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Safety of L-tryptophan technically pure, produced by fermentation with *Escherichia coli* DSM 25084, KCCM 11132P and SARI12091203 for all animal species based on a dossier submitted by FEFANA Asbl

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Abstract

In 2015, the EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) issued an opinion on the safety and efficacy of L-tryptophan produced by fermentation using three strains of *Escherichia coli,* when used as a nutritional additive for all animal species. The Panel concluded that the safety of L-tryptophan produced by *E. coli* SARI12091203 for target animals, consumers, users and the environment could not be assessed because the data submitted did not permit the identity and safety of the strain, and the purity of the additive, to be determined. During the current assessment, the applicant withdrew the application for L-tryptophan produced by *E. coli* SARI12091203. L-Tryptophan produced by *E. coli* DSM 25084 or KCCM 11132P was considered safe for non-ruminant target species, the consumer and the environment. For both products, the level of endotoxins and the possible dusting potential indicated a risk by inhalation for the user. In the absence of data, a potential for dermal sensitisation could not be excluded. The Commission gave the applicant the possibility of submitting complementary information to allow the FEEDAP Panel to complete its assessment. The additional data on the characterisation of the additives and on their potential for inhalation toxicity and as skin sensitisers are the subject of the current opinion. Due to improvements in the manufacturing process, the level of endotoxins present in the L-tryptophan produced by *E. coli* KCCM 11132P has been markedly reduced; consequently, the endotoxin content does not represent a health risk for the user. The additive has a low acute toxicity by inhalation and is not considered as a potential skin sensitiser. L-Tryptophan produced by *E. coli* DSM 25084 is not considered a skin sensitiser. The level of endotoxins in this product, however, represents a risk by inhalation for the user handling the additive.

Keywords: nutritional additive, amino acids, L-tryptophan, safety, efficacy, genetically modified microorganisms

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Summary

Following a request from the European Commission, the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) was asked to deliver a scientific opinion on the safety of L-tryptophan produced by genetically modified strains of *Escherichia coli* (DSM 25084, KCCM 11132P or SARI12091203) for all animal species. L-Tryptophan produced by *E. coli* SARI12091203 was withdrawn from the application during the assessment.

The product under assessment is a nutritional feed additive produced by fermentation using one of three strains of *Escherichia coli*. In 2015, the FEEDAP Panel issued an opinion on the safety and efficacy of the product and concluded that 'L-Tryptophan produced by *E. coli* SARI12091203 could not be assessed because the data submitted did not permit the identity and safety of the strain, and the purity of the additive, to be determined. The EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) could not conclude on the safety of this product for target animals, consumers, users and the environment'. L-Tryptophan produced by *E. coli* DSM 25084 or KCCM 11132P was safe for non-ruminant target species when supplemented to diets in appropriate amount, for the consumer of animal products and the environment. The FEEDAP Panel also concluded that 'the level of endotoxins present in the products of *E. coli* DSM 25084 or KCCM 11132P and its possible dusting potential indicate an inhalation risk for the user. In the absence of data on the potential for dermal sensitisation it is concluded that such potential may exist'.

The Commission gave the applicant the possibility of submitting complementary information to allow the FEEDAP Panel to complete its assessment. The additional data on the characterisation of the additives and on their potential for inhalation toxicity and as skin sensitisers are the subject of the current opinion.

The EFSA FEEDAP Panel assessed the additional data using an approach in line with the principles laid down in Regulation (EC) No 429/2008 and the relevant guidance documents: Guidance on nutritional additives (EFSA FEEDAP Panel, 2012a) and Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012b).

Due to improvements in the manufacturing process, the level of endotoxins present in L-tryptophan produced by *E. coli* KCCM 11132P has been markedly reduced; consequently, the endotoxin content does not represent a health risk for the user. The additive has a low acute toxicity by inhalation and is not considered as a potential skin sensitiser.

L-Tryptophan produced by *E. coli* DSM 25084 is not considered a skin sensitiser. The level of endotoxins in this product, however, represents a risk by inhalation for the user handling the additive.

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1. Introduction

1.1. Background and Terms of Reference as provided by the requestor

Regulation (EC) No 1831/2003[1] establishes rules governing the Community authorisation of additives for animal nutrition and, in particular, article 9 defines the terms of the authorisation by the Commission.

The applicant, AMAC/EEIG – Amino Acids Authorisation Consortium, is seeking a Community authorisation of L-tryptophan, technically pure, to be used as nutritional additive for all animal species (Table 1).

Table 1: Description of the substances

Category of additive	Nutritional additive
Functional group of additive	Amino acids, their salts and analogues
Description	L-tryptophan, technically pure
Target animal category	All animal species
Applicant	FEFANA
Type of request	New opinion

On September 2015, the Panel on Additives and Products or Substances used in Animal Feed of the European Food Safety Authority ("Authority"), in its opinion on the safety and efficacy of the product, considers that L-Tryptophan produced by *E. coli* SARI12091203 could not be assessed because the data submitted did not permit the identity and safety of the strain, and the purity of the additive, to be determined. The EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) could not conclude on the safety of this product for target animals, consumers, users and the environment. The level of endotoxins present in the products of *E. coli* KCCM 11132P or *E. coli* DSM 25084 and its possible dusting potential indicate an inhalation risk for the user. In the absence of data on the potential for dermal sensitisation it is concluded that such potential may exist.

The Commission gave the possibility to the applicant to submit complementary information in order to complete the assessment and to allow a revision of the Authority's opinion.

On 15 July 2016 the Commission has received new data on L-tryptophan, technically pure. The supplementary data are sent to the authority by the applicant.

In view of the above, the Commission asks the Authority to deliver a new opinion on L-tryptophan, technically pure, produced by *Escherichia coli* strains DSM 25084, KCCM 11132P and SARI12091203 as nutritional additive for all animal species based on the additional data submitted by the applicant.

1.2. Additional information

L-Tryptophan was first authorised for use in animal nutrition by Directive 88/485/EEC. It is currently authorised as a nutritional additive (functional group: amino acids, their salts and analogues) for use in all animal species without time limit and without maximum content in feed.

The applicant has provided information on the endotoxin activity and dusting potential of the product of *E. coli* DSM 25084, and information on endotoxin activity and studies on the safety for the user of the product of *E. coli* KCCM 11132P. No information was received on strain SARI12091203 or its product. During the assessment, however, the European Commission informed EFSA that the applicant withdrew the application for the product of *E. coli* SARI12091203. In addition, the applicant withdrew the use in water for drinking of the products of *E. coli* strains DSM 25084 and KCCM 11132P.

2. Data and methodologies

2.1. Data

The present assessment is based on data submitted by the applicant in the form of a technical dossier[2] following a previous application on the same product.[3]

[1] Regulation (EC) No 1831/2003 of the European Parliament and of the Council of 22 September 2003 on additives for use in animal nutrition. OJ L 268, 18.10.2003, p. 29.
[2] FEED dossier reference: FAD-2016-0054.
[3] FEED dossier reference: FAD-2010-0056.

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the characterisation and safety of L-tryptophan produced by *E. coli* DSM 25084 and KCCM 11132P is in line with the principles laid down in Regulation (EC) No 429/2008[4] and the relevant guidance documents: Guidance on nutritional additives (EFSA FEEDAP Panel, 2012a) and Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012b).

3. Assessment

The current application is for the authorisation of L-tryptophan (minimum content of L-tryptophan 98% 'as is' basis) produced by two genetically modified (GM) strains of *Escherichia coli* (DSM 25084 or KCCM 11132P). It is intended to be used in feed for all animal species as a nutritional additive (functional group: amino acids, their salts and analogues).

In 2015, the FEEDAP Panel issued an opinion on the safety and efficacy of L-tryptophan produced by *E. coli* strains DSM 25084 or KCCM 11132P (EFSA FEEDAP Panel, 2015). The Panel concluded that the additives produced by *E. coli* strains DSM 25084 and KCCM 11132P do not give rise to safety concerns with regard to the genetic modifications of the production strain and that they are safe for non-ruminant target species when supplemented to diets in nutritionally appropriate amount, but should not be given to ruminants in an unprotected form because of the formation of skatole (3-methylindole) during ruminal fermentation. The products of *E. coli* strains DSM 25084 and KCCM 11132P are also safe for the consumer of animal products and the environment. For both products, the FEEDAP Panel expressed some concerns regarding safety for the user: 'The level of endotoxins present in the product and its possible dusting potential indicate an inhalation risk for the user. In the absence of data on the potential for dermal sensitisation, it is concluded that such potential may exist'.

The applicant has provided information on the endotoxin activity and the dusting potential of the product of *E. coli* DSM 25084, and information on endotoxin activity and studies on the safety for the user of the product of *E. coli* KCCM 11132P. These new data are the subject of the current opinion.

3.1. L-Tryptophan produced by *Escherichia coli* KCCM 11132P[5]

L-Tryptophan produced by *E. coli* KCCM 11132P has been characterised in a previous scientific opinion (EFSA FEEDAP Panel, 2015).

The applicant stated that an improvement had been made in the production process to reduce the amount of bacterial endotoxins in the final product.[6] To support this claim, additional data on the content of endotoxins were submitted. Additionally, the applicant provided new toxicological studies to support the safety for the user: an acute inhalation toxicity test and a skin sensitisation test.

3.1.1. Characterisation of the product

Before the change in the production process, the endotoxin activity (three batches) of the product was up to 2,900 IU/mg.[7] Three batches of the additive produced by the new method of production were analysed for bacterial endotoxin activity. The values ranged from 0.09 to 0.29 IU/mg, showing a reduction by four orders of magnitude of the upper endotoxin level compared to the former one of 2,900 IU/mg (European Pharmacopoeia 2.6.14 method D).[8]

Data on particle size and dusting potential were already assessed in the previous opinion. The FEEDAP Panel considers that the changes in the manufacturing process would not significantly affect the physical properties of the additive. Particle size distribution analysis (one batch analysed by laser diffraction (v/v)) showed that the percentages of particles below 100, 50 and 10 μm were about 90%,

[4] Commission Regulation (EC) No 429/2008 of 25 April 2008 on detailed rules for the implementation of Regulation (EC) No 1831/2003 of the European Parliament and of the Council as regards the preparation and the presentation of applications and the assessment and the authorisation of feed additives. OJ L 133, 22.5.2008, p. 1.
[5] This section has been amended following the applicable provisions on confidentiality
[6] Technical dossier/Supplementary information October 2016/Annex Qi Justif-Endotox reduced level CONFID.
[7] Technical dossier FAD-2010-0056/Supplementary information May 2015/Annex Q1 Analysis of Microbial Endotoxins.
[8] Technical dossier/endotoxin test results.

67% and 17%, respectively.[9] The dusting potential (Stauber–Heubach) measured in one batch was 2.5 g/m^3.[10]

3.1.2. Safety for the user

3.1.2.1. Effects on the respiratory system

The changes in the manufacturing process made it necessary to reassess the relevance of the bacterial endotoxin activities found in the final product.

The scenario used to estimate the exposure of persons handling the additive to endotoxins in the dust, based on the EFSA Guidance on user safety (2012b), is described in Appendix A. The health-based recommended threshold for the quantity of inhaled endotoxins per working day is 900 IU, derived from provisional occupational exposure limits given by the Dutch Expert Committee on Occupational Safety (DECOS) (Health Council of the Netherlands, 2010) and the UK Health and Safety Executive (HSE, 2013). Based upon the calculation of the potential endotoxin content in dust (Wallace et al., 2016), the inhalation exposure could be up to 407 endotoxin IU per 8-h working day, indicating no risk from the exposure to endotoxins for people handling the additive.

In an acute inhalation toxicity test (in accordance with OECD Guideline 403),[11] a group of five female and five male RccHan:WIST strain rats were exposed to dust of the additive under assessment (98.5% purity) at a limit concentration of 5.1 g/m^3 (60 L/min) for 4 h. After 14 days, the animals were subject to necropsy. Treatment-related clinical abnormalities of the exposure to L-tryptophan consisted of increased respiratory rate and hunched posture. All animals recovered within 4 days after exposure. No macroscopic abnormalities were detected at necropsy. As mortality did not occur, the 4-h median lethal concentration for L-tryptophan was estimated to be higher than 5.1 g/m^3 for male and female rats, indicating that the additive under assessment has a low acute toxicity by inhalation.

3.1.2.2. Effects on skin

A local lymph node assay (OECD Guideline 429) was conducted with the additive under assessment (98.5% purity).[12] The experiment comprised three L-tryptophan treatment groups (5%, 10% and 25% L-tryptophan in a suspension in propylene glycol) of four female CBA/Ca mice each. No mortality occurred during the study period; no systemic signs were observed. There were no significant differences between the L-tryptophan-treated groups and the vehicle control in the LLNA test. All stimulation indexes were below a threefold increase in ^{3}H-thymidine incorporation, the limiting value required for the classification as a skin sensitiser. The results indicate that the additive under assessment is not a skin sensitiser.

3.1.3. Conclusions on the safety of L-tryptophan produced by *Escherichia coli* KCCM 11132P for the users

Due to improvements in the manufacturing process, the endotoxin levels have been markedly reduced; therefore, the endotoxin content of the additive under assessment does not represent a health risk for the user. The results of an acute inhalation study indicate that the additive has a low acute toxicity by this route. The additive is not considered as a potential skin sensitiser.

3.2. L-Tryptophan produced by *Escherichia coli* DSM 25084

L-Tryptophan produced by *E. coli* DSM 25084 has been characterised in a previous scientific opinion (EFSA FEEDAP Panel, 2015).

The applicant stated that the production process has been further developed to decrease the dusting potential of the additive. Additional data have been submitted on endotoxin activity and dusting potential of the additive.

[9] Technical dossier FAD-2010-0056/Supplementary information October 2014/Annexes Qi Particle size distribution.
[10] Technical dossier FAD-2010-0056/Supplementary information October 2014/Annexes Qi Dusting Potential.
[11] Technical dossier/acute inhalation toxicity.
[12] Technical dossier/skin sensitisation.

3.2.1. Characterisation of the product

The endotoxin activity in three batches of the product in the former application ranged from 23.9 to 31.6 IU/mg.[13] New data have been submitted on the bacterial endotoxin activity of the product in this application, measured in three batches. The values ranged from 0.18 to 0.34 IU/mg (European Pharmacopoeia 2.6.14 method).[14] The applicant, however, stated that no improvement had been made in the production process to reduce the amount of bacterial endotoxins in the final product and that the reduction observed may be due to a 'storage-dependent decrease' attributable to the absorption of endotoxins to the storage container (polypropylene).[15] During the assessment the applicant also stated that the endotoxin activity in the batches submitted could not be confirmed and that the previous results on endotoxin activity ranging from 23.9 to 31.6 IU/mg still represent the status quo.[15] Therefore, the FEEDAP Panel considers that the data representative for the endotoxin activity of the L-tryptophan produced with *E. coli* DSM 25084 are those assessed in the former scientific opinion (range 23.9–31.6 IU/mg).

The dusting potential (Stauber–Heubach) of three batches reported in the previous opinion ranged from 2.3 to 2.9 g/m^3.[10] The applicant submitted data on three further batches which showed values from 1.29 to 1.36 g/m^3.[16] The applicant stated that the reduction achieved was obtained through process development, media optimisation and increased carbon source conversion of the fermentation process, which lead to a purer product and resulted in larger particles during crystallisation.[15] However, no new information on particle size distribution was provided.

3.2.2. Safety for the user

3.2.2.1. Effects on the respiratory system

Although a previous assessment was performed in January 2015 (EFSA FEEDAP Panel, 2015), the technological change in the manufacturing process makes it necessary to reassess the relevance of the bacterial endotoxin activities found in the final product for the user.

The bacterial endotoxin activity ranges from 23.9 to 31.6 IU/mg (as in the previous submission; see Section 3.2.1). The dusting potential ranges from 1.29 to 1.36 g/m^3.

The scenario used to estimate the exposure of persons handling the additive to endotoxins in the dust, based on the EFSA Guidance on user safety (2012b), is described in Appendix A. The health-based recommended threshold for the quantity of inhaled endotoxins per working day is 900 IU, derived from provisional occupational exposure limits given by the DECOS (Health Council of the Netherlands, 2010) and the UK Health and Safety Executive (HSE, 2013). Based upon the calculation of the potential endotoxin content in dust (Wallace et al., 2016), the inhalation exposure could be up to 23,900 (rounded figure) endotoxin IU per 8-h working day, which is over one magnitude order greater than occupational exposure limit of 900 IU, indicating a risk from the exposure to endotoxins for people handling the additive.

3.2.2.2. Effects on skin

No new information has been provided on the potential for dermal sensitisation of the product. The FEEDAP Panel noted that additives under assessment differ only in the endotoxin content, which is not considered to be relevant for skin sensitisation; otherwise, the two additives show similar physical characteristics (particle size distribution) and the production strains share a common lineage (*E. coli* K-12). Therefore, the FEEDAP Panel considers that the outcome of the dermal sensitisation study of the product of *E. coli* KCCM 11132P can be extended to the product of *E. coli* DSM 25084.

3.2.3. Conclusions on the safety of L-tryptophan produced by *Escherichia coli* DSM 25084 for the user

The level of endotoxins present in the product and its dusting potential indicate a health risk for the user upon inhalation. The additive is not considered as a dermal sensitiser.

[13] Technical dossier FAD-2010-0056/Supplementary information May 2015/Annexes Qi.
[14] Technical dossier/endotoxins improvement.
[15] Technical dossier/Supplementary information October 2016/Annex Qii Justif-Endotox reduced level.
[16] Technical dossier/dusting potential.

4. Conclusions

Due to improvements in the manufacturing process, the upper level of endotoxins present in L-tryptophan produced by *E. coli* KCCM 11132P has been markedly reduced by four orders of magnitude; consequently, the endotoxin does not represent a health risk for the user. The additive has a low acute toxicity by inhalation and is not considered as a potential skin sensitiser.

L-Tryptophan produced by *E. coli* DSM 25084 is not considered a skin sensitiser. The level of endotoxins in this product, however, represents a risk by inhalation for the user handling the additive.

Documentation provided to EFSA

1) L-Tryptophan produced by *Escherichia coli* for all animal species. September 2016. Submitted by FEFANA Asbl.
2) L-Tryptophan produced by *Escherichia coli* for all animal species. Supplementary information. October 2016. Submitted by FEFANA Asbl.

References[17]

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2012a. Guidance for the preparation of dossiers for nutritional additives. EFSA Journal 2012;10(1):2535, 14 pp. doi:10.2903/j.efsa.2012.2535

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2012b. Guidance on studies concerning the safety of use of the additive for users/workers. EFSA Journal 2012;10(1):2539, 5 pp. doi:10.2903/j.efsa.2012.2539

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2015. Scientific Opinion on the safety and efficacy of L-tryptophan, technically pure, produced by *Escherichia coli* strains DSM 25084, KCCM 11132P or SARI12091203 for all animal species based on a dossier submitted by AMAC EEIG. EFSA Journal 2015;13(9):4238, 29 pp. doi:10.2903/j.efsa.2015.4238

Health Council of the Netherlands, 2010. Endotoxins, 2010. Health-based recommended occupational exposure limit. Publication No 2010/04OSH. Health Council of the Netherlands, The Hague. 100 pp.

HSE (Health and Safety Executive), 2013. Occupational hygiene implications of processing waste at materials recycling facilities (MRFs). RR977 Research Report, HSE, London, UK, 41 pp.

Wallace RJ, Gropp J, Dierick N, Costa LG, Martelli G, Brantom PG, Bampidis V, Renshaw D and Leng L, 2016. Risks associated with endotoxins in feed additives produced by fermentation. Environmental Health, 15, 1–7.

Abbreviations

DECOS	Dutch Expert Committee on Occupational Safety
DSM	German Collection of Microorganisms and Cell Cultures
EC	European Commission
FEEDAP Panel	EFSA Panel on Additives and Products or Substances used in Animal Feed
GM	genetically modified
HSE	British Health and Safety Executive
IU	International unit of endotoxin activity. One IU corresponds to one endotoxin unit (EU)
KCCM	Korean Culture Center of Microorganisms

[17] This section has been amended following the applicable provisions on confidentiality.

Appendix A – Safety for the user of L-tryptophan produced by *E. coli* KCCM 11132P

Calculation of maximum acceptable levels of exposure from feed additives. The probable exposure time according to EFSA guidance (EFSA FEEDAP Panel, 2012b) for additives added in premixtures assumes a maximum of 40 periods of exposure per day, each comprising 20 s = 40 × 20 = 800 s/day. With an uncertainty factor of 2, maximum inhalation exposure would occur for 2 × 800 = 1,600 s = 0.444 h/day. Again, assuming a respiration volume of 1.25 m^3/h, the inhalation volume providing exposure to potentially endotoxin-containing dust would be 0.444 × 1.25 = 0.556 m^3/day. This volume should contain no more than 900 IU endotoxin, so the dust formed from the product should contain no more than 900/0.556 = 1,619 IU/m^3.

Calculation of endotoxin content of dust. Two key measurements are required to evaluate the potential respiratory hazard associated with the endotoxin content of the product (the dusting potential of the product, expressed in g/m^3, and the endotoxin activity of the dust, determined by the Limulus amoebocyte lysate assay (expressed in IU/g)). If data for the dust are not available, the content of endotoxins of the product can be taken instead. If the content of endotoxins of the relevant additive is *a* IU/g and the dusting potential is *b* g/m^3, then the content of endotoxins of the dust, *c* IU/m^3, is obtained by simple multiplication, *a* × *b*. This resulting value is further used for calculation of the potential inhalatory exposure of users to endotoxins from the additives under assessment (Tables A.1 and A.2) (EFSA FEEDAP Panel, 2012b).

Table A.1: Estimation of user exposure to endotoxins from the additive L-tryptophan produced by *Escherichia coli* KCCM11132P, including consideration of using a filter mask FF P2 or FF P3 as a preventative measure

Calculation	Identifier	Description	Amount	Source
	a	Endotoxin content IU/g product	293	Technical dossier
	b	Dusting potential (g/m^3)	2.5	Technical dossier
a × *b*	*c*	Endotoxin content in the air (IU/m^3)	732.5	
	d	No. of premixture batches made/ working day	40	EFSA Guidance on user safety (EFSA FEEDAP Panel, 2012b)
	e	Time of exposure (s) per production of one batch	20	EFSA Guidance on user safety (EFSA FEEDAP Panel, 2012b)
d × *e*	*f*	Total duration of daily exposure/worker (s)	800	
	g	Uncertainty factor	2	EFSA Guidance on user safety (EFSA FEEDAP Panel, 2012b)
f × *g*	*h*	Refined total duration of daily exposure/worker (s)	1,600	
h/3,600	*i*	Refined total duration of daily exposure (h)	0.44	
	j	Inhaled air (m^3) per 8-h working day	10	EFSA Guidance on user safety (EFSA FEEDAP Panel, 2012b)
j/8 × *i*	*k*	Inhaled air during exposure (m^3)	0.56	
c × *k*	*l*	**Endotoxin inhaled (IU) during exposure per 8-h working day**	**407**	
	m	Health-based recommended exposure limit of endotoxin (IU/m^3) per 8-h working day	90	Health Council of the Netherlands (2010)
m × *j*	*n*	**Health-based recommended exposure limit of total endotoxin exposure (IU) per 8-h working day**	**900**	

Calculation	Identifier	Description	Amount	Source
l/10		Endotoxins inhaled (IU) per 8-h working day reduced by filter mask FF P2 (reduction factor 10)	41	
l/20		Endotoxins inhaled (IU) per 8-h working day reduced by filter mask FF P3 (reduction factor 20)	20	

Table A.2: Estimation of user exposure to endotoxins from the additive L-tryptophan produced by *Escherichia coli* DSM 25084, including consideration of using a filter mask FF P2 or FF P3 as a preventative measure

Calculation	Identifier	Description	Amount	Source
	a	Endotoxin content IU/g product	31,600	Technical dossier
	b	Dusting potential (g/m^3)	1.361	Technical dossier
a × *b*	*c*	Endotoxin content in the air (IU/m^3)	43,008	
	d	No. of premixture batches made/ working day	40	EFSA Guidance on user safety (EFSA FEEDAP Panel, 2012b)
	e	Time of exposure (s) per production of one batch	20	EFSA Guidance on user safety (EFSA FEEDAP Panel, 2012b)
d × *e*	*f*	Total duration of daily exposure/worker (s)	800	
	g	Uncertainty factor	2	EFSA Guidance on user safety (EFSA FEEDAP Panel, 2012b)
f × *g*	*h*	Refined total duration of daily exposure/worker (s)	1,600	
h/3,600	*i*	Refined total duration of daily exposure (h)	0.44	
	j	Inhaled air (m^3) per 8-h working day	10	EFSA Guidance on user safety (EFSA FEEDAP Panel, 2012b)
j/8 × *i*	*k*	Inhaled air during exposure (m^3)	0.56	
c × *k*	*l*	**Endotoxin inhaled (IU) during exposure per 8-h working day**	**23,893**	
	m	Health-based recommended exposure limit of endotoxin (IU/m^3) per 8-h working day	90	Health Council of the Netherlands (2010)
m × *j*	*n*	**Health-based recommended exposure limit of total endotoxin exposure (IU) per 8-h working day**	**900**	
l/10		Endotoxins inhaled (IU) per 8-h working day reduced by filter mask FF P2 (reduction factor 10)	2,389	
l/20		Endotoxins inhaled (IU) per 8-h working day reduced by filter mask FF P3 (reduction factor 20)	1,195	