European Food Safety Authority

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SCIENTIFIC OPINION

Scientific Opinion on the safety and efficacy of Coxiril® (diclazuril) as a feed additive for turkeys for fattening[1]

EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP)[2,3]

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

Coxiril®, containing 0.5 % diclazuril, is intended for the control of coccidiosis in turkeys for fattening at concentrations between 0.8 and 1.2 mg diclazuril/kg complete feed. The highest proposed use level (1.2 mg diclazuril/kg feed) is considered safe for turkeys for fattening. Diclazuril has no substantial antibacterial activity. Diclazuril from Coxiril® is considered toxicologically equivalent to the other currently authorised diclazuril. The use of Coxiril® at the highest proposed use level of 1.2 mg diclazuril/kg complete feed in turkeys for fattening is safe for the consumer since Maximum Residue Limits are not exceeded. Coxiril® is considered non-irritant to eyes and skin. It is not a potential skin sensitiser. User exposure to Coxiril®, as a result of normal handling, is unlikely to cause respiratory or systemic toxicity. Based on a Phase I assessment, the use of diclazuril in turkeys for fattening at the high use level does not pose a risk to the environment. Diclazuril from Coxiril® has the potential to control coccidiosis in turkeys for fattening at a minimum concentration of 0.8 mg/kg complete feed. This conclusion is derived from literature data, and from recent studies performed with Coxiril®.

KEY WORDS

coccidiostat, Coxiril®, diclazuril, safety, efficacy, turkeys for fattening

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[1] On request from the European Commission, Question No EFSA-Q-2012-00919, adopted on 22 May 2014.

[2] Panel members: Gabriele Aquilina, Vasileios Bampidis, Maria De Lourdes Bastos, Lucio Guido Costa, Gerhard Flachowsky, Mikolaj Antoni Gralak, Christer Hogstrand, Lubomir Leng, Secundino López-Puente, Giovanna Martelli, Baltasar Mayo, Fernando Ramos, Derek Renshaw, Guido Rychen, Maria Saarela, Kristen Sejrsen, Patrick Van Beelen, Robert John Wallace and Johannes Westendorf. Correspondence: FEEDAP@efsa.europa.eu

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SUMMARY

Following a request from the European Commission, the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) was asked to deliver a scientific opinion on the safety and efficacy of Coxiril® (diclazuril) in turkeys for fattening. Coxiril®, containing 0.5 % diclazuril, is intended for the control of coccidiosis in turkeys for fattening at concentrations between 0.8 and 1.2 mg diclazuril/kg complete feed.

The highest proposed use level (1.2 mg diclazuril/kg feed) is considered safe for turkeys for fattening. None of the parameters measured in the tolerance study showed an adverse effect of the 12-fold use level of diclazuril. However, the tolerance study showed a certain weakness particularly due to a low number of blood samples per treatment and sex. Diclazuril has no substantial antibacterial activity.

Diclazuril from Coxiril® is considered toxicologically equivalent to the other currently authorised diclazuril since the specifications of the European Pharmacopoeia are respected. A residue study with Coxiril® in turkeys for fattening demonstrated compliance with the Maximum Residue Limits for diclazuril without applying a withdrawal period. Consequently, Coxiril® used at the highest dose proposed (1.2 mg diclazuril/kg complete feed) is safe for the consumer.

Coxiril® is considered non-irritant to eyes and skin. It is not a potential skin sensitiser. User exposure to Coxiril®, as a result of normal handling, is unlikely to cause respiratory or systemic toxicity.

The use of diclazuril in turkeys for fattening at the highest use level does not pose a risk to the environment.

Diclazuril from Coxiril® has the potential to control coccidiosis in turkeys for fattening at a minimum concentration of 0.8 mg/kg complete feed. This conclusion is derived from literature data, and from recent studies performed with Coxiril®.

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BACKGROUND

Regulation (EC) No 1831/2003[4] establishes the rules governing the European Union 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 the company Huvepharma N.V.[5] for authorisation of the product Coxiril® (diclazuril) when used as a feed additive for turkeys for fattening (category: coccidiostats and histomonostats) under the conditions mentioned in Table 1.

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.[6] According to Article 8 of that Regulation, 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. The particulars and documents in support of the application were considered valid by EFSA as of 7 December 2012.

The additive Coxiril® (diclazuril) is not authorised in the European Union.

The product Clinacox® 0.5 % containing the same active substance (diclazuril) has been authorised for ten years for use in rabbits (authorisation until 24 October 2018),[7] in chickens for fattening (authorisation until 23 December 2020),[8] in chickens reared for laying (authorisation until 2 August 2023),[9] in turkeys for fattening (authorisation until 26 September 2021),[10] and in guinea fowl (authorisation until 16 March 2021).[11]

The MRLs are part of the respective authorisations of Clinacox® 0.5 % for chickens[12] and turkeys for fattening[13] and for guinea fowl.[14] The values proposed have been enforced at the EU level (Commission Regulation (EC) No 976/2008).[15] Commission Implementing Regulation (EU) No 115/2013 introduced MRLs for poultry (liver, kidney, muscle, skin/fat).[16]

[4] 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.
[5] Huvepharma NV, Uitbreidingstraat 80, Antwerp, Belgium.
[6] EFSA Dossier reference: FAD-2012-0036.
[7] Commission Regulation (EC) No 971/2008 of 3 October 2008 concerning a new use of a coccidiostat as additive in feedingstuffs. OJ L 265, 4.10.2008, p. 3. Corrigendum EN in OJ L 267, 8.10.2008, p. 32.
[8] Commission Regulation (EU) No 1118/2010 of 2 December 2010 concerning the authorisation of diclazuril as feed additive for chickens for fattening (holder of the authorisation Janssen Pharmaceutica NV) and amending Regulation (EC) No 2430/1999. OJ L 317, 3.12.2010, p. 5.
[9] Commission Implementing Regulation (EU) No 667/2013 of 12 July 2013 concerning the authorisation of diclazuril as feed additive for chickens reared for laying (holder of the authorisation Eli Lily and Company Ltd) and repealing Regulation (EC) No 162/2003. OJ L 192, 13.7.2013, p. 35.
[10] Commission Implementing Regulation (EU) No 888/2011 of 5 September 2011 concerning the authorisation of diclazuril as feed additive for turkeys for fattening (holder of the authorisation Janssen Pharmaceutica NV) and amending Regulation (EC) No 2430/1999. OJ L 229, 6.9.2011, p. 9.
[11] Commission Regulation (EU) No 169/2011 of 23 February 2011 concerning the authorisation of diclazuril as feed additive for guinea fowls (holder of the authorisation Janssen Pharmaceutica NV). OJ L 49, 24.2.2011, p. 6.
[12] OJ L 317, 3.12.2010, p. 5.
[13] OJ L 229, 6.9.2011, p. 9.
[14] OJ L 49, 24.2.2011, p. 6.
[15] Commission Regulation (EC) No 976/2008 of 6 October 2008 amending Regulations (EC) No 2430/1999, (EC) No 162/2003 as regards the terms of the authorisation of the feed additive "Clinacox", belonging to the group of coccidiostats and other medicinal substances.OJ L 266, 7.10.2008, p. 3.
[16] Commission Implementing Regulation (EU) No 115/2013 of 8 February 2013 amending the Annex to Regulation (EU) No 37/2010 on pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin, as regard the substance diclazuril. OJ L 38, 9.3.2013, p. 11.

The Scientific Committee on Animal Nutrition (SCAN) issued an opinion on the use of diclazuril in feedingstuffs for chickens for fattening (EC, 1991) and an opinion on extension of use of diclazuril to the feedingstuffs for chickens reared for laying (EC, 1997).

The additive Coxiril® (diclazuril) for turkeys for fattening has never been assessed by EFSA.

The European Food Safety Authority (EFSA) issued an opinion on the Maximum Residue Limits (MRLs) for the additive Clinacox® 0.5 % containing the same active substance (diclazuril) for turkeys for fattening, chickens for fattening and chickens reared for laying (EFSA, 2007a). This opinion was updated by EFSA on 16 April 2008 (EFSA, 2008a). On 23 June 2010 the EFSA adopted an opinion on the safety and efficacy of Clinacox® 0.5 % for chickens for fattening (EFSA FEEDAP Panel, 2010a), on 5 October 2010 an opinion on the safety and efficacy of Clinacox® 0.5 % for guinea fowl (EFSA FEEDAP Panel, 2010b) and on 16 March 2011 an opinion on the safety and efficacy of Clinacox® 0.5 % for turkeys for fattening (EFSA FEEDAP Panel, 2011a). Finally, an opinion on re-evaluation of safety and efficacy of Clinacox® 0.5 % for chickens reared for laying was adopted in 2013 (EFSA FEEADP Panel, 2013). In 2014 EFSA adopted an opinion on the safety and efficacy of Coxiril® for chickens for fattening (EFSA FEEDAP Panel, 2014).

EFSA issued an opinion on the safety and efficacy of Clinacox® 0.5 % based on diclazuril for rabbits for fattening and breeding (EFSA, 2007b), then updated in 2008 (EFSA, 2008b).

TERMS OF REFERENCE

According to Article 8 of Regulation (EC) No 1831/2003, EFSA shall 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 the efficacy of the product Coxiril® (diclazuril), when used under the conditions described in Table 1.

Table 1: Description and conditions of use of the additive as proposed by the applicant

Additive	Diclazuril
Registration number/EC No/No (if appropriate)	Not applicable
Category(-ies) of additive	coccidiostat
Functional group(s) of additive	coccidiostat

Description			
Composition, description	Chemical formula	Purity criteria (if appropriate)	Method of analysis (if appropriate)
Additive composition Diclazuril: 5.0g/kg Starch: binding agent Wheat meal: carrier Calcium carbonate: carrier **International Non-proprietary Name:** Diclazuril. **Chemical name:** **(**RS)-(-4-Chlorophenyl)[2,6-dichloro-4-(3,5-dioxo-4,5-dihydro-1,2,4-triazin-2(3H)-yl)phenyl]acetonitrile. **CAS number:** [101831-37-2]	$C_{17}H_9Cl_3N_4O_2$	**Impurity D:** ≤ 0.1 % **Any other single impurity:** ≤ 0.5 % **Total impurities:** ≤ 1.5 %	**Feed:** reversed phase HPLC with UV detection **Poultry tissues:** LC MS/MS

Trade name (if appropriate)	Coxiril
Name of the holder of authorisation (if appropriate)	Huvepharma NV

Conditions of use				
Species or category of animal	Maximum Age	Minimum content	Maximum content	Withdrawal period (if appropriate)
		mg/kg of complete feedingstuffs		
Turkeys for fattening	/	0.8	1.2	no withdrawal period needed

Other provisions and additional requirements for the labeling	
Specific conditions or restrictions for use (if appropriate)	NA
Specific conditions or restrictions for handling (if appropriate)	Wear suitable protective clothing, gloves and eye/face protection. In case of insufficient ventilation in the premise, wear suitable respiratory equipment.
Post-market monitoring (if appropriate)	NA
Specific conditions for use in complementary feedingstuffs (if appropriate)	NA

Maximum Residue Limit (MRL) (if appropriate)			
Marker residue	Species or category of animal	Target tissue(s) or food products	Maximum content in tissues
diclazuril	poultry	Skin+fat Muscle Liver Kidney	500 µg/kg 500 µg/kg 1500 µg/kg 1000 µg/kg

ASSESSMENT

1. Introduction

The current application concerns the authorisation of Coxiril®, containing the active substance diclazuril, for use as a coccidiostat in turkeys for fattening. This additive is not authorised in the European Union. However, the same active substance, when contained in the additive Clinacox® 0.5 %, is currently authorised for use in chickens for fattening,[17] chickens reared for laying,[18] turkeys for fattening,[19] guinea fowl[20] and rabbits.[21]

The European Food Safety Authority (EFSA) has adopted several opinions on the safety and efficacy of Clinacox® 0.5 % for rabbits for fattening and breeding (EFSA, 2007b, 2008b), chickens for fattening (EFSA FEEDAP Panel, 2010a), guinea fowl (EFSA FEEDAP Panel, 2010b), turkeys for fattening (EFSA FEEDAP Panel, 2011a) and chickens reared for laying (EFSA FEEDAP Panel, 2013). In 2014, EFSA adopted an opinion on the safety and efficacy of Coxiril® for chickens for fattening (EFSA FEEDAP Panel, 2014).

In 2008, EFSA issued an opinion on Maximum Residue Limits (MRLs) for diclazuril when used in turkeys for fattening, chickens for fattening and chickens reared for laying. The MRLs are enforced by Commission Regulation (EC) No 976/2008.[22] Commission Implementing Regulation (EU) No 115/2013 introduced MRLs for poultry (liver, kidney, muscle, skin/fat).[23]

2. Characterisation

The identity of the additive, the characterisation of the active substance and the manufacturing process have been reviewed by the FEEDAP Panel (EFSA FEEDAP Panel, 2014). The conditions of use proposed for turkeys for fattening are identical to those described for chickens for fattening, i.e. Coxiril® is intended for prevention of coccidiosis in turkeys for fattening at a dose range of 0.8 to 1.2 mg diclazuril/kg complete feed. No withdrawal period is proposed by the applicant.

2.1. Evaluation of the analytical methods by the European Union Reference Laboratory (EURL)

The EURL considered that the conclusions and recommendations reached in the previous assessment are valid and applicable for the current application.[24]

3. Safety

3.1. Safety for the target species

3.1.1. Tolerance study in turkeys for fattening

A tolerance study[25] with Coxiril® was carried out in 160 one-day-old turkeys for fattening (Hybrid Grademaker) for eight weeks. The diclazuril concentrations were 0 mg/kg (control), 1.2 mg/kg (highest dose), 3.6 mg/kg (three-fold the highest dose) and 14.4 mg/kg (12-fold the highest dose). The intended concentrations in mash feed (starter diet for the first two weeks, grower diet until completion) were analytically confirmed. Four replicates of male and four replicates of female birds were included

[17] OJ L 317, 3.12.2010, p. 5.
[18] OJ L 192, 13.7.2013, p. 35
[19] OJ L 229, 6.9.2011, p. 9.
[20] OJ L 46, 24.2.2011, p. 6.
[21] OJ L 265, 4.10.2008, p. 3.
[22] OJ L 266, 7.10.2008, p. 3.
[23] OJ L 38, 9.3.2013, p. 11.
[24] The full report is available on the EURL website: http://irmm.jrc.ec.europa.eu/SiteCollectionDocuments/FinRep-FAD-2012-0017.pdf
[25] Technical dossier/Section III/Annex 1.

in each treatment, and the final number of animals per replicate was five (plus two spare birds, which were removed after one week). The diets consisted mainly of soybean meal, wheat and wheat feed. The crude protein content of the starter and the grower diets was calculated to be 28.2 % and 26.5 %, and the total metabolisable energy as 12.2 and 12.0 MJ/kg, respectively.[26]

Body weight and feed intake were measured weekly. On day 55, blood samples were taken from one bird per replicate and were analysed for haematology and routine clinical chemistry.[27] At termination of the study (day 56/57), one bird per replicate was killed and necropsied; organs were weighed (heart, liver, kidneys and spleen) and examined macroscopically and microscopically.[28] No information was provided on how this bird was selected. Statistical evaluation started with a Bartlett's test for variance homogeneity; depending on the outcome, this was followed by either a parametric test (Williams' test with Dunnett's test for group differences) or a non-parametric test (Shirley's test). Organ weight was statistically studied by covariance analysis using body weight as a covariate. The statistical procedure was applied separately to both sexes and to the data of individuals (except feed consumption). Following a request from the FEEDAP Panel, the statistical analysis was repeated using the replicate as the experimental unit for all zootechnical parameters.[29] However, group size for haematological and plasma clinical chemistry was small (four birds per gender and treatment).

Mortality (including culled birds) was low (3 out of a total of 160 birds). One bird died in the use level group and one in the intermediate-dose level group; one bird was euthanised in the control group.

Total body weight gain (average 3 706 g), feed consumption (average 158 g/bird) and feed-to-gain ratio were not statistically different between the treatments. It should be noted that feed intake and body weight gain might be not optimal owing to the use of a mash instead a pelleted diet.

Haematological results revealed no treatment-related effects with the exception of a significant, but not dose-related, reduction in monocytes in all treated groups. No significant changes were seen in the clinical blood chemistry parameters, except for a statistically lower creatinine level in the high-dose group. There were no treatment-related relevant findings regarding absolute and body weight-adjusted organ weights, organ macropathology or histopathology.

3.1.2. Microbial studies

Diclazuril is devoid of antimicrobial activity against a range of fungi and bacteria, including several pathogens (Renshaw, 2013).

Data provided by the applicant on the minimum inhibitory concentration values measured for strains of *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Bacillus subtilis* (ATCC 6633), *Enterococcus faecalis* (ATCC 29212) and *Staphylococcus aureus* (ATCC 25923), all of which are above 64 mg/L, may be used as partial confirmation of the above statement.[30]

3.1.3. Conclusions on the safety for turkeys for fattening

The value of the tolerance study is somewhat limited owing to the low number of replicates/animals per treatment and sex. However, none of the endpoints measured showed a relevant adverse treatment-related effect of diclazuril at 12-fold the use level. The highest proposed use level (1.2 mg diclazuril/kg feed) is considered safe for turkeys for fattening. Diclazuril has no substantial

[26] Supplementary information. February 2014. Reference 1.
[27] Haematocrit, haemoglobin concentration, erythrocyte count, mean corpuscular haemoglobin, mean corpuscular volume , mean corpuscular haemoglobin concentration, total white cell count, differential white blood cell count: heterophils, lymphocytes, basophils, eosinophils, monocytes, thrombocytes, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, lactate dehydrogenase, creatine kinase, bilirubin, bile acids, uric acid, creatinine, glucose, total cholesterol, sodium, potassium, chloride, calcium, phosphorus, magnesium, total protein and albumin.
[28] Caeca, colon, duodenum, ileum, heart, kidneys, liver, lungs and spleen.
[29] Supplementary information. February 2014. Reference 2.
[30] Technical dossier/Section III/Annex 2.

antibacterial activity and, consequently, no microbial risk to the target species or induction of cross-resistance to clinically relevant antibiotics is expected.

3.2. Safety for the consumer

Commission Regulation (EC) No 976/2008 has set the first MRLs for diclazuril used as a feed additive for chickens for fattening, chickens reared for laying and turkeys for fattening. The latest Commission Implementing Regulation (EU) No 115/2013 extends the same values to all poultry, also covering the use of diclazuril for veterinary purposes. These values are 1 500 μg/kg wet liver, 1 000 μg/kg wet kidney and 500 μg/kg wet muscle and skin/fat.

The Regulations[31] concerning the use of diclazuril as a feed additive for different poultry species and categories refer to diclazuril of another applicant (Clinacox® 0.5 %). In these Regulations, diclazuril is specified by thresholds for substance-related impurities, which are quoted through in-house identifiers. The qualitative and quantitative nature of these impurities fully complies with the impurities specified, and has been analytically confirmed for the diclazuril under application (EFSA FEEDAP Panel, 2014; see also section 2.4 and Appendix A). Both specifications comply with the European Pharmacopoeia (Ph. Eur. 7, 2011).

The FEEDAP Panel considers that diclazuril from Coxiril® is chemically and, consequently, toxicologically equivalent to diclazuril from the authorised additive. Therefore, the safety for the consumer of the diclazuril under application can be assessed without additional data on metabolism and toxicology.

However, since the composition of the additive Coxiril® is different to that of Clinacox® 0.5 % (although both contain 0.5 % diclazuril) and the proposed maximum dose of diclazuril from Coxiril® is higher (1.2 vs. 1.0 mg/kg complete feed), the compliance of diclazuril residues from Coxiril® with the MRLs should be demonstrated.

In a residue study,[32] one-day-old turkeys were fed a complete feed supplemented with Coxiril® at the target dose of 1.2 mg diclazuril/kg feed (1.36 mg/kg analysed in finisher feed) for 16 weeks. The marker residue depletion was followed. Birds (three males and three females per withdrawal time point) were slaughtered 0, 3, 6 and 24 hours after withdrawal of the supplemented feed and tissues sampled. Diclazuril residue concentrations were determined in the tissues using a validated liquid chromatography–tandem mass spectrometry (LC-MS/MS) method with a limit of quantification (LOQ) of 50 μg/kg in liver, kidney, muscle and skin/fat (Table 2).

Table 2: Tissue residue kinetics of diclazuril in turkeys administered Coxiril® (1.2 mg diclazuril/kg feed) for 16 weeks

	Diclazuril residues (mg/kg wet tissue) [(a)]			
Withdrawal period (h)	**Liver**	**Kidney**	**Muscle**	**Skin/fat**
0	0.377 ± 0.095 (0.568) [(b)]	0.297 ± 0.061 (0.398)	0.057 ± 0.002 (0.061)	0.100 ± 0.025 (0.149)
3	0.402 ± 0.032 (0.466)	0.314 ± 0.024 (0.362)	0.061 ± 0.006 (0.073)	0.107 ± 0.016 (0.139)
6	0.653 ± 0.102 (0.857)	0.637 ± 0.064 (0.765)	0.061 ± 0.008 (0.077)	0.151 ± 0.014 (0.179)
24	0.310 ± 0.042 (0.394)	0.265 ± 0.031 (0.327)	0.056 ± 0.003 (0.062)	0.087 ± 0.009 (0.105)

(a): Average values ± standard deviation (SD) of three males and three females per withdrawal time point.
(b): Average value + 2 SD.

[31] OJ L 266, 7.10.2008, p. 3; OJ L 317, 3.12.2010, p. 5; OJ L 192, 13.7.2013, p. 35; OJ L 229, 6.9.2011, p. 9; OJ L 49, 24.2.2011, p. 6.
[32] Technical dossier/Section III/Annex 5.

All values measured in tissues at any withdrawal time were below the corresponding MRLs. It is therefore concluded that the use of Coxiril® in diets for turkeys for fattening at the maximum proposed concentration complies with the MRLs. No withdrawal time is required.

The FEEDAP Panel concludes that the use of diclazuril from Coxiril® at the maximum concentration of 1.2 mg/kg feed for turkeys for fattening does not raise concerns for the consumer.

3.3. Safety for the user

Data submitted in the dossier for chickens for fattening are the same as data provided in the application for turkeys for fattening. The FEEDAP Panel has already assessed these data (EFSA FEEDAP Panel, 2014) and concluded: "*Coxiril® is considered as non-irritant to eyes and skin. It is not a potential skin sensitiser. User inhalation exposure to Coxiril®, as a result of normal handling, is unlikely to cause respiratory or systemic toxicity*."

3.4. Safety for the environment

Predicted environmental concentrations (PECs) were calculated for turkeys for fattening receiving a dose of 1.2 mg diclazuril/kg feed in accordance with the technical guidance for assessing the safety of feed additives for the environment (EFSA, 2008c). This yielded a PEC_{soil} value of 5.6 µg/kg and a $PEC_{groundwater}$ value of 0.06 µg/L.

The applicant provided the following information on physical/chemical properties: molecular weight 407.64 g/mol, water solubility 10 µg/L, vapour pressure 1.21×10^{-22} Pa and dissociation constant 5.9. The solubility of diclazuril increases from 0.0026 mg/L at pH 5 to 1.4 mg/L at pH 9. This strong pH dependence indicates the formation of soluble ions. This makes the sorption more complicated than that of a simple neutral molecule. Therefore, the lowest K_{oc} (Freundlich) value should be selected as the most conservative assessment instead of a geometric mean value.

Sorption studies with a radiolabelled diclazuril in 0.01 M calcium chloride in five soils with a pH range from 5 to 7 did not indicate clear pH dependence.[33] The lowest K_{oc} (Freundlich) selected from these soils is 4 986 mL/g with a 1/n of 0.818, corresponding to the soil with a pH 5.1, a cation exchange capacity of 4.3 meq/100 g and a total organic carbon content of 0.65 %.

Since the trigger values of 10 µg/kg for PEC_{soil} and 0.1 µg/L for $PEC_{groundwater}$ are not exceeded, a Phase II assessment is not necessary.

Using diclazuril from Coxiril® in turkeys for fattening at the highest proposed feed concentration would not pose a risk to the environment.

4. Efficacy

For coccidiostats, the efficacy data should derive from three types of target animal experiments: (1) screening for response using artificial single and mixed infections; (2) natural/artificial infection to simulate use conditions (e.g. floor pen studies with poultry), with at least one of the locations being in the EU; and (3) actual use conditions in field trials, all of which should have been carried out in the EU within the last five years.

Sensitivity tests could replace field trials, provided they follow the criteria mentioned in the guidance document on coccidiostats and histomonostats (EFSA FEEDAP Panel, 2011b).[34]

[33] Supplementary information/January 2014.

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

4.1. Battery and floor pen trials with artificial single and mixed *Eimeria* infections

To demonstrate the effect of diclazuril in battery and floor pen trials with turkeys for fattening, the applicant submitted three studies published by Vanparijs et al. (1989a, b, 1990) performed with diclazuril from Clinacox®.

A dose titration study in turkeys (Vanparijs et al., 1989a) indicated that diclazuril at dosages of 0.5 to 2 mg/kg feed was highly active against the three major pathogenic species—*E. adenoides*, *E. gallopavonis* and *E. meleagrimitis*—and effective in terms of reducing lesions, abnormal droppings and oocyst shedding. Similar results were observed for *E. dispersa* (Vanparijs et al., 1989b).

One floor pen trial (Vanparijs et al., 1990) in which turkeys were artificially infected via feed with *E. adenoides*, *E. gallopavonis* and *E. meleagrimitis* demonstrated that diclazuril, at dose levels of 0.5 and 1 mg/kg, completely prevented lesions and oocyst excretion and improved body weight gain and feed-to-gain ratio.

4.2. Floor pen studies with diclazuril from Coxiril®

In study 1, performed in 2011[35] over 12 weeks, 918 'BIG 7' one-day-old male turkeys were randomly allocated to an infected treated (IT, intended concentration 0.8 mg/kg, analysed 0.68–0.93 mg diclazuril/kg feed) group, an infected untreated control (IUC) group or an uninfected untreated control (UUC) group (27 pens with 34 animals). On day 15, six birds (seeder birds) from both the IT and IUC groups were infected with a mixture of *Eimeria* species (*E. meleagrimitis*, *E. adenoides* and *E. dispersa*) isolated from a recent field sample. A total of 32 birds died during the experiment (UUC, 9; IUC, 16; and IT, 7). The average body weight was similar in the three groups during the first four weeks of the study. Overall, the feed-to-gain ratio was significantly better in the IT group (UUC, 2.32; IUC, 2.32; and IT, 2.25) owing to a reduced feed intake (UUC, 290 g; IUC, 288 g; IT, 280 g), while maintaining the daily gain (UUC, 10 630 g/bird; IUC, 10 458 g/bird; and IT, 10 557 g/bird). The oocyst counts per gram faeces (OPGs) on day 21 (i.e. six days after inoculation) were 1 log lower in the IT group than in the IUC group. On days 28 and 35, the OPG results were similar in the IUC and IT groups. By day 42–49, oocyst excretion had decreased to almost zero. Some excretion was observed in the UUC group by day 42–49. Lesion scores were rather mild and similar in the IUC and IT groups.

In study 2, performed in 2012[36] over 85 days, 512 'BUT T9' one-day-old male turkeys were randomly allocated to an infected treated (intended concentration 0.8 mg/kg, analysed 0.6–0.8 mg diclazuril/kg feed) group or an untreated group (16 pens with 32 animals). The infection was natural and therefore no experimental inoculation was performed. Four animals died during the course of the treatment: one from the treated group and three from the untreated group. No significant differences were observed in any of the zootechnical parameters (body weight, feed-to-gain ratio). Peak oocyst excretion was observed on day 22, but no differences were observed between groups, and after this time point oocyst excretion decreased in both groups. Lesion scores were mild and similar in both groups, although lesion scores of 2 were observed only in the unsupplemented group. This study was not considered further.

In study 3, performed in 2011[37] over 12 weeks, 360 male 'BUT8' one-day-old turkeys were randomly allocated to an UUC group, an IUC group or an IT group (intended concentration 0.8 mg/kg, analysed 0.71–0.76 mg diclazuril/kg) (30 floor pens, 12 birds/pen). On day 14, birds in the IUC and IT groups were inoculated with an inoculum based on a recent field isolate from France containing *E. meleagrimitis*, *E. adenoides* and *E. dispersa*. Overall mortality was low (3.1 %). During the acute infection (days 14–28), mortality was significantly higher in the IUC group than in the IT group (4.2 % vs. 0 %). Coxiril® significantly improved the overall feed-to-gain ratio (1.95) compared with

[35] Technical dossier/Section IV/Annex 4.
[36] Technical dossier/Section IV/Annex 5.
[37] Technical dossier/Section IV/Annex 6.

the IUC group (2.05). Additionally, the IT group exhibited similar body weight gain, feed intake and feed-to-gain ratio to the UUC turkeys. On days 7 and 14 (before infection), no oocysts were found in the excreta of any of the treatment groups. The highest OPGs were observed on day 21. IT turkeys excreted significantly fewer *E. meleagrimitis* and *E. dispersa* oocysts than the IUC turkeys. From day 28 to day 63, no significant differences were found in total OPGs between the three treatments. From day 49 until the end of the study, OPGs were very low in all treatment groups.

In study 4, performed in 2013,[38] 600 one-day-old male turkeys (Converter hybrid) were randomly allocated to three groups, an IT group (0.68–0.83 mg diclazuril/kg feed), an IUC group or an UUC group (eight pens/group with 25 animals each). On day 16 of the 12-week study, one bird from each pen allocated to the infected groups (IUC and IT) was inoculated with a mixture of *Eimeria* species (*E. meleagrimitis*, *E. adenoides* and *E. dispersa* sporulated oocysts) isolated from a recent field sample (2013). The overall mortality was high: 17.3 % in the Coxiril® (IT) group and 16.5 % in the IUC group. Both values were significantly higher than in the UUC group (6.2 %). Initially, the *Eimeria* infection caused a severe growth depression (body weight gain on day 33, 958 g/bird and 911 g/bird for the IT and the IUC groups, respectively, vs. 1 312 g/bird in the UUC group). The growth depression disappeared, statistically, on day 44 for the IT group. There were no differences in final body weight among the three groups. On day 27, no oocysts were detected in faecal samples from the UUC group. The total OPG count of the IUC and IT groups was found to be significantly higher than in the UUC group, without a significant difference between the IUC and IT groups. On day 33, all pens of the UUC group were infected. On day 44, overall oocyst shedding was found to be significantly higher in the IUC groups than in the UUC and IT groups. There was no significant difference in total OPG count between the UUC and IT groups. Intestinal lesion score was measured in three birds from each pen after necropsy (seeder birds on day 21). No differences in the total lesion scores between the groups were observed at day 21. On day 33, total intestinal lesion scores of animals were found to be significantly higher in the IUC and IT groups than in the UUC group. There was no significant difference in total intestinal lesion scores between the IUC and IT groups. Intestinal lesion scores for *E. meleagrimitis* were found to be higher in the IUC group than in the IT group, but this difference was not statistically significant. There was no significant difference in average lesion scores for *E. adenoides* between infected groups (IUC and IT).

4.3. Efficacy studies under field conditions - sensitivity tests

Three sensitivity tests were performed following the same experimental design as those described in the previous section, all of them conducted in 2012. In each trial, 108 'BIG 9' male turkeys (18 pens of six 13-day-old birds) were randomly allocated to an UUC group, an IUC group or an IT group (intended concentration diclazuril 0.8 mg/kg, analysed 0.58–0.60 mg diclazuril/kg). At the age of 16 days, birds from the IUC and IT groups were inoculated with recent field isolates containing *E. meleagrimitis* and *E. adenoides* (and *E. dispersa* in the third study). At the end of the experiment (when the turkeys were 28 days old) the birds were killed and evaluated for intestinal lesion scores. The origin of the field isolates was France for study 1, Germany for study 2 and Belgium for study 3.

In sensitivity study 1,[39] the zootechnical performance of the birds was similar until the turkeys reached 23 days of age. The final mean body weight and feed-to-gain ratio in the IUC group (709 g/bird and 2.01, respectively) were significantly lower than in the IT group (771 g/bird and 1.82, respectively) and the UUC group (793 g/bird and 1.67 g/g, respectively). Oocyst excretion in 23-day-old turkeys was significantly lower in the IT group than in the IUC group for all *Eimeria* spp. and no excretion was observed in the UUC group. When the turkeys reached 28 days of age, oocyst excretion was low and similar in the IT and IUC groups. Some excretion was also observed in the UUC group. No significant differences were observed for lesion scores between the IT and IUC groups.

[38] Supplementary information, February 2014/ Reference 3.
[39] Technical dossier/Section IV/Annex 8.

In sensitivity test 2,[40] no significant differences in body weight or feed intake were observed between groups at all time points. During the last week of the study, daily body weight gain in the UUC group (53 g/bird) was significantly higher than in the IT group (48 g/bird) and the IUC group (45 g/bird), and the feed-to-gain ratio in the UUC group (1.39) was significantly better than in the IUC group (1.80), whereas feed-to-gain ratio of the IT group was intermediate. For the overall study period, no significant differences in daily weight gain and feed-to-gain ratio among the groups were observed. The oocyst excretion rate on day 23 was significantly lower in the IT group than in the IUC group for *E. meleagrimitis* and *E. dispersa*, and no excretion was observed in the UUC group. On day 28, oocyst excretion was low and comparable between the IT and IUC groups. Some excretion was observed in the UUC group. No significant differences were observed for lesion scores between the IT and IUC groups.

In sensitivity test 3,[41] no significant differences in body weight, weight gain or feed intake were observed between the IT group and the IUC group, but results were consistently better in the IT group. Lesions scores were not significantly lower in the IT group than in the IUC group, but they were consistently lower. No differences were seen in the OPG count between the IT and IUC groups.

4.4. Conclusions on efficacy

Data published in the literature identify diclazuril from another source as an effective coccidiostat in turkeys for fattening at a dose range of 0.5 to 1.0 mg diclazuril/kg feed. Three floor pen studies and three sensitivity tests with an intended concentration of 0.8 mg diclazuril from Coxiril®/kg feed indicate that the additive has the potential to control coccidiosis from *Eimeria* spp. in turkeys for fattening.

5. Post-market monitoring

The FEEDAP Panel considers that there is no need for specific requirements for a post-market monitoring plan other than those established in the Feed Hygiene Regulation [42] and Good Manufacturing Practice.

Field monitoring of *Eimeria* spp. resistance to diclazuril in turkeys for fattening should be undertaken, preferably during the later part of the period of authorisation.

CONCLUSIONS AND RECOMMENDATIONS

CONCLUSIONS

Coxiril® is considered safe for turkeys for fattening at the highest use level (1.2 mg diclazuril/kg feed) although the value of the tolerance study is somewhat limited due to weaknesses in the design. However, none of the parameters measured showed an adverse effect of diclazuril at 12-fold the use level of diclazuril. Diclazuril has no substantial antibacterial activity.

Diclazuril from Coxiril® is considered toxicologically equivalent to the other currently authorised diclazuril. Since MRLs for diclazuril are not exceeded, the use of Coxiril® at the highest proposed use level (1.2 mg diclazuril/kg complete feed) in turkeys for fattening is safe for the consumer.

Coxiril® is considered non-irritant to eyes and skin. It is not a potential skin sensitiser. User exposure to Coxiril®, as a result of normal handling, is unlikely to cause respiratory or systemic toxicity.

The use of diclazuril in turkeys for fattening at the high use level does not pose a risk to the environment.

[40] Technical dossier/Section IV/Annex 9.
[41] Technical dossier/Section IV/Annex 10.
[42] OJ L 35, 8.2.2005, p. 1.

Diclazuril from Coxiril® has the potential to control coccidiosis in turkeys for fattening at a minimum concentration of 0.8 mg/kg complete feed. This conclusion is derived from literature data and from recent studies performed with Coxiril®.

RECOMMENDATIONS

The specifications of Coxiril® should refer to the substance-related impurities A to I, as listed in the European Pharmacopoeia (Ph. Eur. 7, 2011).

The specifications of diclazuril in Coxiril® should be amended by introducing a maximum content for the residues of a solvent used during the manufacturing process, compliant with the threshold set by the International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products guidelines.

DOCUMENTATION PROVIDED TO EFSA

1. Coxiril® for turkeys for fattening. April 2012. Submitted by Huvepharma HV.
2. Coxiril® for turkeys for fattening. Supplementary information. January 2014.
3. Coxiril® for turkeys for fattening. Supplementary information. February 2014.
4. Evaluation report of the European Union Reference Laboratory for Feed Additives on the Methods(s) of Analysis for Coxiril®.
5. Comments from Member States received through the ScienceNet.

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