SCCS/1519/13
Revision of 12 December 2013

**Scientific Committee on Consumer Safety**

**SCCS**

# OPINION ON

# Ethyl lauroyl arginate HCl - submission IV

# COLIPA n° P95

The SCCS adopted this opinion at its 3rd Plenary meeting
on 19 September 2013

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Three independent non-food Scientific Committees provide the Commission with the scientific advice it needs when preparing policy and proposals relating to consumer safety, public health and the environment. The Committees also draw the Commission's attention to the new or emerging problems which may pose an actual or potential threat.
They are: the Scientific Committee on Consumer Safety (SCCS), the Scientific Committee on Health and Environmental Risks (SCHER) and the Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) and are made up of external experts.

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

SCCS

The Committee shall provide opinions on questions concerning all types of health and safety risks (notably chemical, biological, mechanical and other physical risks) of non-food consumer products (for example: cosmetic products and their ingredients, toys, textiles, clothing, personal care and household products such as detergents, etc.) and services (for example: tattooing, artificial sun tanning, etc.).

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http://ec.europa.eu/health/scientific_committees/consumer_safety/index_en.htm

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This opinion has been subject to a commenting period of four weeks after its initial publication. Comments received during this time have been considered by the SCCS and discussed in the subsequent plenary meeting. Where appropriate, the text of the relevant sections of the opinion has been modified or explanations have been added. In the cases where the SCCS after consideration and discussion of the comments, has decided to maintain its initial views, the opinion (or the section concerned) has remained unchanged. Revised opinions carry the date of revision.

Keywords: SCCS, scientific opinion, preservative, P95, Ethyl lauroyl arginate HCl, directive 76/768/EEC, Regulation 1223/2009, CAS: 60372-77-2 ,EC: 434-630-6

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## 1. BACKGROUND

Submission I for Ethyl Lauroyl Arginate HCl CAS n. 60372-77-2, was submitted in February 2003 by COLIPA[1].

Submission II and II-bis for Ethyl Lauroyl Arginate HCl was submitted respectively in April and October 2006.

Submission III contained a dossier, submitted in December 2008, and additional data submitted in 2010. The aim of the applicant was to demonstrate the safety use of Ethyl Lauroyl Arginate HCl (ELA) in oral hygiene products.

The Scientific Committee on Consumer Safety (SCCS) adopted at 11th plenary meeting of 21 June 2011 the opinion (SCCS/1415/11) with the following conclusion:
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.

Submission IV for Ethyl Lauroyl Arginate HCl was submitted in September 2012.
This submission is intended to demonstrate the safety of the ingredient ELA for use as preservative in oral cosmetic products at a level up to 0.15%

## 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 as preservative up to a maximum concentration of 0.15 % in oral cosmetic products?*

2. *Does the SCCS have any further scientific concerns with regard to the use of Ethyl lauroyl arginate HCl in cosmetic products?*

[1] Cosmetics Europe, ex- 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$^{\alpha}$-dodecanoyl-L-arginate hydrochloride (IUPAC)
Monohydrochloride of L-arginine, N$^{a}$-lauroyl-ethylester

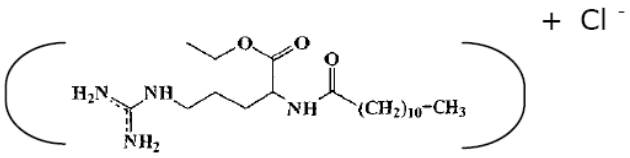
#### 3.1.1.3. Trade names and abbreviations

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: $C_{20}H_{41}N_4O_3Cl$

### 3.1.2. Physical form

White solid

### 3.1.3. Molecular weight

421.02 g/mol

### 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).

Table 1: Specifications from submission II

| **Product** | **Ethyl lauroyl arginate HCl Content** | **Physical form** | **Comments** |
|---|---|---|---|
| Crude Technical product | 74-84% | White solid. $H_2O$ Content: 14-22% | Obtained at the end of the synthesis of Ethyl-$N^{\alpha}$-dodecanoyl-L-arginate HCl |
| LAE (Dehydrated commercial product) | 85-95% | White solid. $H_2O$ 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 $N^{\alpha}$-lauroyl-L-arginine, lauric acid and ethyl laurate. It should be noted that Batch 5159 had a higher water content. It was stated in the submission that it was used in some of the older tests. However, it was only used in the embryo-foetal toxicity studies between 1998 and 1999. The batches used in the studies provided in submission II are included.

Table 2: Ethyl lauroyl arginate HCl content and accompanying contaminants of the batches used in the provided studies

| **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 | |
| $N^{\alpha}$-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 in the studies are in Table 3.

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

| **Batch** | **0000001 4-12-95** | **0000003** | **12 June 1995** | **13 Dec 1995** | **3128** |
|---|---|---|---|---|---|
| | **% (w/w)** | **% (w/w)** | **% (w/w)** | **% (w/w)** | **% (w/w)** |
| Ethyl lauroyl arginate HCl | 20.2 | 20.3 | 20.4 | 20.4 | 20.0 |
| $N^{\alpha}$-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 different submissions 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).

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-$N^{\alpha}$-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.

In the current submission (submission IV), the mouthwashes (0.10% and 0.15% Ethyl lauroyl arginate HCl) have been prepared from Aminat-G (batch number LV110050, 20.0 ± 0.5 % Ethyl lauroyl arginate HCl). The raw LAE employed to produce this batch was dehydrated technical LAE (85 - 95% Ethyl lauroyl arginate HCl, batch LT110001).

***SCCS Comment***
The SCCS notes that the nomenclature used is confusing and inconsistencies exist between the different submissions (see Annex 2 to this opinion).

### 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.

### 3.1.6. Solubility

In water, the solubility is greater than 247 g/l at 20°C. Information provided to JECFA (2008, Ref. 1) and FSANZ (2009, Ref. 2) 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 $P_{ow}$)

Log $P_{ow}$: 1.43 at 20 °C

### 3.1.8. Additional physical and chemical specifications

In previous submissions, no specific characteristics were given for Ethyl lauroyl arginate HCl, only for LAE.

| | |
|---|---|
| Melting point: | 50.5 to 58.0 °C |
| Boiling point: | decomposition from 107 °C |
| Flash point: | / |
| Vapour pressure: | 5.45 x $10^{-4}$ Pa at 25 °C |
| Density: | 1.11 |
| Viscosity: | / |
| pKa: | / |
| Refractive index: | / |
| pH: | / |
| Stability: | not specified but assumed to be 6 months at 4°C in the dark by study authors |

**Ethyl lauroyl arginate - additional physicochemical data**

In the Ethyl lauroyl arginate Chemical and Technical Assessment (JECFA 2008, Ref. 1), 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 in the range of 3.64 to 4.25 in 4 batches.

Ref: 1

In the current submission (submission IV), additional specifications for Aminat-G are provided by the applicant:

Density: 1.22±0.02 g/cm³ (at 20°C)
Viscosity: 4000-6500 cP (at 20°C)

Specifications for the mouthwashes containing 0.10% and 0.15% Ethyl lauroyl arginate HCl and for placebo mouthwash used in the clinical study of submission IV are listed in table 5.

Table 5: Specifications of the mouthwashes used in the clinical study for long term acceptability of Ethyl lauroyl arginate HCl.

| | Ref. | batch | concentration LAE (% m/m) | pH | density (g/ml at 20°C) |
|---|---|---|---|---|---|
| PLACEBO | 001 | 002 | - | 5.10 | 1.0163 |
| | 001 | 003 | - | 5.16 | 1.015 |
| | 001 | 004 | - | 5.11 | 1.016 |
| AMINAT 0.10% | 001 | 002 | 0.10 | 5.06 | 1.0174 |
| | 001 | 003 | 0.10 | 5.15 | 1.016 |
| | 001 | 004 | 0.10 | 5.13 | 1.017 |
| AMINAT 0.15% | 002 | 002 | 0.156 | 5.08 | 1.0180 |
| | 002 | 003 | 0.156 | 5.13 | 0.017 |
| | 002 | 004 | 0.152 | 5.16 | 1.017 |

***SCCS Comment***
Aminat 0.15%, Ref. 002, batch 003 has a strongly deviating density (0.017 g/ml at 20°C)(see annex 2 to this opinion).

### 3.1.9. Homogeneity and Stability

Ethyl-N$^{\alpha}$-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, maleic 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. 1

***SCCS Comment***
In submission IV, no information on the stability of the mouthwashes is provided. All mouthwashes contain 0.9-0.11% (m/m) nipagin (methylparaben) and 0.054-0.066% (m/m) nipasol (propylparaben).

**General Comments to physico-chemical characterisation**
In the previous 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.
From submission I and II, it appears 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. In submission IV, Aminat®-G was 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 meant 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 cosmetic 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 Directive, with the exclusion of use in lip products, oral cosmetic products and spray products.
The current submission intends to use 0.15% Ethyl lauroyl arginate HCl in mouthwash and toothpaste.

EFSA (2007, Ref. 3) 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 foodgrade 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.

***SCCS Comment***
The purpose of using Ethyl lauroyl arginate HCl in oral cosmetic products seems to be antimicrobial rather than as a preservative. Indicative is the use of parabens in the tested mouthwashes.

## 3.3 SUMMARY OF SAFETY DATA ON ETHYL LAUROYL ARGINATE HCL AVAILABLE IN SCCNFP/0837/04, SCCP/1106/07 and SCCS/1415/11 (Ref. 4, 5 and 6)

*Acute toxicity*

In an **acute oral** study on LAE, the acute lethal dose to rats of Ethyl lauroyl arginate HCl was shown to be greater than 1800 mg of Ethyl lauroyl arginate HCl /kg bw. No deaths occurred in an acute oral study on Mirenat (21.6 – 22% (w/w) LAE).

Well-defined irritation (erythema and oedema) was noted in all rats in an **acute dermal** toxicity study. Irritation was resolved by day 9 in 8/10 animals, but persisted to day 12 or 14 in the other two rats. The acute lethal dermal dose to rats of LAE was shown to be greater than 1802 mg/kg bw Ethyl lauroyl arginate HCl.

An **acute inhalation** toxicity study suggested a **mild respiratory tract irritation** if exposure to the aerosol is sufficiently high. However, the exposure to the non-volatile LAE is difficult to assess since much appears to have been lost before reaching the breathing zone. The 4h aerosol $LC_{50}$ is greater than 28150 mg/m³ for the volatile fraction and greater than 5883 mg/m³ for the aerosol fraction.

*Irritation and corrosivity*

The results of a **skin irritation** study indicate that the test item, 90.1% 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.

Several **mucous membrane irritation** studies on New Zealand albino rabbits were performed. In concentrations up to 20.4%, Ethyl lauroyl arginate HCl showed to have an irritating potential and would be classified as "an irritant" under EU labeling regulations. In a study with a concentration up to **0.8%** Ethyl lauroyl arginate HCl was considered as **non-irritant** to the eyes**.**

*Skin sensitisation*

Two Magnusson Kligman studies (OECD 406, EC. B6) were performed. The results indicate that 18% (first study) and 20.4% (second study) Ethyl lauroyl arginate HCl do not induce a sensitisation response in the guinea pig.

*Dermal/percutaneous absorption*

Out of two dermal absorption studies, the SCCS concluded that, according to the SCCS Notes of Guidance (7$^{th}$ revision, Ref. 7), **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 as an **active ingredient at 0.8%.**

*Repeated dose toxicity*

28 day, subchronic and chronic studies are available. Table 6 indicates the achieved doses of Ethyl lauroyl arginate HCl (mg/ kg bw/day) in diet and the NO(A)EL derived by the study authors.

Table 6: achieved doses of Ethyl lauroyl arginate HCl (mg/ kg bw/day) in diet and the derived NO(A)EL values

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

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

*Remark: In the previous opinion of 15 April 2008, the result section of the sub-chronic study on Wistar Han rats states that "There was evidence of neurotoxicity during the weekly functional observational battery tests." This is not correct and changes the interpretation of the outcome of the study. Therefore the SCCS would like to note that the sentence should be "There was no clear evidence of neurotoxicity…"

*Mutagenicity/genotoxicity*

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

*Carcinogenicity*

No data submitted

*Reproductive toxicity*

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

*Toxicokinetics*

Low systemic toxicity of Ethyl lauroyl arginate HCl is supported by its toxicokinetics. In the chronic rat study, the rate and extent of systemic exposure to Ethyl lauroyl arginate HCl and its metabolite LAS appeared to be characterised by dose-independent kinetics. High interindividual variation in plasma LAE concentrations was noted, but this was less marked in plasma LAS concentrations in both sexes.
In human volunteers, the oral pharmacokinetics of Ethyl lauroyl arginate HCl indicated rapid absorption and hydrolysis to LAS and arginine. The terminal half-life of $^{13}$C-LAS (range 2.2 to 3.3 hours) and $^{13}$C-arginine (range 1.6 to 4.0 hours) were similar. Plasma concentrations of $^{13}$C -arginine were generally considerably higher than those of $^{13}$C-LAS.
Thus, even assuming 100% absorption at 0.4% Ethyl lauroyl arginate HCl, it would suggest rapid hydrolysis of Ethyl lauroyl arginate HCl if absorbed through the skin. Therefore, systemic exposure to Ethyl lauroyl arginate HCl and Nα-lauroyl-L-arginine *in vivo* is likely to be very short.

## 3.4. SPECIFIC CONCERNS EXPRESSED IN SCCS/1415/11 WITH RESPECT TO POTENTIAL MUCOSAL IRRITATION OF ETHYL LAUROYL ARGINATE HCL

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

Two *in vitro* studies (testing toothpaste and mouthwash) in which the irritation potential of Ethyl lauroyl arginate HCl was evaluated were provided by the applicant. The *in vitro* method Skinethic RHE, formally validated for human skin irritation (OECD 439, EC. B46) of substances, was applied and its use was extrapolated to study the effects of Ethyl lauroyl arginate HCl on human gingival epithelium.

60 µl/cm² of eacht test substance (solutions, formulations, negative and positive controls) was applied on three tissue replicates for 10 minutes (mouthwash) or 20 minutes (toothpaste) at room temperature (RT, between 18°C to 24°C). Cell viability was assessed by an MTT test. Sodium Dodecyl Sulphate (SDS 5%) and PBS treated epidermis were used as positive and negative controls, respectively.

Tables 7 and 8 provide the results of cell viability after treatment of reconstructed human gingival epithelium and histological analysis of tissues in mouthwash and toothpaste, respectively.

Table 7: Cell viability after treatment of reconstructed human gingival epithelium and histological analysis of tissues in mouthwash

| 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 *stratum corneum* - slight damage |
| Sample 2 – E10.0090 - LAE® 0.75% in deionized water | 91 | 9.9 | Absence of significant cellular alterations | Partial loss of *stratum corneum*; 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 *stratum corneum* – slight damage |
| Positive control - SDS 5% | 64 | 6.3 | Presence of severe cellular alterations and necrotic cells | Severe cellular alterations and necrotic cells |

Table 8: Cell viability after treatment of reconstructed human gingival epithelium and histological analysis of tissues in toothpaste

| 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 *stratum corneum* – moderate damage |
| Sample 2 – E09.4778 – Toothpaste with LAE® 0.75% | 92 | 8.9 | Absence of significant cellular alterations | Alterations in *stratum corneum*– slight damage |
| Sample 3 – E09.4780 – Gingilacer toothpaste | 81 | 10.4 | Absence of significant cellular alterations | Alterations in *stratum corneum* and infiltration of nuclei *into it*– 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 |

The performing laboratory concluded that, on the basis of the obtained results, the analyzed samples (both mouthwash and toothpaste) can be considered as non irritant for the gingival epithelium under the assayed test conditions.

***SCCS Comments***

Skinethic RHE has been formally validated for detecting skin irritation but not for gingival epithelium irritation. No proof was provided that this *in vitro* assay is suitable to assess the potential of chemical substances for mucous membrane irritation. Wurzburger (2011, Ref. 8) reported that the application of reconstructed human gingival epithelium might be a useful screening test prior to human studies. The RhE assay and similar tests, however, are not designed to detect mild irritants. Furthermore, a single application on reconstituted human skin is not comparable to long-term repeated use of oral cosmetic products. Therefore the SCCS could not draw any conclusion from these tests that would be relevant for the safety assessment of Ethyl lauroyl arginate HCl used in oral cosmetic products.

### 3.4.2. Human data

**Clinical and Antibacterial Effect of Toothpastes**

The applicant submitted (submission III) information on three clinical studies with toothpaste and mouth rinse containing Ethyl lauroyl arginate HCl (Ref. 9, 10, 11). The reports of the studies 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 (Ref. 9, 11). Assessment of possible adverse effects was not reported, indirectly suggesting overall excellent oral hygiene and health of the participants. The persons involved were exposed to the test product for periods between 4 and 10 days. These studies were designed to assess the efficacy of the antimicrobial effect of Ethyl lauroyl arginate HCl in formulations in comparison with similar marketed products. The focus of these studies was to evaluate plaque control. Scant information on the test formulations was provided (even in the study reports) and effects on gingival tissue after treatment were not provided.

***SCCS Comments***
The human studies, designed to assess efficacy of plaque control, showed that Ethyl lauroyl arginate HCl reduced plaque significantly. A limitation of the studies was the rigorous inclusion and exclusion criteria of participants, resulting in selection of only participants with excellent oral health. In addition, the group sizes were small, the time frames (4-10 days) short and inadequate information on potentially negative effects, especially on the gingiva, was provided.
These short-term studies did not mirror long term consumer usage, which would consist of twice daily brushing of the teeth 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 did not provide reassurance that no local oral mucosal irritation, in particular of the gingiva, occurs, especially if it would already be compromised. This could be resolved by showing that there would be no local irritation of the oral mucosa and gingiva in long-term studies.

## 3.5. CURRENT APPLICANT REQUEST

In submission IV dated 14 September 2012, the applicant considers that Ethyl lauroyl arginate HCl is safe for use as a preservative in oral cosmetic products up to a maximum concentration of 0.15% and requests an adaptation to Annex VI to Council Directive 76/768/EEC (which corresponds now with Annex V of regulation 1223/2009). This request is made on the basis of new oral care tolerance data obtained from human volunteers studies, along with the safe toxicological profile described in the previous dossiers (summarised under 3.3) and with a new risk assessment of Ethyl lauroyl arginate HCl.

### 3.5.1. Human data

**Verification of the long term acceptability of two mouthwashes versus placebo under normal conditions of use. - In use test in humans controlled by an odontologist.**

A new study in a group of human volunteers was submitted by the applicant, in order to evaluate the tolerance of Ethyl lauroyl arginate HCl in a mouthwash (as an example of an

oral cosmetic product) and to address the limitations identified by the SCCS in the previous submission (SCCS/1415/11).

*Summary of the study*

84 volunteers, of which 6 dropped out, mentioned as 'unconnected to the effect of the test substance' were distributed in three different test groups:

- Test group, sample A (placebo mouthwash): 26 subjects
- Test group, sample B (mouthwash with 0.15% Ethyl lauroyl arginate HCl): 25 subjects
- Test group, sample C (mouthwash with 0.10% Ethyl lauroyl arginate HCl): 27 subjects

A 14-day wash-out period (D -14 to D 0) was foreseen, in which all subjects applied the same commercial neutral toothpaste at home under normal conditions of use, followed by a 6-month study (D 0 to D 168) in which twice daily, after using the commercial neutral toothpaste, all subjects rinsed their mouth with 15 ml of the mouthwash for 30 seconds.

The condition of volunteers' mouth (oral mucosa, gingiva and teeth) was examined by an odontologist on a double blind basis. At both dose levels of Ethyl lauroyl arginate HCl tested (0.15% and 0.10%), the percentage of subjects exhibiting clinical signs attributable to the test products was in all cases 0%.
In addition, an analysis of the sensation of discomfort was reported directly by the test subjects to the odontologist at the time of clinical examination and recorded in their daily logs. The test subjects, at home, had to complete a daily log for each of the 168 consecutive days of the study period, reporting any reaction observed and sensation of discomfort felt. The percentage of subjects reporting sensations of discomfort at the time of examination by the odontologist attributable to the presence of Ethyl lauroyl arginate HCl in the mouthwash (days D8, D14, D28, D56, D84, D112, D140 and D168) was also 0%. No increase in the clinical signs presenting at D0 were recorded in the daily log by any test subject over the duration of the study.

To complete the evaluation of tolerance, the odontologist calculated two inflammation indices that were based on clinical assessment of the gums. These indices were (Annex 1 Tables 1 and 2): the "Loë and Silness" index (to evaluate the degree of inflammation) and the gingival bleeding index (to evaluate the extent of any haemorrhage).

The "Loë and Silness" index considers qualitative changes in the gingiva, providing scores from 0-3. The criteria are:
0= Normal gingiva
1= Mild inflammation – slight change in color, slight oedema but no bleeding on probing;
2= Moderate inflammation – redness, oedema and glazing, bleeding on probing;
3= Severe inflammation – marked redness and oedema, ulceration, tendency to spontaneous bleeding.

The gingival bleeding index was calculated by deviding the number of bleeding areas by the total number of observed areas and multiplying by 100.

Ref. 12, 13, 14

In addition, an efficacy study was performed according to the same conditions as the tolerance study. To assess the effect of the mouthwash on the dental plaque of the volunteers, a coloration of dental plaque and a calculations of the O'Leary index were performed before and after 168 ± 8 consecutive days of product use (on days D0, D8, D14, D28 and D168) and results are present in the submission. Standardized photographs were taken on D0, D8, D14 and D28, which were included in the submission.

The applicant mentioned (related to efficacy and not to risk assessment) that product Sample B (mouthwash with 0.15% Ethyl lauroyl arginate HCl) and Sample C (mouthwash

with 0.10% of Ethyl lauroyl arginate HCl) have a statistically significant effect on dental plaque after 8, 14, 28 and 168 days of treatment, under the experimental conditions adopted and differ from the placebo product Sample A (mouthwash with 0% Ethyl lauroyl arginate HCl), indicating an effect on dental plaque control by the active ingredient after 28 days of treatment.
Furthermore, Sample B (0.15% Ethyl lauroyl arginate HCl) differs in a statistically significant way from the product Sample A (0% Ethyl lauroyl arginate HCl) after 168 days of treatment indicating a higher effect of reducing the dental plaque.

Ref. 14, 15

*Conclusion by the applicant*

No intolerance reaction was noted by the investigator and no sensation of discomfort was described after questioning the test subjects for the duration of the study. Subjects using Samples A (Placebo, 0% Ethyl lauroyl arginate HCl), B (0.15% Ethyl lauroyl arginate HCl) and C (0.10% Ethyl lauroyl arginate HCl) all tolerated the mouthwash well and experienced an improvement of mouth condition.

The maximum level of Ethyl lauroyl arginate HCl administered in a mouthwash under test was 0.15%. In view of the absence of any adverse effects demonstrated by the study at any level of administration, the applicant requests the inclusion of Ethyl lauroyl arginate HCl in Part I of Annex VI of Directive 76/768/EEC (Annex V in regulation 1223/2009) for use as a preservative in oral cosmetic products at levels up to 0.15%.

In terms of efficacy, 0.15% Ethyl lauroyl arginate HCl shows to have a statistically significant higher effect of reducing dental plaque than the placebo after 168 days.

Ref. 14, 15

***SCCS Comments***
The values given for the "Loë and Silness" index and for the gingival bleeding index have a high standard deviation and no other statistical evaluation has been carried out. The measurements of the different samples seem to be of a similar order of magnitude.

No signs of irritancy were reported on a long-term period of 168 consecutive days on 25 subjects, using the mouthwash with 0.15% Ethyl lauroyl arginate HCl twice a day. The requested concentration as preservative in oral cosmetic products is 0.15%. Oral cosmetic products, however, not only consist of mouthwash but also of toothpaste. As in the human volunteer studies presented here, only the use of mouthwash at a concentration of 0.15% was evaluated, it is not possible to predict whether irritation would occur under combined use of both mouthwash and twice daily use of toothpaste (once in the morning, once in the evening), containing both 0.15% Ethyl lauroyl arginate HCl as preservative.

## 3.5.2. Safety evaluation (including calculation of the MoS)

**CALCULATION OF THE MARGIN OF SAFETY**
**Ethyl lauroyl arginate HCl, for a combined application of preservative and active ingredient**

0.15% Ethyl lauroyl arginate HCl as a preservative in oral cosmetic products, 0.4% for other preservative uses and 0.8% as an active ingredient in soap, shampoo and non-spray deodorant

*(i) SED for 0.8% Ethyl lauroyl arginate HCl as an active ingredient in soap, shampoo and non-spray deodorant:*

Amount of the cosmetic product containing ethyl lauryl arginate as active ingredient (a.i.) applied daily (ref. 16):

| | |
|---|---|
| Soap: | 0.2 g/day |
| Deodorants: | 1.5 g/day |
| Shampoo: | 0.11g/day |
| TOTAL | 1.81 g/day |

| | | |
|---|---|---|
| **Estimated daily exposure** | **A** | **= 1.81 g/day** |
| **Dermal absorption per treatment** | **DAp (%)** | **= 2.38%** (Ref. 6) |
| **Typical body weight of human** | | **= 60 kg** |

**SED** **AxCxDApx1000/60kg** **= 0.00574mg/kg bw/d**

*(ii) SED for 0.15% Ethyl lauroyl arginate HCl as a preservative in oral cosmetic products*

| | | |
|---|---|---|
| **Estimated daily exposure** | **A** | **= 2.30 g/day** |
| **Concentration** | **C** | **= 0.15%** |
| **Dermal absorption per treatment** | **DAp (%)** | **= 100%** |
| **Typical body weight of human** | | **= 60 kg** |

**SED** **AxCxDApx1000/60kg** **= 0.0575 mg/kg bw/d**

*(iii) SED for product categories where Ethyl lauroyl arginate HCl can be applied at 0.4% as a preservative*

| | | |
|---|---|---|
| **A** | **$=17.4-(1.81_{[\text{cosmetics a.i.}]}+2.36_{[\text{oral care \& lipstick}]})$** | **= 13.23 g/day** |
| **C** | | **= 0.4%** |
| **DAp (%)** | | **= 3%** (Ref. 6) |
| **Typical body weight of human** | | **= 60 kg** |
| **SED** | **AxCxDApx1000/60kg** | **= 0.02646 mg/kg bw/d** |

*(iv) SED for combined preservative and active ingredient uses when oral cosmetic products at 0.15% are included:*

**SED = 0.0575 + 0.02646 + 0.00574 = 0.08970 mg/kg bw/d**

**MoS for cosmetic uses of Ethyl lauroyl arginate HCl** $= \frac{\textbf{271 mg/kg bw/day}}{\textbf{0.0897 mg/kg bw/day}} = \textbf{3021}$

***SCCS Comment***
No systemic effects are expected at all concentrations and uses requested.
No correction value for the NOAEL was utilized because of the high MoS-value.

### 3.6.2. Discussion

In the previous opinion, the SCCS stated that *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.*

In order to address the limitations in the previously submitted studies, the applicant presented a long-term current study of mouthwashes which contain 0.10% and 0.15% respectively of the substance under investigation. No intolerance reactions were noted during the study and no sensation of discomfort was described after questioning the test subjects for the duration of the study.
Since the study was indeed long term (168 days), and conducted on a varied population of male and female volunteers from 18 to 70 years, with all types of gums and all types of oral hygiene habits, it is reasonable to conclude that the mouthwashes containing 0.10% and 0.15% Ethyl lauroyl arginate HCl cause no local mucosal irritation. The SCCS, however, does not consider a mouthwash as a representative product for all oral cosmetic products.
Indeed, with this study it is not possible to predict whether irritation would occur under combined use of both mouthwash and twice daily use of toothpaste (once in the morning, once in the evening), containing both 0.15% Ethyl lauroyl arginate HCl as preservative. Neither can rinsing one's mouth be compared to the action of brushing one's teeth, the latter not only involving a longer duration of exposure than 30 seconds, but also possibly being more aggravating for the gum.

Systemic safety evaluation and MoS calculations for the use of Ethyl lauroyl arginate HCl as a preservative at 0.15% in oral cosmetic products and 0.4% in other cosmetic products, combined with its use as an active ingredient at 0.8% in soap, shampoo and non-spray deodorant, show that Ethyl lauroyl arginate HCl has a low systemic toxicity. However, since there is no human data concerning local toxicity for combined use of mouthwash and toothpaste at 0.15%, it can only be concluded that Ethyl lauroyl arginate HCl is safe for use in mouthwashes.
The SCCS points out that the use of Ethyl lauroyl arginate HCl in oral cosmetic products appears to be for another function than preservative as parabens were present in all tested formulations.

## 4. CONCLUSION

The SCCS considers Ethyl lauroyl arginate HCl safe for use as a preservative, when used up to a maximum concentration of 0.15% in mouthwashes, though not in oral cosmetic products as a whole.

As no human data concerning local toxicity of Ethyl lauroyl arginate HCl in toothpaste are available, the safety of Ethyl lauroyl arginate HCl in toothpaste cannot be assessed.

## 5. MINORITY OPINION

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## 6. REFERENCES

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## 7. ANNEX 1

Table 1: "Loë and Silness" index

| | SAMPLE A REF: 001 (PLACEBO) | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | D0 | D8 | D14 | D28 | D56 | D84 | D112 | D140 | D168 | D8-D0 | D14-D0 | D28-D0 | D56-D0 | D84-D0 | D112-D0 | D140-D0 | D168-D0 |
| Mean | 0.6 | 0.6 | 0.6 | 0.6 | 0.5 | 0.5 | 0.5 | 0.3 | 0.3 | 0.0 | 0.0 | 0.0 | -0.1 | -0.1 | -0.1 | -0.3 | -0.3 |
| Standard deviation | 0.8 | 0.8 | 0.8 | 0.8 | 0.8 | 0.8 | 0.8 | 0.6 | 0.6 | 0.0 | 0.0 | 0.0 | 0.3 | 0.3 | 0.3 | 0.5 | 0.5 |
| | SAMPLE B REF: 002 (0.15% ACTIVE INGREDIENT) | | | | | | | | | | | | | | | | |
| | D0 | D8 | D14 | D28 | D56 | D84 | D112 | D140 | D168 | D8-D0 | D14-D0 | D28-D0 | D56-D0 | D84-D0 | D112-D0 | D140-D0 | D168-D0 |
| Mean | 0.6 | 0.6 | 0.6 | 0.5 | 0.5 | 0.4 | 0.4 | 0.4 | 0.4 | 0.0 | 0.0 | -0.1 | -0.1 | -0.2 | -0.2 | -0.2 | -0.2 |
| Standard deviation | 0.8 | 0.8 | 0.8 | 0.7 | 0.7 | 0.7 | 0.7 | 0.7 | 0.6 | 0.0 | 0.2 | 0.3 | 0.3 | 0.4 | 0.4 | 0.4 | 0.4 |
| | SAMPLE C REF: 003 (0.10% ACTIVE INGREDIENT) | | | | | | | | | | | | | | | | |
| | D0 | D8 | D14 | D28 | D56 | D84 | D112 | D140 | D168 | D8-D0 | D14-D0 | D28-D0 | D56-D0 | D84-D0 | D112-D0 | D140-D0 | D168-D0 |
| Mean | 0.7 | 0.7 | 0.7 | 0.6 | 0.6 | 0.5 | 0.5 | 0.4 | 0.4 | 0.0 | 0.0 | -0.1 | -0.1 | -0.2 | -0.2 | -0.3 | -0.3 |
| Standard deviation | 0.7 | 0.7 | 0.7 | 0.7 | 0.6 | 0.6 | 0.6 | 0.6 | 0.6 | 0.0 | 0.0 | 0.2 | 0.3 | 0.4 | 0.4 | 0.5 | 0.5 |

Table 2: Gingival bleeding index

| | SAMPLE A REF: 001 (PLACEBO) | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | D0** | D8 | D14 | D28 | D56 | D84 | D112 | D140 | D168 | D8-D0 | D14-D0 | D28-D0 | D56-D0 | D84-D0 | D112-D0 | D140-D0 | D168-D0 |
| Mean | 17.2 | 11.7 | 7.8 | 6.9 | 4.6 | 4.4 | 3.6 | 2.7 | 2.5 | -5.5 | -9.7* | -10.3 | -13.4* | -13.2* | -13.6 | -14.5 | -14.7 |
| Standard deviation | 20.7 | 13.1 | 8.4 | 8.5 | 5.9 | 5.7 | 5.4 | 4.0 | 3.6 | 10.2 | 13.6 | 14.8 | 17.3 | 17.8 | 17.3 | 18.7 | 18.7 |

**Due to the lack of values from subjects 21 and 29 at D14, D56 and D84, these data* may not match with the differences of the means between D14, D56, D84 and D0** (as the column D0 includes the values from subjects 21 and 29).

| | SAMPLE B REF: 002 (0.15% ACTIVE INGREDIENT) | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | D0 | D8 | D14 | D28 | D56 | D84 | D112 | D140 | D168 | D8-D0 | D14-D0 | D28-D0 | D56-D0 | D84-D0 | D112-D0 | D140-D0 | D168-D0 |
| Mean | 13.2 | 10.8 | 7.2 | 7.6 | 4.9 | 3.6 | 2.7 | 3.3 | 3.0 | -2.4 | -6.0 | -5.6 | -8.3 | -9.6 | -10.5 | -9.9 | -10.2 |
| Standard deviation | 12.3 | 9.7 | 7.5 | 7.8 | 6.2 | 4.2 | 3.6 | 4.0 | 4.0 | 8.2 | 8.7 | 11.0 | 11.1 | 10.6 | 10.6 | 9.6 | 10.2 |
| | SAMPLE C REF: 003 (0.10% ACTIVE INGREDIENT) | | | | | | | | | | | | | | | | |
| | D0** | D8 | D14 | D28 | D56 | D84 | D112 | D140 | D168 | D8-D0 | D14-D0 | D28-D0 | D56-D0 | D84-D0 | D112-D0 | D140-D0 | D168-D0 |
| Mean | 12.3 | 10.9 | 7.7 | 8.6 | 4.3 | 4.2 | 3.5 | 2.6 | 2.4 | -1.4 | -4.6 | -3.7 | -8.0 | -8.1 | -8.8 | -9.9* | -9.9 |
| Standard deviation | 9.5 | 9.2 | 8.3 | 9.4 | 4.8 | 4.7 | 4.2 | 3.4 | 3.3 | 4.9 | 7.8 | 11.2 | 7.8 | 6.7 | 7.2 | 7.6 | 8.3 |

**Due to the lack of value from subject 27 at D140, this value* may not match with the difference of the mean between D140 and D0** (as the column D0 includes the value from subject 27).

## 8. ANNEX 2

Concerning trade names of the Ethyl lauryl argenate HCl, the following information has been provided by the applicant after the publication of this opinion:

**Mirenat-N**: this is the trade name of a formulation containing c.a. 20% of Ethyl lauroyl arginate HCl in propylene glycol marketed for use by the food industry. Several toxicological studies have been performed with this product.

**Mirenat-N water dispersed (13-12-95)**: this designation refers to a special batch of Mirenat-N consisting of Ethyl lauroyl arginate HCl c.a. 20% dispersed in water (without any propylene glycol). This special formulation was prepared in order to provide a propylene glycol-free comparator to the standard Mirenat-N for use in studying effects on eye irritation.

**Aminat**: this is the trade name of a formulation containing c.a. 20% of Ethyl lauroyl arginate HCl in propylene glycol marketed for use by the cosmetics industry. Several toxicological studies have been performed with this product. In some of the toxicological studies in which Aminat was used, the test product was diluted in water. In these cases the designation of the test product is given as Aminat X%, where X% indicates the proportion of Aminat (i.e. 20% Ethyl lauroyl arginate HCl in propylene glycol) in water. This is the case in several eye irritation studies.

**Aminat-G**: this is the trade name of a formulation containing c.a. 20% of Ethyllauroyl arginate HCl in glycerine marketed for use by the cosmetics industry.

Additional information provided by the applicant after the publication of this opinion is:
Section 3.1.8: the deviating value of the density 0.017g/ml at 20°C should be 1.017g/ml.