REGULATED PRODUCTS SAFETY ASSESSMENT

Assessment on the Safety and Efficacy of a Feed Additive Consisting of Diclazuril for Chickens Reared for Laying and Pheasants (Huvepharma NV). (RP1780)

Food Standards Agency[1], Food Standards Scotland[2]

[1] Regulated Products Risk Assessment, Food Standards Agency, UK, [2] Risk Assessment, Food Standards Scotland, UK

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FSA Research and Evidence

An application was submitted to the Food Standards Agency in October 2022 from Huvepharma NV ("the applicant") for the authorisation of a new use (extension of species) of an additive consisting of diclazuril. It was submitted under the category of 'coccidiostats and histomonostats' as a coccidiostat for its use in chickens reared for laying and pheasants.

In its previous assessments, the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) could not definitively conclude on the risks associated with the use of diclazuril in acid soils from Coxiril®. However, based on new data provided, the FEEDAP Panel reached the conclusion that no risk to the terrestrial compartment or sediment is expected when diclazuril is used in chickens reared for laying and pheasants under proposed conditions, in both acidic and non-acidic soils. There are no concerns for groundwater, but due to limited data, no conclusions can be drawn for the aquatic compartment. Diclazuril does not bioaccumulate, so the risk of secondary poisoning is unlikely.

FSA/FSS has reviewed the applicant's authorisation application, supporting documentation, and other regulators' risk assessments, most notably the EFSA risk assessment opinion, and considers sufficient evidence has been demonstrated to conclude without the need for further questions or risk assessment.

This is a joint FSA and FSS publication.

1. Introduction

The FSA and FSS have undertaken an assessment of a feed additive submitted by Huvepharma NV, (Uitbreidingstraat 80, 2600 Antwerp, Belgium) consisting of diclazuril (Coxiril®) under Assimilated Regulation (EC) No 1831/2003 (EC, 2003) in each nation of Great Britain (GB). The applicant seeks authorisation under category of 'coccidiostats and histomonostats' for its use in chickens reared for laying and pheasants.

Under Assimilated Regulation (EU) 2015/46 (EC, 2015a) the additive Coxiril® is authorised in chickens for fattening, turkeys for fattening, guinea fowl for fattening and breeding with maximum residue limits (MRLs) of diclazuril of 1,500 µg/kg wet liver, 1,000 µg/kg wet kidney, 500 µg/kg wet muscle and skin/fat. These limits are consistent with those established in Assimilated Regulation (EU) No 115/2013 (EC, 2013) for diclazuril as a veterinary medicine in poultry. In addition, Coxiril® is also authorised in rabbits by Assimilated Regulation (EU) 2015/1417 (EC, 2015b).

In line with Article 8 of Assimilated Regulation (EC) No 1831/2003 (EC, 2003) the assessment has considered and concluded that the feed additive complies with the conditions laid down in Article 5, including: safety considerations for human, animal and environmental health; efficacy of the additive for its intended effect; potential impairment of the distinctive features of animal products. This, and the guidance put in place by the European Food Safety Authority (EFSA) for the evaluation of feed additive applications, has formed the basis and structure for the assessment.

To ensure regulatory systems of the FSA/FSS are risk proportionate and resources are used effectively, the FSA and FSS have used the evidence submitted by the applicant and other information in the public domain, including the EFSA risk assessment opinion, to provide a summary assessment of the evidence of safety presented in this report.

The FEEDAP Panel previously assessed the safety and efficacy of diclazuril from Coxiril® as a feed additive for chickens for fattening (EFSA, 2014a), turkeys for fattening (EFSA, 2014c), guinea fowl (EFSA, 2014b), and rabbits for fattening (EFSA, 2015). Later, the Panel reviewed and updated its conclusions on the use of the additive in chickens reared for laying (EFSA, 2018a) and pheasants (EFSA, 2018b), incorporating new data submitted by the applicant. In the current assessment (EFSA, 2023), the applicant has provided an updated environmental risk assessment following the latest FEEDAP guidelines (EFSA FEEDAP, 2019).

The FSA/FSS risk assessors have reviewed relevant EFSA opinions (EFSA, 2014a, 2015, 2018a, 2018b, 2023). The assessments of safety and efficacy for laying chickens and pheasants were made in 2018 (EFSA, 2018a, 2018b) prior to the UK's exit from the EU. No authorisations were made due to

concerns regarding safety for environment, which were assessed in the latest EFSA opinion (EFSA, 2023). It has been verified that the standard approach taken, when compared to the relevant guidance applied in GB, has been followed and the conclusions made are consistent with the data summarised in the opinion.

The result of the assessment is that there is sufficient evidence of safety and efficacy for the UK to conclude this assessment at this time. This assessment represents the opinion of the FSA and FSS.

Table 1. Table showing products included in this assessment.

Title	Product type	Intended use/s	Intended dose/intake
Diclazuril (Coxiril®)	Feed additive	Coccidiostat feed additive	Chickens reared for laying at doses of 0.8–1.2 mg diclazuril/kg of feed up to 12 weeks of age
			Pheasants at dose of 1.0–1.2 mg diclazuril/kg of feed

2. Assessment

2.1. Details of other regulators opinions

2.1.1. Methodology applied in the EFSA opinion

EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) assessed the safety and the efficacy of diclazuril in accordance with guidance documents:

- Technical Guidance for assessing the safety of feed additives for the environment (EFSA FEEDAP Panel, 2008c);
- Technical Guidance: microbial studies (EFSA FEEDAP Panel, 2008b);
- Technical Guidance: Extrapolation of data from major species to minor species regarding the assessment of additives for use in animal nutrition (EFSA FEEDAP Panel, 2008a);
- 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);
- 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);
- Guidance on the assessment of bacterial susceptibility to antimicrobials of human and veterinary importance (EFSA FEEDAP Panel, 2012c);
- Guidance on the assessment of the safety of feed additives for the environment (EFSA FEEDAP Panel, 2019); and principles in Assimilated Regulation (EC) No 429/2008 (EC, 2008).

These guidance documents were developed and implemented prior to the UK's exit from the EU and were also adopted by FSA and FSS on exit.

2.2. Section II: Identity, characterisation and conditions of use

2.2.1. Characterisation of the active substance and the additive

The FEEDAP Panel has previously reviewed the identity of the additive, identity, characterisation and impurities of the active substance, the manufacturing process, and the stability of Coxiril® (EFSA, 2014a).

The additive contains 0.5% diclazuril as the active substance and is intended for the control of coccidiosis in chickens reared for laying and pheasants. Each kilogram of Coxiril® contains 5 g diclazuril, 15 g starch, 700 g wheat meal, and 280 g calcium carbonate (EFSA, 2014a). The product appears as off-white to beige granules with a tapped density of 0.524 kg/L and a loose density of 0.442 kg/L.

Analysis of six batches showed consistent diclazuril content, averaging 5.05 g/kg (range: 4.98–5.16 g/kg). Levels of As, Cd, Pb, Hg were below the thresholds set by the Directive 2002/32/EC. Values of dioxins, the sum of dioxins and dioxin-like polychlorinated biphenyls (PCBs), Aflatoxin B1, and *Salmonella spp.* were provided.

Sieve analysis indicated that 99% of particles were under 800 µm, with 3% under 100 µm. The dusting potential was analysed using Staubeur-Heubach method and the results ranged from 15 to 35 mg/m³, with diclazuril content in the dust measured at 6.15, 11.39, and 32.36 g/kg across 3 batches.

Diclazuril ($C_{17}H_9Cl_3N_4O_2$) belongs to the triazinone family, has a molecular weight of 407.64, and is synthesized as a racemic mixture of both forms. It has a melting point of 292–297°C, a log partition coefficient (n-octanol/water) of 3.6 and is practically insoluble in water and ethanol. The diclazuril

used in Coxiril® complies with the European Pharmacopoeia standards for impurities based on the data from 3 batches, although no data were provided regarding possible solvent residues from the manufacturing process.

The FSA and FSS agree with the conclusions reached for the characterisation of the additive and active substance. The data was reviewed by EFSA in 2014 and 2018, prior to the UK's exit from the EU; thus, this opinion is applicable to GB. The identity and the manufacturing process of the additive are not changed by the current application made to the FSA and FSS and as such has not been subject to further assessment.

2.2.2. Conditions of use

Coxiril®, containing 0.5% diclazuril, is intended to prevent coccidiosis in chickens reared for laying at doses of 0.8–1.2 mg diclazuril/kg complete feed up to 12 weeks of age, and in pheasants at 1.0–1.2 mg diclazuril/kg complete feed. A zero-day withdrawal period is proposed, consistent with that for chickens and turkeys reared for fattening.

The applicant requested the same conditions of use for GB as EFSA evaluated in their previous opinions (EFSA, 2018a, 2018b).

2.2.3. Conclusion on Section II: Identity, characterisation and conditions of use

The FEEDAP Panel has previously reviewed the identity of the additive, identity, characterisation and impurities of the active substance, the manufacturing process, and the stability of Coxiril® (EFSA, 2014a). The FEEDAP Panel concluded that the amounts of the detected impurities, as well as microbial contamination, do not raise safety concerns.

FSA and FSS agree with the conclusions reached for the characterisation of the additive and active substance. The data was reviewed by EFSA in 2014 and 2018, prior to the UK's exit from the EU; thus, this opinion is applicable to GB. The identity and the manufacturing process of the additive are not changed by the current application made to the FSA and FSS and as such have not been subject to further assessment. The FSA/FSS agree with the conditions of use proposed by the applicant.

2.3. Section III: Safety

The FEEDAP Panel previously assessed the safety of diclazuril from Coxiril® as feed additive for chickens for fattening (EFSA, 2014a), turkeys for fattening (EFSA, 2014c), guinea fowl (EFSA, 2014b), and rabbits for fattening (EFSA, 2015). Later, the Panel reviewed and updated its conclusions on the safety of the additive for the environment in chickens reared for laying (EFSA, 2018a) and pheasants (EFSA, 2018b), incorporating new data

submitted by the applicant. In the current assessment, the applicant has provided an updated environmental risk assessment following the latest FEEDAP guidelines (EFSA FEEDAP Panel, 2019).

2.3.1. Safety for the target species

The FEEDAP Panel previously assessed the safety of diclazuril at use levels up to 1.2 mg/kg complete feed and concluded it is safe for chickens (EFSA, 2014a) and turkeys (EFSA, 2014c) for fattening. Margins of safety could not be established accurately due to limitations with the tolerance studies, particularly the low number of replicates and animals per treatment and sex. However, at threefold and twelvefold the highest use levels of diclazuril for chickens and turkeys for fattening, respectively, no adverse effects were observed.

The FEEDAP Panel concluded that the established safety in chickens for fattening is applicable to chickens reared for laying, as they are same species in the same physiological (growing) stage (EFSA, 2018a). Tolerance studies in chickens and turkeys for fattening identified a sufficiently large range of safe levels for the intended diclazuril concentration in feed. Diclazuril lacks notable antibacterial activity, posing no microbial risk or potential for inducing cross-resistance to relevant antibiotics. The FEEDAP Panel concluded that Coxiril® at 1.2 mg/kg is safe for chickens reared for laying up to 12 weeks of age.

No tolerance study in pheasants was provided for the additive. However, safety data from major species can be extrapolated to physiologically similar minor species if a wide margin of safety (at least 10) is demonstrated in the major species (EFSA FEEDAP Panel, 2008a). The FEEDAP Panel considers Coxiril® at maximum level of 1.2 mg/kg in complete feed safe for pheasants (EFSA, 2018b).

The FSA and FSS agree with the conclusions reached on the data and the use of the extrapolation for pheasants, which is supported by the guidance that is also applicable in GB.

2.3.2. Safety for the consumer

In 2014, the FEEDAP Panel concluded that diclazuril from Coxiril® at the maximum level of 1.2 mg/kg feed for chickens and turkeys for fattening poses no concerns to consumer safety, with no withdrawal periods required (EFSA, 2014a, 2014c). MRLs for diclazuril in chickens and turkeys for fattening, and guinea fowl, were set by Assimilated Regulation (EU) 2015/46 at 1,000 µg/kg wet kidney, 1,500 µg/kg wet liver, and 500 µg/kg wet muscle and skin/fat (EC, 2015a). Same MRLs were established in Assimilated Regulation (EU) No 115/2013 for diclazuril as a veterinary medicine in poultry (EC, 2013). No MRLs have been set for eggs. However,

Assimilated Regulation (EC) No 124/2009 (EC, 2009) sets a maximum limit of 2 µg/kg wet weight for diclazuril in eggs, due to the unavoidable carry-over of coccidiostats or histomonostats in non-target feed.

No residue data for pheasants were submitted. However, based on the FEEDAP Guidance on Extrapolation from major to minor species (EFSA FEEDAP Panel, 2008a), the FEEDAP Panel concludes that residue levels in pheasant tissues would be similar to those in chickens and turkeys for fattening. Therefore, the same MRLs can be applied. The use of diclazuril at 1.2 mg/kg complete feed in pheasant feed is considered safe for consumers, provided MRLs are not exceeded (EFSA, 2018b).

The FSA and FSS agree with the conclusions reached on the data and the use of the extrapolation for pheasants, which is supported by the guidance that is also applicable in GB.

2.3.2.1. Diclazuril carry-over into eggs of laying hens

A study was conducted to assess the potential presence of diclazuril residues in the first eggs after laying onset (EFSA, 2018a). Twenty-two chickens reared for laying (6 weeks of age) were fed a diet containing 1.2 mg diclazuril/kg for six weeks. They were then switched to a control feed until the experiment ended at 20 weeks of age. Eggs from ten experimental hens that began laying earliest (approximately 18th week of age) were collected, and combined yolk and white of egg analysis using liquid chromatography with tandem mass spectrometry (LC-MS/MS) with a limit of quantification of 0.5 µg/kg found diclazuril levels below the quantification limit in all eggs.

2.3.3. Safety for the user

User safety was previously evaluated in the EFSA FEEDAP Panel's opinion on diclazuril from Coxiril® for chickens for fattening (2014a). A study assessing the acute inhalation toxicity of Coxiril® was conducted in line with OECD Guideline 403. Six Harlan Wistar rats (three males, three females) were exposed to a dust atmosphere for 4 hours, followed by 14 days of observation. The mean atmosphere concentration was 5.11 mg/L, and the median diameters were 5.36 µm. The animals were exposed to approximately 2.34 mg of additive/L of particles of diameter <4 µm. No mortalities occurred, though increased respiratory rate, hunched posture, wet fur and pilo-erection were noted, all of which resolved within five to eight days after exposure. Slight body weight fluctuations were observed but normalised during the study. No macroscopic abnormalities were found at necropsy, and the study concluded that Coxiril® has low acute inhalation toxicity. The low dusting potential (15 to 35 mg/m^3) means worker exposure is low, and the associated risks are minimal (EFSA, 2014a).

Studies on Coxiril® assessed its skin and eye irritancy, as well as skin sensitisation potential. In line with OECD Guideline 404, a single four-hour application of Coxiril® to two rabbits' skin showed no irritation. For eye irritancy, a test on New Zealand White rabbits (OECD Guideline 405) revealed redness, swelling, and discharge after applying 0.1 mL (22 mg) Coxiril®; however, no adverse effects were seen at 72 hours. The tested material was classified as a mild eye irritant under the Kay and Calandra classification but not severe enough for regulatory labelling. The skin sensitisation test (OECD Guideline 429) using a local lymph node assay on mice, showed the stimulation index to be less than 1 at all dose levels tested, indicating Coxiril® is not a skin sensitiser.

In 2014, The FEEDAP Panel concluded that Coxiril® is not an irritant to the eyes or skin, nor a potential skin sensitiser. Inhalation exposure during normal handling is unlikely to cause respiratory or systemic toxicity (EFSA, 2014a). The Panel reaffirmed its earlier conclusions in the 2018 opinion (EFSA, 2018a, 2018b).

FSA and FSS agree with the conclusions reached for the safety of the user. The studies were reviewed by EFSA in 2014 and 2018, prior to the UK's exit from the EU; thus, these opinions are applicable to GB.

2.3.4. Safety for the environment

The applicant submitted an updated environmental risk assessment based on FEEDAP guidance (EFSA FEEDAP Panel, 2019). The assessment focused on chickens for fattening, with a maximum diclazuril content of 1.2 mg/kg feed, the same as proposed for the target species. A "replacement pullet scenario" for chickens reared for laying was also included, in which the manure generated up to the 12 weeks of age was diluted with the annual manure produced. The FEEDAP Panel found limitations in its assumptions (e.g., chickens staying in one facility throughout the entire production process). As a result, the Panel concluded that the safety of the worst-case scenario for chickens for fattening also applies to pheasants and chickens reared for laying.

2.3.4.1. Phase I

2.3.4.1.1. Physico-chemical properties

A new study measured the dissociation constant (pKa) of diclazuril, yielding a value of 8.62. However, this result was deemed unreliable as the study was conducted in a mixture of organic solvents rather than water. The previously used pKa value of 5.89 (EFSA FEEDAP Panel, 2018a) is retained for the current assessment. The FEEDAP Panel noted that diclazuril, a triazine, likely deprotonates to a soluble anionic form at pH levels above 5.89, while remaining neutral at lower pH levels. The summary of the physico-chemical properties is provided in Table 2.

Table 2. Physico-chemical properties of diclazuril.

Property	Value	Unit
Octanol/water partition coefficient (log K_{ow})[1]	2.3 (pH 5)	-
	< 0.3 (pH 7)	
	< 0.3 (pH 9)	
Water solubility (20°C)[2]	2.638×10^{-3} (pH 5)	mg/L
	2.334×10^{-2} (pH 7)	
	1.437 (pH 9)	
Vapour pressure[2]	1.21×10^{-22} (20°C)	Pa
	7.94×10^{-22} (25°C)	
Dissociation constant pKa[3]	5.89	-

[1] Technical dossier
[2] EFSA FEEDAP Panel (2015)
[3] EFSA FEEDAP Panel (2018)

2.3.4.1.2. Fate and behaviour

The studies assessed in 2018 (EFSA FEEDAP Panel, 2018a) were re-evaluated for this assessment. In Study 1, the lowest K_{foc} value of 4,986 mL/g was obtained from five soil types with pH values ranging from 5.1 to 7.2. Study 2 was conducted in soils with higher pH ranging from 7.30 to 7.61. Analysis of the adsorption data obtained from 9 soils showed that diclazuril's dissociation has limited impact on its overall adsorption and mobility. Adsorption values of nine soils are summarised in Table 3.

Table 3. Freundlich adsorption coefficients, regression constant and K_{foc} for diclazuril in soils arranged by ascending pH.

Soil ID	pH	Organic carbon content (%)	K_{foc} (mL/g)	1/n	K_{oc} (mL/g)
Sand 2.1 [a]	5.1	0.65	4,986.4	0.818	20,752
Loamy sand 2.2 [a]	5.5	1.77	12,744.6	0.928	23,947
Sandy loam 2.3 [a]	6.8	0.94	20,177.7	1.095	10,037
Clay 6S [a]	7.1	1.66	5,073.3	0.902	9,768
Loam 2.4 [a]	7.2	2.26	8212.8	1.035	6,297
Loam Cl1 [b]	7.3	3.37	13,263.8	1.075	7,536
Sandy loam I2 [b]	7.36	1.53	4,573.2	0.93	7,700
Silty clay loam Fr1 [b]	7.56	1.28	6,024.2	0.985	6,741
Clay Sp3 [b]	7.61	1.14	3,457	0.866	9,495
Geometric mean			**7,412**		**10,146**

[a] Study 1
[b] Study 2

The degradation of diclazuril was studied in two OECD 307-compliant studies. Study 1, previously assessed in 2015, showed pH-dependent degradation. The anionic form is transformed with a DT_{50} of 70 to 97 days at high pH, but no degradation or transformation was observed at a low pH of 5.1 over 120 days.

Study 2, which involved lower-pH soils, found a slow decline in extracted diclazuril levels, with 45–51% of the total applied radioactivity (TAR) reached after 120 days of incubation following application in soils G1, E1 and H2. Very slow decline with levels of TAR after 120 days was observed in soil B1. DT_{50} values derived from single first-order kinetics (SFO) for all soils are summarised in Table 4. Soils with a pH above 5.3 have a DT_{50} ranging from 72.6 to 158.4 days (geometric mean 117 days), while very acidic soils (pH < 5.3) show DT_{50} values between 667.6 and > 1,000 days.

Table 4. DT_{50} values for diclazuril in soils, arranged by ascending pH. All DT_{50} derived assuming single first-order kinetics (SFO).

Soil ID	pH	DT_{50} (d) (20°C)	DT_{90} (d) (20°C)	DT_{50} (d) (12°C)
Sandy loam (B1) [b]	3.7	667.6	>1000	>1000
Sand 2.1 [a]	5.1	>1000	>1000	>1000
Sandy clay loam(G1) [b]	5.3	121.8	404.5	258
Silty clay loam (E1) [b]	5.4	158.4	526.3	336
Clay (H2) [b]	5.4	156.7	520.5	332
Loamy sand 2.2 [a]	5.5	119.4	396.6	253
Sandy loam 2.3 [a]	6.8	72.6	241	154
Clay 6S [a]	7.1	96.5	358.6	205

DT_{50}: time to degradation of 50% of original concentration of the compound in the tested soils.
[a] Study 1
[b] Study 2

In high pH soils, diclazuril exists in an anionic form, while in acidic soils it remains in a neutral form, exhibiting different biodegradation rates and therefore should be considered separately. For exposure assessment, a DT_{50} of 247 days at 12°C is applied for soils with pH above 5.3, and over 1,000 days for acidic soils. A geometric mean K_{foc} of 7,412 mL/g (equivalent to K_{om} of 4,299 mL/g) and an arithmetic 1/n value of 0.959 are used for all soil pH levels.

2.3.4.1.3. Predicted environmental concentrations (PECs)

The input values for diclazuril exposure assessment were: 1.2 mg/kg feed for chickens for fattening, molecular weight 407.64, vapor pressure 1.21×10^{-22} Pa, water solubility 0.023 mg/L, DT_{50} of 247 days at 12°C for soils with pH > 5.3, DT_{50} of 1,000 days (2,120 days normalised to 12°C) for soils with pH < 5.3, and a K_{foc} of 7,412 mL/g. The Phase I predicted

environmental concentrations (PEC) were 18 µg/kg in soil and 0.03 µg/L in groundwater. Phase II assessment is necessary as the Phase I trigger PEC value is exceeded in soils.

2.3.4.2. Phase II

2.3.4.2.1. Exposure Assessment

The DT_{90} for diclazuril was determined to exceed 1 year in both acidic and non-acidic soils. Consequently, PECs at steady state were calculated following the FEEDAP guidance for environmental safety of feed additives (EFSA FEEDAP Panel, 2019) and are shown in Table 5. No concern for groundwater is expected for soils with pH higher than 5.3.

Table 5. Plateau predicted environmental concentrations (PECs) of diclazuril in soil, groundwater, surface water and sediment.

Input	Soils, pH ≥ 5.3	Very acidic soils, pH < 5.3
Dose (mg/kg feed)	1.2	1.2
Molecular weight	407	407
Vapour pressure (Pa) (at 25°C)	1.21×10^{-22}	1.21×10^{-22}
Solubility (mg/L)	0.023	0.023
K_{oc} (L/kg)	7412	7412
DT_{50} in soil at 12°C (days)	247	2120
Output		
Application rate kg/ha	0.014	0.014
PEC_{soil} (µg/kg)	28	161
$PEC_{groundwater}$ (µg/L)	0.048	0.272
$PEC_{surfacewater}$ (µg/L)	0.016	0.091
$PEC_{sediment}$ (µg/kg dry weight)	12	67

K_{oc}: adsorption or desorption coefficient corrected for soil organic carbon content.

The applicant conducted a higher-tier assessment using FOCUS models to evaluate leaching to groundwater. The PEARL model (FOCUS Version 4.4.4.) was used on the relevant avian treatment scenarios, specifically Jokioinen and Piacenza. A worst-case application rate of 0.014 kg/ha for turkeys for fattening was used, along with two DT_{50} values for both acidic and non-acidic soils in the modelling. The model results indicate no concerns for groundwater, with predicted concentrations well below 0.1 µg/L for both soil types. A refinement of the PEC calculation for surface water and sediment was also provided using the same input data as for groundwater (Table 6). Additionally, a worst-case DT_{50} of 1,000 days was used to model degradation in both surface water and sediment.

Table 6. Predicted environmental concentrations (PECs) of diclazuril, surface water and sediment refined with FOCUS models.

Input	Soils, pH ≥ 5.3	Very acidic soils, pH < 5.3
$PEC_{surfacewater}$ (µg/L)	0.01685	0.1668
$PEC_{sediment}$ (µg/kg dry weight)	3.230	19.291

The FEEDAP Panel concluded that no concern is expected for groundwater for either acidic or non-acidic soils. For the purposes of risk assessment, the following PEC values have been used: (i) soil with pH > 5.3: 28 µg/kg for soil, 0.01685 µg/L for surface water and 3.2 µg/kg for sediment, (ii) soil with pH < 5.3: 161 µg/kg for soil, 0.1668 µg/L for surface water and 19 µg/kg for sediment.

2.3.4.2.2. Ecotoxicity studies

Effects on terrestrial plants

Seeds from six plant species (two monocotyledonous and four dicotyledonous) were grown in soil (standard soil 2.3 from LUFA Splyer – sandy loam type, pH 6.8 ± 0.2) amended with five diclazuril concentrations. *Allium cepa*, *Raphanus sativus*, and *Solanum lycopersicum* were exposed to 6.3–99.2 mg/kg of diclazuril, while *Cucumis sativus*, *Hordeum vulgare*, and *Phaseolus vulgaris* were subjected to concentrations of 31.1–503 mg/kg. A reference test using linuron in treatments of 4 mg/kg with one dicotyledonous species (*R. sativus*) and one monocotyledonous species (*A. cepa*) confirmed the system's reliability. Diclazuril had no effect on seedling emergence or survival, even at the highest concentrations. The lowest no observed effect concentration (NOEC) for *S. lycopersicum* shoot length was determined to be 50.3 mg/kg soil.

Effects on terrestrial invertebrates

A study following OECD guideline 207 tested the acute effect of diclazuril on *Eisenia fetida* in artificial soil at concentrations ranging from 31.25 to 500 mg/kg dry weight soil. Both biomass and mortality were assessed after 7 and 14 days. The study was valid, with mortality in the controls below 10% (0% actual) and expected mortality in the toxic reference was observed. Mortality remained below 50% across all treatments, therefore, the 14-day lethal concentration 50% (LC_{50}) was determined to be >500 mg/kg dry weight soil.

Effects on microorganisms

Two studies following OECD guideline 216 were performed to assess the effects of diclazuril on soil microorganisms. In the first study, sandy loam soil was treated with diclazuril at rates of 170.63 and 1,706.3 µg/kg dry weight, corresponding to the maximum $PEC_{soil\ plateau}$ and 10 times that amount. Control and treated soils were incubated for 28 days, with nitrate

concentrations analysed at days 0, 7, 14, and 28. The study was valid, showing less than 15% (≤ 5.62% actual) variation in nitrate concentrations among control replicates for all timepoints. Deviations in nitrate formation rates from controls calculated using both the incremental and overall method at 28 days after treatment were under 25% for the tested PEC concentration but exceeded 25% for the 10x PEC level.

To evaluate long-term effects, the second study investigated the long-term effects of diclazuril on the nitrogen cycling ability of soil microorganisms at the 10 times maximum PEC level (1,706.3 µg/kg soil dry weight) over 100 days. Control and treated soils were sampled on days 0, 28, and 100, with variations in nitrate concentrations among control replicates remaining below 15% (≤ 4.36% actual) for all timepoints. Nitrate formation rate deviations from controls calculated using both the incremental and overall method were less than 25% at both 28 and 100 days for the 10 times maximum $PEC_{soil\ plateau}$.

Overall, at Phase II C, diclazuril exhibited an acceptable effect (less than 25% deviation from untreated controls) at ten times the maximum $PEC_{soil\ plateau}$ of 1,706.3 µg/kg soil dry weight over 100 days, indicating no impact on nitrogen cycling in soil microorganisms.

Effects on algae

No green algae test was submitted. Instead, a previously evaluated study following OECD Guideline 201 examined the effect of diclazuril on the freshwater cyanobacteria *Anabaena flos-aquae*. The cyanobacteria were exposed to diclazuril concentrations of 1.02, 1.49, 3.47 and 10.26 µg/L for 72 hours, with no observed effects up to the highest concentration tested. The 72-hour E_rC_{50} and NOEC were determined to be greater than 10.26 µg/L and 10.26 µg/L, respectively. Upon re-evaluation in line with the FEEDAP guidance on the evaluation of the safety of the additive for the environment (EFSA FEEDAP Panel, 2019), the FEEDAP Panel noted that since the study focused on cyanobacteria, its conclusions cannot be extrapolated to algae.

Effects on aquatic invertebrates

An acute immobilization test with *Daphnia magna* followed OECD Guideline 202 and was previously evaluated by the FEEDAP Panel in 2015. The highest tested concentration of 36 µg/L showed no adverse effects, but due to low water solubility, higher concentrations could not be tested, making it unsuitable for deriving a predicted no effect concentration (PNEC). The study was re-evaluated in the current assessment and the same conclusions were reached.

Additionally, a chronic test following OECD Guideline 211 exposed *D. magna* neonates to diclazuril concentrations ranging from 6.25 to 100 µg/L for 21 days. The study showed no more than 20% mortality in parent animals, with an EC_{10} for reproduction (cumulative offspring per survived parent) of 20.9 µg/L.

Effects on fish

An acute toxicity test with zebrafish (*Danio rerio*) followed OECD Guideline 203 and was previously evaluated by the FEEDAP Panel in 2015. The 96-hour LC_{50} of > 14.5 µg/L was deemed unsuitable for deriving a PNEC. The study was re-evaluated for the current assessment, and the same conclusions were reaffirmed.

Effects on sediment-dwelling organisms

A sediment/water toxicity test, conducted according to OECD Guideline 218, was previously evaluated by the FEEDAP Panel in 2015 (EFSA, 2015). The FEEDAP Panel concluded that the NOEC was 13.3 mg diclazuril/kg artificial sediment dry weight, and the same conclusion applies to the current assessment.

2.2.4.3. Risk characterisation

Data for the terrestrial compartment, including plants, earthworms, and microorganisms were used to evaluate risk, based on a NOEC of 50.3 mg/kg from a plant study and an LC_{50} value of > 500 mg/kg dry weight soil from the earthworm study. For the aquatic compartment, diclazuril's low solubility is noted. The acute studies reported an EC_{50} > 36 µg/L for *Daphnia* and an LC_{50} > 14 µg/L for fish. However, due to the lack of a green algae study, no PNEC can be derived for Phase IIA, and therefore the risk to aquatic compartment cannot be assessed. For the sediment compartment, a NOEC of 13.3 mg/kg artificial soil for *Chironomus riparius* was obtained from the provided study. Risk ratios for terrestrial and sediment compartments are summarised in Tables 7–10.

Table 7. Risk characterisation of diclazuril (PEC/PNEC ratio) for terrestrial compartment – soil with pH ≥ 5.3.

Taxa	PEC_{soil} (µg/kg)	NOEC/LC_{50} (mg/kg)	AF	PNEC (µg/kg)	PEC/PNEC
Plant	28	50.3[1]	10	5,030	0.0056
Earthworm	28	>500[2]	1,000	500	< 0.056

PEC: predicted environmental concentration; NOEC: no observed effect concentration; LC_{50}: lethal concentration, 50%; PNEC: predicted no effect concentration; AF: assessment factor.
(1): NOEC.
(2): LC_{50}.

Table 8. Risk characterisation of diclazuril (PEC/PNEC ratio) for terrestrial compartment – very acidic soils, pH < 5.3.

Taxa	PEC_{soil} (µg/kg)	$NOEC/LC_{50}$ (mg/kg)	AF	PNEC (µg/kg)	PEC/PNEC
Plant	161	50.3[1]	10	5,030	0.032
Earthworm	161	>500[2]	1,000	500	< 0.32

PEC: predicted environmental concentration; NOEC: no observed effect concentration; LC_{50}: lethal concentration, 50%; PNEC: predicted no effect concentration; AF: assessment factor.
(1): NOEC.
(2): LC_{50}.

Table 9. Risk characterisation of diclazuril (PEC/PNEC ratio) for sediment – soil with pH ≥ 5.3.

Taxa	$PEC_{sediment}$ (µg/kg dry weight)	NOEC (mg/kg)	AF	$PNEC_{sed}$ (µg/kg)	PEC/PNEC
Sediment-dwelling invertebrates *Chironomus riparius*	3.2	13.3	100	133	0.02

PEC: predicted environmental concentration; NOEC: no observed effect concentration; PNEC: predicted no effect concentration; AF: assessment factor.

Table 10. Risk characterisation of diclazuril (PEC/PNEC ratio) for sediment - very acidic soils, pH < 5.3.

Taxa	$PEC_{sediment}$ (µg/kg dry weight)	NOEC (mg/kg)	AF	$PNEC_{sed}$ (µg/kg)	PEC/PNEC
Sediment-dwelling invertebrates *Chironomus riparius*	19	13.3	100	133	0.14

PEC: predicted environmental concentration; NOEC: no observed effect concentration; PNEC: predicted no effect concentration; AF: assessment factor.

2.3.4.4. Bioaccumulation and secondary poisoning

With a maximum log octanol–water partition coefficient (log K_{ow}) of 2.3 at pH 5 (and < 0.3 at pH 7 and 9), diclazuril shows no potential for bioaccumulation, making the risk of secondary poisoning unlikely.

2.3.5. Conclusion on Section III: Safety

The FEEDAP Panel concluded that diclazuril from Coxiril® is considered safe for chickens reared for laying at up to 1.2 mg/kg of complete feed until 12 weeks of age and pheasants (it should not be used in laying birds) at 1.2 mg/kg of complete feed, with residue levels in tissues and eggs expected to be comparable to those in chickens and turkeys. The additive is deemed safe for consumers as long as poultry MRLs are not exceeded.

In 2014, The FEEDAP Panel concluded that Coxiril® is not an irritant to the eyes or skin, nor a potential skin sensitiser. Inhalation exposure during normal handling is unlikely to cause respiratory or systemic toxicity.

In high pH soils, diclazuril exists in an anionic form, while in acidic soils it remains in a neutral form, exhibiting different biodegradation rates. The FEEDAP Panel concluded that no concern is expected for groundwater for either acidic or non-acidic soils. Diclazuril had no effect on seedling emergence or survival, even at the highest concentrations.

At Phase II C, diclazuril exhibited an acceptable effect (less than 25% deviation from untreated controls) at ten times the maximum $PEC_{soil\ plateau}$ of 1,706.3 µg/kg soil dry weight over 100 days, indicating no impact on nitrogen cycling in soil microorganisms. No conclusions can be drawn for the aquatic compartment due to insufficient data. Diclazuril shows no potential for bioaccumulation, making the risk of secondary poisoning unlikely.

The FSA and FSS agree with the conclusions reached for the safety of the additive and the use of the extrapolation for pheasants, which is supported by the guidance that is also applicable in GB. The safety data for the target species, consumer and user were reviewed by EFSA in 2014, 2015 and 2018, prior to the UK's exit from the EU; thus, these opinions are applicable to GB. The FSA and FSS agree with the safety assessment for the environment that was carried out in the latest EFSA opinion in 2023.

2.4. Section IV: Efficacy

In 2014, the FEEDAP Panel assessed the efficacy of diclazuril from Coxiril® in preventing coccidiosis in chickens for fattening (EFSA, 2014a). Literature data from dose titration studies in battery cages and floor pens indicated that diclazuril from another source is effective as a coccidiostat in chickens for fattening at doses of at least 0.5 mg/kg feed. Five floor pen studies with Coxiril® confirmed the efficacy of approximately 0.8 mg diclazuril/kg feed in controlling coccidiosis. Two field trials also showed that 0.8 mg diclazuril from Coxiril® was as effective as another diclazuril source. Of the two sensitivity tests, only one clearly demonstrated efficacy against pathogenic *Eimeria* spp. isolates. In the FEEDAP Panel's conclusion, diclazuril from Coxiril® has a potential to control coccidiosis in chickens for fattening at a minimum dose of 0.8 mg/kg feed. These conclusions reached in chickens for fattening can be extended to chickens reared for laying according to the FEEDAP Panel (EFSA, 2018a).

Treatment of pheasants with diclazuril from Coxiril® at a minimum dose of 1 mg/kg reduced intestinal lesion scores, particularly in the caeca, following inoculation with *Eimeria* species specific to pheasants (EFSA, 2018b). This anticoccidial effect also restored feed intake, body weight gain, and feed to

gain ratio, which had been negatively impacted by the inoculation, bringing them back to levels comparable to the uninfected untreated group. The FEEDAP Panel concluded that diclazuril from Coxiril® at a minimum dose of 1 mg/kg complete feed has potential to control coccidiosis in pheasants (EFSA, 2018b).

The FSA/FSS agree with the conclusions reached on the data and the extension to chickens reared for laying, which is supported by the guidance that is also applicable in GB. This demonstrates that the additive has a potential to have coccidiostatic effect.

3. Analytical method evaluation

FSA/FSS evaluated the EURL analytical method evaluations, noting it was carried out in 2012 for authorisation for chickens for fattening and 2013 for authorisation for guinea fowl, when the UK was still part of the EU and would have participated of their approval. No concerns are raised at this stage for the validity of the methods for UK/GB use, and therefore the FSA/FSS accept the EURL analytical method evaluation report (EURL, 2012, 2013). FSA/FSS determined the analytical method as appropriate for official controls for this feed additive.

4. Conclusions

Based on data from chickens and turkeys for fattening, diclazuril from Coxiril® is considered safe for chickens reared for laying at up to 1.2 mg/kg of complete feed until 12 weeks of age. The FEEDAP Panel extended its consumer safety assessment for diclazuril use in chickens for fattening to chickens reared for laying. No detectable residues were found in the first eggs from chickens that had been fed diclazuril at this level from six weeks to 12 weeks of age.

The FEEDAP Panel concluded that diclazuril from Coxiril® is considered safe for pheasants at 1.2 mg/kg of complete feed, with residue levels in tissues and eggs expected to be comparable to those in chickens and turkeys. The additive is deemed safe for consumers as long as poultry MRLs are not exceeded. However, it should not be used in laying birds.

The FEEDAP Panel concluded that Coxiril® is non-irritating to the skin and eyes, not a potential skin sensitiser, and poses low risk of respiratory or systemic toxicity under normal handling.

The FEEDAP Panel concluded that diclazuril from Coxiril® poses no risk to the terrestrial compartment or sediment when used in chickens reared for laying and pheasants under the proposed conditions, regardless of soil type (acidic or non-acidic). Groundwater contamination is also not a concern for either soil type. However, due to a lack of sufficient data,

conclusions cannot be drawn for the aquatic compartment. Diclazuril from Coxiril® is not likely to bioaccumulate, and therefore there is unlikely to be a risk of secondary poisoning.

5. Caveats and uncertainties

Conclusions cannot be drawn for the aquatic compartment, due to the lack of the provided data. This was also concluded by the EFSA FEEDAP Panel in their 2023 opinion.

The FEEDAP Panel previously assessed the safety of diclazuril at use levels up to 1.2 mg/kg complete feed and concluded it is safe for chickens (EFSA, 2014a) and turkeys (EFSA, 2014c) for fattening. However, margins of safety could not be established accurately due to limitations with the tolerance studies, particularly the low number of replicates and animals per treatment and sex.

6. FSA/FSS conclusions for GB risk analysis

The application has been assessed in line with the applicable guidance, which is also the guidance adopted by FSA and FSS and is partially based on considerations of detailed proprietary information available to the Panel, which was also submitted to the FSA and FSS. The EFSA opinion identifies and characterises the hazards present from the use of the feed additive consisting of diclazuril (Coxiril®) for chickens reared for laying and pheasants, and there is sufficient information to enable an assessment of exposure, which is also relevant to GB. The risk characterisation is unchanged from the 2018 opinions for most areas, and appropriate evidence was submitted to support the application, including data on safety to the environment, which is relevant to GB scenarios. The conclusions of the EFSA opinion have been reviewed in detail by FSA and FSS and are considered appropriate and consistent, including the caveats and uncertainties identified in the opinion which are applicable to GB. Sufficient evidence has been demonstrated to conclude without further questions or risk assessment.

Abbreviations

Abbreviation	Definition
AF	Assessment factor
DT_{50}	Time to degradation of 50% of original concentration of the compound in the tested soils
DT_{90}	Time to degradation of 90% of original concentration of the compound in the tested soils
EC	European Commission
EC_{50}	The concentration of a test substance which results in 50% of the test organisms being adversely affected, i.e. both mortality and sublethal effects

Abbreviation	Definition
EU	European Union
EFSA	European Food Safety Authority
EURL	European Union Reference Laboratory
FEEDAP	EFSA Panel on Additives and Products or Substances used in Animal Feed
FSA	Food Standards Agency
FSS	Food Standards Scotland
GB	Great Britain
K_{OC}	Adsorption or desorption coefficient corrected for soil organic carbon content
LC_{50}	Lethal concentration, 50%
Log K_{OW}	Logarithm of octanol–water partition coefficient
MRL	Maximum residue limit
NOEC	No observed effect concentration
OECD	Organisation for Economic Co-operation and Development
PEC	Predicted environmental concentration
PNEC	Predicted no effect concentration
RP	Regulated Product
TAR	Total applied radioactivity
UF	Uncertainty factor
UK	United Kingdom

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