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Safety and efficacy of Coxar® (nicarbazin) for turkeys for fattening

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Abstract

The coccidiostat Coxar® is safe for turkeys for fattening at the use level of 100 mg nicarbazin/kg complete feed, with a margin of safety of about 1.25. Nicarbazin, when ingested, is rapidly split in its two components 2-hydroxy-4,6-dimethylpyrimidine (HDP) and dinitrocarbanilide (DNC), which behave independently. HDP-related residues are much lower than those of DNC. DNC is the marker residue. Liver is the target tissue. Nicarbazin is not genotoxic. The primary toxicity resulting from the oral use of nicarbazin is renal toxicity. The lowest no observed adverse effect level (NOAEL) identified in a 52-week study in rat using DNC+HDP is 20 mg DNC + 8 mg HDP/kg body weight (bw) per day based on the absence of microcrystals in urine and related microscopic renal observations. The use of 100 mg nicarbazin from Coxar®/kg complete feed for turkeys for fattening will not pose a risk to consumers, provided that maximum contents in nicarbazin of 0.1% *p*-nitroaniline (PNA) and 0.4% methyl(4-nitrophenyl) carbamate (M4NPC) would be respected. No withdrawal time is required. Residue data comply with the established maximum residue limits (MRLs). Nicarbazin is not a skin or eye irritant and not a skin sensitiser. These conclusions also apply to the additive Coxar®. Inhalation toxicity of nicarbazin is limited; the granulated additive has a low dusting potential. No risk for users is identified. Based on the available data, the FEEDAP Panel cannot conclude on the safety of Coxar® for the environment. The efficacy of 100 mg nicarbazin from Coxar®/kg feed was demonstrated in three anticoccidial sensitivity tests (AST), but only in one floor pen study. The floor pen study with 75 mg nicarbazin failed to demonstrate evidence of efficacy. No final conclusions on the efficacy of nicarbazin from Coxar® for turkeys for fattening can be drawn.

Keywords: Coxar®, nicarbazin, coccidiostats, turkeys for fattening, safety, efficacy

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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 Coxar® (nicarbazin) when used as a feed additive for turkeys for fattening.

The coccidiostat Coxar® is considered safe for turkeys for fattening at the use level of 100 mg nicarbazin/kg complete feed, with a margin of safety of about 1.25. Nicarbazin has no antimicrobial activity; no incompatibilities or interactions with feedingstuffs, carriers, or other approved additives are expected.

Nicarbazin, when ingested, is rapidly split in its two components 2-hydroxy-4,6-dimethylpyrimidine (HDP) and dinitrocarbanilide (DNC), which behave independently. DNC residues decline rapidly from tissues following nicarbazin withdrawal. HDP-related residues are much lower than those derived from DNC. DNC appears as the marker residue. Liver is the target tissue. The metabolic pathways in the turkey are similar to those in the chicken and rat.

Nicarbazin is not genotoxic. The primary toxicity resulting from the oral use of nicarbazin is renal toxicity. 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 body weight (bw) per day based on the absence of microcrystals in urine and related microscopic renal observations.

The use of Coxar® at the highest proposed dose (100 mg nicarbazin/kg complete feed) will not pose a risk to persons consuming animal products from turkeys for fattening, provided that maximum contents in nicarbazin of 0.1% *p*-nitroaniline (PNA) and 0.4% methyl(4-nitrophenyl) carbamate (M4NPC) would be respected. No withdrawal time is required for Coxar® in turkeys for fattening. Residue data comply with the established maximum residue limits (MRLs).

Nicarbazin is not a skin or eye irritant and not a skin sensitiser. These conclusions also apply to the additive Coxar®. Inhalation toxicity of nicarbazin is limited. Due to low dusting potential of the granulated additive, the risk for users is negligible.

No final conclusions on the safety of nicarbazin for the environment can be made since: (i) DNC refined predicted environmental concentrations (PECs) shows 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 efficacy of 100 mg nicarbazin from Coxar®/kg feed was demonstrated in three anticoccidial sensitivity tests (AST), but only in one floor pen study. The floor pen study with 75 mg nicarbazin failed to demonstrate evidence of efficacy. No final conclusions on the efficacy of nicarbazin from Coxar® for turkeys for fattening can be drawn.

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1. Introduction

1.1. Background and Terms of Reference

Regulation (EC) No 1831/2003[1] establishes the rules governing the Community authorisation of additives for use in animal nutrition. In particular, Article 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 received a request from Huvepharma N.V.[2] for authorisation of the product Coxar® (nicarbazin), when used as a feed additive for turkeys (category: coccidiostats and histomonostats).

According to Article 7(1) of Regulation (EC) No 1831/2003, the Commission forwarded the application to the European Food Safety Authority (EFSA) as an application under Article 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 this application. The particulars and documents in support of the application were considered valid by EFSA as of 7 March 2016.

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 Coxar® (nicarbazin), when used under the proposed conditions of use (see Section 3.1.5).

1.2. Additional information

The feed additive Coxar®, containing nicarbazin as an active substance, has never been assessed by EFSA and it is not authorised in the European Union.

Recently, the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) adopted an opinion on the product Monimax® for turkeys for fattening, containing monensin sodium and nicarbazin as active substances (EFSA FEEDAP Panel, 2017). The holder of Monimax® is Huvepharma N.V., the same applicant as that of the current submission.

There are two other authorised coccidiostats containing nicarbazin: Koffogran[3] (nicarbazin) and Maxiban®[4] (nicarbazin + narasin). Both products are authorised for use with chickens for fattening only.

Koffogran has been assessed by the FEEDAP Panel in 2003 and 2010 (EFSA, 2004 and EFSA FEEDAP Panel, 2010a). Maxiban® has been assessed by the Scientific Committee on Animal Nutrition (SCAN) in 1991 and 1995 (European Commission, 1991, 1995) followed by a FEEDAP opinion in 2010 (EFSA FEEDAP Panel, 2010b).

Maximum residue limits (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 1 day for nicarbazin from Koffogran and 0 day for nicarbazin from Maxiban®.[5]

2. Data and methodologies

2.1. Data

The present assessment is based on data submitted by the applicant in the form of a technical dossier[6] in support of the authorisation request for the use of Coxar® (nicarbazin), as a feed additive. The technical dossier was prepared following the provisions of Article 7 of Regulation (EC) No 1831/2003, Regulation (EC) No 429/2008[7] and the applicable EFSA guidance documents.

[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] Huvepharma N.V. Uitbreidingstraat 80. 2600. Anwerp, Belgium.
[3] 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.
[4] 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.
[5] OJ L 263, 6.10.2010, p. 4. and OJ L 265, 8.10.2010, p. 5.
[6] FEED dossier reference: FAD-2015-0039.
[7] 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.

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 substance in animal feed and marker residue in tissues. The Executive Summary of the EURL report can be found in Annex A.[8]

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of Coxar® (nicarbazin) is in line with the principles laid down in Regulation (EC) No 429/2008[7] and the relevant guidance documents: Guidance for the preparation of dossiers for coccidiostats and histomonostats (EFSA FEEDAP Panel, 2011a), Technical guidance: Tolerance and efficacy studies in target animals (EFSA FEEDAP Panel, 2011b), Technical Guidance for assessing the safety of feed additives for the environment (EFSA, 2008a), Guidance for establishing the safety of additives for the consumer (EFSA FEEDAP Panel, 2012a), Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012b) and Technical Guidance: Microbial Studies (EFSA, 2008b), Guidance on the assessment of bacterial susceptibility to antimicrobials of human and veterinary importance (EFSA FEEDAP Panel, 2012c).

3. Assessment

The present opinion assesses the safety and efficacy of the coccidiostat Coxar® (nicarbazin) when used as feed additive for turkeys for fattening up to 16 weeks of age. The recommended inclusion level of Coxar® in complete feed is 100 mg nicarbazin/kg complete feed. MRLs for edible turkey 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) and a withdrawal period of 0 day is proposed.

3.1. Characterisation

3.1.1. Identity of the additive

Coxar® is specified to contain a minimum of 250 g/kg of nicarbazin from synthetic nicarbazin (minimum 95.1%), 15 g/kg starch, 500 g/kg wheat meal and the rest consisting of calcium carbonate. The analysis of five batches of Coxar® indicated compliance with the specification and batch to batch consistency; mean nicarbazin content was 252.2 g/kg (range: 250.0–259.0 g/kg).[9]

Data on impurities were provided for three batches of the additive.[10] Results showed concentrations of arsenic < 0.04 mg/kg, cadmium between 0.014 and 0.019 mg/kg, lead < 0.05 mg/kg and mercury < 0,005 mg/kg. Levels of aflatoxin B1, B2, G1, G2 were < 1 μg/kg each (total < 1.5 μg/kg). Values for dioxins (polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/F)) were < 0.137 ng WHO-PCDD/F-TEQ per kg, the sum of dioxins and dioxin-like polychlorinated biphenyls (DL-PCBs) was < 0.269 ng WHO-PCDD/F-DL-PCB-TEQ per kg and non-dioxin-like PCBs were < 0.005 mg/kg. *Salmonella* spp. was absent in 25 g samples.[11] None of the amounts of these impurities were of concern.

Coxar® is a granulated product of green-brown colour with a bulk density of 425 kg/m^3 and tapped density of 493 kg/m^3.[12] Sieve analysis of three batches of Coxar® showed that about 4% of particles (w/w) were below 100 μm.[12] Dusting potential was determined in three batches. No dust could be measured in two batches while the third batch indicated a very low dusting potential (0.01 g/m^3).[13]

3.1.2. Characterisation of the active substance

Nicarbazin (CAS-No: 330-95-0) 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, molecular formula $C_{13}H_{10}N_4O_5$, molecular weight 302.25), and 4,6-dimethylpyrimidin-2-ol, also known as 2-hydroxy-4,6-dimethylpyrimidine (HDP,

[8] The full report is available on the EURL website: https://ec.europa.eu/jrc/sites/jrcsh/files/finirep-fad-2015-0039-coxar-turkeys.pdf
[9] Technical dossier/Supplementary information October 2017/Annex 2.
[10] Technical dossier/Supplementary information October 2017/Annex 4.
[11] Technical dossier/Section II/Annex II 7.
[12] Technical dossier/Section II/Annex II 8.
[13] Technical dossier/Section II/Annex II 9.

molecular formula $C_6H_8N_2O$, molecular weight 124.14). The structural formula of nicarbazin is given in Figure 1.

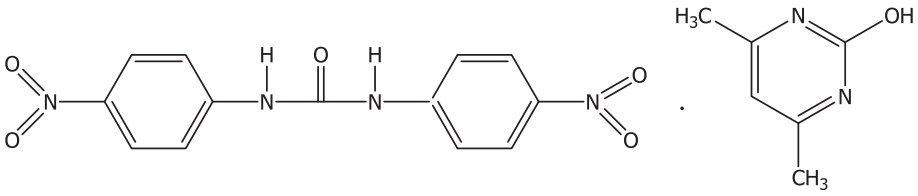

Figure 1: Structural formula of nicarbazin

Nicarbazin is a yellow or yellow green powder having a melting point of 260–265°C. It is slightly soluble in dimethyl formamide and insoluble in water.

[redacted] The specification for *p*-nitroaniline (PNA) is ≤ 0.1%; values measured were 0.02–0.09%. Another impurity was identified (by infrared (IR) and nuclear magnetic resonance (NMR) spectra and mass spectrometric (MS) analysis) as methyl(4-nitrophenyl) carbamate (M4NPC) and specified with ≤ 1.0%; values measured amounted to 0.07–0.34%.

3.1.3. Manufacturing process

The manufacturing process of the active substance via chemical synthesis is fully described in the dossier. [redacted]

Coxar® is obtained by [redacted]

3.1.4. Stability and homogeneity

All the stability studies were performed with three batches of Coxar®.

Coxar® was stored in multiple layer bags with internal polyethylene layer for 48 months at 25°C/60% relative humidity (RH), for 24 months at 30°C/65% RH and for 6 months at 40°C/75% RH.[16] Losses of nicarbazin after 48 months were in the range of 3–4%. Results obtained after 24 months showed losses of 1–2% and 3–4% at 25°C/60% RH and at 30°C/65% RH, respectively. Storage at 40°C/75% RH for 6 months resulted in losses of less than 4%.

Coxar® was incorporated in a vitamin/mineral premixture (containing choline chloride) for chickens for fattening at 20 g nicarbazin/kg premixture. Losses after 6 months were in the range of 2–3% and 5–6% at 25°C/60% RH and at 40°C/75% RH, respectively.[17]

Coxar® was incorporated via premixture in a typical complete feed for chickens for fattening based mainly on wheat and soybean at an inclusion rate of 125 mg nicarbazin/kg. Samples of the mash feed were stored at 25°C/60% RH for 3 months and under 40°C/75% RH for 4 weeks. Feed processing stability of the active substance in Coxar® was examined by pelleting at 85°C, 95°C and 105°C. Samples of the pelleted feed (85°C) were stored under the same conditions as the mash feed.[18]

After 3 months, storage of the mash feed at 25°C/65% RH losses of nicarbazin were in the range of 3–7%. After 4 weeks at 40°C/75% RH, nicarbazin losses were in the range of 2–4%. Nicarbazin concentrations measured following pelleting at 85, 95 and 105°C showed recoveries of 94%, 100% and 91%. Pelleting did not affect the stability of nicarbazin during storage: losses were < 7% after 3 months at 25°C/65% RH and after 4 weeks at 40°C/75% RH.

[redacted]

[16] Technical dossier/Section II/Annex II 20.
[17] Technical dossier/Section II/Annex II 21.
[18] Technical dossier/Section II/Annex II 22.

Ten subsamples from each of the three batches of the mash and pelleted (at 85°C) feed (described above) were analysed for nicarbazin. The coefficients of variation were between 3.4% and 5.6% for the mash batches and between 2.0% and 3.1% for the pelleted batches.[19]

3.1.5. Conditions of use

Coxar® is intended to be used to prevent coccidiosis in turkeys for fattening up to 16 weeks of age. The recommended inclusion level of Coxar® in complete feed for turkeys is 100 mg nicarbazin/kg. The applicant proposes a withdrawal period of 0 day.

3.2. Safety

3.2.1. Safety for the target species

3.2.1.1. Tolerance study in turkeys for fattening

A total of 224 one-day-old male and female turkeys for fattening (hybrid Grademaker) was allocated to four groups with four replicates per sex (five birds + two spare birds for the first week/replicate) each fed pelleted diets containing 0, 75, 100 (1x recommended inclusion level) and 125 (1.25x) mg nicarbazin/kg complete feed, respectively, for 57 days.[20] The basal diet consisted of dehulled extracted toasted soya, wheat, wheat feed, barley, wheat gluten meal, full fat toasted soya, maize gluten meal, and soya oil as feed materials; the starter formulation was calculated to contain 28.2% crude protein (CP), 0.7% methionine (met) and 12.2 MJ metabolisable energy (ME)/kg; the grower formulation 26.5% CP, 0.6% met and 12.0 MJ ME/kg.[21] The starter was fed for 14 days, the grower until the end of the study. The birds had *ad libitum* access to feed and water. The intended concentrations of nicarbazin in starter and grower diets were analytically confirmed.

Clinical observations were made daily; body weight and feed intake were recorded in weekly intervals. On day 55, blood samples were taken from one bird per replicate (four males and four females per treatment) for haematology[22] and clinical blood biochemistry.[23] On day 57/58, 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, heart, and lungs.

Statistical analyses for the zootechnical endpoints were based on the experimental units (birds).[24] Since body weight was significantly different between males and females ($p < 0.0001$) at day 57, separate evaluations were performed. A two-way analysis of variance (ANOVA) (treatment and pen location) was made. In case of significant effects detected by an ANOVA, post hoc analyses were performed, applying a single-way ANOVA or non-parametric tests (Kruskal–Wallis one-way analysis of variance on ranks) when heteroscedasticity was proven. A multiple comparison procedure was then used by applying either the all pairwise multiple comparison procedures (Holm–Sidak method, homoscedasticity demonstrated) or the all pairwise multiple comparison procedures (Tukey Test, non-parametric analysis). Blood chemistry and haematology data as well as organ weights based on single birds were analysed for males and females together by an one-way ANOVA, normality was tested by Shapiro–Wilk test and subsequently equality of variances. In case normality test failed, the Kruskal–Wallis one-way analysis of variance on ranks was used. Multiple comparisons were made versus the control group (Dunnett's Method).

No mortality occurred during the course of the study. The main results are summarised in Table 1.

[19] Technical dossier/Section II/Annex II 21 and 22.
[20] Technical dossier/Section III/Annex III 01.
[21] Technical dossier/Supplementary information February 2017/Annex 06.
[22] Haematocrit (Hct), haemoglobin concentration (Hb), erythrocyte count (RBC), mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCC), total white cell count (WBC), differential WBC count: heterophils (HET), lymphocytes (LYM), basophils (BAS), eosinophils (EOS), monocytes (MON), thrombocyte - avian platelets (THBC).
[23] 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 (URAC), creatinine.
[24] Clarification received in November 2017.

Table 1: Main results of a 57-day tolerance study in turkeys for fattening with Coxar®

Gender	Nicarbazin (mg/kg feed)	Final body weight (g)	Feed intake (g/bird and day)	Feed to gain ratio
Male	0	4,914	197	2.08
	75	4,948	203	2.09
	100	4,834	191	2.05
	125	4,674	193	2.13
Female	0	4,029	155	2.10
	75	4,114	157	2.05
	100	3,867	145	2.03
	125	3,855	153	1.98

Means of males and females in the high-dose group (and of females in the intermediate dose group = use level group) appeared numerically lower than in the control and the 75 mg nicarbazin group. No sex treatment interaction was observed. The mean body weight for the four groups (0, 75, 100 and 125 mg nicarbazin/kg feed) was 4,471, 4,532, 4,384 and 4,295 g, respectively. No significant differences were observed in final body weight. Feed intake and feed to gain ratio were not affected by the treatment.

All haematological and biochemistry parameters as well as organ weights (absolute and relative to body weight) remained unchanged in the supplemented groups when compared to the control group. No treatment-related organ weight changes, macroscopic lesions or histopathological changes were found.

3.2.1.2. Interactions

No incompatibilities or interactions of nicarbazin with feedingstuffs, carriers or other approved additives are to be expected.

3.2.1.3. Microbiological safety of the additive

Antibacterial activity of nicarbazin was studied by analysing the minimum inhibitory concentration of a pool of strains isolated from poultry[25] and human gut.[26] No antimicrobial activity was observed at concentration of 64 mg/L or lower for any of the analysed strains.

3.2.1.4. Conclusions on the safety for the target species

Nicarbazin at a use level of 100 mg/kg complete feed is safe for turkeys for fattening up to 16 weeks of age with a margin of safety of about 1.25. Nicarbazin has no antimicrobial activity; no incompatibilities or interactions with feedingstuffs, carriers or other approved additives are expected.

3.2.2. Safety for the consumer

3.2.2.1. Absorption, distribution, metabolism and excretion

The data submitted concerning the absorption, distribution, metabolism and excretion (ADME) of nicarbazin in the chicken, turkey and rat were assessed by the FEEDAP Panel in an opinion concerning Monimax® for turkeys for fattening (EFSA FEEDAP Panel, 2017). In the absence of new information, the same conclusions can be retained for the current assessment, which are summarised as follow: 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 (by two orders of magnitude) than those derived from DNC. The metabolic pathways in the turkey are similar to those in the chicken and rat.

Nicarbazin as such cannot appear as residue in tissues; only its two individual components may generate residues.

[25] Technical dossier/Section III/Reference III 02. *Staphylococcus* sp., *Pasteurella multocida*, *Salmonella* sp., *Escherichia coli* (3 serotypes: 078K80, O1K1 and O2K1 strains), *Enterococcus* sp. and *Clostridium perfringens.*

[26] Technical dossier/Section III/Reference III 03. *Bifidobacterium* spp., *Eubacterium* spp., *Clostridium* spp., *Peptostreptococcus* spp., *Lactobacillus* spp., *Enterococcus* spp., *Bacteroides* spp., *Escherichia coli* and *Proteus* spp.

3.2.2.2. Residues

Total and marker residue data in chicken and turkey tissues have been recently assessed by the FEEDAP Panel (EFSA FEEDAP Panel, 2017). The data obtained in chickens indicated that total and marker residue concentrations in tissues declined rapidly following nicarbazin withdrawal, DNC residues being much higher than HDP. DNC residues measured in turkey tissues were lower compared to chicken tissues, and it was reasonably assumed that the corresponding total residues would also be lower. This was attributed to lower absorption since no difference was noted in the quantitative metabolic process in the liver (EFSA FEEDAP Panel, 2017).

For the current assessment, a marker residue study (good laboratory practice (GLP)) was performed to measure DNC residues in turkey tissues following the administration of Coxar® for 16 weeks.[27] Groups of six turkeys (3 males and 3 females; 1-day-old) were fed a diet supplemented with 100 mg nicarbazin from Coxar®/kg (analytically confirmed: 109 mg/kg for grower and finisher feed). Groups of birds were slaughtered after 0, 1, 3, 6, 12 and 24 h withdrawal of the supplemented feed. DNC was determined with a validated and further verified reversed-phase high performance liquid chromatography coupled to a triple quadrupole mass spectrometer (RP-HPLC–MS/MS) method with a limit of quantification (LOQ) of 1 mg DNC/kg for all tissues. After 1-h withdrawal, DNC residues (average + 2 SD) were close to the LOQ in the liver (1.22 mg/kg) and < LOQ in all other tissues. All values after 6 h were < LOQ.

3.2.2.3. Toxicological studies

The toxicology of nicarbazin was assessed in a recent opinion of the FEEDAP Panel on the safety and efficacy of Monimax® for turkeys for fattening (EFSA FEEDAP Panel, 2017). The same dataset was submitted for the current application; therefore, the same conclusions can be retained:

> '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 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 body weight (bw) per day. The NOAEL for embryo/fetal 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 occurrence of microcrystals in urine and related microscopic renal observations at higher dose level.'

3.2.2.4. Assessment of consumer safety

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 European Union (EU) level. The extension of these MRLs to turkey tissues has been proposed recently by the FEEDAP Panel (EFSA FEEDAP Panel, 2017).

Residue data obtained for DNC after the use of Coxar® at a level corresponding to 100 mg nicarbazin/kg feed for turkeys for fattening (Section 3.2.2.2) showed that after withdrawal times of 1-h in turkeys all marker residue concentrations were below the respective MRLs in liver, kidney, muscle and skin+fat. The withdrawal time applied is considered equivalent to zero day under practical conditions.

The safety for the consumer of the two nicarbazin impurities, PNA and M4NPC was recently assessed by the FEEDAP Panel for Monimax® containing 50 mg nicarbazin/kg feed (EFSA FEEDAP Panel, 2017). Based on a review of the Health Council of The Netherlands (GR, 2008), on disposition studies of Chopade and Matthews (1984) in laboratory animals[28] and on the absence of new recent data, the FEEDAP Panel concluded that consumer exposure to PNA would be negligible at a maximum level of 0.1% in nicarbazin (EFSA FEEDAP Panel, 2017). Based on (i) an *in vitro* study of the

[27] Technical dossier/Section III/Reference III 07.

[28] The exposure of target animals to PNA when using Monimax® at the highest applied concentration would be below 50 μg PNA/kg complete feed for poultry.

metabolism in the chicken and turkey, (ii) a residue study in chicken and (iii) a battery of mutagenicity tests, the FEEDAP Panel concluded that the impurity M4NPC is considered safe for the consumer provided that a maximum concentration of 0.4% in nicarbazin is not exceeded (EFSA FEEDAP Panel, 2017). The FEEDAP Panel applies the same conclusions to the use of 100 mg nicarbazin from Coxar®/kg feed in turkeys for fattening; no concern for consumer safety would arise from maximum content of 0.1% PNA and 0.4% M4NPC in nicarbazin.

3.2.2.5. Conclusions on the safety for the consumer

The use of Coxar® at the highest proposed dose (100 mg nicarbazin/kg complete feed) will not pose a risk to persons consuming food products from turkeys for fattening, provided that maximum contents in nicarbazin of 0.1% PNA and 0.4% M4NPC would be respected.

No withdrawal time is required for Coxar® in turkeys for fattening.

3.2.3. Safety for the user

Particle size distribution of the granulated additive measured by sieve analysis showed an inhalable fraction (≤ 100 μm) of about 4% (w/w).[29] The highest dusting potential out of three batches was 0.01 g/m^3.[30] The potential exposure of users by inhalation is considered low and therefore the risk by inhalation is considered negligible.

For the current assessment, the applicant provided the same studies performed with nicarbazin as already submitted in the application for Monimax®. These data have been recently assessed by the FEEDAP Panel (EFSA FEEDAP Panel, 2017) as follows:

> 'A study (GLP) was performed to assess the acute inhalation toxicity of nicarbazin according to OECD Guideline 403. A group of ten rats (five males and five females) was exposed to a dust atmosphere. The animals were exposed for four hours using a nose only exposure system, followed by a fourteen day observation period. The mean achieved atmosphere concentration was 5.12 ± 0.20 mg/L. No deaths or major clinical signs were observed, and no macroscopic abnormalities were detected at necropsy (terminal sacrifice). It was therefore considered that the acute inhalation median lethal concentration (4 hr LC50) of nicarbazin (atmosphere concentration of 5.12 mg/L for four hours) in the rat was greater than 5.12 mg/L. [...] The potential of nicarbazin for skin and eye irritation was investigated following OECD TG 404 and 405, respectively. Nicarbazin was very slightly irritant when applied dermally to rabbits. The scores for chemosis, conjunctival redness, iris lesions and corneal opacity following a single ocular administration in rabbits after 24, 48 and 72 hours were all about zero. Nicarbazin is not classified as irritating to the eyes. The skin sensitisation potential of nicarbazin was studied by the murine Local Lymph Node Assay (LLNA) (OECD 429). Neither mortality nor clinical signs were observed during the study. No cutaneous reaction and no increase in ear thickness were observed. No lymphoproliferation was noted. It is concluded that nicarbazin is not a skin sensitiser'.

Conclusions on safety for the user

Nicarbazin is not a skin or eye irritant and not a skin sensitiser. These conclusions also apply to the additive Coxar®. Inhalation toxicity of nicarbazin is limited. Due to low dusting potential of the granulated additive, the risk for users is considered negligible.

3.2.4. Safety for the environment

For the current assessment, the applicant provided the same data set as already submitted in the application for Monimax®.

In its recent opinion on the safety and efficacy of Monimax® for turkeys for fattening, corresponding to a maximum use level of 50 mg nicarbazin/kg feed (EFSA FEEDAP Panel, 2017) the FEEDAP Panel stated that:

> 'A final conclusion on the risk resulting from the use of nicarbazin from Monimax® in turkeys 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

[29] Technical dossier/Section II/Annex II 8.
[30] Technical dossier/Section II/Annex II 9.

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

The same conclusions can be extended to the current assessment considering also that the dose of nicarbazin in Coxar® (100 mg/kg complete feed) is higher than in Monimax® (50 mg/kg feed): based on the available data, the FEEDAP Panel cannot conclude on the safety of Coxar® for the environment.

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: (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 relevant guidance document on coccidiostats and histomonostats (EFSA FEEDAP Panel, 2011a).[31]

The applicant submitted one dose-titration study, four floor pen studies and three ASTs in turkeys for fattening.

3.3.1. Dose–titration study

The applicant submitted a dose–titration study with Coxar® made under controlled conditions in turkeys for fattening artificially infected with mixed *Eimeria* spp.[32] A total of 288 one-day-old male turkeys for fattening (Big 9) were distributed to eight treatment groups (six pens per treatments, six turkeys per pen); an uninfected untreated group (UUC) was compared with an infected untreated (IUC) and six infected treated groups (IT). The treatments were 75, 100, and 125 mg nicarbazin/kg (analytically confirmed). The remaining three groups were treated with a combination of monensin and nicarbazin but results were not reported. On day 16 birds in the IUC and IT groups were inoculated with 108,000 *Eimeria meleagrimitis,* 4,000 *Eimeria dispersa* and 30,000 *Eimeria adenoeides* oocysts. The anticoccidial treatment was provided from day 13 until day 28 (study completion). An ANOVA was performed with the data and group means were compared with least significant difference (LSD) test.

Total intestinal lesion scores did not show improvement due to the coccidiostatic treatment. There was no significant difference in oocyst excretion among the infected groups. The average final body weight per bird and average daily weight gain over the study period (days 13–28) were significantly higher in the UUC and IT 100 treatment groups compared to the IUC group. No significant difference was observed in the average daily feed intake among the treatment groups. The overall feed to gain ratio was found to be significantly higher in the IUC group compared to the UUC group and all nicarbazin treatment groups.

3.3.2. Floor-pen studies

Four floor pen studies in turkeys for fattening, conducted in 2011–2012 and 2016, were submitted.

One of the studies could not be considered for the demonstration of the efficacy.[33] It was noted that only eight out of 25 birds per were inoculated (seeder bird model). Based on the results of intestinal lesion scoring and the number of oocyst in excreta, it was concluded that such model did not appear sensitive in indicating a disease protecting effect of the coccidiostat in all birds used.

The other three studies followed a similar design[34]; birds were penned and distributed into three treatment groups: an UUC group, an IUC group and an IT group. The IT group received feed containing 75 mg nicarbazin/kg feed in trial 1 and 100 mg nicarbazin/kg feed in trials 2 and 3; dosage was analytically confirmed (see Table 2). The duration of the studies was 84 days. All birds in the pen

[31] The FEEDAP Panel stated in its guidance for the preparation of dossiers for coccidiostats and histomonostats (EFSA FEEDAP Panel, 2011a,b) 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.

[32] Technical dossier/Section IV/Reference IV 1.

[33] Technical dossier/Section IV/Reference IV 3.

[34] Trial 1: Technical dossier/Section IV/Reference IV 2. Trial 2: Technical dossier/Section IV/Reference IV 4. and Trial 3: Technical dossier/Supplementary information February 2017/Annex 20.

were inoculated on day 14 with recent field isolates of pathogenic *Eimeria* species.[35] Animal health and mortality were monitored daily. Feed intake and body weight of the animals were measured throughout the study, feed to gain ratio was calculated. Samples of excreta were analysed for oocyst excretion.

Table 2: Experimental design of floor pen studies with turkeys for fattening using Coxar®

Trial	Start date	Replicates per treatment (birds per pen)	Test animal	Diet(1)		
				Type	Period (days)	Results of analysis (IT) Nicarbazin (mg/kg feed)
1	10/2012	8 (7 or 8)	Day-old male turkeys (BGT)	Starter Grower 1 Grower 2	0–28 28–56 56–84	85 64 65
2	04/2012	8 (28 or 29)	Day-old female turkeys (BUT 9)	Starter Grower Finisher	0–28 28–56 56–84	106 101 116
3	06/2016	12 (25)	Day-old female turkeys (BUT 10)	Starter Grower Finisher	0–28 28–56 56–84	83 93 88

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

In all three trials the experimental unit was the pen for the zootechnical parameters and oocysts per gram of excreta (OPG), the statistical unit for intestinal lesion scores was the bird. In all trials, an ANOVA was applied to the data, non-parametric test was used for oocyst counts in trials 2 and 3. The level of significance was set at a p-value ≤ 0.05. In trial 3, group means were compared with LSD test, and in trial 1, Duncan test was used (test not reported for trial 2).

Results of oocysts excretion are presented in Table 3. The treatment with Coxar® did not reduce the oocyst excretion in all trials at the different time points where OPGs were measured. The exception was in trial 1 at days 28 and 42 which showed a significant reduction of OPGs in the IT group compared to IUC group. In trials 1 and 2, the UUC group showed at the end of the trials an oocyst excretion reaching similar values to those of the infected groups.

Table 3: Total number (log_{10}) of *Eimeria* oocysts per gram of excreta (OPG) in floor pen studies

Trial 1	Day 21	Day 28	Day 35	Day 42	Day 49	Day 56	Day 77	Day 87
UUC	0^a	0^a	0^a	0^a	0	0	0	1.18
IUC	5.47^b	3.88^c	3.10^b	1.65^b	0	1.16	0.52	0.84
IT	5.57^b	2.68^b	4.00^b	0.0^a	0.88	1.2	0	0.45

Trial 2	Day 21	Day 25	Day 28	Day 56	Day 84
UUC	2.31	2.12	2.19	2.18	1.88
IUC	4.88	3.93	3.91	2.60	2.14
IT	4.95	4.19	3.69	2.66	2.21

Trial 3	Day 21	Day 25	Day 27	Day 55	Day 83
UUC	nd	nd	nd	nd	nd
IUC	3.76	2.95	2.22	1.92	nd
IT	3.84	2.70	1.97	1.85	nd

nd: not detected.

a,b,c: Means in columns within a study with different superscript are significantly different ($p \leq 0.05$).

[35] Trial 1: French field isolate (07/2011); estimated dosage per bird: 11,740 *E. meleagrimitis*, 4,670 *E. adenoeides*, 920 *E. dispersa*; Trial 2: A recent French field isolate; estimated dose per bird was 60,000 *E. meleagrimitis*, 111,600 *E. adenoeides* + *E. gallopavonis*, 70,000 *E. meleagridis*; Trial 3: A recent European field isolate; estimated dose per bird was 66,628 *E. meleagrimitis*, 13,968 *E. adenoeides*, 1,151 *E. meleagridis* + *E. gallopavonis*.

Table 4 summarises the results concerning mortality and zootechnical endpoints. *Eimeria* infection in trial 1 resulted in a significant decrease of feed intake and final body weight without differences due to the coccidiostatic treatment. Total mortality was highest in the UUC group which reduces the value of this study. In trial 3, no significant differences between the three groups were found concerning the overall performance data and mortality; however, significant improvement of the body weight and average daily gain was seen in the two weeks post-infection period. In trial 2, there were also no significant differences between the groups for the zootechnical parameters at the end of the trial; however, a significant improvement of the body weight and feed to gain ratio was seen in the first 2 weeks post-infection. In the same trial, mortality after infection showed large and significant differences between the groups; coccidiosis-related mortality was significantly reduced by 100 mg nicarbazin from Coxar®/kg feed.

Table 4: Overall performance data and mortality of turkeys in floor pen trials with Coxar®

	Feed intake (kg)	Body weight (kg)	Weight gain (kg)	Feed to gain ratio	Mortality % (n)
Trial 1					
UUC	0.196[b]	9.39[b]	–	1.90	19.6
IUC	0.161[a]	7.60[a]	–	1.85	13.8
IT	0.162[a]	7.57[a]	–	1.87	14.3
Trial 2					
UUC	0.168	6.74	0.080	2.11	2.2 (5)
IUC	0.157	6.40	0.076	2.08	51.7 (118)
IT	0.161	6.57	0.078	2.07	36.2 (83)*
Trial 3					
UUC	0.165	6.67	0.079	2.09	1.9 (6)
IUC	0.162	6.61	0.078	2.08	2.3 (7)
IT	0.165	6.73	0.080	2.08	2.9 (9)

–: not reported.
*: IT mean significantly different from IUC mean ($p \leq 0.05$).
a,b,c: Means in columns within a study with different superscript are significantly different ($p \leq 0.05$).

In two trials performed with 100 mg nicarbazin/kg feed, only one could be considered as indicative for the efficacy (significantly reduced coccidiosis-related mortality). In the trial performed with 75 mg nicarbazin/kg feed, the treatment reduced significantly the oocyst excretion at two time points; however, these findings were not considered indicative for a coccidiostatic effect since they were not consistent for the days of observation and were not supported by the improvement of any other parameter.

3.3.3. Anticoccidial Sensitivity Tests

Three ASTs with a similar experimental design, performed in 2012, were submitted.[36] Each test was made with the groups UUC, IUC and IT, the latter receiving feed supplemented with 100 mg nicarbazin (analysed 106) from Coxar®. The day-old male turkeys for fattening (Big 9) were randomly allocated to the groups; group size was 36 turkeys (6 replicates with 6 birds). Supplemented feed was administered from day 13 until day 28. Birds were artificially infected on day 16 with sporulated oocysts from field isolates.[37] 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 Repérant scoring system for the species relevant to turkeys.

The data were analysed by an ANOVA using the animal as experimental unit (exception pen based data for feed intake). Group differences at $p < 0.05$ level were checked by LSD test.

[36] AST-1: Technical dossier/Section IV/Reference IV 5. AST-2: Reference IV 6. AST-3: Reference IV 7.
[37] AST-1: French field isolate (2011), estimated dosage per bird: 108,000 *E. meleagrimitis*, 4,000 *E. dispersa* 30,000 *E. adenoeides*; AST-2: German field isolate (2011), estimated dose per bird: 57,500 *E. meleagrimitis*, 500 *E. dispersa* 24,000 *E. adenoeides;* and AST-3: Belgian field isolate (2011), estimated dosage per bird: 54,000 *E. meleagrimitis*, 56,000 *E. adenoeides*.

The results are summarised in Table 5. There was no bird mortality in the three tests. Oocyst excretion 7 days after inoculation (day 23) indicated successful *Eimeria* infection in the IUC and IT groups. In AST-1, the intestinal lesion score due to *E. adenoeides* was significantly reduced by the treatment, but OPGs only numerically; both findings could explain the significantly higher body weight and better feed to gain ratio observed in the IT group compared to the IUC, as a result of coccidiosis prevention. Comparable observations were made in AST-2, total intestinal lesion score and OPG were significantly reduced by nicarbazin. No effect on the zootechnical parameters was seen in AST-3; however, oocyst excretion, total intestinal lesion score and lesions score due to *E. meleagrimitis* were significantly lower in the IT group compared to the IUC group.

Table 5: Summary of anticoccidial sensitivity tests performed with Coxar®

AST	Tr. group	Daily feed intake (kg)	Body weight (kg)	Daily weight gain (kg)	Feed to gain ratio	Total OPG	Average lesion scores		
							E. meleagrimitis	*E. adenoeides*	Total
		D13–28	D28	D13–28	D13–28	D23	D23		
1	UUC	0.065	0.793^{a}	0.039^{a}	1.67^{a}	0^{b}	0.3	0^{a}	0.3^{b}
	IUC	0.067	0.709^{b}	0.033^{b}	2.01^{b}	118,967^{a}	0.3	0.6^{b}	0.9^{a}
	IT	0.063	0.800^{a}	0.039^{a}	1.62^{a}	83,300^{a}	0.6	0.1^{a}	0.7^{ab}
2	UUC	0.065	0.793^{ab}	0.039^{ab}	1.67^{a}	0^{c}	0.3^{b}	0^{b}	0.3^{c}
	IUC	0.068	0.756^{b}	0.036^{b}	1.89^{b}	140,667^{b}	1.2^{a}	0.5^{a}	1.7^{a}
	IT	0.069	0.839^{a}	0.041^{a}	1.67^{ab}	65,300^{a}	0.8^{a}	0.2^{ab}	1.0^{b}
3	UUC	0.065	0.793^{b}	0.039^{b}	1.67	0^{c}	0.3^{a}	0^{b}	0.3^{c}
	IUC	0.064	0.725^{a}	0.034^{a}	1.90	90,833^{b}	1.3^{b}	0.9^{a}	2.2^{a}
	IT	0.065	0.778^{ab}	0.037^{ab}	1.75	55,167^{a}	0.5^{a}	0.8^{a}	1.3^{b}

a,b,c: Means in columns within a study with different superscript are significantly different ($p \leq 0.05$).

3.3.4. Conclusions on efficacy

The efficacy of 100 mg nicarbazin from Coxar®/kg feed was demonstrated in three ASTs, but only in one floor pen study. The floor pen study with 75 mg nicarbazin failed to demonstrate evidence of efficacy. No final conclusions on the efficacy of nicarbazin from Coxar® for turkeys for fattening can be drawn.

3.4. Post-market monitoring

Field monitoring of *Eimeria* spp. resistance to 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.

4. Conclusions

Coxar® is considered safe for turkeys for fattening at the use level of 100 mg nicarbazin/kg complete feed with a margin of safety of about 1.25. Nicarbazin has no antimicrobial activity; no incompatibilities or interactions with feedingstuffs, carriers, or other approved additives are expected.

Nicarbazin, when ingested, is rapidly split in its two components HDP and DNC which behave independently. DNC residues decline rapidly from tissues following nicarbazin withdrawal. HDP-related residues are much lower than those derived from DNC. DNC appears as the marker residue. Liver is the target tissue. The metabolic pathways in the turkey are similar to those in the chicken and rat.

Nicarbazin is not genotoxic. The primary toxicity resulting from the oral use of nicarbazin is renal toxicity. 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.

The use of Coxar® at the highest proposed dose (100 mg nicarbazin/kg complete feed) will not pose a risk to persons consuming animal products from turkeys for fattening, provided that maximum contents in nicarbazin of 0.1% PNA and 0.4% M4NPC would be respected. No withdrawal time is required for Coxar® in turkeys for fattening. Residue data comply with the established MRLs.

Nicarbazin is not a skin or eye irritant and not a skin sensitiser. These conclusions also apply to the additive Coxar®. Inhalation toxicity of nicarbazin is limited. Due to low dusting potential of the granulated additive, the risk for users is considered negligible.

No final conclusions on the safety of nicarbazin for the environment can be made since: (i) DNC refined PECs shows 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 efficacy of 100 mg nicarbazin from Coxar®/kg feed was demonstrated in three ASTs, but only in one floor pen study. The floor pen study with 75 mg nicarbazin failed to demonstrate evidence of efficacy. No final conclusions on the efficacy of nicarbazin from Coxar® for turkeys for fattening can be drawn.

Documentation provided to EFSA

1) Coxar® for turkeys for fattening. October 2015. Submitted by Huvepharma N.V.
2) Coxar® for turkeys for fattening. Supplementary information. February 2017. Submitted by Huvepharma N.V.
3) Coxar® for turkeys for fattening. Supplementary information. October 2017. Submitted by Huvepharma N.V.
4) Evaluation report of the European Union Reference Laboratory for Feed Additives on the Methods of Analysis for Coxar®.
5) Comments from Member States.

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Abbreviations

ADME	absorption, distribution, metabolism and excretion
ANOVA	analysis of variance
AST	anticoccidial sensitivity test
bw	body weight
CAS	Chemical Abstracts Service
DNC	dinitrocarbanilide
HDP	2-hydroxy-4,6-dimethylpyrimidine
EURL	European Union Reference Laboratory
FEEDAP	EFSA Panel on Additives and Products or Substances used in Animal Feed
GLP	Good Laboratory Practice
LC50	median lethal concentration
LLNA	local lymph node assay
LOQ	limit of quantification
LSD	least significant difference
M4NPC	methyl(4-nitrophenyl) carbamate
MRL	maximum residue limit
NOAEL	no observed adverse effect level
OECD	Organisation for Economic Co-operation and Development
OPG	oocysts per gram of excreta
PEC	predicted environmental concentration
PNEC	predicted no effect concentration
PNA	*p*-nitroaniline
RH	relative humidity
SCAN	Scientific Committee on Animal Nutrition
SD	standard deviation

Annex A – Executive Summary of the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Methods of Analysis for Coxar®[8]

In the current application authorisation is sought for *Coxar®*, 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 *turkeys for fattening*. *Coxar®* consists of 250 g/kg of *nicarbazin* complemented by starch for granulation, wheat meal and calcium carbonate. *Coxar®* is intended to be incorporated in *feedingstuffs* through *premixtures* for turkeys for fattening at a concentration of *nicarbazin* of 100 mg/kg *feedingstuffs*. Furthermore the Applicant proposed Maximum Residue Limits (MRLs) in turkey tissues ranging from 4000 to 15000 μg *4,4-dinitrocarbanilide (DNC)*/kg of fresh material.

For the quantification of *nicarbazin* in the *premixtures* and *feedingstuffs* the Applicant submitted the EN ISO 15782 method, based on High Performance Liquid Chromatography coupled to spectrophotometric detection (HPLC-UV). Precisions (relative standard deviations for *repeatability* and *reproducibility*) ranging from 2.6 to 12% were reported, together with a limit of quantification (LOQ) of 20 mg/kg *feedingstuffs*. The Applicant submitted for the quantification of *nicarbazin* in the *feed additive* (*Coxar®*) a single-laboratory validated and further verified method based on the above mentioned EN ISO 15782 method using different extraction solvent (dimethylformamide), and reported satisfactory experimental data. Based on the performance characteristics available the EURL recommends for official control the HPLC-UV methods for the quantification of *nicarbazin* in the *feed additive*, *premixtures* and *feedingstuffs*.

For the quantification of *DNC* (target compound for *nicarbazin*) in target turkey 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 *DNC* 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.