SCIENTIFIC OPINION

EFSA Journal

ADOPTED: 2 October 2018

doi: 10.2903/j.efsa.2018.5459

Safety and efficacy of Monimax® (monensin sodium and nicarbazin) for chickens for fattening and chickens reared for laying

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

Abstract

The coccidiostat Monimax® (monensin sodium and nicarbazin) is considered safe for chickens for fattening and chickens reared for laying at the highest use level of 50 mg monensin and 50 mg nicarbazin/kg complete feed. This conclusion is extended to chickens reared for laying. For both active substances, the metabolic pathways in the chicken are similar to those in the turkey and rat. Nicarbazin, when ingested, is rapidly split in its two components dinitrocarbanilide (DNC) and 2-hydroxy-4,6-dimethylpyrimidine (HDP) which behave independently. Monimax® does not represent a genotoxic risk. No safety concerns would arise from the nicarbazin impurities *p*-nitroaniline and methyl (4-nitrophenyl) carbamate. The lowest no observed effect level (NOEL) identified for monensin sodium in a developmental study in rabbits is 0.3 mg monensin sodium/kg body weight (bw) per day for maternal toxicity in rabbits. The lowest no observed adverse effect level (NOAEL) identified in a 52-week study in rat using DNC + HDP was 20 mg DNC + 8 mg HDP/kg bw per day based on the absence of microcrystals in urine and related microscopic renal observations. No significant interaction between monensin sodium and nicarbazin is expected from toxicological studies. The use of Monimax® at the highest proposed dose will not pose a risk to persons consuming animal products from treated chickens for fattening. This conclusion is extended to chickens reared for laying. No withdrawal time is required for Monimax® in chickens for fattening. Residue data comply with the established maximum residue limits (MRLs) for monensin and DNC. Based on the available data, the FEEDAP Panel cannot conclude on the safety of Monimax® for the environment. Monimax® has the potential to control coccidiosis in chickens for fattening at a minimum concentration of 40 mg monensin and 40 mg nicarbazin/kg complete feed.

Keywords: coccidiostat, Monimax, monensin sodium, nicarbazin, safety, efficacy, chickens for fattening, chickens reared for laying

Requestor: European Commission

Question number: EFSA-Q-2012-00791

Correspondence: feedap@efsa.europa.eu

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

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

Suggested citation: EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Bampidis V, Azimonti G, Bastos ML, Christensen H, Dusemund B, Kouba M, Kos Durjava M, López-Alonso M, López Puente S, Marcon F, Mayo B, Pechová A, Petkova M, Ramos F, Sanz Y, Villa R, Woutersen R, Aquilina G, Bories G, Brantom P, Cocconcelli PS, Halle I, Kolar B, van Beelen P, Wester P, Holczknecht O, Vittoria Vettori M and Gropp J, 2018. 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

ISSN: 1831-4732

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

Summary

Following a request from European Commission, the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) was asked to deliver a scientific opinion on the safety and efficacy of Monimax® (monensin sodium and nicarbazin), when used as a feed additive for chickens for fattening and chickens reared for laying.

Monimax® is considered safe for chickens for fattening at the highest use level of 50 mg monensin and 50 mg nicarbazin/kg complete feed. The margin of safety is about 1.5. This conclusion is extended to chickens reared for laying. The simultaneous use of Monimax® and certain antibiotic drugs (i.e. tiamulin) is contraindicated. Monensin has a selective antimicrobial activity against Gram-positive bacterial species while many Enterobacteriaceae are naturally resistant. Induction of cross-resistance with clinically relevant antimicrobials or increased shedding of enteropathogenic bacteria are not reported. Nicarbazin has no antimicrobial activity.

Monensin sodium is absorbed at a limited extent and excreted rapidly, it is extensively metabolised and gives rise to demethylated, oxidised and decarboxylated metabolites. Nicarbazin, when ingested, is rapidly split in its two components dinitrocarbanilide (DNC) and 2-hydroxy-4,6-dimethylpyrimidine (HDP) which behave independently. Liver is the target tissue. DNC residues decline rapidly from tissues following nicarbazin withdrawal. DNC appears as the marker residue. HDP-related residues are much lower than those derived from DNC. For both compounds of Monimax®, the metabolic pathways in the chicken are similar to those in the turkey and rat.

The FEEDAP Panel concludes that the active substances in Monimax®, monensin sodium and nicarbazin, do not represent a genotoxic risk. No safety concerns would arise from the nicarbazin impurities *p*-nitroaniline (PNA) and methyl(4-nitrophenyl) carbamate (M4NPC). Monensin sodium has no structural alert for carcinogenesis. Monensin sodium is not a reproductive or developmental toxicant. The lowest no observed effect level (NOEL) identified in a developmental study in rabbits is 0.3 mg monensin sodium/kg body weight (bw) per day for maternal toxicity in rabbits. The primary toxicity resulting from the oral use of nicarbazin is renal toxicity. The absence of similar findings after treatment with DNC and HDP confirms that this equimolar association of compounds is better tolerated than nicarbazin at equivalent doses. The lowest NOAEL identified in a 52-week study in rat using DNC + HDP was 20 mg DNC + 8 mg HDP/kg bw per day based on the absence of microcrystals in urine and related microscopic renal observations. No significant interaction between monensin sodium and nicarbazin is expected from toxicological studies.

The use of Monimax® at the highest proposed dose (50 mg monensin and 50 mg nicarbazin/kg complete feed) will not pose a risk to persons consuming animal products from treated chickens for fattening. This conclusion is extended to chickens reared for laying up to 16 weeks of age. No safety concern would arise from the impurity PNA if the maximum content in nicarbazin of 0.1% is respected. The impurity M4NPC is considered safe for the consumer provided that a maximum concentration of 0.4% in nicarbazin is not exceeded. No withdrawal time is required for Monimax® in chickens for fattening. Residue data comply with the established maximum residue limits (MRLs) for monensin and DNC.

The monensin sodium contained in Monimax® presents a hazard by inhalation. Monimax® is not a skin irritant; however, no data are available for the eye irritation potential of monensin. Monimax® may also act as a dermal toxicant due to its monensin component. Monimax® is not a skin sensitiser.

The use of monensin sodium from Monimax® in complete feed for chickens for fattening does not pose a risk for the aquatic compartment and sediment, while a risk cannot be excluded for the terrestrial compartment. A final conclusion on the risk resulting from the use of nicarbazin from Monimax cannot be made because (i) DNC refined predicted environmental concentrations (PECs) show uncertainties linked to the very high persistence of the compound, (ii) DNC might accumulate in the sediment compartment, and (iii) DNC can potentially bioaccumulate and may cause secondary poisoning. No concerns would arise for the HDP moiety of nicarbazin excreted from chickens fed Monimax®. These concerns prevent the FEEDAP Panel to draw a final conclusion on the environmental risk resulting from the use of Monimax® in chickens for fattening and chickens reared for laying.

The FEEDAP Panel concludes that Monimax® has the potential to control coccidiosis in chickens for fattening at a minimum concentration of 40 mg monensin and 40 mg nicarbazin/kg complete feed. This conclusion is extended to chickens reared for laying.

Table of contents

1. Introduction

1.1. Background and Terms of Reference

Regulation (EC) No 1831/2003[1] establishes the rules governing the Community authorisation of additives for use in animal nutrition. In particular, Article 4(1) of that Regulation lays down that any person seeking authorisation for a feed additive or for a new use of a feed additive shall submit an application in accordance with Article 7.

The European Commission (EC) received a request from the company Huvepharma N.V.[2] for authorisation of the product Monimax® (monensin sodium and nicarbazin), when used as a feed additive for chickens for fattening and chickens reared for laying (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 application under Article 4(1) (authorisation of a feed additive or new use of a feed additive). EFSA received directly from the applicant the technical dossier in support of the application. The particulars and documents in support of the application were considered valid by EFSA as of 31 January 2013.

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 Monimax® (monensin sodium and nicarbazin) when used under the proposed conditions of use (see Section 3.1).

1.2. Additional information

The feed additive Monimax® containing two active substances, monensin sodium and nicarbazin, is not authorised in the European Union. The safety and efficacy of the additive for turkeys for fattening has been recently assessed by the FEEDAP Panel (EFSA FEEDAP Panel, 2017).

There are two authorised coccidiostats containing monensin sodium: Coxidin®[3] and Elancoban®.[4] Both additives are authorised for their use in chickens for fattening, chickens reared for laying and turkeys. The holder of the Coxidin® authorisation is Huvepharma N.V., the same applicant as that of the current submission.

The Scientific Committee on Animal Nutrition (SCAN) issued two opinions in which monensin sodium for its use in poultry (European Commission, 1981) and in turkeys (European Commission, 1983) was assessed. Coxidin® was evaluated by the EFSA Scientific Panel on Additives and Products or Substances used in Animal Feed (FEEDAP Panel) for chickens and turkeys for fattening (EFSA, 2005, 2006a), for turkeys (EFSA, 2007) and for chickens reared for laying (EFSA FEEDAP Panel, 2011a). Elancoban® was evaluated for chickens for fattening, chickens reared for laying and turkeys for fattening (EFSA, 2004a). Both products are currently under re-evaluation following Article 10(2) of Regulation (EC) No 1831/2003. The maximum residue limits (MRLs) and withdrawal period for monensin sodium in chickens for fattening and turkeys for fattening were evaluated by the FEEDAP Panel in various scientific opinions (EFSA, 2006b, 2008a,b; EFSA FEEDAP Panel, 2013). Provisional MRLs for monensin sodium in chickens for fattening, chickens reared for laying and turkeys for

[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] Uitbreidingstraat 80, 2600 Antwerp, Belgium.

[3] Commission Regulation (EC) No 109/2007 of 5 February 2007 concerning the authorisation of monensin sodium (Coxidin) as a feed additive. OJ L 31, 6.2.2007, p. 6. amended by Commission Regulation (EC) No 1095/2008 of 6 November 2008 amending Regulation (EC) No 109/2007 as regards the terms of the authorisation of the feed additive monensin sodium (Coxidin). OJ L 298, 7.11.2008, p. 3. and Commission Implementing Regulation (EU) No 140/2012 of 17 February 2012 concerning the authorisation of monensin sodium as a feed additive for chickens reared for laying (holder of authorisation Huvepharma NV Belgium). OJ L 47, 18.2.2012, p. 18.

[4] Commission Regulation (EC) No 1356/2004 of 26 July 2004 concerning the authorisation for 10 years of the additive 'Elancoban' in feedingstuffs, belonging to the group of coccidiostats and other medicinal substances. OJ L 251, 27.7.2004, p. 6. amended by Commission Regulation (EC) No 108/2007 of 5 February 2007 amending Regulation (EC) No 1356/2004 as regards the conditions for authorisation of the feed additive Elancoban, belonging to the group of coccidiostats and other medicinal substances. OJ L 31, 6.2.2007, p. 4. and by Commission Regulation (EC) No 1096/2008 of 6 November 2008 amending Regulation (EC) No 1356/2004 as regards the terms of the authorisation of the feed additive 'Elancoban', belonging to the group of coccidiostats and other medicinal substances. OJ L 298, 7.11.2008, p. 5.

fattening, are in force: 25 μg monensin sodium/kg of wet skin + fat and 8 μg monensin sodium/kg of wet liver, kidney and muscle[5] ; the withdrawal time is one day before slaughter.[6]

There are two authorised coccidiostats containing nicarbazin: Koffogran[7] (nicarbazin) and Maxiban®[8] (nicarbazin and narasin); both products are authorised for chickens for fattening only. Koffogran has been assessed by the FEEDAP Panel (EFSA, 2004b and EFSA FEEDAP Panel, 2010a). Maxiban® has been assessed by the SCAN (European Commission, 1991, 1995) followed by a FEEDAP Panel assessment (EFSA FEEDAP Panel, 2010b). MRLs are in force for nicarbazin (dinitrocarbanilide (DNC) as the marker residue) in chicken tissues: 15,000 μg DNC/kg of fresh liver, 6,000 μg DNC/kg of fresh kidney, 4,000 μg DNC/kg fresh muscle and fresh skin + fat. The withdrawal time before slaughter is one day for nicarbazin from Koffogran and zero day for nicarbazin from Maxiban®.[9]

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

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

EFSA has verified the EURL report as it relates to the methods used for the control of the active substances in animal feed and the marker residues in tissues. The Executive Summary of the EURL report can be found in Annex A.[11]

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of Monimax® (monensin sodium and nicarbazin) is in line with the principles laid down in Regulation (EC) No 429/2008[12] and the relevant guidance documents: Guidance for the preparation of dossiers for coccidiostats and histomonostats (EFSA FEEDAP Panel, 2011b), Technical guidance: Tolerance and efficacy studies in target animals (EFSA FEEDAP Panel, 2011c), Technical Guidance for assessing the safety of feed additives for the environment (EFSA, 2008c), Guidance for establishing the safety of additives for the consumer (EFSA FEEDAP Panel, 2012a), Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012b), Technical Guidance: Microbial Studies (EFSA, 2008d), Technical Guidance: Extrapolation of data from major species to minor species regarding the assessment of additives for use in animal nutrition (EFSA, 2008e) and Guidance on the assessment of bacterial susceptibility to antimicrobials of human and veterinary importance (EFSA FEEDAP Panel, 2012c).

3. Assessment

Monimax® (monensin sodium and nicarbazin) is intended to be used as a coccidiostat in feed for chickens for fattening and chickens reared for laying for the control of coccidiosis.

[5] OJ L 31, 6.2.2007, p. 6. and OJ L 47, 18.2.2012, p. 18.
[6] OJ L 298, 7.11.2008, p. 3. and OJ L 47, 18.2.2012, p. 18.
[7] 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.
[8] 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 the authorisation Eli Lilly and Company Ltd) amending Regulation (EC) No 2430/1999. OJ L 265, 8.10.2010, p. 5.
[9] OJ L 263, 6.10.2010, p. 4. and OJ L 265, 8.10.2010, p. 5.
[10] FEED dossier references: FAD-2012-0027.
[11] The full report is available on the EURL website: https://ec.europa.eu/jrc/sites/jrcsh/files/finrep-fad-2012-0027-monimax.pdf
[12] 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.

3.1. Characterisation

The additive Monimax® contains as active substances monensin sodium and nicarbazin. Monensin sodium is a polyether ionophore produced by fermentation from a culture of *Streptomyces* spp.[13] Nicarbazin is an equimolar complex of 1,3-bis(4-nitrophenyl)urea, also known as *N*,*N*′-bi(4-nitrophenyl) urea or 4,4′-dinitrocarbanilide (DNC) and 4,6-dimethylpyrimidin-2-ol (HDP). The composition is reported in Table 1.

Table 1: Composition of Monimax®

Ingredients	g/kg Monimax®
Active ingredients	
Monensin(1)	80
Nicarbazin(2)	80
Other ingredients	
Starch for granulation	15
Wheat meal	580
Calcium carbonate	q.s. 1,000

(1): From monensin sodium technical substance containing ≥ 27% monensin activity.
(2): From nicarbazin containing ≥ 95.1% nicarbazin.

The identity of the additive, characterisation of the active substance, manufacturing process and technological properties of the additive have been recently reviewed by the FEEDAP Panel; the production strain has been also characterised and assessed (EFSA FEEDAP Panel, 2017).

Monimax® is intended to be used to prevent coccidiosis in chickens for fattening and chickens reared for laying. The recommended inclusion level of Monimax® in complete feed for chickens for fattening and for chickens reared for laying up to 16 weeks of age is 40 + 40 to 50 + 50 mg monensin + nicarbazin/kg. The applicant proposes a withdrawal period of 1 day.

MRLs for edible chicken tissues are proposed (15,000 μg DNC/kg of fresh liver, 6,000 μg DNC/kg of fresh kidney, 4,000 μg DNC/kg fresh muscle and fresh skin + fat).

3.2. Safety

3.2.1. Safety for the target species

3.2.1.1. Tolerance study in chickens for fattening

A total of 224 one-day-old male and female chickens for fattening (Ross 308) was randomly allocated to four groups with four replicates per sex (five birds + two spare birds for the first week/replicate) each fed diets containing 0, 60 + 60 (1.2× maximum proposed level), 75 + 75 (1.5x) and 100 + 100 (2x) mg monensin + nicarbazin/kg complete feed, respectively, for 42 days.[14] The basal diet consisted mainly of wheat, soya and maize.[15] Nutrient contents of both starter and grower diets were not provided. The starter diet was fed as crumbles for 14 days; the grower as pellets until the end of the study. The birds had *ad libitum* access to feed and water. The intended concentrations of monensin and nicarbazin in the starter and the grower diet were analytically confirmed.

Clinical observations were made daily; body weight and feed intake were recorded at weekly intervals. On day 39, blood samples were taken from one bird per replicate (four males and four females per treatment) for haematology[16] and clinical blood biochemistry.[17] On day 42, one bird/replicate was killed and subjected to necropsy, organ weights were determined for heart, liver, kidneys and spleen. Histopathology was performed for duodenum, ileum, caeca, colon, liver, kidneys, spleen,

[13] Technical dossier/Section II/Annex II.17.
[14] Technical dossier/Section III/Annex III.1.
[15] Technical dossier/Supplementary information February 2017/Annex 12.
[16] Red blood count (RBC), haematocrit, haemoglobin, mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCC), thrombocyte (TBH), white blood cell (WBC) count: heterophils, eosinophils, basophils, monocytes and lymphocytes).
[17] Aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), creatine kinase (CK), total protein, total bilirubin, bile acids (BIAC), total cholesterol, glucose, calcium, inorganic phosphorus, magnesium, sodium, potassium, chloride, uric acid, creatinine.

heart and lungs. Two separate statistical analyses were provided; the first one based on separate datasets for male and female birds, and the second one on all birds.[18] Both analyses were based on analysis of variance (ANOVA) after verification the normal distribution and the homogeneity of the data. If the conditions for applying an ANOVA were not proved nonparametric procedures (i.e. Kruskal–Wallis one-way analysis of variance on ranks, Shirley's or Steel's test) were applied. Covariance analysis was applied to organ weights, and the Fisher's exact test to clinical pathology. Group comparisons were done by the Tukey's test/Dunnett's method.

Four birds in the high-dose group died without preceding clinical symptoms. The main results of the study are summarised in Table 2.

Table 2: Main results of a 6-week tolerance study in chickens for fattening with Monimax®

Mo + Ni (mg/kg feed)(1)	**0 + 0**		**60 + 60**		**75 + 75**		**100 + 100**	
Sex	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**
Final body weight (g)	2,250	2,124	2,214	2,085	2,305	2,076	2,180	1,869*
Feed intake (g/bird and day)	88	90	89	96	94	92	96	84
Feed to gain ratio	1.62	1.71	1.65	1.83	1.67	1.77	1.81*	1.85
WBC (10^9/L)	18.75	17.81	13.75*	11.65*	11.56*	13.75*	10.31*	11.56*
Lymphocytes (10^9/L)	7.75	8.50	6.64	5.30	5.95	7.27	4.97	6.29
Heterophils (10^9/L)	8.84	7.09	5.29*	4.29*	3.89*	4.90*	4.05*	3.60*
Thrombocytes (10^9/L)	7	9	11	14*	12	12*	15*	23*

(1): Mo: monensin; Ni: nicarbazin.
*: Significant change compared to the control within sex ($p \leq 0.05$).

A significant growth depression was observed in the high-dose female group compared to the control group. Feed to gain ratio was also inferior in the high-dose groups, but significant only for males. However, a combined evaluation of both sexes (mean body weights for the four groups were 2,187, 2,150, 2,191 and 2,025 g, respectively; mean feed to gain ratios were 1.67, 1.74, 1.72 and 1.83, respectively) did not indicate significant differences compared to the control group for both endpoints due to treatment or pen.

All treated groups showed a significantly lower level of white blood cells (WBC), compared with the control group, by both statistical methods. However, it was noted that the control values for WBC are high compared to historical data of the applicant (11.7 and 13.0 × 10^9/L for females and males, respectively).[19] All treated groups showed significantly lower numbers of heterophils by both statistical methods (mean values were 7.97, 4.79, 4.40 and 3.82 10^9/L for control, and the groups treated with 60 + 60, 75 + 75 and 100 + 100 mg monensin + nicarbazin/kg, respectively). Thrombocytes were significantly higher for females in all treated groups and for males in the high-dose group only. Group averages of all treated groups (13.0, 11.0, 13.0 10^9/L) were significantly higher than the control group value (8.5 10^9/L). The differences in heterophils and thrombocytes showed no dose relationship (confirmed by a separate ANOVA followed by Tukey's test for the treated groups (group means and gender subgroups) only). Thus, the statistical differences are considered to reflect a control group with values at the ends of the normal range.

Most clinical biochemical parameters (ALP, ALT, AST, LDH, CK, bilirubin, cholesterol, Na, K, Cl, Mg, uric acid, creatinine, glucose, total proteins, albumin) remained unchanged in treated groups when compared with controls. Significant differences were recorded for bile acid, phosphorus and albumin/globulin ratio. Bile acid and phosphorus values were higher in the intermediate-dose group (17.25 vs

[18] Technical dossier/Supplementary information February 2017/Annex 13.
[19] Technical dossier/Supplementary information June 2015/Annex I.

12.26 μmol/L for bile acid and 2.72 vs 2.35 mmol/L for P) than in the control group but there was no dose-related effect and the values in the use level and high-dose groups (14.83 and 13.43 mg μmol/L for bile acid and 2.38 and 2.35 mmol/L for P, respectively) did not differ when compared with controls. The values remained in the physiological range. The difference is most probably a random effect reaching accidentally significance. The albumin/globulin ratio was significantly higher in the high-dose group when compared with control animals. The difference remained marginal (1.036 vs 0.936 in controls), its toxicological significance is questionable.

No significant differences in organ weights were found (both statistical analyses). Macro- and histopathology did not indicate any potentially relevant treatment effect.

Conclusions

Feeding the twofold of the highest proposed use level of Monimax® (100 + 100 mg monensin + nicarbazin/kg) resulted in a reduction of the zootechnical performance. No such effects were seen at 75 + 75 mg monensin + nicarbazin/kg complete feed. Although some differences were seen between treated and control groups in values from haematology and clinical blood chemistry, the FEEDAP Panel considers that these are within the normal range and of questionable toxicological significance.

Monimax® at the highest proposed dose (50 + 50 mg monensin + nicarbazin/kg complete feed) is considered safe for chickens for fattening with a margin of safety of about 1.5.

3.2.1.2. Interactions

Interactions between the polyether ionophore coccidiostats and the diterpene antibiotic tiamulin as well as other antimicrobials (mainly macrolides) were already described by the FEEDAP Panel in 2004 (EFSA, 2004c). Therefore, the simultaneous use of monensin and certain antibiotic drugs (i.e. tiamulin) is contraindicated. The same contraindications would also apply to Monimax® due to its monensin content. However, since this interaction is dose-dependent, it could be expected that the lower feed concentration of monensin from Monimax® would result in reduced severity of interactions.

3.2.1.3. Microbiological safety of the additive

The microbiological safety of monensin sodium and nicarbazin has been assessed recently (EFSA FEEDAP Panel, 2017). The FEEDAP reiterates the same conclusions for the current assessment: monensin has a selective antimicrobial activity against Gram-positive bacterial species while many Enterobacteriaceae are naturally resistant. The use of monensin as a feed additive is not expected to increase shedding of enteropathogens and to induce resistance or cross-resistance to antimicrobials used in human and animal therapy. Nicarbazin has no antimicrobial activity.

3.2.1.4. Conclusions on the safety for the target species

The highest use level, 50 mg monensin and 50 mg nicarbazin/kg complete feed is considered safe for chickens for fattening with a margin of safety of about 1.5. The conclusions reached in chickens for fattening are extended to chickens reared for laying.

The simultaneous use of Monimax® and certain antibiotic drugs (i.e. tiamulin) is contra-indicated.

Monensin has a selective antimicrobial activity against Gram-positive bacterial species while many Enterobacteriaceae are naturally resistant; induction of cross-resistance with clinically relevant antimicrobials or increased shedding of enteropathogenic bacteria was not reported. Nicarbazin has no antimicrobial activity.

3.2.2. Safety for the consumer

3.2.2.1. Absorption, distribution, metabolism and excretion

Monensin sodium

Data concerning the metabolic fate of monensin sodium in chicken, turkey and rat were submitted in former dossiers of Coxidin® and were assessed by the FEEDAP Panel (EFSA, 2005, 2006a). For the present assessment, the same conclusions can be retained which can be summarised as follows: (i) monensin sodium is absorbed to a limited extent and this fraction is eliminated largely through bile, (ii) monensin sodium is metabolised extensively and gives rise to demethylated, oxidised and decarboxylated metabolites, (iii) unchanged monensin represents about 19% of the whole faecal excretion in chicken, up to 40% in turkey (iv) the same metabolites have been found in the excreta

and tissues where they represent each less than 10% of the total monensin derivatives, and (v) the metabolic pathways in the chicken are similar to those in the turkey and rat.

A recent study of the metabolism of monensin sodium in chickens was submitted.[20] Chickens (3 males and 3 females) were administered for eight consecutive days, by gavage and twice a day, ^{14}C-monensin sodium at a nominal dose corresponding to 125 mg/kg feed (analytically confirmed). The excreta were collected each day and 24 h after the last dose. Tissues were collected from birds slaughtered at 1, 3 and 6 h after the last dose. Monensin and metabolites were analysed by liquid chromatography–mass spectrometry (LC–MS). Monensin amounted to 28–31% of the radioactivity excreted, all metabolites being below 10%. Metabolites in tissues and excreta were identified as demethylated forms of monensin and (mono- and di-) hydroxylated monensin (hydroxylation positions not established). These findings are in line with the previous conclusions.

Nicarbazin

Nicarbazin is entirely split in the intestinal tract of birds into its two constituents, DNC and HDP. Consequently, nicarbazin cannot appear as residue in tissues and is therefore of no concern for consumer safety; only its two individual components may generate residues.

Absorption, distribution, metabolism and excretion (ADME) studies including total residue determination have been performed with [^{14}C]-DNC nicarbazin and [^{14}C]-HDP nicarbazin administered in feed under powder form to avoid splitting of the molecular complex in solution (e.g. water or solvent like dimethyl sulfoxide (DMSO) used for gavage). A comparative *in vitro* study of the metabolism of nicarbazin in chicken, turkey and rat hepatocytes was also provided.

[^{14}C]-DNC nicarbazin[21]

In a Good Laboratory Practice (GLP) study, chickens (24-day-old chickens, 3 males and 3 females per group) were administered nicarbazin (both unlabelled and [DNC-phenyl-U-^{14}C]-nicarbazin) and monensin sodium, each at a nominal level of 55 mg/kg feed (analytically confirmed), for 10 consecutive days to reach steady state. Animals were slaughtered after 0.25-, 1- and 2-day additive withdrawal. Identity of metabolites in tissues was examined using liquid chromatography with tandem mass spectrometry (LC–MS/MS) analysis and synthesised reference compounds. After 0.25-day withdrawal time, the metabolic profile was similar in liver, muscle and skin/fat with DNC being the main component detected (68%, 80% and 95%, respectively). 4-Acetylamino-4′-nitrocarbanilide, resulting from the reduction of one nitro group and its subsequent acetylation, represented 18% of the total radioactivity in liver and 10% in muscle. 4,4′-Diacetylamino-carbanilide, resulting from the reduction and acetylation of the two nitro groups, amounted to 12% of the total radioactivity in muscle, 4-acetylamino-4′-nitrocarbanilide up to 10% and an unknown component 12%. In the kidney, 4,4′-diacetylamino-carbanilide was the main component (54%), followed by *N*,*N*′-1,4-phenylenebis-acetamide (27%) resulting from the split of the molecule and DNC (14%).

[^{14}C]-HDP-nicarbazin[22]

A GLP study following the same experimental design as for the DNC study described above, was performed using [HDP-pyrimidyl-2-^{14}C]-nicarbazin. Identification of HDP and metabolites in tissues was performed using LC–MS/MS analysis. After 0.25-day withdrawal time, HDP appeared to be the major compound in the liver of males and females (60% and 53% of total radioactivity, respectively), one major metabolite representing 40% and 37%, and another metabolite 11% (in females only). HDP prevailed in the kidney (92%, average of male and female), muscle (99%) and skin/fat (88%), metabolites being < 10%. None of the metabolites were identified.

In vitro comparison of the metabolic fate of DNC and HDP in chicken, turkey and rat hepatocytes[23]

An *in vitro* comparison of the metabolic fate of DNC in cryopreserved hepatocytes of chicken, turkey and rat was performed, based on high-performance liquid chromatography with high-resolution tandem mass spectrometry (HPLC-high-resolution MS/MS) analysis. Seven to eight metabolites were isolated from hepatocyte incubations of the three species, separated and tentatively identified. The main metabolites produced *in vivo* and already described were identified *in vitro*. Hydroxylation (position not established) followed by glucuronidation or sulfation and glucuronidation of secondary

[20] Technical dossier/Supplementary information June 2015/Annex VII_Reference 5.
[21] Technical dossier/Supplementary information February 2017/Annex 14.
[22] Technical dossier/Supplementary information February 2017/Annex 15.
[23] Technical dossier/Supplementary information February 2017/Annex 16.

amine function were also identified. A large overlap but also differences were observed between animal species. Qualitatively the main enzymes involved in DNC metabolism are nitro-reductase and N-acetyl transferases in chicken, nitro reductase, *N*-acetyl transferases, oxidase and UGTs in turkey and oxidase and UGTs in rat hepatocytes. Quantitatively, no significant difference was observed in the amount of DNC metabolised over time between chicken and turkey.

The *in vitro* metabolism of HDP by chicken, turkey and rat cryopreserved hepatocytes was investigated in the same study using the same experimental design and the analytical approaches used to characterise DNC and metabolites. Only unreacted HDP was detected in any incubation analysed, indicating the absence of significant biotransformation of HDP in the three species.

In conclusion, the metabolic pathways in the chicken are similar to those in the turkey and rat.

3.2.2.2. Residues in tissues

Residue studies were performed with the additive Monimax® to investigate the total and marker residue concentrations in tissues of chickens for fattening.

Monensin

Monensin residues in poultry tissues were determined by LC–MS/MS analytical method with a limit of quantification (LOQ) of 0.0005 mg/kg for all tissues.

In a monensin residue study, chickens (3 males and 3 females day-old per group) were administered feed containing 50 + 50 mg monensin + nicarbazin from Monimax®/kg (analytically confirmed) for 42 days and slaughtered after 6 h (0.25-day withdrawal).[24] Average monensin concentration was 0.008 ± 0.002 mg/kg in the skin/fat and below the LOQ in the liver, kidney and muscle.

In another monensin residue study, chickens (3 males and 3 females, day-old per group) were administered feed containing 50 + 50 mg monensin sodium + nicarbazin from Monimax®/kg (analytically confirmed) for 36 days.[25] The animals were slaughtered after 3, 18, 24 and 48 h withdrawal. After a 3-h withdrawal period, analysis of monensin in tissues showed values of 0.0015 mg/kg liver, 0.0017 mg/kg kidney, 0.0025 mg/kg muscle and 0.0074 mg/kg skin/fat.

Nicarbazin

DNC

In a GLP study,[26] chickens (24-day-old, 3 males and 3 females per group) were administered nicarbazin (both unlabelled and [DNC-phenyl-U-^{14}C]-nicarbazin) and monensin sodium, each at a nominal level of 55 mg/kg feed (analytically confirmed), for 10 consecutive days. Animals were slaughtered after 6, 12 and 48 h (0.25-, 1- and 2-day withdrawal). Total and marker residue concentrations declined rapidly following nicarbazin withdrawal. The results of total residues and marker residue measured after a 0.25-day withdrawal period are presented in Table 3.

Table 3: Total residues (expressed as mg equivalent DNC/kg fresh tissue) and marker residue measured in tissues from chickens (3 males and 3 females) administered for 10 days 55 mg ^{14}C-DNC-nicarbazin and 55 mg monensin sodium/kg feed in powder form, following a 0.25-day withdrawal period

	Liver	Kidney	Muscle	Skin/fat
TRC(1) (mg/kg) ± SD	14.637 ± 2.149	9.705 ± 1.733	2.154 ± 0.596	2.750 ± 0.600
TRC ± 2SD	18.9	13.2	6.1	4.0
MRC(2) (mg/day) ± SD	6.857 ± 0.920	0.806 ± 0.584	0.761 ± 0.207	1.269 ± 0.326
MRC ± 2SD	8.7	2.0	1.2	1.9
RMTR(3)	0.47	0.08	0.35	0.46

(1): Total residue concentration.
(2): Marker residue concentration.
(3): Ratio marker to total residues at 0.25-day withdrawal time.

[24] Technical dossier/Section III/Annex III.8.
[25] Technical dossier/Section III/Annex III.6.
[26] Technical dossier/Supplementary information February 2017/Annex 18.

Marker residue studies were provided for chickens for fattening.[27] Birds (3 males and 3 females, day-old) were fed a diet containing Monimax® (50 + 50 mg monensin + nicarbazin/kg feed). The duration was 35 days.

DNC and HDP residues were analysed by LC–MS/MS analysis with LOQs of 0.1 and 0.11 mg/kg for all tissues, respectively. Mean DNC concentrations plus 2SD measured in the liver, kidney, muscle and skin/fat after 0.25-day withdrawal were 8.331, 1.514, 1.182 and 1.723 mg DNC/kg tissue, respectively. Average HDP concentrations were 0.117 mg/kg in the liver and below the LOQ in the kidney, muscle and skin/fat.

HDP

The quantification of total residues in tissues resulting from the administration of ^{14}C-HDP-nicarbazin to chickens was addressed in the study described above.[26] The results corresponding to 0.25-day withdrawal period are presented in Table 4.

Table 4: Total residues (expressed as mg equivalent HDP/kg fresh tissue) measured in tissues from chickens administered for 10 days 55 mg ^{14}C-HDP-nicarbazin and 55 mg monensin sodium/kg feed under powder form, following a 0.25-day withdrawal period

	Liver	Kidney	Muscle	Skin/fat
TRC (mg/kg) ± SD	0.065 ± 0.019	0.111 ± 0.032	0.053 ± 0.032	0.060 ± 0.018
TRC ± 2SD	0.103	0.175	0.085	0.096

TRC: total residue concentration.

HDP residues are 221, 87, 41 and 46 times lower than the corresponding DNC residues.

3.2.2.3. Residues in eggs

From a study assessed by the FEEDAP Panel in 2011, monensin residues in eggs of chickens reared for laying fed diets containing monensin from Coxidin® at onset of laying were below the LOQ (EFSA FEEDAP Panel, 2011a).

A study was performed to determine the marker residue of nicarbazin (DNC) in the first eggs laid by chickens reared for laying.[28] Three groups of animals (12 per group, day-old) were fed a blank feed until 8 weeks of age, followed by the administration of a complete feed supplemented with 50 mg nicarbazin from Monimax®/kg (analytically verified) for 4, 6 and 8 weeks ending to 12, 14 and 16 weeks of age, respectively. From the end of the Monimax® feeding period until onset of laying, a blank feed was given. The first 20 eggs after onset of laying were collected of which ten eggs were analysed (mixed yolk and albumen of each egg) by LC–MS/MS (LOQ of 0.005 mg/kg whole egg, limit of detection (LOD) of 0.0005 mg/kg) for DNC content. In the group of 16-week old birds (8 weeks exposure to Monimax®), the first three eggs collected 9, 12 and 14 days after withdrawal showed DNC residues of 0.260, 0.044 and 0.027 mg/kg, respectively, the seven eggs collected later were below the LOQ. No residues were detected in all eggs collected in the groups corresponding to 14- and 12-week animals.

3.2.2.4. Toxicological studies

The toxicity of nicarbazin and monensin sodium was assessed by the FEEDAP Panel in 2017 (EFSA FEEDAP Panel, 2017) and it was concluded that:

> Monensin sodium is not genotoxic in an adequate range of studies and has shown no structural alert for carcinogenesis. Monensin sodium is not a reproductive or developmental toxin based on adequate current studies in rat and rabbit. The lowest NOEL identified in the developmental study in rabbits is 0.3 mg monensin sodium/kg bw per day for maternal toxicity in rabbits.

Nicarbazin showed mutagenic activity in the *Salmonella* Typhimurium TA98 strain in the presence and in the absence of metabolic activation, while the substance was negative in the other bacterial strains. Negative results were reported also in a gene mutation assay in L5178Y TK+/– mouse lymphoma cells and in a chromosome aberration test in human lymphocytes *in vitro*. Moreover nicarbazin did not show any mutagenic activity in an *in vivo* micronucleus test in rat in conditions

[27] Technical dossier/Supplementary information February 2017/Annex 17.
[28] Technical dossier/Supplementary information January 2014/Annex 4.

warranting the exposure of the target cells to the test substance. The primary toxicity resulting from the oral use of nicarbazin is renal toxicity. The absence of similar findings after treatment with DNC and HDP confirms that this equimolar association of compounds is better tolerated than nicarbazin at equivalent doses. At parentally toxic doses (renal effects) there is no impairment of reproductive performance in rats treated with a combination of DNC/HDP at doses up to 580/193 mg/kg bw per day. The NOAEL for embryo/foetal development is 120 mg nicarbazin/kg bw of rabbits per day. The lowest NOAEL identified in a 52-week study in rat using DNC + HDP was 20 mg DNC + 8 mg HDP/kg bw per day based on the occurence of microcrystals in urine and related microscopic renal observations at higher dose level.

When the combination of monensin sodium and DNC + HDP was tested in a bacterial reverse mutation test, the results were consistent with the findings of separate tests with monensin sodium and nicarbazin. The FEEDAP Panel concludes that the active substances in Monimax®, monensin sodium and nicarbazin, do not represent a genotoxic risk. Furthermore, there was no evidence resulting from the toxicological studies for any significant interaction between monensin sodium and nicarbazin'.

The toxicity of the nicarbazin impurities, p-nitroaniline (PNA) and methyl(4-nitrophenyl) carbamate (M4NPC), were assessed in the same opinion (EFSA FEEDAP Panel, 2017) and it was concluded that no safety concerns would arise from the nicarbazin impurities PNA and M4NPC in Monimax®.

3.2.2.5. Assessment of consumer safety

For monensin, the current provisional MRLs in chickens for fattening (25 μg/kg wet skin + fat and 8 μg/kg wet liver, kidney and muscle) ensure consumer safety. Residue data obtained for monensin after the use of the highest proposed level of Monimax® in feed (50 + 50 mg monensin + nicarbazin/kg) for chickens for fattening (Section 3.2.2.2) showed that after withdrawal times of 3 and 6 h in chickens all marker residue concentrations were below the respective MRLs in liver, kidney, muscle and skin + fat. The withdrawal times applied are considered equivalent to 0-day under practical conditions.

As the consumer will not be exposed to nicarbazin but to DNC and HDP, and DNC residues are orders of magnitude higher than HDP residues (see Tables 3 and 4), DNC is considered the marker residue.

A health-based guidance value (acceptable daily intake (ADI)) for the nicarbazin moieties (DNC and HDP) can be derived from the NOAEL of 20 mg DNC and 8 mg HDP/kg body weight (bw) based on the absence of microcrystals in the urine obtained in a 52-week study in rat using DNC + HDP. The ADI is 0.2 mg DNC and 0.08 mg HDP/kg bw applying an uncertainty factor of 100.

Exposure of the consumer to DNC was calculated applying the food basket of Regulation (EC) No 429/2008 (Table 5).[29] Consumer exposure to total DNC residues from chicken tissues showed compliance with the ADI (35%) after practical 0-day withdrawal. Similar calculation carried out with HDP total residues (from Table 5) indicates compliance with the corresponding ADI (dietary intake of HDP total residues is 0.047 mg/day corresponding to about 1% of the ADI).

For nicarbazin, MRLs for DNC of 15 mg/kg liver, 6 mg/kg kidney and 4 mg/kg muscle and skin/fat in chickens for fattening are in force at EU level. Although these MRLs were derived from an ADI of 0.77 mg DNC/kg bw per day, they comply with the newly derived ADI (see Table 5). Calculation of consumer exposure, using the MRLs and applying the ratio marker to total residues (RMTR) of the recent residue study in chickens (Section 3.2.2.2), results in an exposure of about 70% of the new ADI.

Table 5: Consumer exposure to DNC in tissues of chickens for fattening and compliance with the ADI

	Liver	Kidney	Muscle	Skin/fat	Sum
TRC (mg/kg) + 2SD	18.9	13.2	6.1	4.0	
DITR(1) (mg/day)	1.89	0.13	1.83	0.36	4.21 (35% ADI)
RMTR(2)	0.47	0.08	0.35	0.46	
MRL (mg/kg)	15	6	4	4	
DITRMRL(3) (mg/day)	3.2	1	3.4	0.8	8.4 (70% ADI)

(1): Dietary intake of total residues.
(2): Ratio marker to total residues at 0.25 day withdrawal time.
(3): Dietary intake of total residues calculated from the MRLs.

Compliance with the MRLs is given at zero day withdrawal time.

[29] OJ L 133, 22.5.2008, p. 1.

Monensin residues in eggs of chickens reared for laying fed diets containing 125 mg monensin from Coxidin®/kg feed at onset of laying were below the limit of quantification in a study assessed in 2011 (EFSA FEEDAP Panel, 2011a). Considering that the dose of monensin sodium from the use of Monimax® is lower (50 mg monensin/kg feed), no safety concern is expected for the consumer of eggs from chickens fed Monimax® under the proposed conditions of use. Consumer exposure to DNC by the use of Monimax® at the maximum applied use level for chickens reared for laying from the first egg laid 9 days after cessation of the supplemented feed (given up to 16 weeks of age) would be negligibly low compared to the intake from chicken tissues (0.03 mg *vs*. 4.21 mg DNC/person per day applying the food basket of Regulation (EC) No 429/2008 for eggs).

No residues were found in chicken muscle and kidney (< 0.002 mg/kg), when exposed to about the fivefold M4NPC dietary level compared to the highest use level applied for Monimax®; residues in liver and skin/fat were 0.009 and 0.013 mg/kg, respectively. Consequently, M4NPC in Monimax® is considered safe for the consumer provided that a maximum concentration of 0.4% in nicarbazin would not be exceeded. No safety concern would arise from the impurity PNA if the maximum content in nicarbazin of 0.1% is respected.

3.2.2.6. Conclusions on the safety for the consumer

The use of Monimax® at the highest proposed dose (50 + 50 mg monensin + nicarbazin/kg complete feed) will not pose a risk to persons consuming animal products from treated chickens for fattening. This conclusion is extended to chickens reared for laying up to 16 weeks of age.

No safety concern would arise from the impurity PNA if the maximum content in nicarbazin of 0.1% is respected. The other nicarbazin related impurity, M4NPC, is considered safe for the consumer provided that a maximum concentration of 0.4% in nicarbazin would not be exceeded.

No withdrawal time is required for Monimax® in chickens for fattening.

3.2.3. Safety for the user

The safety for the user of Monimax® has been assessed recently (EFSA FEEDAP Panel, 2017). The FEEDAP reiterates the same conclusions for the current assessment.

'In the absence of data performed with Monimax®, the FEEDAP concluded on the safety for the user based on the data available on its individual components. The monensin sodium contained in Monimax® presents a hazard by inhalation. Although the dusting potential of Monimax® is low, users will be exposed to monensin by inhalation indicating a risk to persons handling Monimax®.

Monimax® is not a skin irritant; however, no data are available for the eye irritation potential of monensin. Monimax® may also act as a dermal toxicant due to its monensin component. Monimax® is not a skin sensitiser'.

3.2.4. Safety for the environment

Active substances are not physiological/natural substances of established safety for the environment. The additive is also not intended for companion animals. Consequently, according to Regulation (EC) No 429/2008[29] the Phase I assessment has to be continued to determine the predicted environmental concentration.

In Phase I and II initially a total residues approach will be taken, meaning that the predicted environmental concentrations (PECs) will be calculated, based on the assumption that the additives are excreted 100% as parent compound. Nicarbazin is an equimolar complex of DNC and HDP in a 70:30 w/w ratio, which splits during the intestinal passage. Consequently, the environmental risk assessment should not consider nicarbazin but both components separately. Distribution in the environment is based on the properties of the individual components of Monimax® as long as no data on relevant metabolites and on potential interaction are submitted.

MONENSIN SODIUM

3.2.4.1. Phase I

Physico-chemical properties

The physical–chemical properties of monensin sodium are summarised in Table 6. The dissociation constant pKa was not provided by the applicant but Sun et al. (2016) reported a pKa of 4.5.

Table 6: Physico-chemical properties of monensin sodium

Property	Value	Unit
Octanol/water partition coefficient (log K_{ow})(1)	4.48 (pH 5.2–5.7, 25°C) 3.82 (pH 7, 25°C) 3.82 (pH 10, 25°C)	–
Water solubility (20°C)(2)	8.78	mg/L
Dissociation constant (pKa)	4.5	–
Vapour pressure(3)	3.03×10^{-28}	Pa

(1): Technical dossier/Supplementary information June 2015/Annex 4.
(2): Technical dossier/Supplementary information June 2015/Annex 27.
(3): EPI Suite, 2015.

Studies assessing monensin sodium adsorption/desorption and biodegradation in soil were assessed by the FEEDAP Panel in its opinion on the safety and efficacy of Monimax® for turkeys (EFSA FEEDAP Panel, 2017); the same conclusions on the fate and behaviour of monensin sodium can be retained for the current assessment: 'A K_{oc} of 74.1 L/kg and a DT_{50} of 2.5 days at 20°C will be used for the assessment'.

Predicted environmental concentrations (PECs)

The methodology for the calculation of the maximum predicted environmental concentrations (PECs) in soil, groundwater, surface water and sediment are described in the technical guidance for assessing the safety of feed additives for the environment (EFSA, 2008a).

The input values used for monensin A sodium were: 50 mg/kg broiler feed, molecular weight 693.8, vapour pressure 3×10^{-28} Pa, solubility 8.78 mg/L, DT_{50} 2.5 days and K_{oc} 74.1 L/kg. The calculated values are given in Table 7.

Table 7: Initial predicted environmental concentration (PECs) of monensin sodium, in soil (μg/kg), groundwater (μg/L), surface water (μg/L) and sediment (μg/kg dry weight)

Compartment	PEC
Soil	260
Ground water	182
Surface water	61
Sediment	322

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

PEC_{soil} refined for metabolism

In a former assessment of the environmental impact of monensin from Coxidin® (EFSA FEEDAP Panel, 2011a), the FEEDAP Panel considered that about 20% of the monensin sodium ingested by chickens was excreted unchanged. No information was given on the mode of administration of labelled monensin (single or multidose). In a newly submitted study (see Section 3.2.2.1),[30] ^{14}C-monensin was administered for eight consecutive days at the same time as nicarbazin in order to take account of an eventual interaction of both compounds. About 30% of the ingested monensin sodium was excreted unchanged, numerous metabolites representing each < 10%. As these experimental conditions mimic the practical use of Monimax®, the FEEDAP Panel considered that about 30% of the monensin ingested by chickens was excreted unchanged.

Assuming that the ionophoric activity of monensin sodium and its metabolites in chicken excreta will not exceed in total 30% of the orally administered dose, the refined dose used for PEC calculations was $50 \times 0.3 = 15$ mg/kg feed. The refined PEC_{soil}, $PEC_{groundwater}$, $PEC_{surfacewater}$ and $PEC_{sediment}$ are reported in Table 8.

[30] Technical dossier/Supplementary information June 2015/Annex 15.

Table 8: Refined predicted environmental concentrations (PECs) of monensin sodium, in soil (μg/kg), groundwater (μg/L), surface water (μg/L) and sediment (μg/kg dry weight)

Input	Value
Dose (mg/kg feed)	15
Molecular weight	693.8
Vapour pressure (Pa) (at 25°C)	3×10^{-28}
Solubility (mg/L)	8.78
K_{oc} (L/kg)	74.1
DT_{50} in soil at 20°C (days)	2.5
Output	
Application rate kg/ha	0.234
PEC_{soil}	78
$PEC_{groundwater}$	55
$PEC_{surface\ water}$	18
$PEC_{sediment}$	97

When the $PEC_{groundwater}$ is set equal to the concentration in pore water based on a worst-case assumption (the total residue approach), the concentration exceeds the trigger value of 0.1 μg/L identified by the EU as quality standard.[31]

$PEC_{groundwater}$ refined with FOCUS

Leaching of monensin to groundwater was simulated using the FOCUS recommended leaching model PEARL (FOCUS Version 4.4.3) (EFSA, 2008c). The calculated groundwater concentrations for the scenarios Jokioinen and Piacenza were below 0.001 μg/L.

$PEC_{surface\ water}$ and $PEC_{sediment}$ refined with FOCUS

Concentrations in surface waters for monensin were assessed using the FOCUS Step 3 surface water assessment approach. The FOCUS recommended surface water models PRZM_SW, MACRO and TOXSWA were used (EFSA, 2008c). Generally, run-off was calculated from a soil receiving 0.234 kg monensin/ha which was homogeneously distributed into a 20-cm deep layer. In the sensitive R1-stream run-off scenario from FOCUS 4.4.3 using SPIN 2.2, the global maximum $PEC_{surface\ water}$ was 0.69 μg/L. The $PEC_{sediment}$ value was 0.11 μg/kg dry weight.

Conclusions on PEC used for calculation

The following values are used for the assessment: PEC_{soil} of 78 μg/kg, $PEC_{surface\ water}$ of 0.69 μg/L and $PEC_{sediment}$ 0.11 μg/kg dry weight.

Ecotoxicity studies

The effects of monensin sodium on the aquatic, terrestrial and sediment compartments were studied in a number of ecotoxicity studies already assessed in the FEEDAP opinion on the safety and efficacy of Monimax® for turkeys (EFSA FEEDAP Panel, 2017). The same conclusions can be retained for the current assessment:

'For the terrestrial compartment, data are available for micro-organisms, earthworms and plants. The lowest toxicity value for the terrestrial compartment of EC_{50} 4 mg monensin/kg was found for plant species *Sinapis alba.*

For the aquatic compartment, data are available for algae, aquatic invertebrates, fish and information from public literature on the effect of monensin sodium on zooplankton. The ErC_{50} and ErC_{10} used in the assessment are 3.3 mg/L and 0.91 mg/L for algae, respectively. The 48h EC_{50} for immobilisation of daphnids was determined to be 7.29 mg monensin sodium/L and the 96h LC_{50} for fish was 1.88 mg monensin sodium/L. The NOEL from the microcosmos study on zooplankton of 0.05 mg monensin sodium/L is as well used in the assessment.

Ecotoxicological data for sediment-dwelling invertebrates are provided for the sediment compartment. The NOEC was determined as 5.0 mg monensin sodium/kg sediment (dry weight)'.

[31] Directive 2006/118/EC of the European Parliament and of the Council of 12 December 2006 on the protection of groundwater against pollution and deterioration. OJ L 372, 27.12.2006, p. 19.

Risk characterisation

The risk characterisation ratios for terrestrial, freshwater and sediment compartments are reported in Tables 9, 10 and 11, respectively.

The previous risk characterisation for the terrestrial compartment (EFSA, 2005; EFSA FEEDAP Panel, 2011a) for plants was based on a NOEC. A re-assessment of the original data indicated a large variability between the individual data and showed that the NOEC values would be less reliable than the EC_{50} values. Therefore, the FEEDAP Panel decided to base the risk characterisation of plants on the EC_{50} values.

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

Taxa	PEC_{soil} (µg/kg)	EC_{50}/LC_{50} (mg/kg)	AF	PNEC (µg/kg)	PEC/PNEC
Earthworm	78	108.8(1)	100	1,088	0.07
Plants		4.0(2)	100	40	1.95

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

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

Taxa	$PEC_{surfacewater\ FOCUS}$ (µg/L)	ErC_{50}/ErC_{10}/NOEL (mg/L)	AF	PNEC (µg/kg)	PEC/PNEC
Algae *Selenastrum capricornutum*	0.69	3.3(1) 0.91(2)	1,000 1,000	3.41 0.91	0.2 0.76
Aquatic invertebrates *Daphnia magna*		7.29(3)	1,000	7.3	0.09
Fish *Brachydanio rerio*		1.83(4)	1,000	1.83	0.38
Microcosm	0.69	0.05(5)	10	5	0.14

AF: assessment factor.
(1): ErC_{50}.
(2): ErC_{10}.
(3): 48-h EC_{50}.
(4): 96-h LC_{50}.
(5): NOEL.

Table 11: Risk characterisation (PEC/PNEC ratio) for sediment

Taxa	$PEC_{sediment}$ (µg/kg dry weight)	NOEC (mg/kg)	AF	PNEC (µg/kg)	PEC/PNEC
Sediment-dwelling invertebrates *Chironomus riparius*	0.11	5.0	10	0.485	0.23

AF: assessment factor.

Bioaccumulation

No data on bioaccumulation on monensin sodium were submitted. The log K_{ow} at pH 7 is 3.2 and there is evidence that monensin is degraded in the animal body (see Section 3.2.2.1). Therefore, a risk for bioaccumulation is unlikely.

NICARBAZIN (DNC and HDP)

3.2.4.3. Phase I

Physico-chemical properties

Table 12: Physical–chemical properties of DNC

Property	Value	Unit
Octanol/water partition coefficient (log Kow)(1)	3.25 (pH 5) 3.21 (pH 7) 3.23 (pH 9)	–
Water solubility (20°C)(1)	0.0209 (pH 5–9, 20 ± 0.5°C)	mg/L
Dissociation constant (pKa)(2)	12.44 ± 0.70(3)	–
Vapour pressure(1)	3.1×10^{-10}	Pa

(1): Technical dossier/Supplementary information June 2015/Annex 9.
(2): Technical dossier/Supplementary information June 2015/Annex 3.
(3): Estimated value since the substance exhibits insufficient water solubility and ultraviolet-visible absorptivity to enable experimental determination.

Table 13: Physical–chemical properties of HDP

Property	Value	Unit
Octanol/water partition coefficient (log Kow)(1)	−0.9546 (pH 5) −0.9232 (pH 7) −0.9528 (pH 9)	–
Water solubility (20°C)(1)	66,740 (pH 5, 20°C) 65,400 (pH 7, 20°C) 70,290 (pH 9, 20°C)	mg/L
Dissociation constant (pKa)(1)	3.75 (25°C)	–
Vapour pressure(1)	9.084×10^{-6} (20°C) 1.834×10^{-5} (25°C)	Pa

(1): Technical dossier/Supplementary information June 2015/Annex 8.

The physical–chemical properties of DNC and HDP are summarised in Tables 12 and 13.

Studies assessing DNC and HDP adsorption/desorption and biodegradation in soil were assessed by the FEEDAP Panel in its opinion on the safety and efficacy of Monimax® for turkeys (EFSA FEEDAP Panel, 2017); the same conclusions on the fate and behaviour of DNC and HDP can be retained for the current assessment: 'For DNC, a K_{oc} of 74,128 and a DT_{50} of 1,191 days will be used for the assessment; for HDP a K_{oc} of 102 and a DT_{50} of 2.3 days will be used for the assessment'.

Predicted environmental concentrations (PECs)

The input values for DNC used were: 35.44 mg/kg broiler feed, molecular weight 302.24, vapour pressure 3.1×10^{-10} Pa, solubility 0.0209 mg/L, DT_{50} 1,191 days and K_{oc} 74,128 L/kg. The input values for HDP used were: HDP concentration in turkey feed 14.56 mg/kg, molecular weight 124.14, vapour pressure 9.084×10^{-6} Pa, solubility 65,400 mg/L, DT_{50} 4.9 days and K_{oc} 102 L/kg.

The calculated PEC initial values for both DNC and HDP are given in Table 14.

Table 14: Initial predicted environmental concentrations (PECs) of DNC and HDP (μg/kg), in soil (μg/kg), groundwater (μg/L), surface water (μg/L) and sediment (μg/kg dry weight)

Input	Value	
	DNC	HDP
Dose (mg/kg feed)	35.44	14.56
Molecular weight	35.44	124.14
Vapour Pressure (Pa) (at 25°C)	3E-10	9E-6
Solubility (mg/L)	0.029	65,400
K_{oc} (L/kg)	74,128	102
DT_{50} in soil at 12°C (days)	1,191	4.9
Output		
PEC_{soil}	184	76
$PEC_{groundwater}$	0.141	40
$PEC_{surfacewater}$	0.047	13
$PEC_{sediment}$	174	88

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

3.2.4.4. Phase II

Exposure assessment

PECs calculation refined in Phase II

DNC – refinement of PEC_{soil} for persistent compounds

The DT_{90} for DNC in all four soils was determined to be greater than 1 year, therefore the PECs refined at steady state was calculated (EFSA, 2008a). The results are provided in Table 15.

Table 15: Refined plateau predicted environmental concentrations (PECs) of DNC in soil (μg/kg), groundwater (μg/L), surface water (μg/L), and sediment (μg/kg dry weight)

Compartment	$PEC_{plateau}$
Soil	962
Ground water	0.74
Surface water	0.24
Sediment	909

DNC – $PEC_{surface\ water}$ $PEC_{sediment}$ refined with FOCUS

Concentrations in surface waters for DNC were assessed using the FOCUS Step 3 surface water assessment approach. The FOCUS recommended surface water models PRZM_SW, MACRO and TOXSWA were used.[32] A FOCUS PRZM calculation was performed with an application rate of 552 g/ha, a K_{oc} of 74,128 L/kg and a DT_{50} of 562 days at 20°C. According to the manual, the drainage scenarios D3 and D5 were selected together with the run-off scenarios R1 and R3. For the run-off scenarios a homogeneous distribution of the DNC in 20 cm soil layer was assumed.

After 20 years, 3,600 g/ha was accumulated in the soil. This corresponds to 1,200 μg DNC/kg soil. Due to the high K_{oc}, there was no drainage of DNC to groundwater, surface water or sediment.

The run-off scenarios revealed a different picture. The R3 stream scenario indicated a maximum time weighted average exposure concentration of DNC in surface water to be 0.027 μg/L in the first year. The corresponding sediment concentration was 54 μg/kg sediment. Since the run-off will be 3,600/552 = 6.5 times higher after 20 years, the surface water concentration will be 6.5 x 0.027 = 0.18 μg/L. The sediment concentration can exceed 6.5 × 54=351 μg/kg since DNC might accumulate over the years in sediment. Nitroaromatic compounds can be reduced in sediment to the corresponding anilines (van Beelen and Burris, 1995). Nevertheless, no data on the

[32] Technical dossier/Supplementary information February 2017/Annex 27.

transformation of DNC in sediment were provided by the applicant. Especially in aerobic sediments, there might be a continuous increase in DNC concentrations over the years.

Conclusions on PEC used for calculation

The following values are used for the assessment: for DNC a PEC_{soil} of 1,200 μg/kg, a $PEC_{surface\ water}$ of 0.18 μg/L and a $PEC_{sediment}$ of > 351 μg/kg; for HDP a PEC_{soil} of 76 μg/kg, $PEC_{surface\ water}$ of 13 μg/L and $PEC_{sediment}$ of 88 μg/kg.

Ecotoxicity studies

The effects of DNC and HDP on the aquatic, terrestrial and sediment compartments were studied in a number of ecotoxicity studies already assessed in the FEEDAP opinion on the safety and efficacy of Monimax® for turkeys (EFSA FEEDAP Panel, 2017). The same conclusions can be retained for the current assessment:

'For the terrestrial compartment, data were provided for micro-organisms, earthworms and plants. For the effect of DNC and HDP on the reproduction of earthworms, the NOEC was set on 300 and 123.46 mg/kg (dry weight), respectively. In two of six tested plant species, a statistically significant effect of the equimolar mixture of DNC and HDP was observed, however the effect concentration could not be calculated. The FEEDAP Panel calculated the effect concentration based on information provided in the test report. Assuming the worst case, the EC_{50} for plants is set to 102 mg/kg and 248 mg/kg of HDP and DNC, respectively.

For the aquatic compartment, data for DNC are available for acute and chronic effect on algae, aquatic invertebrates and on acute toxic effects on fish. No effect of DNC could be observed in any of performed tests, thus, the applicant proposes the lowest concentration tested as the NOEC value. The DNC NOEC for algae is set to 10.13 μg/L. The 21-day NOEC for reproduction of daphnids was determined to be 4.51 μg/L. The 96 h LC_{50} in fish was determined to be > 5.4 μg/L. Data for the toxic effect of HDP on the aquatic compartment was studied in tests of acute and long term effect on algae and acute effect on daphnids and fish resulting in NOEC value of 101.5 mg/L for algae. The LC_{50} and EC_{50} for fish and immobilisation for Daphnia were determined to be > 100 mg/L, respectively. Data on the effect of DNC and HDP on cyanobacteria are considered as supportive information.

Ecotoxicological data for the DNC and HDP for sediment-dwelling invertebrates are provided for the sediment compartment. The NOEC for DNC and HDP were determined as 241.1 and 556.7 mg/kg sediment (dry weight), respectively'.

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

The risk characterisation ratios for terrestrial, freshwater and sediment compartments are reported in Tables 16–20.

Table 16: Risk characterisation (PEC/PNEC ratio) for DNC and for HDP for the terrestrial compartment

	Taxa	PEC_{soil} (μg/kg)	EC_{50}/NOEC (mg/kg)	AF	PNEC (μg/kg)	PEC/PNEC
DNC	Earthworm	1,200	300(1)	10	30,000	0.032
	Plants		248(2)	100	2,480	0.5
HDP	Earthworm	76	123.46(1)	10	12,346	0.006
	Plants		102(2)	100	1,020	0.07

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

Table 17: Risk characterisation (PEC/PNEC ratio) for the freshwater compartment for the DNC

Taxa	$PEC_{surfacewater}$ (µg/L)	LC_{50}/NOEC (µg/L)	AF	PNEC (µg/kg)	PEC/PNEC
Algae *Selenastrum subspicatus*	0.18	10.13(1)	50	0.20	0.9
Aquatic invertebrates *Daphnia magna*		4.51(1)	50	0.09	2
Fish *Brachydanio rerio*		> 5.4(2)		/	/

AF: assessment factor.
(1): NOEC.
(2): 96-h LC_{50}.

According to the FEEDAP guidance on the environmental risk assessment for feed additives (EFSA, 2008c), two chronic tests on the aquatic compartment would allow a risk assessment of this compartment. Chronic tests with DNC were provided for algae and aquatic invertebrates, with HDP only for algae.

Table 18: Risk characterisation (PEC/PNEC ratio) for the freshwater compartment for the HDP

Taxa	$PEC_{surfacewater}$ (µg/L)	EC_{50}/LC_{50}/NOEC (mg/L)	AF	PNEC (µg/kg)	PEC/PNEC
Algae *Selenastrum subspicatus*	13	101.5(1)	1,000	101.5	0.13
Aquatic invertebrates *Daphnia magna*		> 100 mg/L(2)	/		
Fish *Brachydanio rerio*		> 100 mg/L(3)	/		

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

Table 19: Risk characterisation (PEC/PNEC ratio) for sediment for the DNC

Taxa	$PEC_{sediment}$ (µg/kg)	NOEC (mg/kg)	AF	PNEC (µg/kg)	PEC/PNEC
Sediment-dwelling invertebrates *Chironomus riparius*	> 351	241	10	24,100	/

AF: assessment factor.

Table 20: Risk characterisation (PEC/PNEC ratio) for sediment for the HDP

Taxa	$PEC_{sediment}$ (µg/kg)	NOEC (mg/kg)	AF	PNEC (µg/kg)	PEC/PNEC
Sediment-dwelling invertebrates *Chironomus riparius*	88	556.7	10	55,670	0.001

AF: assessment factor.

Bioaccumulation

No data on bioaccumulation have been submitted. The log K_{ow} for DNC is > 3 for HDP < 3. The high persistence and hydrophobicity of DNC indicate that there might be a risk for bioaccumulation.

3.2.4.5. Conclusions on safety for the environment

The use of monensin sodium from Monimax® in complete feed for chickens for fattening does not pose a risk for the aquatic compartment and sediment, while a risk cannot be excluded for the terrestrial compartment based on the results of an ecotoxicity test on plants. The bioaccumulation potential of monensin in the environment is low.

A final conclusion on the risk resulting from the use of nicarbazin from Monimax® in chickens for fattening cannot be made for the following reasons: (i) DNC refined PECs showed uncertainties linked to the very high persistence of the compound, (ii) DNC might accumulate in the sediment compartment, and (iii) DNC can potentially bioaccumulate and may cause secondary poisoning. The PEC/PNEC ratios indicate a risk for daphnids but no adverse effect were seen at the concentration tested. This adds further uncertainty to the risk assessment of DNC in the aquatic compartment. No concerns would arise for the HDP moiety of nicarbazin excreted from chickens fed Monimax®.

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 Monimax® for the environmental risk assessment. This conclusion is extended to chickens reared for laying.

3.3. Efficacy

Efficacy data for coccidiostats (following Art. 4 of Reg. (EC) No 1831/2003) should derive from three types of target animal experiments (Reg. (EC) No 429/2008): (a) dose–titration studies (b) natural/artificial infection to simulate use conditions (e.g., floor pen studies with poultry), at least one of the locations should be in the EU, and (c) actual use conditions in field trials; all should be done in the EU within the last 5 years. Anticoccidial sensitivity tests (AST) could replace field trials provided they follow the criteria mentioned in the guidance document on coccidiostats and histomonostats (EFSA FEEDAP Panel, 2011b).[33]

3.3.1. Efficacy studies in chickens for fattening

The applicant submitted two dose–titration studies, six floor pen studies, one field trial and four ASTs performed with chickens for fattening.

3.3.1.1. Dose–titration studies in chickens for fattening

The applicant submitted two dose–titration studies made under controlled conditions in chickens for fattening artificially infected with mixed *Eimeria* spp. The efficacy of Monimax® was also compared with that of the single anticoccidial components of Monimax®, monensin sodium (in both studies) and nicarbazin (in one study). In both studies, oocyst excretion and intestinal lesion scores were determined as specific endpoints in addition to the zootechnical parameters. An ANOVA was performed with the data; group means were compared with the Tukey's test.

In the first dose–titration study, a total of 504 one-day-old male and female chickens for fattening (Ross) were distributed to seven treatment groups (12 pens per treatment, six chickens per pen); an uninfected untreated control group (UUC) was compared with an infected untreated control (IUC) and five infected treated groups (IT).[34] The treatments were 100 mg monensin/kg feed (IT-Mo), 125 mg nicarbazin/kg (IT-Ni) and 30 + 30 (IT-MoNi 30), 40 + 40 (IT-MoNi 40) and 50 + 50 (IT-MoNi 50) mg monensin + nicarbazin/kg feed; dosage was analytically confirmed. On day 16, the groups IUC and IT were inoculated with about 100,000 *Eimeria* oocysts per chicken via water for drinking. The anticoccidial dietary treatment was provided from day 1 until day 35 (study completion). At the end of the study, no differences in body weight were observed. Faecal oocyst counts per gram (OPG) in the infected groups did mostly not differ significantly; an interpretation of these data is limited due to the high variability between pens. Among infected groups, lesion scores in the different intestinal segments showed best values in the IT-MoNi 50 group followed by the IT-MoNi 40, IT-Mo and IT-Ni; however, none of the scores was significantly different to the IUC group. No lesions were seen in the UUC group.

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

[34] Technical dossier/Section IV/Annex IV.1.

In the second dose–titration study, a total of 210 one-day-old chickens for fattening (Ross) was distributed to seven treatment groups (six pens per treatment, five chickens per pen).[35] The original study design could not be followed due to an error in feed attribution to the groups. In fact, there was an IUC group with 12 pens (2 × 6) and five treated groups with six pens each: an uninfected treated group (UTC-Ni) receiving 125 mg nicarbazin/kg feed and four infected treated groups receiving 100 mg monensin/kg feed (IT-Mo), 40 + 40 (IT-MoNi 40), 50 + 50 (IT-MoNi 50) and 60 + 60 (IT-MoNi 60) mg monensin + nicarbazin/kg feed. On day 18, 180 birds were inoculated with about 500,000 sporulated oocysts of *Eimeria* spp. The anticoccidial dietary treatments were provided from day 15 until day 30 (study completion). OPG values measured on day 23 and 30 showed a reduction in oocyst excretion in the IT groups compared to the IUC group. The difference was found to be significant only on day 23. There was no difference among the treatment groups. Lower lesion scores were observed in IT groups with respect to the IUC group but statistical significance was reached only in group IT-MoNi 60. For the total duration of the study, no difference was observed among the infected groups in the performance of the birds. In the first week after inoculation, average daily weight gain in the IT groups was significantly higher than in the IUC.

3.3.1.2. Floor pen studies in chickens for fattening

Three floor pen studies in chickens for fattening, conducted in 2011, were submitted.[36] In each study, male chickens (Ross 308) were penned and distributed into three treatment groups: an UUC group, an IUC group and an IT group. The IT group received feed containing 40 mg monensin and 40 mg nicarbazin/kg feed. In the infected groups, individually tagged seeder birds (10 out of 60 per pen in trial 1, 10 out of 33 per pen in trial 2 and 8 out of 37 in trial 3) were inoculated with recent field isolates of pathogenic *Eimeria* species. The same number of animals were sham-inoculated (water only) in the UUC group. Mortality could not be considered as an endpoint indicating coccidiostatic efficacy since it was low in two studies. In one study the mortality was affected by oocyst inoculation but no improvement was seen due to the treatment. The *Eimeria* species specific lesion scores were determined in three floor pen studies at three different time points (resulting in a total of 27 data sets). The seeder bird model used in the floor pen studies did not appear very sensitive in indicating a disease protecting effect of the coccidiostat in all birds used (the severity of intestinal lesions in contact birds was affected by the infection only in 2 out of 18 data sets while in seeder birds in 6 out of 9 data sets). The coccidiostat reduced significantly the severity of intestinal lesions only in one of 18 cases in contact birds and in one of 9 cases in seeder birds.

Treatment with Monimax® did not significantly reduce oocyst excretion in the three floor pen studies measured at three time points for six different *Eimeria* species each.

Feed intake and body weight gain of birds in the floor pen studies were significantly higher for the infected treated groups compared to the infected non treated birds in the floor pen studies. However, in the view of the FEEDAP Panel, these observations could not be used to derive a coccidiostatic effect, since none of the specific endpoints (mortality, oocyst excretion and intestinal lesions) showed such effect.

Three additional floor pen studies, conducted in 2018, were submitted.[37] The first two studies were conducted in the same research institute, at the same time, with the same feed, and used the same UUC group. Therefore, they would not be considered as independent studies. In general, three independent studies are needed to provide evidence of efficacy, particularly under the different conditions of use (e.g. place, feed and animals). These general criteria were established for all feed additives of which the effect is on the composition or utilisation of feed (EFSA FEEDAP Panel, 2018). Coccidiostats act on *Eimeria* spp. irrespectively of the composition of the feed and are not considered to modulate feed utilisation. Consequently, the inoculum with sporulated oocysts is considered the most critical factor in studies with artificial infection. In these studies, efficacy of the coccidiostat should be assessed by comparing the effect of *Eimeria* inocula observed in the IT group against the IUC. The UUC group (common control in these studies) is used only to verify the growth of the animals under farming conditions similar to those in field. The statistical approach applied in the two studies reflected this concept and compared the IUC against IT. Taking into account the above and the fact that in the two studies inocula with different geographical origin were used, the Panel considers

[35] Technical dossier/Section IV/Annex IV.2.

[36] Trial 1: Technical dossier/Section IV/Annex IV.3. Trial 2: Technical dossier/Section IV/Annex IV.4. and Trial 3: Technical dossier/Section IV/Annex IV.5.

[37] Trial 1: Technical dossier/Supplementary information May 2018/Annex 1 - R-H-2017-132. Trial 2: Technical dossier/Supplementary information May 2018/Annex 2 - R-H-2017-133. and Trial 3: Technical dossier/Supplementary information May 2018/Annex 3 - R-H-2017-138.

that the two trials could be used to assess independently the effect of the additive against different *Eimeria* inocula. Therefore, the two studies could be considered as separate studies.

In each study, male chickens (Ross 308) were penned and distributed into three treatment groups: a UUC, an IUC and an IT group. The IT group received feed containing 40 mg monensin sodium and 40 mg nicarbazin/kg feed; the doses were analytically confirmed. The duration of the studies was 35 days and the experimental diets were fed from day 11 (trials 1 and 2) or from day 12 (trial 3). All birds in the IUC and IT groups were inoculated with recent field isolates of pathogenic *Eimeria* species two days after administration of the experimental diet (see Table 21). In the UUC group of trial 3, all birds were sham-inoculated with drinking water. In trials 1 and 2, the doses of inocula were chosen to give about 25% drop in weight gain of the IUC group compared to UUC. In trial 3, the dose of the inoculum was intended to cause weight gain depression of about 15% and lesion scores (LS) of approximately 2 for *E. acervulina* and *E. tenella* at 6–7 days after inoculation. 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 (results were expressed as OPG). Intestinal lesions were scored using the method of Johnson and Reid (1970) 1, 2 and 3 weeks post inoculation on eight birds per pen in trial 1 and 2 and ten birds in trial 3.

Table 21: Experimental design of three floor pen studies with chickens for fattening using Monimax®

Trial	Replicates per treatment (Birds per replicate)	Inoculum characteristics				Feed analysis (mg/kg feed)(1)	
		Month/year and country of isolation	Intended dose per bird		Day and mode of inoculation	Monensin	Nicarbazin
1	5 (47)	12/2017 Galicia (NW Spain)	50,000	*E. acervulina*	Day 13 orally via feed	< 0.5/41/43	< 1/43/42
			15,000	*E. tenella*			
			20,000	*E. maxima*			
			10,000	*E. brunetti*			
			25,000	*E. mitis*			
			10,000	*E. necatrix*			
2	5 (47)	12/2017 Zaragoza (NE Spain)	75,000	*E. acervulina*	Day 13 orally via feed	< 0.5/41/43	< 1/43/42
			20,000	*E. tenella*			
			30,000	*E. maxima*			
			30,000	*E. mitis*			
			15,000	*E. necatrix*			
			20,000	*E. brunetti*			
3	8 (50)	2017 Europe	105,000	*E. acervulina*	Day 14 via syringe	< 0.5/45	< 1/46
			13,000	*E. tenella*			
			6,000	*E. maxima*			

(1): In trial, 1 and 2, birds received starter diet (crumbles) from day 0 to 11 free of coccidiostat, grower diet (pelleted) from day 11 to 24 and finisher diet (pelleted) from day 24 to 35. In trial 3 birds received starter diet (pelleted) from day 0 to 12 free of coccidiostat and grower diet (pelleted) from day 12 to 35.

In trials 1 and 2, a two-sided significance level $\alpha = 0.05$ was used to detect differences between IT and IUC groups. Lesion scoring data as well as mortality and morbidity data were assessed using categorical data analysis (χ^2 from Kruskal–Wallis test). For statistical analysis of continuous variables, a randomised complete block design was used (ANOVA with treatment as fixed effects and block as random effect). If data could be approximated to a normal distribution, they were analyzed in the same manner as the continuous variables.

In trial 3, performance data and OPG were analysed on a pen basis using a linear regression model (ANOVA) with treatment group as fixed effect. After assessing the statistical significance of the fixed effect considered ($p \leq 0.05$), the difference between the treatment groups was assessed by Bonferroni post hoc test ($p \leq 0.05$). Data on intestinal lesion scores were analysed at bird level using a linear mixed regression model (ANOVA) with treatment group and day after inoculation as fixed effects and pen as random effect. After assessing the statistical significance of the fixed effects considered

($p \leq 0.05$), the difference between the treatment groups within the same day was assessed by a pairwise comparison with Bonferroni adjustment for multiple comparisons ($p \leq 0.05$).

Mortality by coccidiosis after inoculation was significantly reduced by Monimax® treatment in trials 1 and 2. No such effect was seen in trial 3 (see Table 25).

Tables 22 and 23 show the results of intestinal lesion scoring. A significant reduction of LS was seen in birds of IT groups compared to the IUC groups of trials 1 and 2 at day 19 (6 days post inoculation) in upper, middle and lower part of duodenum. A similar observation was made in trial 1 at day 27 (14 days post inoculation) for the middle part of the duodenum and caeca. At 22 days post-inoculation no lesions were found in any group.

Table 22: Results of lesion scoring in the different intestinal sections in trials 1 and 2(1)

	6 days post inoculation				14 days post inoculation			
	Upper	**Middle**	**Lower**	**Caeca**	**Upper**	**Middle**	**Lower**	**Caeca**
Trial 1								
UUC	0	0	0	0	0	0	0	0.1
IUC	1.5	1.7	1.3	2.1	0.2	0.3	0.1	0.4
IT	1.0*	1.0*	0.4*	2.0	0.1	0.1*	0	0*
Trial 2								
UUC	0	0	0	0	0	0	0	0.1
IUC	2.0	2.2	1.6	2.4	0	0	0	0
IT	1.0*	1.1*	0.5*	2.0	0.1	0.1	0	0.1

(1): For the upper (*E. acervulina and E. mitis*), middle (*E. maxima and E. necatrix*), lower (*E. brunetti*) and caecal (*E. tenella*) intestinal sections.
*: IT significantly different from IUC ($p \leq 0.05$).

In trial 3, scores were evaluated for *Eimeria* specific and total *Eimeria* lesions at days 21, 28 and 35 (7, 14 and 21 days post inoculation) (Table 23). A significant reduction of *E. acervulina and E. maxima* lesions was found 7 days post inoculation, of *E. tenella* 14 and 21 days post-inoculation. LS for *E. maxima* was also significantly lower 21 days post inoculation.

Table 23: Coccidiosis lesion scoring based on *Eimeria* species-specific lesions in trial 3(1)

	7 days post inoculation				14 days post inoculation				21 days post inoculation			
	Acer	***Max***	***Ten***	**Total**	***Acer***	***Max***	***Ten***	**Total**	***Acer***	***Max***	***Ten***	**Total**
UUC	0.18^{a}	0.14^{a}	0.03^{a}	0.34^{a}	0.36	0.13^{a}	0^{a}	0.49^{a}	1.40^{a}	0.78^{a}	0.05^{a}	2.23^{a}
IUC	2.00^{b}	0.70^{b}	1.16^{b}	3.86^{b}	0.41	0.69^{b}	1.04^{b}	2.14^{b}	0.44^{b}	0.73^{a}	0.40^{b}	1.56^{b}
IT	1.51^{c}	0.39^{a}	1.03^{b}	2.93^{c}	0.29	0.46^{b}	0.30^{c}	1.05^{a}	0.18^{b}	0.34^{b}	0.04^{a}	0.55^{c}

a,b,c: Means in a column with different superscript are significantly different ($p \leq 0.05$).
(1): *Eimeria acervulina (Acer), Eimeria tenella (Ten), Eimeria maxima (Max).*

Results of oocyst excretion are summarised in Table 24. Oocyst excretion was significantly reduced in the IT group compared to the IUC group on days 21, 28 and 35 (7, 14 and 21 days post-inoculation) in trials 1 and 2. In trial 3, the oocyst excretion was not significantly affected.

Table 24: Total oocyst counts per gram (OPG; geometric means) in floor pen trials

Days post inoculation(1)	6/7	14	21/22
Trial 1			
UUC	nd	nd	nd
IUC	214,000	72,400	2,243
IT	39,800*	2,240*	nd*
Trial 2			
UUC	nd	nd	nd
IUC	251,000	66,100	1,740
IT	49,000*	513*	nd*
Trial 3			
UUC	130^{b}	245^{b}	$121,080^{a}$
IUC	$127,503^{a}$	$6,311^{ab}$	$9,775^{b}$
IT	$81,950^{a}$	$9,724^{a}$	$2,892^{b}$

nd: not detected.
(1): 6/14/22 days in Trial 1 and 2; 7/14/21 days in trial 3.
*: IT significantly different from IUC ($p \leq 0.05$).
a,b: means with different letters in a column are significantly different.

Table 25 shows the performance parameters including mortality. *Eimeria* infection resulted in a significant reduction of final body weight in trial 1. In trials 1 and 2, feed to gain ratio was significantly reduced in the IT group compared with IUC group. In trial 3, the final body weight and daily weight gain (days 12–35) was significantly higher in IT group compared with IUC group.

Table 25: Performance parameters and mortality of chickens for fattening in floor pen studies(1)

	Feed intake (g/day)	Body weight (g)	Weight gain (g/day)	Feed to gain ratio	Mortality(2) (n)
Trial 1					
UUC	105.3	2,729	76.7	1.37	0
IUC	99.3	2,528	70.9	1.40	100
IT	102.7	2,656	74.6	1.37*	11*
Trial 2					
UUC	105.3	2,729	76.7	1.37	0
IUC	103.8	2,645	74.3	1.40	117
IT	101.8	2,621	73.6	1.38	24*
Trial 3					
UUC	121.8	$2,044^{ab}$	73.0^{a}	1.67	0
IUC	118.6	$1,960^{b}$	66.8^{b}	1.78	1
IT	122.6	$2,075^{a}$	72.3^{a}	1.70	0

(1): Results of trial 1 and 2 refer to the study period from 0 to 35 days; results of trial 3 refer to the period days 12–35.
(2): Coccidiosis related mortality; total mortality was: Trial 1: IT 12, IUC 105; UUC 5 birds; Trial 2: UUC 5, IUC 118; IT 5 birds; Trial 3: UUC 1, IUC 3; IT 4 birds.
*: IT significantly different from IUC ($p \leq 0.05$).
a,b: Means with different letters in a column are significantly different.

3.3.1.3. Field trial in chickens for fattening

The applicant provided a trial carried out in 2012 under controlled field conditions.[38] A total of 135,400 one-day-old male chickens for fattening distributed in four houses were allocated to two groups (representing two houses per group). The control group received in the first three weeks of life feed containing a combination of a polyether coccidiostat (50 mg/kg) and a synthetic coccidiostat (50 mg/kg). From the fourth week, birds received feed containing only the polyether coccidiostat (60 mg). The treated group received feed containing Monimax® (40 mg monensin and 40 mg nicarbazin/kg

[38] Technical dossier/Section IV/Annex IV.8.

feed). Study duration was 35 days followed by a 1-week withdrawal period. At the age of 33, 34 or 36 days, around 30% animals of all houses were moved out for slaughtering without individual identification. The same happened with the remaining animals at the age of 40, 41 or 42 days at the end of the study. Body weight was measured weekly in 50 animals per house. Lesion scores were examined four times in ten animals per house. The selection criteria of animals taken for body weight and lesion score measurement were not described. Faecal samples to determine the OPG were taken weekly in each house.

The FEEDAP Panel finally did not consider the results of the field study, due to the following weaknesses in study design and reporting. Sum of dead/culled birds and those slaughtered was not in a constant relation to the initial number of birds. Body weight appeared to depend more on the house than on the anticoccidial treatment. A sample size of 50 birds is considered too small to give a reliable estimate of the body weight of more than 30,000 birds (no selection criteria described). It is also not expected that ten birds out of more than 30,000 will allow an estimate on the prevalence of lesion scores.

3.3.1.4. Anticoccidial sensitivity tests in chickens for fattening

Four ASTs performed in 2011 (AST-1 and AST-2) and 2014 (AST-3 and AST-4) were submitted.[39] Each test was made with the groups UUC, IUC and IT, the latter receiving feed supplemented with Monimax® at an intended concentration of 40 mg monensin and 40 mg nicarbazin/kg feed, dosage was analytically confirmed (see Table 26). The birds (Ross PM3 in AST-1 and Ross 308 in AST-2, AST-3 and AST-4) were randomly allocated to the groups. In three of the studies other anticoccidial additives were also tested (seven in AST-1, five in AST-3 and AST-4). Birds were artificially infected with sporulated oocysts from recent field isolates. In AST-3, also a test with a historical isolate (AST-3h) *vs* a recent isolate (AST-3r) was made. 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).

The data were analysed by ANOVA. Group differences at $p \leq 0.05$ level were checked by Newman–Keuls test or LSD. In AST-2 AST-3 and AST-4 lesion scores were analysed by a non-parametric test.

Table 26: Experimental design of ASTs with chickens for fattening using Monimax®

	Replicates per treatment (Birds per replicate)	Inoculum characteristics			Day of inoculation	Anticoccidial treatment(1) (days of life)	Feed analysis monensin + nicarbazin (mg/kg feed)
		Month/year and country of isolation	Intended dose per bird				
AST-1	3 (6)	8/2011 France	248,600	*E. acervulina*	15	13–22	38 + 36
			21,600	*E. brunetti*			
AST-2	7 (5)	2011 Netherlands	62,000	*E. acervulina*	14	11–24	34 + 33
			29,000	*E. tenella*			
			11,000	*E. maxima*			
			5,000	*E. mitis*			
			10,000	*E. praecox*			
AST-3h	6 (5)	1996/EU	96,120	*E. acervulina*	17	13–23	41 + 39
			16,920	*E. tenella*			
			12,900	*E. maxima*			
			5,040	*E. mitis*			
			11,520	*E. necatrix/ praecox*			

[39] AST-1: Technical dossier/Section IV/Annex IV.6. AST-2: Technical dossier/Section IV/Annex IV.7. AST-3: Technical dossier/Spontaneous Supplementray Information April 2017/2014-09 final. AST-4: Technical dossier/Spontaneous Supplementray Information April 2017/2014-06 final.

	Replicates per treatment (Birds per replicate)	Inoculum characteristics			Day of inoculation	Anticoccidial treatment(1) (days of life)	Feed analysis monensin + nicarbazin (mg/kg feed)
		Month/year and country of isolation	Intended dose per bird				
AST-3r	6 (5)	3/2012 Belgium	133,200	*E. acervulina*			
			12,240	*E. tenella*			
			2,880	*E. maxima*			
			14,040	*E. mitis*			
			25,200	*E. necatrix/ praecox*			
AST-4	6 (5)	2/2013 Belgium	85,600	*E. acervulina*	18	15–25	39 + 44
			35,000	*E. tenella*			
			68,000	*E. maxima*			
			3,200	*E. praecox*			
			64,000	*E. mitis*			

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

Table 27 summarises the results of the four ASTs, giving consideration to the different isolates (historical and recent) used in AST-3.

Mortality was low in AST-2, AST-3 and AST-4 and not coccidiosis related. Increased mortality due to coccidiosis was seen only in IUC of AST-1, 6 birds out of 18 died and 4 deaths were coccidiosis related. Oocyst excretion 6–9 days after inoculation indicated successful *Eimeria* infection on days 14–18. No OPG was found in the UUC of AST-1, AST-2 and AST-3, and only a small number in AST-4. These findings support the validity of the ASTs. OPG was significantly reduced by the Monimax® treatment (comparison of IT vs IUC) in AST-2, AST-3h, AST-3r and AST-4, but not in AST-1. In AST-1 and AST-2, a reduction of *Eimeria* spp. specific lesion scores by treatment (IT) was not observed. In contrast, the total average lesion score reported in AST-3h, AST-3r and AST-4 showed a significant reduction in the IT groups (compared to IUC) down to the level of UUC.

The adverse effect of oocyst inoculation was seen by a significant depression (IUC vs. UUC) of feed intake in AST-1 and AST-3r and of daily body weight gain in AST-1, AST-3r and AST-4. A beneficial effect of the anticoccidial treatment (IT vs. IUC) was observed as a significantly higher feed intake in AST-1 and AST-3r, significantly higher daily weight gain in AST-1, AST-3r and AST-4.

Table 27: Results of anticoccidial sensitivity tests in chickens

	Daily Feed Intake (g)	Body weight (g)	Daily Weight Gain (g)	Feed to gain ratio	Mortality (n)	Total OPG	Mean lesion scores(1)			
							Acer	*Bru*	*Ten*	*Max*
AST-1	**D13-22**	**D22**	**D13-22**	**D13-22**	**Total**	**D20-22**	**D22**			
UUC	107.2^{a}	$1,025.3^{a}$	59.5^{a}	1.80^{c}	0^{b}	0^{b}	0.0^{b}	0.0^{b}	–	–
IUC	79.3^{c}	657.5^{c}	19.2^{c}	4.17^{a}	6^{a}	2.7×10^{8a}	2.7^{a}	2.1^{a}	–	–
IT	98.2^{b}	857.1^{b}	40.0^{b}	2.47^{b}	0^{b}	3.7×10^{8a}	3.1^{a}	2.0^{a}	–	–
AST-2	**D11-24**	**D24**	**D11-24**	**D11-24**	**Total**	**D20**	**D20**			
UUC	81^{a}	870^{a}	47^{a}	1.7^{b}	0	0^{c}	0.00^{b}	–	0.07^{a}	0.86^{a}
IUC	78^{a}	840^{a}	44^{a}	1.8^{ab}	1	1.2×10^{6a}	1.43^{a}	–	0.07^{a}	0.79^{a}
IT	82^{a}	837^{a}	44^{a}	1.8^{a}	0	5.4×10^{5b}	1.43^{a}	–	0.14^{a}	0.57^{a}
AST-3h	**D17-23**	**D23**	**D17-23**	**D17-23**	**Total**	**D21-23**	**Mean lesion score D23**			
UUC	107^{a}	$1,167^{a}$	78^{a}	1.38^{a}	0	0^{c}	0.7^{b}			
IUC	97^{a}	$1,088^{b}$	63^{b}	1.64^{a}	2	9.9×10^{5a}	3.1^{a}			
IT	112^{a}	$1,118^{ab}$	70^{ab}	1.68^{a}	3	4.8×10^{3b}	0.5^{b}			

	Daily Feed Intake (g)	Body weight (g)	Daily Weight Gain (g)	Feed to gain ratio	Mortality (n)	Total OPG	Mean lesion scores(1)			
							Acer	*Bru*	*Ten*	*Max*
AST-3r	**D17-23**	**D23**	**D17-23**	**D17-23**	**Total**	**D21-23**	**Mean lesion score D23**			
UUC	107^{ab}	$1,167^{a}$	78^{a}	1.38^{b}	0	0^{c}	0.7^{b}			
IUC	95^{b}	$1,029^{b}$	51^{b}	1.97^{a}	1	4.4×10^{6a}	4.7^{a}			
IT	115^{a}	$1,225^{a}$	82^{a}	1.41^{b}	0	1.6×10^{5b}	0.7^{b}			
AST-4	**D15-25**	**D25**	**D15-25**	**D15-25**	**Total**	**D25**	**Mean lesion score D23-25**			
UUC	117.3^{a}	$1,281.0^{a}$	81.3^{a}	1.30^{b}	0	3.0×10^{2c}	0.56^{b}			
IUC	116.9^{a}	$1,033.9^{b}$	55.2^{b}	1.92^{a}	1	1.2×10^{6a}	3.72^{a}			
IT	124.4^{a}	$1,318.3^{a}$	83.1^{a}	1.35^{b}	0	1.2×10^{5b}	0.67^{b}			

(1): *Eimeria acervulina (Acer), Eimeria brunetti (Bru), Eimeria tenella (Ten), Eimeria maxima (Max).*
a,b,c: Means within a column within a study with different superscript are significantly different ($p \leq 0.05$).

3.3.1.5. Synopsis of the efficacy studies in chickens for fattening

The synopsis is based on three newly submitted floor pen studies and four anticoccidial sensitivity tests performed with the lowest applied concentration of the coccidiostat Monimax® (40 mg monensin and 40 mg nicarbazin/kg feed).

The treatment with Monimax ® significantly reduced mortality in two floor pen studies and in one AST. In all floor pen studies, the treatment significantly reduced intestinal lesion scores. In two of them, a significantly lower oocyst excretion was observed. The zootechnical data demonstrated the depressive effect of *Eimeria* invasion, the treatment resulted in one study in an improvement of feed to gain ratio and in another study of final body weight compared to the IUC.

In all four ASTs oocyst inoculation resulted in a significant increase of intestinal lesions, two of them showed a significant reduction owing to the treatment with Monimax®. The ASTs showed a significant reduction of oocyst excretion by Monimax® in three of four tests. In three of four ASTs, the anticoccidial efficacy of Monimax® demonstrated by an improvement of coccidiosis-related specific endpoints was further confirmed by an improvement of body weight gain. In ASTs, performance data are regarded as supportive considering the short duration of the test period (10–14 days) and the small number of animals per group (18–35) and per replicate (5–6).

3.3.2. Conclusions on efficacy studies

Based on floor pen studies and anticoccidial sensitivity tests, the FEEDAP Panel concludes that Monimax® has the potential to control coccidiosis in chickens for fattening at a minimum concentration of 40 mg monensin and 40 mg nicarbazin/kg complete feed. The conclusion is extended to chickens reared for laying.

3.4. Post-market monitoring

Field monitoring of *Eimeria* spp. resistance in chickens for fattening and chickens reared for laying to monensin sodium/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 and a field study.

3.4.1. Conclusions

Monimax® is considered safe for chickens for fattening at the highest use level of 50 mg monensin and 50 mg nicarbazin/kg complete feed. The margin of safety is about 1.5. This conclusion is extended to chickens reared for laying. The simultaneous use of Monimax® and certain antibiotic drugs (i.e. tiamulin) is contraindicated. Monensin has a selective antimicrobial activity against Gram-positive bacterial species while many Enterobacteriaceae are naturally resistant. Induction of cross-resistance with clinically relevant antimicrobials or increased shedding of enteropathogenic bacteria are not reported. Nicarbazin has no antimicrobial activity.

Monensin sodium is absorbed at a limited extent and excreted rapidly, it is extensively metabolised and gives rise to demethylated, oxidised and decarboxylated metabolites. Nicarbazin, when ingested, is rapidly split in its two components HDP and DNC which behave independently. Liver is the target tissue. DNC residues decline rapidly from tissues following nicarbazin withdrawal. DNC appears as the marker residue. HDP-related residues are much lower than those derived from DNC. For both compounds of Monimax®, the metabolic pathways in the chicken are similar to those in the turkey and rat. The potential interaction, if any, of monensin and nicarbazin has been taken into consideration.

The FEEDAP Panel concludes that the active substances in Monimax®, monensin sodium and nicarbazin, do not represent a genotoxic risk. No safety concerns would arise from the nicarbazin impurities PNA and M4NPC. Monensin sodium has no structural alert for carcinogenesis. Monensin sodium is not a reproductive or developmental toxicant. The lowest NOEL identified in the developmental study in rabbits is 0.3 mg monensin sodium/kg bw per day for maternal toxicity in rabbits. The primary toxicity resulting from the oral use of nicarbazin is renal toxicity. The absence of similar findings after treatment with DNC and HDP confirms that this equimolar association of compounds is better tolerated than nicarbazin at equivalent doses. The lowest NOAEL identified in a 52-week study in rat using DNC + HDP was 20 mg DNC + 8 mg HDP/kg bw per day based on the absence of microcrystals in urine and related microscopic renal observations. No significant interaction between monensin sodium and nicarbazin is expected from toxicological studies.

The use of Monimax® at the highest proposed dose (50 + 50 mg monensin + nicarbazin/kg complete feed) will not pose a risk to persons consuming animal products from treated chickens for fattening. This conclusion is extended to chickens reared for laying up to 16 weeks of age. No safety concern would arise from the impurity PNA if the maximum content in nicarbazin of 0.1% is respected. The impurity M4NPC is considered safe for the consumer provided that a maximum concentration of 0.4% in nicarbazin is not exceeded. No withdrawal time is required for Monimax® in chickens for fattening. Residue data comply with the established MRLs for monensin and DNC.

The monensin sodium contained in Monimax® presents a hazard by inhalation. Monimax® is not a skin irritant; however, no data are available for the eye irritation potential of monensin. Monimax® may also act as a dermal toxicant due to its monensin component. Monimax® is not a skin sensitiser.

The use of monensin sodium from Monimax® in complete feed for chickens for fattening does not pose a risk for the aquatic compartment and sediment, while a risk cannot be excluded for the terrestrial compartment. A final conclusion on the risk resulting from the use of nicarbazin from Monimax cannot be made because (i) DNC refined PECs show uncertainties linked to the very high persistence of the compound, (ii) DNC might accumulate in the sediment compartment, and (iii) DNC can potentially bioaccumulate and may cause secondary poisoning. No concerns would arise for the HDP moiety of nicarbazin excreted from chickens fed Monimax®. Based on the available data, the FEEDAP Panel cannot conclude on the safety of Monimax for the environment.

Based on the submitted floor pen studies and anticoccidial sensitivity tests evaluated, the FEEDAP Panel concludes that Monimax® has the potential to control coccidiosis in chickens for fattening at a minimum concentration of 40 mg monensin and 40 mg nicarbazin/kg complete feed. The conclusion is extended to chickens reared for laying.

Documentation provided to EFSA

1) Monimax® for chickens for fattening and chickens reared for laying. July 2012. Submitted by Huvepharma N.V.
2) Monimax® for chickens for fattening and chickens reared for laying. Supplementary information. January 2014. Submitted by Huvepharma N.V.
3) Monimax® for chickens for fattening and chickens reared for laying. Supplementary information. June 2015. Submitted by Huvepharma N.V.
4) Monimax® for chickens for fattening and chickens reared for laying. Supplementary information. October 2015. Submitted by Huvepharma N.V.
5) Monimax® for chickens for fattening and chickens reared for laying. Supplementary information. February 2017. Submitted by Huvepharma N.V.
6) Monimax® for chickens for fattening and chickens reared for laying. Spontaneous supplementary information. April 2017. Submitted by Huvepharma N.V.
7) Monimax® for chickens for fattening and chickens reared for laying. Supplementary information. October 2017. Submitted by Huvepharma N.V.

8) Evaluation report of the European Union Reference Laboratory for Feed Additives on the Methods of Analysis for Monimax®.
9) Comments from Member States.

Chronology

Date	Event
26/7/2012	Dossier received by EFSA
29/8/2012	Reception mandate from the European Commission
31/1/2013	Application validated by EFSA – Start of the scientific assessment
13/3/2013	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*
30/4/2013	Comments received from Member States
30/10/2014	Reception of the Evaluation report of the European Union Reference Laboratory for Feed Additives
3/6/2015	Reception of supplementary information from the applicant - Scientific assessment re-started
4/12/2015	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. Issues: characterisation, safety for target species, safety for the consumer, safety for the user and efficacy
3/2/2017	Reception of supplementary information from the applicant - Scientific assessment re-started
25/7/2017	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 target species, safety for the consumer, safety for the user and efficacy
26/10/2017	Reception of supplementary information from the applicant - Scientific assessment re-started
5/12/2017	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 target species, safety for the consumer, safety for the user and efficacy
3/05/2018	Reception of supplementary information from the applicant - Scientific assessment re-started
2/10/2018	Opinion adopted by the FEEDAP Panel. End of the Scientific assessment

References

van Beelen P and Burris DR, 1995. Reduction of the explosive 2,4,6-trinitrotoluene by enzymes from aquatic sediments. Environmental Toxicology and Chemistry, 14,2115–2123.

EFSA (European Food Safety Authority), 2004a. Opinion of the Scientific Panel on additives and products or substances used in animal feed (FEEDAP) on the reevaluation of coccidiostat Elancoban in accordance with article 9G of Council Directive 70/524/EEC. EFSA Journal 2004;2(3):42, 61 pp. https://doi.org/10.2903/j.efsa.2004.72

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), 2004c. Opinion of the scientific Panel on Additives and Products or substances used in Animal Feed on a request from the Commission on the re-evaluation of Sacox 120 microGranulate in accordance with article 9G of Council Directive 70/524/EEC. EFSA Journal 2004;2(7):76, 49 pp. https://doi.org/10.2903/j.efsa.2004.76

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

EFSA (European Food Safety Authority), 2006a. Opinion of the Panel on additives and products or substances used in animal feed (FEEDAP) on the safety of Coxidin® (monensin sodium). EFSA Journal 2006;4(7):283, 10 pp. https://doi.org/10.2903/j.efsa.2006.381

EFSA (European Food Safety Authority), 2006b. Opinion of the Panel on additives and products or substances used in animal feed (FEEDAP) on the Maximum Residue Limit for monensin sodium for chickens and turkeys for fattening. EFSA Journal 2006;4(11):413, 13 pp. https://doi.org/10.2903/j.efsa.2006.413

EFSA (European Food Safety Authority), 2007. Opinion of the Scientific Panel on additives and products or substances used in animal feed (FEEDAP) on efficacy of Coxidin® 25 % (monensin sodium) as a feed additive for turkeys. EFSA Journal 2007;5(9):545, 13 pp. https://doi.org/10.2903/j.efsa.2007.545

EFSA (European Food Safety Authority), 2008a. Scientific Opinion of the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) on a request from the European Commission on withdrawal period for Elancoban® for chickens for fattening, chickens reared for laying and turkeys for fattening. EFSA Journal 2008;6(7):730, 16 pp. https://doi.org/10.2903/j.efsa.2008.730

EFSA (European Food Safety Authority), 2008b. Opinion of the Scientific Panel on additives and products or substances used in animal feed (FEEDAP) on the withdrawal period for Coxidin® for chickens and turkeys for fattening and re-examination of the provisional Maximum Residue Limit. EFSA Journal 2008;6(7):731, 14 pp. https://doi.org/10.2903/j.efsa.2008.731

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

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

EFSA (European Food Safety Authority), 2008e. Technical Guidance: extrapolation of data from major species to minor species regarding the assessment of additives for use in animal nutrition. EFSA Journal 2008;6(9):803, 5 pp. https://doi.org/10.2903/j.efsa.2008.803

EFSA FEEDAP Panel (EFSA Panel on Additives and Products used in Animal Feed), 2010a. 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 used in Animal Feed), 2010b. 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 used in Animal Feed), 2011a. Scientific Opinion on the safety and efficacy of Coxidin® (monensin sodium) as feed additive for chickens reared for laying. EFSA Journal 2011;9(12):2442, 15 pp. https://doi.org/10.2903/j.efsa.2011.242

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

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

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

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

EFSA FEEDAP Panel (EFSA Panel on Additives and Products used in Animal Feed), 2013. Scientific Opinion on the modification of the withdrawal period for Coxidin® (monensin sodium) for chickens for fattening and chickens reared for laying. EFSA Journal 2013;11(1):3045, 15 pp. https://doi.org/10.2903/j.efsa.2013.3045

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, Lopez-Alonso M, Lopez Puente S, Mantovani A, Mayo B, Ramos F, Saarela M, Villa RE, Wallace RJ, Wester P, Brantom P, Halle I, van 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), Rychen G, Aquilina G, Azimonti G, Bampidis V, Bastos ML, Bories G, Chesson A, Cocconcelli PS, Flachowsky G, Gropp J, Kolar B, Kouba M, López-Alonso M, López Puente S, Mantovani A, Mayo B, Ramos F, Saarela M, Villa RE, Wallace RJ, Wester P, Anguita M, Galobart J, Innocenti ML and Martino L, 2018. Guidance on the assessment of the efficacy of feed additives. EFSA Journal 2018;16(5):5274, 25 pp. https://doi.org/10.2903/j.efsa.2018.5274

European Commission, 1981, online. Report of the Scientific Committee for Animal Nutrition on the use of monensin sodium in feedingstuffs for poultry. Available online: https://ec.europa.eu/food/sites/food/files/safety/docs/animal-feed_additives_rules_scan-old_report_antibiotics-26.pdf

European Commission, 1983, online. Report of the Scientific Committee for Animal Nutrition on the use of monensin sodium in feedingstuffs for turkeys. Available online: https://ec.europa.eu/food/sites/food/files/safety/docs/animal-feed_additives_rules_scan-old_report_antibiotics-27.pdf

European Commission, 1991, online. Report of the Scientific Committee for Animal Nutrition on the use of Narasin + Nicarbazin in feedingstuffs for chickens. Available online: https://ec.europa.eu/food/sites/food/files/safety/docs/animal-feed_additives_rules_scan-old_report_antibiotics-31.pdf

European Commission, 1995, online. Complementary report of the Scientific Committee for Animal Nutrition on Question 52 by the Commission on the of Narasin + Nicarbazin in feedingstuffs for chickens. Available online: https://ec.europa.eu/food/sites/food/files/safety/docs/animal-feed_additives_rules_scan-old_report_antibiotics-32.pdf

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

Sun P, Pavlostathis SG and Huang C-H, 2016. Estimation of environmentally relevant chemical properties of veterinary ionophore antibiotics. Environmental Science and Pollution Research 23, 18353–18361.

Abbreviations

ADI	acceptable daily intake
ADME	absorption, distribution, metabolism and excretion
AF	assessment factor
ANOVA	analysis of variance
AST	anticoccidial sensitivity tests
bw	body weight
CAS	Chemical Abstracts Service
DITR	Dietary intake of total residues
DNC	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
ErC_{10}	median effective concentration which results in a 10% reduction in growth rate
ErC_{50}	median effective concentration which results in a 50% reduction in growth rate
EURL	European Union Reference Laboratory
FEEDAP	EFSA Panel on Additives and Products or Substances used in Animal Feed
FOCUS	FOrum for Co-ordination of pesticide fate models and their USe
GLP	Good Laboratory Practice
HDP	2-hydroxy-4,6-dimethylpyrimidine
K_{oc}	adsorption or desorption coefficient corrected for soil organic carbon content
LC_{50}	median lethal concentration
LOD	limit of detection
log K_{ow}	octanol/water partition coefficient
LOQ	limit of quantitation
M4NPC	methyl(4-nitrophenyl) carbamate
MIC	minimum inhibitory concentrations
MRC	marker residue concentration
MRL	maximum residue limit
NOAEL	no observed adverse effect level
NOEC	no observed effect concentration
NOEL	no observed effect level
OECD	Organisation for Economic Co-operation and Development
OPG	oocysts per gram of excreta
PEC	predicted environmental concentration
pK_a	dissociation constant
PNA	*p*-nitroaniline
PNEC	predicted no effect concentration
RMTR	ratio marker to total residues
SCAN	Scientific Committee on Animal Nutrition
SD	standard deviation
TRC	total residue concentration

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

In the current application authorisation is sought for *Monimax*®, under article 4(1), for the category 'coccidiostats and histomonostats', according to the classification system of article 6 of Regulation (EC) No 1831/2003. Authorisation is sought for *chickens for fattening* and *chickens reared for laying*. *Monimax*® consists of 80 g/kg of *monensin sodium* and 80 g/kg of *nicarbazin* complemented by starch for granulation, wheat meal and calcium carbonate.

Monimax® is intended to be incorporated in *feedingstuffs* through *premixtures* and it is not to be mixed with other coccidiostats. For each of the active substances the Applicant proposes a final concentration in *feedingstuffs* ranging from 40 to 50 mg/kg. Furthermore, the Applicant proposed maximum residue limits (MRLs) in skin/fat, muscle, liver and kidney. As these MRLs are not set up by Commission Regulation (EC) No 37/2010, the correspondent methods of analysis have to be evaluated by the EURL.

For the quantification of *monensin sodium* in *premixtures* and *feedingstuffs* the Applicant submitted the multi-analyte ring-trial validated method EN ISO 14183 based on High Performance Liquid Chromatography with post-column derivatisation and VIS detection (HPLC-VIS). The Applicant applied this method for the analysis of the *feed additive* and presented performance characteristics similar to those reported for the EN ISO standard method. Based on the experimental evidence available the EURL recommends for official control the HPLC with post-column derivatisation and VIS detection for the quantification of *monensin sodium* in the *feed additive*, *premixtures* and *feedingstuffs*.

For the quantification of *nicarbazin* in *premixtures* and *feedingstuffs* the Applicant submitted the ring-trial validated method EN ISO 15782 based on HPLC coupled with ultraviolet (UV) detection. A similar method was used by the Applicant for the quantification of *nicarbazin* in the *feed additive*. Based on the performance characteristics presented the EURL recommends for official control the HPLC method with UV detection for the quantification of *nicarbazin* in the *feed additive, premixtures* and *feedingstuffs*.

For the quantification of *monensin sodium* and *nicarbazin* in target tissues (skin/fat, muscle, liver and kidney) the Applicant submitted a single-laboratory and further verified method based on reversed-phase high performance liquid chromatography coupled to a triple quadrupole mass spectrometer (RP-HPLC–MS/MS) in electrospray ionisation mode (ESI) using matrix matched standards. Based on the performance characteristics presented, the EURL recommends for official control the RP-HPLC–MS/MS method proposed by the Applicant or any equivalent other analytical methods complying with the requirements set by Commission Decision 2002/657/EC, to enforce the MRLs of *monensin sodium* and *nicarbazin* in the target *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.