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## Safety for the environment of a feed additive consisting of diclazuril (Coxiril<sup>®</sup>) for chickens reared for laying and pheasants (Huvepharma NV)

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### Abstract

Following a request from the European Commission, EFSA was asked to deliver a scientific opinion on the safety for the environment of diclazuril (Coxiril<sup>®</sup>) as a coccidiostat feed additive for 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 reach a final conclusion on the risk resulting from the use of diclazuril in acid soil from Coxiril<sup>®</sup>. On the basis of the new data provided, the FEEDAP Panel updates the previous conclusions as follows: no risk is expected for the terrestrial compartment and for sediment when diclazuril is used in chickens reared for laying and to pheasants at the proposed condition of use (in both acidic and non-acidic soils). No concern for groundwater is expected for both acidic and non-acidic soils. Due to the lack of data, no conclusions can be drawn for the aquatic compartment. Diclazuril does not have the potential for bioaccumulation; therefore, a risk of secondary poisoning is unlikely.

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## 1. Introduction

### 1.1. Background and Terms of Reference as provided by the requestor

Regulation (EC) No 1831/2003<sup>1</sup> establishes the rules governing the Community authorisation of additives for use in animal nutrition and, in particular, Article 9 defines the terms of the authorisation by the Commission.

The applicant, Huvepharma NV, is seeking a Community authorisation of Diclazuril as a feed additive to be used as a coccidiostats and histomonostats for pheasants (FAD-2017-0030) and chickens reared for laying (FAD-2015-0036) (Table 1).

**Table 1:** Description of the substances

<b>Category of additive</b>	Coccidiostats and histomonostats
<b>Functional group of additive</b>	Coccidiostats and histomonostats
<b>Description</b>	Diclazuril
<b>Target animal category</b>	pheasants (FAD-2017-0030) and chickens reared for laying (FAD-2015-0036)
<b>Applicant</b>	Huvepharma NV
<b>Type of request</b>	New opinion

On 20 February 2018 and 22 March 2018, the Panel on Additives and Products or Substances used in Animal Feed of the European Food Safety Authority ('Authority'), in its opinions on the safety and efficacy of the product, could not conclude on the safety of the additive for the environment: 'A final conclusion on the risk resulting from the use of the additive in acid soil cannot be done due to high uncertainties related to the potential accumulation of diclazuril over time'.

After the discussion with the Member States on the Standing Committee, it was suggested to check the possibility to demonstrate the safety in acid soils.

In view of the above, the Commission asks the Authority to deliver a new opinion on Diclazuril as a feed additive for pheasants (FAD-2017-0030) and chickens reared for laying (FAD-2015-0036) based on the additional data submitted by the applicant.

### 1.2. Additional information

The Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) adopted in 2014 six opinions on the safety and efficacy of Coxiril<sup>®</sup> when used as a feed additive in chickens for fattening (EFSA FEEDAP Panel, 2014a), turkeys for fattening (EFSA FEEDAP Panel, 2014b), guinea fowl (EFSA FEEDAP Panel, 2014c), rabbits for fattening and rabbits for breeding (EFSA FEEDAP Panel, 2015), chickens reared for laying (EFSA FEEDAP Panel, 2018a) and pheasants (EFSA FEEDAP Panel, 2018b).

The additive Coxiril<sup>®</sup> is authorised in chickens for fattening, turkeys for fattening, guinea fowl for fattening and breeding by Regulation (EU) 2015/46<sup>2</sup> 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. The same MRLs are reported in Regulation (EU) No 115/2013<sup>3</sup> for the use of diclazuril as veterinary medicine in poultry. Coxiril<sup>®</sup> is also authorised in rabbits by Regulation (EU) 2015/1417<sup>4</sup>.

<sup>1</sup> 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.

<sup>2</sup> Commission Implementing Regulation (EU) 2015/46 of 14 January 2015 concerning the authorisation of diclazuril as a feed additive for chickens for fattening, for turkeys for fattening and for guinea fowl for fattening and breeding (holder of authorisation Huvepharma NV). OJ L 9, 15.1.2015, p. 5.

<sup>3</sup> 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 regards the substance diclazuril. OJ L 38, 9.2.2013, p. 11.

<sup>4</sup> Commission Implementing Regulation (EU) 2015/1417 of 20 August 2015 concerning the authorisation of diclazuril as a feed additive for rabbits for fattening and for breeding (holder of the authorisation Huvepharma NV). OJ L 220, 21.8.2015, p. 15.

## 2. Data and Methodologies

### 2.1. Data

The present assessment is based on data submitted by the applicant in the form of additional information<sup>5</sup> to a previous application of the same product.<sup>6</sup> The dossier was received on 25/3/2020 and the general information and supporting documentation available on Open.EFSA at <https://open.efsa.europa.eu/questions/EFSA-Q-2020-00343>.

The FEEDAP Panel used the data provided by the applicant together with data from other sources, such as previous risk assessments by EFSA or other expert bodies, to deliver the present output.

### 2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of diclazuril is in line with the principles laid down in Regulation (EC) No 429/2008<sup>7</sup> and the relevant guidance documents: Guidance on the assessment of the safety of feed additives for the environment (EFSA FEEDAP Panel, 2019).

## 3. Assessment

The FEEDAP Panel assessed the safety for the environment of diclazuril from Coxiril<sup>®</sup> when fed to chickens for fattening (EFSA FEEDAP Panel, 2014a), turkeys for fattening (EFSA FEEDAP Panel, 2014b), guinea fowl (EFSA FEEDAP Panel, 2014c) and rabbits for fattening and rabbits for breeding (EFSA FEEDAP Panel, 2015).

The FEEDAP Panel reviewed and updated the previous assessment of the safety of diclazuril from Coxiril<sup>®</sup> for the environment in its opinions on the use of the additive in chickens reared for laying and pheasants (EFSA FEEDAP Panel, 2018a,b). These new opinions took into consideration new information submitted by the applicant and considered the use of the additive in chickens reared for laying and pheasants.

The FEEDAP Panel concluded as follows with regard to the use of Coxiril<sup>®</sup> in chickens reared for laying (EFSA FEEDAP Panel, 2018a): 'The use of diclazuril from Coxiril<sup>®</sup> in chickens reared for laying at the highest proposed feed concentration would not pose a risk to the environment for neutral/alkaline soils (pH  $\geq$  7). A final conclusion on the risk resulting from the use of diclazuril in acid soil from Coxiril<sup>®</sup> cannot be done due to the high uncertainties related to potential accumulation of diclazuril over time'.

The FEEDAP Panel concluded as follows about the use of Coxiril<sup>®</sup> in pheasants (EFSA FEEDAP Panel, 2018b): 'In accordance with the technical guidance on the extrapolation from major species to species regarding the assessment of feed additives for use in animal nutrition, the environmental risk assessment for pheasants can be extrapolated from the assessment done for the use of diclazuril in the major species. Recently, the FEEDAP Panel concluded that the use of diclazuril from Coxiril<sup>®</sup> in chickens reared for laying at 1.2 mg/kg complete feed would not pose a risk to the environment for neutral/alkaline soils (pH  $\geq$  7). A final conclusion on the risk resulting from the use of diclazuril in acid soil from Coxiril<sup>®</sup> cannot be done due to the high uncertainties related to potential accumulation of diclazuril over time (EFSA FEEDAP Panel, 2018a,b). As the conditions of use among poultry species are similar, the same conclusion applies to the use of the additive in pheasants'.

For the current assessment the applicant has submitted an updated environmental risk assessment according to the FEEDAP guidance on the assessment of the safety of the feed additives for the environment (EFSA FEEDAP Panel, 2019).<sup>8</sup>

<sup>5</sup> FEED dossier reference: FAD-2020-0023.

<sup>6</sup> FAD-2017-0030 and FAD-2015-0036.

<sup>7</sup> Commission Regulation (EC) No 429/2008 of 25 April 2008 on detailed rules for the implementation of Regulation (EC) No 1831/2003 of the European Parliament and of the Council as regards the preparation and the presentation of applications and the assessment and the authorisation of feed additives. OJ L 133, 22.5.2008, p. 1.

<sup>8</sup> Technical dossier/Supplementary information May 2022.

### 3.1. Characterisation

The identity of the additive, the characterisation of the active substance, the manufacturing process, the identity of diclazuril impurities and stability of the additive have been previously reviewed by the FEEDAP Panel (EFSA FEEDAP Panel, 2014a).

Coxiril<sup>®</sup>, containing 0.5% diclazuril, is intended for the prevention of coccidiosis in chickens reared for laying at a dose range of 0.8–1.2 mg diclazuril/kg complete feed up to a maximum age of 12 weeks and in pheasants at a dose range of 1.0–1.2 mg diclazuril/kg complete feed.

### 3.2. Safety for the environment

The applicant submitted an updated environmental risk assessment in line with the requirements of the FEEDAP guidance to evaluate the safety of the additives for the environment (EFSA FEEDAP Panel, 2019). The species covered by the environmental risk assessment presented by the applicant are the major species chickens for fattening for which the maximum authorised content is 1.2 mg diclazuril/kg complete feed, equal to the maximum content proposed for the target species object of this application. In addition, the applicant proposed a scenario for chickens reared for laying ('replacement pullet scenario') in which the manure generated up to the 12 weeks of age was diluted with the annual manure produced.

The FEEDAP Panel checked the proposed scenario for chickens reared for laying ('replacement pullet scenario') and noted that it presents a number of limitations (e.g. the assumption that the chickens reared for laying will remain in the same facility for the entire production process, which is not common practice) that prevents it to be used in the current evaluation. Therefore, the Panel assumes that safety of the worst-case scenario 'chickens for fattening' covers the safety of the additive for the species under assessment (pheasants and chickens reared for laying).

#### 3.2.1. Phase I

##### 3.2.1.1. Physico-chemical properties

The physico-chemical properties of diclazuril are summarised in Table 2.

**Table 2:** Physico-chemical properties of diclazuril

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

(1): Technical dossier/Section II.

(2): EFSA FEEDAP Panel (2015).

(3): EFSA FEEDAP Panel (2015).

A new experimental study was provided to measure the dissociation constant (pKa) of diclazuril.<sup>9</sup> The apparent pKa value of 8.62 derived from this study was not considered reliable since the study was performed in a mixture of different organic solvents and not in water. The pKa value of 5.89 used in a previous FEEDAP evaluation (EFSA FEEDAP Panel, 2018a) is used in the present assessment.

The FEEDAP Panel noted that diclazuril is a triazine which can probably be deprotonated to a soluble anionic form at a pH above the pKa value of 5.89. At pH lower than 5.89, diclazuril is present in its neutral form.

<sup>9</sup> Technical dossier/Supplementary information September 2020/Annex-1.

### 3.2.1.2. Fate and behaviour

#### *Fate in soil*

##### Adsorption

The same studies evaluated in 2018 (EFSA FEEDAP Panel, 2018a) were made available for the current assessment.<sup>10</sup> The studies were re-evaluated for the present assessment (Table 3).

From the first study (Study 1),<sup>11</sup> the lowest  $K_{foc}$  value of 4,986 mL/g was obtained from five soil types (sand, loamy sand, sandy loam, loam and clay) at different pH values (5.1, 5.5, 6.8, 7.2 and 7.1).

The second study (Study 2) was performed in soils at higher pH.<sup>12</sup> The Freundlich adsorption of <sup>14</sup>C-labelled diclazuril was studied in four soils with pH values (CaCl<sub>2</sub>) ranging from 7.30 to 7.61. In Table 3, a summary of adsorption values for the nine soils is reported. From the analysis of the adsorption data from nine soils, covering a range of soil pH from 5.1 to 7.61, it may be stated that diclazuril dissociation has limited influence on the overall adsorption and subsequent mobility of diclazuril.

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

Soil ID (study 1 <sup>11</sup> or 2 <sup>12</sup> )	pH	Organic carbon content (%)	$K_{foc}$ (mL/g)	1/n	$K_{oc}$ (mL/g)
Sand 2.1 (1)	5.1	0.65	4,986.4	0.818	20,752
Loamy sand 2.2 (1)	5.5	1.77	12,744.6	0.928	23,947
Sandy loam 2.3 (1)	6.8	0.94	20,177.7	1.095	10,037
Clay 6S (1)	7.1	1.66	5,073.3	0.902	9,768
Loam 2.4 (1)	7.2	2.26	8212.8	1.035	6,297
Loam Cl1 (2)	7.3	3.37	13,263.8	1.075	7,536
Sandy Loam I2 (2)	7.36	1.53	4,573.2	0.93	7,700
Silty clay loam Fr1 (2)	7.56	1.28	6,024.2	0.985	6,741
Clay Sp3 (2)	7.61	1.14	3,457	0.866	9,495
<b>Geometric mean</b>			<b>7,412</b>		<b>10,146</b>

For modelling purposes, the geometric mean value for  $K_{foc}$  of 7,412 mL/g (equivalent to a  $K_{om}$  of 4,299 mL/g) and the arithmetic 1/n of 0.959 are the most appropriate values.

##### Degradation

The degradation of diclazuril was determined in two studies performed according to OECD 307.<sup>13</sup>

The first study (Study 1),<sup>14</sup> performed on 4 soils, was assessed in a previous opinion (EFSA FEEDAP Panel, 2015). The study indicated that the degradation of diclazuril in soil shows marked pH dependence. At high pH, the anionic form of diclazuril is transformed with a  $DT_{50}$  of 70 and 97 days. At low pH, no degradation or transformation was observed at pH 5.1 during 120 days, whereas a slow transformation was observed at pH 5.5 with a half-life of 119 days.

A second study (Study 2)<sup>15</sup> was performed with four soils having different properties (Table 4), all with a lower soil pH. A slow decline in the levels of extracted diclazuril was observed in soils G1, E1 and H2 where diclazuril levels of 45–51% of the total applied radioactivity (TAR) were reached after 120 days incubation following application. In soil B1, diclazuril decline was very slow with levels of 78% TAR after 120 days. Table 4 reports a summary of the  $DT_{50}$ s derived through single first-order kinetics (SFO) for all soils.

<sup>10</sup> Technical dossier/Supplementary information May 2022/Y6CY1030\_Diclazuril\_Sorption and FR000568\_Diclazuril\_Sorption high pH soils.

<sup>11</sup> Technical dossier/Supplementary information May 2022/Y6CY1030\_Diclazuril\_Sorption.

<sup>12</sup> Technical dossier/Supplementary information May 2022/FR000568\_Diclazuril\_Sorption high pH soils.

<sup>13</sup> Technical dossier/Supplementary information May 2022/ Y6CJ1020 - Diclazuril Soil Deg and R002503\_Diclazuril\_Soil degradation (low pH).

<sup>14</sup> Technical dossier/Supplementary information May 2022/Y6CJ1020 - Diclazuril Soil Deg.

<sup>15</sup> Technical dossier/Supplementary information May 2022/R002503\_Diclazuril\_Soil degradation (low pH).

**Table 4:** DT<sub>50</sub> values for diclazuril in soils, arranged by ascending pH. All DT<sub>50</sub> derived assuming single first-order kinetics (SFO)

Soil (study 1 <sup>14</sup> or study 2 <sup>15</sup> )	pH	DT <sub>50</sub> (d) (20°C)	DT <sub>90</sub> (d) (20°C)	DT <sub>50</sub> (d) a (12°C)
Sandy loam (B1) (2)	3.7	667.6	> 1,000	> 1,000
Sand 2.1 (1)	5.1	> 1,000	> 1,000	> 1,000
Sandy clay loam (G1) (2)	5.3	121.8	404.5	258
Silty clay loam (E1) (2)	5.4	158.4	526.3	336
Clay (H2) (2)	5.4	156.7	520.5	332
Loamy sand 2.2 (1)	5.5	119.4	396.6	253
Sandy loam 2.3 (1)	6.8	72.6	241	154
Clay 6S (1)	7.1	96.5	358.6	205

DT<sub>50</sub>: time to degradation of 50% of original concentration of the compound in the tested soils.

Soils with a pH higher than 5.3 have a DT<sub>50</sub> within a similar range between 72.6 to 158.4 days (geometric mean 117 days), while very acidic soils (i.e. pH < 5.3), have a DT<sub>50</sub> which range between 667.6 and > 1,000 days.

Considering the above, the following DT<sub>50</sub>s normalised to 12°C will be used for the evaluation: 247 days for soils with pH > 5.3 and DT<sub>50</sub> > 1,000 days for soils with pH < 5.3.

#### Conclusion on fate and behaviour

In high pH soils, diclazuril is present in an anionic form whereas it is present in a neutral form in acid soils. Based on the data available, the FEEDAP Panel noted that the two forms exhibit a different biodegradation and should be considered separately.

For exposure assessment, a DT<sub>50</sub> normalised to 12°C of 247 days has to be considered for soils with pH higher than 5.3 and a DT<sub>50</sub> > 1,000 days for acidic soils. For all soil pHs, a geometric mean value for K<sub>foc</sub> of 7,412 mL/g (equivalent to a K<sub>om</sub> of 4,299 mL/g) and the arithmetic 1/n of 0.959 are the most appropriate values.

#### 3.2.1.3. Predicted environmental concentrations (PECs)

The input values used for exposure assessment of diclazuril were: 1.2 mg/kg feed for chicken for fattening; molecular weight of 407.64; vapour pressure  $1.21 \times 10^{-22}$  Pa, water solubility 0.023 mg/L, DT<sub>50</sub> of 247 days at 12°C for soils with pH > 5.3 and DT<sub>50</sub> 1,000 days for soil with pH below 5.3 (2,120 days when normalised to 12°C) and a K<sub>foc</sub> of 7,412 mL/g.

The phase I PEC, which does not differ for the two scenarios, is reported in Table 5.

**Table 5:** Phase I predicted environmental concentration of diclazuril

Compartment	PEC
Soil (µg/kg)	18
Groundwater (µg/L)	0.03

The Phase I PEC trigger value is exceeded for soils; therefore, a Phase II assessment is necessary.

### 3.2.2. Phase II

#### 3.2.2.1. Exposure assessment

*PECs calculation refined in Phase II*

##### Refinement of PEC<sub>soil</sub> for persistent compounds.

The DT<sub>90</sub> for diclazuril was determined to be greater than 1 year for both acidic and not acidic soils; therefore, the PECs refined at steady state were calculated according to the FEEDAP technical guidance for assessing the safety of feed additives for the environment (EFSA FEEDAP Panel, 2019) (Table 6).



**Table 6:** 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)	$1.21 \times 10^{-22}$	$1.21 \times 10^{-22}$
Solubility (mg/L)	0.023	0.023
K <sub>oc</sub> (L/kg)	7412	7412
DT <sub>50</sub> in soil at 12°C (days)	247	2120
Output		
Application rate kg/ha	0.014	0.014
PEC <sub>soil</sub> (µg/kg)	28	161
PEC <sub>groundwater</sub> (µg/L)	0.048	0.272
PEC <sub>surfacewater</sub> (µg/L)	0.016	0.091
PEC <sub>sediment</sub> (µg/kg dry weight)	12	67

K<sub>oc</sub>: adsorption or desorption coefficient corrected for soil organic carbon content.

No concern is expected for groundwater for soils with pH higher than 5.3.

#### PEC<sub>groundwater</sub>, PEC<sub>surfacewater</sub> and PEC<sub>sediment</sub> refinement with FOCUS

The applicant provided a higher tier calculation through FOCUS models in order to address the leaching to groundwater. The model PEARL (FOCUS Version 4.4.4.) was used on the FOCUS scenarios that are specified as relevant for avian treatments, i.e. Jokioinen and Piacenza. An application rate of 0.014 kg/ha for turkey for fattening (worst case) and two DT<sub>50</sub> for soil (acidic or not) were considered in modelling. The results of the model show no concern for groundwater, with predicted concentration far below 0.1 µg/L for both acidic and not acidic soils).

The applicant also provided a refinement in PEC calculation for surface water and sediment through the FOCUS Step 3 surface water models (Table 7). The same input data used for groundwater assessment were used for surface water. Furthermore, in a worst-case approach, a DT<sub>50</sub> of 1,000 days was considered to simulate both surface water and sediment degradation.

**Table 7:** Predicted environmental concentrations (PECs) of diclazuril, surface water and sediment refined with FOCUS models

	Most soils, pH ≥ 5.3	Very acidic soil, pH < 5.3
PEC <sub>surfacewater</sub> (µg/L)	0.01685	0.1668
PEC <sub>sediment</sub> (µg/kg dry weight)	3.230	19.291

AF: assessment factor.

### Conclusions

No concern for groundwater is expected for both acidic and not acidic soils.

The following PEC values are used for risk assessment: (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.

### 3.2.2.2. Ecotoxicity studies

#### *Toxicity to soil organisms*

#### Effects on terrestrial plants

The seeds from six plant species (two monocotyledonous and four dicotyledonous) were allowed to emerge and grow in soil (standard soil 2.3 from LUFA Splyer – sandy loam type, pH 6.8 ± 0.2) amended with five different diclazuril concentrations.<sup>16</sup> *Allium cepa*, *Raphnanus sativus* and *Solanum lycopersicum* were exposed to concentrations ranging from 6.3 to 99.2 mg/kg, while *Hordeum vulgare*, *Cucumis sativus* and *Phaseolus vulgaris* were exposed to concentrations ranging from 31.1 to 503 mg/kg

<sup>16</sup> Technical dossier/Supplementary information May 2022/Y6CJ1060 - Diclazuril plant ecotox.

soil. The performance of the test system was assessed using a reference item – linuron in treatments of 4 mg/kg with one dicotyledonous species (*R. sativus*) and one monocotyledonous species (*A. cepa*). The study was well performed, the validity criteria were fulfilled, and the results are reliable. Diclazuril had no effect on either seedling emergence or survival at any treatment concentrations (even the highest applied, 99.2 and 503 mg/kg). The lowest no observed effect concentration (NOEC) of 50.3 mg/kg soil was determined for shoot length of *S. lycopersicum*.

#### Effects on terrestrial invertebrates

A study following OECD guideline 207 was performed to investigate the acute effect of diclazuril on *Eisenia fetida*<sup>17</sup> in an artificial soil at a nominal concentration range of 31.25, 62.5, 125, 250 and 500 mg/kg dry weight soil. Mortality and biomass were assessed after 7 and 14 days. The study was valid; mortality in the controls was < 10% at the end of the test (actual 0%) and the expected mortality was observed in the toxic reference. Mortality in all the treatments including the maximum were less than 50%; therefore, the 14-day LC<sub>50</sub> was determined as > 500 mg/kg dry weight soil.

#### Effects on microorganisms

The applicant submitted two studies performed according to OECD guideline 216 to investigate the effect of diclazuril on soil microorganisms.

In the first study,<sup>18</sup> a sandy loam soil was treated with diclazuril at a rate of 170.63 and 1,706.3 µg/kg soil dry weight, equivalent to the maximum PEC<sub>soil plateau</sub> and 10× maximum PEC<sub>soil plateau</sub>. Control and treated soils were incubated for 28 days, and subsamples were taken on 0, 7, 14 and 28 days after treatment and analysed for the nitrate concentration. The study was valid; variation in nitrate concentration of control replicates was less than 15% (actual ≤ 5.62%) for all timepoints. Nitrate formation rate deviations from the controls calculated using both the incremental and overall method at 28 days after treatment were less than 25% for the PEC concentration tested; however, for the 10× PEC levels, the deviation from controls was more than 25%.

Since the first study was not extended to 100 days, the second study<sup>19</sup> was performed to examine the long-term effects of diclazuril on the nitrogen cycling ability of soil microorganisms at the 10× maximum PEC level (1,706.3 µg/kg soil dry weight). Control and treated soils were incubated for 100 days, and subsamples were taken on 0, 28 and 100 days after treatment and analysed for the nitrate concentration. The study was valid; variation in nitrate concentration of control replicates was less than 15% (actual ≤ 4.36%) for all timepoints. Nitrate formation rate deviations from the controls calculated using both the incremental and overall method at 28 and 100 days after treatment were less than 25% for the 10× maximum PEC<sub>soil plateau</sub>. At Phase IIC, diclazuril demonstrates an acceptable effect (< 25% deviation from the untreated controls) at the maximum PEC<sub>soil plateau</sub> at 10× (1,706.3 µg/kg soil dry weight) when considered over 100 days and will have no impact on the nitrogen cycling of soil microorganisms.

#### Toxicity to aquatic organisms

##### Effects on algae

No test on green algae was submitted. Instead, a study following OECD Guideline 201<sup>20</sup> already evaluated in previous FEEDAP opinion, was provided. The test was performed to investigate effect of diclazuril on cyanobacteria. *Anabaena flos-aquae*, freshwater cyanobacteria species was exposed to 1.02, 1.49, 3.47, 10.26 µg/L diclazuril for up to 72 h. The evaluation of biological endpoints was performed using measured concentrations. No effect of diclazuril on cyanobacteria was observed up to the highest concentration tested. The 72-h E<sub>r</sub>C<sub>50</sub> and NOEC were determined as > 10.26 µg/L and 10.26 µg/L, respectively.

The study was re-evaluated for the current assessment in line with the FEEDAP guidance on the evaluation of the safety of the additive for the environment (EFSA FEEDAP Panel, 2019) and the FEEDAP Panel notes that the study provided is performed on cyanobacteria and the conclusions from this study cannot be extrapolated to algae.

<sup>17</sup> Technical dossier/Supplementary information May 2022/ FR002444\_Diclazuril\_Earthworm acute.

<sup>18</sup> Technical dossier/Supplementary information May 2022/FR002445 Diclazuril\_Nitrate transformation.

<sup>19</sup> Technical dossier/Supplementary information May 2022/ FR002445-R\_Diclazuril\_100 d Nitrate transformation.

<sup>20</sup> Technical dossier/Supplementary information May 2022/13BT2AB\_diclazuril\_algae.

### Effects on aquatic invertebrates

An acute immobilisation test was conducted with *Daphnia magna* in accordance with OECD Guideline 202.<sup>21</sup> The study was already evaluated by the FEEDAP Panel in 2015 and it was concluded that 'The highest tested concentration (36 µg/L) resulted in no adverse effects and, because of the low water solubility, higher concentrations could not be tested. These data cannot be used to derive a PNEC'. The study was re-evaluated for the current assessment in line with the FEEDAP guidance on the evaluation of the safety of the additive for the environment (EFSA FEEDAP Panel, 2019) and the same conclusions are reached.

A study following OECD guideline 211 was performed to investigate the chronic effect of diclazuril on aquatic invertebrates.<sup>22</sup> Neonates of the aquatic invertebrate *D. magna* were exposed to diclazuril at a nominal concentration range of 6.25, 12.5, 25, 50 and 100 µg/L for 21 days in a semi-static exposure test. To assess the stability of the test item, the concentration of diclazuril in the test media was determined at the start and end of three exposure media renewals. The study, which was considered valid, provided the mortality rates of the parent animals (female *Daphnia*) that did not exceed 20% at the end of the test. The EC<sub>10</sub> for reproduction (cumulative offspring per survived parent) was 20.9 µg/L.

### Effects on fish

An acute toxicity test with zebrafish (*Danio rerio*) was conducted in accordance with OECD Guideline 203.<sup>23</sup> The study was already evaluated by the FEEDAP Panel in 2015 and it was concluded that 'The reported 96-hour lethal concentration 50 % (LC<sub>50</sub>) for zebrafish of > 14.5 µg/L is not considered appropriate to derive the PNEC'. The study was re-evaluated for the current assessment in line with the FEEDAP guidance on the evaluation of the safety of the additive for the environment (EFSA FEEDAP Panel, 2019) and the same conclusions are reached.

### Effects on sediment-dwelling organisms

A sediment/water toxicity test was performed in accordance OECD Guideline 218.<sup>24</sup> The study was already evaluated by the FEEDAP Panel in 2015 (EFSA FEEDAP Panel, 2015) and it was concluded that a NOEC of 13.3 mg diclazuril/kg artificial sediment dry weight can be obtained from the study. The FEEDAP Panel notes that the same conclusion applies to the current evaluation.

### 3.2.3. Risk characterisation

For the terrestrial compartment, data are available for plants, earthworms and microorganisms. The risk for terrestrial compartment was evaluated based on a plant study resulting in the NOEC of 50.3 mg/kg and the earthworm study resulting with the LC<sub>50</sub> value of > 500 mg/kg dry weight soil.

For the aquatic compartment, it is noted that diclazuril has a low water solubility. The acute studies submitted by the applicant reported an EC<sub>50</sub> for *Daphnia* of > 36 µg/L and a LC<sub>50</sub> for fish of > 14 µg/L. A study with green algae is missing. Therefore, a PNEC for Phase IIA cannot be derived. Consequently, the risk to this compartment cannot be assessed.

Ecotoxicological data for sediment-dwelling invertebrate *Chironomus riparius* were provided for the sediment compartment resulting in a NOEC of 13.3 mg diclazuril/kg artificial soil.

The risk characterisation ratios for terrestrial compartment and sediment are reported in Tables 8–11.

**Table 8:** Risk characterisation of diclazuril (PEC/PNEC ratio) for terrestrial compartment – soil with pH ≥ 5.3

Taxa	PEC <sub>soil</sub> (µg/kg)	NOEC/LC <sub>50</sub>	AF	PNEC (µg/kg)	PEC/PNEC
Plant	28	50.3 mg/kg <sup>(1)</sup>	10	5030	0.0056
Earthworm		> 500 mg/kg <sup>(2)</sup>	1,000	500	< 0.056

PEC: predicted environmental concentration; NOEC: no observed effect concentration; LC<sub>50</sub>: lethal concentration, 50%; PNEC: predicted no effect concentration; AF: assessment factor.

(1): NOEC.

(2): LC<sub>50</sub>.

<sup>21</sup> Technical dossier/Supplementary information May 2022/13BT2DA\_diclazuril\_daphnia.pdf.

<sup>22</sup> Technical dossier/Supplementary information May 2022/ FR002447\_Diclazuril\_Daphnia reproduction.

<sup>23</sup> Technical dossier/Supplementary information May 2022/13BT2FY\_diclazuril\_fish.

<sup>24</sup> Technical dossier/Supplementary information May 2022/ Y6CJ1080\_Diclazuril\_Chironomid.

**Table 9:** Risk characterisation of diclazuril (PEC/PNEC ratio) for terrestrial compartment – very acidic soils, pH < 5.3

Taxa	PEC <sub>soil</sub> (µg/kg)	NOEC	AF	PNEC (µg/kg)	PEC/PNEC
Plant	161	50.3 mg/kg <sup>(1)</sup>	10	5,030	0.032
Earthworm		> 500 mg/kg <sup>(2)</sup>	1,000	500	< 0.32

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

(1): NOEC.

(2): LC<sub>50</sub>.

**Table 10:** Risk characterisation of diclazuril (PEC/PNEC ratio) for sediment – soil with pH ≥ 5.3

Taxa	PEC <sub>sediment</sub> (µg/kg dry weight)	NOEC (mg/kg)	AF	PNEC <sub>sed</sub> (µg/kg)	PEC/PNEC
<b>Sediment-dwelling invertebrates</b> <i>Chironomus riparius</i>	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 11:** Risk characterisation of diclazuril (PEC/PNEC ratio) for sediment - very acidic soils, pH < 5.3

Taxa	PEC <sub>sediment</sub> (µg/kg dry weight)	NOEC (mg/kg)	AF	PNEC <sub>sed</sub> (µg/kg)	PEC/PNEC
<b>Sediment-dwelling invertebrates</b> <i>Chironomus riparius</i>	19	13.3	100	133	0.14

PEC: predicted environmental concentration; NOEC: no observed effect concentration; LC<sub>50</sub>: lethal concentration, 50%; PNEC: predicted no effect concentration; AF: assessment factor.

### 3.2.4. Bioaccumulation and secondary poisoning

Based on the maximum log octanol–water partition coefficient (log K<sub>ow</sub>) of 2.3 at pH 5 (< 0.3 at pH 7 and pH 9), diclazuril does not have the potential for bioaccumulation. Therefore, the risk of secondary poisoning is unlikely.

## 4. Conclusions

No risk is expected for the terrestrial compartment and for sediment when diclazuril from Coxiril<sup>®</sup> is used in chickens reared for laying and to pheasants at the proposed condition of use (in both acidic and non-acidic soils). No concern for groundwater is expected for both acidic and non-acidic soils. Due to the lack of data, no conclusions can be drawn for the aquatic compartment. Diclazuril from Coxiril<sup>®</sup> does not have the potential for bioaccumulation, therefore, the risk of secondary poisoning is unlikely.

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## Abbreviations

AF	assessment factor
DT <sub>50</sub>	time to degradation of 50% of original concentration of the compound in the tested soils
DT <sub>90</sub>	time to degradation of 90% of original concentration of the compound in the tested soils
EC <sub>50</sub>	the concentration of a test substance which results in 50% of the test organisms being adversely affected, i.e. both mortality and sublethal effects
FEEDAP	EFSA Scientific Panel on Additives and Products or Substances used in Animal Feed
K <sub>OC</sub>	adsorption or desorption coefficient corrected for soil organic carbon content
LC <sub>50</sub>	lethal concentration, 50%
Log K <sub>ow</sub>	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
UF	uncertainty factor