SCIENTIFIC OPINION

EFSA Journal

ADOPTED: 3 October 2018

doi: 10.2903/j.efsa.2018.5460

Safety and efficacy of Monteban® G100 (narasin) for chickens for fattening

EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP), Vasileios Bampidis, Giovanna Azimonti, Maria de Lourdes Bastos, Henrik Christensen, Birgit Dusemund, Maryline Kouba, Mojca Kos Durjava, Marta López-Alonso, Secundino López Puente, Francesca Marcon, Baltasar Mayo, Alena Pechová, Mariana Petkova, Fernando Ramos, Yolanda Sanz, Roberto Edoardo Villa, Ruud Woutersen, Gabriele Aquilina, Georges Bories, Paul Brantom, Pier Sandro Cocconcelli, Ingrid Halle, Boris Kolar, Pieter Wester, Patrick van Beelen, Orsolya Holczknecht, Maria Vittoria Vettori and Jürgen Gropp

Abstract

The feed additive Monteban® G100, containing the active substance narasin, an ionophore anticoccidial, is intended to control coccidiosis in chickens for fattening at a dose of 60–70 mg/kg complete feed. Narasin is produced by fermentation. Limited data on the taxonomic identification of the production strain did not allow the proper identification of strain NRRL 8092 as *Streptomyces aureofaciens*. The FEEDAP Panel cannot conclude on the absence of genetic determinants for antimicrobial resistance in *Streptomyces* spp. under assessment. Based on the available data set, the FEEDAP Panel cannot conclude on the safety of Monteban® G100 for chickens for fattening. The simultaneous use of Monteban® G100 and certain antibiotic drugs (e.g. tiamulin) is contraindicated. Narasin is not genotoxic. No indication of carcinogenicity or developmental toxicity was found at the doses tested in the mouse, rat and rabbit. The lowest no observed effect level (NOEL) identified in the oral toxicity studies was 0.5 mg/kg body weight (bw) per day for the neuropathy seen in a one-year dog study. The acceptable daily intake (ADI) derived from this NOEL is 0.005 mg narasin/kg bw applying a uncertainty factor of 100. Monteban® G100 is safe for the consumer. Maximum residue limits (MRLs) of 50 μg narasin/kg for all wet tissues ensure consumer safety. Monteban® G100 is irritatant to the eyes but not to the skin. It has the potential to induce skin sensitisation. Inhalation exposure would pose a risk to persons handling the additive. Narasin, when used as a feed additive for chickens for fattening at 70 mg/kg feed, is not expected to pose a risk to the environment. The risk for sediment compartment cannot be assessed. The FEEDAP Panel cannot conclude on the efficacy of Monteban® at the minimum applied dose of 60 mg narasin/kg complete feed for chickens for fattening.

Keywords: coccidiostats, narasin, chickens for fattening, safety and efficacy

Requestor: European Commission

Question number: EFSA-Q-2013-00767

Correspondence: feedap@efsa.europa.eu

Panel members: Giovanna Azimonti, Vasileios Bampidis, Maria de Lourdes Bastos, Henrik Christensen, Birgit Dusemund, Maryline Kouba, Mojca Kos Durjava, Marta López-Alonso, Secundino López Puente, Francesca Marcon, Baltasar Mayo, Alena Pechová, Mariana Petkova, Fernando Ramos, Yolanda Sanz, Roberto Edoardo Villa and Ruud Woutersen.

Legal notice: Relevant information or parts of this scientific output have been blackened in accordance with the confidentiality requests formulated by the applicant pending a decision thereon by the European Commission. The full output has been shared with the European Commission, EU Member States and the applicant. The blackening will be subject to review once the decision on the confidentiality requests is adopted by the European Commission.

Acknowledgements The EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed) wishes to thank the following for the support provided to this scientific output: Anguita Montserrat, Jaume Galobart, Gloria López-Gálvez, Matteo Lorenzo Innocenti, Paola Manini, Jordi Tárres-Call.

Suggested citation: EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Bampidis V, Azimonti G, Bastos ML, Christensen H, Dusemund B, Kouba M, Kos Durjava M, López-Alonso M, López Puente S, Marcon F, Mayo B, Pechová A, Petkova M, Ramos F, Sanz Y, Villa RE, Woutersen R, Aquilina G, Bories G, Brantom P, Cocconcelli PS, Halle I, Kolar B, Wester P, van Beelen P, Holczknecht O, Vettori MV and Gropp J, 2018. Scientific Opinion on the safety and efficacy of Monteban® G100 (narasin) for chickens for fattening. EFSA Journal 2018;16(11):5460, 38 pp. https://doi.org/10.2903/j.efsa.2018.5460

ISSN: 1831-4732

The EFSA Journal is a publication of the European Food Safety Authority, an agency of the European Union.

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 and efficacy of Monteban® G100 (narasin) for chickens for fattening.

The feed additive Monteban® G100, containing the active substance narasin, an ionophore anticoccidial, is intended to control coccidiosis in chickens for fattening at a dose of 60–70 mg/kg complete feed. Narasin is present in Monteban® G100 in its granulated form and is produced by fermentation of a strain of *Streptomyces* spp. (NRRL 8092).

Limited data on the taxonomic identification of the production strain did not allow the proper identification of strain NRRL 8092 as *Streptomyces aureofaciens*. The FEEDAP Panel cannot conclude on the absence of genetic determinants for antimicrobial resistance in *Streptomyces* spp. under assessment.

Based on the available data set, the FEEDAP Panel cannot conclude on the safety of Monteban® G100 for chickens for fattening. Narasin is active against Gram-positive bacteria, while Gram-negative bacteria are resistant. The use of narasin as a feed additive is unlikely to induce resistance or cross-resistance to antimicrobials used in human and animal therapy. Narasin may increase *Salmonella*-shedding, but there is no reason to believe that narasin is different from other polyether ionophores in this respect. The simultaneous use of Monteban® G100 and certain antibiotic drugs (e.g. tiamulin) is contraindicated.

Narasin is not genotoxic. No indication of carcinogenicity or developmental toxicity was found at the doses tested in the mouse, rat and rabbit. Effects on reproduction were tested only in one species, the rat. The lowest no observed effect level (NOEL) identified in the oral toxicity studies was 0.5 mg/kg body weight (bw) per day for the neuropathy seen in a 1-year dog study. Since this dose is above the lowest no observed adverse effect level (NOAEL) previously identified of 0.5 mg/kg bw per day, there is no reason to consider acute cardiovascular effects in the risk assessment. The NOAEL of 0.5 mg/kg bw per day is an appropriate base for confirming the acceptable daily intake (ADI) of 0.005 mg narasin/kg bw already established by the FEEDAP Panel in its former opinions applying a uncertainty factor of 100.

The use of Monteban® G100 in chickens for fattening at the maximum dose proposed, and without applying a withdrawal period, is safe for the consumer. Maximum residue limits (MRLs) of 50 μg narasin/kg for all wet tissues ensure consumer safety.

Monteban® G100 is irritant to the eyes but not to the skin. It has the potential to induce skin sensitisation. The acute systemic toxicity following dermal application is low. Inhalation exposure would pose a risk to persons handling the additive.

Narasin, when used as a feed additive for chickens for fattening at 70 mg/kg feed, is not expected to pose a risk to the environment. The risk for the sediment compartment cannot be assessed. Narasin is not considered to have a bioaccumulation potential.

The efficacy of Monteban® G100 was shown in three floor pen studies and only in two anticoccidial sensitivity tests. The FEEDAP Panel is therefore not in a position to conclude on the efficacy of Monteban® at the minimum applied dose of 60 mg narasin/kg complete feed for chickens for fattening.

Table of contents

1. Introduction

1.1. Background and Terms of Reference

Regulation (EC) No 1831/2003[1] establishes the rules governing the Community authorisation of additives for use in animal nutrition. In particular, Article 10(2) of that Regulation also specifies that for existing products within the meaning of Article 10(1), an application shall be submitted in accordance with Article 7, at the latest one year before the expiry date of the authorisation given pursuant to Directive 70/524/EEC for additives with a limited authorisation period, and within a maximum of seven years after the entry into force of this Regulation for additives authorised without a time limit or pursuant to Directive 82/471/EEC.

The European Commission received a request from Eli Lilly and Company Ltd.[2] for re-evaluation of the product Monteban® G100 (narasin) when used as a feed additive for chickens for fattening (category: coccidiostats and histomonostats).

According to Article 7(1) of Regulation (EC) No 1831/2003, the Commission forwarded the application to the European Food Safety Authority (EFSA) as an application under Article 10(2) (re-evaluation of an authorised feed additive). EFSA received directly from the applicant the technical dossier in support of this application. The particulars and documents in support of the application were considered valid by EFSA as of 17 February 2014.

According to Article 8 of Regulation (EC) No 1831/2003, EFSA, after verifying the particulars and documents submitted by the applicant, shall undertake an assessment in order to determine whether the feed additive complies with the conditions laid down in Article 5. EFSA shall deliver an opinion on the safety for the target animals, consumer, user and the environment and on the efficacy of the product Monteban® G100 (narasin), when used under the proposed conditions of use (see Section 3.1.4).

1.2. Additional information

Monteban® G100 has been authorised for 10 years for use in chickens for fattening.[3] The authorisation has been amended as regards the introduction of a maximum residue limit (MRL) for narasin (50 μg narasin/kg for all wet tissues).[4] The authorisation was further amended to change the withdrawal period to 0 day following the request of the applicant.[5]

The same active substance is also present in the product Maxiban® G160. This product (preparation of nicarbazin and narasin) has been authorised for 10 years for use in chickens for fattening (authorisation until 28 October 2020).[6]

EFSA issued an opinion on the safety and efficacy of the coccidiostat Monteban® G100 for chickens for fattening including the setting of MRLs for narasin (EFSA, 2004). A further opinion was issued on the same product concerning the modification of the withdrawal time (EFSA FEEDAP Panel, 2010a).

The same active substance was also evaluated by EFSA's Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) in the product Maxiban® G160 (preparation of narasin and nicarbazin) for chickens for fattening (EFSA FEEDAP Panel, 2010b).

In 2012, the joint FAO/WHO expert committee on food additives evaluated narasin as veterinary drug (JEFCA, 2012).

[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] On 15/6/2018, the applicant informed that the petitioner changed from Eli Lilly and Company Ltd. to Elanco GmbH, Heinz-Lohmann-Str. 4. 27472 Cuxhaven, Germany.

[3] Commission Regulation (EC) No 1464/2004 of 17 August 2004 concerning the authorisation for 10 years of the additive 'Monteban' in feedingstuffs belonging to the group of coccidiostats and other medicinal substances. OJ L 270, 18.8.2004, p.8.

[4] Commission Regulation (EC) No 545/2006 of 31 March 2006 amending Regulation (EC) No 1464/2004 as regards the conditions for authorisation of the feed additive 'Monteban', belonging to the group of coccidiostats and other medicinal substances. OJ L 94, 1.4.2006, p. 26.

[5] Commission Regulation (EC) No 884/2010 of 7 October 2010 amending Regulation (EC) No 1464/2004 as regards the withdrawal time of the additive 'Monteban', belonging to the group of coccidiostats and other medicinal substances. OJ L 265, 8.10.2010, p. 4.

[6] Commission Regulation (EC) No 885/2010 of 7 October 2010 concerning the authorisation of the preparation of narasin and nicarbazin as a feed additive for chickens for fattening (holder of authorisation Eli Lilly and Company Ltd) and amending Regulation (EC) No 2430/1999. OJ L 265, 8.10.2010, p. 5.

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[7] in support of the authorisation request for the use of Monteban® G100 as a feed additive. The technical dossier was prepared following the provisions of Article 7 of Regulation (EC) No 1831/2003 and the applicable EFSA guidance documents.

The FEEDAP Panel used the data provided by the applicant together with data from other sources, such as previous risk assessments by EFSA or other expert bodies, peer-reviewed scientific papers, other scientific reports and experts' knowledge, to deliver the present output.

EFSA has verified the EURL report as it relates to the methods used for the control of the active substance in animal feed/marker residue in tissues. The Executive Summary of the EURL report can be found in Annex A.[8]

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of Monteban® G100 (narasin) is in line with the principles laid down in Regulation (EC) No 429/2008[9] and the relevant guidance documents: Guidance for the preparation of dossiers for coccidiostats and histomonostats (EFSA FEEDAP Panel, 2011a), Technical guidance: Tolerance and efficacy studies in target animals (EFSA FEEDAP Panel, 2011b), Technical Guidance for assessing the safety of feed additives for the environment (EFSA, 2008a), Guidance for establishing the safety of additives for the consumer (EFSA FEEDAP Panel, 2012a), Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012b), Technical Guidance: Microbial Studies (EFSA, 2008b) and Guidance on the assessment of bacterial susceptibility to antimicrobials of human and veterinary importance (EFSA FEEDAP Panel, 2012c).

3. Assessment

The present opinion assesses the safety and efficacy of the coccidiostat Monteban® G100 containing narasin as active principle when used as a feed additive in chickens for fattening.

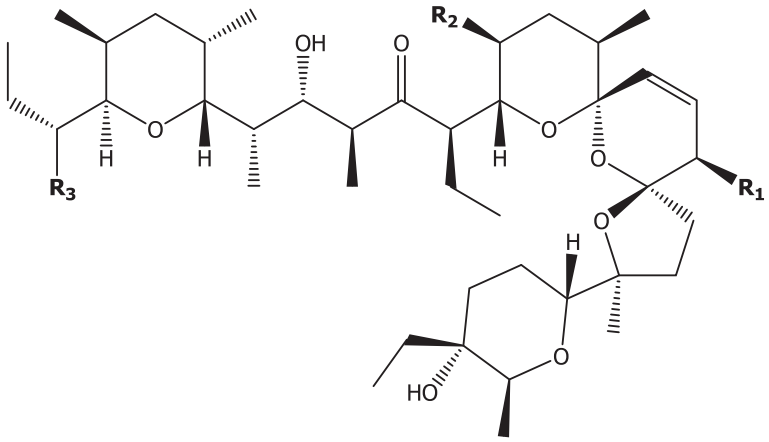
3.1. Characterisation

3.1.1. Characterisation of the active substance

Narasin is a polyether ionophore obtained via fermentation of a strain of *Streptomyces* spp.[10] The manufacturing is fully described in the technical dossier. No changes in the fermentation process have been introduced since the FEEDAP Panel made the first assessment in 2004 (EFSA, 2004). [redacted] The composition of narasin granulated is given as follows: narasin activity (13%), dipotassium hydrogen phosphate (as stabiliser, 6%), mycelial solids (28%), montmorillonite clay (as carrier, 53%). Narasin granulated is specified to contain ≥ 100 g narasin activity per kg. [redacted]

The main component of narasin is narasin A ((2*R*)-2-{(2*R*,3*S*,5*S*,6*R*)-6-[(2*S*,3*S*,4*S*,6*R*)-6-{(2*S*,5*S*,7*R*,9*S*,10*S*,12*R*,15*R*)-2-[(2*R*,5*R*,6*S*)-5-ethyl-5-hydroxy-6-methyltetrahydro-2*H*-pyran-2-yl]-15-hydroxy-2,10,12-trimethyl-1,6,8-trioxadispiro[4.1.5^7.3^5]pentadec-13-en-9-yl}-3-hydroxy-4-methyl-5-oxooctan-2-yl]-3,5-dimethyltetrahydro-2*H*-pyran-2-yl}butanoic acid; $C_{43}H_{72}O_{11}$; molecular mass 764 g/mol; CAS number 55134-13-9). In smaller quantities narasin also contains narasin variants B, D and I. The structural formula of narasin variants is given in Figure 1.

[7] FEED dossier reference: FAD-2013-0041.
[8] The full report is available on the EURL website: https://ec.europa.eu/jrc/sites/default/files/finrep-fad-2013-0041-monteban_0.pdf
[9] 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.
[10] Technical dossier/Section II/Annex II.12 and II.13.



	R_1	R_2	R_3
Narasin A	OH	CH_3	COOH
Narasin B	=O	CH_3	COOH
Narasin D	OH	C_2H_5	COOH
Narasin I	OH	CH_3	$COOCH_3$

Figure 1: Structural formula of narasin variants

Specification for narasin A activity is ≥ 90%. [redacted]

The concentration of narasin is expressed as narasin activity which includes the relative biopotency of the different narasin variants. The relative biopotency is based on the microbiological responses against *Enterococcus faecium* and was determined as 1.000, 0.250, 1.402 and 0.012 for variants A, B, D and I, respectively. Chemical composition of narasin granulated was determined by high-performance liquid chromatography (HPLC) analysis and resulted in 96.35%, 0.94%, 2.09% and 1.37% for variants A, B, D and I, respectively. Narasin activity was determined multiplying the chemical concentrations by the relative biopotency responses resulting in biopotencies of 963.5, 2.35, 29.3 and 0.15 μg/mg for variants A, B, D and I, respectively. Calculating the % of biopotency contributions (96.8%, 0.2%, 2.9% and < 0.1%) the results are similar to the chemical compositions. The FEEDAP Panel noted that the relative activity of narasin was established based on microbiological response against *E. faecium* and not based on its anticoccidial activity.

3.1.1.1. Characterisation of the production organism

The active substance narasin is produced by fermentation of a strain of *Streptomyces* spp. The non-genetically modified strain was originally identified as *Streptomyces aureofaciens* and it is deposited in Agricultural Service Culture Collection (USA) under the deposition number NRRL 8092. [redacted] the assignation of the production strain to *S. aureofaciens* species cannot be confirmed. More detailed data on the taxonomic identification were not provided.

Data on antimicrobial susceptibility of the production strain were not provided. Consequently, the FEEDAP Panel cannot conclude on the absence of genetic determinants for antimicrobial resistance in *Streptomyces* spp. under assessment.

The absence of antimicrobial compounds relevant to the use of antibiotics in humans or animals, other than the narasin in the mycelial product, was assessed comparing the minimum inhibitory concentrations (MIC) of three batches of the fermentation product with three batches of pure narasin.[12] [redacted]

[redacted]

[12] Technical dossier/Supplementary information June 2017/Annex 34.

[redacted]

Since no differences in the inhibitory spectrum and in the MIC values were observed between the pure and mycelial form for any of the strains tested, the product is considered free of antimicrobial activity other than narasin.

A literature review[14] has been carried out to assess the information available on the potential of *S. aureofaciens* to produce secondary metabolites.[15] Since the conclusive identification of the production strain as *S. aureofaciens* has not been provided, these data were not considered relevant for the current assessment.

3.1.2. Characterisation of the additive

[redacted] The final additive is produced by mixing narasin granulated with 10–25 g mineral oil, 10–20 g vermiculite (expanded vermiculite magnesium-aluminosilicate mineral) per kg additive and with rice hulls (the quantity is adjusted to ensure a narasin content of Monteban® G100 within the limit of specification). Monteban® G100 contains by specification 95.0–107.5 g narasin activity/kg. [redacted]

The concentrations of heavy metals and arsenic, dioxins and the sum of dioxins and dioxin-like polychlorinated biphenyls were considered of no concern.

No data were provided regarding the absence of viable cells of the production strain in the final product. In spite of this, the FEEDAP Panel recognises that the manufacturing process [redacted] may eliminate or reduce the number of vegetative cells and spores of the production strain.

Three batches of Monteban® G100 were analysed for mycotoxin and microbial contamination. [redacted] Particle size distribution measured by laser diffraction in three batches of the additive [redacted]

[redacted]

[14] Databases searched: Agriculture Online Access/STN, Biosis Previews/STN, CAB Abstracts/STN, Chemical Abstracts Plus/STN, Derwent Biotechnology Resource/STN, Derwent Drug Backfile/STN, Derwent Drug File/STN, Embase/STN, Embase Alert/STN, Food Science and Technology Abstracts/STN, Foodline Science/STN, International Pharmaceutical Abstracts/STN, Medline/STN, Pascal/STN, ProQuest Science & Technology/STN, Science Citation Index/STN; time span: 1907 – June 2017.

[15] Technical dossier/Supplementary information June 2017/Annex 35.

[redacted]

[redacted]The dusting potential [redacted]
The particle size distribution of the dust [redacted]The narasin content of the dust was similar to that of the additive [redacted]

3.1.3. Stability and homogeneity

3.1.3.1. Shelf-life of the additive

Monteban® G100 was stored at 25 °C/60% relative humidity (RH) [redacted] and at 30°C/65% RH for 24 months [redacted] Narasin activity measured at the end of the study showed losses of 2–3% at 25°C/60% RH and 4–6% at 30°C/65% RH.[23]

3.1.3.2. Stability in premixtures and feedingstuffs

Three batches of Monteban® G100 were incorporated in a vitamin/mineral premixture (with choline chloride) for poultry containing 5 g narasin/kg premixture.[24] The samples were stored at 25°C for up to 6 months and at 40°C for up to 3 months (RH: 60%) in permeable paper bags. No loss was detected during 6 months at 25°C, (recovery rate was 99% with average concentration of 4.948 ± 355 mg/kg). After 3 months at 40°C, the average recovery rate was 93% (4.659 ± 240 mg/kg).

A similar study design was applied using a complete diet for chickens for fattening (60 and 70 mg narasin/kg feed).[25] Mash and pelleted samples (pelleting temperature 77°C) were stored at 25°C for up to 3 months and at 40°C for up to 1 month in permeable paper bags (RH: 60%). No losses were detected. Concentrations measured at the end of the storage periods showed values of 61.5 ± 4.5 mg/kg and 73.6 ± 6.5 mg/kg at 25°C, and 67.2 ± 5.4 and 77.4 ± 5.2 mg/kg at 40°C. The same measurements were performed with pelleted feed. The results were similar to those obtained with mash feed; thus, it was concluded that pelleting did not affect stability.

3.1.3.3. Homogeneity

The capacity of narasin to homogeneously distribute was studied in 10 subsamples of three batches each of the above premixture[26] and mash feed.[25] The coefficients of variation (CVs) for premixture were 5.3%, 10.5% and 20.8% for the three batches, respectively. Regarding mash feed, two concentrations were tested (60 and 70 mg narasin/kg feed). The CVs for the lower dose were 5.8%, 7.2%, 9.8% and 5.5%, 6.4% and 6.3% for the higher dose.

3.1.4. Conditions of use

Monteban® 100G is intended to be used in the prevention of coccidiosis in chickens for fattening at a concentration of narasin in complete feed of 60 mg to 70 mg/kg.

3.2. Safety

3.2.1. Absorption, distribution, metabolism, excretion and residues

3.2.1.1. Absorption, distribution, metabolism and excretion

The data submitted concerning the metabolic fate of narasin in the chicken and rat include the studies already assessed by the FEEDAP Panel and presented in a previous opinion on Monteban® G100 (EFSA, 2004) and on Maxiban® G160 (EFSA FEEDAP Panel, 2010b). The same conclusions can be retained: '(i) The main metabolic pathway of narasin in the chicken and rat involves oxidative processes leading to the formation of di-, tri- and tetra-hydroxynarasins as well as keto-narasins. (ii) Unchanged narasin is a minor component (up to 5%) of chicken excreta in the feed dose range proposed, whereas a great number of metabolites have been identified. Two major di-hydroxy and two major tri-hydroxy narasin metabolites represented together about 30% of the whole narasin related excreted compounds. (iii) Narasin metabolites in tissues and excreta are qualitatively similar. The liver

[redacted]
[23] Technical dossier/Supplementary information June 2014.
[24] Technical dossier/Section II/Annex II.23 and II.24.
[25] Technical dossier/Section II/Annex II.25.
[26] Technical dossier/Section II/Annex II.23.

is the target tissue. A great number of narasin metabolites represent each less than 10% of the whole tissue residues. However, for control purposes skin/fat and narasin should be retained as practical target tissue and marker residue'.

Two additional studies, not submitted in the framework of the former assessments, were provided. The first one concerned the metabolic fate of ^{14}C-narasin in chicken and quails, but after intravenous injection, and was therefore not considered.[27] The second highlighted the ionophoric properties of four narasin di-hydroxy metabolites identified in chicken excreta.[28] These metabolites were isolated and purified from cattle faeces and chicken excreta, then tested on rat liver mitochondria for ATPase activity and oxygen consumption. Based on the action on these two endpoints, it was shown that the most active metabolite exhibited an ionophoric activity 200 times lower than narasin.

3.2.1.2. Residues

The applicant submitted total and marker residue studies in chickens for fattening previously assessed by the FEEDAP Panel (EFSA, 2004; EFSA FEEDAP Panel, 2010a).

An overview of the relevant data for the assessment of the safety for the consumer (see Section 3.2.3) is given below.

Total residues after a 0.25-day withdrawal were measured in a study carried out in chickens for fattening with the maximum dose proposed for use (70 mg [^{14}C]-narasin/kg feed) administered for 5 consecutive days. Total residues at 0.25-day withdrawal are reported in Table 1.

Table 1: Total residues (mg equivalent narasin/kg wet tissue) in tissues from chickens fed a diet supplemented with 70 mg [^{14}C]-narasin for 5 days and slaughtered after 0.25-day withdrawal (EFSA, 2004; EFSA FEEDAP Panel, 2010a)

	Liver	Kidney	Muscle	Skin/fat
TRC(1)	0.272	0.068	< 0.015(3)	0.082
TRC + 2SD(2)	0.416	0.090	< 0.021	0.112

(1): TRC: total residue concentration (average).
(2): TRC (average) + 2 standard deviations.
(3): Limit of quantification (LOQ) = 0.015 mg/kg.

In a marker residue study already assessed (EFSA FEEDAP Panel, 2010a), groups of animals (four of each sex) were fed for 5 days a diet supplemented with Monteban® 100G, then slaughtered after 0, 0.25-, 0.5- and 1.0-day withdrawal. The animals received a diet supplemented with 70 mg narasin from Monteban® 100G/kg (analytically confirmed). The analytical method (high-performance liquid chromatography (HPLC)) exhibited a LOQ of 0.025 mg narasin/kg wet tissue and a LOD of 0.010 mg/kg. After 0.25-day withdrawal residues in all tissues were below the LOQ (0.025 mg/kg). The lowest value (0.01 mg/kg) has been retained as worst case for the calculation of the ratios. The following ratios marker to total residues have been established at 0.25-day withdrawal: 0.037, 0.147, 0.667 and 0.354 for the liver, kidney, muscle and skin/fat, respectively (EFSA FEEDAP Panel, 2010a).

An additional study, already available for the former assessments but not described in the previous opinion, concerned the depletion of narasin residues in chickens for fattening (45 days administration of 80 mg narasin/kg complete feed, different withdrawal periods).[29] Only skin and skin/fat were analysed with a bioautographic method of poor quantitative reliability; therefore, this study was not considered further.

3.2.2. Safety for the target species

3.2.2.1. Tolerance in chickens for fattening

The applicant provided (i) three tolerance studies which were already assessed in 2004 by the FEEDAP Panel and a fourth one that was submitted already in 2004 but not assessed (ii) a literature search performed by the applicant on the tolerance of narasin covering the period 2000–2014 (iii) a review of the pharmacovigilance data of the company.

[27] Technical dossier/Section III/Annex III.26.
[28] Technical dossier/Section III/Annex III.37.
[29] Technical dossier/Section III/Annex III.32.

Tolerance studies

The four tolerance studies have been re-evaluated for the purpose of this assessment taking into consideration current requirements established in the applicable Guidance document.[30] The study design of the four studies is summarised in Table 2.

Table 2: Study design and dosages of the tolerance studies in chickens for fattening

Study	Year of the study	Total no of animals replicates/treatment (birds/replicate)	Strain sex duration (days)	Test item(1)	Intended concentrations (ppm narasin)(2)
1	1978	336 4 (12)	Hubbard by White Mountain Cross ♂/♀ 56	Mycelial narasin and crystalline narasin	0 80 240 400
2	1980	1,696 8 (53)	Hubbard by White Mountain Cross ♂/♀ 56	Mycelial narasin	0 80 240 400
3	1984	1,590 6 (53)	Hubbard by White Mountain Cross ♂/♀ 49 + 3	Mycelial narasin	0 70 80 120 210
4	1999	120 2 (20)	Chankey Ross ♂/♀ 42 + 7	No information provided	0 80 240

(1): The test item was incorporated in the diet containing maize and soybean meal as main feed materials in study 1, 2 and 3 (~ 13.0 MJ metabolisable energy (ME)/kg). No information was included on the diet composition in study 4.
(2): The intended dietary narasin levels were analytically confirmed.

In the first study,[31] chickens were fed two preparations of narasin (mycelial and crystalline) at feed concentrations of 0, 80, 240 or 400 mg/kg for 56 days. Body weight and feed consumption were determined weekly and feed to gain ratio was calculated. On days 28 and 56, five chickens per replicate were selected for blood sampling (haematology[32] and clinical chemistry[33]) and subsequent necropsy.[34] Statistical evaluation was done by multifactorial analysis of variance (ANOVA) followed by the two-sided Dunnett's test for group comparisons.

Mortality was low (0–8%) in the untreated control, the low and intermediate narasin dose, and increased at the 400 mg/kg treatment to 10% and 23% for the two batches. Body weight gain and total feed consumption were 1,721 g and 3,967 g in the control, 1,596 g and 3,756 g for the 80 mg/kg feed, 900 g and 2,344 g for 240 mg/kg feed, and 437 g and 1,164 g for 400 mg/kg feed. These results showed a trend for reduction in growth with increasing doses of narasin. A numerical reduction in body weight gain and feed consumption in the 80 mg/kg feed group and a statistically significant reduction in the 240 and 400 mg/kg feed groups compared to the control were observed. Haemoglobin, haematocrit and serum potassium (following the trend in the growth of the animals) were significantly below the control group values for the intermediate and the high narasin treatments, while AST was increased. In the same groups, organ weight values were significantly below the control group. The histopathology done on samples collected on day 28 and 56, revealed a dose-associated degeneration and acute myositis of skeletal muscles and increased numbers of fat cells in the heart in chickens fed the two higher narasin concentrations. No treatment-associated lesions were found in those chickens receiving narasin at 80 mg/kg.

In the second study,[35] the birds were monitored for clinical signs and mortality. Necropsy was performed only on birds that died during the experiment. Birds in the study were weighed and feed

[30] Technical guidance: Tolerance and efficacy studies in target animals (EFSA FEEDAP Panel, 2011b).
[31] Technical dossier/Section III/Annex III.5.
[32] Haemoglobin, haematocrit.
[33] Glucose, sodium, potassium, total protein, aspartate aminotransferase (AST).
[34] At necropsy, weight of heart, kidney and liver were determined; samples of abdominal, pectoral and leg muscles, heart, liver, gall bladder, kidney, lung, crop, proventriculus, intestines, pancreas were collected and fixed for histopathological evaluation.
[35] Technical dossier/Section III/Annex III.2.

intake was measured at 3 and 8 weeks, feed to gain ratio was calculated. Prothrombin times were determined at 4 days and 8 weeks. The litter condition was observed weekly. Litter feather scores were monitored and recorded weekly after 4 weeks. The data were analysed by Dunnett's T-test.

During the course of the study, there was a dose-associated increase in mortality: 3.0% in the control, 3.5% in 80 mg/kg feed group, 8.8% in 240 mg/kg feed and 26.5% in the 400 mg/kg feed group, respectively. For the other parameters, the actual values were not reported, but only the differences between the groups. After 8 weeks of treatment, there was a significant decrease in body weights for the 80, 240 and 400 mg/kg treatments for both males and female when compared to controls (values not provided). Feed consumption was significantly reduced in female birds receiving 80, 240 and 400 mg/kg treatments but only in the 240 and 400 mg/kg group males during the first three weeks of treatment. This same relationship in feed consumption continued for weeks 4–8. There were no changes in prothrombin times, indicating no treatment-related toxicity. Litter feather counts revealed more feathers found in the control and 80 mg/kg treatment groups than in the 240 and 400 mg/kg treatment groups, showing that there was a dose-associated decrease in feathering. Gross necropsy observations of birds that died or were killed in extremis revealed that the birds were small, dehydrated and cachectic. Microscopically, birds treated with 240 and 400 mg/kg narasin had focal degeneration of skeletal muscles and congestive heart failure. Birds administered 80 mg/kg had no lesions which were regarded as treatment related. The study did not consider haematology (except prothrombin time) and clinical blood chemistry. Necropsy, gross pathological and histopathological examination was done on dead birds only. Since the results were not available, the description of the findings in the study report are informative only if there are significant changes in the parameters assessed, showing the direction of the effect but not its magnitude. Nevertheless, the FEEDAP Panel noted that effects were seen already at 80 mg/kg on body weight and feed consumption.

In the third study,[36] the birds were monitored for clinical signs and mortality. Birds in the study were weighed and feed intake was measured at 4 and 7 weeks and after a 3-day withdrawal, feed to gain ratio was calculated. The litter condition was observed at 4 and 7 weeks. Litter feather scores were monitored and recorded at 4 weeks and at the end of the study. Blood samples were collected from three birds at day 4 for prothrombin time determination and from five birds per pen at 7 weeks for haematology[37] and clinical chemistry[38] determinations. Tissues from all major organ systems were collected for histopathological examination in five birds per pen. Necropsy and gross examination was performed on 13 birds per pen. The data were analysed by Dunnett's T-test.

The results included only the differences between the groups, actual values were not reported. There were no increases in mortality attributed to treatment throughout the study. Weight gain and feed to gain ratio for the 70 and 80 mg/kg narasin treatments were comparable to or better than the controls while the growth was significantly reduced in the groups of the higher concentrations. Litter moisture and floor feather scores were not affected by narasin administration. There were no toxicologically relevant changes in haematology or organ weights of birds treated with narasin. There was, however, a dose-related increase in aspartate amino transferase values. An increased incidence of slight focal regeneration of skeletal muscle in birds fed 120 and 210 mg/kg narasin indicated repair of a previous mild injury. There were no gross or microscopic lesions in the heart attributable to treatment. Six treated chickens had lesions of congestive heart failure: one male each from the 80 and 120 mg/kg groups, one female each from the 120 and 210 mg/kg groups and two males from the 210 mg/kg group. The occurrence of heart failure in this study was considered unrelated to narasin administration. The results were not detailed and therefore the description of the findings in the study report are informative only if there are significant changes in the parameters assessed, showing the direction of the effect but not its magnitude. The FEEDAP Panel noted that the administration of 80 mg/kg did not adversely affect the measured parameters.

In the fourth study,[39] the birds were monitored for clinical signs and mortality. Faecal conditions and feather conditions were observed daily. Birds in the study were weighed and feed intake was measured weakly, feed to gain ratio was calculated. Blood samples were collected from five male and five female birds at the end of the study (at 0 and 7 days withdrawal) for haematology[40] and

[36] Technical dossier/Section III/Annex III.3.
[37] Erythrocyte count, haemoglobin, haematocrit and prothrombin time.
[38] Serum glucose, total protein, total bilirubin, aspartate amino transferase and inorganic phosphorous.
[39] Technical dossier/Section III/Annex III.4.
[40] Erythrocyte count, haemoglobin, packed cell volume, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, total and differentiate leucocyte count.

clinical chemistry[41] determinations. Five male and five female birds were necropsied at the end of the study (at 0 and 7 days withdrawal) for necropsy, organ weight and histopathological analysis. Data were analysed by one-way ANOVA or Kruskall–Wallis test. Pair-wise comparisons were made by Dunnett's or Scheffe's test. No changes related to narasin administration were observed in birds of both sexes receiving 80 mg narasin/kg and in males receiving 240 mg narasin/kg for all parameters. For the females in the 240 mg/kg treatment group, decreased body weight gain and feed intake, and increased total cholesterol and urate levels were observed at the end of the study (0 day withdrawal). However, all of these changes recovered quickly by day 49 (end of the study + 7 days withdrawal). The FEEDAP Panel noted that the number of animals/replicates was small (120/2) and the report was incomplete.

Literature search

The applicant performed a literature search[42] on the tolerance of narasin covering the period 2000–2014 using several databases.[43] The search included the terms 'narasin AND chicken OR turkey OR layer OR broiler OR poultry', 'narasin AND coccidiosis', 'narasin AND toxicity AND chicken OR poultry', 'narasin AND safety AND chicken OR poultry', 'narasin AND tolerance' and 'narasin AND drug interaction'. The applicant claimed that no papers relevant to the safety of the target species were identified. However, no evidence was provided.

Review of pharmacovigilance data

The applicant provided a review of pharmacovigilance case data: the database of the company was queried for the product family Monteban and all cases reported in Europe during the period from 30 June 2004 to 1 July 2014. The query returned a total of 14 cases (3 cases in the target species (chicken) and 11 cases in non-target animal species). Of the cases reviewed in the target species, two cases related to a perceived lack of efficacy and in the third case post mortem indicate that birds died of botulism. The 11 cases in a non-target animal species related to accidental inclusion in feed.

Synopsis and conclusions on the tolerance for chickens for fattening

The literature search indicated no evidence in the databases searched of reported adverse effects for the target species and the company's pharmacovigilance report did not reveal any adverse event related to the use of Monteban® G100.

In the tolerance studies, the proposed maximum use level, 70 mg narasin/kg was tested only in one study (study 3). The results of these four studies showed that birds had reduced growth when fed with concentrations above 120 mg/kg feed. In one of these studies, the birds receiving 80 mg/kg feed showed significant decreases in body weight and feed intake compared to the control (study 1). The number of parameters measured in this study was very limited and consequently did not permit to have evidence of other effects apart from a depression in growth. In the other three studies, birds fed with 80 mg/kg feed showed no statistical differences compared to the control group in the parameters studied, indicating that 80 mg/kg feed may be tolerated by the birds. However, the studies show the following limitations: (i) low number of replicates that does not permit to obtain a reliable estimate and may not be sufficient to evidence differences between the groups, (ii) incomplete data set of parameters evaluated in most of the trials (iii) not sufficient reporting of the results; in two out of the four studies the results were given only descriptively and not showing the actual data, which does not permit to know the magnitude of the effect (study 2 and 3), and (iv) studies conducted before year 2000 with commercial breeds for chickens for fattening that may not be representative of the current breeds in terms of growth performance.

Although the studies have some indications that 70–80 mg/kg feed may be tolerated by the birds, the FEEDAP Panel is not in the position to draw a final conclusion on the tolerable concentration of narasin in feed for chickens for fattening due to the limitations mentioned above.

3.2.2.2. Interactions

The FEEDAP Panel stated in 2004 and confirmed in 2010 (EFSA FEEDAP Panel, 2010b) that the known history of use of narasin has shown that incompatibilities or interactions with feedingstuffs, carriers, or other approved additives are not to be expected.

[41] Lactate dehydrogenase, aspartate aminotransferase, gamma-glutamyl transpeptidase, total protein, albumin, globulin, A/G ratio, total cholesterol, triglyceride, glucose, total bilirubin, urate, creatinine, calcium, inorganic phosphorous, sodium, potassium and chlorine.

[42] Databases searched: ScienceDirect, PubMed: British National Library of Medicine, Google Scholar.

[43] Technical dossier/Supplementary information May 2015.

The FEEDAP Panel summarised in 2004 that 'published data from three experiments (Laczay et al., 1989) on the compatibility of narasin with tiamulin, erythromycin, tylosin, kitasamycin, flumequine, sulfachorpyrazine or sulfaquinoxaline confirmed the incompatibility of narasin with tiamulin, erythromycin, sulfachorpyrazine and sulfaquinoxaline. No incompatibilities were found with tylosin, kitasamycin and flumequine. Clinically important interactions between the ionophore anticoccidials and the antibiotic tiamulin are well known phenomena in chickens, turkeys and other species (Hanrahan et al., 1981; Umemura et al., 1985; Van Vleet et al., 1987; Szucs et al., 2000a,b)'.

The applicant performed a literature search[42] on the interactions of narasin covering the period 2000–2014 using several databases.[43] The search included the terms 'narasin AND chicken OR turkey OR layer Or broiler OR poultry', 'narasin AND coccidiosis', 'narasin AND toxicity AND chicken OR poultry', 'narasin AND safety AND chicken OR poultry', 'narasin AND tolerance' and 'narasin AND drug interaction'. Four papers were found (Appendix A) and one of them was identified as relevant (Islam et al., 2009). This paper confirmed the strong interaction at high dose (even leading to death), between the ionophore coccidiostats monensin, narasin and salinomycin when tiamulin is used at therapeutic levels in poultry. The interaction was found to be dose related, not observed at low doses.

Since no new findings were reported, the FEEDAP Panel reiterates its former conclusion that 'the simultaneous use of Monteban® G100 and certain antibiotic drugs (e.g. tiamulin) is contra-indicated'.

3.2.2.3. Microbial studies

The antimicrobial activity of narasin, as for other ionophoric compounds, is limited to Gram-positive bacteria (see Section 3.1.1.1). In 2004, the FEEDAP Panel (EFSA, 2004) summarised that:

> 'The MICs of narasin for common intestinal bacterial species such as *Enterococcus* spp. and *Clostridium perfringens* are basically low but enterococci may develop resistance to narasin. There is no cross-resistance to other antimicrobials except to salinomycin. Narasin may increase *Salmonella*-shedding, but there is no reason to believe that narasin is different from other polyether ionophores in this respect. There are no data on the influence of narasin on the intestinal microflora other than on *Clostridium perfringens* and *Salmonell*a.
>
> Narasin, at the levels used for treatment of coccidiosis, is also effective in the prevention of necrotic enteritis in chickens'.

The applicant performed a literature search[44] on the emergence of resistance to narasin or other ionophores and on the cross-resistance to antimicrobials covering the period 2000–2014. The search included the terms 'narasin', 'Monteban', 'ionophores', 'resistant', 'crossresistance', 'tolerance', 'adaptation', and 'resistance'. Twenty four papers dealing with narasin were identified[43] (Appendix A) and in none of them evidence of development of resistance to this ionophore or cross-resistance to antimicrobials used for therapy in human or veterinary medicine was reported.

This was confirmed in a study performed on 589 *Enterococcus* strains showing that narasin MIC distribution of strains isolated from chicken supplemented with narasin did not differ from those sampled from non-treated animals.[45]

The literature review did not report studies on the effect of narasin on shedding of *Salmonella* and *Campylobacter*.

Consequently, the FEEDAP Panel concludes, in agreement with its previous conclusions, that narasin is active against Gram-positive bacteria, while Gram-negative bacteria are resistant. The use of narasin as feed additive is unlikely to induce resistance or cross-resistance to antimicrobials used in human and animal therapy. Narasin may increase *Salmonella*-shedding, but there is no reason to believe that narasin is different from other polyether ionophores in this respect.

3.2.2.4. Conclusions

Based on the available data set, the FEEDAP Panel cannot conclude on the safety of Monteban® G100 for chickens for fattening.

The simultaneous use of Monteban® G100 and certain antibiotic drugs (e.g. tiamulin) is contraindicated.

Narasin is active against Gram-positive bacteria, while Gram-negative bacteria are resistant. The use of narasin as feed additive is unlikely to induce resistance or cross-resistance to antimicrobials

[44] Databases searched: PubMed; time span: up to 2014.
[45] Technical dossier/Section III/Annex III_25.

used in human and animal therapy. Narasin may increase *Salmonella*-shedding, but there is no reason to believe that narasin is different from other polyether ionophores in this respect.

3.2.3. Safety for the consumer

3.2.3.1. Toxicological studies

In 2004, the FEEDAP Panel assessed the toxicity of narasin (EFSA, 2004). For the current application, the same data assessed in 2004 was submitted with the addition of two studies not available at the time of the previous assessment and a literature search covering the period 2004–2014.

The FEEDAP Panel reassessed the package of toxicological studies submitted in the frame of a previous application and resubmitted in the current assessment (EFSA, 2004).

The FEEDAP Panel noted that the studies reported were done to standards appropriate to the time but some were not in accordance either with Good Laboratory Practice (GLP) or with previous and current OECD guidelines. However, the quality of the studies was considered sufficient for the assessment. All the *in vivo* and *in vitro* genotoxicity studies performed with pure or mycelial narasin (in combination with crystalline nicarbazin) were GLP compliant and gave negative results. Acute oral toxicity of mycelial narasin was moderate in most species tested. It is reported to be between 15.8 and 16.7 mg/kg in mice and between 21.1 and 18.5 mg/kg in rats. In all the toxicological studies performed in rats (three 90-day studies, a carcinogenicity study and a three-generation study in which the final mating of each generation was used as teratology phase), mice (a 90-day study and a combined chronic toxicity and carcinogenicity study), rabbits (developmental study) and dogs (two 90-day study and 1-year study), the lowest no observed effect level (NOEL) observed was 0.5 mg/kg body weight (bw) per day for neurological observations and histopathological effects seen in the 1-year study in dogs. No indication of carcinogenicity or developmental toxicity was found at the doses tested in rat, mouse and rabbits. Reproduction toxicity was tested in rats giving a lowest no observed adverse effect level (NOAEL) of 0.7 mg/kg bw per day in parental animals. Overall, there was no clear difference in the toxicity between the two forms of narasin used in the studies above (mycelial and crystalline narasin), when the dosed were expressed in terms of narasin content.

For the current application two studies, not available at the time of the previous assessment, were submitted. These studies are: (i) a dose-range-finding study performed in rats,[46] giving NOAELs of 3.2 mg/kg bw per day for males and 5.4 mg/kg bw per day for females, based on decreased body weight gain at 6.2 mg/kg bw per day or 6.3 mg/kg bw per day, respectively and (ii) a three-month dog study[47] giving a NOAEL of 1 mg/kg bw per day, based on signs of neurotoxicity seen at 2 mg/kg bw per day. These two studies are shortly described below.

A dose-range finding study was performed in Sprague–Dawley (Crj:CD(SD)) rats. Groups of four males and four females were fed crystalline narasin at dietary concentrations of 0, 15, 30, 60 120 and 240 mg/kg for 28 days. All of the animals at the top-dose group died; the dosages received by the other groups were equal to 0, 1.6, 3.2, 6.2, 8.2 mg/kg bw per day in males and 0, 1.7, 3.3, 5.4, 6.3 mg/kg bw per day in females. Ophthalmoscopic examinations were performed at the start of the study and on treatment day 24. Urine was collected on day 22. All surviving rats were killed at the end of the treatment period, terminal blood samples were taken for haematology[48] and blood biochemistry[49] and necropsies were performed. Selected organs (heart, liver, kidneys, spleen, thyroid, adrenals, testes, ovaries) were weighed. Mortality was increased at doses of 60 mg/kg (6.2 mg/kg bw per day) or more in males and 120 mg/kg (6.3 mg/kg bw per day) or more in females. There were various signs of toxicity in the top-dose (240 mg/kg) group rats, but not at lower doses (120 mg/kg or less). There was a dose-related decrease in food intake, which was significant at 120 mg/kg or more. Bodyweight gain was decreased in the males given 60 mg/kg or more and females given 30 mg/kg or more. Ophthalmoscopy and urinalysis (colour, pH, turbidity, protein) showed no adverse effects. Haematology showed a significant increase in red blood cell count and haemoglobin the 120 mg/kg in males and females. There were no significant effects on blood biochemistry parameters. Autopsies showed no particular organ changes in survivors of the

[46] Technical dossier/Section II/Annex III.59.
[47] Technical dossier/Section III/Annex III.61.
[48] Red blood cell, haemoglobin, packed cell volume, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, white blood cell, platelets, prothrombin time, activated partial thromboplastin time (APIT).
[49] Lactate dehydrogenase, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, γ-glutamyl transferase, creatine kinase, total protein, albumin, globulin, total cholesterol, triglycerides, glucose, bilirubin, blood urea nitrogen, creatinine, Ca, P, Na, K, Cl.

treatment, although rats given 120 mg/kg or more were emaciated. There were no consistent changes in organ weights. The NOAELs for this study were 30 mg/kg (3.2 mg/kg bw per day) for males and 60 mg/kg (5.4 mg/kg bw per day) for females, based on decreased body weight gain at 60 mg/kg (6.2 mg/kg bw per day) and 120 mg/kg (6.3 mg/kg bw per day), respectively.

In a 3-month dogs study, groups of four Beagles of each sex were given mycelial narasin in capsules at daily oral doses of 0, 0.5, 1 or 2 mg narasin activity/kg bw per day. Ophthalmoscopy was performed at the beginning and end of the treatment period. Electrocardiograms of all dogs in the 2 mg/kg bw per day group were taken in week 17, but no pr-treatment measurements of these dogs were available for comparison. Blood and urine samples were collected predosing and in weeks 1, 2, 4, 8 and 12 of treatment. All animals were killed at the end of the treatment period, autopsies were performed, and organs weighed and preserved for microscopic examination. All dogs survived the study and no effects were seen on bodyweight gain or ophthalmoscopy. Signs of toxicity, including leg weakness, incoordination and ataxia, were observed in the top-dose group only. Electrocardiogram measurements of the top-dose animals showed bradycardia in 6 out of 8 dogs, and normal to markedly aberrant sino-arrhythmias were present, including signs of sino-arrest, short QRS intervals, and deep S-wave. Haematology[50] serum biochemistry[51] and urinalysis[52] showed no treatment-related adverse effects. There was also no effect on the populations of cells in bone marrow. Organ weights, gross pathology and histopathology were unaffected by treatment. The NOAEL for this study was 1 mg/kg bw per day, based on signs of neurotoxicity seen at 2 mg/kg bw per day. Interpretation of the adverse cardiac effects seen in most animals given 2 mg/kg bw per day is difficult as no measurements were made at lower doses and no measurements were made prior to dosing.

The applicant performed a literature search[42] on the toxicology of narasin covering the period 2000–2014 using several databases.[43] The search included the terms 'Narasin AND acute toxicity', 'Narasin AND repeat dose toxicity', 'Narasin AND carcinogenicity', 'Narasin AND reproductive toxicity', 'Narasin AND developmental toxicity', 'Narasin AND genotoxicity OR mutagenicity', 'Narasin and environmental safety'. The outcome of the literature review (Appendix A) did not identify new data requiring consideration in the current opinion.

The FEEDAP Panel noted that positive inotropic effects (an effect seen with some ionophoric coccidiostats) have been previously accepted as being relevant to consumer risk assessment, particularly when occurring at doses lower than identified as a toxicological NOAEL (EFSA, 2005). It should be noted that a NOEL for induction of positive inotropy by oral doses of narasin was not identified in any of the studies assessed in its previous assessments by the FEEDAP Panel (EFSA, 2004), electrocardiogram results for dogs given up to 2 mg narasin/kg bw per day showed no treatment-related effects.[53] Since this dose is above the lowest NOAEL previously identified of 0.5 mg/kg bw per day, there is no reason to consider acute cardiovascular effects in the risk assessment.

Overall, the FEEDAP Panel concluded, in agreement with its previous assessment in 2004, that the NOEL for narasin identified in the oral toxicity studies was 0.5 mg/kg bw per day for the neuropathy seen in a one-year dog study.

3.2.3.2. Other toxicological studies

The applicant submitted the same studies in horses, turkeys and rabbits that were already evaluated by the FEEDAP Panel in 2004 (EFSA, 2004). Based on these studies, the FEEDAP Panel concluded that narasin in doses proposed for feed supplementation in chickens is toxic to horses, turkeys and rabbits. The same conclusions are retained.

3.2.3.3. Conclusions on toxicology

The FEEDAP Panel re-assessed the toxicological studies available and the two studies not submitted for the previous application and concluded that the previously identified lowest NOAEL of 0.5 mg/kg bw per day is an appropriate base for confirming the acceptable daily intake (ADI) of 0.005 mg narasin/kg bw already established by the FEEDAP Panel in its former opinions (EFSA, 2004; EFSA FEEDAP Panel, 2010a) applying an uncertainty factor of 100.

[50] Red blood cell, haemoglobin, packed cell volume, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, white blood cell, differential leucocyte count, prothrombin time, sedimentation rate, blood clotting time, platelets.
[51] Glucose, blood urea nitrogen, creatinine, total bilirubin, Lactate dehydrogenase, γ-glutamyl transferase, alkaline phosphatase, aminotransferase, aspartate aminotransferase.
[52] Specific gravity, pH, protein, sugar, occult blood.
[53] Technical dossier/Section II/Annex III.62.

3.2.3.4. Assessment of consumer safety

The FEEDAP Panel already assessed the safety of the consumer exposed to narasin residues from chicken tissues using the data summarised in Section 3.2.1 of this opinion. An overview of the relevant calculations made in 2010 for the assessment of the safety for the consumer is given below (EFSA FEEDAP Panel, 2010a).

Exposure to total residues, calculated applying the food basket of Regulation (EC) No 429/2008, complied with the ADI (20%) after 0.25-day withdrawal (equivalent to a practical 0-day withdrawal period) (Table 3).[54] Considering the very low ionophoric activity of narasin main metabolites, the residues of toxicological concern (toxicity related to the ionophoric property) would represent a much lower potential risk than total residues retained for calculation as a conservative approach.

Table 3: Consumer exposure to narasin total residues and comparison with the acceptable daily intake (ADI) of 0.005 mg narasin/kg bw per day

	Liver	Kidney	Muscle	Skin/fat	Sum
TRC (mg/kg wet tissue)(1)	0.272	0.068	< 0.015(5)	0.082	
TRC + 2SD(2)	0.416	0.090	< 0.021	0.112	
Consumption (g/day)(3)	100	10	300	90	500
DITR (mg/day)(4)	0.042	0.001	0.006	0.010	0.059
% ADI	14	0.3	2	3	20

(1): TRC: total residue concentration (average).
(2): TRC (average) + 2 standard deviations.
(3): Based on the food basket of Regulation (EC) No 429/2008.
(4): Dietary intake calculated from total residues.
(5): Limit of quantification (LOQ) = 0.015 mg/kg.

MRLs of 50 μg narasin/kg for all wet tissues from chickens for fattening are in force at EU level.[55] The authorised withdrawal period is 0 day.[56]

The consumer exposure to narasin, corresponding to the consumption of chicken tissues containing residues corresponding to the uniform MRL was calculated in 2010 (EFSA FEEDAP Panel, 2010a) and was demonstrated to represent 58% of the ADI (Table 4).[57]

Table 4: Consumer exposure to narasin residues derived from established maximum residue limits (MRLs) and comparison with the acceptable daily intake (ADI) of 0.005 mg narasin/kg bw per day

	Liver	Kidney	Muscle	Skin/fat	Sum
RMTR(1)	0.037	0.147	0.667	0.354	
MRLs (mg/kg wet tissue)	0.05	0.05	0.05	0.05	
Consumption (g/day)(2)	100	10	300	90	500
$DITR_{MRL}$ (mg/day)(3)	135	3	23	13	174

[54] The contribution of total narasin residues in edible tissues of chicken to 20% of the ADI is based on the food basket of the Regulation (EC) No 429/2008. Applying instead European food consumption data from EFSA's Comprehensive European Food Consumption Database (see Guidance on the assessment of the safety of feed additives for the consumer (EFSA FEEDAP Panel, 2017)), this contribution would account only to about 2% of the ADI.

[55] Commission Regulation (EC) No 545/2006 of 31 March 2006 amending Regulation (EC) No 1464/2004 as regards the conditions for authorisation of the feed additive 'Monteban', belonging to the group of coccidiostats and other medicinal substances. OJ L 94, 1.4.2006, p. 26.

[56] Commission Regulation (EU) No 884/2010 of 7 October 2010 amending Regulation (EC) No 1464/2004 as regards the withdrawal time of the additive 'Monteban', belonging to the group of coccidiostats and other medicinal substances. OJ L 265, 8.10.2010, p. 4.

[57] The contribution of MRL-derived narasin residues in edible tissues of chicken to 58% of the ADI is based on the food basket of the Regulation (EC) No 429/2008. Applying instead European food consumption data from EFSA's Comprehensive European Food Consumption Database (see Guidance on the assessment of the safety of feed additives for the consumer (EFSA FEEDAP Panel, 2017)), this contribution would account to about 20% of the ADI.

	Liver	Kidney	Muscle	Skin/fat	Sum
% ADI	45	1	8	4	58

(1): Ratio marker to total residues at a 0.25-day withdrawal time (from EFSA FEEDAP Panel, 2010a: the ratios have been established by default considering the values measured after a 0.25-day withdrawal.
(2): Based on the food basket of Regulation (EC) No 429/2008.
(3): Dietary intake calculated from MRLs.

The FEEDAP Panel reiterates its previous conclusion that the use of Monteban® G100 in chickens for fattening at the maximum dose proposed, and without applying a withdrawal period, is safe for the consumer.

3.2.2.5. Conclusion on safety for the consumer

The ADI of 0.005 mg/kg bw is maintained and supports the setting of MRLs as already in force. Consumer safety is ensured without applying a withdrawal period provided that the maximum recommended dose is not exceeded.

3.2.4. Safety for the user

3.2.4.1. Effects on eyes and skin

Eye and skin irritation and skin sensitisation potential of narasin were already assessed in the FEEDAP opinion in 2004 (EFSA, 2004). The FEEDAP Panel concluded that:

> 'Monteban® G100 can cause irritation to the eyes but not to the skin. In addition, Monteban® G100 has the potential to induce skin sensitisation when applied to the ears of mice. The acute systemic toxicity following dermal application to rabbits and rats was low'.

In the absence of new data, the FEEDAP Panel reiterates its previous conclusions.

3.2.4.2. Effects on the respiratory system

The acute[58] and repeat concentration[59] inhalation toxicity studies submitted in the dossier were already assessed in 2004 (EFSA, 2004). The FEEDAP Panel reassessed these studies. Although the repeat-concentration study in dogs (91 day with 5 days/week of 6-h exposure/day) showed a number of limitations in design (e.g. low number of animals), the lowest NOEL (0.114 mg narasin/m^3) is retained for the assessment.

3.2.4.3. Inhalation exposure

The potential exposure of users by handling the additive to inhaled narasin was calculated according to the Technical Guidance on User safety (EFSA FEEDAP Panel, 2012b) and reported in Appendix B. From dusting potential and narasin content of the dust, the narasin concentration in the inhaled air could be calculated as 50 mg/m^3, resulting in inhalation exposure of 7 mg narasin from Monteban® G100 per person during an 8-h working day. Exposure to narasin by the respirable fraction (< 10 μm) of the dust only (36–45%) would be about 3 mg.

Comparing the narasin concentration in the inhaled air of 50 mg/m^3 and the NOEL in dogs of 0.114 mg/m^3, the inhalation exposure of users handling Monteban® 100G is considered a risk.

Conclusions on safety for the user

Monteban® G100 is irritant to the eyes but not to the skin. It has the potential to induce skin sensitisation. The acute systemic toxicity following dermal application is low.

On the basis of the available information, inhalation exposure would pose a risk to persons handling the additive.

3.2.5. Safety for the environment

The active ingredient is not a physiological/natural substance of established safety for the environment. The additive is also not intended for companion animals only. Consequently, according to Regulation (EC) No 429/2008, the Phase I assessment has to be continued to determine the predicted environmental concentration (PEC), according to the proposed conditions of use in chickens for fattening.

[58] Technical dossier/Section III/Reference 84 and 85.
[59] Technical dossier/Section III/Reference 87 and 88.

The FEEDAP Panel evaluated the new studies provided in the dossier and reassessed the studies already considered in its previous opinions (EFSA, 2004 and EFSA FEEDAP Panel, 2010b). The applicant performed a literature search[42] on the environmental safety of narasin covering the period 2000–2014 using several databases.[43] The search included the terms 'Narasin and environmental safety'. The outcome of the literature review (Appendix A) did not identify new data requiring consideration in the current opinion.

3.2.5.1. Phase I

Physicochemical properties of narasin

The physicochemical properties of narasin are summarised in Table 5.

Table 5: Physicochemical properties of narasin

Property	Value	Unit
Octanol/water partition coefficient (log K_{ow} 25°C)(1)	4.79 (pH 5) 4.85 (pH 7) 5.06 (pH 9)	
Water solubility(2)	102 (pH 7) 681 (pH 9)	mg/L
Vapour pressure(3)	6.2E-24	Pa
Dissociation constant (pKa)(4)	7.9 (in 66% dimethylformamide)	–

(1): Technical dossier/Supplementary information May 2015/Ref. 7.
(2): Technical dossier/Supplementary information May 2015/Ref. 5.
(3): Estimated by EPIweb.
(4): Technical dossier/Supplementary information May 2015/Ref. 6.

Fate and behaviour

No information is available on the hydrolysis or biodegradation of narasin in water.

Fate in soil

Adsorption/desorption in soil

The adsorption and desorption behaviour of narasin was determined in five soils using five test concentrations following OECD 106.[60] The pH of the soils ranged from 5.2 to 7.7, and the soil organic carbon content ranged from 0.7% to 5.0%. Correlation coefficients indicated that the isotherms followed well the Freundlich equation. The K_{oc} values ranged from 873 to 2,576, with a mean value of 1,357 L/kg (Table 6). Narasin is a carboxylic acid with a pKa of 7.9 which shows a low solubility at pH 5 and a much higher water solubility of 102 mg/L at pH 7 and 681 mg/L at pH 9. This indicates that the anionic form of narasin is soluble while the neutral acid is not. Since there is a pH dependence in the solubility, the FEEDAP Panel assumes that there also will be a pH dependence in the sorption. In the case of a pH-dependent sorption, the lowest K_{oc} value (873 L/kg) is selected for further calculations.

Table 6: Adsorption of narasin in different soils(1)

	Properties of the soils		Adsorption		Desorption	
Soil	%OC	pH	K_d	K_{oc}	K_d	K_{oc}
Clay loam (TB_PF)	5.0	7.2	44	873	49	983
Sandy clay loam (MSF-Pf)	1.9	6.2	18	927	39	2,060
Clay loam (DU-Loam)	4.1	5.2	106	2,576	151	3,677
Loamy sand (Roger Myron)	1.3	5.7	15	1,149	24	1,878
Clay (Montana Clay)	0.7	7.7	8.8	1,263	20	2,844
Geometric mean				1,248		2,089

(1): %OC: % of organic carbon; K_d: soil adsorption coefficient; K_{oc}: adsorption or desorption coefficient corrected for soil organic.

[60] Technical dossier/Supplementary information May 2015/Ref. 4.

Biodegradation in soil

A good laboratory practice (GLP)-compliant study, following the SETAC (1995) guideline recommended for the aerobic degradation of pesticides, was performed on the aerobic degradation of [^{14}C]-narasin in three soils (sandy loam, a silt loan and a clay loam).[61] HPLC and thin-layer chromatography (TLC) were applied for the identification of potential metabolites and a CO_2 trap for quantifying mineralisation. The duration of the study was 84 days with samples analysed on days 0, 7, 14, 21 28, 42, 56, 70 and 84 days after application. Several metabolites where found but not identified. Only one metabolite was found at concentrations higher than 10% of the total radioactivity with a maximum of 16% in silt loam at 70 days after application decreasing to 14% at 84 days. Mineralisation to CO_2 was the main degradation process, accounting for 64% in sandy loam, 19% in silt loam and 54% in clay loam. Non-extractable residues accounted for 18, 20 and 25%, respectively. The DT_{50} values for sandy loam, silt loam and clay loam were 21, 49, and 29 days, the DT_{90} values were 69, 162 and 96 days, respectively. When the mean DT_{50} of 33 days is adjusted to an incubation temperature of 12°C using the Arrhenius equation,[62] the new DT_{50} is 70 days. When the mean DT_{90} of 109 days is adjusted to 12°C, the new DT_{90} is 231 days. These values are used for further assessment.

Fate in manure

A study on the aerobic mineralisation and transformation of narasin in chicken manure was performed in 2011.[63] The methods were based on the OECD guideline 307, aerobic and anaerobic transformation in soil. Instead of soil, chicken manure was used obtained from a commercial poultry farm. At the time of collection, the chickens were fed an industry-typical corn and soybean meal-based commercial broiler finishing ration containing 63 g (narasin from Monteban®)/tonnes feed and no other medications. The experiments were performed with either fresh manure or with fresh manure amended with aged chicken litter containing wood shavings. The microbial biomass increased from day 0 up to day 17 and then decreased till the end of the test at day 38. The temperature and moisture were controlled. Radiolabelled ^{14}C narasin was added at 0.5 mg/kg dry weight manure. Only a small proportion (1–3%) was eventually transformed into $^{14}CO_2$. In the first week, half of the parent narasin was degraded into three major pools of radioactivity. Two major chromatographic peaks clearly separated from the parent compound were formed with 30% and 20% of the radioactivity. These were identified as narasin without the A ring and trihydroxylated narasin. Also a water-soluble and a non-extractable residue were formed. After the first week the conversion of narasin into degradation products stopped. The conclusion that 50% of the narasin can be degraded in chicken manure was used in the risk assessment below.

Conclusion on fate and behaviour

The following values will be used for the assessment: K_{oc} of 873 L/kg and an average DT_{50} of 70 days (at 12°C).

Predicted environmental concentrations (PECs)

Based on the proposed use of 70 mg narasin/kg feed for chickens for fattening the calculated PEC_{soil} (364 μg/kg) and $PEC_{groundwater}$ (23 μg/L) exceeded the trigger values of 10 μg/kg and 0.1 μg/L, respectively, as indicated in the FEEDAP Technical guidance for assessing the safety of feed additives for the environment (EFSA, 2008a). Therefore, the environmental risk assessment of narasin requires a Phase II assessment.

3.2.5.2. Phase II

Exposure assessment

In its opinion on Maxiban® (narasin and nicarbazin) for chickens for fattening (EFSA FEEDAP Panel, 2010b), the FEEDAP Panel considered that the metabolism data on narasin indicated that only 5% of the administered dose was excreted as a parent compound and 30% was excreted as metabolites having not more than 20% of the ionophoric activity (as a worst-case assumption). In addition, 50% of the 5% parent compound is degraded in manure rapidly, leaving only 2.5% parent compound and 2.5% degradation products. These new data on degradation of narasin in manure allowed a further refinement of the amount of environmentally relevant ionophoric activity to 9% (2.5 + (30+2.5) × 0.20).

[61] Technical dossier/Supplementary information May 2015/Ref. 8.
[62] The temperature correction was performed according to the scientific opinion of the Panel on Plant Protection Products and their Residues on a request from EFSA related to the default Q10 value used to describe the temperature effect on transformation rates of pesticides in soil (EFSA, 2007).
[63] Technical dossier/Supplementary information May 2015/Ref. 18.

PECs calculation refined in Phase II

According to EFSA guidance (EFSA, 2008a), if a high persistence in soil is anticipated (DT_{90} > 1 year), the potential for residues to accumulate in soil should be considered. This is not the case for narasin, having a DT_{90} at 12°C < 1 year.

Considering the degradation of narasin in manure and assuming that the ionophoric activity of narasin and its metabolites in chicken excreta would not exceed in total 9% of the orally administered dose, the refined dose used for PEC calculations was 6.3 mg/kg feed. The PEC_{soil}, $PEC_{surfacewater}$ and $PEC_{sediment}$ are reported in Table 7.

Table 7: Predicted environmental concentrations of narasin in soil (μg/kg), groundwater (μg/L), surface water (μg/L) and sediment (μg/kg dry weight) refined for metabolism and degradation

Input	Value
Dose (mg narasin/kg feed)	70 × 9% = 6.3
Molecular weight (narasin)	756
VP (Pa)	6E-24
Solubility (mg/L)	102
K_{oc} (L/kg)	873
DT_{50} at 12°C (days)	70
Output	
PEC_{soil}	33
$PEC_{groundwater}$	2.1
$PEC_{surfacewater}$	0.7
$PEC_{sediment}$	32

VP: vapour pressure; K_{oc}: adsorption or desorption coefficient corrected for soil organic carbon content; DT_{50}: disappearance Time 50 (the time within which the concentration of the test substance.

The FOCUS (FOrum for Co-ordination of pesticide fate models and their Use) groundwater exposure calculation on the parent compound performed by the applicant[64] resulted in predicted concentrations of narasin in groundwater lower than the quality standard in the EU.[65]

Ecotoxicity studies

Toxicity to terrestrial compartment

Effects on plants

The effects of soil incorporated narasin on the emergence and growth of three species of plants was determined in accordance with OECD 208 (1984).[66] The study was already assessed in 2010 by the FEEDAP Panel (EFSA FEEDAP Panel, 2010b) and re-evaluated for the current assessment. Winter oats, radish and mung bean were exposed to 0, 0.38, 3.38 or 29.26 mg narasin per kg soil (nominal levels of 0, 0.35, 3.5 or 35 mg/kg).

For winter oats, the EC_{50} (median effective concentration) for emergence and growth were both greater than highest tested concentration. The no observed effect concentration (NOEC) for emergence was estimated to be 29.26 mg/kg, the one for growth 3.38 mg/kg.

For radish, an EC_{50} for emergence was estimated at 5.07 mg/kg; although no confidence limits could be determined, the EC_{50} for growth was 6.18 mg/kg. The NOEC for emergence and growth were 3.38 and 0.38 mg/kg, respectively.

For mung bean, an EC_{50} for emergence was estimated to be greater than 29.26 mg/kg; an EC_{50} for growth was estimated at 8.99 mg/kg.

The results on mung bean indicated that the mean shoot weights of seedlings in the control treatment and at 0.37, 3.38 and 29.26 mg/kg were 0.58, 0.49, 0.42 and 0.19 g per plant, respectively. The percent reduction at 0.37, 3.38 and 29.26 mg/kg was 14.8%, 27.2% and 66.6%, respectively. The mean shoot weights from each of the three treatments were found to be significantly different from

[64] Directive 2006/118/EC of the European Parliament and of the Council of 12 December 2006 on the protection of groundwater against pollution and deterioration. OJ L 372, 27.12.2006, p. 19.
[65] Technical dossier/Supplementary information June 2017/Annex 46.
[66] Technical dossier/Supplementary information May 2015/Ref. 9.

the mean control value according to the applicant. However, a re-analysis of the data with the Dunnett's test indicated no significant differences between the control and the 0.38 mg/kg concentration. The NOEC for this endpoint was established at 0.38 mg/kg. A growth reduction for mung bean has been observed at all tested levels; hence, a NOEC could not be determined (< 0.38 mg/kg).

Table 8 summarises the phytotoxicity of narasin in the three species of plants.

Table 8: Ecotoxicological effects data for terrestrial plants (mg narasin/kg)

	Emergence		Growth	
Plant species	**EC_{50}**	**NOEC**	**EC_{50}**	**NOEC**
Winter oat	> 29.26	29.26	> 29.26	3.38
Radish	5.07	3.38	6.18	0.38
Mung bean	> 29.26	0.38	8.99	< 0.38

Across all three species, the lowest EC_{50} is 5.07 mg/kg and the lowest NOEC is lower than 0.38 mg/kg.

Effects on earthworms

The acute toxicity of nominal concentrations of 5, 40, 80, 160 and 320 mg/kg of narasin to the earthworm *Eisenia foetida* was assessed in a study conducted in accordance with OECD 207 (1984) (11).[67] The study was already assessed in 2010 by the FEEDAP Panel (EFSA FEEDAP Panel, 2010b) and re-evaluated for the current assessment. Results indicated that LC_{50} at 7 and 14 days was 51.1 and 46.4 mg/kg, respectively, and the NOEC based on both bodyweight and survival was 34.3 and 4.3 mg/kg narasin for day 7 and day 14, respectively. At day 14, there was 100% mortality in the two highest groups and these are excluded from the bodyweight analysis.

A chronic earthworm *E. foetida* study conducted in accordance with OECD 222 (2004) assessed nominal narasin levels of 3.1, 6.3, 13, 25 and 50 mg/kg narasin.[68] According to this guideline, the substance is mixed into the soil and the earthworms are fed with clean manure without any toxicants. This is different from the situation in the field where the substance is present in the manure at much higher concentrations than the final concentration in soil. The 28 day LC_{50} was determined as 41 mg/kg. The NOEC for F_0 earthworm survival was 25 mg/kg. The NOEC for biomass and reproduction were both determined as 50 mg/kg. The EC_{50} for reproduction was > 50 mg/kg, as no test level resulted in more than 50% inhibition of reproduction.

The FEEDAP Panel noted that in the study of acute effects of narasin, the subacute effects on the bodyweight were analysed, resulting a NOEC of 4.3 mg/kg narasin at day 14. This concentration is significantly lower than the concentrations provided in the reproduction tests and it is selected for the assessment.

Effects on soil microorganisms

The potential effects of narasin on the rate of microbial respiration and on the nitrification and nitrogen-mineralisation capacity of soil microflora under aerobic conditions were investigated in a study conducted according to OECD guidelines 216.[69] The study was already assessed in 2010 by the FEEDAP Panel (EFSA FEEDAP Panel, 2010b) and re-evaluated for the current assessment. Sandy loam soil samples were treated with narasin using quartz sand as a carrier. Treated sand was added to soil samples to provide nominal concentrations of narasin of 3.5 and 17.5 mg/kg, resulting in actual exposure concentrations of 3.3, and 17.4 mg/kg. The results show that exposure to nominal narasin, concentrations of 3.5 or 17.5 mg/kg (3.3 or 17.3 mg/kg exposure level) does not affect the microbial respiration, mineralisation of organic nitrogen or nitrification activity associated with the soil microflora.

Toxicity to aquatic organisms

Effects on algae

A static toxicity test was conducted in accordance with OECD Guideline 201 (1984) and the GLP in order to evaluate the effects of narasin on the green alga *Selenastrum capricornutum* (with a recent

[67] Technical dossier/Supplementary information May 2015/Ref. 11.
[68] Technical dossier/Supplementary information May 2015/Ref. 12.
[69] Technical dossier/Supplementary information May 2015/Ref. 13.

taxonomic name of *Pseudokirchneriella subcapitata* (Korshikov)).[70] The study was already assessed in 2010 by the FEEDAP Panel (EFSA FEEDAP Panel, 2010b) and re-evaluated for the current assessment. Algal cells were cultured for approximately 72 h in a liquid nutrient medium that contained narasin at average assayed concentrations of 4.17, 2.16, 1.06, 0.54, 0.23 (calculated) and 0.035 (calculated) mg/L and an untreated control. The lower two concentrations were calculated as the analysis of the 0.23 test was not quantifiable by HPLC and the 0.035 level was below the LOD of the analytical method. The algal concentration was quantified at 24, 48 and 72 h. The temperature and pH ranged from 21 to 23°C and 7.92 to 10.42, respectively, throughout the test.

The effect on growth rate resulting in the E_rC_{50} (median effective concentration which results in 50% reduction in growth rate) of 2.92 mg/L has been used for this purpose.

Effects on crustaceans

The toxicity of narasin to *Daphnia magna* has been investigated in a 48-h static test.[71] The study was already assessed in 2010 by the FEEDAP Panel (EFSA FEEDAP Panel, 2010b) and re-evaluated for the current assessment. The study was performed in 1985 and is not conducted to current OECD guidelines, however it is a GLP study and the data are considered to be acceptable. Daphnids were exposed to assayed concentrations of 0.0, 4.69, 7.86, 12.45, 18.96, 35.08, and 42.18 mg/L of narasin for 48 h. Test solutions temperature averaged 20.4°C and had the following water quality characteristics: average dissolved oxygen 7.2 mg/L and pH averaged 8.2 and ranged from 7.7 to 8.5. No physical signs of toxicity were observed in the control populations. Exposure-related signs of toxicity ranging from hypoactivity to immobilisation were observed at all treatment levels. Immobilisation frequencies were 3%, 13%, 0%, 30%, 87% and 100% at the assayed concentrations of 4.69, 7.86, 12.45, 18.96, 35.08 and 42.18 mg/L. The 48-h EC_{50} was 20.56 mg/L.

Effects on fish

The toxicity of narasin to fish has been investigated in a 96-h static test.[72] The study was already assessed in 2010 by the FEEDAP Panel (EFSA FEEDAP Panel, 2010b) and re-evaluated for the current assessment. While this study was performed in 1985 and is not conducted according to current OECD guidelines, it is a GLP study and the data are considered to be acceptable. Juvenile rainbow (*Oncorhynchus mykiss*) trout were exposed to test solutions with assayed narasin concentrations of 0, 0.103, 0.561, 1.00, 1.82, 3.04 and 5.26 mg/L. Concentration related responses were found ranging from hypoactivity to laboured respiration at narasin concentrations greater $\geq$ 0.316 mg/L. The 96-h LC_{50} was 2.23 mg/L. No mortalities or behavioural signs of toxicity were found at concentrations $\leq$ 0.190 mg/L. The water quality characteristics were as follows: pH, 8.2 to 8.6; dissolved oxygen averaged 10.2 mg/L and temperature 13°C.

Effects on sediment-dwelling invertebrates

No studies submitted.

Conclusions on the ecotoxic effect on soil and water

The FEEDAP panel noted that toxicity tests on earthworms (OECD 222; 2006), plants (OECD 208; 1984) and green algae (OECD 201; 1984) were performed according to the standard methods which were replaced with more recent guidelines. Nevertheless, the new methods obtained are not substantially different in comparison to the previous ones; thus, the results provided can be accepted and evaluated. In the case of fish and daphnids, the ecotoxicity data were obtained in 1985, according to the ASTM standard methods which can be seen as complementary to the OECD methods.

For the terrestrial compartment, toxicity data on microorganisms, earthworms and plants are provided. Narasin does not affect the nitrogen transformation in soil when microflora is exposed up to 17.43 mg/kg narasin. The effect of narasin on earthworms was established at the concentration of 4.3 mg/kg as a NOEC for the subacute endpoint such as bodyweight loss. The reproduction study on earthworms was not used in the assessment as it is shown to be less sensitive than the acute one. Assessment of the effect of narasin on plants is based on the concentration that inhibits the emergence of radish, considering the EC_{50} value of 5.07 mg/kg.

For the aquatic compartment, data on acute toxicity of narasin are available for algae (E_rC_{50} of 2.92 mg/L), daphnids (EC_{50} of 20.56 mg/L) and fish LC_{50} (median lethal concentration) of 2.2 mg/L. The

[70] Technical dossier/Supplementary information May 2015/Ref. 16.
[71] Technical dossier/Supplementary information May 2015/Ref. 14.
[72] Technical dossier/Supplementary information May 2015/Ref. 15.

results of the test on algae are questionable as the pH value at the end of the test exceeded 10. Nevertheless, the lowest acute endpoint was observed in fish which are then used in the risk characterisation.

The applicant did not provide the data on toxic effects of narasin on the sediment compartment.

Risk characterisation (PEC/PNEC ratio)

The risk characterisation ratios for terrestrial, freshwater and sediment compartments are reported in Tables 9 and 10.

Table 9: Risk characterisation (PEC/PNEC ratio) for terrestrial compartment

Taxa	PEC_{soil} (µg/kg)	$NOEC/EC_{50}$ (mg/kg)	AF	PNEC (µg/kg)	PEC/PNEC
Earthworm(1)	33	4.3(2)	100	43	0.8
Plants		5.07(3)	100	50.7	0.65

AF: assessment factor.
(1): The AF of 1,000 is normally chosen for acute toxicity endpoints for substances of DT_{50} > 60 days. In this case, a subacute endpoint is taken from an acute test and an AF of 100 is used.
(2): NOEC.
(3): EC_{50}.

Table 10: Risk characterisation (PEC/PNEC ratio) for freshwater compartment

Taxa	$PEC_{surfacewater}$ (µg/L)	ErC_{50}/48-h EC_{50}/LC_{50} (mg/L)	AF	PNEC (µg/L)	PEC/PNEC
Algae *Pseudokirchneriella subcapitata (Scenedesmus subspicatus)*	0.7	2.92(1)	1,000	2.2	0.3
Aquatic invertebrates *Daphnia magna*		20.56(2)			
Fish *Oncorhynchus mykiss*		2.2(3)			

AF: assessment factor.
(1): E_rC_{50}.
(2): 48-h EC_{50}.
(3): LC_{50}.

3.2.5.3. Bioaccumulation

The FEEDAP Panel noted that the high octanol/water partition coefficient (log K_{ow} = 4.85 at pH 7) of narasin does not rule out bioconcentration in environmental food chains. However, since narasin is extensively metabolised in the chicken (Section 3.2.2.1), bioaccumulation is not expected.

3.2.5.4. Conclusion on environmental risk assessment

Narasin, when used as a feed additive for chickens for fattening at 70 mg/kg feed, is not expected to pose a risk to the environment.

The risk for sediment compartment cannot be assessed as no data were provided.

Narasin is not considered to have a bioaccumulation potential.

3.3. Efficacy

For coccidiostats under re-evaluation, efficacy data should derive from two types of target animal experiments: a) natural/artificial infection to simulate use conditions (e.g., floor pen studies with poultry), at least one of the locations should be in the EU, b) actual use conditions in field trials, all should be done in the EU within the last five years. Anticoccidial sensitivity tests (AST) could replace

field trials provided they follow the criteria mentioned in the relevant guidance document on coccidiostats and histomonostats (EFSA FEEDAP Panel, 2011a).[73]

The applicant submitted three floor pens studies, three ASTs performed with recent field isolates and two ASTs performed with laboratory strains. These last two studies were not considered for the demonstration of efficacy because the laboratory strains do not represent field conditions (EFSA FEEDAP Panel, 2011a).

Floor pen studies

Three floor pen studies in chickens for fattening, conducted in 2012, were submitted.[74] In each study, chickens (male Ross 308 in trial 1, female Ross cobs in trial 2, breed and gender not reported for trial 3) were penned and distributed into three treatment groups: an uninfected untreated control (UUC) group, an infected untreated control (IUC) group and an infected treated (IT) group. The IT group received feed containing 60 mg narasin/kg feed. The dosage was analytically confirmed (see Table 11). The experimental diets were fed for 42 days. In the infected groups, all birds were inoculated with recent field isolates of pathogenic *Eimeria* species; in trial 3, two parallel experiments with different inoculates were performed. Animals in the UUC group were sham-inoculated (water only). Animal health and mortality were monitored daily. Feed intake and body weight of the animals were measured, feed to gain ratio was calculated. Samples of excreta were analysed for oocyst excretion. Intestinal lesions were scored on three birds per pen in trial 1, and one bird per pen in trial 2 and on four birds per pen in trial 3 following the method of Johnson and Reid (1970) (0 = no lesion, 1 = very mild, 2 = mild, 3 = moderate and 4 = severe).

In all trials, an ANOVA was performed with the data, in trial 1, oocyst counts and lesions scores were analysed by Kruskal–Wallis test. Differences between the IUC and the IT groups were compared with post-hoc tests (least significant difference (LSD) test in trials 1 and 3, not reported in trial 2). Level of significance was set at a p value ≤ 0.05.

Table 11: Experimental design of floor pen studies with chickens for fattening using Monteban® G100

Trial	Replicates per treatment (birds per replicate)	Inoculum characteristics				Feed analysis narasin (mg/kg feed)(1)
		Year and country of isolation	Intended dose (number of oocysts) and strain per bird		Day and mode of inoculation	
1	12 (41–42)	2012 Spain	100,000	*E. acervulina*	Day 14 via feed	58.7/55.6/57.0
			10,000	*E. tenella*		
			50,000	*E. maxima*		
2	12 (12)	2010 UK	33,991	*E. acervulina*	Day 16 orally via syringe	55.3/58.9
			25,349	*E. tenella/necatrix*		
			2,916	*E. maxima*		
			15,432	*E. mitis*		
			714	*E. praecox/brunetti*		

[73] The FEEDAP Panel stated in its guidance for the preparation of dossiers for coccidiostats and histomonostats (EFSA FEEDAP Panel, 2011a) that studies with artificial infection would be preferred over field trials due to their inherent weaknesses. These short term studies should use field strains of *Eimeria*, recently confirmed as pathogenic/resistant by a sensitivity test or recognised problems in the poultry operation (confirmed by veterinary certificate). The *Eimeria* field strains should ideally undergo one, but in any case not more than two passage(s) before use in such trials.

[74] Trial 1: Technical dossier/Section IV/Annex IV.8. Trial 2: Technical dossier/Section IV/Annex IV.9. and Trial 3: Technical dossier/Section IV/Annex IV.10.

Trial	Replicates per treatment (birds per replicate)	Inoculum characteristics				Feed analysis narasin (mg/kg feed)(1)
		Year and country of isolation	Intended dose (number of oocysts) and strain per bird		Day and mode of inoculation	
3a	8 (20)	2011 The Netherlands	104,000	*E. acervulina*	Day 14 orally via syringe	50/54.6
			30,000	*E. tenella*		
			86,000	*E. maxima*		
			12,000	*E. praecox/necatrix*		
			4,000	*E. mitis*		
3b	8 (20)	2011 Belgium	77,000	*E. acervulina*		
			12,000	*E. tenella*		
			20,000	*E. maxima*		
			3,000	*E. praecox/necatrix*		
			2,000	*E. mitis*		

(1): In trial 1, birds received starter diet from day 0 to 14, grower diet from day 14 to 29 and finisher diet from day 29 to 42. In trial 2, birds received starter diet from day 0 to 10, grower diet from day 10 until study completion. In trial 3 birds received starter diet from day 0 to 14 and grower diet from day 14 to 42.

Tables 12 and 13 show the results of intestinal lesion scoring in trials 1, 2 and 3. A significant reduction of the lesion scores was observed in trial 1 (upper and middle intestine). Lesion scores in trials 2 and 3 were comparable in the IT and IUC groups.

Table 12: *Eimeria* infection related intestinal lesion scores in different intestinal sections 6 days post-infection in trials 1 and 2(1)

	Upper	Middle	Caecal	Total
Trial 1				
UUC	0	0	0	–
IUC	1.9	1.4	2.1	–
IT	1.3*	0.6*	1.8	–
Trial 2				
UUC	0	0	0	1
IUC	2.3	2.4	1.3	2.4
IT	1.8	2.3	1.0	2.1

– : not reported.
*: IT mean significantly different from IUC mean ($p \leq 0.05$).
(1): Lesions in the upper intestine were probably due to *E. acervulina*, in the middle intestine to *E. maxima* and in the caecal intestine to *E. tenella*.

Table 13: *Eimeria* infection related intestinal lesion scores in different intestinal sections 6 days post-infection in Trial 3

	Upper	Middle	Lower	Caecal
Trial 3a				
UUC	0.7	0.4	0.1	0.1
IUC	1.2	0.5	0.1	1.0
IT	1.0	0.4	0.1	1.1
Trial 3b				
UUC	0.7	0.4	0.1	0.1
IUC	1.0	0.4	0.2	1.6
IT	0.9	0.5	0.0	1.5

UUC: uninfected untreated control; IUC: infected untreated control; IT: infected treated.

Oocyst excretion on day 23 was significantly reduced in the IT group compared to the IUC group for all three different *Eimeria* species in trial 1 (See Appendix C). In trial 2, species-specific results were not reported. Total oocyst counts measured on days 22, 24, 27, 29 and 31 showed numerically lower counts in the IT group compared to the IUC group. Significant differences were seen only on the last day of the trial (IT 139 vs IUC 39841). In trial 3, oocyst excretion for *E. maxima* was significantly lower in IT compared to IUC at all time points (day 20, 22 and 28) when inoculum A was used. For inoculum B, OPG was significantly reduced by the treatment on day 20 for *E. necatrix/praecox*. A tendency for the reduction of *E. acervulina*, *E. tenella* and *E. maxima* oocyst excretion was seen on days 22 and 28.

Table 14 summarises the results concerning mortality and zootechnical endpoints. Mortality in the IT groups was lower than in the IUC groups (except in trial 3b), albeit not significant. In all three trials, weight gain of the IT birds was significantly higher compared to the IUC birds and reached the level of the UUC groups in trials 1 and 2. In trial 3, increased feed intake of the IT groups resulted in higher body weight gain than in the UUC groups but without significant improvement of the feed to gain ratio.

Table 14: Performance parameters and mortality of chickens for fattening in floor pen studies

	Feed intake(1) (g)	Final body weight (g)	Weight gain(2) (g)	Feed to gain ratio(3)	Mortality(4) n
Trial 1					
UUC	126	3,057	73.5	1.72	16
IUC	126	2,924	70.3	1.79	10
IT	125	3,063*	73.7*	1.70*	7
Trial 2					
UUC	496/806/1,960	2,557	2,046	1.36/1.47/1.73	19
IUC	384/803/1,944	2,427	1,886	1.93/1.63/1.63	23
IT	428*/862*/2,270*	2,617*	2,086*	1.88/1.62/1.72	15
Trial 3a					
UUC	3,348	–	1,538	2.178	5
IUC	3,690	–	1,684	2.194	11
IT	3,728	–	1,805*	2.066	7
Trial 3b					
UUC	3,348	–	1,538	2.178	5
IUC	3,365	–	1,551	2.177	6
IT	3,606*	–	1,702*	2.120	9

– : not reported.
*: IT mean significantly different from IUC mean ($p \leq 0.05$).
(1): Results of trial 1 refer to daily feed intake during the whole study duration; results of trial 2 refer to overall feed intake during days 16–22/22–29/29–42; results of trial 3 refer to overall feed intake during the whole study duration.
(2): Results of trial 1 refer to the average daily weight gain considering the whole study duration; results of trial 2 refer to the overall weight gain during the post-inoculation period (day 16–42); results of trial 3 refer to overall weight gain during the whole study duration.
(3): Results of trials 1 and 3 refer to the feed to gain ratio calculated for the whole study duration; results of trial 2 refer to the ratios calculated for the periods 16–22/22–29/29–42 days.
(4): Results of trials 1 and 2 refer to total mortality in the post-infection period; results of trial 3 refer to the whole study duration.

3.3.1. Anticoccidial Sensitivity Tests

Three ASTs performed in 2012 were submitted.[75] Each test was made with the groups UUC, IUC and IT, the latter receiving feed supplemented with Monteban® at an intended concentration of 60 mg narasin/kg feed (analytically confirmed, see Table 15). The birds (female Ross 308 in AST-1 and AST-2; Cobb 500 in AST-3) were randomly allocated to the groups. Three other anticoccidial additives were also tested in all studies. Birds were artificially infected with sporulated oocysts from recent field isolates. Animal health and mortality were monitored. Feed intake and body weight of the animals were measured, feed to gain ratio was calculated. Samples of excreta were analysed for oocyst

[75] AST-1: Technical dossier/Section IV/Annex IV.13. AST-2: Technical dossier/Section IV/Annex IV.14. AST-3: Technical dossier/Section IV/Annex IV.15.

excretion. Intestinal lesions were scored following the method of Johnson and Reid (1970) (0 = no lesion, 1 = very mild, 2 = mild, 3 = moderate and 4 = severe).

The statistical tests were two-sided; the level of significance was set at a p value ≤ 0.05. IUC and IT group means were compared applying two-sided t-tests and Fisher's exact tests for lesions scores in AST-1 and AST-2.

Table 15: Experimental design of ASTs with chickens for fattening using Monteban® G100

Trial	Replicates per treatment (birds per replicate)	Inoculum characteristics				Anticoccidial treatment(1) (days of life)	Feed analysis narasin (mg/kg feed)
		Month/year and country of isolation	Intended dose (number of oocysts) per bird and strain		Day of inoculation		
1	4 (5)	03/2012 UK	296,375	*E. acervulina*	14	7–21	57
			23,399	*E. maxima*			
			31,198	*E. tenella*			
			31,198	*E. praecox*			
			7,799	*E. mitis*			
2	4 (5)	12/2011 France	218,548	*E. acervulina*	14	7–21	59.5
			29,802	*E. maxima*			
			21,855	*E. tenella*			
			11,722	*E. praecox*			
			3,974	*E. mitis*			
3	4 (8)	03/2012 Spain	100,000	*E. acervulina*	16	9–22	63
			25,000	*E. maxima*			
			25,000	*E. brunetti*			
			10,000	*E. tenella*			
			10,000	*E. necatrix*			

(1): Birds in the IT group were fed a basal diet supplemented with Monteban® G100. Animals in the control groups UUC and IUC received the same basal diet without inclusion of the coccidiostat.

Table 16 summarises the results of the ASTs.

There were no mortalities in AST-1, and one IUC bird died in AST-2. Increased mortality due to coccidiosis was seen only in IUC of AST-3; 12 birds died which is significantly different to the IT group in which no mortality occurred.

Significantly lower OPG value in IT group, showing the effect of the coccidiostatic treatment, was seen only in AST-3. A reduction of lesion scores by treatment (IT) was observed in all tests; however, significance was reached only in AST-1 (upper intestine) and AST-3 (upper, lower intestine and caeca).

A beneficial effect of the anticoccidial treatment (IT vs. IUC) was observed as a significantly higher weight gain and improved feed to gain ratio in AST-3.

Table 16: Results of anticoccidial sensitivity tests

Group	Feed intake (g)		Weight gain(2) (g)		Feed to gain ratio	Total OPG	Mean lesion scores			
							Upper	*Mid*	*Low*	*Caeca*
	D14–21	D7–21	D14–21	D7–21	D7–21	D17–21	D21			
AST-1										
UUC(1)	2,518	4,106	355	568	1.56	0	0	0	0	0
IUC	2,073	3,673	194	398	1.85	448,955	2.6	1.1	0	0.7
IT	2,163	3,773	223	440	1.72	481,163	0.7*	0.8	0	0.1
AST-2										
UUC(1)	2,433	4,244	214	343	2.49	0	0	0	0	–
IUC	2,015	3,548	187	360	1.98	3,209,608	2.16	0.90	2.71	–
IT	2,130	3,733	224	414	1.83	3,317,853	2.10	0.55	2.60	–
	D16–22		D16–22		D16–22	D25	D25			
AST-3										
UUC	NR		289*		1.29*	0	0	0	0	0
IUC	NR		98		1.71	117,611	2.6	1.9	2.1	2.8
IT	NR		208*		1.37*	23,782*	2.2*	1.5	0.8*	2.4*

-: not measured.
NR: measured, but not reported.
*: IT mean/UUC mean significantly different from IUC mean ($p \leq 0.05$).
(1): The cages of the UUC group were kept in another building than those of IUC and IT groups. The zootechnical data of UUC group are therefore not directly comparable to IUC and IT.
(2): The results in AST-1 and 2 refer to total average weight gain per bird and in AST-3 to average daily gain per pen.

Synopsis on efficacy studies

The synopsis is based on three floor pen studies and three ASTs made with the lowest applied dietary concentration of the coccidiostat Monteban® G100 (60 mg narasin/kg feed).

Mortality in the floor pen studies did not indicate a coccidiostatic effect since mortality rates were not significantly different and appeared even not to be influenced by *Eimeria* inoculation (mean UUC 13%, mean IUC 14%). In the ASTs, mortality was very low in two trials; however, it was affected by oocyst inoculation in a third trial (AST-3) and significantly reduced by narasin treatment.

Lesion scores in the upper, mid and lower intestine of *Eimeria* inoculated birds were numerically reduced by narasin in all three floor pen studies and ASTs. However, this reduction reached significance only in one floor pen study (trial 1) for the upper and mid intestine and in two ASTs for the upper intestine (AST-1 and 3, in AST-3 also for the low intestine and caeca).

Oocyst excretion of all tested four *Eimeria* species was significantly reduced by Monteban® in one floor pen study (trial 1), for *Eimeria maxima* in one part (inoculum A) of the second study (trial 3), but not by inoculum B of the same study. A third study (trial 2) showed only numerical reductions of oocyst excretion (except on day 26 after inoculation where significance was reached). In AST-1 and AST-2, oocyst excretion on days 3–7 post-*Eimeria* inoculation was higher in the Monteban® groups than in the untreated groups, whereas it was significantly reduced in a third AST (AST-3).

Body weight gain of birds in the floor pen studies was significantly higher for the Monteban® treated groups compared to the infected non treated birds. However, this effect cannot doubtlessly be traced back to an anticoccidial effect when the specific endpoints fail to support such a relation. This was not the case for all floor pen studies even if the reduction in oocyst excretion was not significant in trial 2. In the ASTs, body weight gain (or any other performance parameter) cannot be taken into account as an indication of an anticoccidial efficacy of Monteban® (i) considering the short study duration of the relevant observation period (7 days) and (ii) because the two control groups (UUC and IUC) in AST-1 and AST-2 were hold in different rooms.

In summary, the anticoccidial efficacy of 60 mg narasin/kg feed is demonstrated in floor pen study 1 by the endpoints lesion scores and oocyst excretion, in floor pen study 2 by improved body weight as a follow up of reduced oocyst excretion, in floor pen study 3 by oocyst excretion; in AST-1 by lesion scores and in AST-3 by reduced mortality, lesion scores and oocyst excretion. AST-2 failed to demonstrate any significant improvement of the coccidiosis related endpoints.

Studies on the quality of the animal products where this is not the effect claimed

The applicant submitted two studies which were already assessed in the previous opinion on Monteban® (EFSA, 2004).[76] The Panel reassessed the studies and reiterates its previous conclusions that 'Monteban® G100 at recommended concentrations in feed does not influence the organoleptic and nutritional quality of chicken meat'.

Conclusions on efficacy for the target species

The efficacy of Monteban® G100 was demonstrated in three floor pen studies and in two ASTs, a third AST lacking to show anticoccidial efficacy. The FEEDAP Panel is therefore not in a position to conclude on the efficacy of Monteban® at the minimum applied dose of 60 mg narasin/kg complete feed for chickens for fattening.

Monteban® G100 at recommended concentrations in feed does not influence the organoleptic and nutritional quality of chicken meat.

3.4. Post-market monitoring

Field monitoring of *Eimeria* spp. resistance to narasin should be undertaken, preferably during the latter part of the period of authorisation.

4. Conclusions

Monteban® G100 contains the active substance narasin which is produced by fermentation. Limited data on the taxonomic identification of the production strain does not allow the proper identification of strain NRRL 8092 as *S. aureofaciens*. The FEEDAP Panel cannot conclude on the absence of genetic determinants for antimicrobial resistance in *Streptomyces* spp. under assessment.

Based on the available data set, the FEEDAP Panel cannot conclude on the safety of Monteban® G100 for chickens for fattening. Narasin is active against Gram-positive bacteria while Gram-negative bacteria are resistant. The use of narasin as a feed additive is unlikely to induce resistance or cross-resistance to antimicrobials used in human and animal therapy. Narasin may increase *Salmonella*-shedding, but there is no reason to believe that narasin is different from other polyether ionophores in this respect. The simultaneous use of Monteban® G100 and certain antibiotic drugs (e.g. tiamulin) is contraindicated.

Narasin is not genotoxic. No indication of carcinogenicity or developmental toxicity was found at the doses tested in the mouse, rat and rabbit. The lowest NOEL identified in the oral toxicity studies was 0.5 mg/kg bw per day for the neuropathy seen in a 1-year dog study. Since this dose is above the lowest NOAEL previously identified of 0.5 mg/kg bw per day, there is no reason to consider acute cardiovascular effects in the risk assessment. The NOAEL of 0.5 mg/kg bw per day is an appropriate base for confirming the ADI of 0.005 mg narasin/kg bw already established by the FEEDAP Panel in its former opinions applying a uncertainty factor of 100.

The use of Monteban® G100 in chickens for fattening at the maximum dose proposed, and without applying a withdrawal period, is safe for the consumer. MRL of 50 μg narasin/kg for all wet tissues ensure consumer safety.

Monteban® G100 is irritant to the eyes but not to the skin. It has the potential to induce skin sensitisation. The acute systemic toxicity following dermal application is low. Inhalation exposure would pose a risk to persons handling the additive.

Narasin, when used as a feed additive for chickens for fattening at 70 mg/kg feed, is not expected to pose a risk to the environment. The risk for sediment compartment cannot be assessed. Narasin is not considered to have a bioaccumulation potential.

The efficacy of Monteban® G100 was shown in three floor pen studies and only in two AST. The FEEDAP Panel is therefore not in a position to conclude on the efficacy of Monteban® at the minimum applied dose of 60 mg narasin/kg complete feed for chickens for fattening.

5. Recommendations

Narasin is toxic to horses, turkeys and rabbits at levels below those used in the prevention of coccidiosis in chickens.

[76] Technical dossier/Supplementary information June 2017/Annex 30 and 31.

Documentation provided to EFSA

1) Monteban® G100 for chickens for fattening. August 2013. Submitted by Eli Lilly and Company Ltd.
2) Monteban® G100 for chickens for fattening. Supplementary information. June 2014. Submitted by Eli Lilly and Company Ltd.
3) Monteban® G100 for chickens for fattening. Supplementary information. September 2014 (not complete). Submitted by Eli Lilly and Company Ltd.
4) Monteban® G100 for chickens for fattening. Supplementary information. April 2015 (not complete). Submitted by Eli Lilly and Company Ltd.
5) Monteban® G100 for chickens for fattening. Supplementary information. May 2015. Submitted by Eli Lilly and Company Ltd.
6) Monteban® G100 for chickens for fattening. Supplementary information. October 2015 (not complete). Submitted by Eli Lilly and Company Ltd.
7) Monteban® G100 for chickens for fattening. Supplementary information. June 2017. Submitted by Eli Lilly and Company Ltd.
8) Evaluation report of the European Union Reference Laboratory for Feed Additives on the methods of analysis for Monteban® G100.
9) Comments from Member States.

Chronology

Date	Event
22/8/2013	Dossier received by EFSA
16/9/2013	Reception mandate from the European Commission
17/2/2014	Application validated by EFSA – Start of the scientific assessment
16/4/2014	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. *Issues: Characterisation*
5/5/2014	Reception of the Evaluation report of the European Union Reference Laboratory for Feed Additives
17/5/2014	Comments received from Member States
12/6/2014	Reception of supplementary information from the applicant - Scientific assessment re-started
24/7/2014	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended *Issues: Safety for the target species, consumer and environment*
24/9/2014	Reception of supplementary information from the applicant - The information was considered not complete. Applicant was informed via email dated 14/10/2014. The scientific assessment remained suspended
23/10/2014	Request of additional supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003. *Issues: Safety for the environment*
24/04/2015	Reception of supplementary information from the applicant - The information was considered not complete. Applicant was informed via email dated 19/05/2015. The scientific assessment remained suspended
26/5/2015	Reception of supplementary information from the applicant - Scientific assessment re-started
25/6/2015	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended *Issues: Characterisation, Efficacy*
27/10/2015	Reception of supplementary information from the applicant - The information was considered not complete. Applicant was informed via email dated 30/11/2015. The scientific assessment remained suspended
17/5/2016	Request of additional supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003. *Issues: Safety for the environment*
28/6/2017	Reception of supplementary information from the applicant - Scientific assessment re-started
3/10/2018	Opinion adopted by the FEEDAP Panel. End of the Scientific assessment

References

EFSA (European Food Safety Authority), 2004. Opinion of the Scientific Panel on Additives and Products or Substances used in Animal Feed on the re-evaluation of efficacy and safety of the coccidiostat Monteban® G100 in accordance with article 9G of Council Directive 70/524/EEC. EFSA Journal 2004;90, 1–44. Available online: http://www.efsa.europa.eu/en/efsajournal/doc/90.pdf

EFSA (European Food Safety Authority), 2005. Opinion of the Scientific Panel on additives and products or substances used in animal feed (FEEDAP) on the evaluation of the coccidiostat COXIDIN® (Monensin Sodium). EFSA Journal 2005;3(11):283, 53 pp. https://doi.org/10.2903/j.efsa.2005.283

EFSA (European Food Safety Authority), 2007. Scientific opinion of the Panel on Plant Protection Products and their Residues on a request from EFSA related to the default Q10 value used to describe the temperature effect on transformation rates of pesticides in soil. EFSA Journal 2007;5(12):622, 32 pp. https://doi.org/10.2903/j.efsa.2007.622

EFSA (European Food Safety Authority), 2008a. Technical Guidance of the Scientific Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) for assessing the safety of feed additives for the environment. EFSA Journal 2008;6(10):842, 28 pp. https://doi.org/10.2903/j.efsa.2008.842

EFSA (European Food Safety Authority), 2008b. Technical Guidance: microbial studies. EFSA Journal 2008;6(10):836, 3 pp. https://doi.org/10.2903/j.efsa.2008.836

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2010a. EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP); Scientific Opinion on the modification of authorisation of the feed additive Monteban® G100 (narasin) for chickens for fattening on request from European Commission. EFSA Journal 2010;8(3):1549, 10 pp. https://doi.org/10.2903/j.efsa.2010.1549

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2010b. EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP); Scientific Opinion on the safety and efficacy of Maxiban® G160 (narasin and nicarbazin) for chickens for fattening. EFSA Journal 2010;8(4):1574, 45 pp. https://doi.org/10.2903/j.efsa.2010.1574

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2011a. Guidance for the preparation of dossiers for coccidiostats and histomonostats. EFSA Journal 2011;9(5):2174, 12 pp. https://doi.org/10.2903/j.efsa.2011.2174

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2011b. Technical guidance: tolerance and efficacy studies in target animals. EFSA Journal 2011;9(5):2175, 15 pp. https://doi.org/10.2903/j.efsa.2011.2175

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2012a. Guidance for establishing the safety of additives for the consumer. EFSA Journal 2012;10(1):2537, 12 pp. https://doi.org/10.2903/j.efsa.2012.2537

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. https://doi.org/10.2903/j.efsa.2012.2539

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2012c. Guidance on the assessment of bacterial susceptibility to antimicrobials of human and veterinary importance. EFSA Journal 2012;10(6):2740, 10 pp. https://doi.org/10.2903/j.efsa.2012.2740

EFSA FEEDAP Panel (EFSA Panel on Products or Substances used in Animal Feed), 2017. Guidance on the assessment of the safety of feed additives for the consumer. EFSA Journal 2017;15(10):5022, 17 pp. https://doi.org/10.2903/j.efsa.2017.5022

Hanrahan LA, Corrier DE and Naqi SA, 1981. Monensin toxicosis in broiler chickens. Veterinary Pathology, 18, 665–671.

Islam KM, Klein U and Burch DG, 2009. The activity and compatibility of the antibiotic tiamulin with other drugs in poultry medicine–A review. Poultry Science, 88, 2353–2359.

JEFCA (Joint FAO/WHO Expert Committee on Food Additives), 2012. Evaluation of certain veterinary drug residues in food: seventy-fifth report of the Joint FAO/WHO Expert Committee on Food Additives. (WHO technical report series; no. 969).

Johnson J and Reid WM, 1970. Anticoccidial drugs: lesion scoring techniques in battery and floor pen experiments with chickens. Experimental Parasitology, 28, 30–36.

Laczay P, Simon F, Mora Z and Lehel J, 1989. The compatibility of the new ionophore-coccidiostats with of chemotherapeutics in broilers. DTW. Deutsche Tierarztliche Wochenschrift, 96, 449–451.

Szucs G, Bajnogel J, Varga A, Mora Z and Laczay P, 2000a. Studies on the toxic interaction between monensin and tiamulin in rats: toxicity and pathology. Acta Veterinaria Hungarica, 48, 209–219.

Szucs G, Laczay P, Bajnogel J and Mora Z, 2000b. Studies on the toxic interaction between monensin and tiamulin in rats: effects on P450 activities. Acta Veterinaria Hungarica, 48, 361–368.

Umemura T, Kawaminami A, Goryo M and Itakura C, 1985. Enhanced myotoxicity and involvement of both type I and II fibers in monsensin-tiamulin toxicosis in pigs. Veterinary Pathology, 22, 409–414.

Van Vleet JF, Runnels LJ, Cook JR Jr and Scheidt AB, 1987. Monensin toxicosis in swine: potentiation by tiamulin administration and ameliorative effect of treatment with selenium and/or vitamin E. American Journal of Veterinary Research, 48, 1520–1524.

Abbreviations

ADI	acceptable daily intake
AF	assessment factor
AST	anticoccidial sensitivity tests
bw	body weight
CV	coefficient of variations
DT_{50}	Disappearance Time 50 (the time within which the concentration of the test substance is reduced by 50%)
DT_{90}	Disappearance Time 90 (the time within which the concentration of the test substance is reduced by 90%)
EC_{50}	median effective concentration
E_rC_{50}	median effective concentration which results in 50% reduction in growth rate
EURL	European Union Reference Laboratory
FEEDAP	EFSA Panel on Additives and Products or Substances used in Animal Feed
FOCUS	FOrum for Co-ordination of pesticide fate models and their USe
GLP	Good Laboratory Practice
K_d	soil adsorption coefficient
K_{oc}	adsorption or desorption coefficient corrected for soil organic carbon content
LC_{50}	median lethal concentration
LOD	limit of detection
log K_{ow}	octanol/water partition coefficient
LOQ	limit of quantification
MIC	minimum inhibitory concentrations
MRL	maximum residue limit
NOAEL	no observed adverse effect level
NOEC	no observed effect concentration
NOEL	no observed effect level
OC	organic carbon
OPG	oocysts per gram of excreta
PEC	predicted environmental concentration
pKa	dissociation constant
PNEC	predicted no effect concentration
RMTR	ratio marker to total residue
TRC	total residue concentration
VP	vapour pressure

Appendix A – List of references retrieved from the literature search provided by the applicant

Interaction with other drugs

Brennan J, Skinner J, Barnum DA and Wilson J, 2003. The efficacy of bacitracin methylene disalicylate when fed in combination with narasin in the management of necrotic enteritis in broiler chickens. Poultry Science, 82, 360–363.
EFSA, 2009. Safety and efficacy of Miya-Gold®S (*Clostridium butyricum*) as feed additive for chickens for fattening.
Islam KM, Klein U and Burch DG, 2009. The activity and compatibility of the antibiotic tiamulin with other drugs in poultry medicine–A review. Poultry Sciences, 88, 2353–2359.
Vissiennon T, Kröger H, Köhler T and Kliche R, 2000. Effect of avilamycin, tylosin and ionophore anticoccidials on *Clostridium perfringens* enterotoxaemia in chickens. Berl Munch Tierarztl Wochenschr, 113, 9–13.

Resistance and cross-resistance

Aarestrup FM, Bager F, Jensen NE, Madsen M, Meyling A and Wegener HC, 1998. Surveillance of antimicrobial resistance in bacteria isolated from food animals to antimicrobial growth promoters and related therapeutic agents in Denmark. APMIS, 106, 606–622.
Bowen RE, 1973. Studies designed to determine the effect of passing bacteria forty times in media containing monensin on their resistance to monensin and thirteen other antimicrobials. Elanco Internal Pooled Study Reports 460-G107-203.
Butaye P, Devriese LA and Haesebrouck F, 2001. Differences in antibiotic resistance patterns of *Enterococcus faecalis* and *Enterococcus faecium* strains isolated from farm and pet animals. Antimicrobial Agents and Chemotherapy, 45, 1374–1378.
Callaway TR and Russell JB, 1999. Selection of a highly monensin-resistant *Prevotella bryantii* subpopulation with altered outer membrane characteristics. Applied and Environmental Microbiology, 65, 4753–4759.
Callaway TR, Erdington TS, Rychlik JL, Genovese KJ, Poole TL, Yung YS, Blachoff KM, Anderson RC and Nisbet DJ, 2003. Ionophores: their use as ruminant growth promotants and impact on food safety. Current Issues in Intestinal Microbiology, 4, 43–51.
Chen M and Wolin MJ, 1979. Effect of monensin and lasalocid-sodium on the growth of methanogenic and rumen saccharolytic bacteria. Applied and Environmental Microbiology, 38, 72–77.
DANMAP, 1998. Consumption of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from food animals, food and humans in Denmark. Available online: http://www.dfvf.dk
Dawson KA and Boling JA, 1987. Effects of potassium ion concentrations on the antimicrobial activities of ionophores against ruminal anaerobes. Applied and Environmental Microbiology, 53, 2363–2367.
Devriese LA, Daube G, Hommez G and Haesebrouck F, 1993. *In vitro* susceptibility of *Clostridium perfringens* isolated from farm animals to growth-enhancing antibiotics. Journal of Applied Bacteriology, 75, 55–57.
Edrington TS, Callway TR, Varey PD, Jug YS, Bischoff KM, Elder RO, Anderson RC, Kutter E, Brabban AD and Nisbet NJ, 2003. Effects of the antibiotic ionophores monensin, lasalocid, laidlomycin propionate and bambermycin on *Salmonella* and *E. coli* O157:H7 *in vitro*. Journal of Applied Microbiology, 94, 207–213.
Houlihan AJ and Russell JB, 2003. The susceptibility of ionophore-resistant *Clostridium aminophilum* F to other antibiotics. Journal of Antimicrobial Chemotherapy, 52, 623–628.
Johansson A, Greko C, Engström BE and Karlsson M, 2004. Antimicrobial susceptibility of Swedish, Norwegian and Danish isolates of *Clostridium perfringens* from poultry, and distribution of tetracycline resistance genes. Veterinary Microbiology, 99, 251–257.
Martel A, Devriese LA, Cauwerts K, De Gussem K, Decostere A and Haesebrouck F, 2004. Susceptibility of *Clostridium perfringens* strains from broiler chickens to antibiotics and anticoccidials. Avian Pathology, 33, 3–7.
McConville M, Gate J and Shryock TR, 2003. Lack of selection of monensin-resistant bacteria. 13th European Congress of Clinical Microbiology and Infectious Disease.
Morehead MC and Dawson KA, 1992. Some growth and metabolic characteristics of monensin-sensitive and monensin-resistant strains of *Prevotella* (*Bacteroides*) *ruminicola*. Applied and Environmental Microbiology, 58, 1617–1623.
Newbold CJ, Wallace RJ and Watt ND, 1992. Properties of ionophore-*resistant Bacteriodes ruminicola* enriched by cultivation in the presence of tetronasin. Journal of Applied Bacteriology, 72, 65–70.
Nilsson O, Greko C, Bengtsson B and Englund S, 2012. Genetic diversity among VRE isolates from Swedish broilers with the coincidental finding of transferable decreased susceptibility to narasin. Journal of Applied Microbiology, 112, 716–722.
Russell JB and Houlihan JB, 2003. Ionophore resistance of ruminal bacteria and its potential impact on human health. FEMS Microbiology Reviews, 27, 65–74.
Russell JB and Strobel HJ, 1989. Effect of ionophores on ruminal fermentation. Applied and Environmental Microbiology, 55, 1–6.
Rychlik JL and Russell JB, 2002 The adaptation and resistance of Clostridium aminophilum F to the butyrivibriocin-like substance of *Butyrivibrio fibrisolvens* JL5 and monensin. FEMS Microbiology Letters, 209, 93–98.

Simjee S, Heffron A-L, Pridmore A and Shryock TR, 2012. Reversible monensin adaptation in *Enterococcus faecium*, *Enterococcus faecalis* and *Clostridium perfringens* of cattle origin: potential impact on human food safety. Journal of Antimicrobial Chemotherapy, 67, 2388–2395.

Smith II CK, Galloway RB and White SL, 1981. Effect of ionophores on survival, penetration and development of *Eimeria tenella* sporozoites *in vitro*. Journal of Parasitology, 67, 511–516.

Smith II CK and Galloway RB, 1983. Influence of monensin on cation influx and glycolysis of *Eimeria tenella* sporozoites *in vitro*. Journal of Parasitology, 69, 666–670.

Watkins KL, Shryock TR, Dearth RN and Saif YM, 1997. In-vitro antimicrobial susceptibility of *Clostridium perfringens* from commercial turkey and broiler chicken origin. Veterinary Microbiology, 54, 195–200.

Toxicology and environmental safety of narasin

Eggen T, Normann Asp T, Grave K and Hormazabal V, 2011. Uptake and translocation of metformin, ciprofloxacin and narasin in forage- and crop plants. Chemosphere, 85, 26–33.

Hansen M, Krogh KA, Böjrklund E, Halling-Sørensen B, Brandt A, 2009. Environmental risk assessment of ionophores. Trends in Analytical Chemistry, 28, 534–542.

Dorne JLCM, Fernández-Cruz, Bertelsen U, Renshaw DW, Peltonen A, Anadon A, Feil A, Sanders P, Wester P, Fink-Gremmels J, 2013. Risk Assessment of coccidostatics during cross-contamination: Animal and human health effects. Toxicology and Applied Pharmacology, 270, 196–208.

Sun P, Barmaz D, Cabrera M, Pavlostathis SG, Huang CH, 2013. Detection and quantification of ionophore antibiotics in run-off, soil and poultry litter. Journal of Chromatography A, 1312, 10–17.

Bohn P, Bak SA, Björklund E, Krough KA, Hansen, 2013. Abiotic degradation of antibiotic ionophores. Environmental Pollution, 177–183.

Appendix B – Estimation of user exposure to narasin from the additive Monteban® 100G, including consideration of using a filter mask FF P2 or FF P3 as a preventative measure

Calculation	Identifier	Description	Amount	Source
	a	Narasin in the dust (mg/g)	[redacted]	Technical dossier
	b	Dusting potential (g/m^3)	[redacted]	Technical dossier
a × *b*	*c*	Narasin in the air (mg/m^3)	50.4	
	d	No of premixture batches prepared/working day	10	EFSA Guidance on user safety (EFSA FEEDAP Panel, 2012b)
	e	Time of exposure per production of one batch (s)	20	EFSA Guidance on user safety (EFSA FEEDAP Panel, 2012b)
d × *e*	*f*	Total duration of daily exposure/worker (s)	200	
	g	Uncertainty factor	2	EFSA Guidance on user safety (EFSA FEEDAP Panel, 2012b)
f × *g*	*h*	Refined total duration of daily exposure/worker (s)	400	
h/3 600	*i*	Refined total duration of daily exposure (h)	0.11	
	j	Inhaled air per hour (m^3)	1.25	EFSA Guidance on user safety (EFSA FEEDAP Panel, 2012b)
j × *i*	*k*	Inhaled air during exposure (m^3)	0.14	
c × *k*	*l*	Narasin inhaled during exposure per eight-hour working day (mg)	7.0	
	m	Particles below 10 μm in the dust (%) generated during the Stauber–Heubach measurement	45	Technical dossier
l x *m*/100	*n*	Narasin inhaled per eight-hour working day (mg) reduced by respirable fraction	3.1	
n/10	*o*	Narasin inhaled per eight-hour working day (mg) reduced by filter mask FF P2 (reduction factor 10)	0.31	
n/20	*p*	Narasin inhaled per eight-hour working day (mg) reduced by filter mask FF P3 (reduction factor 20)	0.16	

Appendix C – Total number of *Eimeria* oocysts per gram of faeces (OPG) in floor pen trials with chickens for fattening(1)

	Day 23 Log_{10} OPG			
	E. acervulina	***E. maxima***	***E. tenella***	***Total***
Trial 1				
UUC	nd	nd	nd	nd
IUC	4.38	4.07	4.29	4.75
IT	4.02*	3.78*	3.92*	4.40*

	Geometric mean of faecal oocyst counts at different days						
	22	**24**	**27**	**29**	**31**	**36**	**42**
Trial 2							
UUC	0	0	1	0	0	2	12
IUC	16,682,947	12,338,450	3,377,339	740,500	100,091	10,987	39,841
IT	12,976,779	8,019,309	943,011	685,024	99,698	18,457	139*

	OPG x 10^3						
	***E. acer*(2)**	***E. ten***	***E. max***	***E. bru***	***E. nec/prae***	***E. mit***	**Total**
Trial 3 Inoculum A							
	Day 20						
UUC	302	8.1	0	0	0.3	3.2	313
IUC	420	12.0	51.6	0	6.9	27.1	517
IT	389	11.5	3.0*	0	11.12	17.9	432
	Day 22						
UUC	79	0	0.3	0	0	6.6	86
IUC	136	14.5	18.7	0	28.6	25.9	223
IT	155	30.4	1.7*	0	31.6	33.6	252
	Day 28						
UUC	111	7.9	0	0	23.8	22.1	165
IUC	26	2.67	1.47	0	5.2	5.8	41
IT	56.9	7.5	0.15*	0	21.4	6.9	93

	OPG x 10^3						
	E. acer	***E. ten***	***E. max***	***E. bru***	***E. nec/prae***	***E. mit***	**Total**
Trial 3 Inoculum B							
	Day 20						
UUC	302	8.1	0	0	0.3	3.2	313
IUC	677	8.2	0	0	6.0	19.5	710
IT	752	4.5	0	0	16.0*	33.8	806
	Day 22						
UUC	79	0	0.3	0	0	6.6	86
IUC	576	188	19.2	0	564	134	1,481
IT	396	55	16.5	0	194	59	721
	Day 28						
UUC	111	7.9	0	0	23.8	22.1	165
IUC	34	1.3	10.3	0	4.33	3.3	53
IT	77	3.4	6.5	0.1	4.68	6.3	98

UUC: uninfected untreated control; IUC: infected untreated control; IT: infected treated.
(1): IT with * is significantly different from control (IUC).
(2): *Eimeria* species are *acervulina* (*acer*), *tenella* (*ten*), *maxima* (*max*), *brunetti* (*bru*), *necatrix*/*praecox* (*nec*/*prae*), *mitis* (*mit*).

Annex A – Executive Summary of the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Methods of Analysis for Monteban® G100

Monteban® G100 is a feed additive currently authorized for *chickens for fattening* by Commission Regulation (EC) No 1464/2004 belonging to the group "Coccidiostats and other medicinal substances" listed in Chapter I of Annex B of Directive 70/524/EEC. In the current application an authorisation of an existing product under article 10 (2) of the Regulation (EC) No 1831/2003 is requested. *Monteban® G100* consists of 10% (w/w) of *narasin* (active substance), rice hulls as base material, mineral oil as antidusting oil and verxite as anti-caking agent. The Applicant suggested a concentration of narasin in *feedingstuffs* ranging from 60 to 70 mg/kg.

Furthermore the Applicant suggests maximum residue limits (MRLs) of 50 μg/kg for all wet tissues from *chicken for fattening* as already established by Commission Regulation (EC) No 545/2006.

For the quantification of *narasin* in the *feed additive* and *feedingstuffs*, the Applicant submitted single-laboratory validated methods based on the EN ISO 14183 using high-performance liquid chromatography with post-column derivatisation coupled to ultraviolet detection (HPLC-PCD-UV). Based on the provided performance characteristics, the EURL recommends for official control the HPLC-PCD-UV method for the quantification of *narasin* in the *feed additive*, and the EN ISO 14183 for the quantification of *narasin* in *premixtures* and *feedingstuffs*.

For the quantification of *narasin* in chicken *tissues* the Applicant submitted a single laboratory validated (in muscle, kidney, skin/fat and liver) and further verified (in muscle) method based RP-HPLC coupled to a triple quadrupole mass spectrometer (MS/MS) in electrospray ionisation (ESI) mode using matrix matched standards, similar to the one developed and validated by the European Union Reference Laboratory for Pharmacologically Active Substances (BVL). The satisfactory performance characteristics provided by the Applicant for the four tissues of concern demonstrate that (i) the method proposed by the Applicant is equivalent to the BVL method, and (ii) the Applicant method is also applicable to kidney and skin/fat tissues. Based on the performance characteristics presented, the EURL recommends for official control the single laboratory validated and further verified RP-HPLC-MS/MS method proposed by the Applicant to enforce the *narasin* MRLs in the relevant *tissues*.

Further testing or validation of the methods to be performed through the consortium of National Reference Laboratories as specified by Article 10 (Commission Regulation (EC) No 378/2005) is not considered necessary.