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Modification of the terms of authorisation regarding the maximum inclusion level of Maxiban® G160 (narasin and nicarbazin) for chickens for fattening

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Abstract

Following the 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 proposed modification of the terms of the authorisation regarding the maximum inclusion level of Maxiban® G160. The FEEDAP Panel cannot conclude on the safety of Maxiban® G160 at a dose level of 70 + 70 mg/kg feed for the target species. The use of Maxiban® G160 in diets for chickens for fattening at the maximum proposed dose complies with the maximum residue levels (MRLs) in force of narasin and 4,4′-dinitrocarbanilide (DNC) at 0-day withdrawal except for DNC in kidney which was slightly above the MRL. Compliance with DNC MRLs was seen in all tissues at 1-day withdrawal. Based on the available data, the FEEDAP Panel cannot conclude on the safety of Maxiban® G160 for the environment due to the risk identified for the terrestrial organisms due to DNC. Moreover, the high persistence and hydrophobicity of DNC indicate that there might be a risk for bioaccumulation but the risk for secondary poisoning was not identified. The potential of DNC to accumulate in soil over the years should be investigated by monitoring in a field study. The FEEDAP Panel would not be in the position to conclude on the efficacy of Maxiban® G160 in chickens for fattening based on the data provided for the dose of 40 + 40 mg narasin + nicarbazin/kg feed.

Keywords: Maxiban® G160, narasin, nicarbazin, chickens for fattening, maximum inclusion level

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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 13(3) of that Regulation lays down that if the holder of an authorisation proposes changing the terms of the authorisation by submitting an application to the Commission, accompanied by the relevant data supporting the request for the change, the Authority shall transmit its opinion on the proposal to the Commission and the Member States.

The European Commission received a request from Eli Lilly and Company Ltd[2] for a modification of the authorisation of the product Maxiban® G160 (narasin and nicarbazin), 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 13(3) (modification of the authorisation of a feed additive). The particulars and documents in support of the application were considered valid by EFSA as of 12 May 2015.

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 Maxiban® G160 (narasin and nicarbazin), when used under the proposed conditions of use (see Section 3.1.1).

1.2. Additional information

The coccidiostat Maxiban® G160 is composed of two active substances, narasin and nicarbazin, and is authorised for chickens for fattening until 28 October 2020 at a dose range of 40–50 mg narasin/kg plus 40–50 mg nicarbazin/kg complete feed.[3]

In 2010, EFSA issued an opinion on the safety and efficacy of Maxiban® G160 for chickens for fattening (EFSA FEEDAP Panel, 2010a). In 2016, the FEEDAP Panel adopted an opinion concerning the modification of the terms of the authorisation regarding the formulation of Maxiban® G160 (EFSA FEEDAP Panel, 2016).

The active substance narasin, as the only active substance of the product Monteban® G100, was assessed by the FEEDAP Panel in 2004 (EFSA, 2004a) and re-evaluated in 2018 (EFSA FEEDAP Panel, 2018a). Monteban® G100 is intended to be used for chickens for fattening at a dose range of 60–70 mg narasin/kg complete feed.[4] The holder of the Monteban® G100 and the Maxiban® G160 authorisations is the same company (Elanco GmbH).

The active substance nicarbazin is the active substance of the authorised coccidiostat Koffogran for chickens for fattening,[5] which was assessed by EFSA in 2004 and 2010 (EFSA, 2004b; EFSA FEEDAP Panel, 2010b). Nicarbazin has also been assessed by EFSA as one of the two active substances of Monimax® (monensin sodium and nicarbazin) for chickens for fattening and chickens reared for laying (EFSA FEEDAP Panel, 2018b) and turkeys (EFSA FEEDAP Panel, 2017).

[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 EFSA that the applicant changed from Eli Lilly and Company Ltd. to Elanco GmbH, Heinz-Lohmann-Str. 4. 27472 Cuxhaven, Germany.

[3] Commission Regulation (EU) 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. amended by Commission Implementing Regulation (EU) 2018/1957 of 11 December 2018. OJ L 315, 12.12.2018, p. 23.

[4] 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. amended by 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. And amended by Commission Implementing Regulation (EU) 2019/138 of 29 January 2019 as regards the name of the holder of the authorisation for feed additives. OJ L 26, 30.1.2019, p. 1.

[5] Commission Regulation (EU) No 875/2010 of 5 October 2010 concerning the authorisation for 10 years of a feed additive in feedingstuffs. OJ L 263, 6.10.2010, p. 4.

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[6] in support of the authorisation request for the use of Maxiban® G160 (narasin and nicarbazin) as a feed additive.

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 and peer-reviewed scientific papers to deliver the present output.

EFSA has verified the European Union Reference Laboratory (EURL) report as it relates to the methods used for the control of the active substances in animal feed and marker residues in tissues. The Executive Summary of the EURL report can be found in Annex A.[7]

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of Maxiban® G160 (narasin and nicarbazin) is in line with the principles laid down in Regulation (EC) No 429/2008[8] 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, 2008), Guidance for establishing the safety of additives for the consumer (EFSA FEEDAP Panel, 2012a) and Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012b).

3. Assessment

The additive Maxiban® G160, containing as active substances narasin and nicarbazin, is currently authorised in feed for chickens for fattening for the control of coccidiosis. The applicant has requested the modifications of the current authorisation to increase the maximum dose in complete feedingstuffs from the currently authorised 50 mg/kg to 70 mg/kg complete feed for both narasin and nicarbazin.

3.1. Characterisation

The applicant submitted the same data for the identity of the additive, characterisation of the active substance and the manufacturing process that had already been reviewed by the FEEDAP Panel (EFSA FEEDAP Panel, 2010a) when assessing safety and efficacy of Maxiban® G160 for use in chickens for fattening.

Maxiban® G160 contains two active ingredients, narasin (an ionophoric coccidiostat produced by fermentation of a *Streptomyces* spp.) and nicarbazin (a synthetic coccidiostat), at a level of 80 g/kg each. The authorised composition of Maxiban® G160 is summarised in Table 1.

Table 1: Composition of Maxiban® G160 according to Regulation EC (EU) No 885/2010 amended by Regulation (EU) No 2018/1957

Ingredients	g/kg Maxiban® G160
Active ingredients	
Narasin activity	80
Nicarbazin	80
Other ingredients	
Vegetal or mineral oil	10–30

[6] FEED dossier reference: FAD-2014-0045.

[7] The full report is available on the EURL website: https://ec.europa.eu/jrc/sites/jrcsh/files/finrep-fad-2014-0036_0045_maxiban160.pdf

[8] 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.

Ingredients	g/kg Maxiban® G160
Micro tracer red	4–11
Vermiculite	0–20
Corn cob grits or rice hulls	q.s. 1000

q.s.: *quantum satis.*

New data were submitted on the content of *p*-nitroaniline (PNA) from five recent batches of nicarbazin.[9] All five samples of nicarbazin had a PNA content of 0.1% which is in line with the provisions set in the current authorisation for Maxiban® G160, i.e. the level of PNA should be ≤ 0.1% as of 26 October 2013.[10]

In its opinion on the safety and efficacy of Monteban® G100 containing narasin produced by the same microorganism (EFSA FEEDAP Panel, 2018a), the FEEDAP Panel concluded that: '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'. The same limitations in the characterisation of the fermentation strain of narasin apply to the current assessment.

3.1.1. Conditions of use

Maxiban® G160 is intended to be used in the prevention of coccidiosis in chickens for fattening at a concentration of 40 + 40 mg to 70 + 70 mg narasin + nicarbazin/kg feed with a withdrawal time of zero day.

3.2. Safety

3.2.1. Safety for the target species

The applicant provided (i) two tolerance studies in chickens for fattening, (ii) a literature search on the tolerance of narasin, nicarbazin and Maxiban® G160 in chickens for fattening and (iii) a review of the pharmacovigilance data of the company on narasin and Maxiban.

3.2.1.1. Tolerance in chickens for fattening

Tolerance studies

The first tolerance study submitted[11] was reviewed and was considered not suitable for the safety assessment in target species for the following reasons: (i) the design of the study did not allow to derive a margin of safety for the proposed use level of the additive, (ii) the insufficient numbers of replicates per gender, (iii) the absence of analytically confirmed dietary concentrations of narasin and nicarbazin, (iv) the study failed to replicate histopathological changes seen previously (treatment-related increase in the incidence and severity of congestive heart failure, myocardial degeneration and skeletal muscle degeneration and regeneration) (EFSA FEEDAP Panel, 2010a) and (v) inaccurate reporting.

The applicant provided a new tolerance study in which Maxiban® G160 was administered to 1-day-old chickens at dietary levels of 1, 1.5, 2 and 2.5 times the proposed maximum use level for at least 35 consecutive days.[12] A total of 210 male and 210 female chickens (LABEL chicken, a slow growing breed) was allotted to five groups: 4 groups were fed diets supplemented with 70 mg narasin + 70 mg nicarbazin/kg (1×), 105 mg narasin + 105 mg nicarbazin/kg (1.5×), 140 mg narasin + 140 mg nicarbazin/kg (2×) and 175 mg narasin + 175 mg nicarbazin/kg (2.5×); the other group was fed the un-supplemented diet. The intended treatment levels were analytically confirmed. Group size was 6 replicates per sex per treatment. Animals were housed in pens of seven birds up to day 8, after which there were five birds per pen. Spare animals were initially considered in order to cover early stage mortality. Males and females were separated in different rooms. Feed was given as starter (crumbles) for the first 14 days and as grower (pelleted) from day 15 to the end of the study. The wheat-soybean-barley type complete feed was calculated to contain as a starter 12.4 MJ metabolisable energy (ME)/kg,

[9] Technical dossier/Supplementary information March 2018/Section II.
[10] OJ L 265, 8.10.2010, p. 4.
[11] Technical dossier/Section III/Annex III.6.
[12] Technical dossier/Supplementary information March 2018/Section III/Annex_III_25.

21.4% crude protein (CP) and 0.93% methionine + cysteine, and 12.8 MJ ME/kg, 19.5% CP and 0.81% methionine + cysteine as a grower. Feed and water were offered for *ad libitum* access.

General health and behaviour, appearance and signs of illness were monitored. Dead animals were necropsied. Detailed clinical signs were recorded once a week. Mean pen and group body weight were recorded at arrival and then together with feed consumption at weekly intervals until day 35. On days 35, 36, 37, 38 and 39, blood samples for haematology[13] and blood biochemistry[14] were collected daily from one animal per replicate (a total of 6 males and 6 females each treatment group) selected at random. On the same days, a total of four birds per replicate randomly chosen (24 males and 24 females per treatment) were killed and subject to gross pathology and necropsy. Control and high dose group slides were examined (heart, liver, to be completed); intermediate- and low-dose groups were not examined since no treatment-related microscopic abnormalities were found at the high dose.

All statistical analyses were carried out separately for males and females. The pen was considered as the experimental unit. The following parameters were analysed at each time point: body weight gain, feed consumption, haematology, blood chemistry, organ weights, final body weight and organ weights adjusted for body weight, and macroscopic findings (for the number of pens with and without each finding). Significant difference was established at $p < 0.05$ level.

A model was fitted with fixed effect of group and heterogeneous covariance terms for each group. The treated groups were compared against the control using Dunnett's test. When the model failed to converge, the treated groups were compared against the control using the Mann–Whitney *U* test. When only two groups were found different, the treated groups were compared against the control using Fisher's exact test. For organ weight data, analysis of covariance was performed using final body weight as covariate. All statistical tests were two-tailed except for the Fisher's exact tests applied to macroscopic findings which were one-tailed for an increase.

Final body weight and cumulative final performance data are reported in Table 2. In the groups supplemented with 1.5×, 2× and 2.5×, the maximum recommended dose of both sexes final body weight was significantly lower than in the control group. Female birds had significantly lower body weight also at the maximum recommended dose (1.0×).

Table 2: Effects of Maxiban® G160 on the performance of chickens for fattening

Sex	Narasin + nicarbazin (mg/kg feed)	Final body weight (g)	Feed intake(1) (g)	Feed to gain ratio(1)	Mortality(2) (n)
M	0	1,512	2,408	1.6	1
M	70 + 70	1,513	2,471	1.7	3
M	105 + 105	1,436*	2,359	1.7	2
M	140 + 140	1,387*	2,317	1.7	1
M	175 + 175	1,221*	2,058	1.7	0
F	0	1,335	2,233	1.7	0
F	70 + 70	1,269*	2,226	1.8	1
F	105 + 105	1,257*	2,121	1.7	2
F	140 + 140	1,184*	1,995	1.7	0
F	175 + 175	1,031*	1,841	1.9	2

Mean values with * are significantly different from the control group ($p < 0.05$).
(1): Statistical analysis was performed on experimental periods only but not for cumulative data.
(2): Dead and culled animals of a total of 30.

The haematological endpoints showing significant differences between the control group and some of the treated groups were: haemoglobin, lymphocytes and consequently white blood cell counts. The differences observed were not dose-related. Therefore, haematological parameters do not seem to be influenced by the administration of the test item Maxiban® G160.

[13] Haemoglobin (Hb), packed cell volume/haematocrit (MHCT), erythrocyte count (manual MRBC, absolute ARBC), thrombocyte count (manual ATRC, absolute TRCA), total leucocyte count (manual MWBC, absolute WBCA), differential leucocyte count (heterophils, lymphocytes, monocytes, eosinophils, basophils), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC).

[14] Glucose (Glu), uric acid (URIC/URAC), creatinine (Creat), bilirubin, total (Bili), cholesterol, total (Chol), triglycerides (Trig), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), creatine kinase (CK), bile acids (Bi Ac), lactic dehydrogenase (LDH), calcium (Ca), phosphorus inorganic (Phos), sodium (Na), potassium (K), chloride (Cl), magnesium (Mg), protein, total (Total prot), albumin (Alb), globulin (Glob), albumin/globulin ratio (A/G).

The blood biochemistry endpoints showing significant differences between the control group and treated groups are summarised in Table 3. A dose-related significant decrease in the ALP values was recorded in males (groups 1.5×, 2.0×, 2.5×) and females (groups 1.0×, 1.5×, 2.0×, 2.5×) and a treatment-related increase of albumin in males (groups 1.0×, 1.5×, 2.5×) and females (groups 2.0× and 2.5×) and of the A/G ratio in females (group 2.0×, 2.5×). The other statistical differences between the control animals and treated animals were not considered relevant because of the magnitude of the change or/and the absence of a clear dose-related response.

Table 3: Selected(1) blood biochemistry endpoints

Sex	Narasin + nicarbazin (mg/kg feed)	ALP (U/L)	Ca (mmol/L)	Creat (μmol/L)	Alb (g/L)	Glob (g/L)	A/G ratio	Triglyc (mmol/L)
M	0	2,809*	2.73	8	12*	18*	0.67*	1.18*
M	70 + 70	1,612*	2.77	10*	14*	20*	0.70*	1.07*
M	105 + 105	1,520*	2.79	11*	15*	18*	0.82*	1.12*
M	140 + 140	1,164*	2.87*	10*	14*	19*	0.71*	1.22*
M	175 + 175	1,324*	2.81	10*	14*	18*	0.78*	0.93*
F	0	2,021*	2.69*	8*	13*	19*	0.66*	1.44*
F	70 + 70	1,418*	2.83*	10*	13*	21*	0.64*	1.27*
F	105 + 105	1,266*	2.76*	10*	14*	19*	0.77*	1.03*
F	140 + 140	1,145*	2.78*	10*	15*	17*	0.84*	1.06*
F	175 + 175	1,074*	2.79*	13*	15*	16*	0.98*	0.96*

ALP: alkaline phosphatase; Ca: calcium; Creat: creatinine; Alb: albumin; Glob: globulin (Glob); A/G: albumin/globulin ratio; Triglyc: triglycerides.
Mean values with * are significantly different from the control group ($p \leq 0.05$).
(1): Only endpoints with significant differences to control group.

The results of the macroscopic examination of the organs were considered to be within the range of normal background lesions that may be seen in chickens of this strain and age and under the experimental conditions used in this study. No relevant differences in the absolute heart and liver weight were recorded between control animals and treated birds. However, treatment resulted in a significant increase of the adjusted liver weight in all male groups (34.7, 34.6, 35.6 and 35.6 g for the 1×, 1.5×, 2×, 2.5× groups vs 29.9 g) and in the 2.5× female group (28.8, 31.1, 30.9 and 31.3 g for the 1×, 1.5×, 2×, 2.5× groups vs 28.7 g). The microscopic examination (of 24 male and female birds of the control and the 2.5× groups, each) did not reveal any test-item-related findings.

The administration of Maxiban® G160 to chickens for fattening of a slow growing breed resulted in a dose related reduction of body weight gain and blood ALP, reaching significance already at use level in female birds. The adjusted liver weight increases at all Maxiban® G160 levels in males and at the 2.5-fold level in females; however, not dose related and without histopathological alterations.

The FEEDAP Panel also notes that the use of a slow-growing breed would not allow to extend any conclusions to rapidly growing, commonly used breeds.

Literature search

Narasin

The holder of the authorisation of Maxiban® G160 and Monteban® G100 is the same and narasin from Maxiban® G160 is applied at the same maximum concentration as narasin from Monteban® G100. The applicant submitted the same literature search[15] on the tolerance of narasin in poultry covering the period 2000–2014 using several databases that has been assessed by the FEEDAP Panel in 2018 in its opinion on the safety and efficacy of Monteban® G100 for chickens for fattening (EFSA FEEDAP Panel, 2018a).[16] The applicant claimed that no papers relevant to the safety of the target species were identified.[17]

[15] Technical dossier/Supplementary information March 2018/Annex III_1 and 2.
[16] Databases searched: ScienceDirect, PubMed: British National Library of Medicine, Google Scholar.
[17] 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'.

Nicarbazin

The applicant performed a literature search[18] on target animal tolerance (chickens and turkeys) of nicarbazin and drug interactions covering the period 2010–2015 using several databases.[19] The search included nicarbazin and its individual components 4,4′-dinitrocarbanilide (DNC) and 2-hydroxy-4,6-dimethyl-pyrimidine (HDP). Sixteen papers were identified (Appendix A) from a total of 93 references from the search strategy run. None of them identified concerns on nicarbazin safety for poultry for fattening. The FEEDAP Panel noted that nicarbazin when used alone at use level of 125 mg/kg feed is well known to aggravate symptoms of heat stress in chickens for fattening. The findings of Aslian et al. (2014) suggest that these adverse events may be a result of oxidative stress in birds.

Review of pharmacovigilance data

The applicant provided the same review of pharmacovigilance case data on narasin assessed by the FEEDAP Panel in 2018 (EFSA FEEDAP Panel, 2018a)[20]: 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.

For Maxiban® G160, the applicant submitted two recent periodic safety update reports (PSUR) generated for reporting in Canada.[21] These summarise the adverse reports made to the company following the use of Maxiban globally in the period February 2015–January 2016. These reports suggest that there are no safety concerns, associated with the current use of Maxiban® G160.

Overall, the literature search indicated no evidence of reported adverse effects of narasin and nicarbazin for the target species in the databases searched; the company's pharmacovigilance report and safety update reports did not reveal any adverse event related to the use of Maxiban® G160.

3.2.1.2. Interactions

Data on the interactions of narasin with feed materials, other approved additives or medicinal products have been assessed by the FEEDAP Panel in the past (EFSA, 2004a; EFSA FEEDAP Panel, 2010b). In its recent opinion on the re-evaluation of Monteban® G100 (containing narasin) for chickens for fattening (EFSA FEEDAP Panel, 2018a) the FEEDAP Panel concluded that 'the simultaneous use of Monteban® G100 and certain antibiotic drugs (e.g. tiamulin) is contra-indicated'. Since the data assessed in the opinion in 2018 are also available for the current assessment, the FEEDAP Panel applies the same conclusions to the narasin contained in Maxiban® G160.

The applicant performed a literature search[18] on target animal tolerance (chickens and turkeys) of nicarbazin and drug interactions covering the period 2010–2015 using several databases.[19] The search included nicarbazin and its individual components (DNC and HDP). Sixteen papers were identified (Appendix A) from a total of 93 references from the search strategy run. None of them contained relevant data on nicarbazin interactions.

The FEEDAP Panel concludes that the contra-indications identified for narasin would apply to Maxiban® G160, particularly since the highest narasin administration from Monteban® G100 (60–70 mg/kg complete feed) and Maxiban® G160 (40–70 mg/kg complete feed) do not considerably differ.

3.2.1.3. Microbial studies

In 2010, the FEEDAP Panel (EFSA FEEDAP Panel, 2010a) concluded on the microbiological safety of Maxiban® G160 as follows: 'Nicarbazin does not display any antimicrobial properties and consequently no microbiological safety concern is associated with this compound. However, narasin has an antimicrobial activity against several Gram-positive intestinal bacterial species (0.25–4.0 mg/L)'.

The applicant submitted data from studies performed in 1981 and 2001[22] and a published paper (Lanckriet et al., 2010) indicating that nicarbazin does not show antibacterial activity when tested against a

[18] Technical dossier/Supplementary information March 2018/Annex III_6.
[19] Agricola, BIOSIS Previews, CAB Abstracts, Chemical Abstracts Plus, Elsevier Biobase, Embase, Food Science and Technology Abstracts, IPA, Medline, ProQuest Science & Technology, Registry, SciSearch and Toxcenter.
[20] Technical dossier/Supplementary information March 2018/Annex III_23 and 24.
[21] Technical dossier/Supplementary information March 2018/Annex III_4.
[22] Technical dossier/Supplementary information March 2018/Annex III_16 and 22.

pool of Gram-positive and Gram-negative bacteria.[23] Consequently, the FEEDAP Panel concludes, in agreement with its previous conclusions, that the use of nicarbazin as a feed additive is unlikely to induce resistance or cross-resistance to antimicrobials used in human and animal therapy.

The microbial safety of narasin has been assessed by the FEEDAP Panel in the past (EFSA, 2004a; EFSA FEEDAP Panel, 2010a). In its recent opinion on the re-evaluation of Monteban® G100 (containing narasin) for chickens for fattening (EFSA FEEDAP Panel, 2018a), the FEEDAP Panel concluded 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'. Since the data assessed in the opinion in 2018 have been made available for the current assessment, the FEEDAP Panel applies the same conclusions to the narasin contained in Maxiban® G160.

For the current assessment, the applicant submitted a study demonstrating that the presence of nicarbazin did not significantly increase the antimicrobial activity of narasin against some dominant and some indicator (*Escherichia coli*, *Staphylococcus aureus*) bacterial isolates from faeces.[24]

3.2.1.4. Conclusions on the safety for the target species

The FEEDAP Panel cannot conclude on the safety of Maxiban® G160 at a dose level of 70 + 70 for the target species due to effects observed in a tolerance study at the use level. The Panel also notes that the use of a slow-growing breed would not allow to extend any conclusions to rapidly growing, commonly used breeds.

The simultaneous use of Maxiban® G160 and certain antibiotic drugs (e.g. tiamulin) is contraindicated.

Nicarbazin has no antimicrobial activity. Narasin is active against Gram-positive bacteria, while Gram-negative bacteria are resistant. The use of Maxiban® G160 as a feed additive is unlikely to induce resistance or cross-resistance to antimicrobials used in human and animal therapy.

3.2.2. Safety for the consumer

Commission Regulation (EU) No 885/2010[25] sets maximum residue levels (MRLs) for narasin (0.05 mg/kg for all wet tissues) and nicarbazin expressed as DNC (15 mg/kg of fresh liver; 6 mg/kg of fresh kidney; 4 mg/kg for fresh muscle and fresh skin + fat) in chickens for fattening. These MRLs were set as a follow-up of several FEEDAP Panel opinions assessing the safety and efficacy of narasin and nicarbazin (DNC and HDP) for chickens for fattening (EFSA, 2004a; EFSA FEEDAP Panel, 2010a,b).

In a residue depletion study,[26] narasin and nicarbazin residues were measured in tissues of chickens (1-day-old, three of each sex per group) administered a feed supplemented with targeted levels of 70 mg narasin + 70 mg nicarbazin from Maxiban® G160/kg (analysed: 70.5 (± 9.0) + 61.9 (± 18.2) mg/kg, respectively) for 42 days. Groups of animals were slaughtered after 0 (3 h), 1, 2 and 5 days withdrawal and tissues (liver, kidney, muscle and skin/fat) sampled. Narasin residues were measured using a liquid chromatography with tandem mass spectrometry (LC–MS/MS) analytical method with a limit of quantification (LOQ) of 1.2 μg/kg liver and muscle and 1.5 μg/kg kidney and skin/fat. DNC residues were measured using the same analytical method with a LOQ of 20 μg/kg. Residue concentrations of both active substances are reported in Table 4.

Table 4: Narasin and DNC (nicarbazin) residues (mg/kg wet tissue)[(1)] in tissues of chickens administered 70 mg narasin and 70 mg nicarbazin from Maxiban® G160/kg feed for 42 days, without applying a withdrawal period (3 h) (narasin and DNC) and at 1-day withdrawal (DNC)

	Liver	Kidney	Muscle	Skin/fat
Narasin (3 h)	0.003 ± 0.003 (0.009)	0.003 ± 0.003 (0.009)	0.001 ± 0 (0.001)	0.026 ± 0.012 (0.050)

[23] *Bacteroides, Campylobacter, Clostridium, Clostridium perfringens, Enterococcus, Escherichia coli, Klebsiella pneumoniae, Lactobacillus, Proteus mirabilis, Salmonella, Salmonella* Typhimurium*, Staphylococcus aureus*.

[24] Technical dossier/Supplementary information March 2018/Annex III_9.

[25] Commission Regulation (EU) 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. 1.

[26] Technical dossier/Section III/Annex_III_7.

	Liver	Kidney	Muscle	Skin/fat
DNC (3 h)	8.988 ± 1.965 (12.918)	3.515 ± 1.485 (6.485)	1.813 ± 0.430 (2.673)	2.018 ± 0.660 (3.338)
DNC (24 h)	5.377 ± 0.963 (7.303)	1.811 ± 1.140 (4.091)	1.279 ± 0.518 (2.315)	1.611 ± 0.372 (2.355)

(1): Average ± SD (average + 2SD).

After a withdrawal time of 3 h, all narasin residue concentrations were below or at the level of the respective MRLs in liver, kidney, muscle and skin + fat. The withdrawal time applied is considered equivalent to zero day under practical conditions. In its recent opinion on the re-evaluation of Monteban® G100, containing narasin at a maximum level of 70 mg/kg feed, the FEEDAP Panel confirmed that the above-mentioned MRLs ensure consumer safety without applying a withdrawal period (EFSA FEEDAP Panel, 2018a). Results from the residue study performed for the current assessment confirmed the above conclusions.

Residue data obtained for DNC after the use of the highest proposed level of Maxiban® G160 in feed (70 + 70 mg narasin + nicarbazin/kg) for chickens for fattening showed that after a withdrawal time of 3 h, DNC concentrations (average + 2SD) comply with the MRLs in liver, muscle and skin/fat, and were slightly above the MRL of 6 mg/kg for kidney. Compliance with the DNC MRLs was seen in all tissues at 1-day withdrawal.

On the basis of the above results, the FEEDAP Panel concludes that the use of Maxiban® G160 in diets for chickens for fattening at the maximum proposed dose complies with the MRLs in force of narasin and DNC at 0-day withdrawal except for DNC in kidney which was slightly above the MRL of 6 mg/kg. Compliance with the DNC MRLs was seen in all tissues at 1-day withdrawal.

3.2.2.1. Conclusions on safety for the consumer

The use of Maxiban® G160 in diets for chickens for fattening at the maximum proposed dose complies with the MRLs in force of narasin and DNC at 0-day withdrawal except for DNC in kidney which was slightly above the MRL of 6 mg/kg. Compliance with the DNC MRLs was seen in all tissues at 1-day withdrawal.

3.2.3. Safety for the user

The present request concerns the increase of the maximum inclusion level to 70 + 70 mg narasin + nicarbazin/kg feed, the formulation of the additive is not affected. Therefore, the conclusions reached for the user safety in previous opinions (EFSA FEEDAP Panel, 2010a, 2018a) still apply and can be summarised as follows: Maxiban® G160 is a slight skin irritant and should be considered as an eye irritant and a skin sensitiser. The inhalation exposure would pose a risk to persons handling the additive due to its narasin content.

3.2.4. Safety for the environment

The applicant performed an updated environmental risk assessment including studies already assessed in a former EFSA FEEDAP opinion (EFSA FEEDAP Panel, 2010a) with the addition of new studies. The previous studies, the new studies and the outcome of a literature search[27] performed by the applicant covering the period 2004–2015, using various databases[28] were assessed by the FEEDAP Panel following Regulation (EC) No 429/2008[29] and the FEEDAP technical guidance for assessing the safety of feed additives for the environment (EFSA, 2008).

The active substance is not a physiological/natural substance of established safety for the environment. Consequently, according to Regulation (EC) No 429/2008[29] the Phase I assessment has to be continued to determine the predicted environmental concentration (PEC).

In Phase I and Phase II, initially a total residues approach will be taken, meaning that the PECs will be calculated, based on the assumption that the additive is excreted 100% as parent compound.

[27] Technical dossier/Supplementary information March 2018/Annex III_5.
[28] Agricola, BIOSIS Previews, CAB Abstracts, Chemical Abstracts Plus, Elsevier Biobase, Embase, Food Science and Technology Abstracts, IPA, Medline, ProQuest Science & Technology, Registry, RTECS, SciSearch and Toxcenter.
[29] OJ L 133, 22.5.2008, p. 1.

NARASIN

In its opinion on the use of Monteban® G100 (narasin) for chickens for fattening (EFSA FEEDAP Panel, 2018a), the FEEDAP Panel evaluated the safety of narasin for the environment when used as a feed additive in chickens for fattening at a concentration of 70 mg/kg complete feed. In that opinion, it is concluded that: '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'.

For the current assessment, no studies were submitted on sediment dwelling organisms with narasin, but the predicted no effect concentration in sediment ($PNEC_{sed}$) was calculated based on equilibrium partitioning to be 99.6 μg/kg. The calculation was performed with a organic carbon–water partitioning coefficient (K_{oc}) of 873 L/kg and a $PNEC_{surfacewater}$ of 2.2 μg/L (EFSA FEEDAP Panel, 2018a). Considering the predicted environmental concentration in sediment (PEC_{sed}) value of 32 μg/kg dry weight (EFSA FEEDAP Panel, 2018a), the PEC/PNEC ratio is < 1; therefore no risk is expected for sediment.

On the basis of these calculations, the former conclusion on the safety of narasin can be updated as follow: 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. Narasin is not considered to have a bioaccumulation potential.

NICARBAZIN (DNC and HDP)

3.2.4.1. Phase I

Physico-chemical properties

The physico-chemical properties of DNC and HDP are summarised in Tables 5 and 6.

Table 5: Physico-chemical properties of DNC

Property	Value	Unit
Octanol/water partition coefficient (log K_{ow})(1)	3.62 (pH 5) 3.61 (pH 7) 3.56 (pH 9)	–
Water solubility (20°C)(2)	< 0.2 (water, buffer pH 4, pH 7, pH 9)	mg/L
Dissociation constant (pKa)	Not given	–
Vapour pressure(3)	3.1 × 10E-10	Pa

(1): Technical dossier/Supplementary information March 2018/Annex_III_3.4_ERA_8.
(2): Technical dossier/Supplementary information March 2018/Annex_III_3.4_ERA_5.
(3): EPI Suite (2015).

Table 6: Physical–chemical properties of HDP

Property	Value	Unit
Octanol/water partition coefficient (log K_{ow})(1)	−0.95 (pH 5) −0.91 (pH 7) −0.94 (pH 9)	–
Water solubility (20°C)(2)	69,230 (water) 70,720 (pH 4) 66,320 (pH 7) 71,450 (pH 9)	mg/L
Dissociation constant (pKa)	Not given	–
Vapour pressure(3)	9.084 × 10E-6 (20°C) 1.834 × 10E-5 (25°C)	Pa

(1): Technical dossier/Supplementary information March 2018/Annex_III_3.4_ERA_8.
(2): Technical dossier/Supplementary information March 2018/Annex_III_3.4_ERA_5.
(3): EPI Suite (2015).

Fate and behaviour

Fate in soil

Adsorption/desorption in soil

DNC

The study submitted was already assessed in 2010 by the FEEDAP Panel (EFSA FEEDAP Panel, 2010a). The adsorption/desorption behaviour of [^{14}C]-DNC was investigated in three soil types: sandy loam, clay loam and silty clay loam.[30] The FEEDAP Panel reassessed the same study for the current application and agreed with its previous assessment (Table 7). Since just three soils were tested, the lowest K_{oc} value of 16,137 dm^3/kg C is used for the risk assessment.

Table 7: Adsorption of 0.02 µg/L DNC in different soils

Soil identification	%OC	pH ($CaCl_2$)	K_d dm^3/kg	K_{oc} dm^3/kg
Sandy loam	1.3	5.8	286	21,962
Clay loam	3.3	5.3	533	16,137
Silty clay loam	2.5	4.7	423	16,900

K_d: sorption/desorption coefficient; Koc: organic carbon–water partitioning coefficient.

HDP

The study submitted was already assessed in 2010 by the FEEDAP Panel (EFSA FEEDAP Panel, 2010a). The adsorption/desorption behaviour of [^{14}C]-HDP was investigated in three soil types: sandy loam, clay loam and silty clay loam.[31] The FEEDAP Panel reassessed the same study for the current application and agreed with its previous assessment (Table 8). Since just three soils were tested, the lowest K_{oc} value of 33 dm^3/kg C is used for the risk assessment.

Table 8: Adsorption of 5 mg/L HDP in different soils

Soil identification	%OC	pH ($CaCl_2$)	K_d dm^3/kg	K_{oc} dm^3/kg
Sandy loam	1.3	7.5	1.6	119
Clay loam	3.3	7.3	1.1	33
Silty clay loam	2.5	6.1	2.9	114

Kd: sorption/desorption coefficient; Koc: organic carbon–water partitioning coefficient.

Degradation in soil

DNC

The study submitted was already assessed in 2010 by the EFSA FEEDAP Panel (2010a).[32] The aerobic degradation of DNC in accordance with OECD 307 was evaluated in sandy loam, sandy clay loam and silt loam soils using [^{14}C]-DNC and included the estimation of the fate and behaviour in soil. Evolved [^{14}C]-O_2 was low throughout the study period, for all soils, accounting for 1–2% of applied radioactivity after 120 days. Chromatographic analyses indicated that DNC was the only significant component present in all soil types. At 64 and 120 days, up to four minor components (< 3% each) were detected but not identified. The dissipation was mainly attributed to the formation of bound residues accounting for 27% in the sandy loam. In the other soil types, the non-extractable residues were not determined. The mass balance is above 95% just for the sandy loam soil, while for the other two, it is in the range 64–97% (sandy clay loam soil) and 67–95% (silt loam soil), quite below the standard acceptable criteria. The DT_{50}, recalculated according FOrum for Co-ordination of pesticide fate models and their Use (FOCUS) single first-order kinetics (SFO),[33] was 233 days, 187 days and

[30] Technical dossier/Supplementary information March 2018/Annex_III_3.4_ERA_2.
[31] Technical dossier/Supplementary information March 2018/Annex_III_3.4_ERA_3.
[32] Technical dossier/Supplementary information March 2018/Annex_III_3.4_ERA_10.
[33] FOCUS (2006) 'Guidance Document on Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration' Report of the FOCUS Work Group on Degradation Kinetics, EC Document Reference Sanco/10058/2005 version 2.0.

240 days in sandy loam, sandy clay loam and silt loam soil, respectively. The degradation is quite slow, especially in the second phase. Evaluating DT_{50} with biphasic kinetics, as suggested by FOCUS guidance, the values obtained are also higher, indicating a high persistence in soil for DNC. Taking into account the low recovery, the high persistence in soil and the need to recalculate DT_{50} to 12°C, a standard DT_{50} of 1,000 days is proposed to calculate soil accumulation.

The degradation of uniformly ^{14}C ring-labelled DNC present in nicarbazin was studied in a greenhouse study[34] and a field plot[35] with a silt loam soil at Greenfield, Indiana, USA. The radioactivity concentration in the test plot remained constant over the period of 1 year in which the experiment was conducted indicating that DNC does not leach below the 15-cm sampling depth and does not degrade. In the greenhouse study, the degradation of DNC proceeded slowly for the first 18 weeks and then stopped for the remaining year. Up to 20% of DNC was still extractable after 1 year. These older studies confirm that the degradation of DNC is very low and proceeds by irreversible binding.

HDP

The study submitted was already assessed in 2010 by the EFSA FEEDAP Panel (2010a).[36] The aerobic degradation of $[^{14}C]$-HDP was studied as described above for DNC, using the same soil types. The formation of $[^{14}C]$-O_2 was relatively high throughout the study, accounting for 22–31% of applied radioactivity after 120 days. HDP was the only significant component present in all samples from all soil types; no metabolites representing more than 10% of the total radioactivity applied were found. The mass balance is above 95% just for the sandy loam soil, while for the other two, it is in the range 27–99% (sandy clay loam soil) and 25–90% (silt loam soil), too below the standard acceptable criteria. Dissipation was strongly attributed to the fast formation of non-extractable residues as demonstrated in sandy loam soil (not determined in the other soil types). The degradation behaviour of HDP is biphasic and the DT_{50} for HDP was calculated according FOCUS guidance using the Double First-Order in Parallel (DFOP) kinetics, the best fit for calculating persistence. DT_{50} was calculated as 12 days for sandy loam soil, 10 days for silt loam soil and 14 days for sandy clay loam soil. Since just three soils were tested and considering the low recovery, the highest DT_{50} of 14 days was selected for further calculations. The corresponding highest value considering the SFO kinetics is 4.3 days.

For the simple calculation of soil accumulation, according to the Technical Guidance for assessing the safety of feed additives for the environment (EFSA, 2008), the DT_{50} of 14 days can be recalculated to a DT_{50} at 12°C considering a factor of 2.12. The recalculated DT_{50} at 12°C is about 30 days. This indicates that HDP is not persistent in soils.

Fate in water

Neither DNC nor HDP are susceptible to hydrolysis.[37] Definitive data on the susceptibility of DNC and HDP to photolysis are not available.

Conclusion on fate and behaviour

For DNC, a K_{oc} of 16,137 dm^3/kg and a DT_{50} of 1,000 days will be used for the assessment; for HDP a K_{oc} of 33 dm^3/kg and a DT_{50} of 30 days will be used for the assessment.

Predicted environmental concentrations

The calculated PEC initial values for both DNC and HDP are given in Table 9.

Table 9: Predicted environmental concentrations (PECs) of DNC and HDP, in soil, groundwater, surface water and sediment

Input	Value	
	DNC	HDP
Dose (mg/kg feed)	49.62	20.38
Molecular weight	302.24	124.14
Vapour pressure (Pa) (at 25°C)	10^{-10}	10^{-6}
Solubility (mg/L)	0.02	65,400

[34] Technical dossier/Supplementary information March 2018/Annex_III_3.4_ERA_12.
[35] Technical dossier/Supplementary information March 2018/Annex_III_3.4_ERA_13.
[36] Technical dossier/Supplementary information March 2018/Annex_III_3.4_ERA_11.
[37] Technical dossier/Supplementary information March 2018/Annex_III_3.4_ERA_19.

Input	Value	
	DNC	HDP
K_{oc} (L/kg)	16,137	33
DT_{50} in soil at 12°C (days)	1,000	30
Output		
PEC_{soil} (μg/kg)	258	106
$PEC_{groundwater}$ (μg/L)	0.8	133
$PEC_{surfacewater}$ (μg/L)	0.3	44
$PEC_{sediment}$ (μg/kg dry weight)	244	164

The Phase I PEC trigger values are exceeded; therefore, a Phase II assessment is considered necessary.

3.2.4.2. Phase II

Exposure assessment

PECs calculation refined in Phase II

DNC – refinement of PEC_{soil} for persistent compounds

The DT_{90} for DNC was determined to be greater than 1 year; therefore, the PECs refined at steady state was calculated (Table 11) according to the FEEDAP technical guidance for assessing the safety of feed additives for the environment (EFSA, 2008) (Table 10).

Table 10: Plateau predicted environmental concentration (PECs) of DNC in soil (μg/kg), groundwater (μg/L), surface water (μg/L) and sediment (μg/kg)

Compartment	$PEC_{plateau}$ (DNC)
Soil	1,150
Ground water	4.0
Surface water	1.3
Sediment	1,090

DNC and HDP – $PEC_{groundwater}$ refined

The relationship $K_{OM} > -5.9 + 3.8\ DT_{50}$ can be used to ensure that the leaching concentrations is below the trigger value of 0.1 μg/L. For both DNC and HDP, taking into account the application rate, the highest DT_{50} calculated according SFO at 20°C, the lowest K_{oc}, the inequality is respected. Therefore, no risk of leaching into groundwater is expected either for DNC or for HDP.

Conclusions on PEC used for calculation

The following values are used for the assessment: for DNC, a PEC_{soil} of 1,150 μg/kg, a $PEC_{surface\ water}$ of 1.3 μg/L and a $PEC_{sediment}$ of 1,090 μg/kg; for HDP, a PEC_{soil} of 106 μg/kg, $PEC_{surface\ water}$ of 44 μg/L and $PEC_{sediment}$ of 164 μg/kg.

Ecotoxicity studies

Ecotoxicity studies performed with DNC and HDP are assessed below. The applicant submitted several additional ecotoxicity studies evaluating the effects of nicarbazin and the combination of nicarbazin and narasin on terrestrial and aquatic compartment. These additional studies are reported in Appendix B.

Toxicity of DNC and HDP to soil organisms

Effects on plants – DNC

A phytotoxicity study,[38] already assessed by the FEEDAP Panel in 2010 (EFSA FEEDAP Panel, 2010a), was conducted on the toxicity of DNC to ryegrass, oats, mung bean, lettuce, radish and turnip. Plants were exposed to DNC incorporated in a loamy sand soil. The OECD guideline was not completely followed; effects were measured at concentrations of 800, 4,000 and 8,000 μg/kg (1, 5 and 10 times the

[38] Technical dossier/Supplementary information March 2018/Annex_III_3.4_ERA_37.

estimated PEC of 800 μg/kg). Endpoints were number of seedlings that emerged and the fresh and dry weight of seedlings (shoot only). DNC did not have any effect on the emergence and growth of ryegrass, oats, mung bean, lettuce, radish and turnip. All the no observed effect concentration (NOEC) values for growth are ≥ 8,000 μg/kg. This conclusion is in compliance with the previous Panel opinion (EFSA FEEDAP Panel, 2010a).

The applicant submitted a new phytotoxicity study[39] in accordance with the OECD guideline 208. Study was conducted on the toxicity of DNC to ryegrass, wheat and corn to evaluate whether the effects in the ryegrass were reproducible. In this study, plants were exposed to DNC in an artificial soil at concentrations of 2,900, 4,300, 6,500, 9,700, 14,600 and 21,900 μg/kg. There were no significant phytotoxic effects observed other than normal variation in seedling emergence, survival, dry shoot weight or height for any treatment level in any species. In the study, also visible phytotoxic effects on seedlings were observed, particularly in maize, the incidence of chlorosis, necrosis and leaf curl was observed. Visible phytotoxic effects were not described in a previous study, but according to the OECD guideline they are relevant for the validity of the study. A NOEC of 2.9 mg/kg can be accepted for further assessment of DNC based on the incidence of these effects.

Effects on plants – HDP

A phytotoxicity study,[38] already assessed by the FEEDAP Panel in 2010 (EFSA FEEDAP Panel, 2010a), was conducted on the toxicity of HDP to ryegrass, oats, mung bean, lettuce, radish and turnip. Plants were exposed to DNC incorporated into loamy sand soils. The OECD guideline 208 was not completely followed, effects were measured at 350, 1,750 and 3,500 μg/kg HDP did not have any effect on the emergence and growth of lettuce, oats, ryegrass and turnip seedlings. HDP did have a phytotoxic effect on the emergence of both radish and mung bean, resulting in LC_{50} values of 2,780 and 2,890 μg/kg, respectively. The mean radish shoot fresh weight was higher in the ×10 rate group compared with the controls although this was not confirmed with the dry weight analysis. For mung bean, the total dry weight at 3.5 mg/kg (×10) was significantly lower than the controls. However, fewer plants emerged in the 3.5 mg/kg group than in the controls. The NOEC for both plants was 1.75 mg/kg.

The applicant submitted a new phytotoxicity study[40] in accordance with the OECD guideline 208. Study was conducted to further investigate the effect of HDP on radish and mung beans and two other dicots (soybean and peas) from the mung bean family. In this study, plants were exposed to HDP in soil at concentrations ranging from 1,100 to 5,900 μg/kg. HDP did not have any effects on the emergence, survival, dry shoot weight or height of radish, soybeans and peas HDP did not have any effect in emergence, survival, or dry shoot weight of mung bean. However, HDP did reduce the mung bean height at the two highest concentrations, 3,900 and 5,900 μg/kg. The NOEC for mung bean of 2,630 μg/kg can be accepted in further assessment of HDP. The overall median effective concentration (EC_{50}) for plants is > 3,500 μg/kg based on the highest concentration tested in monocots. The NOEC for mug bean is 2.63 mg/kg.

Effect on earthworms – DNC

The acute effects of DNC on earthworms have been evaluated.[41] Study following the OECD guideline 207 was performed with earthworms (*Eisenia fetida*) that were placed in artificial soil at DNC concentrations ranging from 95 to 1,000 mg/kg of DNC for 14 days. The endpoints were survival and body weight. There were no mortalities in the test and there were no significant effects on body weight compared to the controls. The LC_{50} value for the study was > 1,000 mg/kg of DNC. For the further assessment of DNC, a LC_{50} of 1,000 mg/kg for the earthworms is used.

Effect on earthworms – HDP

The acute effects of HDP on earthworms have been evaluated.[42] Study following the OECD guideline 207 was performed with earthworms (*E. fetida*) that were placed in artificial soil at HDP concentrations ranging from 95 to 1,000 mg/kg for 14 days. The endpoints were survival and body weight. There were no mortalities and no significant effects on body weight observed when compared to the control. The LC_{50} value for the study was > 1000 mg/kg. For the further assessment of HDP, a LC_{50} of 1,000 mg/kg for the earthworms is used.

[39] Technical dossier/Supplementary information March 2018/Annex_III_3.4_ERA_38.
[40] Technical dossier/Supplementary information March 2018/Annex_III_3.4_ERA_42.
[41] Technical dossier/Supplementary information March 2018/Annex_III_3.4_ERA_39.
[42] Technical dossier/Supplementary information March 2018/Annex_III_3.4_ERA_43.

Effects on soil micro-organisms – DNC

In a study following the OECD guideline 216,[43] soil was amended with DNC at two concentrations, 800 and 8,000 μg/kg. After 28 days, the amount of nitrate in the treated soils did not differ from that in the control soil by more than 25%. Therefore, there were no biologically important effects on nitrogen transformation by soil microflora at either concentration. However, it should be noted that the highest concentration tested is below 10 × PEC as required by the OECD 216 guideline.

Effects on soil micro-organisms – HDP

In a study following the OECD guideline 216,[44] soil was amended with HDP at two concentrations, 350 and 3,500 μg/kg. After 28 days, the amount of nitrate in the treated soils did not differ from that in the control soil by more than 25%. Therefore, there were no biologically important effects on nitrogen transformation by soil microflora at either concentration.

Toxicity of DNC and HDP to aquatic organisms

Effects on algae – DNC

A toxicity study with the green alga *Pseudokirchneriella subcapitata* (formerly *Selenastrum capricornutum*) was submitted.[45] Following the OECD guideline 201, algal organisms were exposed to DNC under static conditions (test duration = 72 h). During the test, DNC was not stable in the treated solutions with losses in concentrations ranging from 47 to 83% of the nominal concentrations (nominal concentrations = 13–100 μg/L; geometric mean of measured concentrations = 8.29–42.25 μg/L). Results indicated no decreases of yield or growth rates up to DNC concentration of 42.25 μg/L. Consequently, the EC_{50} and NOEC values were > 42.25 and 42.25 μg/L, respectively. The NOEC value of 0.042 mg/L will be used for the assessment.

Effects on algae – HDP

In the study,[46] the green alga, *P. subcapitata* (formerly *S. capricornutum*), was exposed to HDP. The study was performed following the OECD guideline 201, where the algal organisms were exposed under static conditions (test duration 72 h). HDP concentrations were stable all over the duration of the test and approximately close to nominal concentrations. Mean measured concentrations of HDP ranged from 5,084 to 46,362 μg/L. No significant differences in yield or growth rate were found between controls and treatments and the EC_{50} and NOEC values were > 46,362 and 46,362 μg/L, respectively. The EC_{50} value of 46.4 mg/L for HDP will be used for the assessment.

Effects on crustaceans – DNC

Following to the OECD guideline 202, specimens of *Daphnia magna* were exposed to DNC for 48-h in a static acute toxicity test.[47] The treatment levels ranged from 17 to 93 μg/L of DNC (mean measured concentrations). The EC_{50} for immobilization was > 93 μg/L (48 h). Some lethargy was noticed at concentrations of 40, 64 and 93 μg/L. Furthermore, at 40, 64 and 93 μg/L the percent of immobility was 5%, 25% and 5%, respectively. No toxicity was noticed when organisms were exposed to 27 μg/L of DNC.

In a full life-cycle test (OECD guideline 211), the effects of chronic exposure to DNC in *Daphnia magna* were recorded.[48] Daphnids (< 24 h old) were selected for tests which lasted 21 days. The measured endpoints were: (i) survival, (ii) growth and (iii) reproduction. For the exposure, five concentrations of DNC were considered and the mean measured concentrations were: 2.7, 5.9, 14, 35 and 85 μg/L. At the highest concentration of exposure (85 μg/L) no survival was recorded (0% of surviving organisms); however, no significant mortality was recorded at the lower concentrations. After 21 days, reproduction and weight were reduced significantly at exposure concentration of 35 μg/L (the percent of decrease was 44% and 16%, respectively). In addition, at 35 and 14 μg/L, the length of daphnids was decreased, by 13.5% and a 1.8%, respectively. However, even if the reduction in length at 14 μg/L was statistically significant, the small decrease is not considered to have any ecological consequences. The lack of

[43] Technical dossier/Supplementary information March 2018/Annex_III_3.4_ERA_36.
[44] Technical dossier/Supplementary information March 2018/Annex_III_3.4_ERA_40.
[45] Technical dossier/Supplementary information March 2018/Annex_III_3.4_ERA_44.
[46] Technical dossier/Supplementary information March 2018/Annex_III_3.4_ERA_50.
[47] Technical dossier/Supplementary information March 2018/Annex_III_3.4_ERA_45.
[48] Technical dossier/Supplementary information March 2018/Annex_III_3.4_ERA_46.

biological significance is also supported by the absence of effects in the other considered endpoints. Consequently, the NOEC for DNC is considered to be 14 μg/L and will be used for the assessment.

Effect on crustaceans – HDP

In a 48-h static acute toxicity test (OECD guideline 202), specimens of *D. magna* were exposed to HDP at mean measured concentrations ranging from 15,000 to 107,000 μg/L.[49] No effects were recorded up to the highest concentration, therefore, the EC_{50} (48 h) and the NOEC values of HDP are both > 107,000 μg/L. The EC_{50} value of 107 mg/L for HDP is used for the assessment.

Effects on fish – DNC

In a 96-h static toxicity test (OECD guideline 203), individuals of rainbow trout (*Oncorhynchus mykiss*) were exposed to DNC at a mean measured concentration of 69 μg/L.[50] No sublethal effects or mortalities were recorded. Therefore, the LC_{50} value is > 69 μg/L.

In another 96-h static toxicity test (OECD guideline 203), bluegill individuals (*Lepomis macrochirus*), were exposed to DNC at a mean measured concentration of 72 μg/L.[51] No sublethal effects or mortalities were recorded. Consequently, the LC_{50} is > 72 μg/L.

A third study[52] reports, the results of a reproductive study conducted on fathead minnows exposed to five concentrations (mean measured concentrations of 0.80, 2.6, 8.9, 28 and 91 μg/L) of DNC and using a protocol similar to OECD 229. Groups (2 males and 4 females per group) of sexually mature (proven spawners) were selected for the test and exposed for 4 weeks under flow-through conditions. Each week, eggs were incubated to monitor the percent of hatching. No significant effects were observed on selected toxicological endpoints (survival, fecundity, egg fertility or egg hatchability. Therefore, the NOEC in this study was 91 μg/L. The NOEC of 0.091 mg/L for DNC from long subacute fish studied is used for the assessment.

Effects on fish – HDP

In a 96-h static toxicity test (OECD guideline 203), individuals of rainbow trout (*O. mykiss*) were exposed at mean measured concentration of 110,000 μg/L.[53] No effects (sublethal or mortalities) were recorded for the exposed organisms. Therefore, a LC_{50} value > 110,000 μg/L was assigned.

In a 96-h static toxicity test (OECD guideline 203), individuals of bluegill (*L. macrochirus*), were exposed to HDP at mean measured concentration of 122,000 μg/L.[54] No effects (sublethal or mortalities) were noted for the exposed bluegill. Consequently, the LC_{50} value was considered to be > 122,000 μg/L. The EC_{50} value of 110 mg/L for HDP from acute fish studies is used in the assessment.

Effects on sediment dwelling organisms

No studies submitted. $PNEC_{sed}$ calculated based on equilibrium partitioning is 2,260 μg/kg for DNC. Calculation was performed with a K_{oc} of 16,137 L/kg and a $PNEC_{surfacewater}$ of 1.4 μg/L for DNC. $PNEC_{sed}$ for HDP was not calculated with the equilibrium partitioning because according to REACH (ECHA, 2008) a log K_{oc} or log K_{ow} ≥ 3 for an organic chemical is used as a trigger value for sediment effect assessment. Based on this, it is not expected that HDP will pose a risk to sediment.

Risk characterisation (PEC/PNEC ratio) for DNC and HDP

The risk characterisation ratios for terrestrial, freshwater and sediment compartments are reported in the tables below (Tables 11–14).

[49] Technical dossier/Supplementary information March 2018/Annex_III_3.4_ERA_51.
[50] Technical dossier/Supplementary information March 2018/Annex_III_3.4_ERA_47.
[51] Technical dossier/Supplementary information March 2018/Annex_III_3.4_ERA_48.
[52] Technical dossier/Supplementary information March 2018/Annex_III_3.4_ERA_49.
[53] Technical dossier/Supplementary information March 2018/Annex_III_3.4_ERA_52.
[54] Technical dossier/Supplementary information March 2018/Annex_III_3.4_ERA_53.

Table 11: Risk characterisation (PEC/PNEC ratio) for DNC and for HDP for the terrestrial compartment

	Taxa	PEC_{soil} (µg/kg)	LC_{50} or NOEC (mg/kg)	AF	PNEC (µg/kg)	PEC/PNEC
DNC	Earthworm	1,150	1,000$^{(1)}$	1,000	1,000	1.2
	Plants		2.9$^{(2)}$	10	290	4.0
HDP	Earthworm	106	1,000$^{(1)}$	100	10,000	0.01
	Plants		1.75$^{(2)}$	10	175	0.6

AF: assessment factor.
(1): LC_{50}.
(2): NOEC.

Table 12: Risk characterisation (PEC/PNEC ratio) for the freshwater compartment for the DNC

Taxa	$PEC_{surfacewater}$ (µg/L)	NOEC (µg/L)	AF	PNEC (µg/kg)	PEC/PNEC
Algae	1.3	0.042	10	1.4	0.9
Aquatic invertebrates		0.014			
Fish		0.091			

AF: assessment factor.

Table 13: Risk characterisation (PEC/PNEC ratio) for the freshwater compartment for the HDP

Taxa	$PEC_{surfacewater}$ (µg/L)	EC_{50} (µg/L)	AF	PNEC (µg/kg)	PEC/PNEC
Algae	44	46.4	1,000	464	0.09
Aquatic invertebrates		107			
Fish		111			

AF: assessment factor.

Table 14: Risk characterisation (PEC/PNEC) for the sediment compartment

	PEC_{sed} (µg/kg)	NOEC (mg/kg)	AF	$PNEC_{sed, EqP}$ (µg/kg)	PEC/PNEC
DNC	1,090	–	–	2,260	0.48

AF: assessment factor.

Bioaccumulation and secondary poisoning

HDP, with log $K_{ow} < 3$, does not have the potential for bioaccumulation; hence, there is no risk for secondary poisoning for this substance. Based on the Log K_{ow} of 3.6 and high persistence, DNC has a potential for bioaccumulation, but no bioconcentration factor (BCF) values for earthworm and fish have been provided for this substance.

The FEEDAP Panel made an assessment on secondary poisoning of DNC in its opinion on Maxiban® G160 in 2010 (EFSA FEEDAP Panel, 2010a). Based on the data presented there, $PNEC_{oral}$ for DNC is 0.83 mg/kg feed. This value is higher than the estimated concentration in the worms and fish of 0.29 and 0.30 mg/kg, respectively, which are based on PECs presented in Table 10. The PEC/PNEC ratios for surface water and soil are given in Table 15. A risk for secondary poisoning for worm/fish eating birds and mammals is not likely to occur for DNC.

Table 15: Risk assessment for DNC based on the 100% of the proposed recommended dose

	$PEC_{oral, sw}$ (mg/kg)	$PEC_{oral, soil}$ (mg/kg)	PNEC (mg/kg)	$PEC/PNEC_{sw}$	$PEC/PNEC_{soil}$
DNC	0.30	0.29	0.83	0.36	0.35

3.2.4.3. Conclusions on safety for the environment

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. Narasin is not considered to have a bioaccumulation potential.

The two components of nicarbazin (DNC and HDP) have a different impact on the environment. DNC and HDP do not pose a risk for the groundwater. No risk for the terrestrial and aquatic compartment is associated to the HDP component of nicarbazin. The bioaccumulation potential of HDP in the environment is low. The risk for secondary poisoning is not identified for HDP. DNC is very persistent in soil and a risk for the terrestrial compartment cannot be excluded based on the results of ecotoxicity tests on terrestrial organisms. DNC does not pose a risk for the aquatic compartment and sediment. The high persistence and hydrophobicity of DNC indicate that there might be a risk for bioaccumulation but the risk for secondary poisoning was not identified. The potential of DNC to accumulate in soil over the years should be investigated by monitoring in a field study.

In summary, based on the available data, the FEEDAP Panel cannot conclude on the safety of Maxiban® G160 for the environment due to the risk identified for terrestrial organisms for DNC.

3.3. Efficacy

Maxiban® G160 is currently authorised at a dose range of 40 + 40 mg to 50 + 50 mg narasin + nicarbazin/kg complete feed.[55] The present request concerns the increase of the maximum inclusion level to 70 + 70 mg narasin + nicarbazin/kg feed. Since efficacy should be demonstrated for the lowest recommended level, and this has not been changed, efficacy studies are not required. Nevertheless, the applicant submitted a total of seven studies which were assessed by the FEEDAP Panel as follows.

3.3.1. Floor pen studies

Four floor pen studies in chickens for fattening, conducted in 2014, were submitted (see Table 16).[56] In trial 2, two parallel experiments with different inoculates were performed.[57] In each study, 1-day-old chickens were penned and distributed into the following treatment groups: an uninfected untreated control group (UUC), an infected untreated control group (IUC) and two infected treated groups (IT) with a lower dose of the additive (40 mg narasin + 40 mg nicarbazin/kg feed in trials 2 and 3 (IT40) and 50 mg narasin + 50 mg nicarbazin/kg feed in trial 1 (IT50)) and a higher dose (70 mg narasin + 70 mg nicarbazin/kg feed (IT70)). The doses were analytically confirmed (see Table 16). The experimental diets were fed for 42 days. In the infected groups, all birds were inoculated with field isolates of pathogenic *Eimeria* species. 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 in trials 1, 2a and 2b. Intestinal lesions were scored on three birds per pen in trial 1, on four birds per pen in trial 2, and on two (day 20) and four birds (day 21) 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 trial 1, mortality between treatment groups was analysed by chi-squared test. Oocysts counts and lesions scores were analysed with non-parametric Kruskal–Wallis test. Performance parameters were analysed in a randomised complete block design. The treatment was considered as a fixed effect and the block (situation of the cage) as a random effect, taking the pen as experimental unit. Comparisons between treatments were established by least significant differences (LSD). In the other trials, the data were analysed by analysis of variance (ANOVA). Results were compared between the IUC and IT groups using least squares corrected means.

55 Commission Regulation (EU) 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.

56 Trial 1: Technical dossier/Section IV/Annex IV.1. Trial 2a: Technical dossier/Section IV/Annex IV.2. Trial 2b: Technical dossier/Section IV/Annex IV.3. and Trial 3: Technical dossier/Section IV/Annex IV.4.

57 Trial 2a and 2b were conducted in the same research institute, at the same time, with the same feed, and used the same UUC group. Taking into account that the inoculum with sporulated oocysts is the most critical factor in this type of studies and that the UUC group (common control) is used only to verify the growth of the animals under farming conditions, 2a and 2b can be considered as separate studies in the assessment of the coccidiostatic efficacy of the additive against *Eimeria* infection.

Table 16: Experimental design of floor pen studies with chickens for fattening using Maxiban® G160

Trial	Replicates per treatment (Birds(1) per replicate)	Inoculum characteristics				Feed concentration (narasin + nicarbazin mg/kg feed)(3)	
		Year and place of isolation	Intended dose (number of oocysts) and *Eimeria* strain per bird		Day and mode of inoculation	Intended	Analysed
1	16 (30–31)	2013 Spain	80,000	*E. acervulina*	day 14 via feed	50 + 50 70 + 70	47.6 + 49.2/48.8 + 47.9/ 52.0 + 49.8 68.7 + 68.5/69.4 + 64.2/ 68.7 + 64.4
			37,000	*E. tenella*			
			25,000	*E. maxima*			
2a	9 (30)	2013 Italy	100,000	*E. oocysts*(2)	day 14 orally via syringe	40 + 40 70 + 70	32.3 + 39.1/23.6 + 19.6 66.3 + 65.7/44.8 + 35.2 32.3 + 35.6/27.0 + 23.3 68.0 + 56.0/49.9 + 36.6
2b	9 (30)	2013 EU	55,000	*E. acervulina*			
			30,000	*E. tenella*			
			20,000	*E. maxima*			
			15,000	*E. mitis, E. brunetti, E. necatrix*			
3	9 (35)	2014 Spain	36,000	*E. acervulina*	day 14 orally	40 + 40 70 + 70	30.8 + 25.8/34.4 + 25.7/ 37.9 + 27.6/38.2 + 29.9 58.9 + 48.1/62.6 + 49.7/ 65.3+53.3/58.7 + 53.9
			28,000	*E. tenella*			
			20,000	*E. maxima*			
			20,000	*E. necatrix*			
			16,000	*E. mitis*			
			16,000	*E. brunetti*			

(1): Male Ross 308 in trials 1 and 2; male and female Cobb x Cobb 500 in trial 3.
(2): The applicant used a mixture of four isolates. The predominant species were *E. maxima* and *E. tenella.*
(3): In trial 1, birds received starter diet from day 0 to 14, grower diet from day 14 to 28 and finisher diet from day 28 to 42. In trials 2a and b, birds received starter diet from day 0 to 14, grower diet from day 14 until study completion. In trial 3, birds received pre-starter diet from day 0 to 7, starter diet from 7 to 21, grower diet from 21 to 33 and finisher diet from 33 to 42.

Mortality in IT groups of trials 1 and 3 was significantly lower than in the IUC groups whatever the dose (Table 17). In trial 2a, mortality was very low (total of 13 including 7 culls) and not affected by treatment. In trial 2b, five birds died in the IUC group related to coccidiosis, whereas none were found in the treated groups.

Table 17: Mortality (n) in floor pen trials(1)

	Trial 1	Trial 2a	Trial 2b	Trial 3
UUC	18 (0)	7 (1)	7 (1)	6 (0)
IUC	162* (153)	2 (0)	9 (5)	118 (104)
IT40	–	2 (0)	3 (0)	7* (1)
IT50	31* (1)	–	–	–
IT70	19* (0)	2 (0)	10 (0)	11* (4)

Mean values with * are significantly different from IUC ($p \leq 0.05$).
(1): In brackets coccidiosis-related mortalities are indicated.

In trial 1, IT birds had significantly lower intestinal lesion scores compared to IUC birds in the upper and middle intestine and in the caeca 6 days post-inoculation (Table 18). On day 27 (day 13 post-infection), only 7 IUC birds presented lesions.

Table 18: Intestinal lesion scores in different intestinal sections 6 days post-inoculation in trial 1[(1)]

	Upper	Middle	Caecal
Trial 1			
UUC	0	0.1	0.1
IUC	1.5	1.4	2.1
IT50	0.1*	0*	0.4*
IT70	0.1*	0.1*	0.1*

Mean values with * are significantly different from IUC ($p \leq 0.05$).
(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*.

The very low lesion scores reported in trial 2a (Table 20) are probably due to low pathogenicity of the inoculum (reduced viability of oocysts) and are in line with the low mortality observed (see Table 19). In trial 2b, birds of the IT groups had significantly lower scores in different intestinal segments (in colon and caeca for IT40 and IT70). The total lesion scores were also significantly lower in IT than in IUC birds.

Table 19: Intestinal lesion scores 6 days post-inoculation in trials 2a and 2b

	Duodenum (*E. acervulina*)	Ileum/jejunum (*E. maxima*)	Colon (*E. brunetti*)	Caeca (*E. tenella*)	Total
Trial 2a					
UUC	0.2	0.2	0	0.3	0.7
IUC	0	0.3	0.3	0.4	1.0
IT40	0.1	0*	0*	0.1*	0.3*
IT70	0	0*	0*	0*	0*
Trial 2b					
UUC	0.2	0.2	0	0.3	0.7
IUC	0	0.2	0.3	1.1	1.6
IT40	0.1	0.1	0*	0.1*	0.3*
IT70	0.1	0*	0*	0.1*	0.2*

Mean values with * are significantly different from IUC ($p \leq 0.05$).

In trial 3 (Table 20), coccidiosis lesion scoring was carried out on 6 and 7 days post-inoculation. On both days, IT birds had significantly lower scores compared to IUC birds in the caeca (*E. tenella*) which also resulted in a significant difference in total lesion scores at both doses.

Table 20: Intestinal lesion scores 6 and 7 days post-inoculation in trial 3

	Duodenum (*E. acervulina*)	Ileum/jejunum (*E. maxima*)	Caeca (*E. tenella*)	Total
6 days PI				
UUC	0.4	0	0	0.4
IUC	0.1	0.1	1.8	1.9
IT40	0	0	0.1*	0.1*
IT70	0	0.1	0*	0.1*
7 days PI				
UUC	0.2	0	0	0.2
IUC	0.0	0.0	1.4	1.5
IT40	0.0	0.0	0.1*	0.1*
IT70	0	0	0*	0*

PI: post-inoculation.

Oocyst excretion was measured only in trials 1, 2a and 2b. In trial 1, on day 20 and 28 (6 and 14 days post-inoculation), species-specific oocysts counts per gram of faeces (OPG) were significantly lower in both IT groups compared to the IUC group (Table 21). On day 35, there were no significant differences in the total oocyst excretion values between groups.

Table 21: Total number of *Eimeria* oocysts per gram of faeces ($\log_{10}$ OPG) in floor pen trial 1

	E. acervulina		*E. maxima*		*E. tenella*		Total	
	Day 20	Day 28	Day 20	Day 28	Day 20	Day 28	Day 20	Day 28
UUC	1.92	1.96	0	0	0	0	1.92	1.96
IUC	5.34	3.11	4.45	2.31	5.05	2.82	5.58	3.31
IT50	1.9*	0*	0*	0*	0*	0*	1.9*	0*
IT70	0*	0*	0*	0*	0*	0*	0*	0*

Mean values with * are significantly different from IUC ($p \leq 0.05$).

In trials 2a and 2b, only total OPG counts were reported. On days 20 and 22 (6 and 8 days post-inoculation), OPGs were significantly lower in IT40 and IT70 compared to IUC group in trial 2b, while in trial 2a only IT70 showed significantly lower OPGs. On day 28 (14 days post-inoculation), there were no significant differences in the total oocyst excretion values between groups (Table 22).

Table 22: Total number of *Eimeria* oocysts per gram of faeces (OPG $\times$ 10^3) in floor pen trials 2a and 2b

	D20 (6 day PI)	D22 (8 day PI)	D28 (14 day PI)
Trial 2a			
UUC	52.2	5.9	6.5
IUC	78.2	22.9	3.8
IT40	82.1	24.2	2.8
IT70	0.6*	1.9*	0.3
Trial 2b			
UUC	52.2	5.9	6.5
IUC	211.3	23.7	1.0
IT40	9.1*	4.7*	11.1
IT70	34.0*	5.5*	0.5

PI: post-inoculation.
Mean values with * are significantly different from IUC ($p \leq 0.05$).

Table 23 summarises the results concerning the zootechnical endpoints. In all four experiments, the zootechnical performance of the IT birds were significantly improved compared to the IUC birds.

Table 23: Performance parameters of chickens for fattening in floor pen trials

	Feed intake(1) (g)	Body weight (g)	Weight gain(2) (g)	Feed to gain ratio
Trial 1				
UUC	119	3,153	74	1.61
IUC	104	2,706	63	1.63
IT50	120*	3,120*	73*	1.64
IT70	120*	3,102*	73*	1.65
Trial 2a				
UUC	4,713	–	2,240	2.10
IUC	4,690	–	2,139	2.12
IT40	4,857	–	2,403*	2.02*
IT70	5,137*	–	2,591*	1.99*
Trial 2b				
UUC	4,713	–	2,240	2.10
IUC	4,228	–	1,923	2.20

	Feed intake(1) (g)	Body weight (g)	Weight gain(2) (g)	Feed to gain ratio
IT40	4,833*	–	2,383*	2.03*
IT70	4,934*	–	2,522*	1.96*
Trial 3				
UUC	4,782	–	2,744	1.74
IUC	5,055	–	2,619	1.93
IT40	4,785*	–	2,880*	1.66*
IT70	4,792*	–	2,873*	1.67*

–: not reported.
Mean values with * are significantly different from IUC ($p \leq 0.05$).
(1): Mean results of trial 1 refer to daily feed intake per bird; those of trials 2 and 3 to total feed intake per bird during the whole study duration.
(2): Mean results of trial 1, refer to daily weight gain per bird considering the whole study duration; those of trials 2 and 3 refer to the total weight gain per bird during the whole study duration.

3.3.2. Anticoccidial sensitivity tests

The applicant submitted three anticoccidial sensitivity tests (ASTs) performed with field isolates and one AST performed with laboratory strains. This last study was not considered for the demonstration of efficacy because the laboratory strains do not represent field conditions (EFSA FEEDAP Panel, 2011a).

Two ASTs performed in 2014 were submitted.[58] Birds were artificially infected with sporulated oocysts from recent field isolates. In AST-1, two parallel experiments with different inoculates were performed.[59] The birds were randomly allocated to the groups UUC, IUC and two IT groups with two Maxiban® G160 concentrations. The high dose (70 + 70 mg narasin+nicarbazin/kg feed) was the same in AST-1a, AST-1b and AST-2, whereas the lower dose was different (50 + 50 mg narasin+nicarbazin/kg feed in AST-1a and AST-1b and 40 + 40 mg narasin+nicarbazin/kg feed in AST-2). Dosages were analytically confirmed (see Table 24). 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 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).

In AST-1a and AST-1b, for performance parameters a generalised linear mixed model was applied at a 5% significance level. Group comparisons were made by an LSD test adjusted for multiple comparisons (Tukey). Oocysts counts, lesion scores and mortality in the different treatments were compared using a non-parametric Kruskal–Wallis test, and differences between groups were tested by Wilcoxon-Mann-Whitney test. The pen was considered the statistical unit. In AST-2, an ANOVA with a 0.05 level of significance was used. Group differences were analysed by Tukey test.

[58] AST-1a: Technical dossier/Section IV/Annex IV.6. AST-1b: Technical dossier/Section IV/Annex IV.7. AST-2: Technical dossier/Section IV/Annex IV.8. and IV.9.
[59] Trial 1a and 1b were conducted in the same research institute, at the same time and with the same feed. Taking into account that the inoculum with sporulated oocysts is the most critical factor in this type of studies and that the UUC group is used only to verify the growth of the animals under farming conditions, 1a and 1b can be considered as separate studies in the assessment of the coccidiostatic efficacy of the additive against *Eimeria* infection.

Table 24: Experimental design of ASTs with chickens for fattening using Maxiban® G160

Trial	Replicates per treatment (Birds[(1)] per replicate)	Inoculum characteristics			Anticoccidial treatment[(2)] (days of life)	Feed concentration (narasin + nicarbazin mg/kg feed)	
		Year and country of isolation	Intended dose (number of oocysts) per bird and strain	Day of inoculation		Intended	Analysed
1a	4 (8)	2013 Spain	50,000 *E. acervulina* 50,000 *E. maxima* 30,000 *E. tenella*	15	8–24	50 + 50 70 + 70	47.8 + 51.0 67.6 + 62.5
1b	4 (8)	2013 Germany and France	100,000 *E. acervulina* 35,000 *E. maxima* 35,000 *E. tenella*				
2	4 (5)	2012 UK	172,299 *E. acervulina* 21,103 *E. maxima* 40,234 *E. tenella*	14	7–21	40 + 40 70 + 70	38.4 + 33.3 70.7 + 60.1

(1): Cobb 500 in AST-1a and AST-1b and female Ross 308 in AST-2.
(2): Birds in the IT group were fed a basal diet supplemented with Maxiban® G160. Animals in the control groups UUC and IUC received the same basal diet without inclusion of the coccidiostat.

There was no mortality in the IT and UUC groups in AST-1a and AST-1b. In the IUC groups, six and seven birds (each out of 32) died due to coccidiosis in the two ASTs, respectively. The statistical analysis indicated a significant difference between IUC and the IT groups. In AST-2, no mortality occurred.

Table 25 summarises the results of the ASTs. Significantly lower OPG values in the IT groups compared to IUC groups showing the effect of the coccidiostatic treatment, were seen in AST-1a and AST-1b. A reduction of lesion scores by treatment (IT) was observed in all tests; however, no statistical analysis was made in AST-2. In AST-1a and AST-1b, the analysis showed that significance was reached in all part of the intestinal tracts examined.

In AST-1a and AST-1b, bodyweight gain and feed to gain ratio (the two parameters analysed statistically) were significantly better in both IT groups than in the IUC group over the seven days post-inoculation. In the same period of AST-2 the results showed significantly higher bodyweight gain in IT groups compared to the IUC groups (feed to gain ratio improved significantly only in IT70).

Table 25: Results of anticoccidial sensitivity tests

Group	Final body weight[(1)] (g)	Feed intake (g)	Average daily gain (g)	Feed to gain ratio	Total $\log_{10}$ OPG	Intestinal lesion scores			
						Upper	Mid	Low	Caeca
	D21	D15–21	D15–21	D15–21	D21–24	D21			
AST-1a									
UUC	760	–	401*	1.30*	1.53*	0	0	–	0
IUC	637	–	227	1.88	5.63	2.1	2.4	–	2.5
IT50	749	–	381*	1.30*	3.96*	0.3*	0.3*	–	0.5*
IT70	762	–	403*	1.33*	2.94*	0*	0*	–	0*
AST-1b									
UUC	757	–	410*	1.30*	1.53	0	0	–	0
IUC	606	–	243	1.90	5.49	2.1	1.9	–	2.5
IT50	739	–	390*	1.33*	2.37*	0.4*	0.3*	–	0.6*
IT70	751	–	410*	1.31*	1.53*	0.25*	0*	–	0.1*

Group	Final body weight[(1)] (g)	Feed intake (g)	Average daily gain (g)	Feed to gain ratio	Total log_{10} OPG	Intestinal lesion scores			
						Upper	Mid	Low	Caeca
	D21	D14–21	D14–21	D14–21	D17–21	D21			
AST-2[(2)]									
UUC[(3)]	931	3,405	509	1.33	0	0	0	0	0
IUC	660	2,395	247	2.13	5.63	3	1	0.7	3.2
IT40	684	2,630	293*	1.79	5.51	1.2	0.4	< 0.1	0.6
IT70	703	2,420	295*	1.66*	5.57	0.9	0.2	< 0.1	0.2

–: not reported.
*: IT mean/UUC mean significantly different from IUC mean ($p \leq 0.05$).
(1): per bird, no statistical analysis was performed.
(2): Statistical analysis was performed with the data on weight gain, feed to gain ratio and oocyst counts.
(3): 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.

3.3.2.1. Conclusions on efficacy

The FEEDAP Panel noted that efficacy studies are not required to support the application since the lower dose is currently authorised. Nevertheless, the applicant did provide newly performed studies.

The primary parameters of coccidiostatic effects (mortality, intestinal lesion score and oocyst excretion) were affected by feed supplemented with Maxiban® G160 in four floor pen studies (low concentration 40 + 40 in trials 2a, 2b and 3, 50 + 50 mg narasin+nicarbazin/kg feed in trial 1; high concentration in all trials 70 + 70 mg narasin+nicarbazin/kg feed). A reduced mortality and lower intestinal lesion scores for both Maxiban doses were observed in trials 1 and 3, as well as reduced oocyst excretion in trial 1 (not measured in trial 3). Intestinal lesion scores and oocyst excretion were also reduced in trial 2b for both Maxiban concentrations, whereas in trial 2a effects on these endpoints were mainly seen for the high concentration.

In two ASTs mortality was reduced by 50 + 50 and 70 + 70 mg narasin+nicarbazin/kg feed (AST-1a and AST-1b). These tests showed also lower intestinal lesion scores and reduced oocyst excretions by both Maxiban concentrations. AST-2 showed no effect on mortality and oocyst excretion. This test could not be fully assessed since intestinal lesion scores were only given as a mean without statistics.

Since three floor pen studies and three ASTs, including a treated group with the lowest recommended dose, are required to conclude on a coccidiostatic effect of an additive, the FEEDAP Panel would not be in the position to conclude on the efficacy of Maxiban® G160 in chickens for fattening based on the data provided for the dose of 40 + 40 mg narasin+nicarbazin/kg feed.

3.4. Post-market monitoring

Field monitoring of *Eimeria* spp. resistance in chickens for fattening to narasin and nicarbazin should be undertaken, preferably during the latter part of the period of authorisation.

The potential of DNC to accumulate in soil over the years should be investigated by monitoring in a field study.

4. Conclusions

Maxiban® G160 contains the active substances narasin and nicarbazin. Narasin 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 *Streptomyces aureofaciens*. The FEEDAP Panel cannot conclude on the absence of genetic determinants for antimicrobial resistance in *Streptomyces* spp. under assessment.

The FEEDAP Panel cannot conclude on the safety of Maxiban® G160 at a dose level of 70 + 70 mg/kg feed for the target species.

The simultaneous use of Maxiban® G160 and certain antibiotic drugs (e.g. tiamulin) is contra-indicated.

Nicarbazin has no antimicrobial activity. Narasin is active against Gram-positive bacteria, while Gram-negative bacteria are resistant. The use of Maxiban® G160 as a feed additive is unlikely to induce resistance or cross-resistance to antimicrobials used in human and animal therapy.

The use of Maxiban® G160 in diets for chickens for fattening at the maximum proposed dose complies with the MRLs in force of narasin and DNC at 0-day withdrawal except for DNC in kidney which was slightly above the MRL of 6 mg/kg. Compliance with DNC MRLs was seen in all tissues at 1-day withdrawal.

Maxiban® G160 is a slight skin irritant and should be considered as an eye irritant and a skin sensitiser. The inhalation exposure would pose a risk to persons handling the additive due to its narasin content.

Narasin is not expected to pose a risk to the environment. Narasin is not considered to have a bioaccumulation potential. The two components of nicarbazin (DNC and HDP) have a different impact on the environment. DNC and HDP do not pose a risk for the groundwater. No risk for the terrestrial and aquatic compartment is associated to the HDP component of nicarbazin. The bioaccumulation potential of HDP in the environment is low. The risk for secondary poisoning is not identified for HDP. DNC is very persistent in soil and a risk for the terrestrial compartment cannot be excluded based on the results of ecotoxicity tests on terrestrial organisms. DNC does not pose a risk for the aquatic compartment and sediment. The high persistence and hydrophobicity of DNC indicate that there might be a risk for bioaccumulation but the risk for secondary poisoning was not identified. The potential of DNC to accumulate in soil over the years should be investigated by monitoring in a field study. Based on the available data, the FEEDAP Panel cannot conclude on the safety of Maxiban® G160 for the environment due to the risk identified for the terrestrial organisms due to DNC.

The FEEDAP Panel noted that efficacy studies are not required to support the application since the lower dose is currently authorised. Nevertheless, the applicant did submit newly performed studies which were assessed by the Panel as follows: since three floor pen studies and three ASTs, including a treated group with the lowest recommended dose, are required to conclude on a coccidiostatic effect of an additive, the FEEDAP Panel would not be in the position to conclude on the efficacy of Maxiban® G160 in chickens for fattening based on the data provided for the dose of 40 + 40 mg narasin+nicarbazin/kg feed.

Documentation as provided to EFSA/Chronology

Date	Event
18/12/2014	Dossier received by EFSA. Maxiban® G160 submitted by Eli Lilly and Company Ltd.
13/1/2015	Reception mandate from the European Commission
12/5/2015	Application validated by EFSA – Start of the scientific assessment
4/6/2015	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. *Issues: methods of analysis*
9/11/2015	Reception of supplementary information from the applicant – Scientific assessment re-started
12/8/2015	Comments received from Member States
13/11/2015	Reception of the Evaluation report of the European Union Reference Laboratory for Feed Additives
13/5/2016	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, safety for the target species, safety for the consumer and safety for the environment*
13/3/2018	Reception of supplementary information from the applicant – Scientific assessment re-started
2/7/2019	Opinion adopted by the FEEDAP Panel. End of the Scientific assessment

References

Aslian S, Boojar MMA, Yaghmaei P and Amiri S, 2014. Nicarbazin induce oxidative response and colony stimulating factor production in mouse lung cells in vitro. Biosciences Biotechnology Research Asia, 11, 141–149.

ECHA (European Chemicals Agency), 2008. Guidance on information requirements and chemical safety 1398 assessment Chapter R.10: Characterisation of dose [concentration]-response for environment. 1399 https://echa.europa.eu/documents/10162/13632/information_requirements_r10_en.pdf

EFSA (European Food Safety Authority), 2004a. Opinion of the Scientific Panel on additives and products or substances used in animal feed (FEEDAP) 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;2(7):90, 44 pp. https://doi.org/10.2903/j.efsa.2004.90

EFSA (European Food Safety Authority), 2004b. Opinion of the Scientific Panel on additives and products or substances used in animal feed (FEEDAP) on the efficacy and safety of the coccidiostat Koffogran. EFSA Journal 2004;2(3):16, 40 pp. https://doi.org/10.2903/j.efsa.2004.16

EFSA (European Food Safety Authority), 2008. 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 FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2010a. 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), 2010b. Scientific Opinion on the safety and efficacy of Koffogran (nicarbazin) as a feed additive for chickens for fattening. EFSA Journal 2010;8(3):1551, 40 pp. https://doi.org/10.2903/j.efsa.2010.1551

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), Rychen G, Aquilina G, Azimonti G, Bampidis V, Bastos ML, Bories G, Chesson A, Cocconcelli PS, Flachowsky G, Kolar B, Kouba M, López Puente S, López-Alonso M, Mantovani A, Mayo B, Ramos F, Saarela M, Villa RE, John Wallace RJ, Wester P, Brantom P, Halle I, van Beelen P, Holczknecht O, Vettori MV and Gropp J, 2016. Scientific Opinion on the modification of the terms of the authorisation regarding the formulation of Maxiban® G160 (narasin and nicarbazin) for chickens for fattening. EFSA Journal 2016;14(11):4614, 7 pp. https://doi.org/10.2903/j.efsa.2016.4614

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Rychen G, Aquilina G, Azimonti G, Bampidis V, Bastos ML, Bories G, Chesson A, Cocconcelli PS, Flachowsky G, Kolar B, Kouba M, López-Alonso M, López Puente S, Mantovani A, Mayo B, Ramos F, Saarela M, Villa RE, Wallace RJ, Wester P, Brantom P, Halle I, Beelen P, Holczknecht O, Vettori MV and Gropp J, 2017. Scientific Opinion on the safety and efficacy of Monimax® (monensin sodium and nicarbazin) for turkeys for fattening. EFSA Journal 2017;15(12):5094, 49 pp. https://doi.org/10.2903/j.efsa.2017.5094

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, 2018a. 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

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 R, Woutersen R, Aquilina G, Bories G, Brantom P, Cocconcelli PS, Halle I, Kolar B, van Beelen P, Wester P, Holczknecht O, Vettori MV and Gropp J, 2018b. Scientific Opinion on the safety and efficacy of Monimax® (monensin sodium and nicarbazin) for chickens for fattening and chickens reared for laying. EFSA Journal 2018;16(11):5459, 34 pp. https://doi.org/10.2903/j.efsa.2018.5459

Lanckriet A, Timbermont L, De Gussem M, Marien M, Vancraeynest D, Haesebrouck F, Ducatelle R and Van Immerseel F, 2010. The effect of commonly used anticoccidials and antibiotics in a subclinical necrotic enteritis model. Avian Pathology, 39, 63–68.

Abbreviations

AF	assessment factor
ALP	alkaline phosphatase
ANOVA	analysis of variance
AST	anticoccidial sensitivity test
BCF	bioconcentration factor
BW	body weight
DFOP	Double First-Order in Parallel
DNC	4,4′-dinitrocarbanilide
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
EqP	equilibrium partitioning
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
HDP	2-hydroxy-4,6-dimethyl-pyrimidine
K_d	sorption/desorption coefficient
K_{oc}	organic carbon–water partitioning coefficient
K_{OM}	organic-matter/water distribution coefficient (L/kg). It corresponds to K_{oc}/1.724
LC_{50}	lethal concentration, 50%
log K_{ow}	octanol/water partition coefficient
LOQ	limit of quantification
LSD	least significant differences
MRL	maximum residue limit
NOEC	no observed effect concentration
OECD	Organisation for Economic Co-operation and Development
OPG	oocysts counts per gram of faeces
PEC	predicted environmental concentration
PEC_{sed}	predicted environmental concentration in sediment
pKa	dissociation constant
PNA	*p*-nitroaniline
$PNEC_{sed}$	predicted no effect concentration in sediment
PSUR	periodic safety update reports
q.s	*quantum satis*
SD	standard deviation
SFO	single first-order

Appendix A – Literature search

Tolerance – nicarbazin

de Domingues CH, Sgavioli S, Praes MFFM, Castiblanco DMC, Marchizeli PCA and Pereira AA, 2014. Use of nicarbazin, salinomycin and zinc oxide as alternative molting methods for commercial laying hens. Brazilian Journal of Poultry Science, 16, 25–30.

Clarke L, Fodey TL, Crooks SR, Moloney M, O'Mahony J, Delahaut P, O'Kennedy R and Danaher M, 2014. A review of coccidiostats and the analysis of their residues in meat and other food. Meat Science, 97, 358–374.

Wang, X-Y, Wang L, Chen G-C, Nie Y-M, Sun Z-Y and Zhang L-J, 2013. Efficiency of combination of maduramicin ammonium and nicarbazin in coccidiosis. Siliao Yanjiu, 10, 33–35 CODEN: SYIABZ; ISSN: 1002-2813.

Aslian S, Yaghmaei P, Amiri S and Boojar MMA, 2014. Nicarbazin induce oxidative response and colony stimulating factor production in mouse lung cells in vitro. Biosciences Biotechnology Research Asia; Biosciences Biotechnology Research Asia, 11, 141–149, 39 refs. ISSN 0973-1245.

Dorne JLCM, Fernandez-Cruz ML, Bertelsen U, Renshaw DW, Peltonen K, Anadon A, Feil A, Sanders P, Wester P and Fink-Gremmels J, 2013. Risk assessment of coccidostatics during feed cross-contamination: animal and human health aspects. Toxicology and Applied Pharmacology, 270, 196–208. ISSN: 0041-008X Published by: Elsevier Inc. Source Note: 2013 Aug. 1, v. 270, no. 3.

Wu CB, Zheng WJ, Chen JX, Huang JL, Wu CB, Zheng WJ, Chen, JX, Huang JL, 2013. Drug resistance of *Eimeria tenella* isolated from Putian. Journal of Economic Animal, 17, 19–22, 26, 12 refs. ISSN: 1007-7448 Published by: Journal of Economic Animal, Changchun City URL (Availability): http://jdxb.jlau.edu.cn

Peebles ED [Reprint Author], Bafundo KW, Womack SK, Zhai W, Pulikanti R and Bennettt LW, 2012. Effects of nicarbazin on the blood glucose and liver glycogen statuses of male broilers. Poultry Science, 91, 2183–2188. CODEN: POSCAL. ISSN: 0032-5791.

Lee K-W, Lillehoj HS, Jang SI, Lee S-H, 2012. Effects of various field coccidiosis control programs on host innate and adaptive immunity in commercial broiler chickens. Han'guk Kagum Hakhoechi, 39, 17–25. CODEN: HKHAAE. ISSN: 1225-6625.

Bienenmann-Ploum ME, Huet AC, Campbell K, Fodey TL, Vincent U, Haasnoot W, Delahaut P, Elliott C, Nielen MWF Editor(s) and Schilt R, 2012. Fiveplex flow cytometric immunoassay for the simultaneous detection of six coccidiostats in feed and eggs. [Conference poster]. Residues of veterinary drugs in food. Proceedings of the EuroResidue VII Conference, Egmond aan Zee, The Netherlands, 14–16 May, 2012. Volume 1, 2 and 3 (2012), pp. 899-902, 5 refs. ISBN: 978-94-6173-318-4 Published by: Board of the EuroResidue Conferences Foundation, Egmond aan Zee Conference: Residues of veterinary drugs in food. Proceedings of the EuroResidue VII Conference, Egmond aan Zee, The Netherlands, 14-16 May, 2012. Volume 1, 2 and 3.

Lee KW, Lillehoj HS (correspondence), Jang SI, Pagès M, Bautista DA, Pope CR, Ritter GD, Lillehoj EP, Neumann AP and Siragusa GR, 2012. Effects of *in ovo* vaccination and anticoccidials on the distribution of *Eimeria* spp. in poultry litter and serum antibody titers against coccidia in broiler chickens raised on the used litters. Research in Veterinary Science, 93, 177–182. Refs: 33 ISSN: 0034-5288; E-ISSN: 1532-2661 CODEN: RVTSA9.

Gerhold RW, Fuller AL, Lollis L, Parr C and McDougald LR, 2011. The efficacy of anticoccidial products against *Eimeria* spp. in northern bobwhites. Avian Diseases, 55, 59–64, 21 refs. CODEN: AVDIAI ISSN: 0005-2086 https://doi.org/10.1637/9572-101310-reg.1 Published by: American Association of Avian Pathologists, 382 West Street Road, Kenneth Square, PA 19348-1692 (US).

Olejnik M (Reprint), Szprenger-Juszkiewicz T and Jedziniak P, 2011. Depletion study on nicarbazin and narasin in tissues and eggs of hens housed in deep litter. Bulletin of the Veterinary Institute in Pulawy, 55, 761–766. ISSN: 0042-4870.

Bilandzic N, Varenina I, Bozic and Kolanovic BS, 2011. Monitoring the concentration of nicarbazin in poultry eggs and liver. Veterinarska Stanica 42, 407–413, 27 refs. ISSN: 0350-7149 Published by: Hrvatski Veterinarski Institut, Centar za Peradarstvo, Zagreb.

Steinhauserova I, Svobodova I, Borilova G and Gallas L, 2010. Impact of technological operations on microbiological parameters of poultry carcasses. Maso, 21, 40–43, 9 refs. ISSN: 1210-4086.

Aquilina G, Bories G, Brantom P, Chesson A, Cocconcelli PS, de Knecht J, Dierick, NA, Gralak MA, Gropp J, et al. 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.

Aquilina G, Bories G, Chesson A, Cocconcelli PS, de Knecht J, Dierick NA, Gralak MA, Gropp J, Halle I, Kroker R, Leng L, Lindgren S, Lundebye Haldorsen A-K, Mantovani A, Mézes M, Renshaw D and Saarela M, 2010. Scientific opinion on the safety and efficacy of Koffogran (nicarbazin) as a feed additive for chickens for fattening. EFSA Journal, 2010;8(3):1551, 40 pp.

Interaction with other drugs – narasin

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.

Toxicology and ERA – nicarbazin

Kang J, Park HC, Gedi V, Park SJ, Kim MA, Kim MK, Kwon HJ, Cho BH, Kim TW, Lee KJ and Lim CM, 2015. Veterinary drug residues in domestic and imported foods of animal origin in the Republic of Korea. Food Additives and Contaminants Part B Surveillance, 8, 106–112.
Albonetti P, Marletta A, Repetto I and Sasso EA, 2015. Efficacy of nicarbazin (Ovistop®) in the containment and reduction of the populations of feral pigeons (*Columba livia* var. domestica) in the city of Genoa, Italy: a retrospective evaluation. Veterinaria italiana, 51, 63–72.
Liu RL, Wang L, Zhang LQ, Cai XM, Zheng MX, Gu SP and Bai R, 2014. Determination of sensitivity of *Eimeria acervulina* precocious strain to anticoccidials. Zhongguo Xumu Shouyi, 41, 232–235. CODEN: ZXSHA5; ISSN: 1671-7236.
Clarke L, Moloney M, O'Mahony J, Danaher M, Fodey TL, Crooks SRH, Delahaut P, Clarke L and O'Kennedy R, 2014. A review of coccidiostats and the analysis of their residues in meat and other food. Meat Science, 97, 358–374. ISSN: 0309-1740.
Dorne JL, Fernandez-Cruz ML, Bertelsen U, Renshaw DW, Peltonen K, Anadon A, Feil A, Sanders P, Wester P and Fink-Gremmels J, 2013. Risk assessment of coccidostatics during feed cross-contamination: animal and human health aspects. Toxicology and Applied Pharmacology, 270, 196–208. ISSN: 0041-008X Published by: Elsevier Inc. Source Note: 2013 Aug. 1, v. 270, no. 3.
Bilandzic N, Dolenc J, Gacnik KS, Varenina I and Kolanovic BS, 2013. Feed additives diclazuril and nicarbazin in egg and liver samples from Croatian farms. Food Additives & Contaminants. Part B, Surveillance, 6, 90–97.
MacDonald A and Wolf E, 2013. THE political and social barriers for contraception in pest birds: a case study of ovocontrol® (nicarbazin). Journal of Zoo and Wildlife Medicine, 44, S132–S134. ISSN: 1042-7260.
Woodward K, 2013. Consumer Safety – Maximum Residue Limits. Toxicological Effects of Veterinary Medicinal Products in Humans, Vol 1. Royal Society of Chemistry, Cambridge, UK. pp. 40–80.
Olejnik M, Jedziniak P, Juszkiewicz TS and Zmudzki J, 2013. In-house quality control material of nicarbazin and narasin in eggs: preparation and inter-laboratory evaluation. Accreditation and Quality Assurance, 18, 421–427, 23 refs. ISSN: 0949-1775.
Mano H, Murayama K, Suzuki Y, Nakada N and Minamiyama M, 2013. Initial environmental risk assessment of chemicals contained in treated wastewater by using PRTR data. Gesuido Kyokaishi, 50, 85–93. CODEN: GSKSAQ. ISSN: 0021-4639.
McGarrity M, 2013. Anthelmintic drugs and coccidiostats: anti-parasitic drug residues in meat. International Meat Topics, 4, 7–9. ISSN: 2045-6948 Published by: Positive Action Publications Ltd, Driffield.
Lee KW, Lillehoj HS, Jang SI, Pages M, Bautista DA, Pope CR, Ritter GD, Lillehoj EP, Neumann AP and Siragusa GR, 2012. Effects of in ovo vaccination and anticoccidials on the distribution of *Eimeria* spp. in poultry litter and serum antibody titers against coccidia in broiler chickens raised on the used litters. Research in Veterinary Science, 93, 177–182. CODEN: RVTSA9; ISSN: 0034-5288.
Jones SRM, Forster I, Liao X and Ikonomou MG, 2012. Dietary nicarbazin reduces prevalence and severity of *Kudoa thyrsites* (Myxosporea: Multivalvulida) in Atlantic salmon *Salmo salar* post-smolts. Aquaculture, 342–343, 1-6 CODEN: AQCLAL; ISSN: 0044-8486.

Piatkowska M, Jedziniak P and Zmudzki J, 2012. Residues of veterinary medicinal products and coccidiostats in eggs - causes, control and results of surveillance program in Poland. Polish Journal of Veterinary Sciences, 15, 803–812.

Broekaert N, Daeseleire E, Delezie E, Vandecasteele B, De Beer T and Van Poucke C, 2012. Can the use of coccidiostats in poultry breeding lead to residues in vegetables? An experimental study. Journal of Agricultural and Food Chemistry, 60.

Brachwitz F, Schwaegele F and Scheuer R, 2012. Residues of coccidiostats in eggs – detection and metabolism. Mitteilungsblatt der Fleischforschung Kulmbach, 51, 227–237.

Iglesias A, Nebot C, Miranda JM, Vazquez BI and Cepeda A, 2012. Detection and quantitative analysis of 21 veterinary drugs in river water using high-pressure liquid chromatography coupled to tandem mass spectrometry. Environmental Science and Pollution Research, 19, 3235–3249.

Lee K-W, Lillehoj HS, Jang SI and Lee S-H, 2012. Effects of various field coccidiosis control programs on host innate and adaptive immunity in commercial broiler chickens. Han'guk Kagum Hakhoechi, 39, 17–25.

MacLachlan DJ and Mueller U, 2012. A refined approach to estimate exposure for use in calculating the Maximum Residue Limit of veterinary drugs. Regulatory Toxicology and Pharmacology, 62, 99–106.

Lim CM, Kang JW, Park SJ, Kim MA, Bong YH, Kim MK, Kwon HJ, Woon JH, Cho BH, Son SW, Lim CM, Kang JW, Park SJ, Kim MA, Bong YH, Kim MK, Kwon HJ, Woon JH, Cho BH, Son SW Editor(s): Schilt R, 2011 Exploratory testing of veterinary drug residues in domestic and imported foods of animal origin in Republic of Korea. [Conference poster]. Residues of veterinary drugs in food. Proceedings of the EuroResidue VII Conference, Egmond aan Zee, The Netherlands, 14-16 May, 2012. Volume 1, 2 and 3 (2012), 999–1004, 2 refs. ISBN: 978-94-6173-318-4 Published by: Board of the EuroResidue Conferences Foundation, Egmond aan Zee Conference: Residues of veterinary drugs in food. Proceedings of the EuroResidue VII Conference, Egmond aan Zee, The Netherlands, 14-16 May, 2012. Volume 1, 2 and 3.

Broekaert N, Daeseleire E, Delezie E, Vandecasteele B, van Poucke C, van Poucke, C. Editor(s): Schilt R, 2012. Is there a necessity to establish maximum residue limits for coccidiostats in vegetables? An experimental study. Residues of veterinary drugs in food. Proceedings of the EuroResidue VII Conference, Egmond aan Zee, The Netherlands, 14-16 May, 2012. Volume 1, 2 and 3 (2012), 77–82, 7 refs. ISBN: 978-94-6173-318-4 Published by: Board of the EuroResidue Conferences Foundation, Egmond aan Zee Conference: Residues of veterinary drugs in food. Proceedings of the EuroResidue VII Conference, Egmond aan Zee, The Netherlands, 14-16 May, 2012. Volume 1, 2 and 3.

Olejnik M, Szprengier-Juszkiewicz T, Jedziniak, P, Sledzinska, E, Szymanek-Bany, I, Korycinska, B, Pietruk, K and Zmudzki, J, 2011. Residue control of coccidiostats in food of animal origin in Poland during 2007–2010. Food Additives & Contaminants. Part B: Surveillance, 4, 259–267. ISSN: 1939-3229 Published by: Taylor & Francis Group Source Note: 2011 Dec. 1, v. 4, no. 4.

Guo B, Yao L-X, Liu Z-Z, He Z-H, Zhou C-M, Li G-L, Yang B-M and Huang L-X, 2011. Environmental residues of veterinary antibiotics in Guangzhou City, China. Journal of Agro-Environment, 30, 938–945.

Olejnik M, Szprenger-Juszkiewicz T and Jedziniak P, 2011. Depletion study on nicarbazin and narasin in tissues and eggs of hens housed in deep litter. Bulletin of the Veterinary Institute in Pulawy, 55, 761–766. ISSN

Guo B, Yao LX, He ZH, Zhou CM, Li GL, Yang BM, Huang LX, Guo B, Yao LX, He ZH, Zhou CM, Li GL, Yang BM and Huang LX, 2011. Determination of nicarbazin in fowl dung by high performance liquid chromatography. Environmental Science & Technology, 34, 97–99.

Abbas RZ, Iqbal Z, Blake D, Khan MN and Saleemi MK, 2011. Anticoccidial drug resistance in fowl coccidia: the state of play revisited. Worlds Poultry Science Journal, 67, 337–349.

Zhao Q, Li T, Dong H, Han H, Kong C, Jiang L, Zhu S and Huang B, 2011. Sensitivity of precocious lines of *Eimeria maxima* and *Eimeria tenella* to eight anticoccidial drugs. Zhongguo Dongwu Chuanranbing Xuebao, 19, 57–61.

Furtula V, Farrell EG, Diarrassouba F, Rempel H, Pritchard J and Diarra MS, 2010. Veterinary pharmaceuticals and antibiotic resistance of *Escherichia coli* isolates in poultry litter from commercial farms and controlled feeding trials. Poultry Science, 89, 180–188.

Kawakami T, Yamashita N, Nakada N and Tanaka, 2010. Ecotoxicological evaluation of pharmaceuticals and personal care products using aquatic organisms of different trophic levels. Kankyo Eisei Kogaku Kenkyu, 24, 132–135.

Scientific opinion on the safety and efficacy of Maxiban® G160 (narasin and nicarbazin) for chickens for fattening. EFSA Journal 2010;8(4):1574, 16 refs. ISSN: 1831-4732 Published by: European Food Safety Authority, Parma.

Lanckriet A, Timbermont L, de Gussem M, Marien M, Vancraeynest D, Haesebrouck F, Ducatelle R, and van Immerseel F, 2010. The effect of commonly used anticoccidials and antibiotics in a subclinical necrotic enteritis model. Avian Pathology, 39, 63–68. ISSN: 0307-9457 Source Note: 2010 Feb., v. 39, no. 1

Fagerstone KA, Miller LA, Killian G and Yoder CA, 2010. Review of issues concerning the use of reproductive inhibitors, with particular emphasis on resolving human-wildlife conflicts in North America. Integrative Zoology, 5, 15–30.

Scientific opinion on the safety and efficacy of Koffogran (nicarbazin) as a feed additive for chickens for fattening. EFSA Journal 2010;8(3):1551, 13 refs. ISSN: 1831-4732 Published by: European Food Safety Authority.

Appendix B – Additional information on the ecotoxicity of nicarbazin, and the combination of narasin and nicarbazin

Terrestrial compartment

Effect of nicarbazin on earthworms

Earthworms (*Eisenia fetida*) were exposed to soil fortified with nicarbazin at five concentrations ranging from 95,000 to 1,000,000 μg/kg for 14 days.[60] Methods followed those described in the OECD guideline 207 and EEC Directive 87/302/EEC, Part C. Endpoints were survival and body weight. Concentrations of nicarbazin (as DNC or HDP) were not verified. There were no treatment related effects on survival or body weight. The LC_{50} was determined to be greater than 1,000,000 μg/kg.

In an older study, earthworms (*Lumbricus terrestris*) were exposed to soil fortified with nicarbazin at concentrations of 10,000 and 100,000 μg/kg for 14 days (Study W01382, (62)).[61] Methods followed those described by Karnak and Hamelink (1982). Endpoints were physical signs of toxicity, changes in body weight and mortality. Concentrations of nicarbazin (as DNC or HDP) were not verified. There were no signs of toxicity or mortalities due to nicarbazin and there was no apparent effect on body weight.

Effect of narasin and nicarbazin on earthworms

Earthworms (*Lumbricus terrestris*) were exposed to soil fortified with narasin and nicarbazin at five nominal concentrations ranging from 700 to 25,000 μg/kg for 14 days.[62] Methods followed those described by Karnak and Hamelink (1982). Endpoints were physical signs of toxicity and body weight. Concentrations of nicarbazin (as DNC or HDP) were not verified. Significant decreases in growth and sublethal signs of toxicity were observed at 16,000 μg/kg and higher. No treatment-related decreases in body weight or other physical signs of toxicity were observed at 2,750 μg/kg or below. Based on nominal concentrations, the NOEC was established as 2,750 μg/kg.

Aquatic compartment

Effects of nicarbazin on crustaceans

Daphnids (*Daphnia magna*) were exposed to nicarbazin at a nominal concentration of 100,000 μg/L under static conditions for 48 h.[63] Methods generally followed those described in ASTM E729-80 (ASTM 1980). Endpoints were physical signs of toxicity and immobility. Concentrations were verified by measuring concentrations of HDP only. DNC was not measured due to its low water solubility. HDP represented 26.8% of nicarbazin by assay. The measured concentrations of HDP were consistent during the study and the mean measured concentration was 24,200 μg/L. There were no observations of immobility or toxicity.

Effects of nicarbazin on fish

Rainbow trout (*Oncorhynchus mykiss*) were exposed to nicarbazin at a nominal concentration of 100,000 μg/L under static conditions for 96 h.[64] Methods generally followed those described in ASTM E729-80 (ASTM 1980). Concentrations were verified by measuring HDP only. DNC was not measured due to its low water solubility. HDP represented 26.82% of nicarbazin. The measured concentrations of HDP were consistent during the study and mean measured concentrations was 26,700 μg/L. There were no observations of mortality or toxicity.

Effects of narasin and nicarbazin on crustaceans

Daphnia magna were exposed to eight nominal narasin and nicarbazin concentrations ranging from 500 to 25,000 μg/L for 48 h under static conditions.[65] Methods followed those described in ASTM E729-80 (ASTM 1980). Endpoints were immobilization and sublethal signs of toxicity. Concentrations were verified by measuring narasin and HDP; DNC was not measured due to its low water solubility. HDP represented 26.72% of nicarbazin, by assay. The measured concentrations of narasin and HDP

[60] Technical dossier/Supplementary information March 2018/Annex_III_3.4_ERA_54.
[61] Technical dossier/Supplementary information March 2018/Annex_III_3.4_ERA_55.
[62] Technical dossier/Supplementary information March 2018/Annex_III_3.4_ERA_58.
[63] Technical dossier/Supplementary information March 2018/Annex_III_3.4_ERA_56.
[64] Technical dossier/Supplementary information March 2018/Annex_III_3.4_ERA_57.
[65] Technical dossier/Supplementary information March 2018/Annex_III_3.4_ERA_59.

were consistent during the study. Mean measured concentrations ranged from 160 to 7,800 μg/L for HDP and 459 to 24,000 μg/L for narasin. No physical signs of toxicity observed at 500 μg/L (nominal) or below. Based on nominal concentrations, the NOEC was 500 μg/L (459 μg/L based on mean measured narasin concentrations) and the LC_{50} was 21,260 μg/L (20,650 μg/L based on mean measured narasin concentrations).

Effects of narasin and nicarbazin on crustaceans

Rainbow trout (*O. mykiss*) were exposed to 10 nominal narasin and nicarbazin concentrations ranging from 450 to 5,600 μg/L for 96 h under static conditions.[66] Methods followed those described in ASTM E729-80 (ASTM 1980). Endpoints were survival and sublethal signs of toxicity. Concentrations were verified by measuring narasin and HDP; DNC was not measured due to its low water solubility. HDP represented 26.72% of the nicarbazin, by assay. The measured concentrations of narasin and HDP were consistent during the study. Mean measured concentrations ranged from 140 to 1,810 μg/L for HDP and 390 to 5,380 μg/L for narasin. Physical signs of toxicity were observed at all concentrations; therefore, a NOEC was not established. Mortality ranged from 20 to 100% at concentrations 0.80–5.6 μg/L (nominal). Based on nominal concentrations, the LC_{50} was determined to be 1,610 μg/L (1,480 μg/L based on mean measured narasin concentrations).

A second study with rainbow trout (*O. mykiss*) was conducted to establish a NOEC.[67] Trout were exposed to four nominal narasin and nicarbazin concentrations ranging from 160 to 800 μg/L for 96 h under static conditions. Endpoints were survival and sublethal signs of toxicity. Concentrations were verified by measuring narasin and HDP; DNC was not measured due to its low water solubility. HDP represented 26.72% of the nicarbazin, by assay. The measured concentrations of narasin and HDP were consistent during the study. Mean measured concentrations ranged from 43 to 250 μg/L for HDP and from 133 to 732 μg/L for narasin. The NOEC was established as 160 μg/L (nominal), where no physical signs of toxicity were observed, or 133 μg/L based on mean measured narasin concentrations.

[66] Technical dossier/Supplementary information March 2018/Annex_III_3.4_ERA_60.
[67] Technical dossier/Supplementary information March 2018/Annex_III_3.4_ERA_61.

Annex A – Executive Summary of the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Method(s) of Analysis for Maxiban® G160

Maxiban® G160 is a *feed additive* – belonging to the group "Coccidiostats and histomonostats" listed in Regulation (EC) No 1831/2003 – initially authorized for chickens for fattening by Regulation (EC) 2430/1999 and further re-authorised by Commission Regulation (EC) No 885/2010. In the current applications authorisation is sought under article 13(3)[68,69] of the Regulation (EC) No 1831/2003 for chickens for fattening. *Maxiban® G160* consists of 80 g/kg of *narasin* and 80 g/kg of *nicarbazin* (as active substances) antidusting oil, anticaking agent, microtrazer-F-Red and rice hulls. It is intended to be incorporated directly into *feedingstuffs* or through *premixtures.* The Applicant proposes (1) the inclusion of a lower amount of the microtrazer-F-Red[1] and (2) to increase the maximum level in the complete *feedingstuffs.*[2] Consequently the Applicant proposed a concentration of *narasin+nicarbazin* in *feedingstuffs* ranging from 40 + 40 mg/kg to 70 + 70 mg/kg for chickens for fattening. Furthermore maximum residue limits (MRLs) in chicken *tissues* of 50 μg/kg for *narasin*[2] and ranging from 4000 to 15000 μg/kg (depending on the *tissue*) for *4,4-dinitrocarbanilide (DNC)* – marker residue for *nicarbazin*[1,2] have been already established by Commission Regulation (EC) No 885/2010.

For the quantification of *narasin* in the *feed additive, premixtures* and *feedingstuffs* the Applicant submitted two single-laboratory validated and further verified methods based on EN ISO 14183 using High Performance Liquid Chromatography with post-column derivatisation coupled to spectrophotometric detection (HPLC-PCD-UV-Vis.). For the quantification of *nicarbazin* in the *feed additive, premixtures* and *feedingstuffs* the Applicant submitted two single-laboratory validated and further verified methods based on EN 15782 using HPLC-UV.

Based on the performance characteristics available the EURL recommends for official control the two single-laboratory validated methods for the quantification of *narasin* and *nicarbazin* in the *feed additive* together with the EN methods for the quantification of the two active substances in *premixtures* and *feedingstuffs*.

For the quantification of *narasin* and *nicarbazin* in chicken *tissues* the Applicant submitted methods based on Reversed Phase High Performance Liquid Chromatography coupled to a triple quadrupole mass spectrometer (RP-HPLC-MS/MS) in electrospray ionisation mode validated according to the requirements set by Commission Decision 2002/657/EC. Based on the performance characteristics available the EURL recommends for official control these methods or any equivalent methods complying with the requirements set by Commission

Decision 2002/657/EC, to enforce the *narasin* and *4-4′-dinitrocarbanilide (DNC)*-marker residue for *nicarbazin* 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.

[68] FAD 2014-0036.
[69] FAD 2014-0045.