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SCIENTIFIC OPINION

Scientific Opinion on the safety and efficacy of diclazuril (Clinacox® 0.5 %) as feed additive for chickens reared for laying[1]

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

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ABSTRACT

Clinacox® 0.5 % is a feed additive containing 0.5 % diclazuril and is used to control coccidiosis in poultry. The previous conclusion on the safety of diclazuril in chickens for fattening at the use level of 1 mg/kg complete feed is extended to chickens reared for laying. Chickens reared for laying are in a similar physiological stage as chickens for fattening. The use level proposed for diclazuril in feed is the same for both categories of birds. Consequently, the metabolic fate and residue status of this additive described for chickens for fattening can be extrapolated to chickens reared for laying. Feeding diclazuril at 1 mg/kg complete feed to chickens reared for laying for the first 16 weeks of life would result in negligibly low or absent residues of diclazuril in the first eggs for production. Therefore, maximum residue levels (MRLs) for eggs are not necessary. The previous assessment of consumer safety for the use of diclazuril in chickens for fattening is confirmed and extended to chickens reared for laying. The same MRLs for tissues as already established can be applied. Clinacox® has a low irritation potential for the eye and skin. Skin sensitisation was not observed. Considering the particle size (particle of respirable size) and dusting potential, exposure by inhalation cannot be excluded, although workers' exposure has been calculated to be low in comparison with the ADI. In the absence of any data on respiratory toxicity, a hazard from inhalation of dust cannot be entirely excluded and appropriate protective measures should be taken. The use of Clinacox® at 1 mg/kg complete feed does not pose a risk to the terrestrial or aquatic environment. One mg diclazuril/kg complete feed is effective in controlling coccidiosis in chickens reared for laying.

KEY WORDS

Coccidiostat, diclazuril, Clinacox® 0.5 %, chickens reared for laying, safety, MRL, efficacy

[1] On request from the European Commission, Question No EFSA-Q-2012-00303, adopted on 31 January 2013.

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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 Clinacox® 0.5 % for chickens reared for laying.

Clinacox® 0.5 % is a feed additive containing 0.5 % of the active substance diclazuril and is used to control coccidiosis in poultry. The FEEDAP Panel extended the conclusion on the safety of diclazuril in chickens for fattening at the use level of 1 mg/kg complete feed to chickens reared for laying.

Regarding the longer administration time of diclazuril to chickens reared for laying compared to chickens for fattening the extrapolation was considered acceptable since the only experiments (two studies) performed for the same administration time (16 weeks) in poultry (turkey for fattening) did not show any apparent negative effects on performance, haematology, serum biochemistry, gross pathology and histopathology.

Chickens reared for laying are at a similar physiological stage (growth) as chickens for fattening. The use level proposed for diclazuril in feed is the same for both categories of birds. Consequently, the metabolic fate and residue status of this additive described for chickens for fattening can be extrapolated to chickens reared for laying.

Feeding diclazuril at 1 mg/kg complete feed to chickens reared for laying for the first 16 weeks of life would result in negligibly low or absent residues of diclazuril in the first eggs for production. Therefore, maximum residue levels (MRLs) for eggs are not necessary.

The FEEDAP Panel confirms its previous assessment of consumer safety for the use of diclazuril in chickens for fattening and extends it to chickens reared for laying. The same MRLs for tissues as already established by Regulation (EC) No 976/2008 can be applied.

Clinacox® 0.5 % has a low irritation potential for the eye and skin. Skin sensitisation was not observed. Considering the particle size (particle of respirable size) and dusting potential, exposure by inhalation cannot be excluded, although workers' exposure has been calculated to be low in comparison with the average daily intake (ADI). In the absence of any data on respiratory toxicity, a hazard from inhalation of dust could not be entirely excluded and appropriate protective measures should be taken.

The use of Clinacox® 0.5 % at 1 mg/kg complete feed does not pose a risk to the terrestrial or aquatic environment.

One mg diclazuril/kg complete feed is effective in controlling coccidiosis in chickens reared for laying. This conclusion is based on the already demonstrated effect of the additive in chickens for fattening and on the results of one battery study, three floor pen trials and one field trial in chickens reared for laying.

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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 10(2) of that Regulation also specifies that for existing products within the meaning of Article 10(1), an application shall be submitted in accordance with Article 7, at the latest one year before the expiry date of the authorisation given pursuant to Directive 70/524/EEC for additives with a limited authorisation period, and within a maximum of seven years after the entry into force of this Regulation for additives authorised without time limit or pursuant to Directive 82/471/EEC.

The European Commission received a request from the company Janssen Pharmaceutica NV[5] for re-evaluation of the product Clinacox® 0.5 %, diclazuril, to be used as a feed additive for chickens reared for laying (category: coccidiostats and histomonostat) 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 10(2) (re-evaluation of an authorised 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 13 August 2012.

The additive Clinacox® 0.5 % is a coccidiostat containing 0.5 % of the active substance diclazuril. This product has been authorised for ten years for use in chickens for fattening (authorisation until 23 December 2020),[7] in chickens reared for laying (authorisation until 20 January 2013),[8] in turkeys for fattening (authorisation until 26 September 2021),[9] and in guinea fowl (authorisation until 16 March 2021).[10]

The Scientific Committee on Animal Nutrition (SCAN) issued an opinion on the extension of use of diclazuril to the feedingstuffs for chickens reared for laying (EC, 1997). The European Food Safety Authority (EFSA) issued an opinion on the Maximum Residue Limits (MRLs) for Clinacox® 0.5 % (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 issued an opinion on the safety and efficacy of Clinacox® 0.5 % for chickens for fattening (EFSA, 2010a), on 5 October 2010 an opinion on the safety and efficacy of Clinacox® 0.5 % for guinea fowl (EFSA, 2010b) and on 16 March 2011 an opinion on the safety and efficacy of Clinacox® 0.5% for turkeys for fattening (EFSA, 2011).

[4] Regulation (EC) No 1831/2003 of the European Parliament and Council of 22 September 2003 on additives for use in animal nutrition. OJ L 268, 18.10.2003, p. 29.
[5] Turnhoutseweg 30, B-2340 Beerse, Belgium.
[6] EFSA Dossier reference: FAD-2012-0004.
[7] 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.
[8] Commission Regulations (EC) No 976/2008 of 6 October 2008 amending Regulation (EC) No 2430/1999, (EC) No 418/2001 and (EC) No 162/2003 as regards the terms of the authorisation of the feed additive "Clinacox" belonging to the group of coccidiostats and histomonostats. OJ L 266, 7.10.2008, p. 3.
[9] 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, 6.09.2011, p. 9.
[10] 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.

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 Clinacox® 0.5 % (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	Clinacox® 0.5 %
Registration number/EC No/No (if appropriate)	E711
Category(-ies) of additive	Coccidiostats and histomonostat
Functional group(s) of additive	n/a

Description			
Composition, description	Chemical formula	Purity criteria (if appropriate)	Method of analysis (if appropriate)
Active substance Diclazuril **Additive composition** Diclazuril 0.545% w/w Protein-poor soybean meal 99.25% w/w Polyvidone K30 0.20% w/w Sodium hydroxide 0.05% w/w	$C_{17}H_9Cl_3N_4O_2$	100.0-110.0% Diclazuril	HPLC

Trade name (if appropriate)	Clinacox® 0.5%
Name of the holder of authorisation (if appropriate)	Janssen Pharmaceutica N.V.

Conditions of use				
Species or category of animal	Maximum Age	Minimum content	Maximum content	Withdrawal period (if appropriate)
		mg/kg of complete feedingstuffs		
Chickens reared for laying	16 weeks	1	1	0 days

Other provisions and additional requirements for the labeling	
Specific conditions or restrictions for use (if appropriate)	Clinacox 0.5% should not be mixed or used simultaneously with any other anticoccidal.
Specific conditions or restrictions for handling (if appropriate)	Operators should wear protective clothing, gloves, dust mask and goggles when mixing or handling CLINACOX 0.5%
Post-market monitoring (if appropriate)	Post Market monitoring will be conducted using an already established pharmacovigilance system.
Specific conditions for use in complementary feedingstuffs (if appropriate)	-

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

ASSESSMENT

1. Introduction

Diclazuril is intended to control coccidiosis in poultry. The use of Clinacox® 0.5 % (diclazuril) is currently authorised in chickens reared for laying by Regulation (EC) 162/2003.[11] The authorisation expired on 20 January 2013. The applicant submitted a dossier for re-evaluation of Clinacox® 0.5 % (diclazuril) at a dose of 1 mg/kg complete feed for chickens reared for laying under the category coccidiostats and histomonostats.

In 2010, the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) issued an opinion on the safety and efficacy of Clinacox® 0.5 % (diclazuril) for chickens for fattening (EFSA, 2010a). By Regulation (EC) No 1118/2010,[12] Clinacox 0.5 % has been authorised for use in chickens for fattening (authorisation until 23 December 2020). Following a FEEDAP opinion on diclazuril for guinea fowl (EFSA, 2010b), its use has been authorised by Regulation (EC) No 169/2011 (authorisation until 16 March 2021).[13] In 2011, the FEEDAP Panel released an opinion on the safety and efficacy of Clinacox® 0.5 % for turkeys for fattening (EFSA, 2011): its use has been authorised by Regulation (EC) No 888/2011 (authorisation until 26 September 2021).[14]

Based on a scientific opinion of the FEEDAP Panel (EFSA, 2008a) on maximum residue limits (MRLs) for Clinacox® 0.5 % (diclazuril) for turkeys for fattening, chickens for fattening and chickens reared for laying, the European Commission issued Regulation (EC) No 976/2008,[15] in which the MRLs for the above-mentioned animal categories are introduced.

2. Characterisation

2.1. Characterisation of the product

The identity of the additive, the characterisation of the active substance and the manufacturing process have been reviewed by the FEEDAP Panel (EFSA, 2010a). The conditions of use proposed for chickens reared for laying are identical to those described for chickens for fattening and for turkeys for fattening.

The only newly provided data refer to the shelf-life of the additive.

2.2. Shelf-life of the additive

The applicant has previously proposed a shelf-life of the additive of 36 months based on data provided with the application for chickens for fattening (EFSA, 2010a). With the current application the applicant provided new data confirming the already proposed shelf-life of the additive (36 months, when stored in a dry place).

[11] Commission Regulation (EC) No 162/2003 of 30 January 2003 concerning the authorisation of an additive in feedingstuffs. OJ L 26, 31.1.2003, p. 3.
[12] Commission Regulation (EC) No 1118/2010 of 2 December 2010 concerning the authorisation of diclazuril as feed additives for chickens for fattening (holder of the authorisation Janssen Pharmaceutica NV) and amending Regulation (EC) 2430/1999. OJ L 317, 3.12.2010, p. 5.
[13] Commission Regulation (EC) No 169/2011 of 23 February 2011 concerning the authorisation of diclazuril as feed additives for guinea fowls (holder of the authorisation Janssen Pharmaceutica NV). OJ L 49, 24.2.2011, p. 6.
[14] 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 authorisation Janssen Pharmaceutica NV) and amending Regulation (EC) No 2430/1999. OJ L 229, 6.9.2011, p. 9.
[15] Commission Regulation (EC) No 976/2008 of 6 October 2008 amending Regulations (EC) No 2430/1999, (EC) No 418/2001 and (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.

2.3. Conditions of use

Clinacox® 0.5 % (diclazuril) is intended to prevent coccidiosis in chickens reared for laying at a concentration of 1 mg/kg of complete feed up to a maximum age of 16 weeks. A withdrawal period of zero days is proposed, in line with that for chickens and turkeys for fattening.

2.4. 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.[16]

3. Safety

3.1. Safety for the target species

The safety of diclazuril at a dose of 1 mg/kg complete feed was assessed by the FEEDAP Panel for chickens for fattening (EFSA, 2010a) and turkeys for fattening (EFSA, 2011). The applicant submitted studies conducted in broiler breeder hens and cockerels in 1988 and until now not assessed by the FEEDAP Panel. Since the animal category (breeder hens) used in this study is different from that under application and the main endpoints of the study (hatchability and fertility of eggs) are not related to growing poultry, this study was not considered.

In its opinion on Clinacox 0.5 % for chickens for fattening the Panel concluded that:

"Chickens for fattening tolerated a 25-fold overdose of diclazuril for 37 days without any observable negative effects on performance, haematology, necropsy and histopathology. The FEEDAP Panel considers the histopathological data, which is not required for substances with a comparable high margin of safety, compensates for the lack of routine blood biochemistry.

The diclazuril preparation used in the tolerance study is not fully identified. Although it is required that this study should be performed with a product identical to that for which authorisation is sought, the FEEDAP Panel would not expect a different outcome considering the high margin of safety of diclazuril derived from the tolerance study.

Clinacox® 0.5% does not have antibacterial properties and consequently no microbial risk for the target species or induction of cross-resistance to clinically relevant antibiotics is expected.

The FEEDAP Panel considers Clinacox® 0.5% safe for chickens for fattening at the recommended dose."

The FEEDAP Panel extends the above conclusions on the safety of diclazuril for chickens for fattening at the use level of 1 mg/kg complete feed to chickens reared for laying. Regarding the longer administration time of diclazuril to chickens reared for laying compared to chickens for fattening the extrapolation is considered acceptable since in two studies with turkeys for fattening, no apparent negative effects were observed on performance, haematology, serum biochemistry, gross pathology and histopathology during the same administration period (16 weeks) (EFSA, 2011).

3.2. Safety for the consumer

No new studies were provided. No new data were available in the published literature which would require reconsideration of the previous opinions.

The FEEDAP Panel has already concluded (EFSA, 2008a) that diclazuril (i) is absorbed and biotransformed to a very limited extent, (ii) is the major residue in all tissues tested (70–90 % of total

[16] The full report is available on the EURL website. http://irmm.jrc.ec.europa.eu/SiteCollectionDocuments/FinRep-FAD-2008-0053.pdf.

residues) and (iii) is the marker residue in poultry tissues and products. This is in agreement with previous conclusions of the Scientific Committee for Animal Nutrition (SCAN) (EC, 1991), the Joint FAO/WHO Expert Committee on Food Additives (JECFA, 1999) and the European Medicines Agency (EMEA, 1996, 2004). The available data on total and marker residues in chicken tissues were considered satisfactory for the assessment of consumer safety.

All toxicological studies submitted have already been assessed (EFSA, 2007b, 2008a). It was found that (i) diclazuril exhibited very low acute toxicity; (ii) there was no evidence of mutagenicity or carcinogenicity; and (iii) the overall no observed adverse effect level (NOAEL) in the chronic toxicity/carcinogenicity study was 2.9 mg/kg body weight (bw) per day in male mice, with liver changes (swelling of parenchymal and sinusoidal cells with fatty vacuolation) as the critical effect at 11 mg/kg bw per day or greater. An ADI of 0.029 mg/kg bw per day (corresponding to 1.7 mg diclazuril/person per day) was derived from that NOAEL, applying a safety factor of 100. The same overall NOAEL and ADI were proposed by JECFA (1999) and EMEA (1996, 2004).

The FEEDAP Panel calculated, on the basis of total residues, that the maximum consumer exposure at zero days withdrawal would comply with the ADI (13 %), and therefore no MRLs would be needed (EFSA, 2008a). However, the FEEDAP Panel proposed, should MRLs be considered necessary, values of 1.5, 1, 0.5 and 0.5 mg diclazuril/kg wet tissue for liver, kidney, muscle and skin/fat, respectively. These figures comply with the most recent proposal of EMA for MRLs (EMA, 2012). The maximum theoretical exposure of the consumer corresponding to those MRLs would represent 30 % of the ADI. A withdrawal time for chickens for fattening and turkeys for fattening was not considered necessary.

3.2.1. Diclazuril carry-over into the eggs of laying hens

The potential carry-over of diclazuril into the first eggs at onset of laying from hens fed a diet containing diclazuril during the rearing period until the end of the 16th week of age was assessed by SCAN in its opinion on diclazuril for this poultry category (EC, 1997). For this purpose, a study performed in laying hens that received 0, 1 and 5 mg diclazuril/kg feed for 32 days followed by a 20-day withdrawal period was reviewed. SCAN concluded that at a supplementation rate of 1 mg diclazuril/kg complete feed (i) steady-state concentrations of diclazuril (limit of quantification 0.05 mg/kg egg yolk and albumen) were reached after day 9 in yolk and day 11 in albumen; (ii) the diclazuril concentration in yolk was about four times higher than in albumen; and (iii) withdrawal was followed by a rapid decline in diclazuril in albumen (< LOQ after 4-day withdrawal) and by a much slower decline in yolk (< LOQ after 15-day withdrawal)..

Considering that there are at least two weeks between the end of the rearing period and the onset of laying, the FEEDAP Panel considers that the concentration of diclazuril in the yolk of the first eggs would be negligibly low and absent in the albumen.

3.2.2. Conclusions

Chickens reared for laying are at a similar physiological stage (growth) as chickens for fattening. The use level proposed for diclazuril in feed is the same for both categories of birds. Consequently, the metabolic fate and residue status of this additive described for chickens for fattening (EFSA, 2008a, 2010a) can be extrapolated to chickens reared for laying.

Feeding diclazuril at 1 mg/kg complete feed to chickens reared for laying for the first 16 weeks of life would result in negligibly low or absent diclazuril residues in the first eggs for production. Therefore, MRLs for eggs are not applicable.

The FEEDAP Panel confirms its previous assessment of consumer safety for the use of diclazuril in chickens for fattening and extends it to chickens reared for laying. The same MRLs for tissues as already established by Regulation (EC) No 976/2008[17] can be applied.

3.3. Safety for the user

The FEEDAP Panel assessed the safety for the user of Clinacox® 0.5 % in its opinion on Clinacox® 0.5 % in chickens for fattening (EFSA, 2010a). No new information has been provided in this dossier. The FEEDAP Panel reiterates its previous assessment that Clinacox 0.5 % has a low irritation potential for the eye and skin. Skin sensitisation was not observed. Considering the particle size (particle of respirable size) and dusting potential, exposure by inhalation cannot be excluded, although workers' exposure has been calculated to be low in comparison with the ADI, indicating a low risk of systemic toxicity resulting from swallowing of dust captured in the mucous lining the respiratory tract. In the absence of any data on respiratory toxicity, a hazard from inhalation of dust cannot be entirely excluded and appropriate protective measures should be taken.

3.4. Safety for the environment

Predicted environmental concentrations (PECs) were calculated in accordance with EFSA technical guidance for assessing the safety of the feed additives for the environment (EFSA, 2008b). The PEC of both soil (5 µg/kg) and groundwater, calculated using the lowest K_{oc} of 34 600 (EFSA, 2007b, 2010a) (0.008 µg/L), were below the thresholds of concern.

The FEEDAP Panel confirms its former conclusion (EFSA, 2007b, 2010a) that the use of Clinacox® 0.5 % at 1 mg/kg complete feed does not pose a risk for the terrestrial or aquatic environment.

4. Efficacy

In the case of coccidiostats, efficacy data should derive from three types of target animal experiments: (a) screening for response using artificial single and mixed infections; (b) studies of natural/artificial infection to simulate use conditions (e.g. floor pen studies with poultry), the location of at least one of which should be in the EU; and (c) actual use conditions in field trials, all should have been done in the EU within the last five years.

The FEEDAP Panel notes that the efficacy of diclazuril demonstrated in chickens for fattening (EFSA, 2010a) can be extended to chickens reared for laying. The applicant submitted one battery study, four floor pen studies and three field trials carried out in chickens reared for laying to demonstrate efficacy of diclazuril in preventing coccidiosis. The submitted studies reflect the sensitivity of recent *Eimeria* field strains to diclazuril.

4.1. Battery trials

In battery trial 1,[18] which utilised a randomised block design, the efficacy of Clinacox® 0.5 % (diclazuril) was tested at 1 mg/kg feed (analytical value 1.1 mg/kg) against recent field isolates of chicken coccidia from Spain. A total of 192 one-day-old pullets (Hy-Line Brown) reared for laying were randomly allocated to six groups (four replicates/group, eight birds/cage). After seven days, half of the birds were treated with Clinacox® 0.5 % at 1 mg/kg feed for 14 days; the other half were fed a blank diet and served as an infected untreated control (IU). Chickens were infected on day 15 with 117 000, 225 000 or 335 000 sporulated oocysts from *E. acervulina*, *E. maxima*, *E. brunette*, *E. tenella* and *E. necatrix*. An infected treated (IT) group (1 mg diclazuril/kg) was compared with an infected untreated control (IU).

The mixed infection caused severe coccidiosis in the IU group, resulting in a mortality rate of 6, 9 and 25 %, respectively, in the groups infected at a dosage of 117 000, 225 000 and 335 000 sporulated

[17] OJ L 266, 7.10.2008, p. 3.
[18] Technical dossier/Section IV/Annex 3.

oocysts and an excretion of 0.3, 6.7 and 5.3 × 10^5 oocysts per gram faeces, respectively. In the IT group, no mortality was observed and oocyst excretion was not detected. Body weight gain was significantly reduced by the infection in the IU group compared with the diclazuril groups. Feed to gain ratio reflected the differences in weight gain between the groups.

4.2. Floor pen studies

One study was not considered because of the small number of animals and the short duration.[19] The remaining three studies share a common design. A treated group (fed 1 mg diclazuril/kg complete feed (analytically confirmed) for at least 112 days) and an untreated group were infected with sporulated *Eimeria* oocysts (recent field isolates from European countries) after 14 or 15 days. These groups (IT and IU) were compared with an uninfected untreated group (UU). Zootechnical performance, mortality, oocyst excretion, faecal score (faeces consistency) and lesion score in different intestinal parts were taken as endpoints.

In study 1,[20] a total of 432 one-day-old female chickens reared for laying (Hy-Line Brown) were randomly allocated to the IT, IU and UU groups (144 birds per group, 36 pens with 12 animals). Each bird was inoculated with 328 000 oocysts (73 % *E. acervulina*, 13 % *E. tenella*, 2 % *E. maxima*, 6 % *E. necatrix* and 6 % *E. mitis*). On days 29, 36 and 57, body weight in the IT group was significantly higher than in the IU group. No significant differences in body weights were observed between infected groups on days 16, 22, 85 and 112. On days 22, 24, 30, 32, 35, 63 and 77, faecal oocyst counts were found to be significantly lower in the IT group than in the UU group. No significant differences in oocyst counts between the infected groups were observed on days 20, 24, 28, 49. No significant differences in faecal scores were observed between treatment groups between days 15 and 22. Lesion score data showed that the IT group had lower mean lesion scores than the IU group, although the difference was not significant.

In study 2,[21] a total of 900 one-day-old female chickens reared for laying (Hy-Line Brown) were randomly allocated to 30 pens of 30 birds each. Each bird was inoculated with 225 000 sporulated oocysts via feed (44.4 % *E. acervulina*, 4.4 % *E. tenella*, 24.4 % *E. maxima*, 22.2 % *E. brunetti* and 4.4 % *E. necatrix*). Overall mortality was 6, 68 and 3 out of 300 animals in the IT, IU, and UU groups, respectively. Body weight at day 56 was significantly improved in IT group compared with the IU group (704 g vs 682 g), whereas no significant differences were seen at completion. No significant differences in body weight were observed between IT and UU birds on day 56 and day 113. Feed to gain ratio was significantly improved in IT compared with IU birds up to day 56 (2.67 vs 2.80). Only six, two, one and zero IT birds presented lesions in the upper, middle, lower and caecal intestinal sections, respectively, whereas 28, 24, 25 and 30 IU birds showed lesions in the corresponding intestinal sections. Moreover, only two IT birds presented lesions in more than one intestinal section, whereas 29 IU birds presented lesions in more than one intestinal section and 2l IU birds presented lesions in all four intestinal sections. Differences between IT and IU in lesion scoring were significant in all intestinal sections. Total and species-specific oocyst counts per gram of faeces were significantly lower in IT than IU pens at 8, 11 and 14 days post inoculation.

In study 3,[22] a total of 576 one-day-old female chickens reared for laying (Lohmann Brown) were randomly allocated to 36 pens with 16 pullets each. Sporulated *Eimeria* spp. oocysts were given by oral gavage (268 000 sporulated oocysts per bird, consisting of 74.6 % *E. acervulina*, 9.7 % *E. tenella*, 2.2 % *E. maxima*, 5.2 % *E. mitis* and 8.2 % *E. necatrix*). No differences in mortality between groups were seen. Significant differences in body weight between the IT and the IU group were seen only on day 14. Significant differences in feed to gain ratio between the IT and IU group were not observed. Significantly lower *E. acervulina* lesion scores were measured in birds of the IT group compared to IU group on days 19 and 21. Total oocyst excretion in the IU group 5 and 7 days after inoculation

[19] Technical dossier/Section IV/Annex 6.
[20] Technical dossier/Section IV/Annex 5.
[21] Technical dossier/Section IV/Annex 7.
[22] Technical dossier/Section IV/Annex 8.

amounted to 1.7 and 1.2 million/g of faeces, respectively; the corresponding data for the IT group were 0.4 and 0.3 million, respectively.

4.3. Field trials

Birds were not infected and the exposure depended on the natural prevalence of *Eimeria* spp.

Three field trials were submitted for the assessment. Assessment of two of the trials is difficult since in the first one a polyether control was used and in the second one diclazuril was administered to chickens already vaccinated against coccidiosis. Additionally, in these two field trials two different breeds were used for the control and treated groups. Because of these deficiencies field trials 1 and 2 were not further considered.

Field trial 3[23] was conducted at a multi-age layer rearing facility with untreated control birds (22 278 female Lohmann Classic Brown pullets) in one house and the diclazuril-treated group (22 596 Lohmann Classic Brown pullets; 1 mg diclazuril/kg feed) in another house. All animals were fed a starter diet until three weeks of age, followed by a grower feed up to eight weeks of age, then by another grower replacement feed until 16 weeks of age (end of study). Feed analysis confirmed the intended diclazuril supplementation (0.98–1.5 mg/kg). Body weight was measured at weekly intervals in 50 randomly selected individuals from each house. Intestinal lesions were scored (10 animals per group) on days 28, 35, 42 and 49. Oocyst excretion was determined in at least 10 intestinal and five caecal samples, at weekly intervals beginning on day 14.

Mortality was 2.6 % in the control group and 3.4 % in the diclazuril group and was not coccidiosis related. Body weight was significantly higher in the diclazuril-treated birds than in control birds on days 14, 56, 84, 98 and 112. Feed to gain ratio was 4.07 and 4.32 in diclazuril group and the control group, respectively. On day 28 no significant differences in intestinal lesion scores were found between the groups (mild lesions caused by *E. acervulina* and *E. maxima*, no lesions caused by *E. necatrix*, *E. tenella* or *E. brunetti*). These findings were not essentially different from those made on days 35, 49 and 84. On day 42, the lesion score of *E. tenella* was found to be significantly higher in the control group than in the diclazuril group. Total oocyst per gram counts were 0 for the first 49 days. They were higher in the control group than in the diclazuril group on days 63, 70, 77 and 84, but lower on day 56. Differences between days 91 and 112 showed a high degree of variability and seemed to be random. *Eimeria* species-specific oocyst excretion varied from one day of observation to another and between groups; no clear coccidiostat-related finding could be observed.

4.4. Conclusions on efficacy

Conclusions on the efficacy of diclazuril from Clinacox® 0.5 % in chickens reared for laying are based on one battery experiment, three floor pen trials and one field trial. All floor pen studies and the battery experiment were conducted with artificial inoculation of recent field isolates of *Eimeria* spp. from different countries of the EU, providing evidence that the product is still effective. Results from a field trial are considered indicative for the efficacy of diclazuril in preventing coccidiosis under field conditions. These results support the extrapolation of data obtained in chickens for fattening to chickens reared for laying.

The FEEDAP Panel concludes that 1 mg diclazuril/kg complete feed is effective in the control of coccidiosis in chickens reared for laying.

[23] Technical dossier/Section IV/Annex 11.

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[24] and Good Manufacturing Practice.

CONCLUSIONS AND RECOMMENDATIONS

CONCLUSIONS

The FEEDAP Panel extends the conclusion on the safety of diclazuril in chickens for fattening at the use level of 1 mg/kg complete feed to chickens reared for laying. Regarding the longer administration time of diclazuril to chickens reared for laying compared to chickens for fattening the extrapolation is considered acceptable since in two studies with turkeys for fattening, no apparent negative effects were observed on performance, haematology, serum biochemistry, gross pathology and histopathology during the same administration period (16 weeks).

Chickens reared for laying are in a similar physiological stage (growth) as chickens for fattening. The use level proposed for diclazuril in feed is the same for both categories of birds. Consequently, the metabolic fate and residue status of this additive described for chickens for fattening can be extrapolated to chickens reared for laying.

Feeding diclazuril at 1 mg/kg complete feed to chickens reared for laying for the first 16 weeks of life would result in negligibly low or absent residues of diclazuril in the first eggs for production. Therefore, MRLs for eggs are not necessary.

The FEEDAP Panel confirms its former assessment of consumer safety for the use of diclazuril in chickens for fattening and extends it to chickens reared for laying. The same MRLs for tissues as already established by Regulation (EC) No 976/2008 can be applied.

Clinacox® 0.5 % has a low irritation potential for the eye and skin. Skin sensitisation was not observed. Considering the particle size (particles of respirable size) and dusting potential, exposure by inhalation cannot be excluded, although workers' exposure has been calculated to be low in comparison with the ADI. In the absence of any data on respiratory toxicity, a hazard from inhalation of dust cannot be entirely excluded.

The FEEDAP Panel confirms its former position that the use of Clinacox® 0.5 % at 1 mg/kg complete feed in poultry does not pose a risk for the terrestrial or aquatic environment.

The use of Clinacox® 0.5 % at 1 mg diclazuril/kg complete feed is considered to be effective in controlling coccidiosis in chickens reared for laying. This conclusion is based on the already demonstrated effect of the additive in chickens for fattening and on the results of one battery study, three floor pen trials and one field trial in chickens reared for laying.

RECOMMENDATIONS

To protect persons handling the additive from inhalation exposure appropriate protective measures are recommended.

Field monitoring of *Eimeria* spp. resistance in chickens reared for laying to diclazuril should be undertaken, preferably during the latter part of the period of authorisation.

[24] Regulation (EC) No 183/2005 of the European Parliament and of the Council of 12 January 2005 laying down requirements for feed hygiene. OJ L 35, 8.2.2005, p. 1.

DOCUMENTATION PROVIDED TO EFSA

1. Clinacox 0.5 % (diclazuril) for chickens reared for laying. January 2012. Submitted by Janssen Pharmaceutica NV.

2. Evaluation report of the European Union Reference Laboratory for Feed Additives on the Methods(s) of Analysis for diclazuril.

3. Comments from Member States received through the ScienceNet.

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