60 \text{ kg}}}$$

A (g/day): Amount of the cosmetic product applied daily

C (%): the Concentration of the ingredient under study in the finished cosmetic product on the application site

DAp (%): Dermal Absorption expressed as a percentage Systemic Exposure Dose

SED, dermal	$17.79 \times 1000 \times 0.4\% \times 100\% / 60$	= 1.19 mg/kg/d
NOAEL	(mg/kg bw/day)	= 271 mg/kg bw/d

<b>Margin of Safety – preservative use only</b>	<b>NOAEL / SED</b>	<b>= 228</b>
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**Combined 0.4% preservative and 0.8 % a.i .in soap, shampoo and non-spray deodorant**

$$\boxed{\text{SED} = \frac{\mathbf{A} (\text{g/day}) \times 1000\text{mg/g} \times \mathbf{C} (\%) / 100 \times \mathbf{DA}_p (\%) / 100}{60 \text{ kg}}}$$

Amount of the cosmetic product as active ingredient applied daily

Soap:	0.24 g/day
Deodorants:	0.5 g/day
Shampoo:	0.08 g/day
TOTAL	0.82 g/day

Total SED ( $16.97 \times 1000 \times 0.4\% + (0.82 \times 1000 \times 0.8\%) \times 100\% / 60$ )	= 1.24 mg/kg/d
NOAEL (mg/kg bw/day)	= 271 mg/kg bw/d

<b>Margin of Safety – preservative and a.i. use</b>	<b>NOAEL / SED</b>	<b>= 219</b>
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**3.3.14. Discussion**

Submission II on Ethyl lauroyl arginate HCl included studies on acute inhalation, chronic and reproductive toxicity together with a new mutation and two toxicokinetic studies as well as two studies on human volunteers. The SCCP reassessed the data provided in Submission I, in conjunction with the new studies provided in Submission II.

The earlier SCCP opinion (SCCP/0837/04) expressed concern that in the sub-chronic LAE study, Ethyl lauroyl arginate HCl caused mucosal irritation in the non-glandular region of the stomach. This concern was reinforced by a letter from the head of Reproductive Studies Group attached to Reference 31, commenting on the respiratory effects following gavage. He considered that the respiratory effects following gavage were due to the route of administration and not a systemic toxic response but said "to base the NOAEL on results of dietary studies should be considered in the light of the proposed route of administration for use in the material in cosmetics. It should be acceptable if the material is applied as a lotion to the body skin, but might be less acceptable if applied as a spray or to the lip/face."

**Opinion on ethyl lauroyl arginate HCl**

In Submission II, the acute inhalation study and chronic toxicity study further corroborated the potential for mucosal irritation. The authors of the acute inhalation study stated that 'combined experimental evidence would suggest that the test substance has a mild respiratory tract irritation potential if exposure to the aerosol is sufficiently high'.

The EFSA AFC panel (additives, flavourings, processing aids and materials in contact with food) in its opinion on ethyl lauroyl arginate as a food additive considered the non-glandular forestomach lesions were not indicative of systemic toxicity. However, they stated concerns 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 could not 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.' They considered that the NOAEL should be more in line with that seen in the 90 day Mirenat-N study (ref 18), equivalent to mean doses of 44 mg Ethyl lauroyl arginate HCl/kg body weight/day for males and 53 mg Ethyl lauroyl arginate HCl/kg body weight/day for females.

The effect of Ethyl lauroyl arginate HCl on peripheral haematological parameters is equivocal, making interpretation difficult. There was no consistent pattern of white blood cells affected when different doses, time points and rat strains were compared.

Comparison of the total and differential white cell counts with historical control haematological data generated for Sprague Dawley Crl:CD SD rats aged between weeks 13-22 and 23-47, (Charles Rivers, 2006) showed that the study animals at treatment weeks 13 -14 (age 21 -22 weeks old) and at treatment weeks 26 week (age 31 weeks old) fell within the control range even at the highest doses. The most consistent response observed in the different studies was the reduced numbers of LUC (large unstained cells) in all treated groups;. the concurrent controls were at or above the high end of the range and the treated groups towards the low end of the range.

In the chronic study, the lack of pre-dosing data for the individual animals did not permit an assessment as to whether the changes seen over time were within the normal variation or if they were treatment related. In addition, data for replacement animals were not included until after inclusion in the study. The ranges of haematological parameters in the control group were wider than in the treated animals at most doses. However, the percentages of the differential WBC of all animals were similar to the historical controls, with the exception of a higher percentage of monocytes and LUC in both the concurrent controls and dosed animals at 26 weeks. Throughout the study, there were no consistent outliers within the control animals. Similar high inter-individual variation was also noted in plasma LAE concentrations in both sexes in the toxicokinetic section of the chronic study.

A more feasible cause in the lower WBC counts and the lack of consistency of cell types affected would be a migration of cells to the areas of damaged tissue. Similar gastric mucosal lesions were seen at post mortem in the subchronic and chronic LAE studies. The study authors considered these lesions in the non-glandular forestomach to be local irritation, induced by Ethyl lauroyl arginate HCl and unrelated to a systemic toxicity. The fourfold higher dose in the subchronic study would seem to counterweight the time frame of the chronic study. In the subchronic study, the post mortem effects were seen only at the highest dose. In the chronic study, gastric mucosal lesions seemed to be dose-related: ulceration and epithelial hyperplasia were seen in animals in all dose groups and accompanied by subepithelial/submucosal inflammation and subepithelial fibrosis of the non-glandular epithelium, along with some inflammation of the underlying muscle and serosal layer, especially at the high dose. The peripheral blood responses at necropsy in both studies show similar trends (statistically significant decreases in the total WBC in males and a downward trend in the total WBC counts in females). Migration of white blood cells would occur at the onset of the irritation. In the chronic study this would seem to occur mainly between the two blood sampling times at Week 14 and 26. The changes in the white

**Opinion on ethyl lauroyl arginate HCl**

blood cell counts in individual animals would be dependent on the severity and time the lesions developed.

This interpretation is also supported by an additional expert statement reviewing the repeated dose study data on white blood cells, which was provided by the applicant (Brown 2007). The conclusion was that the reduction of mature white cells in the peripheral blood is almost certainly part of a normal physiological response to the changes seen in the stomach and associated tissues. This was summarized by 3 points:

1. Normal myeloid cell production appears to be taking place as indicated by the myelograms at the end of the 52-week study (total myeloid cells, myeloid left-shift index, M:E Ratio).
2. There is no evidence from the peripheral blood results in any of the studies of excessive cell destruction or damage, neither by marked changes in the actual numbers, nor in morphology of white blood cells.
3. The most likely reason for the lack of mature blood cells in the circulation, therefore, is migration to the tissues.

Low systemic toxicity of Ethyl lauroyl arginate HCl is supported by its toxicokinetics. In the chronic rat study, the rate and extent of systemic exposure to Ethyl lauroyl arginate HCl and its metabolite LAS appeared to be characterised by dose-independent kinetics. High inter-individual variation in plasma LAE concentrations was noted, but this was less marked in plasma LAS concentrations in both sexes.

In human volunteers, the oral pharmacokinetics of Ethyl lauroyl arginate HCl indicated rapid absorption and hydrolysis to LAS and arginine. The terminal half-life of <sup>13</sup>C-LAS (range 2.2 to 3.3 hours) and <sup>13</sup>C -arginine (range 1.6 to 4.0 hours) were similar. Plasma concentrations of <sup>13</sup>C -arginine were generally considerably higher than those of <sup>13</sup>C -LAS.

Thus, even assuming 100% absorption at 0.4% Ethyl lauroyl arginate HCl, it would suggest rapid hydrolysis of Ethyl lauroyl arginate HCl if absorbed through the skin. Therefore, systemic exposure to Ethyl lauroyl arginate HCl and N $\alpha$ -lauroyl-L-arginine *in vivo* is likely to be very short.

If a restriction is placed on Ethyl lauroyl arginate HCl for solely dermal application in cosmetics, the local gastric mucosal effects need not be considered. The NOAEL/NOEL for Ethyl lauroyl arginate HCl in diet from the subchronic and chronic studies were within the same range. The NOAEL from the chronic study of 271 mg Ethyl lauroyl arginate HCl /kg bw/day was used for risk assessment.

The NOAEL for maternotoxicity and foetotoxicity of Ethyl lauroyl arginate HCl were 207 mg/kg bw/day and 691 mg/kg bw/day respectively. No treatment related effects were seen in a two-generation reproduction toxicity study.

Ethyl lauroyl arginate HCl (up to 0.8%) was considered to be non-irritant to the eyes under the test conditions of the studies. It also appeared not to have sensitisation potential.

Ethyl lauroyl arginate HCl did not appear to have any mutagenic potential under the experimental conditions.

The EFSA AFC panel stated in its opinion on the use of ethyl lauroyl arginate as a food additive, that exposure to this substance through food products is at or above the ADI level. Therefore, and in the light of the MoS for cosmetics, there are concerns that additional exposure to Ethyl lauroyl arginate from non-cosmetic sources might lead to exposure levels that could not be considered safe.

#### 4. CONCLUSION

The SCCP considers, with the additional data provided, that Ethyl Lauroyl Arginate HCl is safe for the consumers, when used:

- up to a maximum authorised 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 authorised concentration of 0.8 % in soap, anti-dandruff shampoos, and non-spray deodorants.

This opinion is based on the use of ethyl lauroyl arginate HCl in the specified cosmetic products only. It takes no account of other possible and probable sources of exposure by the consumer of this substance.

#### 5. MINORITY OPINION

Not applicable

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