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Safety of ethyl lauroyl arginate (E 243) as a food additive in the light of the new information provided and the proposed extension of use

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Abstract

The present scientific opinion deals with the evaluation of the safety of the food additive ethyl lauroyl arginate (E 243) in the light of a new interpretation of the available toxicological data and with respect to the proposed changes to the currently authorised conditions of use. Ethyl lauroyl arginate (E 243) is an already authorised food additive in the EU for use in heat-treated meat products only, with some exceptions. The safety of ethyl lauroyl arginate (E 243) as a food additive has been evaluated in 2007 by EFSA and an acceptable daily intake (ADI) of 0.5 mg/kg body weight (bw) was set. The present assessment is based on a new interpretation of the available data elaborated by the applicant and on exposure estimates calculated by the Panel for both the current and the proposed changes to the authorised uses of this food additive. The Panel considered the new information provided, including the re-examination of some of the results from the toxicological studies included in the original application dossier submitted for the initial evaluation of ethyl lauroyl arginate (E 243) in 2007. The Panel concluded that it does not contain new scientific evidence. The concerns and uncertainties expressed in the previous scientific opinions of the Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Foods (AFC) and Panel on Food additives and Nutrient Sources added to Food (ANS) remain to be addressed and there is no justification for changing the current ADI. Based on the above, the Panel concluded that the current ADI of 0.5 mg/kg bw would be reached in toddlers and children at the 95th percentile already for exposure estimates calculated using the currently permitted maximum level (ML) for ethyl lauroyl arginate (E 243). At the proposed new uses and use levels, the ADI would be exceeded at mean level of consumption in all age groups.

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Summary

The European Commission asked the European Food Safety Authority (EFSA) to provide a scientific opinion as regards the safety of ethyl lauroyl arginate (E 243) as a food additive in the light of a new interpretation of the available toxicological data and on the proposed extension of use of ethyl lauroyl arginate (E 243) to 20 additional food categories and changes to the currently authorised use, in accordance with Regulation (EC) No 1331/2008 establishing a common authorisation procedure for food additives, food enzymes and flavourings.

Ethyl lauroyl arginate (E 243) is currently an authorised food additive in the European Union (EU) under Annex II of Regulation (EC) 1333/2008 for use in a single food category, i.e. heat-treated meat products with the exception of emulsified sausages, smoked sausages and liver pastes with a maximum limit in food of 160 mg/kg.

The currently permitted use of ethyl lauroyl arginate (E 243) follows the evaluation of a dossier by EFSA and the conclusions of the scientific opinion on the use of ethyl lauroyl arginate as a food additive (EFSA, 2007a). In that opinion, the former Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Foods (AFC) concluded that, after ingestion, ethyl lauroyl arginate is broken down into products of normal metabolism and that toxicity studies did not suggest a carcinogenic potential. Based on effects on white blood cells (WBC) observed in toxicity studies conducted on rats, the AFC Panel identified the lowest No Observed Adverse Effect Levels (NOAELs) as 47 and 56 mg/kg body weight (bw) per day for males and females, respectively, and established an acceptable daily intake (ADI) of 0.5 mg/kg bw for ethyl lauroyl arginate (based on the proposed specifications) (EFSA, 2007a). Although the toxicological relevance of the haematological findings observed could not be elucidated by the Panel, these effects were still selected for the derivation of the ADI because they had been consistently observed in different strains of rats and in different sexes in two 90-day studies and in a 52-week study (EFSA, 2007a).

In addressing the current mandate, the Panel noted that no new toxicological data have been provided by the applicant in support of the application for the proposed extension of use of the food additive ethyl lauroyl arginate (E 243) in several food categories, rather a new interpretation of the currently available toxicological data.

The applicant has reanalysed previously evaluated toxicological studies confirming the previously established statistically significant changes in WBC counts and the effects on delayed vaginal opening.

According to the applicant, the effects observed in the toxicological studies can be explained with the increased dietary intake of arginine over several weeks or months, resulting from the exposure to ethyl lauroyl arginate. The applicant, however, did not provide sufficient evidence for the proposed mode of action of the purported role of arginine in the adverse effects observed in the animal studies.

The applicant is proposing to increase the ADI for ethyl lauroyl arginate to 5 mg/kg bw per day by applying an uncertainty factor of 100 to the NOAEL for delayed vaginal opening (502 mg/kg bw per day) observed in a reproductive toxicity study in rats. The corresponding ADI for the active substance ethyl-N- α -lauroyl-L-arginate HCl, would be 4.4 mg/kg bw per day.

The Panel noted that this derivation of a NOAEL is inconsistent with the assessment made by the applicant that the observed delay in vaginal opening is of no long-term toxicological relevance. The Panel considered the effect on vaginal opening as a toxicologically relevant endpoint. However the Panel noted that the NOAEL from this effect would be higher than the NOAEL for the observed effects on WBC, and therefore maintained the previous position of both AFC and Food additives and Nutrient Sources added to Food (ANS) Panels that the lowest NOAEL should be used for deriving an ADI.

Jointly with the proposed revision of the current ADI of 0.5 mg/kg bw per day of ethyl lauroyl arginate (E 243), the applicant is also proposing several changes to the currently permitted use and use level of this food additive. Ethyl lauroyl arginate (E 243) is currently authorised in a single food category (08.3.2, heat-treated meat products, with the exception of emulsified sausages, smoked sausages and liver paste) at a maximum level (ML) of 160 mg/kg food. The applicant is proposing to increase the level permitted for use in this food category to 200 mg/kg food and to remove existing restrictions, as well as extending the use of this food additive to 20 additional food categories.

In the latest exposure assessment performed, EFSA estimated exposure to ethyl lauroyl arginate using five scenarios each using very narrow, specific descriptions of the types of food to which it was proposed to add ethyl lauroyl arginate (i.e. meat specialities, pastes, pâtés and terrines, preserved meat, sausages) (EFSA, 2013).

In the current opinion, an exposure estimate was calculated by the Panel for ethyl lauroyl arginate (E 243) in its currently permitted single use and use level, taking into account the applicable

restrictions and exceptions. According to this new exposure estimate, maximum exposure to ethyl lauroyl arginate (E 243) at the mean level would be 0.1 mg/kg bw per day in all age groups. At the 95th percentile, the current ADI would be reached in toddlers and children.

For the proposed new uses, the Panel has performed an exposure assessment to ethyl lauroyl arginate (E 243) using the Food Additives Intake Model (FAIM) tool (version 2). According to this assessment, the estimated exposure to ethyl lauroyl arginate (E 243) from its new proposed uses and use levels would range from 0.1 mg/kg bw per day in infants to 2.8 mg/kg bw per day in toddlers at the mean exposure level and from 0.4 mg/kg bw per day in infants to 4.2 mg/kg bw per day in toddlers at the high exposure level.

In principle, it would have been possible for the Panel to refine its exposure assessment to ethyl lauroyl arginate (E 243) at the new proposed uses and use levels, however, in the light of the results from the refined exposure estimate performed at the current ML and in the single authorised use, showing that the current ADI of 0.5 mg/kg is already reached in some of the age groups at the 95th percentile and that this more refined estimate does not take into account the additional exposure resulting from the use in cosmetics, the Panel considered that there is no need to further refine the exposure estimate to ethyl lauroyl arginate (E 243) at the proposed new uses and use levels.

The Panel considered the new information provided, including the re-examination of some of the results from the toxicological studies included in the original application dossier submitted for the initial evaluation of ethyl lauroyl arginate (E 243) in 2007. The Panel concluded that it does not contain new scientific evidence.

The concerns and uncertainties expressed in the previous scientific opinions of the AFC and ANS Panels remain to be addressed and there is no justification for changing the current ADI.

Based on the above, the Panel concluded that the current ADI of 0.5 mg/kg bw would be reached in toddlers and children at the 95th percentile already for exposure estimates calculated using the currently permitted ML for ethyl lauroyl arginate (E 243). At the proposed new uses and use levels, the ADI would be exceeded at mean level of consumption in all age groups.

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1. Introduction

Ethyl lauroyl arginate (E 243) is currently an authorised food additive in the European Union (EU) under Annex II of Regulation (EC) No 1333/2008¹ for use in the food category '08.3.2 Heat-treated meat products' except for emulsified sausages, smoked sausages and liver paste.

The present scientific opinion deals with the evaluation of the safety of ethyl lauroyl arginate (E 234) in the light of new information provided by the applicant and of the proposed extension of use in several food categories and changes to the currently authorised use.

1.1. Background and Terms of Reference as provided by the European Commission

1.1.1. Background

The use of food additives is regulated under the European Parliament and Council Regulation (EC) No 1333/2008 on food additives. Only food additives that are included in the Union list, in particular in Annex II to that regulation, may be placed on the market and used in foods under the conditions of use specified therein.

Ethyl lauroyl arginate (E 243) is currently an authorised food additive in the European Union under Annex II to Regulation (EC) No 1333/2008 for use in the food category '08.3.2 Heat-treated meat products' for use in heat-treated meat products except for emulsified sausages, smoked sausages and liver paste.

In 2007 the former Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Foods (AFC Panel) issued an opinion on the use of ethyl lauroyl arginate as a food additive (EFSA, 2007a). The AFC Panel established an Acceptable Daily Intake (ADI) of 0.5 mg ethyl lauroyl arginate of the proposed specifications/kg bw and concluded that potential dietary exposure for the proposed uses were at or above the ADI.

In 2009 the Panel on Food additives and Nutrient Sources added to Food (ANS Panel) assessed the new information provided as to whether it constitutes new scientific evidence compared to that originally submitted and considered in the AFC Panel opinion and concluded that the concerns and uncertainties expressed in the opinion of the AFC Panel had not been addressed (EFSA ANS Panel, 2009).

In 2012 and 2013 refined exposure assessments were carried out. In the latter the European Food Safety Authority concluded that the anticipated dietary exposure from the proposed use in the selected meat products was below the ADI (EFSA, 2012, 2013).

The Health and Food Safety Directorate-General has received a request from Laboratorios Miret S.A. (LAMIRSA) and considers appropriate to consult EFSA as regards:

- i) The amendment of Annex II to Regulation (EC) 1333/2008 to extend the use of ethyl lauroyl arginate (E 243) to several food categories as detailed in the application dossier.
- ii) A re-evaluation of the safety of ethyl lauroyl arginate (E 243) as a food additive taking into account the new information provided by the applicant.

1.1.2. Terms of Reference

The European Commission requests the European Food Safety Authority to provide a scientific opinion on the safety of ethyl lauroyl arginate (E 243) as a food additive in the light of the new information provided on the proposed extension of use to several food categories as detailed in the application dossier in accordance with Regulation (EC) No 1331/2008 establishing a common authorisation procedure for food additives, food enzymes and flavourings.

1.1.3. Interpretation of the Terms of Reference

In addressing the current mandate, the Panel noted that no new toxicological data have been submitted by the applicant in support of the application for the proposed extension of use of the food additive ethyl lauroyl arginate (E 243) in several food categories, rather the applicant has provided a new interpretation of the existing toxicological studies.

¹ Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives OJ L 354 31.12.2008.

1.2. Information on existing evaluations and authorisations

In accordance with Regulation (EC) No 1333/2008, as amended by Commission Regulation (EU) No 506/2014², ethyl lauroyl arginate (E 243) is currently authorised as a food additive for use in heat-treated meat products (FC 08.3.2), with the exception of emulsified sausages, smoked sausages and liver paste, at a maximum level (ML) of 160 mg/kg.

The currently permitted use of ethyl lauroyl arginate (E 243) follows the evaluation of a dossier by European Food Safety Authority (EFSA) and the conclusions of the scientific opinion on the use of ethyl lauroyl arginate as a food additive (EFSA, 2007a). In that opinion, the former AFC Panel concluded that, after ingestion, ethyl lauroyl arginate is broken down into products of normal metabolism and that toxicity studies did not suggest a carcinogenic potential. Based on effects on white blood cells (WBC) observed in toxicity studies conducted on rats, the AFC Panel identified the lowest No Observed Adverse Effect Levels (NOAELs) as 47 and 56 mg/kg body weight (bw) per day for males and females, respectively, and established an ADI of 0.5 mg/kg bw for ethyl lauroyl arginate (based on the proposed specifications) (EFSA, 2007a). Although the toxicological relevance of the haematological findings observed could not be elucidated by the Panel, these effects were still selected for the derivation of the ADI because they had been consistently observed in different strains of rats and in different sexes in two 90-day studies and in a 52-week study (EFSA, 2007a).

In 2009, the ANS Panel was requested to review new information on ethyl lauroyl arginate provided by the applicant, consisting of a re-examination of some of the original results from the toxicological studies submitted with the original application dossier (EFSA ANS Panel, 2009). The ANS Panel noted the three expert opinions providing hypothesis for the observed alterations in WBC counts in rats; however, the scientific evidence provided at the time was considered to be inadequate to support a plausible underlying mechanism(s) of action and therefore to overrule the concerns and the uncertainties expressed in the AFC opinion. The ANS Panel, therefore, confirmed the previously established ADI of 0.5 mg/kg bw per day (EFSA ANS Panel, 2009).

In 2012 and 2013, EFSA was requested to perform refined exposure assessments of ethyl lauroyl arginate (E 243) based on revised use levels proposed by the applicant (EFSA, 2012, 2013). In its latter assessment, EFSA estimated that the anticipated dietary exposure resulting from the proposed use in selected meat products at a ML of 160 mg/kg would be below the ADI, even for high levels of consumption (95th percentile) in any of the population groups considered in the assessment (EFSA, 2013).

The safety of ethyl lauroyl arginate was evaluated by Joint Expert Committee on Food Additives (JECFA) at its 69th meeting when an ADI of 0–4 mg/kg bw expressed as active ingredient ethyl-N- α -lauroyl-L-arginate-HCl was established based on a NOAEL of 442 mg/kg bw per day identified in studies of reproductive toxicity in rats. The critical endpoint selected by JECFA for deriving the ADI was delayed vaginal opening observed among the female offspring (JECFA, 2009).

According to the applicant, ethyl lauroyl arginate (E 243) is also listed in the *Codex Alimentarius* General Standard for Food Additives (GSFA, Codex STAN 192-1995) approved for use in several food categories.

The Panel noted that the applicant has provided information on existing uses, authorisations and evaluations in the EU and in other countries (Australia, Canada, Columbia, Chile, Israel, Mexico, Turkey, United Arab Emirates, USA and Vietnam).

1.2.1. Use in cosmetic products

The safety of ethyl lauroyl arginate has been evaluated by the Scientific Committee on Consumer Products (SCCP, 2008), for its use as preservative in cosmetic products. It was concluded that ethyl lauroyl arginate is safe for consumers for the specified uses, which however excluded oral hygiene products and a NOAEL of 271 mg/kg bw per day from a chronic study in rats was used for the calculation of the Margin of Safety.

An addendum to this scientific opinion was subsequently adopted by the Scientific Committee on Consumer Safety (SCCS) in 2011, evaluating new data on irritation and dermal absorption (SCCS, 2011). In its 2011 opinion, the SCCS maintained the earlier opinion of the SCCP on ethyl lauroyl arginate HCl, confirming the concern with respect to local mucosal irritation further to regular use of

² Commission Regulation (EU) No 506/2014 of 15 May 2014 amending Annex II to Regulation (EC) No 1333/2008 of the European Parliament and of the Council and the Annex to Commission Regulation (EU) No 231/2012 as regards Ethyl lauroyl arginate as a preservative in certain heat-treated meat products. OJ L 145, 16.5.2014, p. 35–37.

toothpaste and possible additional use of a mouthwash containing ethyl lauroyl arginate HCl in the general population.

In 2013, the SCCS expressed an opinion with respect to the use of ethyl lauroyl arginate HCl as a preservative in oral cosmetic products at a level up to 0.15%. The SCCS concluded that the use up to a maximum concentration of 0.15% was considered to be safe in mouthwashes alone, but not in oral cosmetic products as a whole. As no human data concerning local toxicity of ethyl lauroyl arginate HCl in toothpaste were available, the safety of the ingredient in toothpaste could not be assessed. In that opinion, the SCCS had estimated a Systemic Exposure Dosage (SED) resulting from all the proposed uses in cosmetic products of 0.0897 mg/kg bw per day (SCCS, 2013).

An addendum to the 2013 SCCS opinion was prepared to address the safety of ethyl lauroyl arginate HCl in cosmetics product, also taking into account dietary exposure from its use as food additive. The estimated SED resulting from the use of ethyl lauroyl arginate HCl in oral cosmetics product for children 3 years old and 9 years old was 0.29 mg/kg bw per day and 0.14 mg/kg bw per day, respectively. These values were added to the dietary exposure estimated by EFSA in its 2013 refined exposure assessment of 0.442 mg/kg bw day in children in the 95th percentile, resulting in values of 0.58 and 0.73 mg/kg bw per day, both not covered by the ADI of 0.5 mg/kg bw per day set by EFSA. The SCCS concluded that the use of mouthwashes for children at the concentration of 0.15% for longer time periods was not safe (SCCS, 2015).

2. Data and methodologies

2.1. Data

The applicant has submitted a dossier in support of its application for the safety evaluation and extension of the approved uses (additional food categories) of ethyl lauroyl arginate (E 243) in the EU ('Documentation provided to EFSA' n. 1).

The EFSA Comprehensive European Food Consumption Database was used to estimate dietary exposure.

2.2. Methodologies

This opinion was formulated following the principles described in the EFSA Guidance of the Scientific Committee on transparency with regard to scientific aspects of risk assessment (EFSA, 2009) and following the relevant existing Guidances from the EFSA Scientific Committee.

The current 'Guidance for submission for food additive evaluations' (EFSA ANS Panel, 2012) has been followed by the ANS Panel for assessing the safety and the proposed extension of use of ethyl lauroyl arginate (E 243) in several food categories.

Dietary exposure to ethyl lauroyl arginate (E 243) from its proposed extension of use as a food additive was estimated combining the food consumption data available within the EFSA Comprehensive European Food Consumption Database with the proposed use levels provided by the applicant using the Food Additives Intake Model 2.0 (FAIM) (output generated between 8 and 10 January 2019).³

3. Assessment

3.1. Technical data

3.1.1. Identity of the substance and specifications

Current EU specifications for ethyl lauroyl arginate (E 243) are listed in the Commission Regulation (EU) No 231/2012⁴, as amended by Commission Regulation (EU) No 506/2014. The chemical name of ethyl lauroyl arginate (E 243) as given in the Regulation is ethyl-N- α -dodecanoyl-L-arginate-HCl and synonyms include lauric arginate ethyl ester; lauramide arginine ethyl ester; ethyl-N- α -lauroyl-L-arginate-HCl; LAE.

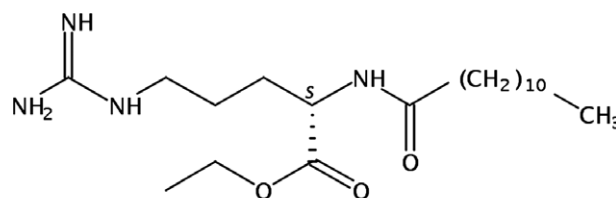
According to the current EU specifications, ethyl lauroyl arginate is defined as being synthesised by esterifying arginine with ethanol, followed by reacting the ester with lauroyl chloride, in aqueous media

³ <https://www.efsa.europa.eu/en/applications/foodingredients/tools>

⁴ Commission Regulation (EU) No 231/2012 of 9 March 2012 laying down specifications for food additives listed in Annexes II and III to Regulation (EC) No 1333/2008. OJ L 83, 22.3.2012, p. 1–295.

at a controlled temperature between 10 and 15°C and at a pH between 6.7 and 6.9. The resultant ethyl lauroyl arginate is recovered as the hydrochloride salt, which is filtered and dried. Ethyl lauroyl arginate (E 243) consists of not less than 85% and not more than 95% ethyl-N- α -lauroyl-L-arginate·HCl. In the current specifications, limits are set for: N- α -Lauroyl-L-arginine (not more than 3%); lauric acid (not more than 5%); ethyl laurate (not more than 3%); L-arginine·HCl (not more than 1%) and ethyl arginate·2HCl (not more than 1%). Limits are also set for lead, cadmium and mercury (not more than 1 mg/kg) and arsenic (not more than 3 mg/kg).

The molecular formula of ethyl-N- α -lauroyl-L-arginate·HCl is C₂₀H₄₁N₄O₃Cl; the molecular weight is 421.02 g/mol. The structural formula is presented in Figure 1.



• HCl

Figure 1: Structural formula of ethyl-N- α -lauroyl-L-arginate·HCl (SciFinder, online)

The Panel considered that the present application is related to proposed extension of use of the already authorised food additive ethyl lauroyl arginate (E 243). No changes to the identity, characterisation or specification of the food additive are proposed by the applicant nor to the manufacturing process are proposed.

3.1.2. Methods of analysis in food

According to the applicant, the content of the active ingredient, ethyl-N- α -lauroyl-L-arginate·HCl and its by-products in food can be quantified by reversed-phase, high-performance liquid chromatography with ultraviolet (UV) detection at 215 nm. The analytical methods involve the use of different sample preparation techniques depending on the type of food matrix to be analysed. A method for solid and semi-solid matrices and another for liquid matrices were described in the original dossier evaluated by EFSA in 2007 (EFSA, 2007a).

3.1.3. Stability of the substance, and reaction and fate in food

The stability of ethyl lauroyl arginate (E 243) was already assessed by EFSA at the time of the evaluation of the original dossier (EFSA, 2007a). Ethyl lauroyl arginate (E 243) was shown to be stable for more than 2 years at room temperature in a closed container, whereas in aqueous solution at 25°C, its half-life decreased from more than 1 year at pH 4, to 57 days at pH 7 and 34 h at pH 9. Other studies have investigated the combined effect of temperature with low pH conditions, showing these factors markedly influence the hydrolysis of ethyl-N- α -lauroyl-L-arginate to its by-products N- α -lauroyl-L-arginine (LAS) and products resulting from further hydrolysis including arginine and lauric acid (EFSA, 2007a).

The fate of ethyl lauroyl arginate (E 243) in food, its possible incompatibilities with different hydrocolloids, food preservatives and antioxidants (e.g. nitrites), enzymes, colour additives and proteins or protein extracts have also been considered by EFSA at the time of the evaluation of the original dossier. Ethyl-N- α -lauroyl-L-arginate was found to be stable throughout the duration of the study, in all processed food matrices tested with the exception of dried and salted cod, marinated meat and chickpeas. In these three cases, a decrease in ethyl-N- α -lauroyl-L-arginate was observed, due to enzyme-mediated hydrolysis resulting in the formation of LA as the main metabolite product (EFSA, 2007a).

The Panel noted that, in the context of the current application, although the proposed ML for use in meat preparations as defined by Regulation (EC) No 853/2004 restricted to prepacked preparations of fresh minced meat and meat preparations to which other ingredients than additives or salt have been added is of 200 mg/kg, the applicant has claimed that this level would be halved due to enzymatic degradation. In the absence of data supporting this claim, the Panel used the proposed ML of 200 mg/kg

for this food category in the exposure estimate calculated for this scientific opinion (see Table 1 and Section 3.3.2).

3.2. Proposed uses and use levels

The applicant has submitted an application to propose extension of the use of ethyl lauroyl arginate (E 243) as a food additive at MLs as displayed in Table 1. The applicant is also proposing an increase of the ML permitted in the only food category in which ethyl lauroyl arginate (E 243) is currently authorised (FC 08.3.2). Heat-treated meat products, with the exception of emulsified sausages, smoked sausages and liver paste) from 160 mg/kg to 200 mg/kg.

Table 1: Currently authorised maximum levels (MLs) of ethyl lauroyl arginate (E 243) in foods according to the Annex II of Regulation (EC) No 1333/2008 and new proposed uses and MLs

Food category	Currently authorised		Proposed in the application	
	ML (mg/kg or mg/L as appropriate)	Restrictions/exceptions	ML (mg/kg or mg/L as appropriate) ^(a)	Restrictions/exceptions
01.7.1. Unripened cheese excluding products falling in category 16	–		200	
01.7.2. Ripened cheese	–		200	Only cheese prepacked, sliced and cut; layered cheese and cheese with added foods
01.7.4. Whey cheese	–		200	Only cheese prepacked, sliced; layered cheese and cheese with added foods
01.7.5. Processed cheese	–		200	
01.7.6 Cheese products (excluding products falling in category 16)	–		200	Only unripened products; ripened products, prepacked, sliced; layered ripened products and ripened products with added foods
02.2.2. Other fat and oil emulsions including spreads as defined by Regulation (EC) No 1234/2007 and liquid emulsions	–		200	
04.2.5.1. Extra jam and extra jelly as defined by Directive 2001/113/EC	–		200	Only low sugar and similar low calorie or sugar-free products, <i>mermeladas</i>
04.2.5.2. Jam, jellies and marmalades and sweetened chestnut puree as defined by Directive 2001/113/EC	–		200	Only low sugar and similar low calorie or sugar-free products, <i>mermeladas</i>
04.2.5.3 Other similar fruit or vegetable spreads	–		200	Other fruit-based spreads, <i>mermeladas</i>

Food category	Currently authorised		Proposed in the application	
	ML (mg/kg or mg/L as appropriate)	Restrictions/exceptions	ML (mg/kg or mg/L as appropriate) ^(a)	Restrictions/exceptions
05.2. Other confectionery including breath refreshing microsweets	–		200	
05.3. Chewing gum	–		200	
08.2. Meat preparations as defined by Regulation (EC) No 853/2004	–		200	Only prepacked preparations of fresh minced meat and meat preparations to which other ingredients than additives or salt have been added
08.3.1. Non-heat-treated meat products	–		200	
08.3.2. Heat-treated meat products	160	Except emulsified sausages, smoked sausages and liver paste	200	
08.3.3. Casings and coatings and decorations for meat	–		200	
09.2. Processed fish and fishery products including molluscs and crustaceans	–		200	Only salted, dried fish; only semi-preserved fish and fisheries products including crustaceans, molluscs, surimi and fish/crustacean paste; cooked crustaceans and molluscs
09.3. Fish roe	–		200	
12.6. Souces	–		200	
12.7. Salads and savoury-based sandwich spreads	–		100	
14.1.4. Flavoured drinks	–		50	
14.1.5.2. Other	–		50	Only liquid tea concentrates and liquid fruit and herbal infusion concentrates

– : currently not authorised.

(a): expressed as ethyl-N- α -lauroyl-L-arginate·HCl.

3.3. Exposure data

3.3.1. Food consumption data used for exposure assessment

EFSA Comprehensive European Food Consumption Database

Since 2010, the EFSA Comprehensive European Food Consumption Database (Comprehensive Database) has been populated with national data on food consumption at a detailed level. Competent authorities in the European countries provide EFSA with data on the level of food consumption by the individual consumer from the most recent national dietary survey in their country (cf. Guidance of EFSA on the 'Use of the EFSA Comprehensive European Food Consumption Database in Exposure

Assessment' (EFSA, 2011a). New consumption surveys added in the Comprehensive database were also taken into account in this assessment.⁵

The food consumption data gathered by EFSA were collected by different methodologies and thus direct country-to-country comparisons should be interpreted with caution. Depending on the food category and the level of detail used for exposure calculations, uncertainties could be introduced owing to possible subjects' underreporting and/or misreporting of the consumption amounts. Nevertheless, the EFSA Comprehensive Database represents the best available source of food consumption data across Europe at present.

Food consumption data from the following population groups: infants, toddlers, children, adolescents, adults and the elderly were used for the exposure assessment. For the present assessment, food consumption data were available from 33 different dietary surveys carried out in 19 European countries (Table 2).

Table 2: Population groups considered for the exposure estimates of ethyl lauroyl arginate (E 243)

Population	Age range	Countries with food consumption surveys covering more than 1 day
Infants	From more than 12 weeks up to and including 11 months of age	Bulgaria, Denmark, Finland, Germany, Italy, UK
Toddlers	From 12 months up to and including 35 months of age	Belgium, Bulgaria, Denmark, Finland, Germany, Italy, Netherlands, Spain, UK
Children ^(a)	From 36 months up to and including 9 years of age	Austria, Belgium, Bulgaria, Czech Republic, Denmark, Finland, France, Germany, Greece, Italy, Latvia, Netherlands, Spain, Sweden, UK
Adolescents	From 10 years up to and including 17 years of age	Austria, Belgium, Cyprus, Czech Republic, Denmark, Finland, France, Germany, Italy, Latvia, Spain, Sweden, UK
Adults	From 18 years up to and including 64 years of age	Austria, Belgium, Czech Republic, Denmark, Finland, France, Germany, Hungary, Ireland, Italy, Latvia, Netherlands, Romania, Spain, Sweden, UK
The elderly ^(a)	From 65 years of age and older	Austria, Belgium, Denmark, Finland, France, Germany, Hungary, Ireland, Italy, Romania, Sweden, UK

(a): The terms 'children' and 'the elderly' correspond, respectively, to 'other children' and the merge of 'elderly' and 'very elderly' in the Guidance of EFSA on the 'Use of the EFSA Comprehensive European Food Consumption Database in Exposure Assessment' (EFSA, 2011a).

Consumption records were codified according to the FoodEx classification system (EFSA, 2011b). Nomenclature from the FoodEx classification system has been linked to the food categorisation system (FCS) as presented in Annex II of Regulation (EC) No 1333/2008, part D, to perform exposure estimates. In practice, the FoodEx food codes were matched to the FCS food categories.

3.3.2. Exposure to ethyl lauroyl arginate (E 243) from its proposed use as food additive

Estimate of exposure based on the FAIM tool

The applicant has provided an estimate of the exposure to ethyl lauroyl arginate (E 243) based on the output obtained using the new Comprehensive European Dietary Exposure Model ('Documentation provided to EFSA' n.1). According to the applicant, the exposure model used was based on the EFSA FAIM tool and used data from the 2015 release of the EFSA Comprehensive European Food Consumption Database.

In accordance with the applicable guidance for submission for food additive evaluations (EFSA ANS Panel, 2012) for estimating exposure to the food additive from the proposed changes to the authorised conditions of use, the Panel used the FAIM tool that has been developed with the purpose of supporting calculation of exposure estimates.

A new estimated exposure was, therefore, performed by EFSA using the FAIM tool (output generated on 8–10 January 2019). The results of the estimate exposure are reported in Table 3.

⁵ Available online: <http://www.efsa.europa.eu/en/datexfoodcdb/datexfooddb.htm>

Table 3: Summary of dietary exposure to ethyl lauroyl arginate (E 243) from its use as a food additive in the ML scenario and in the proposed extension of use scenario, in six population groups (minimum–maximum across the dietary surveys in mg/kg bw per day) calculated using the FAIM tool

	Infants (12 weeks– 11 months)	Toddlers (12–35 months)	Children (3–9 years)	Adolescents (10–17 years)	Adults (18–64 years)	The elderly (≥ 65 years)
Currently authorised ML						
• Mean	0.02–0.2	0.1–0.8	0.2–0.7	0.1–0.4	0.1–0.3	0.1–0.3
• 95th percentile	0.2–0.6	0.5–1.3	0.5–1.3	0.2–0.8	0.2–0.7	0.1–0.6
Proposed extension of uses						
• Mean	0.1–0.9	0.4–2.8	0.9–2.2	0.4–1.5	0.4–1.3	0.4–1.1
• 95th percentile	0.4–2.6	1.1–4.2	1.9–4.0	0.9–2.6	0.8–2.4	0.7–1.8

From the currently authorised use of ethyl lauroyl arginate (E 243) as a food additive, the calculated exposure using the FAIM template ranges from 0.02 mg/kg bw per day in infants to 0.8 mg/kg bw per day in toddlers at the mean exposure level and from 0.1 mg/kg bw per day in the elderly to 1.3 mg/kg bw per day in toddlers and children at the high exposure level.

From the proposed changes to the authorised use of ethyl lauroyl arginate (E 243) as a food additive, the calculated exposure using the FAIM tool ranges from 0.1 mg/kg bw per day in infants to 2.8 mg/kg bw per day in toddlers at the mean exposure level and from 0.4 mg/kg bw per day in infants to 4.2 mg/kg bw per day in toddlers at the high exposure level.

For the exposure estimate to the proposed new uses of ethyl lauroyl arginate (E 243) calculated with the FAIM tool using the data indicated in the current application dossier, EFSA decided not to include the proposed uses in food categories 04.2.5.1, 04.2.5.2 and 04.2.5.3 in the estimate because in this case, the tool only allows the use of the more general category 04.2 (Processed fruit and vegetables) which would have introduced a significant overestimation in the results. The Panel further noted that the many exceptions proposed by the applicant in its application for extending the use of the authorised food additive ethyl lauroyl arginate (E 243) in several food categories may have also resulted in a general overestimation of exposure.

The proposed new use in food category 01.7.6 could not be taken into account in the calculation of exposure because this food category is currently missing from the FAIM tool.

For the exposure estimate calculated using the single use currently authorised for this food additive, for which the FAIM tool only allows selection of the whole food category 08.3, even though the food additive is authorised only in the subcategory 08.3.2 with some restrictions. The output generated via the FAIM tool may have resulted therefore in an overestimation of exposure.

The main food categories that contribute to total exposure to ethyl lauroyl arginate (E 243) from the new proposed uses calculated using FAIM tool are reported in Table 4.

Table 4: Main food categories contributing to total exposure to ethyl lauroyl arginate (E 243) calculated with proposed ML across dietary surveys using FAIM for the proposed uses. Results are shown as range of contribution (%) and number of surveys

Food Category	Infants (12 weeks– 11 months)	Toddlers (12–35 months)	Children (3–9 years)	Adolescents (10–17 years)	Adults (18–64 years)	The elderly (≥ 65 years)
01.7.1 Unripened cheese excluding products falling in category 16	6–49% (3)	10–29% (8)	6–21% (11)	6–21% (3)	5–21% (7)	5–16% (5)
01.7.2 Ripened cheese	6–29% (3)	5–21% (8)	5–14% (11)	6–13% (11)	5–14% (9)	6–18% (6)

Food Category	Infants (12 weeks– 11 months)	Toddlers (12–35 months)	Children (3–9 years)	Adolescents (10–17 years)	Adults (18–64 years)	The elderly (≥ 65 years)
02.2 Fat and oil emulsions mainly of type water-in-oil	8–36% (3)	5–22% (7)	5–17% (11)	5–16% (7)	7–9% (6)	5–14% (10)
08.3 Meat products	7–83% (6)	17–67% (10)	18–59% (18)	13–51% (17)	9–44% (17)	8–38% (14)
14.1.4.1 Flavoured drinks with sugar	8–19% (2)	9–32% (7)	6–33% (18)	10–35% (17)	6–18% (14)	8–13% (2)
14.1.5 Coffee, tea, herbal and fruit infusions, chicory; tea, herbal and fruit infusions and chicory extracts; tea, plant, fruit and cereal preparations for infusions, as well as mixes and instant mixes of these products	17–54% (4)	7–22% (3)	7–24% (8)	6–26% (11)	11–53% (17)	26–64% (14)

The main food category that contributes to total exposure to ethyl lauroyl arginate (E 243) across all populations groups is 08.3 (Meat products). Significant contributions are also given by the food categories related to the proposed use of the food additive in flavoured drinks (14.1.1) and in other drinks (14.1.5). For infants and children also the proposed use in cheese products (01.7.1 and 01.7.2) may also contribute significantly to the exposure.

Refined estimate of exposure to ethyl lauroyl arginate for the currently authorised use

In order to quantify the possible overestimation resulting from the use of the generic food category 08.3 in the FAIM tool, an additional exposure estimate was calculated by EFSA for the currently authorised use of ethyl lauroyl arginate (E 243) in food category 08.3.2 (heat-treated meat products) at the ML of 160 mg/kg with the exception of emulsified sausages, smoked sausages and liver paste, selecting food from the nomenclature of the EFSA Comprehensive database at the most detailed level possible. The output is shown in Table 5.

Table 5: Additional exposure estimate to the food additive ethyl lauroyl arginate (E 243) in its currently authorised use ML (160 mg/kg) in food category 08.3.2 (heat-treated meat products) and applicable exceptions

	Infants (12 weeks– 11 months)	Toddlers (12–35 months)	Children (3–9 years)	Adolescents (10–17 years)	Adults (18–64 years)	The elderly (≥ 65 years)
Maximum level exposure assessment scenario						
– Mean	< 0.1–0.1	< 0.1–0.1	< 0.1–0.1	< 0.1–0.1	< 0.1–0.1	< 0.1–0.1
– 95 th percentile	0.0–0.3	0.1–0.5	0.1–0.5	0.1–0.3	0.1–0.3	0.1–0.2

The Panel noted that the additional exposure estimate performed for ethyl lauroyl arginate (E243), at the currently permitted ML (160 mg/kg) in food category 08.3.2 and taking into account the applicable exceptions, resulted in a lower estimate compared to the one obtained using the FAIM tool. At mean levels, the values were below the ADI of 0.5 mg/kg bw in all age groups, whereas the ADI was reached at the 95th percentile in toddlers and children.

The foods listed in Appendix A were considered for the additional exposure estimate to the currently permitted use of ethyl lauroyl arginate (E 243) at the permitted ML.

3.3.3. Summarised data extracted from the Mintel's Global New Products Database

The Mintel's GNPD is an online database which monitors new introductions of packaged goods in the market worldwide. It contains information of over 2.9 million food and beverage products of which more than 1,100,000 are or have been available on the European food market. Mintel started covering EU's food markets in 1996, currently having 20 out of its 28 member countries and Norway presented in the Mintel GNPD.

For the purpose of this Scientific Opinion, Mintel's GNPD was used for checking the labelling of food and beverages products and food supplements for ethyl lauroyl arginate (E 243) within the EU's food market as the database contains the compulsory ingredient information on the label.

According to Mintel's GNPD, ethyl lauroyl arginate (E 243) was labelled on 54 products belonging to six food subcategories: meat products (n = 42), poultry products (n = 9), sandwiches/wraps (n = 2) and pizzas (n = 1). The products were published in this database between January 2014⁶ and January 2019.

Table 6 lists the percentage of the food products labelled with ethyl lauroyl arginate (E 243) out of the total number of food products per food subcategories according to the Mintel's GNPD food classification. The percentages ranged from 0.02% to 0.2% in Mintel's GNPD food subcategory 'Meat Products'. The average percentage of foods labelled to contain ethyl lauroyl arginate (E 243) was 0.1%.

Table 6: Number and percentage of food products labelled with ethyl lauroyl arginate (E 243) out of the total number of food products present in the Mintel GNPD per food subcategory between 2013 and 2018

Mintel subcategory(a)	Total number of products	Products labelled with ethyl lauroyl arginate E 243)	
		Number	%
Meat Products	22,248	42	0.2
Poultry Products	9,090	9	0.1
Sandwiches/Wraps	2,648	2	0.1
Pizzas	5,044	1	0.02
Total Sample	39,030	54	0.1

3.3.4. Uncertainty analysis

Uncertainties in the exposure assessment of ethyl lauroyl arginate (E 243) have been discussed above. In accordance with the guidance provided in the EFSA opinion related to uncertainties in dietary exposure assessment (EFSA, 2007b), the following sources of uncertainties have been considered and summarised in Table 7.

Table 7: Qualitative evaluation of influence of uncertainties on the dietary exposure estimate

Sources of uncertainties	Direction (a)
Consumption data: different methodologies/representativeness/underreporting/misreporting/no portion size standard	+/-
Methodology used to estimate high percentiles (95th) long-term (chronic) exposure based on data from food consumption surveys covering only a few days	+
Food categories selected for the FAIM exposure assessment: exclusion of very specific food categories to avoid a huge overestimation (n=3 sub food categories belonging to FC 04.2)	-
Food categories selected for the FAIM exposure assessment: inclusion of food categories without considering the restriction/exception (n=5 food categories in the FAIM estimate)	+
Food categories selected for the FAIM exposure assessment: whole food category taken into account instead of subcategory (n=1 FC 02.2 instead of FC 02.2.2 in the FAIM estimate)	+

⁶ The use of ethyl lauroyl arginate as a food additive in the EU was only authorised in 2014.

Sources of uncertainties	Direction ^(a)
Food categories selected for the exposure assessment: exclusion of food categories due to missing FoodEx linkage (n=1 FC 01.7.6)	–
Maximum level exposure assessment scenario: - exposure calculations based on the maximum proposed use levels (from the applicant)	+

(a): +, uncertainty with potential to cause overestimation of exposure; –, uncertainty with potential to cause underestimation of exposure.

Additionally, the Panel noted that use in cosmetic products for oral use (see Section 1.2.1) could be a potential source of exposure to the ethyl lauroyl arginate, although the Panel was not able to quantify it.

Considering the exposure estimates based on the current authorised MPLs, the Panel considered that the uncertainties identified would result in an overestimation of the exposure to ethyl lauroyl arginate (E 243) as a food additive in European countries considered in the EFSA European database as it is assumed that all foods with the food category 08.3.2 with the restriction provided will contain the food additive ethyl lauroyl arginate (E 243) at the MPL of 160 mg/kg.

Considering the exposure estimates based on the uses and use levels proposed by the applicant and performed with the FAIM template, uncertainties would lead to an overestimation of exposure to ethyl lauroyl arginate (E 243) as a food additive in European countries considered in the EFSA European database. This is also due to the fact that it is assumed that all foods within the proposed food categories would contain the food additive ethyl lauroyl arginate (E 243) and that its levels would always be at the MPL (i.e. equal to 200 or 50 mg/kg depending on the food category). Added to that, proposed restrictions to food categories are not taken into account.

3.4. Biological and toxicological data

A document elaborating a new interpretation of the currently available toxicological data has been submitted (Documentation provided to EFSA n. 1) in support of a proposal for revising the current ADI of 0.5 mg/kg bw previously set by the AFC Panel in 2007.

3.4.1. Toxicological endpoints identified in previous evaluations

In the new interpretation provided by the applicant, the critical endpoints identified in previous evaluations by either EFSA or JECFA are re-examined, in particular the two selected for the derivation of the current ADIs applicable to ethyl lauroyl arginate (E 243).

3.4.1.1. Effects on haematology

In its initial evaluation of the application for use of ethyl lauroyl arginate (E 243) as a new food additive, the former EFSA AFC Panel had noted effects on WBC counts reported in two 13-week and in one 52-week toxicity studies in rats. The AFC Panel, at the time, concluded that these effects could not be disregarded because they were observed in different rat strains and in different sexes, and therefore selected these as the critical toxicological endpoint for deriving the ADI (EFSA, 2007a).

The lowest NOAELs from the 90-day study in rats (47 and 56 mg/kg bw per day for males and females, respectively) was selected as the point of reference for deriving the current ADI of 0.5 mg ethyl lauroyl arginate (E 243)/kg bw per day by applying an uncertainty factor of a 100. An extra factor for extrapolation to chronic use was not deemed necessary because the effect on WBC did not become more severe upon prolonged exposure.

The selection of this critical endpoint for the derivation of the ADI was already challenged by the applicant in 2009, when the opinions of three individual experts, re-examining the results on WBC from the toxicological studies previously evaluated by EFSA, were submitted for evaluation by the ANS Panel (ANS Panel, 2009).

The three experts had concluded separately that the haematological findings were of no toxicological relevance based on the fact that these were inconsistent both within and between the studies considered and without a dose–response relationship and because the effects were not accompanied by histopathological changes in any of the studies considered. The ANS Panel, having reviewed the arguments provided by the applicant, at the time was of the opinion that: ‘given the fact that the alterations in WBC counts were found consistently in different strains of rats and in different sexes in

two 90-day studies and in a 52-week study, the toxicological relevance of these findings cannot be assessed as long as the mechanism leading to these abnormalities is not elucidated' (EFSA, 2009).

In the same scientific opinion, the ANS Panel evaluated the hypotheses postulated in the expert opinions for a possible mechanism that could explain the alterations in WBC observed in the toxicological tests and considered them to be speculative and inconsistent. The Panel therefore concluded that 'scientific evidence of a plausible mechanism for the alterations in WBC counts has not been provided and that the concerns and uncertainties expressed in the opinion of the AFC Panel have not been addressed by the new information provided' (EFSA, 2009).

The same arguments provided by the applicant for disregarding the effects on WBC were instead supported by JECFA in its 2009 opinion (JECFA, 2009), in which effects on leucocytes were noted but considered to be inconsistent within and between studies and not likely to be biologically significant. JECFA further noted that: 'changes were not accompanied by histopathological changes in the progenitor cell populations of the bone marrow or lymphoid tissue, which would be expected if the effect were due to systemic toxicity' (JECFA, 2009), and therefore, this endpoint was not considered as the critical one for the derivation of the ADI by JECFA.

As part of the current submission for evaluation, the applicant has provided an updated statistical analysis of the individual data on haematology from the three toxicity studies evaluated as part of the original application dossier (Documentation provided to EFSA n.1):

- Study No LMA 3/962342: Mirenat-N toxicity to rats for 13 weeks. Huntingdon Life Sciences Ltd, 1996
- Study No LMA 031/004276: LAE toxicity study by dietary administration to Han Wistar rats for 13 weeks. Huntingdon Life Sciences Ltd, 2001
- Study No LMA 050/042556: Lauric arginate toxicity study by dietary administration to CD rats for 52 weeks. Huntingdon Life Sciences Ltd, 2005

The statistical re-analyses conducted on the data from these three studies have confirmed the previous observations of a statistically significant dose-dependent decrease in WBC and lymphocyte counts.

The current submission also contains a re-analysis of the data from the following two short-term toxicity studies, reporting no effects on WBC parameters:

- Study No LMA 02/952124: Mirenat-N preliminary toxicity to rats by dietary administration for 4 weeks. Huntingdon Life Sciences Ltd, 1995
- Study No LMA 030/000063: LAE dose range finding/palatability study by dietary administration to Han Wistar rats for 4 weeks. Huntingdon Life Sciences Ltd, 2000.

In both of the 4-week studies that were re-analysed for the current application, no statistically significant effects were reported, only a non-significant trend towards a dose-dependent decrease in WBC and lymphocyte counts. The lower number of animals included in the studies is, however, acknowledged by the authors.

In addition, data at week 14 from study No LMA 050/042556 and those at week 13 from the two 90-day studies No LMA 031/004276 and No LMA 3/962342 were pooled and analysed.

Data were first normalised to 100% corresponding to the control group and pooled to compare the effect of intake of ethyl lauroyl arginate on WBC and lymphocyte count. The results of this additional, pooled analysis showed a decrease of more than 10% compared to control at doses higher than 445 mg/kg bw per day of ethyl lauroyl arginate (of the defined specifications). Data on WBC count from this pooled analysis were input into the United States Environmental Protection Agency (EPA) Benchmark Dose Software (BMDS) (2.6.0.1) to evaluate dose response.

The two models used, linear and polynomial, resulted in a Benchmark Dose Lower bound (BMDL) of 984 mg/kg bw per day and 391 mg/kg bw per day, respectively.

The Panel noted that there appeared to be divergences in this BMDL modelling from the EFSA guidance and that the reporting was incomplete. For example, the rationale for selection of the time-points used for the data taken from the chronic toxicity study and used for the data modelling, along with pooling procedures used were not given. The Panel also noted that in the chronic study, statistically significant effects on WBC were observed, at 26 and 52 weeks, however, at the time point taken into account in the Benchmark Dose (BMD) analysis (week 14), these effects were not yet statistically significant.

In the light of the results from the updated statistical evaluation of this toxicological endpoint, the applicant has provided a further reconsideration of the experts' assessment previously submitted to EFSA, carried out by a fourth expert (Documentation provided to EFSA n.1). In its assessment, the

points previously brought forward by the applicant for reconsidering the ADI set by the AFC Panel for the food additive ethyl lauroyl arginate (E 243) are further elaborated. Possible explanations for these observations, previously provided to EFSA, were further commented on in the current submission, however eventually acknowledging that a decrease in WBC and lymphocyte counts is observed with increasing doses of ethyl lauroyl arginate, above 400 mg/kg bw per day, albeit in the absence of any observations of toxicity in bone marrow histopathology or myeloid cells evaluation that could be related with the decline in WBC counts.

3.4.1.2. Reproductive toxicity

In its initial evaluation of the application for use of ethyl lauroyl arginate (E 243) as a new food additive, the former EFSA AFC Panel had noted effects on the delay (average of 4 days) in vaginal opening in the female offspring observed in two reproductive toxicity studies. In both studies, the effect was observed in the highest dose group, corresponding to a dietary concentration of 15,000 mg ethyl lauroyl arginate/kg feed. The AFC Panel had estimated that the achieved dosage would be in excess of 1,900 mg/kg bw per day. The AFC Panel, at the time, noted that the effect was not accompanied by other functional changes, but was of the opinion that it could not be disregarded, and therefore identified a NOAEL of 6,000 mg/kg diet (corresponding to an intake of 434 mg ethyl lauroyl arginate/kg bw per day and 502 mg ethyl lauroyl arginate/kg bw per day for males and females, respectively). In its opinion, the AFC Panel further noted statistically significant increase in body weight of the offspring just before weaning in the high-dose group compared to control (EFSA, 2007a).

In its 2009 evaluation, JECFA identified this effect as the critical toxicological endpoint for deriving its current ADI of 0–4 mg/kg bw per day (expressed as ethyl-N- α -lauroyl-L-arginate HCl), by applying a safety factor of 100 to the NOAEL of 6,000 mg/kg diet (calculated by JECFA as corresponding to 442 mg/kg bw per day) identified from the two-generation reproductive toxicity study (JECFA, 2009).

As part of the current submission for evaluation (Documentation provided to EFSA n.1), the applicant has provided an updated statistical analysis of the individual data on delayed vaginal opening from the two reproductive toxicity studies evaluated as part of the original application dossier:

- Study No LMA 041/032575: LAE preliminary study on the effects on reproductive performance in CD rats by dietary administration. Huntingdon Life Sciences Ltd, 2003
- Study No LMA 042/032553: LAE two-generation reproductive performance study by dietary administration to CD rats. Huntingdon Life Sciences Ltd, 2005

The applicant has repeated an analysis of the results from Study No LMA 042/032553 using analysis of variance (ANOVA)/post hoc tests, confirming the previously reported findings of a statistically significant change both in the timing of vaginal opening and in the increase in body weight. Because the latter was measured in all groups at the time of vaginal opening, in the expert opinion that accompanies the current submission, it is noted that the observed effect on body weight may be possibly due to the fact that animals in the higher dose groups were on average 4 days older than those in the negative control group, and that no differences in weight of the F1 generation females across treatment groups were appreciated during the 10-week period prior to mating. The applicant, therefore, concludes that delay in vaginal opening seems to be a treatment-related effect that is not mediated by changes in body weight. The lack of observed effects on other reproductive toxicity endpoints (such as oestrus cycle pre-mating or pre-termination, fertility, primordial follicle counts, percentage of males in each litter and measurement of anogenital distance in the F2 offspring) is also deemed by the applicant as supporting evidence for considering the delay in vaginal opening as a transient effect at the highest exposure, and of no long-term toxicological relevance.

3.4.2. Postulated Mode of Action

Within the current application, the applicant has submitted an additional expert opinion, postulating a possible mode of action for the effects observed in the toxicological studies conducted with ethyl lauroyl arginate (E 243) (Documentation provided to EFSA n.1).

It has been previously demonstrated that further to oral administration, ethyl lauroyl arginate is fully metabolised to L-arginine. Hydrolysis of ethyl lauroyl arginate is expected to increase the levels of arginine in blood and tissues.

According to the applicant, arginine intake resulting from dietary exposure to ethyl lauroyl arginate would therefore be responsible for the adverse effects observed in the animal studies.

With respect to the effects reported on WBC, the applicant has listed a number of publications reporting a direct, 'immunomodulatory' effect for arginine, possibly mediated via nitric oxide synthase and nitric oxide. Also in the case of the reproductive toxicity effects, the Panel noted that no detailed explanation of a plausible underlying mechanism of action for arginine is given, beside the fact that arginine, through the formation of nitric oxide, is involved in a wide range of reproductive functions.

The Panel, however, noted that this statement made by the applicant is not formulated following an appropriate framework for listing the available data in support of a particular mode of action (see, for example, Meek et al., 2014). The Panel also noted that no explanation has been given by the applicant on how the relevant publications have been identified or selected, and no information is provided on the scope or the criteria applied to the literature searches performed.

Moreover, the Panel noted that the published literature that has been brought in support of a possible role of arginine in the observed effects is inconclusive.

3.5. Discussion

In addressing the current mandate, the Panel noted that no new toxicological data have been provided by the applicant in support of the application for the proposed extension of use of the food additive ethyl lauroyl arginate (E 243) in several food categories, rather a new interpretation of the currently available toxicological data.

The applicant has re-analysed previously evaluated toxicological studies confirming the previously established statistically significant changes in WBC counts and the effects on delayed vaginal opening.

According to the applicant, the effects observed in the toxicological studies can be explained with the increased dietary intake of arginine over several weeks or months, resulting from the exposure to ethyl lauroyl arginate. The applicant, however, did not provide sufficient evidence for the proposed mode of action of the purported role of arginine in the adverse effects observed in the animal studies.

The applicant is proposing to increase the ADI for ethyl lauroyl arginate to 5 mg/kg bw per day by applying an uncertainty factor of 100 to the NOAEL for delayed vaginal opening (502 mg/kg bw per day) observed in a reproductive toxicity study in rats. The corresponding ADI for the active substance ethyl-N- α -lauroyl-L-arginate HCl would be 4.4 mg/kg bw per day.

The Panel noted that this derivation of a NOAEL is inconsistent with the assessment made by the applicant that the observed delay in vaginal opening is of no long-term toxicological relevance. The Panel considered the effect on vaginal opening as a toxicologically relevant endpoint. However, the Panel noted that the NOAEL from this effect would be higher than the NOAEL for the observed effects on WBC, and therefore maintained the previous position of both AFC and ANS Panels that the lowest NOAEL should be used for deriving an ADI.

Jointly with the proposed revision of the current ADI of 0.5 mg/kg bw per day of ethyl lauroyl arginate (E 243), the applicant is also proposing several changes to the currently permitted use and use level of this food additive. Ethyl lauroyl arginate (E 243) is currently authorised in a single food category (08.3.2, heat-treated meat products, with the exception of emulsified sausages, smoked sausages and liver paste) at a ML of 160 mg/kg food. The applicant is proposing to increase the level permitted for use in this food category to 200 mg/kg food and to remove existing restrictions as well as extending the use of this food additive to 20 additional food categories.

In the latest exposure assessment performed, EFSA estimated exposure to ethyl lauroyl arginate using five scenarios each using very narrow, specific descriptions of the types of food to which it was proposed to add ethyl lauroyl arginate (i.e. meat specialities, pastes, pâtés and terrines, preserved meat, sausages) (EFSA, 2013).

In the current opinion, an exposure estimate was calculated by the Panel for ethyl lauroyl arginate (E 243) in its currently permitted single use and use level, taking into account the applicable restrictions and exceptions. According to this new exposure estimate, maximum exposure to ethyl lauroyl arginate (E 243) at the mean level would be 0.1 mg/kg bw per day in all age groups. At the 95th percentile, the current ADI of 0.5 mg/kg bw would be reached in toddlers and children.

For the proposed new uses, the Panel has performed an exposure assessment to ethyl lauroyl arginate (E 243) using the FAIM tool. According to this assessment, the estimated exposure to ethyl lauroyl arginate (E 243) from its new proposed uses and use levels would range from 0.1 mg/kg bw per day in infants to 2.8 mg/kg bw per day in toddlers at the mean exposure level and from 0.4 mg/kg bw per day in infants to 4.2 mg/kg bw per day in toddlers at the high exposure level.

In principle, it would have been possible for the Panel to refine its exposure assessment to ethyl lauroyl arginate (E 243) at the new proposed uses and use levels, however, in the light of the results

from the refined exposure estimate performed at the current ML and in the single authorised use, showing that the current ADI of 0.5 mg/kg is already reached in some of the age groups at the 95th percentile and that this more refined estimate does not take into account the additional exposure resulting from the use in cosmetics, the Panel considered that there is no need to further refine the exposure estimate to ethyl lauroyl arginate (E 243) at the proposed new uses and use levels.

4. Conclusions

The Panel considered the new information provided, including the re-examination of some of the results from the toxicological studies included in the original application dossier submitted for the initial evaluation of ethyl lauroyl arginate (E 243) in 2007. The Panel concluded that it does not contain new scientific evidence.

The concerns and uncertainties expressed in the previous scientific opinions of the AFC and ANS Panels remain to be addressed and there is no justification for changing the current ADI.

Based on the above, the Panel concluded that the current ADI of 0.5 mg/kg bw would be reached in toddlers and children at the 95th percentile already for exposure estimates calculated using the currently permitted ML for ethyl lauroyl arginate (E 243). At the proposed new uses and use levels, the ADI would be exceeded at mean level of consumption in all age groups.

Documentation provided to EFSA

- 1) Food additive application dossier – Ethyl lauroyl arginate (E 243). 6 July 2017. Submitted by Laboratorios Miret S.A. (LAMIRSA).

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Abbreviations

ADI	Acceptable Daily Intake
AFC	Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Foods
ANOVA	Analysis of variance
ANS	EFSA Panel on Food Additives and Nutrient Sources added to Food
BMD	Benchmark Dose
BMDL	Benchmark Dose Lower bound
BMDS	Benchmark Dose Software
bw	Body weight
EPA	Environmental Protection Agency
EU	European Union
FAIM	Food Additives Intake Model
GSFA	General Standard for Food Additives
JECFA	The FAO/WHO Joint Expert Committee on Food Additives
ML	Maximum level
NOAEL	No Observed Adverse Effect Level
SCCS	Scientific Committee on Consumer Safety
SCCP	Scientific Committee on Consumer Products
SED	Systemic Exposure Dosage
UV	ultraviolet
WBC	White blood cells

Appendix A – List of foods considered for the additional exposure estimate to the currently permitted use of ethyl lauroyl arginate (E 243) at the permitted ML

FoodEx code	Foodex name	Level
A.06.08	Preserved meat	3
A.06.08.001	Ham, pork	3
A.06.08.002	Ham, beef	3
A.06.08.006	Ham, turkey	3
A.06.08.007	Bacon	3
A.06.08.014	Luncheon meat	3
A.06.08.015	Preserved poultry	3
A.06.09.003	Cooked sausage	3
A.06.09.003.001	Blood sausage	4
A.06.09.003.002	Blood and tongue sausage	4
A.06.09.003.003	Liver sausage, liverwurst	4
A.06.10	Meat specialities	2
A.06.10.001	Pork meat loaf	3
A.06.10.002	Beef loaf	3
A.06.10.003	Meat in aspic	3
A.06.10.004	Ham and cheese loaf	3
A.06.10.005	Liver cheese or liver loaf	3
A.06.10.008	Head cheese (Brawn)	3
A.06.10.009	Sulze	3
A.06.11	Pastes, pâtés and terrines	3
A.06.11.001	Meat paste	3
A.06.11.005	Terrine	3