

**EUROPEAN COMMISSION**  
HEALTH & CONSUMER PROTECTION DIRECTORATE-GENERAL  
Directorate C - Public Health and Risk Assessment  
**C7 - Risk assessment**

**SCIENTIFIC COMMITTEE ON CONSUMER PRODUCTS**

**SCCP**

**OPINION ON**

**ETHYL LAUROYL ARGINATE**

**COLIPA n° P95**

Adopted by the SCCP during the 3<sup>rd</sup> plenary meeting  
of 15 March 2005

**TABLE OF CONTENTS**

1.	BACKGROUND	3
2.	TERMS OF REFERENCE	3
3.	OPINION	4
4.	CONCLUSION	41
5.	MINORITY OPINION	41
6.	REFERENCES	41
7.	ACKNOWLEDGEMENTS	43

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## 1. BACKGROUND

According to Article 4, 1. (e) and (f) of Cosmetics Directive 76/768/EEC, Member States shall prohibit the marketing of cosmetic products containing preservatives other than listed in Annex VI, Part 1, beyond the limits and outside the conditions laid down, unless other concentrations are used for specific purposes apparent from the presentation of the product.

Ethyl lauroyl arginate HCl (COLIPA<sup>1</sup> No P 95) is currently not listed in Annex VI and therefore cannot be used as preservative.

The European Commission has received a request from COLIPA for the inclusion of Ethyl lauroyl arginate HCl in Annex VI and Annex III (for other than preservative purposes) in order to allow the use of Ethyl lauroyl arginate HCl in certain cosmetic products.

Industry has submitted a documentation to support the proposal.

## 2. TERMS OF REFERENCE

The SCCP is requested to answer the following questions:

1. *On the basis of provided data the SCCP is asked to assess the risk to consumers when Ethyl lauroyl arginate HCl is used as a preservative in cosmetic products up to a maximum authorised concentration of 0.2 %.*
2. *On the basis of provided data, the SCCP is asked to assess the risk to consumer when Ethyl lauroyl arginate HCl is used up to a maximum authorised concentration of 0.4 % in the following cosmetic products: soap, anti-dandruff shampoos, deodorants and oral hygiene products.*
3. *Does the SCCP recommend any further restrictions with regard to its use in cosmetic products?*

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

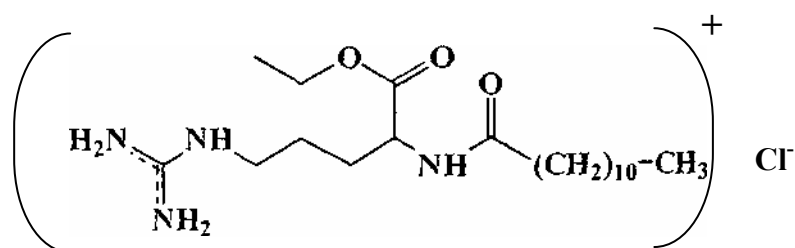
##### 3.1.1.3. Trade names and abbreviations

LAE  
Mirenat-N  
Aminat  
LAE-P (abbreviation for pure compound)

##### 3.1.1.4. CAS / EINECS / ELINECS number

CAS : 60372-77-2  
ELINECS : 00-11-0173-00

##### 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

Lumpy white powder

## 3.1.3. Molecular weight

Molecular weight : 421.02

## 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 production of LAE, the aqueous paste contains 74-84% Ethyl lauroyl arginate HCl. When this paste is dehydrated, Ethyl lauroyl arginate HCl content is between 90-95%.

The Table 1 lists the Ethyl lauroyl arginate HCl content and accompanying contaminants of the batches used in the provided studies. The main impurities are N<sup>α</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.

Table 1: Ethyl lauroyl arginate HCl content and accompanying contaminants in dehydrated LAE

Batch name/number	LAE-P	3036	5733	2625	5159
Ethyl lauroyl arginate HCl	99.0	93.2	90.3	90.1	69.1
Water		4.1	0.9	0.4	23.1
Ethyl laurate		1.5	2.0	0.7	1.0
Lauric acid		2.7	3.0	4.2	1.7
N <sup>α</sup> --lauroyl-L-arginine		1.5	2.1	3.3	1.0
L-arginine ethyl ester		0.3	0.4	0.3	0.2
L-arginine		0.3	0.3	0.2	0.2
Salts (mostly NaCl)		0.7	0.9	0.8	1.6
Ethanol					1.9

**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 2.

Table 2: Ethyl lauroyl arginate HCl content (%) and accompanying contaminants in Mirenat.

Batch	0000001 4-12-95	0000003	12 June 1995	13 Dec 1995	3128
Ethyl lauroyl arginate HCl	20.2	20.3	20.4	20.4	20.0
N <sup>α</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 (Ref 3, 17, 20, 21, 23).

**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.

### 3.1.5. Impurities / accompanying contaminants

The accompanying contaminants are listed in the tables above.

### 3.1.6. Solubility

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

### 3.1.7. Partition coefficient (Log P<sub>ow</sub>)

Log P<sub>ow</sub> : 1.43 @ 20 °C

### 3.1.8. Additional physical and chemical specifications

No specific characteristics were given for Ethyl lauroyl arginate HCl but 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 x 10 <sup>-4</sup> Pa @ 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.”

The use concentration of Ethyl lauroyl arginate HCl is 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.

Mirenat has been developed as a food preservative at concentrations ranging from 0.50 – 2.00 g/l, as a liquid additive, to increase durability of meat products and semi-products, dishes, salads and mayonnaise. However, neither ethyl lauroyl arginate HCl nor Mirenat are in the last amendment (2003/114/EC) or have been included in the proposed amendment (6th).

### 3.3. Toxicological Evaluation

#### 3.3.1. Acute toxicity

##### 3.3.1.1. Acute oral toxicity

#### 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 no	:	2625
Dose	:	1800 mg/kg bw Ethyl lauroyl arginate HCl in 1% aqueous methylcellulose by gavage (10 ml/kg bw)
Controls	:	None
Observ. 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 no	:	000003
Stability	:	6 months
Dose	:	2000 mg/kg bw by gavage
Controls	:	None
Observ. 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

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
Observ. 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 Ethyl lauroyl arginate HCl mg/kg bw.

Ref.: 2

### 3.3.1.3. Acute inhalation toxicity



No data submitted

### 3.3.2 Irritation and corrosivity

#### 3.3.2.1. Skin irritation

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 cottonwool. 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. No control animals were included in this study.

#### 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 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

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 no	:	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

### 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 no	:	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 HC1 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

### **Mirenat-N, study 2**

Guideline	:	OECD 405 (1987)
Species/strain	:	New Zealand albino rabbit
Group size	:	1
Active ingredient:	:	20.4% Ethyl lauroyl arginate HC1
Test substance	:	Mirenat -N
Batch no	:	13 December-9
Purity	:	21.6% LAE
Dose	:	0.1 ml equivalent to 20.4 mg Ethyl lauroyl arginate HC1
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 24-h.

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 HC1 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

### **Aminat, study 1**

Guideline	:	OECD 405 (1987)
Species/strain	:	New Zealand albino rabbit
Group size	:	3 male
Active ingredient:	:	0.02%. Ethyl lauroyl arginate HC1
Test substance	:	Aminat 0.1%: [Mirenat-N & deionised water]

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Batch no	:	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 were 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

### **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 no	:	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

### 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 no	:	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.

**Aminat, study 4**

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 2.0%: [Mirenat-N & deionised water]
Batch no	:	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.

<b>3.3.3. Skin sensitisation</b>
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**Magnusson and Kligman study****LAE**

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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 no	:	2625
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.

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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 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

#### 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 no	:	12 June-95, 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



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		Topical induction: 50% v/v 10.2% Ethyl lauroyl arginate HC1
Resting phase	:	21 days
Challenge	:	50% v/v 10.2% Ethyl lauroyl arginate HC1
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 HC1. 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 HC1 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 HC1.

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 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 HC1, 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:

<u>Injection site (paired)</u>	<u>Treatment</u>
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 HC1 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 HC1 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 HC1 was applied to the left shorn flank. The patches were occluded. After 24 hours, the dressing was 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 HC1.

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 HC1 produced 0 % sensitisation (0/10) at 50% dilution. The results indicate that 20.4% Ethyl lauroyl arginate HC1 does not induce a sensitisation response in the guinea pig.

Ref.: 7

### 3.3.4. Dermal / percutaneous absorption

Guideline	:	According to the 'Guidelines for <i>in vitro</i> methods to assess percutaneous absorption of cosmetic ingredients' adopted by SCCNFP
Species/strain	:	Female pig skin, unboiled back. Animal weight: about 80 kg.
Active ingredient:	:	90.3% Ethyl lauroyl arginate HC1
Test Substance	:	LAE
Batch no	:	5733
Purity	:	90.3% Ethyl lauroyl arginate HC1
Test formulations:	:	0.39% and 1.93% Ethyl lauroyl arginate HC1 in propylene glycol/water 30/70 solution
Dose application	:	4.8 µl/cm <sup>2</sup> of test formulation
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 formulation (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 formulations were allowed 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 formulation 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 formulation, this is 4.8 mg formulation/cm<sup>2</sup> and 18.7 µg/cm<sup>2</sup> of active substance. This represents the maximum dose resulting from the proposed use as active ingredient in cosmetics.

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

**Recovery of test substance:** In the 0.39% Ethyl lauroyl arginate HCl formulation, the quantities of active ingredient were below LOQ in all the compartments analysed.

In the 1.96% Ethyl lauroyl arginate HCl formulation, the quantity 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
<u>Total Recovery</u>	<u>90.13 ± 7.21</u>
Total Absorbed	5.24 ± 12.29

Under the experimental conditions of this study, the percutaneous absorption of 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>. This is 5 times higher than the maximum dose for the proposed use as active ingredient in cosmetics. For this reason the amount absorbed is an overestimate.

Ref.: 4

### 3.3.5. Repeated dose toxicity

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

##### LAE, study 1

Guideline : /  
Species/strain : Rat, Han Wistar

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Group size	:	5 males + 5 females per dose
Active ingredient	:	90.1% Ethyl lauroyl arginate HCl
Test substance	:	LAE
Batch no	:	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/day (equivalent to 2120, 3098 and 3850 mg Ethyl lauroyl arginate HCl/kg/day) for males and 2379, 3329 and 4641 mg/kg/day (equivalent to 2143, 2999 and 4182 mg Ethyl lauroyl arginate HCl/kg/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.

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/day).

Ref.: 15

### **Mirenat-N**

Guideline	:	/
Species/strain	:	Rat, CrI: CD BR

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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 no	:	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/day (equivalent to Ethyl lauroyl arginate HCl of 68, 283 and 1070 mg kg/day) for males and 352, 1400 and 5846 mg kg/day (equivalent to Ethyl lauroyl arginate HCl of 71, 284 and 1187 mg kg/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

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 bodyweight/day.

Ref.: 17

3.3.5.2.	Sub-chronic (90 days) oral / dermal / inhalation toxicity
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### LAE study

Guideline	:	OECD 408 (revised 1998)
Species/strain	:	Rat, Han Wistar
Group size	:	20 males + 20 females per dose
Active ingredient:	:	90.1% and 90.3% Ethyl lauroyl arginate HCl
Test substance	:	LAE
Batch no	:	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 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/day (equivalent 346, 1030 and 3346 mg Ethyl lauroyl arginate HCl/kg/day) for males and 445, 1286, 3915 mg/kg/day (equivalent to 401, 1159 and 3527 mg Ethyl lauroyl arginate HCl/kg/day) for the 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.

## Results

Animals were killed in the first four days of Week 13. There was no recovery period. There were no deaths.

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 in the high dose males; slight increases in mean cell haemoglobin, mean cell haemoglobin concentration, mean cell volume and reduced total white blood cell and lymphocyte counts were noted. 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, eg, adjacent to the entry of the oesophagus.

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

Ref.: 16

### **Mirenat-N**

Guideline	:	OECD No.408 (1981)
Species/strain	:	Rat, CrI: 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)
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/day (equivalent to 44, 183 and 671 Ethyl lauroyl arginate HCl mg kg/day) for males and 262, 1067 and 3927 mg kg/day (equivalent to 53, 216 and 793 Ethyl lauroyl arginate HCl mg kg/day) for females.

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

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 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.

No ocular lesions were 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 weight were seen compared with concurrent controls.

An increased incidence of alopecia was noted amongst mid and high dose females. This was previously not considered treatment-related by the study authors!

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

The study authors concluded that there was evidence of toxicity was noted in the high dose male and female rats, receiving 50000 ppm Mirenat-N, (1010 ppm Ethyl lauroyl arginate HCl ppm Ethyl lauroyl arginate HCl equivalent to mean doses of 671 and 793 mg Ethyl lauroyl arginate HCl/kg/day males and females respectively). 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 body weight/day for males and 216 mg Ethyl lauroyl arginate HCl /kg body weight/day for females). However, as females of all treated groups showed reduced bodyweight gains and alopecia at the mid dose, the SCCP consider the no-effect level should be 3200 ppm Mirenat-N, (646 ppm Ethyl lauroyl arginate HCl 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).

Ref.: 18

### 3.3.5.3. Chronic (> 12 months) toxicity

No data submitted

### 3.3.6. Mutagenicity / Genotoxicity

#### 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 no	:	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	:	<b>without S9 mix:</b> 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	
	<b>with S9 mix:</b> 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

## 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 no	: 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	: <b>without S9 mix:</b> 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 <b>with S9 mix:</b> 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 S-9 mix and TA 1537 only in the presence of S-9 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 S-9 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 S-9 mix

#### Conclusion

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

Ref.: 20

#### *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 no : 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 S-9 : 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 S-9 : 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 S-9, 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 S-9 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 S-9 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

#### ***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 no	:	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		
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 S-9: 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 S-9 : 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	:	<b>without S9 mix:</b> Mitomycin C 0.1µg/ml <b>with S9 mix:</b> : 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

### 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 no	:	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 S-9 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).

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	with S-9 32 h : 250, 500 and 1000 µg Mirenat/ml (50.8, 101.5 and 203 µg Ethyl lauroyl arginate HCl/ml)
	without S-9 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 S-9 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 S-9 18 h 125, 250 and 500 µg Mirenat/ml
	Test 2 with S-9 18 h 700, 800 and 1000 µg Mirenat/ml
	Test 2 without S-9 32 h 500 µg Mirenat/ml
	Test 2 with S-9 32 h 1000 µg Mirenat/ml
Positive controls :	w/out 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

#### 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 no	:	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 postmortem 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 no	:	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 inter-group 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)

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

#### 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 no	:	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



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	(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 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 was 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 lives foetuses.

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

Dose (mg/kg/day)	0	138	415	1382
Life 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

### 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 no	:	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 postmortem 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. Postmortem: 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

#### Preliminary embryo-foetal toxicity study

Guideline	:	/
Species/strain	:	New Zealand White rabbits female
Group size	:	18 pregnant females, 4 per dose, 6 control
Active ingredient:	:	69.1% Ethyl lauroyl arginate HCl
Test substance	:	LAE
Batch no	:	5159
Purity	:	69.1% ethyl lauroyl arginate HCl
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 fetuses 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
Live 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.

Ref.: 30

**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 no	:	5159
Purity	:	69.1% ethyl lauroyl arginate HCl
Vehicle	:	1% w/v aqueous methyl cellulose

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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 of 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

## 3.3.8.2. Teratogenicity

Data in the previous section

## 3.3.9. Toxicokinetics

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

Guideline	:	/
Species/strain	:	Rat, Sprague DawleyCrI: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 no	:	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)

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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>α</sup>-lauroyl-L-arginine ethyl ester, arginine, orthonine and urea were identified. Orthonine 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>α</sup>-lauroyl-L-arginine) and arginine. Arginine was further metabolised to orthonine. 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 orthonine (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>α</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 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 *in vitro* and *in vivo* the pathway that Ethyl lauroyl arginate HCl is metabolised. It is rapidly hydrolysed by the ethyl ester and lauroyl amide to arginine and then further catabolism to orthonine 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

#### 3.3.10. Photo-induced toxicity

No data submitted

#### 3.3.11. Human data

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No data submitted

**3.3.12. Special investigations**

No data submitted

**3.3.13. Safety evaluation (including calculation of the MoS)****CALCULATION OF THE MARGIN OF SAFETY**

Not applicable

**3.3.14. Discussion**

The sub-chronic and chronic toxicity studies were based on Ethyl lauroyl arginate HCl, incorporated in feed. The study authors considered that in rats, the No Observed Adverse Effect Level (NOAEL) to be 346 and 401 mg Ethyl lauroyl arginate HCl/kg/day for males and females respectively. In rat studies, based on dosing by gavage, the NOAEL for the dam was taken to be 138 mg Ethyl lauroyl arginate HCl/kg/day.

Mucosal irritation was indicated during both dosing procedures. Excessive salivation, frequently with staining of the fur, was noted with both dosing regimens. This suggests local irritation of the mucosa. This was more marked in females.

In the sub-chronic toxicity studies in rats, the main treatment-related pathological changes with Ethyl lauroyl arginate HCl were seen in the non-glandular region of the stomach, specifically in the area adjacent to the entry of the oesophagus in the mid and high dose animals. The study authors considered that these changes in the stomach indicated the irritant action of the test substance on mucosal tissue, but commented that it was unusual for the changes to be restricted to a specific area.

In the animals dosed by gavage, adverse respiratory reactions to dosing were seen in both rats (415, 1382 mg ethyl lauroyl arginate HCl /kg/day) and rabbits (207, 691 mg ethyl lauroyl arginate HCl/kg/day). The study authors believed this to be associated with a higher risk of irritation being induced during the dosing procedure when high concentrations of test material were used. They reported that the difficulties with dosing were reduced when the surface of the catheter was washed clean and used damp rather than wiped dry before dose administration but did not provide the data. Since this was a reproductive study, the histopathology of the stomach was not investigated. In reply to a letter requesting interpretation of the embryo-foetal studies, the head of Reproductive Studies Group of the Testing Laboratory stated that the effect was not a systemic toxic response. He went on to say ‘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.’



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

Ethyl lauroyl arginate HCl (99%) was considered to cause serious damage to eyes under the test conditions of the study. Ethyl lauroyl arginate HCl (20.4%), as MIRENAT-N was also classified as a irritant to the rabbit eye. Ethyl lauroyl arginate HCl (0.4%) as AMINAT 2%, was considered to cause no ocular irritation under the test conditions.

4.5% Ethyl lauroyl arginate HCl did not induce a sensitisation response in the guinea pig.

The percutaneous absorption of Ethyl lauroyl arginate HCl in propylene glycol/water 30/70 after an exposure time of 24 h may be considered to be  $5.24 \pm 2.29 \mu\text{g}/\text{cm}^2$ .

Ethyl lauroyl arginate HCl showed no evidence of mutagenic or clastogenic activity *in vitro*. The increased frequency of polyploid metaphases could indicate the possibility of aneugenic activity.

Ethyl lauroyl arginate HCl is rapidly metabolised to ornithine and urea. Due to the rapid hydrolytic degradation in liver and plasma, systemic exposure to Ethyl lauroyl arginate HCl and N $\alpha$ -lauroyl-L-arginine *in vivo* is likely to be very short.

#### 4. CONCLUSION

The SCCNFP 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.

An opinion based on the information available at that time will be given.

#### 5. MINORITY OPINION

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