FOOD STANDARDS Australia New Zealand
Te Mana Kounga Kai – Ahitereiria me Aotearoa

# Supporting document 1

# Hazard Assessment Report – Application A1015

## Ethyl Lauroyl Arginate as a Food Additive

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### Background

#### *Chemistry*

Details of the physicochemical properties of ethyl lauroyl arginate (abbreviation: ELA)[1], including product specifications and the impurity profile, are included in the Food Technology Report. The compound is prepared as a hydrochloride salt (molecular weight 421.0) which is a white solid at room temperature (CAS number 60372-77-2). The active ingredient is ethyl-$N^{\alpha}$-lauroyl-L-arginate·HCl. In solution, ethyl lauroyl arginate acts as a cationic surfactant and its preservative properties are reported to be due to disruption of bacterial cell membranes. The structural formula of ethyl lauroyl arginate is shown in Figure 2.1.

### *Consideration of ethyl lauroyl arginate by various expert committees*

The WHO Joint Expert Committee on Food Additives (JECFA) first considered ethyl lauroyl arginate at its 69th meeting in June 2008 (FAO/WHO 2008). The Committee established an ADI of 0-4 mg/kg bw for ethyl lauroyl arginate, expressed as the active ingredient ethyl-$N^{\alpha}$-lauroyl-L-arginate·HCl, based on the NOAEL of 442 mg/kg bw per day identified in studies of reproductive toxicity and a safety factor of 100 (WHO 2009). The NOAEL was based on delayed vaginal opening observed in two reproductive toxicity studies in rats. The NOAEL for this effect was a dietary concentration of 6000 mg/kg, corresponding to an ethyl lauroyl arginate intake of 502 mg/kg bw/day (442 mg/kg bw per day expressed as ethyl-$N^{\alpha}$-lauroyl-L-arginate·HCl).

The European Food Safety Authority (EFSA) published their opinion on ethyl lauroyl arginate in April 2007. EFSA established an ADI of 0-0.5 mg/kg bw for ethyl lauroyl arginate. The ADI was based on effects observed on white blood cell counts in repeat dose toxicity studies (EFSA 2007). The ADI derived by EFSA was based on an NOAEL of approximately 50 mg ELA/kg bw/day, which was the lowest dose tested in the 13-week study with Mirenat-N (20-25% w/w ethyl lauroyl arginate dissolved in propylene glycol) as test article. EFSA considered ELA prior to the availability of three expert reviews on the white blood cell findings. The expert reviews concluded that the white blood cell findings are unlikely to be of toxicological significance.

[1] Ethyl lauroyl arginate is the official proposed name for the compound according to Codex (December 2008). In many of the study titles quoted in this Attachment, lauric arginate and LAE are used as synonyms for ethyl lauroyl arginate.

The EU Scientific Committee on Cosmetic Products Intended for Consumers (SCCP) issued an opinion on the safety of ethyl lauroyl arginate when used as a preservative in cosmetics, on 15 March 2005 (SCCP 2005). The SCCP considered that ethyl lauroyl arginate was safe for consumers, when used:

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

The SCCP opinion was based on the use of ethyl lauroyl arginate in the specified cosmetic products only and took no account of other sources of exposure.

The US Food and Drug Administration has issued a Letter of No Objection regarding the submission that ethyl lauroyl arginate is Generally Recognised as Safe (GRAS) for use as an antimicrobial at levels up to 225 mg/kg of ethyl lauroyl arginate in the food categories specified (USFDA 2005).

**Scope of the current hazard assessment**

FSANZ has not previously assessed the safety of ethyl lauroyl arginate. Therefore, the aims of the current assessment were to:

- review all of the available data on the kinetics and toxicology of ethyl lauroyl arginate to determine its safety as a food additive; and

- establish an ADI for ethyl lauroyl arginate.

## Evaluation of Submitted Data

FSANZ has assessed the submitted evidence on the safety of ethyl lauroyl arginate including studies on absorption, metabolism, acute toxicity, repeat-dose toxicity, genotoxicity and reproductive toxicity. The submitted data, comprising a relatively comprehensive set of high quality studies, were considered suitable for hazard assessment and assignment of an ADI for ethyl lauroyl arginate.

Absorption studies on ethyl lauroyl arginate were conducted in rats and humans. *In vitro* metabolism studies were conducted with S9 liver fractions from rats, simulated gastric fluid (with and without pepsin), simulated intestinal fluid (with and without pancreatin), and with human plasma and human hepatocytes. *In vivo* metabolism studies were conducted in rats. An excretion study was also conducted in rats.

Acute toxicity, repeat dose toxicity, genotoxicity studies were performed with: (i) ethyl lauroyl arginate (active ingredient content 88-90% w/w), and (ii) Mirenat-N (20-25% w/w ethyl lauroyl arginate dissolved in propylene glycol). An acute toxicity study and two genotoxicity studies were also performed with $N^{\alpha}$-lauroyl-L-arginine (LAS)[2], the major metabolite of ethyl lauroyl arginate. Reproductive toxicity studies were performed with ethyl lauroyl arginate containing the active ingredient at 88% w/w. Developmental toxicity studies were performed with ethyl lauroyl arginate containing the active ingredient at 69% w/w.

[2] The reason for abbreviating $N^{\alpha}$-lauroyl-L-arginine as LAS in the submitted study reports is not known but is retained for consistency throughout this report.

The relatively low content of active ingredient in this batch was due to high water content (23% w/w) because the synthesis product was not subject to a drying step. Because this batch does not meet the JECFA specifications for the content of the active ingredient (85 to 95% w/w), a correction factor was applied to the doses in the developmental toxicity studies to enable comparison with the studies that used batches conforming to the JECFA specifications.

Detailed descriptions of the absorption, metabolism and excretion studies, single and repeat dose toxicity, genotoxicity, reproductive and developmental toxicity studies considered in this assessment are given below.

***Absorption, Metabolism and Excretion studies***

In the studies below, the terms $C_{max}$, $t_{max}$ and AUC refer to maximum plasma concentration, time of maximum plasma concentration, and area under the plasma concentration versus time curve, respectively.

**Absorption**

*Rats*

HLS (2005c) **Study title**: Lauric arginate pharmacokinetics in rats. **Report no.**: LMA 057/053626 **Report date**: 21 Dec 2005 **Laboratory**: Huntingdon Life Sciences Ltd, Huntingdon, Cambridgeshire, England. **GLP**: Yes (OECD)

Sprague-Dawley rats in the fed state received single oral gavage doses of ethyl lauroyl arginate as shown in the table below.

**Table 2.1: Treatment groups and dosing details**

| Group no. | ELA dose (mg/kg bw) | No. animals/group | Vehicle |
|---|---|---|---|
| 1 (Pilot phase) | 40 | 4/sex | Propylene glycol/water |
| 2 (Main phase) | 40 | 4 males | Propylene glycol/water |
| 3 (Main phase) | 120 | 4 males | Propylene glycol/water |
| 4 (Main phase) | 320 | 4 males | Propylene glycol/water |
| 5 (Main phase) | 120 | 4 males | Glycerol/water |
| 6 (Main phase) | 120 | 4 males | Water |

Blood was sampled at 15, 30, 60, 90, 120 and 240 min post-dose for the pilot phase animals and at 30, 60, 90, 120, 240 min with a final sampling time at 8 h post-dose for animals in the main phase of the study. Plasma concentrations of ethyl lauroyl arginate and the metabolite LAS were measured by a partially validated LC-MS/MS method. Acceptable recovery, linearity, precision, accuracy and specificity were observed over the concentration range 1-200 ng/mL for ethyl lauroyl arginate and 5-1000 ng/mL for LAS. The limits in these ranges correspond to the lower and upper limits of quantification. The use of a dilution factor of x10 for ethyl lauroyl arginate was not validated primarily due to degradation during the procedure. The analytes were found to be unstable during one freeze-thaw cycle. Therefore this method is only applicable to rat plasma which is analysed immediately after sampling which was the case in this pharmacokinetics study.

Mean plasma $C_{max}$, $t_{max}$ and $AUC_{0\text{-}8\ h}$ values for ethyl lauroyl arginate and LAS (propylene glycol/water vehicle, main phase) are shown in the table below.

**Table 2.2: Mean plasma $C_{max}$ and $AUC_{0\text{-}8\ h}$ values for ethyl lauroyl arginate and LAS**

| | ELA dose (mg/kg bw) | | | | | |
|---|---|---|---|---|---|---|
| | 40 | | 120 | | 320 | |
| Analyte | ELA | LAS | ELA | LAS | ELA | LAS |
| $C_{max}$ (ng/mL) | 2.02 | 24.2 | 1.23 | 23.2 | 2.60 | 96.9 |
| $t_{max}$ (h) | 0.5 [a] | 1.0 [a] | 1.0 [a] | 0.75 [a] | 3.0 [a] | 1.5 [a] |
| $AUC_{0\text{-}8\ h}$ (ng.h/mL) | - | 52.5 | - | 103 | 7.50 | 315 |

[a] Median - Could not be calculated (insufficient data above the lower limit of quantification)

A comparison of the $C_{max}$ and $AUC_{0\text{-}8\ h}$ values for ethyl lauroyl arginate in the presence of the three different vehicles at an ethyl lauroyl arginate dose of 120 mg/kg bw are shown below.

**Table 2.3: $C_{max}$ and $AUC_{0\text{-}8\ h}$ values for ethyl lauroyl arginate in the presence of the three different vehicles**

| | ELA dose = 120 mg/kg bw | | | | | |
|---|---|---|---|---|---|---|
| Vehicle | Propylene glycol/water | | Glycerol/water | | Water | |
| Analyte | ELA | LAS | ELA | LAS | ELA | LAS |
| $C_{max}$ (ng/mL) | 1.23 | 23.2 | 9.42 | 28.8 | 10.6 | 31.2 |
| $t_{max}$ (h) | 1.0 [a] | 0.75 [a] | 0.75 [a] | 1.0 [a] | 0.75 [a] | 0.5 [a] |
| $AUC_{0\text{-}8\ h}$ | - | 103 | 12.6 | 115 | 8.78 | 109 |

[a] Median- Could not be calculated (insufficient data above the lower limit of quantification)
Note that ethyl lauroyl arginate is freely soluble in water (greater than 247 g/kg at 20°C) and soluble up to 20% w/w in propylene glycol, glycerol and ethanol.

Conclusions: Ethyl lauroyl arginate was rapidly metabolised to LAS. The AUC for LAS was approximately proportional to the ethyl lauroyl arginate dose and was similar in the presence of all three vehicles at an ethyl lauroyl arginate dose of 120 mg/kg bw.

*Humans*

CentraLabS (2005a) **Study title**: LAE: An open label, single-dose study to determine the feasibility of measuring LAE and its breakdown products in plasma after oral administration of LAE to healthy male volunteers. **Report no.**: LMA 047/033421, **Report date**: 12 Jan 2005 **Laboratory**: CentraLabS Clinical Research Ltd., Alconbury, Cambridgeshire, England. (Clinical phase at PPD Development Clinic, Leicester, UK) **GLP**: Yes (OECD), for analytical phase of the study

Two healthy male volunteers each received an oral dose of 5 mg/kg bw $^{13}$C-ELA dissolved in 15 mg/kg bw propylene glycol made up to a volume of 1 mL/kg bw with purified water. It was not stated whether the volunteers were in a fed or fasted state and whether the solution was consumed as a bolus or gradually ingested. Blood samples were taken pre-dose and at 5, 10, 15 and 30 min, and 1, 2, 4, 8, 12 and 24 h after dosing. Plasma concentrations of $^{13}$C-ELA, $^{13}$C-LAS and $^{13}$C-arginine were measured by an LC-MS/MS method (lower limit of quantification 1 ng/mL for ethyl lauroyl arginate and LAS; 10 ng/mL for arginine). $C_{max}$ and $t_{max}$ values are shown in the table below. AUC values were not presented in the report.

**Table 2.4: $C_{max}$ and $t_{max}$ values for $^{13}$C-ELA, $^{13}$C-LAS and $^{13}$C-arginine**

| | $^{13}$C-ELA | | $^{13}$C-LAS | | $^{13}$C-arginine | |
|---|---|---|---|---|---|---|
| | Male 1 | Male 2 | Male 1 | Male 2 | Male 1 | Male 2 |
| $C_{max}$ (ng/mL) | 4.80 | 44.0 | 154 | 140 | 428 | 680 |
| $t_{max}$ (min) | 30 | 15 | 120 | 120 | 60 | 60 |

The approximately 9-fold difference in $C_{max}$ for $^{13}$C-ELA observed in the two subjects may be due to a difference in the fed state of the subjects.

ELA appeared to be well tolerated except for a mild burning throat sensation on administration reported by both subjects and nausea in one subject. It was stated that the burning sensation, and possibly nausea, may have been due to the use of propylene glycol (15 mg/kg bw) as the solvent.

CentraLabS (2005b) **Study title**: LAE an open-label, single-dose study to determine the plasma levels of LAE and its breakdown products after a single oral dose to healthy male volunteers. **Report no.**: LMA 049/034017 **Report date**: 12 Jan 2005 **Laboratory**: CentraLabS Clinical Research Ltd., Alconbury, Cambridgeshire, England. (Clinical phase conducted at PPD Development Clinic, Leicester, UK) **GLP**: Yes (OECD), for analytical phase of study **GCP**: Yes

Approximately 15 min after the completion of a "standard" breakfast, six healthy male volunteers each received an oral dose of $^{13}$C-ELA at dose levels of 2.5 mg/kg bw (subjects 1 and 2) or 1.5 mg/kg bw (subjects 3 to 6). Respective doses of propylene glycol vehicle were 7.5 and 4.5 mg/kg bw. Doses were made up to a volume of 1 mL/kg bw with purified water. Subjects swallowed the solution and the interior of the individual dosing vessel was rinsed twice with 50 mL purified water. Subjects swallowed each rinse. Blood samples were taken pre-dose and at 5, 10, 15 and 30 min, and 1, 2, 4, 8, 12 and 24 h after dosing. Standard meals were provided at 4 h and 10 h post dose and water was available *ad libitum* throughout the study. Plasma concentrations of $^{13}$C-ELA, $^{13}$C-LAS and $^{13}$C-arginine were measured by a validated LC-MS/MS method with a lower limit of quantification of 1 ng/mL for ethyl lauroyl arginate and LAS and 20 ng/mL for arginine.

Due to rapid metabolism no meaningful pharmacokinetic data for ethyl lauroyl arginate were obtained. Plasma concentrations of $^{13}$C-ELA were below the limit of quantification at all sampling times in all subjects, with the exception of subject 2 for whom quantifiable concentrations of $^{13}$C-ELA were found in two samples. Mean pharmacokinetic parameters for the metabolites $^{13}$C-LAS and $^{13}$C-arginine are shown in the table below. AUC values for both LAS and arginine increased with dose.

**Table 2.5: Mean pharmacokinetic parameters for $^{13}$C-LAS and $^{13}$C-arginine**

| | $^{13}$C-LAS | | $^{13}$C-arginine | |
|---|---|---|---|---|
| Dose (mg/kg bw) | 1.5 | 2.5 | 1.5 | 2.5 |
| $C_{max}$ (ng/mL) | 18.2 | 23.9 | 124 | 240 |
| $t_{max}$ (h) | 2 [a] | 1.5 [a] | 0.75 [a] | 1.25 [a] |
| $AUC_{0-\tau}$ (ng.h/mL) [b] | 90.6 | 118 | 383 | 764 |
| $\tau$ (h) [b] | 12 | 8 | 4 | 8 |
| $AUC_{0-\infty}$ (ng.h/mL) | 96.4 | 128 | 556 | 864 |
| $t_{½}$ (h) | 2.5 | 2.4 | 2.4 | 2.4 |

[a] Median
[b] Time intervals for these AUC values varied depending on the time of the last quantifiable sample.

No serious adverse events were reported during the study and no subject withdrew because of an adverse event. Three adverse events were reported by two subjects (one subject at each dose level): headache after the 2.5 mg/kg bw dose, and diarrhoea and flatulence 30 h after the 1.5 mg/kg bw dose. These adverse events were of mild severity and considered unlikely to be related to treatment. There were no clinically significant abnormalities in any of the laboratory data (clinical chemistry, haematology and urinalysis), no notable changes in vital signs during the study, and no clinically significant ECG findings.

**Metabolism**

***In vitro***

HLS (2003a) **Study title**: Nα-Lauroyl-L-arginine ethyl ester monohydrochloride *in vitro* stability. **Report no.**: LMA 043/032898 **Report date**: 29 July 2003 **Laboratory**: Huntingdon Life Sciences Ltd, Huntingdon, Cambridgeshire, England **GLP**: Yes (OECD)

This study investigated the stability of ethyl lauroyl arginate in simulated gastric fluid (pH 0.95), simulated intestinal fluids (at pH 6.8 and 7.5), human plasma, and in a preparation of human hepatocytes. Ethyl lauroyl arginate, radiolabelled with $^{14}C$ at the arginine carbons, was used at concentrations of 0.25 mg/mL (gastric and intestinal fluids) or 10 µg/mL (plasma and hepatocytes). Fluids were incubated at 37º C for 2 h (simulated gastric fluid with and without porcine pepsin) or 4 h (all other incubations). For incubations with simulated gastric fluid, samples were taken for HPLC analysis at the following time-points (min): 0, 1, 5, 15, 30, 60, 120. An additional sample was taken at 4 h for incubations with simulated intestinal fluid. Sampling time-points for incubations with human plasma were 0, 1, 2 and 4 h, and with human hepatocytes were 0, 0.25, 0.5 and 3 h.

In simulated gastric fluid, with and without porcine pepsin, ethyl lauroyl arginate was stable over the 2 h period.

In simulated intestinal fluids (at both pH 6.8 and pH 7.5, and with porcine pancreatin), ethyl lauroyl arginate was immediately degraded to LAS (95 – 98% of total radioactivity immediately after mixing) and then to arginine (90 – 93% of total radioactivity at 60 min). This degradation was enzyme-mediated: in simulated intestinal fluids without porcine pancreatin, ethyl lauroyl arginate was stable at pH 7.5 (over 4 h), while at pH 6.8 degradation to LAS was not detectable until the 60 min sampling time and reached only 19% of total radioactivity by 4 h.

ELA was degraded to LAS (but not to arginine) by human plasma (40 – 50 % of total radioactivity at 4 h) and human hepatocytes (77 – 85 % of total radioactivity at 3 h).

HLS (2001d) **Study title**: N-α-Lauroyl-L-arginine ethyl ester monohydrochloride *in vivo* and *in vitro* metabolism in the rat. **Report no.**: LMA 033/012117 **Report date**: 8 May 2001 **Laboratory**: Huntingdon Life Sciences Ltd, Huntingdon, Cambridgeshire, England **GLP**: Yes (OECD)

In both the *in vitro* and *in vivo* parts of this study, ethyl lauroyl arginate was labelled with $^{14}C$ at the arginine carbon atoms.

*In vitro*: The S9 fraction from the liver of an untreated Sprague-Dawley rat was incubated with $^{14}C$-ethyl lauroyl arginate for up to 24 h at 37º C. Unchanged ethyl lauroyl arginate, $N^{\alpha}$-lauroyl-L-arginine (LAS), arginine ethyl ester, arginine, ornithine and urea were identified in the S9 treated samples as shown in the table below. Analysis was conducted by TLC, HPLC and LC-MS. Ornithine, an endogenous human amino acid, was the major metabolite.

In a control incubation in the absence of S9 liver fraction no significant degradation of ethyl lauroyl arginate was observed.

**Table 2.6: Radioactive components *in vitro* as a percentage of added radioactivity**

| Radioactive component (% of administered radioactivity) | Time (hours after start of incubation) 4 | 6 | 24 |
|---|---|---|---|
| Ornithine / arginine | 25.0 [a] | 28.3 | 29.3 |
| M3 [b] | 1.8 | 2.1 | 2.9 |
| M4 [b] | 2.0 | 3.0 | 9.8 [c] |
| Arginine ethyl ester | 2.7 | 1.5 | |
| LAS | 3.4 | 2.9 | 1.8 |
| ELA | 46.7 | 40.1 | 25.0 |
| Urea | 3.8 | 5.4 | 7.8 |
| Others | 2.0 | 2.3 | 4.1 |
| Not extracted | 9.2 | 14.4 | 12.6 |
| Total | 96.6 | 100 | 93.3 |

[a] By HPLC, ornithine and arginine were equivalent to 23.5% and 1.5%, respectively, of the added radioactivity at 4 h. The relative amounts of these amino acids were not quantified at 6 or 24 h.
[b] M3 co-chromatographed with a minor unknown impurity in the radiolabelled $^{14}$C-arginine reference substance. M4 was also uncharacterised.
[c] Total of M4 and arginine ethyl ester.

*In vivo*: Six male Sprague-Dawley rats (un-fasted) received $^{14}$C-ELA (200 mg/kg bw) as a single oral (gavage) dose. The vehicle was 1% w/v methylcellulose in water. Pairs of rats were sacrificed and blood sampled at 0.5, 1 and 4 h after dosing. Concentrations of radioactivity in the plasma increased from 14.2 µg-equivalents ethyl lauroyl arginate/mL at 0.5 h to 118 µg-equivalents ethyl lauroyl arginate/mL at 4 h after dosing. Proportions of radioactive components in plasma for each rat are shown in the table below. Components were resolved using the same methods as in the above *in vitro* study.

**Table 2.7: Radioactive components in plasma as a percentage of total radioactive residue[a]**

| Radioactive component (% of total radioactive residue[a]) | Time (hours after administration) 0.5 | | 1 | | 4 | |
|---|---|---|---|---|---|---|
| | *Male 1* | *Male 2* | *Male 3* | *Male 4* | *Male 5* | *Male 6* |
| Polar material (lowest retention time)[b] | 17.2 | 13.0 | 13.2 | 21.5 | 7.4 | 7.3 |
| Ornithine | 8.5 | 6.8 | 6.9 | 2.6 | 1.5 | 1.6 |
| Arginine | 46.0 | 50.7 | 20.8 | 22.7 | 7.5 | 11.7 |
| LAS | <1.9 | <1.3 | 4.1 | <1.2 | <0.1 | 0.7 |
| ELA | 7.4 | <1.3 | 11.1 | 4.0 | 0.5 | <0.2 |
| Others [c] | <1.9 | <1.3 | 3.0 | <1.2 | 0.6 | 0.7 |
| Not extracted | 20.3 | 22.8 | 37.9 | 48.9 | 73.1 | 72.8 |

[a] % of total radioactive residue was defined as the fraction of total radioactivity administered that was extracted from plasma. The increase with time in the fraction not extracted is consistent with the extensive degradation of arginine to smaller carbon-containing compounds units and incorporation into other biological components.
[b] Possibly urea.
[c] Arginine ethyl ester, which was observed *in vitro*, was not observed in rat plasma.

**Excretion**

HLS (1998g) **Study title**: N-α-lauroyl-L-arginine ethyl ester monohydrochloride metabolism in the rat. **Report no.**: LMA 017/983416 **Report date**: 26 August 1998 **Laboratory**: Huntingdon Life Sciences Ltd, Huntingdon, Cambridgeshire, England **GLP**: Yes (OECD)

Four male Sprague-Dawley rats (un-fasted) received $^{14}$C-labelled ethyl lauroyl arginate (180 mg/kg bw) as a single oral (gavage) dose. Ethyl lauroyl arginate was labelled with $^{14}$C at the arginine carbon atoms. The vehicle was 1% w/v methylcellulose in water. During the 5 days after dosing a mean of 36.6% of the dose was excreted as carbon dioxide in expired air, 11.8% in urine, 4.3% in faeces and 0.5% in the cage-wash. The HPLC radiochromatogram of urine showed a single peak which co-eluted with urea. Components in faeces were not analysed. A mean of 46.4% of the dose was retained in the carcass at sacrifice. The mean recovery of the administered radioactivity was 99.5%.

The proposed metabolic pathway, based on the results of the above metabolism and excretion studies, is shown below. The proposed degradation products lauric acid (a fatty acid found in various vegetable oils and in human milk) and ethanol were not identifiable in the submitted studies because they would not have contained a radiolabelled carbon. An unpublished review of the above metabolism and excretion studies was submitted by the applicant (Hawkins, 2005). The conclusions of this review are consistent with the results of the above studies and the proposed metabolic pathway.

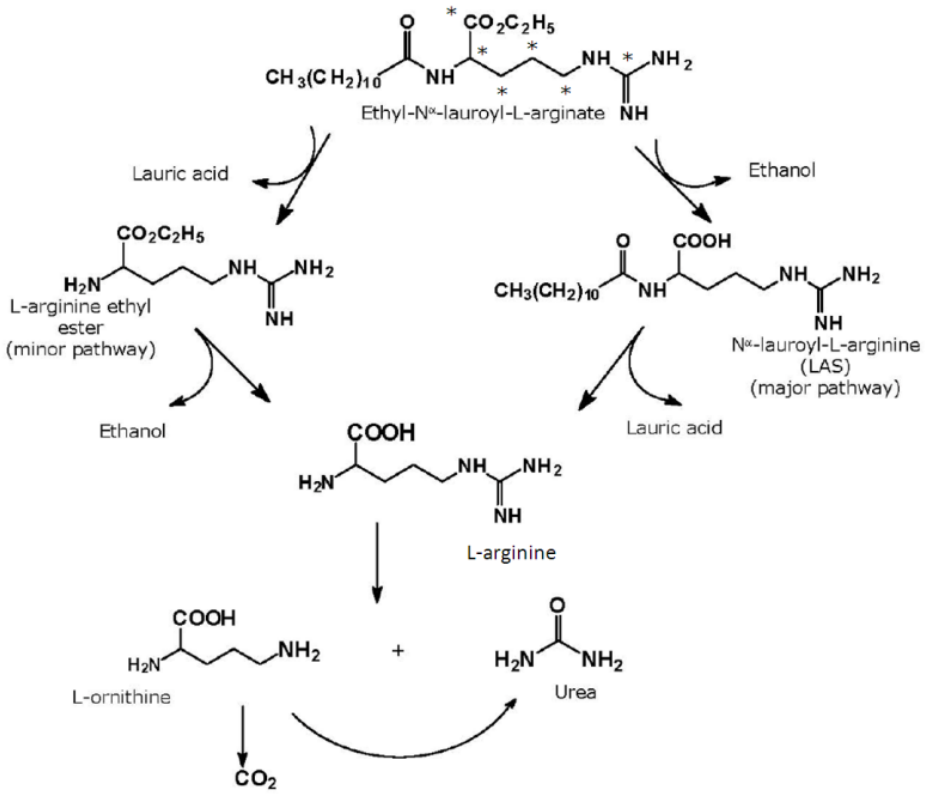


*Figure 2.1: Proposed metabolic pathway for ethyl lauroyl arginate. The positions of the $^{13}$C or $^{14}$C labels in the studies with radiolabelled ethyl lauroyl arginate are indicated with asterisks*

**Single-dose toxicity studies**

**Rats**

Single dose toxicity studies in rats were conducted using Mirenat-N, ethyl lauroyl arginate and LAS as test articles.

(i) Ethyl lauroyl arginate

HLS (2000a) **Study title**: L.A.E. acute oral toxicity to the rat. **Report no.**: LMA 018/002881/AC **Report date**: 27 July 2000 **Laboratory**: Huntingdon Life Sciences Ltd, Huntingdon, Cambridgeshire, England **GLP**: Yes (OECD)

A group of six fasted Sprague-Dawley rats (3/sex) received a single oral gavage dose of ethyl lauroyl arginate (2000 mg/kg bw) formulated in 1% w/v aqueous methylcellulose. Animals were observed for 15 days post-dose. There were no deaths. Treatment-related clinical signs consisted of piloerection and increased salivation which were both evident in all rats within 5 min of dosing. During the same period unsteady gait was observed in all females and hunched posture in all males. All clinical signs had resolved by day 3 or 4. All animals achieved satisfactory weight gain during the study period. No abnormalities were evident in any of the animals at necropsy on day 15.

(ii) LAS

Cidasal (2003a) **Study title**: Determination of acute toxicity in rats by oral route dose limit test. **Report no.**: CD02/8399T **Report date**: 31 January 2003 from **Laboratory**: Cidasal, Barcelona, Spain **GLP**: Yes (OECD)

The ethyl lauroyl arginate metabolite LAS was administered as a single dose to Sprague-Dawley rats (5/sex) by oral gavage at 2000 mg/kg bw. The observation period was 15 days followed by necropsy. There were no unscheduled deaths and no clinical signs of toxicity. Body weight gain was normal. No macroscopic alterations were observed at necropsy.

(iii) Mirenat-N

The formulation termed Mirenat-N is ethyl lauroyl arginate (20-25% w/w) dissolved in propylene glycol.

HRC (1995a) **Study title**: Mirenat-N acute oral toxicity to the rat. **Report no.**: LMA 4/951314/AC **Report date**: 17 July 1995 **Laboratory**: Huntingdon Research Centre Ltd, Huntingdon, Cambridgeshire, England. **GLP**: Yes (OECD)

A group of 10 fasted Sprague-Dawley rats (5/sex) received a single oral gavage dose of Mirenat-N (2000 mg/kg bw equivalent to 500 mg/kg bw ethyl lauroyl arginate). Animals were observed for 15 days post-dose. There were no deaths. Treatment-related clinical signs were confined to piloerection which was observed in all rats and resolved by day 2. All animals achieved satisfactory weight gain during the study period. No abnormalities were evident in any of the animals at necropsy on day 15.

**Rabbits**

RTC (2000) **Study title**: LAE Acute dermal irritation study in the rabbit **Report no.**: 7978/T/171/2000 **Report date**: 15 Dec 2000 **Laboratory**: Research Toxicology Centre, Rome, Italy **GLP**: Yes (OECD)

ELA (0.5 g + 0.5 mL water) was applied as a paste to the clipped dorsal skin of 3 female New Zealand White rabbits. The paste was spread out over a 2.5 x 2.5 $cm^2$ gauze square and applied to the skin as semi-occlusive barrier. After a period of 4 h, the patches were removed and the treated sites cleaned with cotton wool soaked in water. Reaction to treatment was assessed at approximately 1, 24, 48 and 72 h, and 7 and 14 days after the end of the exposure period. Slight to well-defined erythema was observed in all 3 treated animals approximately 1 h after the end of the 4 h exposure period. A slight erythema was still present 7 days after the end of the exposure period in 2 rabbits, with a slight oedema also present in one of these 2 animals. One of the 3 rabbits exhibited a slight erythema at the day 15 examination. Desquamation of the treated skin was also noted at 7 and 14 days after the end of the exposure. There was no indication of a systemic effect of treatment. No significant changes in body weight occurred during the course of the study.

**Repeat dose toxicity studies**

Repeat dose toxicity studies were conducted in two rat strains: Sprague-Dawley and Han Wistar. The test articles (i) ethyl lauroyl arginate and (ii) Mirenat-N (25% w/w solution of ethyl lauroyl arginate in propylene glycol) were administered via diet.

(i) Ethyl lauroyl arginate.

HLS (2000b) **Study title**: LAE dose range finding/palatability study by dietary administration to Han Wistar rats for 4 weeks. **Report no.**: LMA 030/000063 **Report date**: 17 July 2000 **Laboratory**: Huntingdon Life Sciences Ltd, Huntingdon, Cambridgeshire, England **GLP**: No

Groups of Han Wistar rats (5/sex/group) received ethyl lauroyl arginate (90.1% w/w active ingredient) in the diet at concentrations of 0, 25,000, 37,500 or 50,000 ppm for 4 weeks. The estimated intakes of ethyl lauroyl arginate were not presented in the report.

There were no deaths. Piloerection was observed in all females given 50,000 ppm. Ungroomed coats were observed in two females receiving 37,500 and all females receiving 50,000 ppm. Salivation was observed in all treated females and in most males receiving 50,000 ppm. Brown staining of the muzzle, probably dried saliva, was evident for most animals in each treated group. Body weight gain and food consumption were reduced in a dose-dependent manner in all treated animals during week 1. During weeks 2-4, body weight gain was similar to controls while food consumption remained low for treated animals. Reduced food consumption and body weight gain may be attributable to reduced palatability of the diet. Females receiving 50,000 ppm had slightly elevated haemoglobin parameters. There were no other notable haematology findings. Clinical chemistry findings indicated slight effects on the liver as indicated by low total protein, albumin and calcium concentrations in males receiving 37,500 and 50,000 ppm. Females receiving 50,000 ppm had high alkaline phosphatase, alanine aminotransferase, and aspartate aminotransferase. Females receiving 37,500 ppm had slightly higher aspartate aminotransferase and alanine aminotransferase levels compared to controls.

There were no treatment-related findings for organ weights or gross pathology. Histopathology was not investigated. A maximum diet level of 50,000 ppm ethyl lauroyl arginate was considered acceptable for a subsequent 13-week study.

HLS (2001c) **Study title**: LAE toxicity study by dietary administration to Han Wistar rats for 13 weeks. **Report no.**: LMA 031/004276 **Report date**: 28 March 2001 **Laboratory**: Huntingdon Life Sciences Ltd, Huntingdon, Cambridgeshire, England **GLP**: Yes (OECD)

Groups of Han Wistar rats (20/sex/group) received ethyl lauroyl arginate (batches contained 90.1-93.2% w/w active ingredient) in the diet at concentrations of 0, 5000, 15,000 or 50,000 ppm for 13 weeks. The control group received normal untreated diet. These diet levels resulted in ethyl lauroyl arginate intakes (calculated from weekly food consumption) of 0, 384, 1143 and 3715 mg/kg bw/day for males; and 0, 445, 1286 and 3915 mg/kg bw/day for females. Acceptable stability and homogeneity of ethyl lauroyl arginate in the 5000 ppm and 50,000 ppm diets was confirmed by analysis.

There were no deaths. Evidence of mild toxicity was observed at 15,000 and 50,000 ppm with adverse effects on appearance (ungroomed coat, brown staining on the muzzle), body weight gain, food consumption, urinalysis, clinical chemistry and haematology parameters. Findings at 5000 ppm were restricted to slightly lower body weight gain and food consumption for males only during the first week of treatment. These changes were considered to be due to reduced palatability of the diet and not a toxic effect of treatment.

Functional observational battery tests gave no indication of neurotoxicity. There were no treatment-related ophthalmic findings. Urinalysis revealed a low pH for males receiving 15,000 or 50,000 ppm relative to the control group ($p < 0.01$ for both concentrations).

Clinical chemistry findings consisted of decreased total protein for animals receiving 50,000 ppm, slightly decreased albumin for animals receiving 50,000 ppm and females receiving 15,000 ppm, and slightly decreased cholesterol for females receiving 50,000 ppm (see Table below).

**Table 2.8: Clinical chemistry findings**

| Parameter | Males | | | | Females | | | |
|---|---|---|---|---|---|---|---|---|
| | *Group 1* | *Group 2* | *Group 3* | *Group 4* | *Group 1* | *Group 2* | *Group 3* | *Group 4* |
| Total protein (g/L) | 65 | 65 | 64 | 63* | 70 | 67 | 67 | 64** |
| Albumin (g/L) | 37 | 37 | 37 | 36** | 41 | 40 | 39* | 38** |
| Cholesterol (mmol/L) | 1.58 | 1.71 | 1.35 | 1.65 | 1.86 | 1.64 | 1.55 | 1.49* |

Groups 1 to 4 refer to the 0, 5000, 15,000 and 50,000 mg/kg diet groups, respectively.
* $p \leq 0.05$; ** $p \leq 0.01$ (relative to control group).

Haematology findings consisted of slightly increased mean cell haemoglobin, mean cell haemoglobin concentration, and mean cell volume; and slightly decreased white blood cell and lymphocyte counts for males receiving 50,000 ppm (females were unaffected). See table below.

**Table 2.9: Haematology findings**

| Parameter | Males | | | | Females | | | |
|---|---|---|---|---|---|---|---|---|
| | *Group 1* | *Group 2* | *Group 3* | *Group 4* | *Group 1* | *Group 2* | *Group 3* | *Group 4* |
| Mean cell haemoglobin (pg) | 17.5 | 17.7 | 17.4 | 18.3** | 18.8 | 18.7 | 18.5 | 18.7 |
| Mean cell haemoglobin conc. (g/dL) | 35.0 | 35.1 | 35.0 | 35.4* | 35.2 | 35.4 | 35.6 | 35.3 |
| Mean cell volume (fL) | 50.1 | 50.3 | 49.7 | 51.7* | 53.4 | 52.9 | 52.0 | 52.8 |
| White blood cell counts (x $10^9$/L) | 6.97 | 6.87 | 7.15 | 5.15* | 4.00 | 4.04 | 3.51 | 3.24 |
| Lymphocyte counts (x $10^9$/L) | 5.31 | 5.24 | 5.22 | 3.82* | 2.96 | 3.07 | 2.30 | 2.51 |

Groups 1 to 4 refer to the 0, 5000, 15,000 and 50,000 mg/kg diet groups, respectively.
* $p \leq 0.05$; ** $p \leq 0.01$ (relative to control group).

There were no adverse gross pathology or organ weight findings.

Histopathological findings considered to be related to treatment were restricted to the forestomach of rats receiving 15,000 or 50,000 ppm. These findings, tabulated below, comprised parakeratosis, ulceration, erosions, and epithelial hyperplasia.

**Table 2.10: Histopathology findings for the forestomach (non-glandular region of the stomach)**

| Incidence of histopathology findings [a] | Males | | | | Females | | | |
|---|---|---|---|---|---|---|---|---|
| | *Group 1* | *Group 2* | *Group 3* | *Group 4* | *Group 1* | *Group 2* | *Group 3* | *Group 4* |
| *No. of animals examined* | 20 | 20 | 20 | 20 | 20 | 20 | 20 | 20 |
| No. of animals with: | | | | | | | | |
| Parakeratosis - *minimal* | 0 | 0 | 0 | 2 | 0 | 0 | 1 | 2 |
| *- slight* | 0 | 0 | 0 | 9 | 0 | 0 | 0 | 7 |
| *- moderate* | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 4 |
| *- total* | 0 | 0 | 0 | 13** | 0 | 0 | 1 | 13** |
| Erosion, non-glandular region - *minimal* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 |
| Ulceration, non-glandular region - *minimal* | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 |
| *- slight* | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| *- total* | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 2 |
| Epithelial hyperplasia, non-glandular region - *slight* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |

Groups 1 to 4 refer to the 0, 5000, 15,000 and 50,000 mg/kg diet groups, respectively.
** $p \leq 0.01$ (relative to control group).
[a] Histopathology findings were graded in order of increasing severity as follows: minimal, slight, moderate, marked, severe.

Because of the known surfactant activity of ethyl lauroyl arginate, it is likely that these forestomach findings are due to a direct effect on epithelial cells and are not attributable to systemic toxicity.

Because of the clinical chemistry, haematology and forestomach findings at the high dietary level of 50,000 ppm, the NOAEL was considered to be 15,000 ppm which corresponds to an ethyl lauroyl arginate dose of 1143 mg/kg bw/day in males and 1286 mg/kg bw/day in females. However, despite the low incidence (1/20) of some forestomach findings at 15,000 ppm, it is possible that these findings are treatment related and that this dietary concentration may approximate the threshold for the onset of adverse effects on the forestomach.

HLS (2005a) **Study title**: Lauric arginate toxicity study by dietary administration to CD rats for 52 weeks. **Report no.**: LMA 050/042556 **Report date**: 25 November 2005 **Laboratory**: Huntingdon Life Sciences Ltd, Huntingdon, Cambridgeshire, England. **GLP**: Yes (OECD)

Groups of Crl:CD(SD)IGS BR rats (20/sex/group) received diets containing ethyl lauroyl arginate (88.2% w/w active ingredient) at concentrations of 0, 2000, 6000 or 18,000 mg/kg diet for 52 weeks (referred to as groups 1 to 4 in tables below). The control group received normal untreated diet. Acceptable stability of ethyl lauroyl arginate in samples from all treatment diets was confirmed by analysis.

Homogeneity was analysed only for the 2000 mg/kg diet and shown to be acceptable. The diet levels resulted in ethyl lauroyl arginate doses of 0, 106, 307 and 907 mg/kg bw/day for males; and 0, 131, 393 and 1128 mg/kg bw/day for females. Clinical signs, body weight, and food and water consumption were recorded during the treatment period. During weeks 14, 26 and 52, haematological, clinical chemistry, urinalysis and ophthalmology were performed. During week 49, neurobehavioural screening (sensory reactivity, grip strength and motor activity) was performed on 10 males and 10 females from each group.

Gross pathology, organ weights, histopathology, bone marrow smears, and toxicokinetics were investigated at the end of the 52-week dosing period.

There were six unscheduled deaths during the study, one control male, 3 low dose animals and one high dose female. None of the deaths were considered to be attributable to treatment. There were no clinical signs of toxicity at 2000 ppm. At 6000 ppm, females exhibited increased incidences of brown fur staining in the period of week 1 to 13. At 18,000 ppm, females exhibited increased incidences of brown fur staining in the period of weeks 1 to 13 and increased incidences of ungroomed coat during weeks 4 to 12.

Body weight gain was unaffected at 2000 ppm but was reduced in both sexes at 6000 and 18,000 ppm, most notably in the early weeks of the study. Decreased food consumption was evident in the 6000 and 18,000 ppm groups in the first week of the study. These effects are likely to be due to reduced palatability of the diet and not toxicologically relevant.

Haematology findings: there were treatment-related effects on white cell parameters for both sexes as shown in the table below. However, in the absence of any treatment-related effects on the bone marrow and the lack of any histopathology associated with the lymphoid tissues, the white cell changes were not considered to be of toxicological importance.

**Table 2.11: White blood cell counts**

| Cell count (x $10^9$/L) | Week | Males | | | | Females | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | *Group 1* [a] | *Group 2* | *Group 3* | *Group 4* | *Group 1* | *Group 2* | *Group 3* | *Group 4* |
| White blood cells | 14 | 11.99 | 11.24 | 11.09 | 10.19 | 7.74 | 8.14 | 7.31 | 7.13 |
| | 26 | 12.24 | 9.49* | 10.49* | 9.09** | 7.15 | 6.49 | 5.12* | 4.67** |
| | 52 | 11.48 | 9.10 | 10.39 | 8.72** | 6.87 | 6.85 | 6.92 | 5.13 |
| Neutrophils | 14 | 1.45 | 1.45 | 1.28 | 1.46 | 0.93 | 0.90 | 0.82 | 0.59* |
| | 26 | 1.68 | 1.56 | 1.71 | 1.39 | 0.96 | 0.92 | 1.16 | 0.59* |
| | 52 | 2.38 | 2.19 | 1.97 | 1.23** | 1.85 | 1.34 | 2.07 | 0.87* |
| Lymphocytes | 14 | 9.80 | 9.11 | 9.15 | 8.15 | 6.24 | 6.82 | 6.03 | 6.17 |
| | 26 | 9.49 | 7.16* | 7.94* | 7.00** | 5.59 | 5.17 | 3.60** | 3.77** |
| | 52 | 7.89 | 6.07 | 7.37 | 6.68 | 4.23 | 4.90 | 4.17 | 3.75 |
| Basophils | 14 | 0.04 | 0.03 | 0.04 | 0.04 | 0.02 | 0.02 | 0.02 | 0.02 |
| | 26 | 0.04 | 0.03 | 0.04 | 0.03 | 0.02 | 0.01 | 0.01 | 0.01 |
| | 52 | 0.04 | 0.02** | 0.03** | 0.02** | 0.01 | 0.02 | 0.01 | 0.01 |
| Monocytes | 14 | 0.31 | 0.27 | 0.24* | 0.23* | 0.24 | 0.16* | 0.18* | 0.14** |
| | 26 | 0.37 | 0.29 | 0.30 | 0.26* | 0.24 | 0.15** | 0.14** | 0.12** |
| | 52 | 0.58 | 0.41* | 0.49* | 0.38** | 0.43 | 0.29 | 0.37 | 0.24** |
| Large unstained cells | 14 | 0.29 | 0.26 | 0.27 | 0.19** | 0.20 | 0.13* | 0.15* | 0.11** |
| | 26 | 0.50 | 0.31** | 0.37** | 0.27** | 0.23 | 0.13** | 0.10** | 0.11** |
| | 52 | 0.43 | 0.28* | 0.33* | 0.25** | 0.23 | 0.16 | 0.17 | 0.15* |

[a] Groups 1 to 4 refer to the 0, 2000, 6000 and 18,000 mg/kg diet groups, respectively.
* $p < 0.05$; ** $p < 0.01$ (relative to control group).

Clinical chemistry findings were limited to increased urea concentration (by 26% over control group, $p < 0.05$) observed at week 52 in females receiving 18,000 ppm. Urinalysis findings were limited to decreased urine volume at week 12 in males receiving 18,000 ppm (by 38%, $p < 0.01$) and at week 52 in females receiving 18,000 ppm (by 49%, $p < 0.01$).

Gross pathology and histopathology findings were considered to be treatment-related only for the forestomach as shown in the table below. The severity of these findings was described as minimal or slight.

**Table 2.12: Gross pathology and histopathology findings for the forestomach (non-glandular region of the stomach)**

| Incidence of gross pathology and histopathology findings | Males | | | | Females | | | |
|---|---|---|---|---|---|---|---|---|
| | *Group 1* | *Group 2* | *Group 3* | *Group 4* | *Group 1* | *Group 2* | *Group 3* | *Group 4* |
| **Gross pathology** [a] *No. examined* | 19 | 18 | 20 | 19 | 20 | 19 | 20 | 19 |
| No. of animals with: | | | | | | | | |
| Forestomach depression(s) | 0 | 1 | 5 | 12 | 2 | 5 | 6 | 9 |
| **Histopathology** [b] *No. examined* | 20 | 20 | 20 | 20 | 20 | 20 | 20 | 20 |
| No. of animals with: | | | | | | | | |
| Epithelial hyperplasia, non-glandular- *minimal* | 0 | 1 | 2 | 1 | 0 | 0 | 0 | 1 |
| - *slight* | 0 | 0 | 1 | 8 | 1 | 3 | 5 | 7 |
| - *total* | 0 | 1 | 3 | 9** | 1 | 3 | 5 | 8* |
| Sub-epithelial/mucosal inflammation, non-glandular - *minimal* | 0 | 1 | 5 | 5 | 1 | 4 | 3 | 7 |
| - *slight* | 0 | 0 | 0 | 1 | 1 | 1 | 2 | 1 |
| - *total* | 0 | 1 | 5 | 6 | 2 | 5 | 5 | 8 |
| Sub-epithelial fibrosis, non-glandular - *minimal* | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 0 |
| Muscle layer inflammation- *minimal* | 0 | 0 | 1 | 1 | 1 | 3 | 3 | 4 |
| Serosal inflammation - *minimal* | 0 | 0 | 0 | 0 | 0 | 4 | 2 | 1 |
| - *slight* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| - total | 0 | 0 | 0 | 0 | 0 | 4 | 2 | 2 |
| Erosion, non-glandular epithelium - *slight* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| Ulceration, non-glandular epithelium - *minimal* | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 3 |
| - *slight* | 0 | 0 | 2 | 0 | 1 | 2 | 3 | 3 |
| - *total* | 0 | 0 | 3 | 0 | 1 | 3 | 3 | 6 |
| Re-epithelialisation, non-glandular - *minimal* | 0 | 0 | 0 | 3 | 0 | 1 | 1 | 3 |
| - *slight* | 0 | 0 | 1 | 0 | 1 | 2 | 1 | 2 |
| - *total* | 0 | 0 | 1 | 3 | 1 | 3 | 2 | 5 |

Groups 1 to 4 refer to the 0, 2000, 6000 and 18,000 mg/kg diet groups, respectively.
* $p < 0.05$; ** $p < 0.01$ (relative to control group).
[a] Statistical analysis was not conducted on the gross pathology findings.
[b] Histopathology findings were graded in order of increasing severity as follows: minimal, slight, moderate, marked, severe.

There was no correlation between the individual animals which showed lower body weight gain, poor grooming and/or brown fur staining and the presence of these forestomach findings. Nor was there any correlation between the animals which showed forestomach lesions and those which exhibited white blood cell and/or biochemical disturbances.

Because ethyl lauroyl arginate is a cationic surfactant which affects the integrity of cell membranes it is likely that these forestomach findings are due to a direct effect of ethyl lauroyl arginate on epithelial cells and are not attributable to systemic toxicity.

There were no treatment-related effects on neurobehavioural parameters, ophthalmology, bone marrow smears, and organ weights.

Blood samples were taken over a 24 h period during week 52 in order to assess systemic exposure to ethyl lauroyl arginate and the metabolite LAS. Blood samples were taken from 3 satellite rats per sex from each dose group at 6:00 pm, 10:00 pm, 2:00 am, 6:00 am, 10:00 am and 2:00 pm and analysed using a validated LC-MS/MS method. Toxicokinetics results shown in the table below indicate that exposure of males to ethyl lauroyl arginate, as indicated by maximum plasma concentrations, was approximately proportional to dietary concentration. Females exhibited greater exposure than males to ethyl lauroyl arginate at all concentrations. The increase in exposure with dietary concentration was less than proportional for both sexes. Exposure to the metabolite LAS was greater than exposure to ethyl lauroyl arginate for both sexes. Maximum plasma concentrations of ethyl lauroyl arginate and LAS usually occurred during the hours of darkness (corresponding to periods of nocturnal feeding activity).

**Table 2.13: $C_{max}$ and $AUC_{0\text{-}24\ h}$ values for ethyl lauroyl arginate and LAS**

| Dietary concentration | ELA | | | | LAS | | | |
|---|---|---|---|---|---|---|---|---|
| | $C_{max}$ (ng/mL) | | $AUC_{0\text{-}24\ h}$ (ng.h/mL) | | $C_{max}$ (ng/mL) | | $AUC_{0\text{-}24\ h}$ (ng.h/mL) | |
| (ppm) | *males* | *females* | *males* | *females* | *males* | *females* | *males* | *females* |
| 2000 | 1.15 | 11.3 | 19.5 | 78.3 | 10.5 | 12.7 | 46.8 | 169 |
| 6000 | 6.92 | 22.9 | 66.8 | 130 | 18.7 | 26.4 | 286 | 368 |
| 18000 | 17.6 | 26.3 | 190 | 244 | 62.2 | 59.6 | 960 | 1130 |

In conclusion, the NOAEL was considered to be 6000 ppm corresponding to estimated ethyl lauroyl arginate intakes of 307 mg/kg bw/day for males and 393 mg/kg bw/day for females. This NOAEL was based on the local irritant changes in the forestomach at the high dietary concentration of 18,000 ppm. The forestomach findings at 6000 ppm were not considered to be of sufficient severity or incidence to be regarded as toxicologically adverse.

(ii) Mirenat-N

HLS (1995) **Study title**: Mirenat-N preliminary toxicity to rats by dietary administration for 4 weeks **Report no.**: LMA 2/952124 **Report date**: 14 December 1995 **Laboratory**: Huntingdon Life Sciences Ltd, Huntingdon, Cambridgeshire, England **GLP**: Yes (OECD)

Groups of Crl:CD(SD)BR rats (5/sex/group) received diets containing Mirenat-N at concentrations of 0, 3200, 12,800 or 50,000 mg Mirenat-N/kg diet for 4 weeks. These diet levels resulted in ethyl lauroyl arginate intakes (calculated from weekly food consumption) of 0, 84, 348 and 1317 mg/kg bw/day for males; and 0, 88, 350 and 1462 mg/kg bw/day for females. Control animals received normal untreated diet. Clinical signs, body weight, and food and water consumption were recorded during the treatment period.

During week 4, haematological and clinical chemistry analyses were performed. Gross pathology and organ weights were also investigated.

There were no deaths or treatment-related findings at any dose level. It was concluded that a maximum level of 50,000 mg Mirenat-N/kg diet was acceptable for a subsequent 13-week study.

HLS (1996) **Study title**: Mirenat-N toxicity to rats by dietary administration for 13 weeks. **Report no.**: LMA 3/961342. **Report date**: 4 November 1996 **Laboratory**: Huntingdon Life Sciences Ltd, Huntingdon, Cambridgeshire, England. **GLP**: Yes (OECD, FDA).

Groups of Crl:CD(SD)BR rats (10/sex/group) received diets containing Mirenat-N at concentrations of 0, 3200, 12,800 or 50,000 mg Mirenat-N/kg diet for 13 weeks. These diet levels resulted in ethyl lauroyl arginate intakes (calculated from weekly food consumption) of 0, 55, 226 and 831 mg/kg bw/day for males; and 0, 66, 267 and 982 mg/kg bw/day for females. Control animals received normal untreated diet. Clinical signs, body weight, and food and water consumption were recorded during the treatment period. During week 13, haematological, clinical chemistry, urinalysis and ophthalmology were performed. Gross pathology, organ weights and histopathology were also investigated.

One control male and one control female died during the treatment period. The male collapsed and died during week 1 on being returned to the cage following the daily clinical examination. No clinical signs were noted during its lifetime. Post mortem examination revealed a ruptured liver, probably incurred during handling, as the likely cause of death. The female died under anaesthesia during laboratory investigations. 'Anaesthetic trauma' was listed as the possible cause of death.

There were no treatment-related clinical signs observed during the study. Body weight gain for treated females was lower than the controls (88, 79 and 86% that of the controls for the three doses, respectively). The lower body weight gain was statistically significant ($p \leq 0.01$) at the mid (12,800 ppm) and high (50,000 ppm) dietary levels. In the absence of a dose-response relationship and any effect in the males, the lower body weight gain in females is of uncertain relationship to treatment. Food intake was unaffected by treatment for males and females. Males receiving 50,000 mg/kg diet had a slightly higher water consumption (117% of the control value); however this was not statistically significant. There were no treatment-related ophthalmology findings.

As shown in the table below, there was a slightly lower total white blood cell count in females receiving 12,800 and 50,000 mg/kg diet, predominantly due to reduced counts for lymphocytes, monocytes and large unstained cells. In males, there was a slightly lower neutrophil count at 12,800 and 50,000 mg/kg diet. Because there was no consistency in the type of white blood cell contributing to the lower total cell count these findings are unlikely to be toxicologically significant. There were no changes in the other haematological parameters investigated.

**Table 2.14: White blood cell counts**

| Cell count ($\times 10^9$/L) | Males | | | | Females | | | |
|---|---|---|---|---|---|---|---|---|
| | *Group 1* | *Group 2* | *Group 3* | *Group 4* | *Group 1* | *Group 2* | *Group 3* | *Group 4* |
| White blood cells | 14.58 | 14.03 | 13.54 | 12.65 | 8.65 | 8.01 | 6.71* | 6.25** |
| Neutrophils | 1.79 | 1.52 | 1.30** | 1.29** | 0.97 | 0.92 | 0.68 | 0.92 |
| Lymphocytes | 11.86 | 11.69 | 11.41 | 10.34 | 7.06 | 6.63 | 5.68* | 4.98** |

| Cell count (x $10^9$/L) | Males | | | | Females | | | |
|---|---|---|---|---|---|---|---|---|
| | *Group 1* | *Group 2* | *Group 3* | *Group 4* | *Group 1* | *Group 2* | *Group 3* | *Group 4* |
| Eosinophils | 0.17 | 0.13 | 0.16 | 0.18 | 0.16 | 0.12 | 0.09 | 0.12* |
| Basophils | 0.04 | 0.03 | 0.03 | 0.03 | 0.01 | 0.02 | 0.01 | 0.01 |
| Monocytes | 0.22 | 0.20 | 0.22 | 0.34 | 0.16 | 0.13 | 0.09** | 0.08** |
| Large unstained cells | 0.50 | 0.46 | 0.42 | 0.47 | 0.28 | 0.20** | 0.16** | 0.13** |

Groups 1 to 4 refer to the 0, 3200, 12,800 and 50,000 mg/kg diet groups, respectively.
* $p \leq 0.05$; ** $p \leq 0.01$ (relative to control group).

There were no treatment-related clinical chemistry changes. Slightly higher urine volume (121% of the control value, but not statistically significant) was observed in males receiving 50,000 mg/kg diet. This is consistent with the increased water intake recorded for this group. In contrast, decreased urine volume was observed in a repeat-dose study in rats with ethyl lauroyl arginate as the test article [HLS (2005a), above]. The slightly higher water intake and urine volume observed in the present study may be related to the high concentration of propylene glycol in the Mirenat-N test article.

Females receiving 50,000 mg/kg diet had a slightly higher group mean liver weight (body weight relative, 112% of control value, $p \leq 0.05$). However, no microscopic changes were detected in the liver and there were no associated clinical chemistry findings. The slightly increased mean relative liver weight is therefore not considered to be an adverse effect.

At necropsy, a high incidence (50 and 80%, respectively) of alopecia was observed in females at 12,800 and 50,000 mg/kg diet. In isolation this change is considered to be of uncertain biological significance. No other treatment-related changes were observed macroscopically or microscopically.

The NOAEL for Mirenat-N was concluded in the study report to be 12,800 mg/kg diet (equal to 226 mg ethyl lauroyl arginate/kg bw/day) based on evidence of slight changes in males and females at 50,000 mg/kg diet, when compared with controls (lower body weight gain of females, increased water consumption and urine volume of males, and higher group mean adjusted liver weights of females). However, as discussed above, all of these findings are unlikely to be toxicologically relevant, especially since animals in the control group received normal untreated diet. Comparisons between groups receiving Mirenat-N and the control group would be more robust if control animals had received propylene glycol vehicle in the diet.

In the absence of any adverse effects, a NOAEL of 50,000 mg/kg diet (equivalent to 831 and 982 mg ethyl lauroyl arginate/kg bw/day for males and females, respectively) is considered appropriate.

## Genotoxicity studies

Ethyl lauroyl arginate was tested in several *in vitro* genotoxicity assays and the major metabolite LAS was tested in an *in vitro* and an *in vivo* assay as summarised in the table below. These studies were conducted in compliance with GLP (OECD). The *in vitro* assays were performed both in the presence and absence of liver preparations from Aroclor 1254-induced rats (S9 mix, as indicated by ±S9 in the table). An appropriate high dose was tested in the *in vivo* study.

In the preliminary cytotoxicity tests for the *in vitro* assays, ethyl lauroyl arginate was cytotoxic at relatively low concentrations consistent with the cell membrane disrupting activity of the compound. The main tests in these assays used appropriate lower concentrations as shown in the table below. These *in vitro* concentrations of ethyl lauroyl arginate, while lower than those typically recommended for these assays, are far greater than the ethyl lauroyl arginate concentrations observed systemically in *in vivo* studies.

ELA and LAS showed no evidence of mutagenic or clastogenic activity in these assays. Some evidence of polyploidy was observed at cytotoxic concentrations in study HLS (2001b), but this is unlikely to be of biological significance. Negative and positive controls were used in all studies and gave expected results.

**Table 2.15: Genotoxicity assays**

| Test type | Test system | Test article | Mirenat-N, ELA, LAS concentrations /dosages | Result | Reference |
|---|---|---|---|---|---|
| Bacterial reverse mutation | *S. typhimurium* TA 1535, TA 1537, TA 98, TA 100 *E. coli* WP2 uvrA pKM 101 (±S9) | ELA (93.2% w/w active ingredient) dissolved in DMSO | 5 – 5000 µg/plate (preliminary) then 0.15 – 150 µg/plate (plate incorporation) and 1.5 – 150 µg/plate (pre-incubation) | Negative See footnote[a] regarding cytotoxicity | HLS (2001a) |
| Mammalian cell mutation | Mouse lymphoma L5178Y cells (±S9). 3 h (±S9) and 24 h (-S9) treatment. | ELA (88.2% w/w active ingredient) dissolved in DMSO | 0.20 – 600 µg/mL (prelim. toxicity test) then 1 – 50 µg/mL | Negative. See footnote[b] regarding cytotoxicity | HLS (2004) |
| Chromosome aberration | Human lymphocytes *in vitro* (±S9). Test (i): 3 h treatment, 17 h recovery; (ii) 20 h treatment. | ELA (93.2% w/w active ingredient) dissolved in DMSO | 50 – 200 µg/mL | Negative. Some evidence of polyploidy at cytotoxic concs (See footnote[c]) | HLS (2001b) |
| Bacterial reverse mutation | *S. typhimurium* TA 1535, TA 1537, TA 98, TA 100 (±S9) | Mirenat-N (ELA 25% w/w in propylene glycol) dissolved in water | 5 – 5000 µg/plate (preliminary) then 5 – 500 µg/plate (pre-incubation) | Negative See footnote[d] regarding cytotoxicity | HRC (1995b) |
| Mammalian cell mutation | Mouse lymphoma L5178Y cells (±S9). 3 h (±S9) and 24 h (-S9) treatment. | Mirenat-N dissolved in water | 15 – 2000 µg/mL (prelim. toxicity test) then 100 – 300 µg/mL (-S9) 100 – 500 µg/mL (+S9) | Negative. See footnote[e] regarding cytotoxicity | HRC (1995c) |
| Chromosome aberration | Human lymphocytes *in vitro* | Mirenat-N dissolved in water | 125 – 1000 µg/mL | Negative. See footnote[f] regarding | HRC (1995d) |

| Test type | Test system | Test article | Mirenat-N, ELA, LAS concentrations /dosages | Result | Reference |
|---|---|---|---|---|---|
| | (±S9). Test (i): 3 h treatment, 15 h recovery; (ii) 3 h treatment, 29 h recovery | | | cytotoxicity | |
| Bacterial reverse mutation | *S. typhimurium* TA 1535, TA 1537, TA 98, TA 100 *E. coli* WP2 uvrA pKM 101 (±S9) | LAS ($N^{\alpha}$-lauroyl-L-arginine) dissolved in DMSO | 156 – 5000 µg/plate | Negative See footnote[g] regarding cytotoxicity | Cidasal (2003b) |
| Micronucleus formation | Mouse (CD-1) bone marrow (sampled 24 h and 48 h post-dose). N = 5 males/group. | LAS dissolved in water | 2000 mg/kg bw, single oral gavage dose | Negative | Cidasal (2003c) |

[a] The maximum tested concentration for bacterial reverse mutation assays usually extends up to 5000 µg/plate, but testable concentrations were limited because of the antibacterial properties of the compound. Cytotoxicity, observed as an absence or thinning of the bacterial lawn or a reduction in the number of revertants, was observed in all strains at ≥ 150 µg/plate. Therefore, a maximum concentration of 150 µg/plate was therefore selected for the subsequent tests.
[b] Relative suspension growth was negligible at ELA concentrations ≥ 50 µg/mL.
[c] In the absence/presence of S9, ELA (200 µg/mL) caused a reduction in the mitotic index to 31%/32% of the solvent control value. Mitotic index reduction of > 50% is usually considered to represent an appropriate level of cytotoxicity at the maximum concentration for this assay.
[d] Cytotoxicity was observed in all strains at Mirenat-N concentrations ≥ 500 µg/plate. A maximum concentration of 500 µg/plate was therefore selected for the subsequent tests.
[e] Relative suspension growth was negligible at Mirenat-N concentrations ≥ 500 µg/mL.
[f] In the absence/presence of S9, Mirenat-N (1000 µg/mL) caused a reduction in the mitotic index to 7%/18% of the solvent control value.
[g] For LAS, cytotoxicity was observed in the strains TA-1535, TA-1537 and TA-100 only at the maximum tested concentration of 5000 µg/plate.

### Carcinogenicity studies

No carcinogenicity studies were submitted. This is considered acceptable because the compound did not exhibit genotoxicity and there was no evidence of treatment-related pre-neoplasia or neoplasia in the 52-week repeat-dose toxicity study in rats.

### Reproductive toxicity studies

HLS (2003b) **Study title**: LAE preliminary study of effects on reproductive performance in CD rats by dietary administration. **Report no.**: LMA 041/032575 **Report date**: 3 July 2003 **Laboratory**: Huntingdon Life Sciences Ltd, Huntingdon, Cambridgeshire, England. **GLP**: Yes (OECD)

Sprague-Dawley rats (n = 8/sex/group, age 9-10 weeks at commencement of treatment) were fed diets containing ethyl lauroyl arginate (88.2% w/w active ingredient) at concentrations of 0, 1500, 5000 or 15,000 ppm for 4 weeks prior to mating. Animals were terminated after weaning of the litters. Pups selected to form the F1 generation (n = 12/sex/group) were continuously treated from the time of weaning until terminated at approximately 8 weeks of age.

The general condition of animals receiving diets containing ethyl lauroyl arginate was similar to that of controls and no unscheduled deaths occurred. Food consumption and body weight gain of F0 males and females were not adversely affected by treatment and there were no adverse effects on body weight gain for females during gestation and lactation. Pup body weight at day 1 of again, body weight gain to weaning, and body weight of selected F1 males and females to 8 weeks of age, were unaffected by treatment. Food consumption by selected F1 animals was similar to that of controls. Calculated intakes were proportional to the dietary concentrations as shown in the table below.

**Table 2.16: Average intakes for F0 animals before pairing and F1 animals**

| Dietary concentration (ppm) | 1500 | 5000 | 15,000 |
|---|---|---|---|
| Average F0 intake before pairing (mg/kg bw/day) | | | |
| Males | 113 | 380 | 1151 |
| Females | 123 | 432 | 1295 |
| Average intake for F1 animals (mg/kg bw/day) | | | |
| Males | 173 | 589 | 1750 |
| Females | 169 | 586 | 1734 |

Mating performance, fertility, litter size and growth were unaffected by the presence of ethyl lauroyl arginate in the diet at 1500 and 5000 ppm. At 15,000 ppm, the litters of 2 of the 8 females lost weight in the first 4 days after birth and when killed for humane reasons at day 4 of age were found to have no milk in their stomachs. Survival of pups within remaining litters at 15,000 ppm was slightly below that of controls.

Sexual maturation in males was unaffected by treatment but vaginal opening was delayed by approximately 4 days in females treated at 15,000 ppm as shown in the table below. Subsequent establishment of the normal oestrous cycle was demonstrated in all groups.

**Table 2.17: Age at vaginal opening for pups selected as the F1 generation (days)[a]**

| Dietary concentration (ppm) | 0 | 1500 | 5000 | 15,000 |
|---|---|---|---|---|
| Age at vaginal opening (days)[b] | 35.6 (2.2) | 36.8 (2.7) | 36.3 (2.4) | 39.5 (2.0) |

[a] No analyses of statistical significance were performed in this preliminary study.
[b] Mean (Standard deviation)

Necropsy of F0 parental animals and pups (killed at approximately 8 weeks of age) did not reveal any effects related to treatment.

It was concluded that a dietary concentration of 15,000 ppm could be used as the highest treatment level for a two-generation study.

HLS (2005b) **Study title**: LAE two generation reproductive performance study by dietary administration to CD rats. **Report no.**: LMA 042/032553 **Report date**: 6 April 2005 **Laboratory**: Huntingdon Life Sciences Ltd, Huntingdon, Cambridgeshire, England. **GLP**: Yes (OECD)

Sprague-Dawley rats (n = 28/sex/group, age 6 weeks at commencement of treatment) were fed diets containing ethyl lauroyl arginate (88.2% w/w active ingredient) at concentrations of 0, 2500, 6000 or 15,000 ppm for 10 weeks prior to pairing, throughout pairing, gestation, lactation and until termination. All females were pregnant in the control group, 27 were pregnant in the 2500 and 6000 ppm groups and 26 in the 15,000 ppm group. Animals selected for the F1 generation comprised 24 male and 24 female progeny from each group (typically one animal/sex/litter). After weaning, F1 animals received the relevant diet as per the F0 generation throughout the study until termination.

The F1 generation were mated to produce the F2 generation which was raised to weaning and then the study was terminated. Mean achieved dosages (mg/kg bw/day) during the study were as follows:

**Table 2.18: Mean achieved dosages (mg/kg bw/day)**

| | Males | | | Females | | |
|---|---|---|---|---|---|---|
| **Dietary concentration (ppm)** | **2500** | **6000** | **15,000** | **2500** | **6000** | **15,000** |
| F0 generation | | | | | | |
| Before pairing | 181 | 434 | 1073 | 207 | 502 | 1226 |
| During gestation | - | - | - | 231 | 585 | 1518 |
| During lactation | - | - | - | 402 | 1018 | 2600 |
| F1 generation | | | | | | |
| Before pairing | 224 | 537 | 1356 | 246 | 582 | 1489 |
| During gestation | - | - | - | 215 | 535 | 1430 |
| During lactation | - | - | - | 409 | 898 | 2353 |

The general condition of F0 and F1 animals receiving ethyl lauroyl arginate was similar to that of controls. Body weight and body weight gain of adult F0 and F1 animals were not affected by treatment. Food consumption was unaffected by treatment for both generations.

There were no adverse effects in either generation on pre-mating oestrous cycles, mating performance, fertility, litter size, pup survival and day 1 body weight. Pre-weaning reflex tests for F1 and F2 pups were not affected by treatment.

Pups were weaned on day 21 of age. Body weight gain was not affected by treatment during the periods 1-7 and 1-14 days of age. However, body weight gain over the full pre-weaning period (age 1-21 days) was reduced by approximately 10% for F1 males and females receiving 15,000 ppm compared to controls as shown in the table below. The relative magnitude of this reduced body weight gain did not significantly change in the several days following weaning (*i.e.* the relative reduction in body weight gain was similar for the periods 1-21 days and 1-25 days). Thus, most of the reduction in body weight gain occurred in the week prior to weaning (days 14 to 21 of age).

**Table 2.19: Body weight gain of F1 pups**

| **Dietary concentration (ppm)** | **0** | **2500** | **6000** | **15,000** |
|---|---|---|---|---|
| **Males** | | | | |
| Body weight gain (grams): 1-7 days of age | 7.9 (1.3) | 7.8 (2.8) | 7.1 (2.6) | 7.9 (1.6) |
| 1-14 days of age | 24.2 (2.5) | 25.0 (3.6) | 22.4 (4.9) | 24.4 (2.2) |
| 1-21 days of age | 41.9 (5.5) | 42.5 (5.7) | 38.5 (6.0) | 38.0 (2.8)** |
| 1-25 days of age | 60.6 (6.7) | 61.5 (7.7) | 58.4 (6.4) | 55.8 (3.7)** |
| **Females** | | | | |
| Body weight gain (grams): 1-7 days of age | 7.5 (1.2) | 7.7 (2.3) | 7.1 (2.3) | 7.6 (1.5) |
| 1-14 days of age | 23.6 (2.2) | 24.1 (3.3) | 23.0 (2.9) | 23.1 (2.5) |
| 1-21 days of age | 40.1 (4.5) | 40.6 (4.4) | 38.9 (3.3) | 36.1 (2.4)** |
| 1-25 days of age | 56.9 (5.2) | 57.8 (7.3) | 55.9 (5.9) | 52.0 (3.7)** |

[a] Mean (Standard deviation)
** Significant ($p < 0.01$) when compared with the control group.

Balano-preputial separation was unaffected at all dosage levels. A delay in vaginal opening of approximately 4 days was recorded at 15,000 ppm as shown in the table below. Treatment had no impact on estrous cycles pre-pairing or pre-termination, fertility or primordial follicle counts. Anogenital distance in the F2 pups was also unaffected by treatment.

**Table 2.20: Age at vaginal opening for pups selected as the F1 generation**

| Dietary concentration (ppm) | 0 | 2500 | 6000 | 15,000 |
|---|---|---|---|---|
| Age at vaginal opening (days) | 33.0 (2.3) | 33.9 (2.6) | 34.3 (2.7) | 37.0 (2.0)** |
| No. aged ≥ 39 days at vaginal opening | 0 | 0 | 1 | 5 |

[a] Mean (Standard deviation)
** Significant ($p < 0.01$) when compared with the control group.

Terminal investigations of F0 and F1 adult animals showed no effects on pre-termination oestrous cycles or on sperm assessments. Macroscopic examination of adult animals and pups revealed no changes attributable to treatment. In the 15,000 ppm group, spleen weights (absolute and/or body weight relative) of F0 and F1 females at scheduled termination and of F1 male and F1 female weanlings and F2 female weanlings on day 30 of age were significantly lower than controls. The magnitude of the difference reduced with age and was not accompanied by any macroscopic or microscopic changes in F0 or F1 adult animals. This effect was therefore considered to be of no toxicological importance.

Because of the delay in vaginal opening observed at 15,000 ppm in both the preliminary study and the main study, the NOAEL was considered to be 6000 ppm which corresponds to an ethyl lauroyl arginate intake of 502 mg/kg bw/day. This dose level corresponds to the calculated intake before pairing of males and females. Higher dietary intakes of ethyl lauroyl arginate were observed in females during gestation (585 mg/kg bw/day) and lactation (1018 mg/kg bw/day); however, because the relevant time of exposure for the delayed puberty effect is not known, the lower intake (before pairing) is considered to be the appropriate NOAEL.

**Developmental toxicity studies**

Developmental toxicity studies were conducted in rats and rabbits by the oral (gavage) route as summarised below. The active ingredient content in the ethyl lauroyl arginate batch used in these studies was 69.1% w/w. Therefore, this material does not meet the JECFA specifications for content of the active ingredient (85 to 95% w/w). However, the lower content of ethyl lauroyl arginate in this batch was due to a high water content (23% w/w) because the synthesis product was not subject to a drying step, not due to higher impurity levels. In the developmental toxicity studies below, the administered doses have been corrected for water content such that the active ingredient content corresponds to 90% w/w. This allows the doses used in these studies to be compared more readily to the doses in studies which used batches that conform to the JECFA specifications.

## RATS

HLS (1998a) **Study title**: LAE study of tolerance in the rat by oral gavage administration. **Report no.**: LMA011/980114 **Report date**: 6 August 1998 **Laboratory**: Huntingdon Life Sciences Ltd, Huntingdon, Cambridgeshire, England. **GLP**: Yes (OECD)

ELA (69.1% w/w active ingredient) was administered by oral gavage to four non-pregnant Sprague-Dawley rats at an initial dosage of 250 mg/kg bw/day (corrected dose 192 mg/kg bw/day) for two days. The dosage was doubled every two days up to a maximum of 2000 mg/kg bw/day (corrected dose 1536 mg/kg bw/day). A separate group of presumed pregnant females (n = 4) received ethyl lauroyl arginate at 2000 mg/kg bw/day (corrected dose 1536 mg/kg bw/day) from days 6 to 13 of gestation.

There were no deaths during the treatment phase. Salivation was recorded on a number of occasions in both groups for a short period immediately after dosing. The frequency of salivation was increased at corrected doses of 768 and 1536 mg/kg bw/day. The general condition and body weights of the two groups of rats were not significantly affected by treatment and there were no treatment-related adverse gross pathology findings. All presumed-pregnant females were pregnant and embryo survival to gestation day 13 was unaffected by treatment.

HLS (1998b) **Study title**: LAE preliminary study of embryo-foetal toxicity in the CD rat by oral gavage administration. **Report no.**: LMA013/980140 **Report date**: 6 August 1998 **Laboratory**: Huntingdon Life Sciences Ltd, Huntingdon, Cambridgeshire, England. **GLP**: Yes (OECD)

ELA (69.1% w/w active ingredient) was administered by oral gavage at doses of 200, 600 or 2000 mg/kg bw/day to groups of presumed pregnant Sprague-Dawley rats (n = 6/group) from days 6 to 19 of gestation. The corrected doses are 154, 461 and 1536 mg/kg bw/day. Control animals received the vehicle, 1% w/v methylcellulose, throughout the same period.

All presumed-pregnant females were pregnant. One female in the lowest dose group (154 mg/kg bw/day) exhibited minimal food intake (2 g/day) and a body weight loss of 40 g on days 18-19 of gestation. This female was killed *in extremis* on day 19 of gestation after showing signs of pallor, piloerection, brown staining around one eye, red urine and perigenital discharge. Necropsy revealed a large amount of dark red fluid within the vagina and both uterine horns. The uterus contained 15 late resorptions. In the absence of similar findings in animals in the higher dosage groups it is considered that the findings in this animal were unrelated to treatment.

Salivation after dosing was observed occasionally at 461 mg/kg bw/day and frequently at 1536 mg/kg bw/day. Respiratory noises were noted for one animal in each of the treated groups. There were no other significant clinical signs observed in animals in the control group or the treatment groups.

ELA had no significant treatment-related effects on food consumption, body weight, gross pathology, fetal survival or fetal development.

HLS (1998c) **Study title**: LAE study of embryo-foetal toxicity in the CD rat by oral gavage administration. **Report no.**: LMA 014/984183 **Report date**: 24 November 1998 **Laboratory**: Huntingdon Life Sciences Ltd, Huntingdon, Cambridgeshire, England. **GLP**: Yes (OECD)

ELA (69.1% w/w active ingredient) was administered by oral gavage at doses of 200, 600 or 2000 mg/kg bw/day to groups of presumed pregnant Sprague-Dawley rats (n = 22/group) from days 6 to 19 of gestation. The corrected doses are 154, 461 and 1536 mg/kg bw/day. Control animals received the vehicle, 1% w/v methylcellulose, throughout the same period.

Three animals receiving 1536 mg/kg bw/day were humanely sacrificed on days 7 or 8 of gestation following severe signs of respiratory distress and salivation after dosing. Two of the animals had shown body weight loss prior to sacrifice. Necropsy revealed large amounts of gaseous material in the stomach of two females while in one female the entire GI tract was distended with gas.

Two animals receiving 461 mg/kg bw/day were humanely sacrificed towards the end of gestation. These animals also exhibited respiratory distress, salivation, body weight loss and GI tract distention. Necropsy of the high and mid dose animals exhibiting respiratory distress did not indicate damage to the lungs. Accumulation of gas in the stomach and GI tract may be due to gasping respiration following possible aspiration of increased secretions and or traces of the dosing material following treatment with the more concentrated/viscous solutions at the higher doses. The observed respiratory distress is not considered to be a systemic toxic response to oral ingestion of ethyl lauroyl arginate but may suggest possible bronchial irritation if the test material is inhaled. Respiratory distress is also commonly observed in animals dosed using gavage due to intubation errors.

The general condition of the surviving animals was satisfactory and all the females were pregnant. Noisy respiration was observed in some animals from all ethyl lauroyl arginate treatment groups but not in controls. Salivation at the time of dosing was observed in all animals receiving 1536 mg/kg bw/day on approximately 50% of dosing occasions. At 461 mg/kg bw/day, salivation was observed in 14/22 animals on 1-3 dosing occasions, while at 154 mg/kg bw/day only one animal showed salivation on one dosing occasion. Salivation at the time of dosing was not observed in control animals.

There were no overall treatment-related effects on body weight or food consumption, although occasional animals in treatment groups showed periods of body weight loss and reduced food intake which were related to respiratory distress.

Apart from the gaseous distention of the GI tract in three high dose and two mid dose animals, there were no other maternal necropsy findings which were considered related to treatment.

There were no treatment-related effects on fetal survival, growth or development.

Although transient effects were observed on body weight and food consumption associated with animals exhibiting respiratory distress, they were not considered to be systemic toxic responses to oral ingestion of ethyl lauroyl arginate. Based on the absence of any compound related adverse effects a more appropriate NOAEL for dams and fetuses is considered to be 1536 mg/kg/day.

## RABBITS

HLS (1998d) **Study title**: LAE study of tolerance in the rabbit by oral gavage administration. **Report no.**: LMA012/980115 **Report date**: 6 August 1998 **Laboratory**: Huntingdon Life Sciences Ltd, Huntingdon, Cambridgeshire, England. **GLP**: Yes (OECD)

ELA (69.1% w/w active ingredient) was administered by oral gavage to two non-pregnant NZW rabbits at an initial dosage of 60 mg/kg bw/day (corrected dose 46.1 mg/kg bw/day) for two days (group I). The dosage was doubled every two days up to a maximum of 1000 mg/kg bw/day (corrected dose 768 mg/kg bw/day). A separate group (group II) of two pregnant females received ethyl lauroyl arginate at 1000 mg/kg bw/day (corrected dose 768 mg/kg bw/day) from days 6 to 12 of gestation.

Group I: There were no deaths during the treatment phase. The general condition, clinical signs and body weights of the animals were not significantly affected by treatment and there were no treatment-related adverse gross pathology findings.

Group II: There were no deaths during the treatment phase. There was a transient reduction in water and food intake from about day 3 of treatment, associated with marked weight loss.

One female showed abnormal stress reaction to the dosing procedure at the second dose and the second female showed marked respiratory distress after the third dose. One female exhibited continuous weight loss until termination but the other female showed some partial recovery in body weight towards the end of the dosing period. At necropsy, both animals showed some evidence of collapse of areas of the lung which was more extensive and accompanied by suggestions of infection in the lungs of the animal which had shown signs of respiratory distress during treatment. Both animals showed prominent dark vessels on the surface of the kidneys, but the significance of this observation was uncertain. Embryo survival was not affected by maternal treatment.

HLS (1998e) **Study title**: LAE preliminary study of embryo-foetal toxicity in the rabbit by oral gavage administration **Report no.**: LMA015/980169 **Report date**: 6 August 1998 **Laboratory**: Huntingdon Life Sciences Ltd, Huntingdon, Cambridgeshire, England **GLP**: Yes (OECD)

ELA (69.1% w/w active ingredient) was administered by oral gavage at doses of 250, 500 or 1000 mg/kg bw/day to presumed pregnant NZW rabbits (4/group) from day 6 to 19 after mating. The corrected doses are 192, 384 and 768 mg/kg bw/day. Six control presumed pregnant females received vehicle (1% w/v methylcellulose in water) for the same period. All females from each group were pregnant and were killed on day 29 after mating for examination of their uterine contents.

There were no pre-terminal deaths and no treatment-related clinical signs. Small losses in body weight were recorded during gestation days 6 to 12 for 3/4 animals at 768 mg/kg bw/day and for a lower proportion of control, low, and intermediate dose groups. There were no meaningful inter-group body weight differences by the end of pregnancy. Food consumption was lower in the 384 and 768 mg/kg bw/day groups.

There were no necropsy findings related to treatment and no effects on fetal survival or fetal anomalies.

HLS (1998f) **Study title**: LAE study of embryo-foetal toxicity in the rabbit by oral gavage administration. **Report no.**: LMA 016/992096 **Report date**: 26 March 1999
**Laboratory**: Huntingdon Life Sciences Ltd, Huntingdon, Cambridgeshire, England **GLP**: Yes (OECD)

ELA (69.1% w/w active ingredient) was administered by oral gavage at doses of 100, 300 or 1000 mg/kg bw/day to groups of presumed pregnant NZW rabbits (n = 22/group) from day 6 to 19 after mating. The corrected doses are 77, 230 and 768 mg/kg bw/day. Control females (n = 22) received the vehicle (1% w/v/ methylcellulose) throughout the same period. Surviving females were killed on day 29 after mating for examination of uterine contents followed by detailed fetal examination.

Signs of reaction to treatment, largely associated with dosing difficulty and respiratory signs (irregular, gasping, noisy), were observed in 5 animals per group at 230 and 768 mg/kg bw/day. These signs were largely alleviated by using a clean moist catheter (rather than a clean dry catheter) for dose administration. One animal at each of 230 and 768 mg/kg bw/day was killed for human reasons and necropsy revealed some lung congestion. One animal at 768 mg/kg bw/day aborted on day 24 of gestation.

Body weight gain and food consumption were unaffected at 77 and 230 mg/kg bw/day. Body weight gain of rabbits receiving 768 mg/kg bw/day was 40% that of controls during the treatment period ($p < 0.01$) while food consumption was low during the second week of treatment.

Necropsy revealed no treatment related effects on dams, litter parameters or fetuses. The number of females actually pregnant ranged from 19 to 22 per group.

Because of reduced body weight gain and the observation that respiratory signs were not completely alleviated at the high dose after modification of the gavage method, the NOAEL was considered to be 230 mg/kg bw/day. However, as for rats dosed with ethyl lauroyl arginate by gavage (see study LMA014/984183, above), the observed respiratory distress is not considered to be a systemic toxic response to oral ingestion of ethyl lauroyl arginate but may suggest possible bronchial irritation if the test material is inhaled.

For fetuses, the NOAEL was the high dose of 768 mg/kg bw/day.

## Discussion

The submitted data were considered suitable for hazard assessment and assignment of an ADI. The lack of a long term carcinogenicity study was not considered to be a deficiency as discussed below.

The overall quality of the submitted kinetics and toxicology studies was high. Adequate numbers of animals per group were evaluated in the main toxicity studies and appropriate investigations were conducted in these studies. All of the submitted kinetics and toxicology studies, except one preliminary study, were conducted according to Good Laboratory Practice (GLP).

*Absorption, metabolism and excretion*

Absorption studies were conducted in rats and humans administered ethyl lauroyl arginate orally. In a study in rats receiving ethyl lauroyl arginate by gavage at single doses of 40, 120 or 320 mg/kg bw, both ethyl lauroyl arginate and the metabolite LAS were assayed in plasma.

At doses of 40 and 120 mg/kg bw, the area under the plasma concentration versus time curve over 8 h ($AUC_{0\text{-}8\ h}$) for ethyl lauroyl arginate could not be calculated because of rapid metabolism to LAS. At 320 mg/kg bw, the highest dose tested, the $AUC_{0\text{-}8\ h}$ for ethyl lauroyl arginate was only 2.4% of the value for LAS (7.50 and 315 ng.h/mL, respectively). Thus, systemic exposure of rats to ethyl lauroyl arginate was very low at the doses used in this study. This study also investigated the absorption of ethyl lauroyl arginate (at a dose of 120 mg/kg bw) in three different vehicles: (i) propylene glycol/water, (ii) glycerol/water, and (iii) water. Ethyl lauroyl arginate was rapidly metabolised to LAS in the presence of each of the three vehicles. The AUC for LAS was approximately proportional to the ethyl lauroyl arginate dose and was similar for all three vehicles at an ethyl lauroyl arginate dose of 120 mg/kg bw.

Metabolism of ethyl lauroyl arginate to LAS was also rapid in humans. In the main human study, ethyl lauroyl arginate (radiolabelled with $^{13}C$) was administered orally at single doses of 1.5 mg/kg bw (to 4 subjects) and 2.5 mg/kg bw (to 2 subjects). Ethyl lauroyl arginate, LAS and arginine were assayed in plasma over a 24 h period and ELA was shown to be rapidly metabolised to LAS and arginine. No meaningful AUC data could be obtained for ethyl lauroyl arginate because plasma concentrations of $^{13}C$-ELA were below the limit of quantification at all sampling times in all subjects, with the exception of subject 2 (2.5 mg/kg bw) for whom quantifiable concentrations of $^{13}C$-ELA were found in two samples. $AUC_{0\text{-}24\ h}$ values for LAS were 90.6 and 118 ng.h/mL at doses of 1.5 and 2.5 mg/kg bw, respectively. For arginine, the corresponding $AUC_{0\text{-}24\ h}$ values were 4- to 6-fold greater at 382 and 764 ng.h/mL, respectively.

A preliminary human absorption study using a single oral dose of 5 mg/kg bw also indicated rapid metabolism of ethyl lauroyl arginate to LAS and arginine; however, this study was limited by the use of only two subjects (fed state undocumented) and AUC values were not presented.

Studies specifically investigating metabolite formation were conducted *in vitro* and in rats *in vivo.* Rapid metabolism of ethyl lauroyl arginate to LAS and then to arginine was demonstrated to occur *in vitro* in simulated intestinal fluids but only in the presence of the pancreatic enzyme mixture pancreatin. In contrast, in simulated gastric fluid (with and without pepsin) ethyl lauroyl arginate was stable over the 2 h period investigated. Thus, negligible metabolism of ethyl lauroyl arginate is likely in the stomach, while rapid metabolism to LAS and subsequent slower metabolism to arginine likely occurs in the intestine.

Incubations of ethyl lauroyl arginate with human plasma and human hepatocytes also resulted in substantial conversion to LAS but not to arginine. Incubation of $^{14}$C-ELA with the S9 fraction from rat liver resulted in the formation of LAS, arginine, ornithine, arginine ethyl ester, urea and several uncharacterised metabolites. However, substantial amounts of $^{14}$C-ELA (25% of the added radioactivity) remained unchanged after 24 h.

An *in vivo* metabolism study in rats receiving $^{14}$C-ELA as a single gavage dose also showed rapid metabolism of ethyl lauroyl arginate to LAS, arginine, ornithine and urea. At the first analysis time point (0.5 h), approximately 50% of the administered radioactivity was present in arginine, 8% in ornithine, and 15% in small molecular weight polar material (probably urea based on retention time). It is not apparent why relatively larger concentrations of ethyl lauroyl arginate were observed in plasma in this study (greater than the LAS concentrations) compared to the other rat study.

As indicated above, a comparison of AUC values for ethyl lauroyl arginate in rats and humans is not possible because of the negligible systemic exposure to ethyl lauroyl arginate observed in the human studies. For the metabolite LAS, a comparison of the available rat and human AUC values is shown in the table below.

This comparison shows that a human ethyl lauroyl arginate dose of approximately 2 mg/kg bw would result in an AUC value for LAS which is similar to that in rats receiving ethyl lauroyl arginate at approximately 120 mg/kg bw (*i.e.* a 60-fold lower dose in humans, on a body weight basis, results in a similar systemic exposure to LAS).

However, systemic exposure to arginine in humans (arising from the degradation of LAS) was 4- to 6-fold greater than exposure to LAS (based on plasma AUC values). Thus, in relative terms, systemic exposure to LAS is small because of rapid degradation to arginine. Data on systemic exposure to arginine arising from LAS were not available for rats.

**Table 2.21: Plasma AUC data for the metabolite LAS**

| | **Rat** | | | | | | **Human** | |
|---|---|---|---|---|---|---|---|---|
| | **Single dose (mg/kg bw)** | | | **Repeat dose (mg/kg bw/day)** [a] | | | **Single dose (mg/kg bw)** | |
| | 40 | 120 | 320 | 119 | 350 | 1018 | 1.5 | 2.5 |
| $AUC_{0-\tau}$ (ng.h/mL) | 52.5 | 103 | 315 | 108 | 327 | 1045 | 90.6 | 118 |
| $\tau$ (h) | 8 [b] | 8 [b] | 8 [b] | 24 | 24 | 24 | 12 [b] | 8 [b] |

[a] The dose levels of 119, 350, and 1018 are mean values calculated from food intake in the 52 week repeat dose toxicity study (dietary).
[b] The time intervals for these AUC values varied depending on the time of the last quantifiable sample.

An excretion study in rats administered an oral gavage dose of $^{14}$C-ELA indicated that approximately 46% of the applied radioactivity was retained in the carcass at sacrifice, 5 days after administration. This finding is consistent with the formation of arginine as a metabolite which can be subsequently incorporated into endogenous proteins and undergo catabolism into smaller molecules. A large fraction of the dose (37%) was excreted as carbon dioxide in expired air which is also consistent with normal amino acid catabolism resulting in urea and carbon dioxide. Smaller fractions of the applied radioactivity were excreted in urine (12%) and faeces (4%).

The submitted absorption and metabolism studies on ethyl lauroyl arginate do not indicate any important differences between rats and humans with respect to these properties. The rat is therefore considered to be an appropriate animal species for toxicity studies.

Note that some reproductive toxicity studies were conducted in rabbits, a species for which the absorption, metabolism and excretion of ethyl lauroyl arginate has not been studied.

*Single dose toxicity*

Single dose toxicity studies in rats were conducted using Mirenat-N, ethyl lauroyl arginate and LAS as test articles. For Mirenat-N, administered by oral gavage at an effective ethyl lauroyl arginate dose of 500 mg/kg bw, there were no deaths and treatment-related clinical signs were confined to transient piloerection. Body weight gain was normal and no abnormalities were evident in any of the animals at necropsy on day 15. For ethyl lauroyl arginate, administered by oral gavage at a dose of 2000 mg/kg bw, there were also no deaths. Treatment-related clinical signs consisted of piloerection, increased salivation, unsteady gait and hunched posture. All clinical signs of toxicity had resolved by day 3 or 4. All animals achieved satisfactory weight gain and no abnormalities were evident in any of the animals at necropsy on day 15.

The metabolite LAS, also administered at a dose of 2000 mg/kg bw by oral gavage, exhibited lower acute toxicity than ethyl lauroyl arginate as indicated by an absence of clinical signs of toxicity. There were no deaths, body weight gain was normal and no macroscopic alterations were observed at necropsy 15 days after dosing.

While of limited relevance for a food additive, a dermal irritation study in rabbits showed that ethyl lauroyl arginate applied as an aqueous paste caused slight to well-defined erythema followed by desquamation of the treated skin. There was no indication of a systemic effect of treatment.

Finally, in the preliminary absorption studies in humans (also discussed above), ethyl lauroyl arginate at a dose of 5 mg/kg bw appeared to be well tolerated except for a burning sensation and nausea reported on administration. These effects may have been due to the use of propylene glycol as the solvent in this study. These effects were not observed in the subsequent human study which employed lower ethyl lauroyl arginate and propylene glycol doses. In this study there were no clinically significant abnormalities in any of the laboratory investigations (clinical chemistry, haematology and urinalysis), no notable changes in vital signs during the study, and no clinically significant ECG findings.

*Repeat dose toxicity*

Three main repeat dose toxicity studies were conducted in rats. Two additional studies were preliminary studies of 4 weeks dosing duration. All of the studies were conducted according to GLP. The test articles, Mirenat-N (25% w/w solution of ethyl lauroyl arginate in propylene glycol) and ethyl lauroyl arginate, were administered via the diet.

ELA was generally well tolerated in these studies. There were no deaths in animals that received ethyl lauroyl arginate in the 4 and 13-week studies. There were 6 unscheduled deaths in the 52-week study; however, none of the deaths were attributable to treatment. Clinical signs of toxicity included piloerection, ungroomed coats and salivation in the 4-week study with ethyl lauroyl arginate at the high dietary concentration (50,000 ppm), and ungroomed coat and brown staining of the muzzle in the 13 and 52-week studies with ethyl lauroyl arginate predominantly at the high concentration in each study (50,000 and 18,000 ppm, respectively). Neurobehavioural parameters (sensory reactivity, grip strength, motor activity) were investigated in the 52-week study with no significant findings.

Transient and relatively small reductions in body weight gain and food consumption were observed at the high dietary concentrations in 4 out of 5 studies. These findings may be attributable to reduced palatability of the diet at high ethyl lauroyl arginate concentrations.

Potentially treatment related effects on clinical chemistry parameters were observed in one 13-week study and in the 52-week study; however, these effects were not consistent across the studies and are unlikely to be toxicologically relevant. In the 13-week study with ethyl lauroyl arginate, decreased total protein was observed for animals receiving the high dietary concentration of 50,000 ppm, slightly decreased albumin was observed for animals receiving 50,000 ppm and females receiving 15,000 ppm, and slightly decreased cholesterol was observed for females receiving 50,000 ppm. In the 52-week study, clinical chemistry findings were limited to increased urea concentration in females receiving the high dietary concentration of 18,000 ppm.

Potentially treatment related effects on white blood cell parameters were observed in both of the 13-week studies and in the 52-week study. The applicant provided expert opinions on the haematological findings from three scientists (Brown 2008; Escolar 2008; Maronpot 2008).

Escolar (2008) and Maronpot (2008) considered that the haematological findings are unlikely to be toxicologically significant based on the following: (i) the absence of a clear dose-effect relationship; (ii) the findings were dependent on rat strains; (iii) the responses varied according to sex; (iv) there were no associated effects on bone marrow (investigated in the 52 week study); and (v) inconsistent effects both within and between studies. These arguments are consistent with the findings in the submitted studies and are considered to be valid. Brown (2008) considered that the changes may be treatment related, and if so, are likely to be a result of the local effect of ethyl lauroyl arginate on the forestomach (discussed below). Brown considered that the most likely reason for a reduction in mature white blood cells in the circulation was due to migration to the tissues.

However, there was no correlation between the animals which showed forestomach lesions and those which exhibited reduced white blood cell counts. Brown also stated that normal myeloid cell production was not disturbed and that there was no evidence of excessive cell destruction or damage. EFSA considered ELA before these expert reviews were available and concluded that the white blood findings may be related to treatment and that the ADI should therefore be based on these findings (EFSA 2007). The ADI derived by EFSA was based on the NOAEL of approximately 50 mg ELA/kg bw/day which was the lowest dose tested in the 13 week study with Mirenat-N as test article. Based on this NOAEL and a safety factor of 100, EFSA established an ADI of 0-0.5 mg ELA of the proposed specifications /kg bw.

Treatment related gross pathology and histopathology findings were limited to the forestomach and were observed in one of the 13-week studies and in the 52-week study. The findings, of generally minimal or slight severity even at the high dietary levels, were restricted to the non-glandular region of the stomach (the forestomach), and consisted of inflammation, erosion, parakeratosis, ulceration, epithelial hyperplasia and re-epithelialisation.

The increase in the incidence of these findings was statistically significant only at the high dietary levels in each study. These findings are considered to arise from a direct effect on epithelial cells due to the surfactant action of ethyl lauroyl arginate. The effects are not considered to be indicative of systemic toxicity. Moreover, the rodent forestomach does not possess a protective mucus lining and has no counterpart in humans.

*Genotoxicity*

An appropriate set of genotoxicity studies was submitted comprising bacterial reverse mutation assays with Mirenat-N, ethyl lauroyl arginate and the metabolite LAS, mammalian cell mutation and chromosome aberration assays with Mirenat-N and ethyl lauroyl arginate, and a micronucleus formation study with the metabolite LAS. Relatively low maximum concentrations were tested in the *in vitro* assays because of cytotoxicity at higher ethyl lauroyl arginate concentrations. The cytotoxicity was particularly evident in the bacterial reverse mutation assay, this study is therefore of limited value for the evaluation of mutagenicity. None of the test articles showed evidence of mutagenic or clastogenic activity in the submitted assays while negative and positive controls gave expected results. There was some evidence of polyploidy induced by ethyl lauroyl arginate in a chromosome aberration assay; however, this was observed only at cytotoxic concentrations and is unlikely to be of biological significance.

*Carcinogenicity*

A long term carcinogenicity study was not submitted which is considered acceptable because ethyl lauroyl arginate was not genotoxic and has no chemical structural alert and did not show evidence of pre-neoplasia or neoplasia in the repeat dose toxicity studies.

In addition, ethyl lauroyl arginate is rapidly metabolised to endogenous compounds or compounds naturally present in the diet and there were no significant systemic toxic effects observed in any of the studies.

*Reproductive and developmental toxicity*

A total of 5 main reproductive and developmental toxicity studies were conducted in rats and rabbits. The studies investigated fertility, reproductive performance, embryofetal development and postnatal development. Three additional studies were preliminary studies. All of the studies were conducted according to GLP.

The only notable finding potentially attributable to ethyl lauroyl arginate was the observation of delayed onset of puberty in female rats in two studies. The age at vaginal opening, which is an indicator of pubertal onset, was delayed by approximately 4 days at the high dietary ethyl lauroyl arginate level in a preliminary reproductive study in rats and also by 4 days in the main study. In the main study the difference was significant at the $p < 0.01$ level when compared to the control group (mean age at vaginal opening 33.0 days compared to 37.0 days in the high dietary level group). A possible mechanism for this effect is not known.

Body weight gain from birth to day 14 of age was not affected by treatment suggesting that palatability of milk from treated dams was not reduced. However, body weight gain during the week before weaning (days 14 to 21 of age) was reduced by approximately 10% in males and females receiving the high dietary concentration of 15,000 ppm. It is possible that the observed delayed onset of puberty may be related to this reduced body weight gain; however, males also showed reduced body weight gain of similar magnitude but their development was not delayed.

Another potentially relevant consideration is that rat pups are coprophagic and begin to consume the maternal faeces in their second postnatal week. However, an excretion study showed that only approximately 4% of an administered dose of $^{14}C$-labelled ethyl lauroyl arginate was excreted in faeces. The delay in vaginal opening was not associated with any deficit in other markers of development or subsequent reproductive parameters in these animals. The NOAEL for this effect was the mid dietary level of 6000 ppm ethyl lauroyl arginate which corresponds to 502 mg/kg bw/day. This dose level is the calculated intake before pairing of males and females. Higher dietary intakes of ethyl lauroyl arginate were observed in females during gestation (585 mg/kg bw/day) and lactation (1018 mg/kg bw/day); however, because the relevant time of exposure for the delayed puberty effect is not known, the lower intake (before pairing) is considered to be the appropriate NOAEL.

*Toxicology studies relevant to hazard assessment*

The repeat dose toxicity, reproductive toxicity and developmental toxicity studies relevant to the hazard assessment of ethyl lauroyl arginate are summarised in the table below.

**Table 2.22: Levels relevant to hazard assessment**

| Species / strain | Dosing duration (weeks) | No. animals per group | Dose levels [a] (mg/kg bw/day) | NOAEL [b] (mg/kg bw/day) | LOAEL [c] (mg/kg bw/day) | Study no. |
|---|---|---|---|---|---|---|
| Rat, Han Wistar | 13 weeks | 20/sex | 0, 384, 1143, 3715 (males) 0, 445, 1286, 3915 (females) | 1143 (males) 1286 (females) | 3715 (males) 3915 (females) | LMA 031/004276 |
| Rat, SD | 52 weeks | 20/sex | 0, 106, 307, 907 (males) 0, 131, 393, 1128 (females) | 307 (males) 393 (females) | 907 (males) 1128 (females) | LMA 050/042556 |
| Rat, SD | See footnote[d] | 28/sex | 0, 181, 434, 1073 (males) 0, 207, 502, 1226 (females) | 1073 (males)[e] 502 (females) | 1226 (females) | LMA 042/032553 |
| Rat, SD | Gestation days 6 to 19 | 22 females | 0, 154, 461, 1536 | 1536 [e] (dams and fetuses) | - | LMA 014/984183 |
| Rabbit, NZW | Gestation days 6 to 19 | 22 females | 0, 77, 230, 768 | 230 (dams) 768 (fetuses) [e] | 768 (dams) | LMA 016/992096 |

[a] Dose levels were calculated based on measured food consumption and dietary concentration of ethyl lauroyl arginate (corrected for active ingredient content only for study numbers LMA 014/984183 and LMA 016/992096 which used an ethyl lauroyl arginate batch containing 69.1% w/w of the active ingredient).
[b] No Observed Adverse Effect Level
[c] Lowest Observed Adverse Effect Level
[d] Two generation reproductive toxicity study. Animals were dosed for 10 weeks prior to pairing, throughout pairing, gestation, lactation and until termination. After weaning, F1 animals received the relevant diet as per the F0 generation throughout the study until termination. The F1 generation were mated to produce the F2 generation which was raised to weaning.
[e] Highest dose tested.

The forestomach effects observed in the 13-week and 52-week repeat dose toxicity studies are probably due to a local irritant effect arising from the cationic surfactant activity of ethyl lauroyl arginate. There were no adverse findings for the glandular region of the stomach. The rodent fore stomach has no protective mucus lining and has no anatomical equivalent in humans.

It is therefore not considered appropriate to base the ADI on the forestomach findings. Effects on white blood cell counts observed in repeat dose toxicity studies were not consistent across studies and are not likely to be of biological significance. The white blood cell findings are also not considered to be appropriate for setting the ADI.

Because the delay in vaginal opening was observed in two reproductive toxicity studies, and the magnitude of the effect was similar in each case, it is considered that this finding may be due a systemic effect of ethyl lauroyl arginate and is thus suitable for assigning an ADI for ethyl lauroyl arginate. The NOAEL for this effect was 502 mg/kg bw/day. Applying safety factors of 10 for inter-species differences and 10 for inter-individual differences results in an ADI of 0-5 mg/kg bw for ethyl lauroyl arginate.

*Intolerance*

Ethyl lauroyl arginate has been approved for use and commercialised in the USA since 2005, with no published reports of intolerance associated with consumption.

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