



Scientific Committee on Consumer Safety

SCCS

OPINION ON
Ethyl lauroyl arginate HCl

COLIPA n° P95

ADDENDUM to the SCCP Opinion on Ethyl lauroyl arginate HCl
(SCCP/1106/07, 15 April 2008)



The SCCS adopted this opinion at its 11th plenary meeting
of 21 June 2011

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SCCS

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Opinion on ethyl lauroyl arginate HCl

1. BACKGROUND

Submission I for ethyl lauroyl arginate HCl was submitted in February 2003 by COLIPA¹.

The Scientific Committee on Cosmetic Products (SCCP) adopted its opinion SCCNFP/0837/04 at the 3rd plenary meeting of 15th March 2005 with the conclusion, that *"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."*

With the submission II and II-bis for ethyl lauroyl arginate HCl in April and October 2006, respectively, the additional data were provided by the applicant.

The Scientific Committee on Consumer Products (SCCP) adopted its opinion SCCP/1106/07 at the 15th plenary meeting of 15th April 2008 with the conclusion, that

"Ethyl Lauroyl Arginate HCl is safe for the consumers, when used:

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

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

The exclusion of the use of ethyl lauroyl arginate HCl for oral hygiene products is contested by the applicant. An update to the dossier addressing this point was submitted in December 2008, containing expert statements in relation to the irritant potential of ethyl lauroyl arginate as well as a report of the joint FAO/WHO Expert Committee on Food Additives that arrived at a higher ADI than the EFSA evaluation of 2007.

Moreover, in the light of concerns that were raised in relation to combined exposure of consumers to ethyl lauroyl arginate HCl from cosmetics and food, the applicant in 2010 provided a new dermal absorption study to allow revision of the worst case assumption-based exposure assessment in opinion SCCP/1106/07.

2. TERMS OF REFERENCE

- 1. In the light of the data provided, does the SCCS consider that ethyl lauroyl arginate HCl is safe for the consumers, when used up to a maximum concentration of 0.75% in toothpaste and 0.2% in mouthwash products in addition to the currently recommended uses as mentioned above?*
- 2. Taking into account the dermal absorption data submitted, the SCCS is requested to revise the exposure assessment for ethyl lauroyl arginate HCl in cosmetics made in opinion SCCP/1106/07.*
- 3. Does the SCCS have any other scientific concerns of use for ethyl lauroyl arginate HCl in cosmetic products based on the toxicological profile and foreseeable exposure?*

¹ COLIPA - the European Cosmetics 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^α-dodecanoyl-L-arginate hydrochloride (IUPAC)
Monohydrochloride of L-arginine, N^α-lauroyl-ethylester

3.1.1.3. Trade names and abbreviations

LAE-P abbreviation for pure compound

LAE

Lauric arginate

Mirenat-N

Aminat

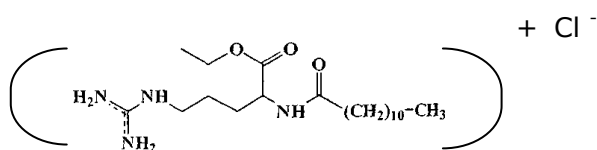
Lauramide arginine ethyl ester

3.1.1.4. CAS / EC number

CAS: 60372-77-2

EC: 434-630-6

3.1.1.5. Structural formula



3.1.1.6. Empirical formula

Formula: C₂₀H₄₁N₄O₃Cl

3.1.2. Physical form

White solid

3.1.3. Molecular weight

Molecular weight: 421.02 g/mol

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3.1.4. Purity, composition and substance codes

Ethyl lauroyl arginate HCl is the active ingredient in the commercial product, LAE. In the crude technical product the aqueous paste contains 74-84% Ethyl lauroyl arginate HCl. LAE is the dehydrated crude product containing 85-95% Ethyl lauroyl arginate HCl.

Table 1: Specifications from submission II

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

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

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

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

| Batch name/number | LAE-P | 3036 | 5733 | 2625 | 5159 | 7446 | 10234 | 12547 | LV090081* |
|--|-------|------|------|------|------|------|-------|-------|-------------|
| | % | % | % | % | % | % | % | % | % |
| Ethyl lauroyl arginate HCl | 99.0 | 93.2 | 90.3 | 90.1 | 69.1 | 88.2 | 88.2 | 91.87 | 86.6 |
| Water | | 4.1 | 0.9 | 0.4 | 23.1 | 3.7 | 2.8 | 1.72 | |
| Ethyl laurate | | 1.5 | 2.0 | 0.7 | 1.0 | 1.0 | 1.4 | 0.83 | |
| Lauric acid | | 2.7 | 3.0 | 4.2 | 1.7 | 2.7 | 2.5 | 2.11 | |
| N ^α -lauroyl-L-arginine (LAS) | | 1.5 | 2.1 | 3.3 | 1.0 | 1.9 | 1.6 | 1.83 | |
| L-arginine ethyl ester | | 0.3 | 0.4 | 0.3 | 0.2 | | | | |
| L-arginine | | 0.3 | 0.3 | 0.2 | 0.2 | | | | |
| Arginate HCl | | | | | | 0.1 | 0.4 | 0.18 | |
| Ethyl arginate 2HCl | | | | | | <0.1 | <0.1 | | |
| Salts (mostly NaCl) | | 0.7 | 0.9 | 0.8 | 1.6 | 1.5 | 0.8 | | |
| Ethanol | | | | | 1.9 | | | | |

*No other data provided

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

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 Table 3.

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

| Batch | 0000001 4-12-95 | 0000003 | 12 June 1995 | 13 Dec 1995 | 3128 |
|------------------------------------|--------------------|---------|-----------------|----------------|---------|
| | % (w/w) | % (w/w) | % (w/w) | % (w/w) | % (w/w) |
| Ethyl lauroyl arginate HCl | 20.2 | 20.3 | 20.4 | 20.4 | 20.0 |
| N ^α -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 | 1.2 | 1.2 |
| Propylene glycol | 73.0 | 73.5 | 73.3 | 0.2 | 73.7 |
| LAE in formulation | 21.6 | 21.6 | 21.6 | 21.6 | 21.2 |

There are some inconsistencies between the submission and the study reports. Batch 0000003 was given as 25% N-Lauroyl ethyl arginate monochlorohydrate.

Batch 13 Dec 1995 differs from the other batches of Mirenat since it is an aqueous formulation rather than a propylene glycol formulation as the other batches of Mirenat (~73% propylene glycol).

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

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

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

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

Aminat 4%, in the mucous membrane irritation test from submission II, was prepared from Aminat, batch JMR-672. This was described as 20% LAE, Ethyl-N^α-dodecanoyl-L-arginate HCl on the certificate of analysis. No other information on the formulation of Aminat was provided. It is not stated whether batch JMR-672 was formulated in water or propylene glycol.

In Submission III, a new formulation, Aminat-G (INCI name: Glycerin and ethyl lauroyl arginate HCl), was used in the gingival irritation studies. Aminat-G was described as 20% LAE in glycerin in the technical data sheet supplied, October 2011, No information on solubility of LAE in glycerin was given.

3.1.5. Impurities / accompanying contaminants

The accompanying contaminants are listed in 3.1.4 for most batches of ethyl lauroyl arginate used in the toxicological studies.

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3.1.6. Solubility

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

Information provided to JECFA (2008, Ref. 45) and FSANZ (2009, Ref. 53) indicates that ethyl lauroyl arginate is soluble up to 20% in propylene glycol, glycerine and ethanol, but no substantiating data was provided to the SCCS.

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

In the acute inhalation toxicity study, the test substance was described as LAE in ethanol. According to the applicant, LAE is soluble in ethanol up to 30%, but no documentation was provided for this.

3.1.7. Partition coefficient (Log Pow)

Log P_{ow}: 1.43 at 20 °C

3.1.8. Additional physical and chemical specifications

No specific characteristics were given for Ethyl lauroyl arginate HCl, only for LAE

Organoleptic properties:

Melting point: 50.5 to 58.0 °C

Boiling point: decomposition from 107 °C

Flash point: /

Vapour pressure: 5.45×10^{-4} Pa at 25 °C

Density: 1.11

Viscosity: /

pKa: /

Refractive index: /

Stability: not specified but assumed to be 6 months at 4°C in the dark by study authors

Mirenat

Stability: 6 months at 4°C in the dark

Ethyl lauroyl arginate - additional physicochemical data

In the Ethyl lauroyl arginate Chemical and Technical Assessment (JECFA 2008, Ref. 45), the chemical characterisations of six ethyl lauroyl arginate batches are included; four are in common with the earlier opinion, SCCP/1106/07. There are some minor variations in the composition of the batches. It also states that commercial products are formulated as 20-25% solutions in appropriate food-grade solvents.

The pH of 1% aqueous solution is the range of 3.64 to 4.25 in 4 batches.

Ethyl-N^α-lauroyl-L-arginate HCl present in ethyl lauroyl arginate is stable for more than 2 years at room temperature when protected in a closed container. The aqueous stability of ethyl lauroyl arginate has been evaluated under acid conditions and at varying temperatures. The acids employed to evaluate the stability were phosphoric, citric, tartaric, malic and fumaric acids and the temperatures were 4, 25 and 50 °C. The results indicate that the stability of ethyl lauroyl arginate decreases with increasing temperature and

reducing pH. In general, the strong inorganic acids affected stability more than the organic acids studied.

Ref: 45

General Comments

In the new study dossiers, ethyl lauroyl arginate HCl and LAE appear to be considered equivalent. For LAE[®] used in the *in vitro* irritation studies, only information on purity and metal content was available.

Whereas the chemistry of the pure chemical is well characterised, in many studies, there is uncertainty as to the purity, dilution and solvent used.

In Submission I and II, the applicant implied that the only formulation for cosmetics was Aminat, 20% ethyl lauroyl arginate HCl in propylene glycol. However, according to information supplied in October 2011, Aminat[®]-G (20% LAE in glycerin) was the formulation used.

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 used as a multi-functional component in the formulation of cosmetic products, with claimed 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 to microbial contamination.

In Submission II, the application was for inclusion of Ethyl lauroyl arginate HCl in annex VI as a preservative with a new maximum concentration of 0.4% in all cosmetic products, and in addition as an antimicrobial in soap, as anti-plaque in oral care products, as deodorant in deodorant products and antidandruff agent in shampoos up to a maximum concentration of 0.8%. Following SCCP opinion SCCP/1106/07, adopted in April 2008, these uses were introduced into the Cosmetics Directive², with the exclusion of use in lip products, oral products and spray products. The current submission is intended to support the use of Ethyl lauroyl arginate HCl in toothpastes at a concentration of 0.75% and in mouth washes at a concentration of 0.2%.

EFSA (2007) established an ADI of 0.5 mg/kg bw ethyl lauroyl arginate for ethyl lauroyl arginate as a food additive for use in non-alcoholic drinks and fruit juices, salted fish, specified meat products, toppings and prepared salads. Commercial products are formulations comprising 20-25% solutions of ethyl lauroyl arginate in appropriate food-grade solvents. In an updated application, uses in dried and salted fish, heat-treated meat products, meat-based prepared salads and surface treatment of cheese are stated.

² Council Directive 76/768/EEC as amended, entries III/207, VI/58

3.3. Toxicological Evaluation

In this opinion, only the irritation and dermal absorption are re-evaluated, based on new information received.

3.3.1. Irritation and corrosivity

3.3.1.1. Skin irritation

Taken from SCCP/0837/04

Guideline: OECD 404 (1992)
 Species/strain: New Zealand albino rabbit
 Group size: 3 females
 Active ingredient: 90.1% Ethyl lauroyl arginate HCl
 Test substance: 0.5 g LAE, moistened with 0.5 ml sterile water
 Batch: 2625
 Dose: 0.07 mg Ethyl lauroyl arginate HCl/cm²
 GLP: in compliance

A paste, (0.5 g LAE with 0.5 ml water), was applied evenly to 6.25 cm² gauze square. This was applied to the dorsum of the rabbit. Semi-occlusive patches were applied and left in place for 4 hours. The test site was cleaned by gently swabbing with cotton wool. The skin was examined for erythema, eschar formation and oedema at 1, 24, 48 and 72 hours, 7 and 14 days after removal of the patches.

Results

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

3.3.1.2. Mucous membrane irritation

Taken from SCCP/0837/04

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

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

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

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

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

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

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

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

Ref.: 8

Taken from SCCP/0837/04

Mirenat-N, study 1

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

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

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

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

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

Ref.: 9

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

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

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

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

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

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

Ref.: 10

Comment

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

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

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

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Batch: Mirenat 3128
Purity: 21.2% LAE
Dose: 0.1 ml equivalent to 20 µg Ethyl lauroyl arginate HCl
GLP: in compliance

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

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

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

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

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

| | |
|-----------------|------|
| Corneal opacity | 0.11 |
| Iridial lesions | 0.00 |
| Hyperaemia | 0.22 |
| Oedema | 0.11 |

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

Ref.: 11

Taken from SCCP/0837/04

Aminat, study 2

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

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

Opinion on ethyl lauroyl arginate HCl

| | |
|-----------------|------|
| Corneal opacity | 0.00 |
| Iridial lesions | 0.00 |
| Hyperaemia | 0.22 |
| Oedema | 0.11 |

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

Ref.: 12

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

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

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

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

| | |
|-----------------|------|
| Corneal opacity | 0.11 |
| Iridial lesions | 0.00 |
| Hyperaemia | 0.56 |
| Oedema | 0.11 |

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

Ref.: 13

Taken from SCCP/0837/04**Aminat, study 4**

| | |
|-----------------|---------------------------|
| Guideline: | OECD 405 (1987) |
| Species/strain: | New Zealand albino rabbit |
| Group size: | 3 male |

Opinion on ethyl lauroyl arginate HCl

Active ingredient: 0.4% Ethyl lauroyl arginate HCl
 Test substance: Aminat 2.0%: [Mirenat-N in deionised water]
 Batch: Mirenat 3128
 Purity: 21.2% LAE
 Dose: 0.1 ml equivalent to 400 µg Ethyl lauroyl arginate HCl
 GLP: in compliance

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

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

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

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

| | |
|-----------------|------|
| Corneal opacity | 0.00 |
| Iridial lesions | 0.00 |
| Hyperaemia | 1.00 |
| Oedema | 0.56 |

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

Ref.: 14

From submission II

Guideline: OECD 405 (2002)
 Species/strain: New Zealand albino rabbit
 Group size: 3 males
 Active ingredient: 0.8% Ethyl lauroyl arginate HCl
 Test substance: Aminat 4% in water
 Batch: JMR-684
 Purity: /
 Dose: 0.1 ml
 GLP: in compliance

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

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

Opinion on ethyl lauroyl arginate HCl

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

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

| | |
|-----------------|------|
| Corneal opacity | 0.11 |
| Iridial lesions | 0 |
| Hyperaemia | 1.22 |
| Oedema | 0.33 |

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

Ref.: 41

Comment: The certificate of analysis showed that Aminat 4% was prepared from Aminat, batch JMR-672. This was described as 20% LAE, Ethyl-N^α-dodecanoyl-L-arginate HCl. This has been confirmed as 20% LAE in propylene glycol, diluted to 0.8% in water.

3.3.2. Gingival irritation – in vitro reconstructed human gingival epithelium (3D)

Two *in vitro* studies (testing toothpaste and mouthwash) have been performed using reconstructed human gingival epithelium. According to the manufacturer, the reconstructed human gingival epithelium used shows a comparable profile of biomarkers as in the *in vivo* situation such as the expression of filaggrin in the granular cell layers, involucrin, keratin 6, keratin 10, keratin 13 and keratin 16 in the supra basal cell layers and Ki67 (proliferation marker) in the basal cells.

LAE[®] containing mouthwash

| | |
|-------------------------|--|
| Guideline: | In-house <i>in vitro</i> evaluation of the irritation potential on reconstructed human gingival epithelium (3D). |
| Date of test: | 20-28 January 2010 |
| Experimental system: | Skinethic (Niza) reconstructed human gingival epithelium |
| Test size: | 3 replicates per test substance and concentration |
| Test substance: | LAE [®] (Ethyl-N ^α -lauroyl-L-arginate HCl) |
| Batch: | LV090081 |
| Purity: | 86.6% |
| Test formulation: | Aminat-G, 20% LV090081 in glycerin |
| Test formulations used: | 1) LAE [®] at 0.20% in deionized water 2) LAE [®] at 0.75% in deionized water 3) Mouthwash containing 0.20% LAE [®] 4) 'Gingilacer mouthwash' 200 ml |
| Positive control: | 5% SDS (Sodium Dodecyl Sulfate) |
| Negative control: | PBS (Phosphate Buffer Saline) |
| GLP/QAU: | not GLP compliant |

60 µl/cm² of each test substance (solutions, formulations, negative and positive controls) was applied on three tissue replicates for 10 minutes at room temperature (RT, between 18°C to 24°C). One section of the report states that no rinsing occurred, whereas another section mentions rinsing with phosphate buffer saline (PBS) and mechanical drying after exposure.

Cell viability was assessed by incubating three replicates of the four treated tissues for 3 hours with MTT solution (300µl for 24 wells plate). The precipitated formazan was then extracted using Isopropanol (IPA) (1500 µl per well) and incubated at room temperature

Opinion on ethyl lauroyl arginate HCl

during 2 hours by shaking. Each solution was transferred to a 96 well microplate and quantified spectrophotometrically at 570 nm. IPA solution was used as a blank.

For each treated tissue, the cell viability is expressed as the percentage of the mean negative control tissues. The OD is corrected by extracting the value obtained from the incubation before IPA extraction (non-specific OD, due to the residual chemical staining).

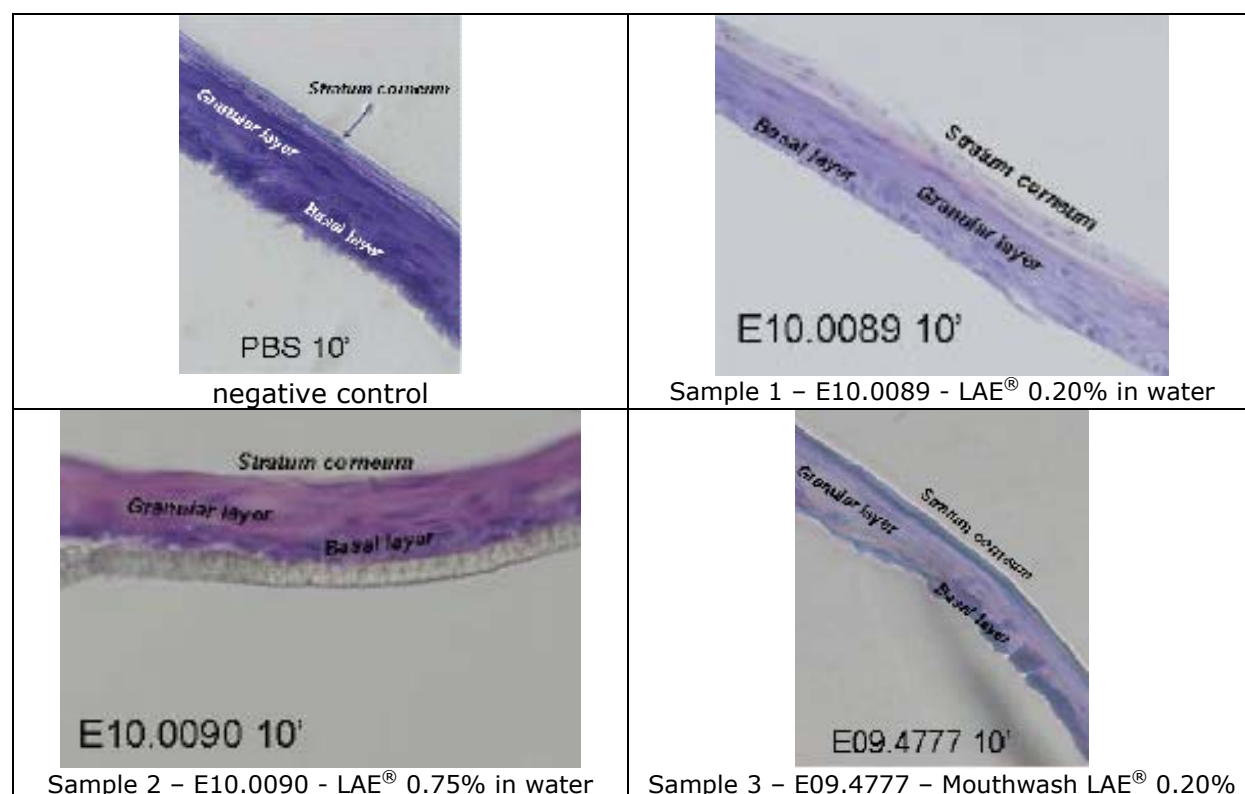
Sodium Dodecyl Sulphate (SDS 5%) and PBS treated epidermis are used as positive and negative controls, respectively.

Results

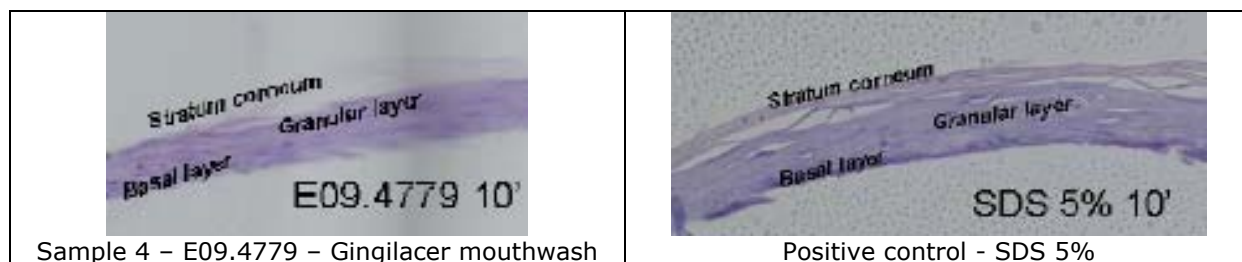
The following table compares the results of cell viability after treatment of reconstructed human gingival epithelium and histological analysis of tissues.

| Sample | Cellular viability Contact time: 10 minutes | | Histological analysis of the tissue | |
|---|--|------------------------|--|---|
| | % Cellular viability (average) | Standard deviation (%) | Tabulated in study report | As characterised in Annex VI of the study |
| Sample 1 – E10.0089 - LAE [®] 0.20% in deionized water | 105 | 8.7 | Absence of significant cellular alterations | Infiltration of nuclei in <i>stratum corneum</i> - slight damage |
| Sample 2 – E10.0090 - LAE [®] 0.75% in deionized water | 91 | 9.9 | Absence of significant cellular alterations | Partial loss of <i>stratum corneum</i> ; necrosis in granular layer – moderate damage |
| Sample 3 – E09.4777 - Mouthwash with LAE [®] 0.20% | 82 | 9.6 | Absence of significant cellular alterations | Similar to negative control |
| Sample 4 – E09.4779 - Gingilacer mouthwash | 110 | 29.9 | Absence of significant cellular alterations | Alterations in <i>stratum corneum</i> – slight damage |
| Positive control - SDS 5% | 64 | 6.3 | Presence of severe cellular alterations and necrotic cells | Severe cellular alterations and necrotic cells |

It was not possible to corroborate the differences in the interpretation between the study authors and the histologists due to the low magnification of histological images provided. .



Opinion on ethyl lauroyl arginate HCl



Conclusion

The performing laboratory considers the positive control (SDS) a moderate irritant at the tested concentration and contact time, as it reduced the cell viability and the treated tissue showed severe cellular alterations in the different layers. The negative control showed no epithelial cellular alteration.

Comparing the results from the test samples (sample 1 to sample 4) the study authors considered that no significant differences were detected. Sample 4, Gingilacer mouthwash, was used as a reference product that is considered safe for use, based on years of in-market experience.

The MTT result for mouthwash containing 0.20% LAE (Sample 3), was marginally lower than the Gingilacer mouthwash (Sample 4), but the authors do not consider the small degree of change in clear absence of any histological abnormality to be biologically meaningful.

The performing laboratory concludes that, on the basis of the obtained results, the analyzed samples can be considered as non irritant for the gingival epithelium under the assayed test conditions.

Comments

The mouthwash formulations used with and without LAE[®] had different compositions. While the study summary states the absence of significant cellular alterations, the detailed histological assessment concluded slight to moderate tissue damage for samples 1, 2, and 4. There appears to be no correlation between the MTT results and the results of the histological analysis of these tissues.

Ref.:46

LAE[®] containing toothpaste

| | |
|-------------------------|--|
| Guideline: | In-house <i>in vitro</i> evaluation of the irritation potential on reconstructed human gingival epithelium (3D). |
| Date of test: | 20-28 January 2010 |
| Experimental system: | Skinethic (Niza) reconstructed human gingival epithelium |
| Test size: | 3 replicates per test substance and concentration |
| Test substance: | LAE [®] (Ethyl-N ^α -lauroyl-L-arginate HCl) |
| Batch: | LV090081 |
| Purity: | 86.6% |
| Formulation: | Aminat-G, 20% LV090081 in glycerin |
| Test formulations used: | 1) LAE [®] at 0.75% in deionized water 2) Toothpaste containing 0.75% LAE [®] 3) 'Gingilacer toothpaste' |
| Positive control: | 5% SDS (Sodium Dodecyl Sulfate) |
| Negative control: | PBS (Phosphate Buffer Saline) |
| GLP/QAU: | not GLP compliant |

60 µl/cm² of each test substance (solutions, formulations, negative and positive controls) was applied on three tissue replicates for 20 minutes at room temperature (RT, between

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18°C to 24°C), after which the epithelia are rinsed with 25 ml phosphate buffer saline (PBS).

Cell viability was assessed by incubating two or the three treated tissues for 3 hours with MTT solution (300µl for 24 wells plate). The precipitated formazan was then extracted using Isopropanol (IPA) (1500 µl per well) and incubated at room temperature during 2 hours by shaking. Each solution was transferred to a 96 well microplate and quantified spectrophotometrically at 570 nm. IPA solution was used as a blank.

For each treated tissue, the cell viability is expressed as the percentage of the mean negative control tissues. The OD is corrected by extracting the value obtained from the incubation incubated before IPA extraction (non-specific OD, due to the residual chemical staining).

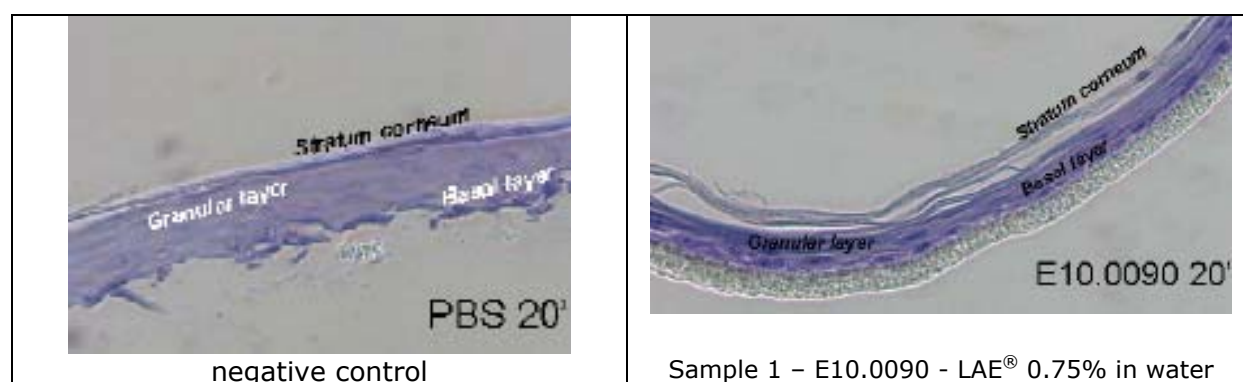
Sodium Dodecyl Sulphate (SDS 5%) and PBS treated epidermis are used as positive and negative controls, respectively.

Results

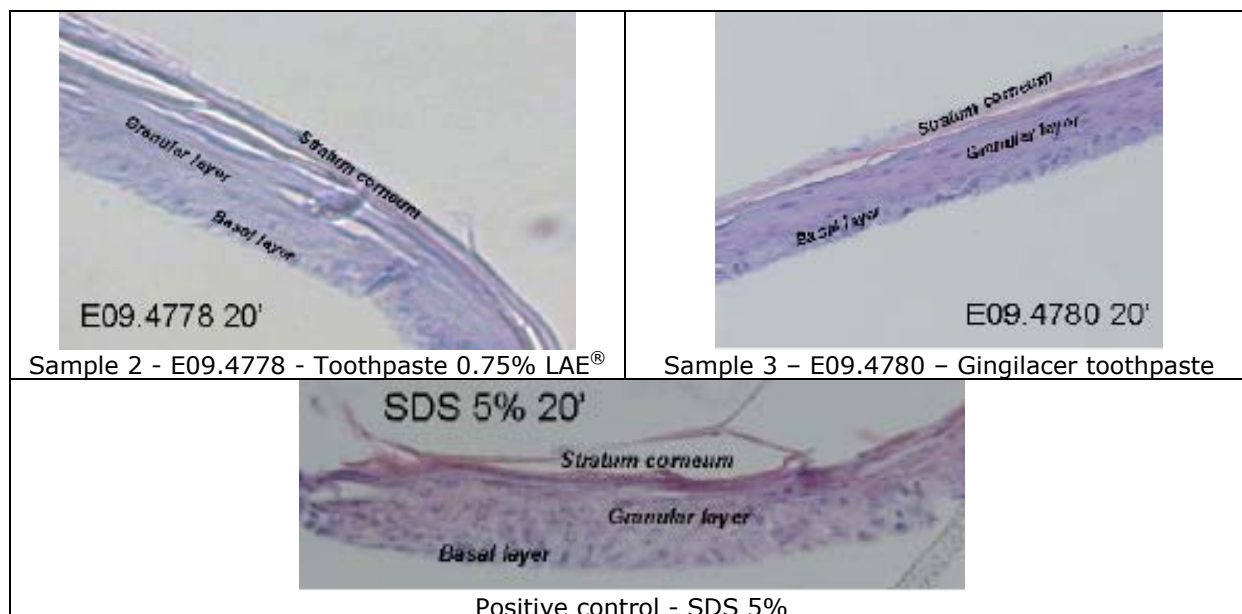
The following table compares the results of cell viability after treatment of reconstructed human gingival epithelium and histological analysis of tissues.

| Sample | Cellular viability Contact time: 20 minutes | | Histological analysis of the tissue | |
|---|--|------------------------|---|---|
| | % Cellular viability (average) | Standard deviation (%) | Tabulated in study report | As characterised in Annex VI of the study |
| Sample 1 – E10.0090 - LAE® 0.75% in deionized water | 88 | 2.4 | Absence of significant cellular alterations – slight damage | Partial loss of <i>stratum corneum</i> – moderate damage |
| Sample 2 – E09.4778 – Toothpaste with LAE® 0.75% | 92 | 8.9 | Absence of significant cellular alterations | Alterations in <i>stratum corneum</i> – slight damage |
| Sample 3 – E09.4780 – Gingilacer toothpaste | 81 | 10.4 | Absence of significant cellular alterations | Alterations in <i>stratum corneum</i> and infiltration of nuclei <i>into it</i> – slight damage |
| Positive control - SDS 5% | 49.8 | 1.8 | Presence of severe cellular alterations and necrotic cells | Presence of severe cellular alterations and necrotic cells |

It was not possible to corroborate the differences in the interpretation between the study authors and the histologists due to the low magnification of histological images provided.



Opinion on ethyl lauroyl arginate HCl



Conclusion

The performing laboratory considers the positive control (SDS) a moderate irritant at the tested concentration and contact time, as it reduced the cell viability and as the treated tissue showed severe cellular alterations in the different epithelial layers. The negative control showed no epithelial cellular alteration.

Comparing the results from the test samples (sample 1 to sample 3), the study authors concluded that no significant differences were detected between them.

The performing laboratory concludes that, on the basis of the obtained results, the analyzed samples can be considered as non irritant for the gingival epithelium under the assayed test conditions.

Ref.:47

Comment

The toothpaste formulations used had different compositions. While the study summary states the absence of significant cellular alterations, the detailed histological assessment concluded slight to moderate tissue damage for samples 1-3. There appears to be no correlation between the MTT results and the results of the histological analysis of these tissues.

General comments on gingival irritation studies

In these studies, a commercially available reconstructed human gingival epithelium has been used, a derivative of the SkinEthic 3-D reconstituted human epidermis (RHE). Several reconstituted human epithelium systems are validated for the assessment for skin irritation and corrosion (Testing guidelines (EC B.46/OECD 439) for *in vitro* skin irritation: reconstructed human epidermis (RhE)). In the guideline for the reconstructed human epidermis (RhE) test, the inability to detect mild irritants is given as a limitation of the method. The test has been designed for the purpose of distinguishing between skin irritating and non-irritating substances in relation to classification of chemical substances. The SCCS, in its memorandum on Episkin³, stated that it must be noted that for cosmetic ingredients, in order to assess the risk in terms of skin contact, exposure time, frequency of use, etc., it

³ Memorandum (addendum) on the *in vitro* test episkin™ for skin irritation testing (SCCS/1392/10), 14 December 2010

Opinion on ethyl lauroyl arginate HCl

is also important to obtain information on possible irritative properties below this initial threshold for classification.

This is supported by a recent publication, Wurzbürger et al (2011, ref. 48), who suggested that 3-D human oral reconstructed tissues may provide useful predictive information for screening oral care formulations for potential irritancy prior to human testing.

Gingival irritation is not included in guideline EC B.46/OECD 439. The study reports do not substantiate the sensitivity of the test system and the potential of the reconstructed gingival tissue assay to detect *in vivo* mucous membrane irritants.

Interpretation of the results of the two studies was also hampered since the study reports included contradictory information on the study design and interpretation of the results.

- LAE® is described as ethyl-N^α-lauroyl-L-arginate HCl. This differs from the information in the earlier submissions where the ethyl lauroyl arginate HCl concentration ranged from 85-95% in LAE
- Interpretation of the histological results differed between the study report and the Annex VI: histological assessment.
- There appeared to be slight or no correlation between the MTT results and the histological analysis of the tissues.

Moreover, essential information was missing. In particular the following points were noted by the SCCS:

- the positive control (5% SDS) remained above or only just reached the cut-off value of 50% cell viability set in the RhE test for skin irritation. This was not discussed in the assessment of the study.
- Test substances are applied at 60 µl/cm², whereas the guideline advises 25 µl/cm². No justification was given
- Contact times and incubation periods are short in comparison with the guideline and no rationale is given.
- No information on the assessment of the barrier function of the epithelium was given
- According to the guideline, the optical density (OD) of the extracted (solubilised) dye from the tissue treated with the negative control (NC) should be at least 20 fold greater than the OD of the extraction solvent alone. Such quantitative checks are not included in the presented reports.
- The reports mention that the test facility is GLP certified by the competent authority (Generalitat de Catalunya) for "*in vitro* toxicological studies" (Certificate BPLI/0912/015/CAT), but that the sponsor did not request these particular studies to be conducted in compliance with GLP.
- The information provided on the benchmark product is limited

Therefore the results of these studies cannot be taken into account for the safety assessment of Ethyl-N^α-lauroyl-L-arginate HCl for the use in oral hygiene products.

3.3.3. Dermal / percutaneous absorption

From SCCP/0837/04 and reassessed in SCCP/1106/07

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

Opinion on ethyl lauroyl arginate HCl

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 $\text{g}/\text{m}^2\cdot\text{h}$ were replaced.

Application

The test solution (9 μl) was applied by micro-pipette to the entire epidermal surface delimited by the upper cell (1.86 cm^2 of exposed area; 4.8 μl formulation/ cm^2 of skin).

The solutions were in contact with the skin for 24 h. At the end of the contact period, the receptor fluid was recovered into a 5 ml volumetric flask.

Then, both the skin bottom and the lower section of the diffusion cell were washed with distilled water, which was added to the receptor fluid taken to a final volume of 5 ml.

The test solution remaining on the skin surface treated was washed off with water. Water aliquots, all tips, all cotton swabs as well as the top of the cell were collected together constituting the fraction of the active compound remaining in the surface.

Skin Stripping

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

Dose levels

In the 0.39% Ethyl lauroyl arginate HCl solution, this is 4.8 mg solution/ cm^2 and 18.7 $\mu\text{g}/\text{cm}^2$ of active substance.

In the 1.96% Ethyl lauroyl arginate HCl solution, it means 4.9 mg solution/ cm^2 and 96.5 $\mu\text{g}/\text{cm}^2$ of active substance.

Recovery of test substance

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

Under the experimental conditions of this study, the percutaneous absorption of 1.96% Ethyl lauroyl arginate HCl in propylene glycol/water 30/70 after an exposure time of 24 h may be considered to be $5.24 \pm 2.29 \mu\text{g}/\text{cm}^2$. The quantities of active ingredient found in all the compartments analysed were as follows:

| <u>Compartments</u> | <u>$\mu\text{g}/\text{cm}^2$</u> |
|-----------------------|---|
| Surface | 56.09 \pm 10.79 |
| Stratum Corneum | 28.80 \pm 9.04 |
| Epidermis | 3.78 \pm 1.84 |
| Dermis | 1.46 \pm 1.65 |
| Receptor Fluid | not detected |
| <u>Total Recovery</u> | <u>90.13 \pm 7.21</u> |
| Total Absorbed | 5.24 \pm 2.29 |

Ref.: 4

Comment

The 1.96% Ethyl lauroyl arginate HCl solution is 5 times higher than the maximum dose for the proposed use as preservative in cosmetics. Back extrapolation of the percutaneous

Opinion on ethyl lauroyl arginate HCl

absorption from 1.96% Ethyl lauroyl arginate HCl to 0.4% Ethyl lauroyl arginate HCl was not considered to be appropriate.

New Dermal absorption study

| | |
|-------------------------|--|
| Guideline: | OECD 428 |
| Species/strain: | human, female abdomen skin discs, frozen dermatomed (~500 µm) |
| Test Substance: | [Arginine-U- ¹⁴ C]LAE·HCl |
| Active ingredient: | 90.3% Ethyl lauroyl arginate HCl |
| Batch: | 3766CJW008-10 |
| Purity: | 99% Ethyl lauroyl arginate HCl |
| Radiochemical purity | 98% |
| Specific activity | 540 MBq/mmol (1.28 MBq/mg) |
| Total activity | 46.6 Mbq |
| Concentrations applied: | 0.4% (4035 mg/L) and 0.8% (7992 mg/L) |
| Application volume | 6.4 µL |
| Donors | 10 skin discs per application (4 donors 0.4%, 5 donors 0.8%) |
| Exposure time | 24 h |
| GLP: | in compliance |
| Date | Nov 2010 |

Skin discs from 5 donors were used, with 3 donors in common to both applied concentrations. The integrity of each skin disc was checked by determination of the permeation of tritiated water and was within the acceptability criteria. Over the duration of the experiment (24 h), the stability of the test solution [Arginine-U-¹⁴C]-LAE·HCl at both concentrations (0.4% and 0.8%) and in the receptor fluid was >99%. The receptor fluid consisted of saline supplemented with 0.5% BSA.

The active ingredient applied to the skin was 25.3 (0.4%) and 50.1 µg (0.8%). Radioactivity was 5.1 MBq/mL for 0.4% solution of and 10.2 MBq/mL for the 0.8% test solution. The donor compartment was non-occluded during the exposure period.

The results are summarized in Table 5.

Conclusion

The percentages absorbed were lower for [Arginine-U-¹⁴C]-LAE·HCl at the 0.8% concentration compared with the 0.4% concentration. The average percentage absorbed was $0.82 \pm 0.78\%$ for the 0.8% test solution and $2.1 \pm 0.9\%$ for the 0.4% test solution

Table 5: In vitro dermal absorption of [Arginine-U-¹⁴C]-LAE·HCl in human skin (averaged from 10 skin discs)

| Concentrations applied | 0.4% (4035 mg/L) | 0.8% (7992 mg/L) |
|-------------------------------------|-------------------------|-------------------------|
| exposure time | 24 h | 24 h |
| Dermal absorption parameters | | |
| Lag time (h) | 0-2 | - |
| Flux (µg/cm ² /h) | 0.002 ± 0.002 | 0.0012 ± 0.0006 |
| Recovery data (%) | | |
| Receptor fluid fractions | 0.089 ± 0.073 | 0.027 ± 0.010 |
| Receptor chamber | 0.005 ± 0.005 | 0.005 ± 0.003 |
| Dermis | 0.13 ± 0.09 | 0.30 ± 0.60 |
| Epidermis | 1.8 ± 0.83 | 0.49 ± 0.46 |
| Absorbed¹ | 2.1 ± 0.9 | 0.82 ± 0.78 |
| Stratum corneum (tape strips 1-8) | 34.7 ± 9.4 | 20.2 ± 5.2 |
| Donor chamber | 58.4 ± 9.3 | 74.3 ± 4.3 |
| Non-absorbed² | 93.1 ± 2.2 | 94.5 ± 4.2 |
| Total recovery | 95.2 ± 2.6 | 95.3 ± 3.9 |

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- 1) Absorbed = fractions + receptor chamber + epidermis + dermis
- 2) Non-absorbed = Tape strips 1-8 from the stratum corneum + donor chamber

Ref.: 49

Comment

According to the SCCS Notes of Guidance⁴ 3.0% (mean absorption of 2.1% +1 SD, 0.9) is used for the calculation of the MOS as a preservative at 0.4% and 2.38% (mean absorption of 0.82% +2 SD, 2 x 0.78, due to high variability) for use of active ingredient at 0.8%.

3.3.4. Human data**Clinical and Antibacterial Effect of Toothpastes**

The applicant submitted information on three clinical studies with toothpaste and mouth rinse containing ethyl lauroyl arginine (Ref. 50, 51, 52). The study reports were covered by confidentiality clauses. Published abstracts were available only for a toothpaste study and a mouth rinse study. In these studies, small groups of subjects (9-16) were selected based on rigorous inclusion and exclusion criteria (except ref. 51, which, however, did not report assessment of possible adverse effects), suggesting overall excellent oral hygiene and health of the participants. They were exposed to the test product for periods between 4 and 10 days. These studies were designed to assess the efficacy of antimicrobial effect of ethyl lauroyl arginine in formulations in comparison with similar marketed formulations. The focus was to evaluate plaque control. Scant information on the test formulations was provided even in the study reports. Effects on gingival tissue after treatment were not provided.

General comment

The clinical studies indicated good plaque control in small sample numbers of individuals with good oral hygiene and health over a short time frame (4–10 days). However, these studies are short term in contrast to long term normal consumer usage. These studies do not provide reassurance that no oral mucosal irritation occurs, especially if it is already compromised.

⁴ The SCCS's Notes of Guidance for the Testing of Cosmetic Ingredients and their Safety Evaluation, 7th Revision, 14 December 2010. http://ec.europa.eu/health/scientific_committees/consumer_safety/docs/sccs_s_004.pdf

| | | |
|---|--------------------|---------------|
| Margin of Safety – preservative and a.i. use | NOAEL / SED | = 8500 |
|---|--------------------|---------------|

3.4. Discussion

From the previous submission, the SCCP concluded that ethyl lauroyl arginate HCl was safe for the consumers when used:

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

The Margin of Safety indicates that ethyl lauroyl arginate HCl has low toxicity. However, the use on lips and in oral hygiene and spray products was of concern due to local irritation effects. These were seen in the acute eye irritation and inhalation studies and as mucosal irritation in the non-glandular region of the stomach in the sub-chronic rat study. Respiratory effects in the rat embryo-foetal gavage study were also considered as a local irritation effect by the study authors. This concern was reinforced by comments included with two studies by the study authors. Both suggested that ethyl lauroyl arginate HCl was not toxic but had a potential for mucosal irritation.

The EFSA AFC panel (additives, flavourings, processing aids and materials in contact with food) in its opinion on ethyl lauroyl arginate as a food additive also considered the non-glandular forestomach lesions were not indicative of systemic toxicity, but local irritation accompanied by subepithelial/submucosal inflammation.

In support of their application for the use of ethyl lauroyl arginate HCl in oral hygiene products, the applicant provided an extensive reappraisal of the data by independent experts. They also asserted that there was a distinction between sensory irritation and systemic toxicity. As well, they indicated that there are no standardized guidelines for tests that examine for existing lesions such as gingivitis. Their conclusion was 'There is no toxicologically-based criterion to prevent use in mouthwash and other oral hygiene products at 0.8% of LAE'. This was based on the lack of gingivitis noted in the chronic feeding trials.

The SCCS agrees that there is a distinction between sensory irritation and systemic toxicity and acknowledges that there are no standardized guidelines for tests that examine for existing lesions such as gingivitis. However, there is a distinction between consumption of food and the longer effect of substances in oral hygiene products in the buccal cavity. Retention in the biofilm for other ingredients of oral hygiene products is up to 4-5 hours. Thus, there is a potential for increased irritation particularly if there is poor oral hygiene. There has to be consideration of the balance between reducing dental plaque and the effects of local irritation on the oral and gingival mucosa. Persistent irritation could lead to local inflammation and thus might be of concern despite the benefits of plaque reduction.

The new *in vitro* studies evaluated the irritation potential on reconstructed human gingival epithelium. However, this extrapolation of the Skinethic RHE method for skin irritation has not been formally validated and no proof was provided that this assay is suitable to assess the potential of chemical substances for mucous membrane irritation. Wurzbürger (2011) commented that reconstructed human gingival epithelium might be a useful screening test prior to human studies. The RhE assay and similar tests are not designed to detect mild irritants. A single application on the reconstituted skin is not comparable to long term repeated use of oral care products. Therefore the SCCS cannot draw any conclusion from these tests relevant for the safety assessment of ethyl lauroyl arginate HCl for the use in oral hygiene products.

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No adequate studies to investigate the effect of ethyl lauroyl arginate HCl (or its metabolites possibly formed during the retention period in the biofilm) on oral mucosa have been provided. The new human studies, designed to assess efficacy of plaque control, showed that ethyl lauroyl arginate HCl reduced plaque significantly. However, a limitation of the studies was the rigorous inclusion and exclusion criteria of participants, resulting in selection of only those with excellent oral health. In addition, the group sizes were small, the time frames (4–10 days) were short and inadequate information on potentially negative effects, especially on the gingiva, was provided.

These short term studies do not mirror long term consumer usage, twice daily brushing with toothpaste and possibly also similar daily usage with a mouthwash. In addition, the oral hygiene of a high percentage (>50%) of consumers would be considered poor in comparison with those having been selected to take part in these studies. Therefore, these studies do not provide reassurance that no local oral mucosal irritation, in particular of the gingiva, occurs, especially if it is already compromised. This could be resolved by showing that there is no local irritation of the oral mucosa and gingiva in longer term studies.

4. CONCLUSION

In the light of the data provided, does the SCCS consider that ethyl lauroyl arginate HCl is safe for the consumers, when used up to a maximum concentration of 0.75 % in toothpaste and 0.2% in mouthwash products in addition to the currently recommended uses as mentioned above?

The SCCS considers the additional data provided on mucosal irritation does not alter its earlier opinion on ethyl lauroyl arginate HCl. The concern that in the general population, regular use of toothpaste and possible additional use of a mouthwash containing ethyl lauroyl arginate HCl could cause local mucosal irritation, was not addressed by the submitted studies.

Taking into account the dermal absorption data submitted, the SCCS is requested to revise the exposure assessment for ethyl lauroyl arginate HCl in cosmetics made in opinion SCCP/1106/07.

The SCCS has revised the Margin of Safety calculation for ethyl lauroyl arginate HCl in cosmetics based on a new dermal absorption study and maintains its conclusion that there are no systemic safety concerns at the currently authorised use concentrations.

Does the SCCS have any other scientific concerns of use for ethyl lauroyl arginate HCl in cosmetic products based on the toxicological profile and foreseeable exposure?

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5. MINORITY OPINION

Not applicable

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