SCIENTIFIC OPINION

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Safety of L-tryptophan produced by fermentation using *Escherichia coli* CGMCC 3667, for all animal species based on a dossier submitted by GBT Europe GmbH

EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP)

Abstract

L-Tryptophan, technically pure, is a feed additive produced by fermentation using a genetically modified strain of *Escherichia coli* (*E. coli*). The Panel on Additives and Products or Substances used in Animal Feed (FEEDAP Panel) of the European Food Safety Authority (EFSA), issued an opinion on the safety and efficacy of the product, in which it could not conclude on the safety of this additive for target animals, consumer, user and the environment, due to the insufficient characterisation of the genetic modification. The European Commission asked EFSA to deliver an opinion on the safety of L-tryptophan, technically pure, as nutritional additive for all animal species based on additional data submitted by the applicant. The new information provided on the genetic modification, including the presence/absence of an antibiotic resistance gene in the production strain, is contradictory. Consequently, the FEEDAP Panel cannot conclude on the safety of the L-tryptophan produced using *E. coli* CGMCC 3667 for target animals, consumers, users and the environment. The FEEDAP Panel reiterates its concern on the use of unprotected tryptophan to ruminants and on the safety of the amino acid L-tryptophan for target species when administered simultaneously via water for drinking. As the estimated maximum exposure to endotoxins by inhalation is below the provisional occupational exposure limit, no risk from the exposure to endotoxins for people handling the additive is expected.

Keywords: nutritional additive, amino acids and their salts and analogues, L-tryptophan, safety, efficacy, genetically modified microorganisms

Requestor: European Commission

Question number: EFSA-Q-2015-00251

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Summary

Following a request from the European Commission (EC), the European Food Safety Authority (EFSA) was asked to deliver a scientific opinion on the safety of L-tryptophan, technically pure, produced by fermentation using the genetically modified strain *Escherichia coli* (*E. coli*) CGMCC 3667 for all animal species.

In 2014, the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP Panel) of the European Food Safety Authority (EFSA) adopted an opinion on the safety and efficacy of L-tryptophan produced by fermentation using *E. coli* CGMCC 3667. The FEEDAP Panel could not conclude on the safety of that product for target animals, consumer, user and the environment because the genetic modification, including the presence/absence of recombinant deoxyribonucleic acid (DNA) and of antibiotic resistance genes in the product, was insufficiently characterised.

The applicant provided additional information in relation to the characterisation of the production microorganism, the characterisation of the additive and its stability. The FEEDAP Panel has performed the assessment of those new data following an approach in line with the principles laid down in Regulation (EC) No 429/2008 and the relevant guidance documents.

The new information provided on the genetic modification, including the presence/absence of antibiotic resistance genes in the production strain, is contradictory. Consequently, the FEEDAP Panel could not conclude on the safety of the L-tryptophan produced by fermentation with *E. coli* CGMCC 3667 for the target animals, the consumers, the users and the environment.

The FEEDAP Panel reiterates its concern on the use of unprotected tryptophan to ruminants and on the safety of the amino acid L-tryptophan for target species when administered simultaneously via water for drinking.

As the estimated maximum exposure to endotoxins by inhalation is below the provisional occupational exposure limit, no risk from the exposure to endotoxins for people handling the additive is expected.

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1. Introduction

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

Regulation (EC) No 1831/2003 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, GBT Europe GmbH, is seeking a Community authorisation of L-tryptophan, technically pure, produced by fermentation with *Escherichia coli* (*E. coli*) to be used as a 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, produced by fermentation with *Escherichia coli*
Target animal category	All animal species
Applicant	GBT Europe GmbH
Type of request	New opinion

On 10 April 2014, the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP Panel) of the European Food Safety Authority ('Authority'), in its opinion on the safety and efficacy of the product could not conclude on the safety of the L-tryptophan produced by fermentation with this recombinant strain of *E. coli* for target animals, consumer, user and the environment. Regardless of the assessment of the genetic modification, the FEEDAP Panel had concerns on the use of unprotected forms of L-tryptophan in ruminants, and on the safety of the amino acid L-tryptophan for target species when administered simultaneously via water for drinking.

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

The Commission has now received new data on the safety of L-tryptophan, technically pure, produced by fermentation with *E. coli*.

In view of the above, the Commission asks the Authority to deliver a new opinion on the safety of L-tryptophan, technically pure, produced by fermentation with *E. coli* as a nutritional additive for all animal species based on the additional data submitted by the applicant.

1.2. Additional information

The applicant has provided additional information on the characterisation of the production microorganism, the characterisation of the additive and its stability and homogeneity.

2. Data and Methodologies

2.1. Data

The present assessment is based on the data submitted by the applicant in the form of additional information[1] following a previous application on the same product.[2]

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the characterisation and safety of L-tryptophan produced by *E. coli* CGMCC 3667 is consistent with the principles laid down in Regulation (EC) No

[1] Dossier reference: FAD-2015-0011.
[2] Dossier reference: FAD-2010-0290.

429/2008[3] and the relevant guidance documents: Guidance on nutritional additives (EFSA FEEDAP Panel, 2012a), Guidance on the risk assessment of genetically modified microorganisms and their products intended for food and feed use (EFSA GMO Panel, 2011), Technical guidance: Update of the criteria used in the assessment of bacterial resistance to antibiotics of human or veterinary importance (EFSA, 2008, revised in 2012b), Technical Guidance: Microbial Studies (EFSA, 2008) and Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012c).

3. Assessment

L-Tryptophan, technically pure, (minimum content of L-tryptophan 98%, 'as is' basis) was first authorised for use in animal nutrition by Directive 88/485/EEC.[4] It is currently included in the European Union Register of Feed Additives pursuant to Regulation (EC) No 1831/2003 concerning additives in feedingstuffs.

The current application is for the authorisation of L-tryptophan, technically pure, produced by a genetically modified (GM) strain of *E. coli* (CGMCC 3667), a derivative of *E. coli* K-12. It is intended to be used in all animal species as a nutritional additive in feed and in water for drinking. The characterisation of the additive and the description of the genetic modification were assessed in the previous opinion (EFSA FEEDAP Panel, 2014). The genetic modification, including the presence/absence of production organism and recombinant DNA genes in the product, was insufficiently characterised. Therefore, the present opinion focuses on the new data provided by the applicant addressing these uncertainties.

3.1. Characterisation

This product has been characterised in a previous opinion (EFSA FEEDAP Panel, 2014). Additional data have been submitted on the characterisation of the production strain, on the physical properties of the additive and its stability and homogeneity in complete feed, and on the manufacturing process.

3.1.1. Characterisation of the production organism[5]

The technical dossier contains information on the susceptibility of the production strain against the list of antibiotics proposed for *E. coli* in the technical guidance on the assessment of bacterial susceptibility to antimicrobials of human and veterinary importance (EFSA FEEDAP Panel, 2012).[6]

Although the parent strain *E. coli* K-12 is not included on EFSA qualified presumption of safety (QPS) list (EFSA BIOHAZ Panel, 2013), it is not considered a human or animal pathogen, it has a long history of apparent safe use in industrial production and is scientifically recognised as a safe bacterial strain not producing toxic substances (US Environmental Protection Agency, 1997; Gorbach, 1978). *E. coli* K-12 has been used widely as a model organism in research in microbial genetics and physiology, and has widespread use in industrial applications. *E. coli* K-12 is one of the most extensively studied of all microorganisms. Its genome sequence was published in 1997 (Blattner et al., 1997) and confirmed the absence of toxigenic potential. It does not appear in the most comprehensive review of pathogenic *E. coli* published by Nataro and Kaper (1998). Indeed, strain K-12 is commonly used as a 'basemodel' (safety reference strain) against which the safety of other E. coli strains is assessed, (see Kaper et al. (2004)). It can be therefore assumed that there is no antibiotic or enterotoxin production from *E. coli* K-12.

[3] 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.

[4] Commission Directive 88/485/EEC of 326 July 1988 amending the Annex to Council Directive 82/471/EEC concerning certain products used in animal nutrition. OJ L 239/36, 30.8.1988, p. 4.

[5] This section of the scientific output is temporarily unavailable to the public pending a final decision on the confidentiality request submitted by the applicant . A revised version of the opinion will be made available as soon as this decision-making process is concluded.

[6] Technical dossier/Supplementary information April 2015/Annex 2.2.5.

Characterisation of the genetic modification[7]

In the former assessment of the production strain (EFSA FEEDAP Panel, 2014), the genetic modification, including the presence/absence of recombinant DNA and of antibiotic resistance genes in the product, was insufficiently characterised.

The new information provided on the genetic modification, including the presence/absence of antibiotic resistance genes in the production strain, is contradictory. A gene coding for resistance to an antibiotic which is claimed to be carried by the production strain was shown to be present by polymerase chain reaction (PCR) but absent by Southern blot analysis.

Therefore, uncertainty remains about the identity and the genetic elements actually present in the production strain.

3.1.2. Manufacturing process[8]

The applicant declared that no antimicrobial compounds (including antibiotics) were used in the production process.[9] It has been demonstrated that the final product does not contain antimicrobial compounds.[10]

Neither viable cells of the production strain,[11] nor its (as claimed by the applicant) recombinant DNA was detected in three batches of the final product.[12] However, because of the uncertainties concerning the identity and the genetic elements actually present in the production strain, no conclusion can be drawn.

3.1.3. Physical properties

The applicant submitted data on dusting potential measured in three batches of the final product (Stauber–Heubach). The values ranged between 0.12 and 0.14 g/m^3.[13]

3.1.4. Stability and homogeneity

In the previous opinion the stability of the additive in complete feed and the capacity of the additive to distribute homogeneously in feed were evaluated by measuring total tryptophan (protein-bound plus free L-tryptophan) instead of free L-tryptophan. The FEEDAP Panel noted that the results obtained might not fully represent the stability of the added free amino acid or its ability to homogeneously distribute in compound feedingstuffs.

A new stability study in complete feed for piglets was provided. L-Tryptophan (three different batches) was used to supplement with 0.04% a mash and pelleted complete feed for piglets (three batches; total tryptophan content: 0.24%). Samples were collected after mixing and after pelleting at 80ºC. The feed was stored in paper bags for 3 months at 5–30ºC and 30–75% relative humidity. No losses were detected.[14]

Free tryptophan was analysed in 10 subsamples of the above pelleted complete feed for piglets to determine the capacity of the additive to homogeneously distribute in feed. The coefficient of variation of supplemental tryptophan was 3%.[15]

[7] This section of the scientific output is temporarily unavailable to the public pending a final decision on the confidentiality request submitted by the applicant. A revised version of the opinion will be made available as soon as this decision-making process is concluded.
[8] This section of the scientific output is temporarily unavailable to the public pending a final decision on the confidentiality request submitted by the applicant . A revised version of the opinion will be made available as soon as this decision-making process is concluded.
[9] Technical dossier/Supplementary information April 2015/Annexes 2.2.1 and 2.2.2.
[10] Technical dossier/Supplementary information April 2015/Annexes 2.1.9–2.1.12.
[11] Technical dossier/Supplementary information April 2015/Annex 2.2.4 and Supplementary information September 2015/Annex II.
[12] Technical dossier/Supplementary information September 2015/Annex III.
[13] Technical dossier/Supplementary information April 2015/Annex 2.1.14.
[14] Technical dossier/Supplementary information April 2015/Annex 2.1.13.
[15] Technical dossier/Supplementary information April 2015/Annex 2.1.13.

3.2. Safety[16]

In its previous scientific opinion (EFSA FEEDAP Panel, 2014a), in relation to the safety of the genetic modification of the production strain, the Panel concluded: '…the genetic modification, including the presence/absence of recombinant DNA and of antibiotic resistance genes in the product, is insufficiently characterised. Consequently, the FEEDAP Panel cannot conclude on the safety of the L-tryptophan produced by fermentation with *E. coli* (CGMCC 3667) for target animals, consumers, users and the environment'.

The new information provided on the genetic modification, including the presence/absence of antibiotic resistance genes in the production strain, is contradictory. Based on this lack of consistency, the FEEDAP Panel cannot conclude on the safety of the L-tryptophan produced by fermentation with *E. coli* CGMCC 3667, with respect to the genetic modification of the production strain, for target animals, consumers, users and the environment.

In relation to the concerns of the FEEDAP Panel on the use of unprotected forms of L-tryptophan in ruminants or on the safety of the amino acid L-tryptophan for target species when administered simultaneously via water for drinking (EFSA FEEDAP Panel, 2014), no information has been provided that would make the Panel reconsider its opinion.

The applicant provided new data on the dusting potential of the additive that allows an assessment of the potential risk posed by the bacterial endotoxin activity of the final product for the user. The production species, *Escherichia coli*, is a Gram-negative bacterium. Although the K-12 strain and its derivatives are safe from the point of view of enterotoxins and other virulence factors (Gorbach, 1978; EPA, 1997; Bauer et al., 2007), *E. coli* K-12 retains lipopolysaccharide in its cell envelope (Luchi and Morrison, 2000; Svensson et al., 2005; Gao et al., 2006) which may potentially result in endotoxin activity in the final product. The user can suffer from occupational respiratory disease depending on the level of endotoxins in the air and dust (Rylander, 1999; Thorn, 2001). The bacterial endotoxin activity was analysed in three batches and ranged from 0.06 to 0.14 IU/mg.[17] Dusting potential was measured in three batches and ranged between 0.12 and 0.14 g/m^3. The fraction of particles having a diameter below 100 µm was 50% and the fraction below 50 µm was up to 7%. [18]

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, the inhalation exposure could be up to 11 endotoxin IU per eight-hour working day, indicating no risk from the exposure to endotoxins for people handling the additive.

4. Conclusions

The new information provided on the genetic modification, including the presence/absence of antibiotic resistance genes in the production strain is contradictory. Consequently, the FEEDAP Panel could not conclude on the safety of the L-tryptophan produced by fermentation with *E. coli* CGMCC 3667 for target animals, consumers, users and the environment.

The FEEDAP Panel reiterates its concern on the use of unprotected tryptophan to ruminants and on the safety of the amino acid L-tryptophan for target species when administered simultaneously via water for drinking.

As the estimated maximum exposure to endotoxins by inhalation is below the provisional occupational exposure limit, no risk from the exposure to endotoxins for people handling the additive is expected.

[16] This section of the scientific output is temporarily unavailable to the public pending a final decision on the confidentiality request submitted by the applicant. A revised version of the opinion will be made available as soon as this decision-making process is concluded.
[17] Technical dossier FAD-2010-0290/Supplementary information December 2012/Annex 4.
[18] Technical dossier FAD-2010-0290/Section II.1.5/Annex II.1.2.

Documentation provided to EFSA

1. L-Tryptophan, technically pure, produced by fermentation with *E. coli*. Supplementary information. April 2015. Submitted by Global Bio-Chem (GBT Europe GmbH).

2. L-Tryptophan, technically pure, produced by fermentation with *E. coli*. Supplementary information. Sept 2015. Submitted by Global Bio-Chem (GBT Europe GmbH).

References

Bauer AP, Dieckmann SM, Ludwig W and Schleifer KH, 2007. Rapid identification of *Escherichia coli* safety and laboratory strain lineages based on Multiplex-PCR. Federation of European Microbiological Societies Microbiology Letters, 269, 36–40.

Blattner FR, Plunkett G 3rd, Bloch CA, Perna NT, Burland V, Riley M, Collado-Vides J, Glasner JD, Rode CK, Mayhew GF, Gregor J, Davis NW, Kirkpatrick HA, Goeden MA, Rose DJ, Mau B and Shao Y, 1997. The complete genome sequence of *Escherichia coli* K-12. Science, 277, 1453–1462

EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP), 2008. Guidance on microbial studies. The EFSA Journal (2008) 836, 1-3.

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 the assessment of bacterial susceptibility to antimicrobials of human and veterinary importance. EFSA Journal 2012;10(6):2740. 10 pp. doi:10.2903/j.efsa.2012.2740

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2012c. 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 BIOHAZ Panel (EFSA Panel on Biological Hazards), 2013. Scientific Opinion on the maintenance of the list of QPS biological agents intentionally added to food and feed (2013 update). EFSA Journal 2013;11(11):3449, 105 pp. doi:10.2903/j.efsa.2013.3449

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2014a. Scientific Opinion on the safety and efficacy of L-tryptophan technically pure produced by fermentation with *Escherichia coli* for all animal species, based on a dossier submitted by HELM AG on behalf of Global Bio-Chem Technology. EFSA Journal 2014;12(5):3673, 2 pp. doi:10.2903/j.efsa.2014.3673

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 produced by *Escherichia coli* CGMCC 7.59 for all animal species based on a dossier submitted by HELM AG on behalf of Meihua Holdings Co. Ltd. EFSA Journal 2015;13(2):4015, 17 pp. doi:10.2903/j.efsa.2015.4015

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), 2011. Scientific Opinion on Guidance on the risk assessment of genetically modified microorganisms and their products intended for food and feed use. EFSA Journal 2011;9(6):2193, 54 pp. doi:10.2903/j.efsa.2011.2193

EPA (US Environmental Protection Agency), 1997. *Escherichia coli* K-12 derivatives final risk assessment. Available online: http://epa.gov/biotech_rule/pubs/fra/fra004.htm

Gao B, Wang Y and Tsan MF, 2006. The heat sensitivity of cytokine-inducing effect of lipopolysaccharide. Journal of Leukocyte Biology, 80, 359–366.

Gorbach SL, 1978. Risk assessment of recombinant DNA experimentation with *Escherichia coli* K12. Proceedings from a workshop at Falmouth, Massachusetts. Journal of Infectious Diseases, 137, 613–714.

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

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

Kaper JB, Natro JP and Mobley HLT, 2004. Pathogenic *Escherichia coli*. Nature Reviews Microbiology, 2, 123–140.

Luchi M and Morrison DC, 2000. Comparable endotoxic properties of lipopolysaccharides are manifest in diverse clinical isolates of Gram-negative bacteria. Infection and Immunity, 68, 1899–1904.

Nataro JP and Kaper JB, 1998. Diarrheagenic *Escherichia coli*. Clinical Microbiology Reviews, 11, 142-201.

Rylander R, 1999. Health effects among workers in sewage treatment plants. Occupational Environmental Medicine, 56, 354–357.

Svensson M, Han L, Silfersparre G, Haggstrom L and Enfors SO, 2005, Control of endotoxin release in Escherichia coli feed-batch cultures. Bioprocess and Biosystems Engineering, 27, 91–97.

Thorn J, 2001. The inflammatory response in humans after inhalation of bacterial endotoxin: a review. Inflammatory Response, 50, 254–261.

Abbreviations

CGMCC	China general microbiological culture collection centre
DECOS	Dutch Expert Committee on Occupational Safety
DNA	deoxyribonucleic acid
EC	European Commission
EPA	US Environmental Protection Agency
FEEDAP	EFSA Panel on Additives and Products or Substances used in Animal Feed
GM	genetically modified
GMO	EFSA Panel on Genetically Modified Microorganisms
HBROEL	health-based recommended exposure limit
HSE	British Health and Safety Executive
IU	International unit of endotoxin activity. One IU corresponds to one EU.
LPS	lipopolysaccharide
NDA	EFSA Panel on Dietetic Products, Nutrition and Allergies

Appendix A – Safety for the user

The Effects of endotoxin inhalation and the exposure limits have been described in a previous opinion (EFSA FEEDAP Panel, 2015).

Calculation of maximum acceptable levels of exposure from feed additives

The likely exposure time according to EFSA guidance (EFSA FEEDAP Panel, 2012c) for additives added in premixtures assumes a maximum of 40 periods of exposure per day, each comprising 20 s = 40 × 20 = 800 s per day. With an uncertainty factor of 2, maximum inhalation exposure would occur for 2 × 800 = 1 600 s = 0.444 h per 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 per 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 endotoxin content of the product (the dusting potential of the product, expressed in g/m^3; 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 used 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 the simple multiplication a × b. This resulting value is further used for calculation of potential inhalatory exposure by users to endotoxin from the additive under assessment (Table 2) (EFSA FEEDAP Panel, 2012c).

Table 2: Estimation of user exposure to endotoxins from the additive L-tryptophan produced by *Escherichia coli* CGMCC 3667, including consideration of using filter mask FF P2 or FF P3 as preventative measure

Calculation	Identifier	Description	Amount	Source
	a	Endotoxin content (IU/g product)	140	Technical dossier
	b	Dusting potential (g/m^3)	0.14	Technical dossier
a × b	c	Endotoxin content in the air (IU/m^3)	19.6	
	d	No of premixture batches made per working day	40	EFSA FEEDAP Panel (2012c)
	e	Time of exposure (s) per production of one batch	20	EFSA FEEDAP Panel (2012c)
d × e	f	Total duration of daily exposure/worker (s)	800	
	g	Uncertainty factor	2	EFSA FEEDAP Panel (2012c)
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-hour working day	10	EFSA FEEDAP Panel (2012c)
j/8 × i	k	Inhaled air during exposure (m^3)	0.56	
c × k	l	Endotoxin (IU) inhaled during exposure per 8-hour working day	11	
	m	Health-based recommended exposure limit of endotoxin (IU/m^3) per 8-hours working day	90	Health Council of the Netherlands (2010)
m × j	n	Health-based recommended exposure limit of total endotoxin exposure (IU) per 8-hour working day	900	
l/10		Endotoxins inhaled (IU) per 8-hour working day reduced by filter mask FF P2 (reduction factor 10)	1.1	
l/20		Endotoxins inhaled (IU) per 8-hour working day reduced by filter mask FF P3 (reduction factor 20)	0.54	