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Safety and efficacy of Deccox® (decoquinate) for chickens for fattening

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Abstract

Deccox®, containing decoquinate as the active substance, is a feed additive intended to be used for the prevention of coccidiosis in chickens for fattening at a dose range of 20–40 mg/kg complete feed. Decoquinate from Deccox® is safe for chickens for fattening at the highest applied concentration in complete feed of 40 mg/kg. No practically relevant interactions with other additives or veterinary drugs exist except with bentonite. Decoquinate does not have antibacterial action. Decoquinate is not genotoxic and not carcinogenic. Deccox® is safe for the consumer under the proposed conditions of use. No withdrawal period is required to ensure consumer safety. No maximum residue limits are considered necessary. The inhalation risk for users is considered negligible since inhalation toxicity and exposure are very low. Deccox® is not an irritant to skin and eyes and has no sensitisation potential. The ratios predicted environmental concentration/predicted no effect concentration (PEC/PNEC) for terrestrial, aquatic compartment and sediment are below 1, indicating that decoquinate used in chickens for fattening up to the highest proposed dose, does not pose a risk for these compartments; as well, no risk is expected neither for secondary poisoning nor for groundwater contamination. Due to insufficient evidence, the potential of decoquinate to prevent coccidiosis in chickens for fattening cannot be established.

Keywords: Deccox®, decoquinate, coccidiostats, chickens for fattening, safety, efficacy

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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 Deccox® (decoquinate) for chickens for fattening.

Deccox®, containing decoquinate as the active substance, is a feed additive applied for the prevention of coccidiosis in chickens for fattening at a dose range of 20–40 mg/kg complete feed.

Based on a tolerance study and supported by a published safety study, the FEEDAP Panel concludes that decoquinate from Deccox® is safe for chickens for fattening at the highest applied concentration in complete feed of 40 mg/kg. The margin of safety is between 5 and 10.

No practically relevant interactions with other additives or veterinary drugs exist except with bentonite. Decoquinate does not have antibacterial action.

Decoquinate is not genotoxic and is not carcinogenic. Deccox® is safe for the consumer under the proposed conditions of use. No withdrawal period is required to ensure consumer safety. No maximum residue levels are considered necessary. The inhalation risk for user is considered negligible since inhalation toxicity and exposure are very low. Deccox® is not an irritant to skin and eyes and has no sensitisation potential.

The ratios predicted environmental concentration/predicted no effect concentration (PEC/PNEC) for terrestrial, aquatic compartment and sediment are below 1, indicating that decoquinate used in chickens for fattening up to the highest proposed dose, does not pose a risk for these compartments; as well, no risk is expected neither for secondary poisoning nor for groundwater contamination.

Due to insufficient evidence, the potential of decoquinate to prevent coccidiosis in chickens for fattening cannot be established.

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1. Introduction

1.1. Background and Terms of Reference

Regulation (EC) No 1831/2003[1] establishes the rules governing the Community authorisation of additives for use in animal nutrition. In particular, Article 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 a time limit or pursuant to Directive 82/471/EEC.

The European Commission received a request from Pfizer Ltd.[2] for re-evaluation of the product Deccox® (decoquinate), when used as a feed additive for chickens for fattening (category: coccidiostats and histomonostats).

According to Article 7(1) of Regulation (EC) No 1831/2003, the Commission forwarded the application to the European Food Safety Authority (EFSA) as an application under Article 10(2) (re-evaluation of an authorised feed additive). EFSA received directly from the applicant the technical dossier in support of this application. The particulars and documents in support of the application were considered valid by EFSA as of 13 September 2013.

According to Article 8 of Regulation (EC) No 1831/2003, EFSA, after verifying the particulars and documents submitted by the applicant, shall undertake an assessment in order to determine whether the feed additive complies with the conditions laid down in Article 5. EFSA shall deliver an opinion on the safety for the target animals, consumer, user and the environment and on the efficacy of the product Deccox® (decoquinate), when used as a feed additive for chickens for fattening, when used under the proposed conditions of use (see Section 3.1.5).

1.2. Additional information

The product Deccox® containing 60 g/kg decoquinate as active substance is a feed additive intended to be used in chickens for fattening for the control of coccidiosis caused by *Eimeria* spp. Deccox® was authorised for use in chickens for fattening until July 2014 by Commission Regulation (EC) No 1289/2004.[3] Maximum residue limits (MRLs) and withdrawal time were introduced by Commission Implementing Regulation (EU) No 291/2014.[4]

EFSA issued an opinion on the safety and efficacy of Deccox® in accordance with Article 9G of Council Directive 70/524/EEC (EFSA, 2003).[5] In 2013, the EFSA FEEDAP Panel issued an opinion on the modification of the authorisation of Deccox® for chickens for fattening (EFSA FEEDAP Panel, 2013) concerning the reduction of the withdrawal time from 3 days to zero days and the introduction of MRLs in edible tissues of chickens for fattening. In 2014, the Panel issued an opinion on a new formulation (Avi-Deccox® 60G) of the additive (EFSA FEEDAP Panel, 2014).

2. Data and methodologies

2.1. Data

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

[1] Regulation (EC) No 1831/2003 of the European Parliament and of the Council of 22 September 2003 on additives for use in animal nutrition. OJ L 268, 18.10.2003, p. 29.
[2] On 27/09/2013 the applicant informed EFSA that the petitioner changed from Pfizer Ltd. to Zoetis Belgium SA, Rue Laid Burniat 1, 1348 Louvain-la-Neuve, Belgium.
[3] Commission Regulation (EC) No 1289/2004 of 14 July 2004 concerning the authorisation for 10 years of the additive Deccox© in feedingstuff, belonging to the group of coccidiostats and other medicinal substances, OJ L 243.15.7.2004, p. 15.
[4] Commission Implementing Regulation (EU) No 291/2014 of 21 March 2014 amending Regulation (EC) No 1289/2004 as regards the withdrawal time and maximum residue limits of the feed additive decoquinate. OJ L 87, 22.3.2014, p. 97.
[5] Council Directive 70/524/EEC of 23 November 1970 concerning additives in feedingstuff, OJ L 270/1, p. 840.
[6] FEED dossier reference: FAD-2013-0034.
[7] Commission Regulation (EC) No 429/2008 of 25 April 2008 on detailed rules for the implementation of Regulation (EC) No 1831/2003 of the European Parliament and of the Council as regards the preparation and the presentation of applications and the assessment and the authorisation of feed additives. OJ L 133, 22.5.2008, p. 1.

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

EFSA has verified the European Union Reference Laboratory (EURL) report as it relates to the methods used for the control of the active substance in animal feed and marker residue in tissues. The Executive Summary of the EURL report can be found in Annex A.[8]

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of Deccox® (decoquinate) is in line with the principles laid down in Regulation (EC) No 429/2008 and the relevant guidance documents: Guidance for the preparation of dossiers for coccidiostats and histomonostats (EFSA FEEDAP Panel, 2011a), Technical guidance: Tolerance and efficacy studies in target animals (EFSA FEEDAP Panel, 2011b), Technical Guidance for assessing the safety of feed additives for the environment (EFSA, 2008a), Guidance for the preparation of dossiers for the re-evaluation of certain additives already authorised under Directive 70/524/EEC (EFSA, 2008b), Guidance for establishing the safety of additives for the consumer (EFSA FEEDAP Panel, 2012a), Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012b) and Technical Guidance: Microbial Studies (EFSA, 2008c).

3. Assessment

The current opinion is aimed at assessing the safety and efficacy of the coccidiostat Deccox® containing decoquinate as an active principle when used as a feed additive in chickens for fattening.

3.1. Characterisation

3.1.1. Characterisation of the additive

Deccox®, containing decoquinate (ethyl-6-decyloxy-7-ethoxy-4-hydroxyquinoline-3-carboxylate) as an active substance, is intended to control coccidiosis caused by *Eimeria* spp. in chickens for fattening.

The additive is a blend of micronised decoquinate (60 g/kg) with colloidal silica (0.6 g/kg), soybean oil (28.5 g/kg) and wheat middlings as carrier up to 1,000 g.

The analysis of five batches of Deccox® indicated product consistency; mean decoquinate content was 62.5 g/kg (range: 62.2–62.7 g/kg).[9]

Data on impurities were provided for three batches of the additive.[10] Results showed concentrations of arsenic between < 0.020 and < 0.040 mg/kg, cadmium between 0.059 and 0.108 mg/kg, lead between 0.07 and 0.13 mg/kg and mercury below 0.005 mg/kg. Levels of aflatoxin B1 were < 1 μg/kg, aflatoxins B2, G1, G2 (one batch) < 1 μg/kg. Dioxins (polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/F)) ranged between 0.14 and < 0.17 ng WHO-PCDD/F-TEQ/kg, and the sum of dioxins and dioxin-like polychlorinated biphenyls (DL-PCBs) was between 0.28 and < 0.49 ng WHO-PCDD/F-DL-PCB-TEQ/kg. Non-DL-PCBs were < 10 μg/kg in all three batches. Data on microbial contamination was given for total aerobic count (49,000–220,000 cfu/g), for Enterobacteriaceae (130–24,000 cfu/g), for *Escherichia coli*, (< 10 cfu/g), for *Staphylococcus aureus* (< 10 cfu/g), for yeasts (< 10 cfu/g) and for moulds (100–1,200 cfu/g). *Salmonella* were absent in 25 g. None of these impurities are of concern. The Panel notes that the microbial contamination in some batches is high.

Deccox® is a buff-coloured coarse powder preparation with an average density of 1,352 kg/m^3 and a bulk density of 408 kg/m^3.[11] Particle size distribution measured in five batches by sieve analysis indicated that only a minor fraction of 0.1% (w/w) passed the 90 μm mesh.[9] The dusting potential (measured by Stauber–Heubach method in three batches) was 0.005 g/m^3.[12]

[8] The full report is available on the EURL website: https://ec.europa.eu/jrc/sites/jrcsh/files/FinRep-FAD-2013-0034-Deccox.doc_.pdf
[9] Technical dossier/Section II/Annex II.1.3.1.
[10] Technical dossier/Section II/Annex II.1.4.1.3.
[11] Technical dossier/Section II/Annex II.1.5.2.
[12] Technical dossier/Supplementary information April 2015/Annex 1.1.

3.1.2. Characterisation of the active substance

Decoquinate (ethyl-6-decyloxy-7-ethoxy-4-hydroxyquinoline-3-carboxylate, $C_{24}H_{35}NO_5$, molecular weight 417.6 g/mol, Chemical Abstracts Service No 18507-89-6), is a synthetic compound of the quinoline family. Its structural formula is given in Figure 1.

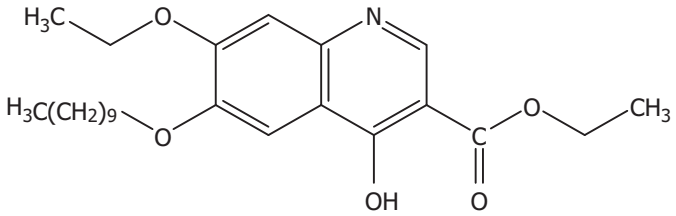

Figure 1: Structural formula of decoquinate

Decoquinate is a cream to buff-coloured, microcrystalline powder with slight odour with a melting point between 242 and 245°C.[13] The applicant refers to three decoquinate-related impurities originating from the chemical synthesis (tested routinely in the active substance). Total impurities are specified to be < 2%. Individual specifications are given with < 0.5% for the impurity 6-decyloxy-7-ethoxy-4-hydroxyquinoline-3-carboxylic acid, < 0.5% for diethyl-4-decyloxy-3-ethoxyanilinomethylenemalonate (anilino compound) and < 1% for methyl-6-decyloxy-7-ethoxy-4-hydroxyquinoline-3-carboxylate. The analytical data from five batches of the active substance confirmed compliance with the specifications: 98.7–99.6% decoquinate, 0.08–0.11% 6-decyloxy-7-ethoxy-4-hydroxyquinoline-3-carboxylic acid, < LOQ for the anilino compound and 0.14 to 0.23% for methyl-6-decyloxy-7-ethoxy-4-hydroxyquinoline-3-carboxylate.[14]

The residual solvents [redacted] were not detected in five batches.[14] [redacted]

3.1.3. Manufacturing process

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

3.1.4. Stability and homogeneity

3.1.4.1. Shelf-life of the additive

The stability of the additive (three batches) was investigated under long-term conditions (at 25°C/60% relative humidity (RH)) up to 36 months and under accelerated conditions (at 40°C/75% RH up to 6 months.[15] After 36 months at long-term conditions, decoquinate recovery rate was 107%, 100% and 100%, respectively, for the three batches. Recovery rates after 6 months at accelerated conditions were 105, 93 and 107%.

Another study was carried out on three other batches to confirm the stability data derived from the above study under long-term conditions up to 36 months and under accelerated conditions up to 12 months.[16] The content of decoquinate and related impurities (see Section 3.1.2) were measured. After 36 months at long-term conditions, recovery rates ranged between 95% and 98%. Recovery rates after 12 months under accelerated conditions were between 96% and 103%. No related impurities were detected.

[13] Technical dossier/Supplementary information April 2015/Annex 1.5
[14] Technical dossier/Section II/Annex II.1.4.1.2.
[15] Technical dossier/Section II/Annex II.4.1.1.1.
[16] Technical dossier/Section II/Annex II.4.1.1.2.

3.1.4.2. Stability of the additive in premixtures and feedingstuffs

The stability of decoquinate (one batch) was studied in a vitamin–mineral premixture (without choline chloride, intended concentration 4,000 mg decoquinate/kg premixture)[17] and in complete feed (maize, soybean, pea type; intended concentration 20 mg decoquinate/kg feed)[18] for chickens for fattening. Samples were stored under long-term conditions (25°C/60% RH) and under accelerated conditions (40°C/75% RH) (premixture only).

The analysed values for the premixture and the feed samples at 0 time point were below the intended concentrations (3,731 mg/kg premixture and 18 mg mg/kg feed). The recovery rates in premixture samples showed that decoquinate content decreased with time until 6 months under long-term conditions (87% at 6 months). Results obtained later were unexpectedly higher (up to 106% at 18 months). A similar trend was noted when premixture samples were stored under accelerated conditions: recovery rate was 62% after 1 month, followed by an increase which could not be interpreted as an increasing stability. The recovery rates in feed samples appeared to be stable under long-term conditions with values of 111%, 106% and 111% after 3, 6 and 12 weeks of storage.

3.1.4.3. Homogeneity

Samples from the premixture and the feed taken for stability studies were used to study the homogeneous distribution of decoquinate in the respective batch. Decoquinate was analysed in eight subsamples for the premixture and the complete feed (mash and pelleted). The coefficient of variation of the decoquinate concentration in the premixture was 2%, in the mash feed 5% and in the pelleted feed 7%.

3.1.5. Conditions of use

Deccox® is a feed additive for the prevention of coccidiosis in chickens for fattening at a dose range of 20–40 mg decoquinate/kg complete feed. The withdrawal period is zero day.

3.2. Safety

3.2.1. Safety for the target species

3.2.1.1. Tolerance study in chickens for fattening

A total of 240 one-day-old chickens (Ross) was randomly distributed to 4 groups (6 replicates of 5 birds per sex) and fed corn-soybean-type diets supplemented with 0, 40 (1x maximum proposed dose), 200 (5x) and 400 (10x) mg decoquinate/kg for 35 days.[19] A starter diet (crumbles, calculated contents: 21.7% crude protein (CP), 12.8 MJ of metabolisable energy (ME)/kg) was given until day 21, followed by a grower diet (pellets, calculated contents: 20% CP, 13.0 MJ ME/kg). The supplemented feed used in the tolerance study contained 39, 203 and 389 mg decoquinate/kg starter diet and 50, 280 and 596 mg decoquinate/kg grower feed (for 40, 200 and 400 mg intended decoquinate/kg complete feed, respectively).

Bird health, litter conditions and mortality were recorded daily. Body weight and feed consumption were measured on days 0, 21 and 35 on a pen basis. Zootechnical parameters (average daily weight gain, and feed to gain ratio) were calculated. On day 35 following final pen weights, blood samples were collected from one randomly selected bird per pen for haematology[20] and clinical blood chemistry.[21] The same bird per pen was subsequently necropsied and selected tissues were collected on day 35.

The pen was the experimental unit for statistical purposes. Statistical analyses using a general linear mixed model were conducted at the 0.10 level of significance using two-sided test.

[17] Technical dossier/Section II/Annex II.4.1.2.1 and 4.1.2.2.
[18] Technical dossier/Section II/Annex II.4.1.2.3 and 4.1.2.4.
[19] Technical dossier/Supplementary information April 2015/Annex 2_1.
[20] Red blood cell count (RBC), haemoglobin, haematocrit, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), red cell distribution width (RDW), thrombocyte check, eosinophils, heterophils, basophils, lymphocytes, monocytes.
[21] Aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), gamma-glutamyltranspeptidase (GGT), creatine kinase (CK), total protein (TP), total cholesterol, blood urea nitrogen (BUN), uric acid, glucose, calcium, phosphorus, magnesium, sodium, potassium, chloride.

No biologically relevant treatment effects were identified during the course of this study. There were only four mortalities (one in each group) and two culls (one at 40 and another at 400 mg decoquinate/kg). No statistically significant differences were identified in any of the performance parameters evaluated (means: 1,969 g body weight, 55 g average daily gain, 1.50 feed to gain ratio, and 84 g daily feed intake) between any of the dose levels tested or when compared to the control group.

Gross pathology of necropsied bird organs did not identify any abnormal findings. There were no statistically significant differences between treatment groups in absolute organ weight for the bursa of Fabricius, heart, kidneys, liver or spleen; and in organ weights relative to body weight for the heart, kidneys and liver. Owing to the absence of findings by macroscopy, no histology was performed.

The statistically significant differences identified in haematology (absolute eosinophils, absolute heterophils, mean corpuscular haemoglobin concentration and red cell distribution width), serum chemistry (gamma-glutamyl transferase and uric acid) and in organ weight relative to body weight (bursa of Fabricius and spleen) were considered to be not biologically relevant as they were identified in a non-dose dependent manner and/or were identified in only one sex.

The other haematology and the blood chemistry parameters were not affected by the treatment.

The results of this study indicate that about 400 mg decoquinate from Deccox®/kg feed – the tenfold of the highest use level – were tolerated by chickens for fattening for 35 days.

The literature review made by the applicant for the time period 2000–2014 (Appendix A) identified two publications in support of the safety of decoquinate for chickens for fattening.[22]

Zhou et al. (2009) investigated the safety of decoquinate as a liquid preparation (3 g decoquinate/100 mL) administered to chickens (28-day-old Yangzhou AA chicken for fattening per group; average body weight 119 g). The groups received water for drinking containing 0, 15, 45, 75 and 150 mg decoquinate/L for 21 days. The decoquinate use level in water for drinking of 15 mg/L can directly be compared with the applied highest use level in feed (40 mg/kg), the ratio of the two doses (2.67:1) is within the range (2–3:1) given by the EFSA FEEDAP Panel Statement on the use of feed additives authorised/applied for use in feed when supplied via water (EFSA FEEDAP Panel, 2010) for the ratio water to feed intake. Parameters studied were body weight, feed consumption and feed to gain ratio, haematology[23] and clinical blood chemistry.[24] At the end, chickens of the groups with 75 and 150 mg decoquinate/mL water for drinking were slaughtered for necropsy (visual inspection, histology of liver and kidney). The t-test was used for statistical examination of group differences.

Since feed consumption and feed composition were not reported and the study was conducted without replicates, the results and particularly the performance data can be considered with reservation only. Body weight gain and feed to gain ratio did not comply with data expected under European husbandry conditions. The groups with 15, 45 and 75 mg decoquinate/L water for drinking showed a significant improvement of body weight gain at 21-day body weight gain (average about 361 g) and feed to gain ratio (average about 2.53) compared to the groups with 0 and 150 mg (average about 262 g and 3.33, respectively). No significant differences were seen in haematology and clinical blood chemistry. At the end of the experiment, for some chickens (exact number not given) of the group with 150 mg decoquinate/L water for drinking, mild clinically adverse reactions were reported (mental depression, decreased feed intake and dishevelled feathers) and necrosis in renal tubular epithelium and focal inflammatory cell infiltration in the liver. The data indicate that the fivefold overdose of decoquinate is tolerated by chickens for fattening.

Although the study does not comply with appropriate reporting, has several weaknesses in design (lack of replicates, short duration) and cannot be considered to reflect European poultry management conditions, the data provide worthwhile evidence of potential adverse side effects found by the clinical examination and microscopical examination of liver and kidney, not performed in the tolerance study provided by the applicant.

A second study found by literature search (Lee et al., 2012) was not considered since decoquinate was not tested alone but in combination with certain drugs and the experimental criteria referred only to the immune status.

[22] Technical dossier/Supplementary information April 2015 Annex 5_I. Databases searched: Medline, Embase, Biosis, CAB Abstracts, Derwent Veterinary Drug File, Agricola, Pascal, Toxcenter, SciSearch, Chemical Abstracts, Dissertation Abstracts, ProQuest SciTech Collection; Period searched: until 2015.

[23] RBC, lymphocytes, heterophils, eosinophils, basophils, monocytes, haemoglobin, MCH and platelet count.

[24] Total protein, albumin, globulin, AST, ALT, LDH, ALP, glucose, BUN, creatinine, calcium, phosphorus, sodium, potassium and chloride.

3.2.1.2. Interactions

In its first assessment, the FEEDAP Panel (EFSA, 2003) summarised that decoquinate, since its introduction as a feed additive, has been extensively used in combination with a variety of other medicinal compounds and ingredients in animal feed with only one incompatibility with bentonite reported.[25] In the only documented study in which compatibility was considered, no incompatibility between decoquinate and the antibiotics chlortetracycline, oxytetracycline or spiramycin and furazolidone was found (Hodgson, 1968).

The applicant made a literature search for interactions of decoquinate with other additives and veterinary drugs (Appendix A).[26] Since no new findings were reported, the FEEDAP Panel maintains its former conclusion that no incompatibilities or interactions with feedingstuffs, carriers, other approved additives or medicinal drugs are expected with the exception of bentonite.

3.2.1.3. Microbial studies

The Committee for Medicinal Products for Veterinary Use (CVMP) of the European Medicines Agency (EMA) acknowledged in 2000 (EMEA CVMP, 2000) that decoquinate does not have antibacterial action. The FEEDAP Panel concluded in 2003 (EFSA, 2003) on a different database that decoquinate does not have marked antibacterial properties.

For the current assessment, the FEEDAP Panel re-assessed the studies available in 2003[27] and evaluated the outcome of the literature search[28] on the emergence of resistance to decoquinate and on the cross-resistance to antimicrobials performed by the applicant (Appendix A).[29] Since no new findings were reported, the FEEDAP Panel reiterates its former conclusion: 'Most of the strains of bacteria investigated occurring in the digestive tract of chickens appear resistant to the effects of decoquinate at concentrations > 64 mg/L a concentration substantially higher than that expected in the digestive tract of poultry. Consequently, effects on the bacterial flora of chickens are likely to be minimal. In the absence of demonstrable effects on the major species of gut bacteria it can be reasonably concluded that the use of decoquinate would not select for strains resistant to clinically important antibiotics or lead to the development of any form of cross-resistance'.

3.2.1.4. Conclusions on the safety for the target species

Based on a tolerance study and supported by a published safety study, the FEEDAP Panel concludes that decoquinate from Deccox® is safe for chicken for fattening at the highest applied concentration in complete feed of 40 mg/kg. The margin of safety is between 5 and 10.

No practically relevant interactions with other additives or veterinary drug exist except with bentonite.

Decoquinate does not have antibacterial action.

3.2.2. Safety for the consumer

3.2.2.1. Absorption, distribution, metabolism, excretion and residues

For the present assessment, the applicant makes reference to the same studies assessed in the former EFSA FEEDAP opinions (EFSA, 2003; EFSA FEEDAP Panel, 2013). An overview of the absorption, distribution, metabolism, excretion and residues of decoquinate firstly assessed by the FEEDAP Panel in 2003 (EFSA, 2003) and then updated in 2013 (EFSA FEEDAP Panel, 2013) is given below.

In its previous opinion on the use of Deccox® for chickens for fattening (EFSA, 2003), the FEEDAP Panel concluded that the limited studies made in chickens and laboratory animals effectively preclude any comparison of the metabolic profile of the chicken with those of the rat. However, the limited data available indicates that there are differences in the number and nature of the metabolites produced in the two species. As none of the metabolites have been identified or quantified (in either species), the

[25] The United States FDA had recorded the incompatibility with bentonite used as a binder in pelleting. The FEEDAP Panel notes that in a more recent FDA publication (e-CFR of March 23, 2017 § 558.195 Decoquinate as (d) Special considerations) it is indicated that 'Bentonite should not be used in decoquinate feeds' (FDA (Federal Register on 12/27/2016) uses this warning for decoquinate, robenidine and carbadox).
[26] Technical dossier/Supplementary information April 2015/Annex 5_III.
[27] Technical dossier/Supplementary information April 2015/3_2.
[28] Databases searched: PubMed; time span: up to 2014.
[29] Technical dossier/Supplemetary information April 2015/Annex 5_III.

risk for the consumer exposed to decoquinate residues in chicken tissues cannot be adequately assessed on the basis of the existing data.

For the assessment in 2013, the applicant submitted new metabolism/residue studies in the chicken and rat, carried out using more advanced analytical tools (radio-high-performance liquid chromatography (HPLC), liquid chromatography–mass spectrometry (LC–MS) and liquid chromatography–tandem mass spectrometry (LC–MS/MS)) than in the former studies (EFSA, 2003). The same studies are submitted for the current assessment.[30]

The FEEDAP Panel reiterates its previous conclusions:

i) Most of the radioactivity administered was recovered in the faeces (82–87%), urine being a minor excretion route (0.2–0.4%). Decoquinate was the major component in the faeces (51 and 60% of the dose excreted in male and female animals, respectively).
ii) Tissue metabolic profiles showed that decoquinate was the major component in liver, kidney, muscle, skin and fat (accounting for 19.6% of liver and kidney radioactivity and 23.9% of muscle, 31.2% of skin and 76.5% of fat total radioactivity). Six metabolites, three of which each accounted for more than 10% of total radioactivity, were isolated from the liver. One or more of these metabolites were isolated from the other tissues.
iii) In terms of comparative metabolism, it appeared that the absorbed fraction of decoquinate is metabolised more extensively in rat than in chicken, with the production of different metabolites.

3.2.2.2. Toxicological studies

For the present assessment, the applicant makes reference to the same studies assessed in the former EFSA FEEDAP opinions (EFSA, 2003 and EFSA FEEDAP Panel, 2013) and performed a literature search on the toxicology of decoquinate (Appendix A).

An overview of the toxicological profile of decoquinate firstly assessed by the FEEDAP Panel in 2003 (EFSA, 2003) and then updated in 2013 (EFSA FEEDAP Panel, 2013) is given below.

The mutagenicity studies presented for the assessment in 2003 were carried out in accordance with Good Laboratory Practice (GLP) or were published in peer-reviewed journals, and the results suggested that decoquinate is not genotoxic. A mouse lymphoma assay found that decoquinate caused gene mutation *in vitro* at the highest concentration tested in the presence of metabolic activation, but it was questionable whether it should be regarded as a positive result as most of the cells were killed at this concentration. Other *in vitro* mutagenicity tests (*Salmonella*/microsome reverse mutation assay, a bacterial rec assay and a cytogenetics test in mammalian cells) gave negative results.

An *in vivo* mammalian erythrocyte micronucleus test was submitted in for the assessment in 2013 and for the current assessment.[31] Rats were given two daily intraperitoneal doses of up to 800 mg decoquinate/kg body weight (bw) in accordance with OECD Guideline 474 and GLP. The study gave negative results for genotoxicity.

Acute oral toxicity was low in rats. The repeated-dose oral toxicity studies and developmental studies reported were done to standards appropriate to the time but some were not in accordance either with GLP or with previous and current OECD guidelines. However, the quality of the studies was considered sufficient for the assessment. Repeat-dose oral toxicity studies were performed in rats (several studies of duration 16 days to 2 years) and dogs (12-week and 2-year studies), with the lowest no observed effect level (NOEL) seen in these studies being 15 mg/kg bw per day for subdued behaviour in dogs given 62.5 mg/kg bw per day for 12 weeks. No adverse effects were seen in rats at doses of up to 37.7 mg/kg bw per day for up to 2 years. No carcinogenicity studies were available, but the two-year chronic toxicity study showed no effects on tumour incidences. A three-generation reproduction study in rats showed no adverse effects up to the highest dose tested (60.6 mg/kg bw per day). A rat developmental toxicity study showed fetotoxicity (retarded skeletal development) at 300 mg/kg bw per day, with a NOEL of 100 mg/kg bw per day, but showed no embryotoxicity or teratogenicity. A rabbit developmental toxicity study showed embryotoxicity at 100 mg/kg bw per day, with a NOEL of 60 mg/kg bw per day, but with no fetotoxic or teratogenic effects (EFSA, 2003).

No treatment-related adverse effects were seen in any of the studies at doses of 15 mg/kg bw per day or less and this dose was therefore adopted as the overall NOEL.

[30] Technical dossier/Section III/Annex_III_2_1_1_2 and 2_1_1_3.
[31] Technical dossier/Section III/Annex_III_2_2_2_1.

In the opinion in 2013, it was then concluded that: 'The ADI of decoquinate, already established by the FEEDAP Panel in 2003, is confirmed. The value is 0.075 mg/kg bw based on the above NOEL, applying an uncertainty factor of 200'.

The FEEDAP Panel noted that the literature search on the toxicology profile of decoquinate did not reveal any new data relevant to consumer safety and reiterates its previous conclusions.

3.2.2.3. Safety for the consumer

In 2013 the FEEDAP Panel noted that (EFSA FEEDAP Panel, 2013):

> 'On the basis of new metabolism/residue studies in chicken and rat, major metabolites appeared to be dissimilar in these two species. Nevertheless, the assessment of consumer safety refers to the ADI of decoquinate, considering the limited exposure of consumers to metabolites from chicken tissues. Decoquinate is the marker residue.
>
> A conservative consumer exposure estimate based on total residues measured two or six hours after withdrawal indicates that it would be about 2% of the ADI. Since a withdrawal period of two or six hours corresponds to a withdrawal time of zero days under practical husbandry conditions, a zero day withdrawal time does not compromise consumer safety.
>
> The residue data submitted confirm that no MRLs are considered necessary.'

In the absence of new data, the FEEDAP Panel confirms its previous conclusions on the safety of decoquinate for the consumer.

3.2.2.4. Conclusion on the safety for the consumer

Decoquinate is not genotoxic and is not carcinogenic. The lowest NOEL was 15 mg/kg bw per day, observed in a 12-week oral toxicity study in dogs. The acceptable daily intake (ADI) is 0.075 mg decoquinate/kg bw per day applying an uncertainty factor of 200 to the NOEL. No withdrawal period is required to ensure consumer safety. No MRLs are considered necessary.

3.2.3. Safety for the user

No new study has been submitted by the applicant.

In a former opinion on Deccox® (EFSA, 2003), two inhalation toxicity studies in rats were assessed: the studies were performed with the active substance (nose only) and did not show signs of irritation up to the highest concentration in air ($\geq$ 4 g/m^3) after a 4–6 h exposure.

Deccox® was considered not irritant to skin and eyes and has no sensitisation potential. Recent data confirmed very low dusting potential of Deccox® (0.005 g/m^3) (see Section 3.1.1).

The literature search provided by the applicant on the toxicology profile of decoquinate did not reveal any new data relevant to user safety (Appendix A).[32]

In line with its former assessment, the FEEDAP Panel concludes that exposure of users and workers from handling the additive would be very low and inhalation risk for the user is considered negligible. Deccox® is not irritant to skin and eyes and has no sensitisation potential.

3.2.4. Safety for the environment

The applicant made reference to the same studies assessed in a former EFSA FEEDAP opinion (EFSA, 2003) with the addition of new studies. The previous studies, the new studies and the outcome of a literature search performed by the applicant covering the period 2004–2014[33] were assessed by the FEEDAP Panel following Regulation (EC) No 429/2008[34] and the FEEDAP technical guidance for assessing the safety of feed additives for the environment (EFSA, 2008a).

[32] Technical dossier/Supplementary information April 2015/Annex 5_IV; Databases searched: Medline, Embase, Biosis, CAB Abstracts, Derwent Veterinary Drug File, Agricola, Pascal, Toxcenter, SciSearch, Chemical Abstracts, Dissertation Abstracts, ProQuest Sci tech Collection. Period searched: until 2015.

[33] Technical dossier/Supplementary information April 2015 Annex 5_V; Databases searched: Medline, Embase, Biosis, CAB Abstracts, Derwent Veterinary Drug File, Agricola, Pascal, Toxcenter, SciSearch, Chemical Abstracts, Dissertation Abstracts, ProQuest Sci tech Collection.

[34] OJ L 133, 22.5.2008, p.1

The active substance is not a physiological/natural substance of established safety for the environment. Consequently, according to Regulation (EC) No 429/2008[34] the Phase I assessment has to be continued to determine the predicted environmental concentration (PEC).

In Phase I and II initially, a total residues approach will be taken, meaning that the PECs will be calculated, based on the assumption that the additive is excreted 100% as a parent compound.

3.2.4.1. Phase I

Physico-chemical properties of decoquinate

The physico-chemical properties of decoquinate are summarised in Table 1. The low vapour pressure indicates that the substance will not volatilise to any great extent.

Table 1: Physico-chemical properties of decoquinate

Property	Value	Unit
Octanol/water partition coefficient (log K_{ow} 25°C)(1)	≥ 5.7 at pH 5, 7, 9	–
Water solubility (20°C)(2)	0.06	mg/L
Dissociation constant pKa(3)	–	–
Vapour pressure(4)	$3x10^{-9}$	Pa at 25°C

(1): Technical dossier/Section III/Annex III_4_1.
(2): EFSA, 2003.
(3): Technical dossier/Section III/Annex III_4_1.
(4): American Chemical Society, https://scifinder.cas.org.

Fate and behaviour

No data were provided on the fate of decoquinate in manure. No information is available on the hydrolysis or biodegradation of decoquinate in water.

Fate in soil

Adsorption

The adsorption of decoquinate to soil organic carbon (K_{oc}) was measured in a GLP-compliant study using [^{14}C]-decoquinate and three soil types.[35] However, preliminary solubility testing demonstrated that decoquinate was insufficiently soluble in 0.01 M aqueous calcium chloride and as such, the OECD 106 guideline for adsorption/desorption was not used. Instead, the K_{oc} determination was undertaken for decoquinate in accordance with the screening method OECD guideline 121, in compliance with GLP.[36] The log K_{oc} of decoquinate was estimated by comparing its retention time, using two different mobile phases, to that of a range of substances for which log K_{oc} values have been determined. Under both sets of conditions, the retention time for decoquinate was longer than that for the most strongly retained reference substance (DDT; log K_{oc} = 5.63) and as such, it was established that the log K_{oc} value for decoquinate was > 5.63 (K_{oc} = 426,580). OECD guideline 121 may provide unreliable K_{oc} values especially when log K_{oc} > 5.63; nevertheless, the study highlight the very high adsorption characteristics of the substance. Moreover, this very high adsorption characteristic is also highlighted in the soil degradation study (see below). A K_{oc} value of 400,000 L/kg is considered an appropriate reference value for exposure calculation.

Degradation

The degradation of [^{14}C]-decoquinate was investigated in two GLP-compliant studies. The older one,[37] from 2000, was performed to meet the regulatory requirements which were in force at the time (Commission Directive 2001/79/EC). The rate and route of degradation were evaluated in sandy loam soil treated with [^{14}C]-decoquinate at an application rate of 1.28 mg/kg soil (dry weight); the rate of degradation was determined in two further soil types (loamy sand and clay loam) at the same application rate. The samples were incubated in the dark at 20°C for up to 120 days, under aerobic conditions. The test system was maintained under negative pressure throughout the incubation period. At intervals throughout the incubation period, samples were removed and extracted and the extracts submitted to chromatographic analysis; non-extractable residues and volatile evolution were also

[35] Technical dossier/Section III/Annex III_4_1_Reference 17.
[36] Technical dossier/Section III/Annex III_4_1_Reference 7.
[37] Technical dossier/Section III/Annex III_4_1_Reference 16.

quantified. Quantitative mass balance was obtained from the route soil (95–109% recovery). It was evident from the outset that decoquinate, or its transformation products, adsorbed very strongly to each of the soils under investigation, as freely extractable radioactivity only accounted for up to 9% of that applied (ethanol was used as the extractant). By using a more vigorous extractant (1 M sodium hydroxide and refluxing), additional radioactivity was extracted from the samples, although this was shown to vary over the course of the 120-day incubation period (up to 79%). Chromatographic analysis of the radioactivity extracted from each soil sample indicated that decoquinate degraded to an unidentified component, which accounted for 35–40% of applied radioactivity at study termination and was more polar than the parent material. Two further minor unidentified components were also detected (up to 5% of applied radioactivity). In each soil type, low levels of $^{14}CO_2$ (up to 2% of applied radioactivity) were released over the course of the incubation period, indicating that decoquinate or its degradation products were not mineralised[38] to a significant extent. The DT_{50} values for decoquinate transformation were estimated to be 96, 116 and 140 days. DT_{90} values could not be reliably calculated, but it is evident that decoquinate is transformed in soil. However, as it cannot be demonstrated experimentally that the DT_{90} value of decoquinate in soil is less than 1 year, the potential for residues to accumulate in soil will be factored into the environmental risk assessment.

The aerobic degradation of [^{14}C]-decoquinate in loamy sand and clay loam soil was examined in a new GLP-compliant study[39] investigating the contribution and nature of the transformation products of [^{14}C]-decoquinate formed in soil (OECD guideline 307). The aim of the study was to relate the findings of the new study with those from the old study described above. [^{14}C]-Decoquinate was applied at a nominal concentration of 1.28 mg/kg dry weight soil to the same soil types as in the study above. The soils were incubated under aerobic conditions and maintained in the dark at about 50% maximum water holding capacity at 20 ± 2°C for up to 120 days (re-calculate at 12°C). For each soil, duplicate samples were taken for analysis at 0, 14, 28, 42, 56, 70, 91 and 120 days after treatment. At each sampling time, samples were extracted and analysed for decoquinate, degradation products and non-extractable residues. Any volatile radioactivity was continuously flushed from the vessels and collected in traps. A mass balance was determined for each sample.

The mean mass balance in loamy sand and clay loam was 97% and 100% of applied radioactivity, respectively. Radioactivity recovered in organic soil extracts declined slightly as the incubation progressed, but was much improved compared with that observed in the first study, particularly in the early sampling intervals. In loamy sand, 101% of applied radioactivity was extracted at day 0, decreasing to 78% at day 120 and in clay loam, 102% of applied radioactivity was extracted at day 0, decreasing to 65% at day 120. A concurrent increase in non-extractable residues was evident, accounting for means of 15% and 30% at day 120 in loamy sand and clay loam, respectively. Subsequent organic matter fractionation of these residues demonstrated that most of the radioactivity was associated with the humin fraction (up to 23%). Radioactivity evolved as $^{14}CO_2$ non-specific organic [^{14}C]-volatile compounds was negligible (< 0.4%), which demonstrates that decoquinate and its degradation products are not mineralised in soil.

Combined sample extracts were submitted for chromatographic analysis using HPLC. For both soils, [^{14}C]-decoquinate was the principal component detected at day 0, accounting for 100% of applied radioactivity in loamy sand and 102% in clay loam. [^{14}C]-Decoquinate levels declined as the incubation progressed, accounting for 52% and 53% at day 120 in loamy sand and clay loam, respectively. The degradation slowed down significantly during the experiments; however, the decline from day 56 to the end of the study was very much lower than the initial decline.

A number of minor unknown components (up to 12) were detected as the incubation progressed in each of the soils. With the exception of a single soil sample (day 70 loamy sand, replicate B), each of the unknown components represented less than 10% of applied radioactivity. Concentrations of the degradation products were too low to permit structural identification.

The results of the new study correlate well with the old study and show that decoquinate is partly transformed to a number of degradation products, which are not mineralised in soil. Considering both studies the DT_{50} of decoquinate transformation in soil ranges between 96 and 140 days.

Very hydrophobic substances like decoquinate might sorb so strongly to soil that the degradation is hampered. In this case, the highest DT_{50} is selected for further calculations since degradation is absent or extremely slow after more than 56 days of incubation.

[38] Degradation to CO_2, salts and water.
[39] Technical dossier/Supplementary information September 2016/Annex 2.

When the DT_{50} of 140 days is adjusted to an incubation temperature of 12°C using the Arrhenius equation,[40] the new DT_{50} is about 300 days, which will be used for further calculations.

Conclusion on fate and behaviour

A K_{oc} of 400,000 is used for the further risk assessment.

Decoquinate is not mineralised in soil. A DT_{50} for transformation of decoquinate of 300 days is used for further calculations.

Predicted environmental concentrations

The methodology for the calculation of the maximum PEC soil, groundwater and surface water are described in the technical guidance for assessing the safety of feed additives for the environment (EFSA, 2008a). For a highly persistent compound like decoquinate, a refined PEC in soil should be used according to the guidance, giving a $PEC_{plateau}$ in soil, groundwater, surface water and sediment (Table 2).

Table 2: Initial plateau predicted environmental concentrations of decoquinate in soil (μg/kg), groundwater, surface water (μg/L) and sediment (μg/kg dry weight)

Input	Value
Dose (mg/kg decoquinate in feed)	40
Molecular weight (decoquinate)	417.53
VP (Pa)	3.3E-9
Solubility (mg/L)	0.06
K_{oc} (L/kg)	400,000
DT_{50} at 12°C (days)	300
Output	
PEC_{soil}	365
$PEC_{groundwater}$	0.052
$PEC_{surfacewater}$	0.017
$PEC_{sediment}$	345

The Phase I PEC trigger value for soil is exceeded. Therefore, a Phase II assessment is considered necessary. Predicted concentration in groundwater is below the trigger value of 0.1 μg/L. No further refinement is necessary for this compartment.

3.2.4.2. Phase II

Effect assessment – Ecotoxicity studies

Toxicity to terrestrial compartment

Effects on plants

The effect of decoquinate on terrestrial plants was investigated in a GLP-compliant study;[41] the study followed the OECD guideline 208 which was in force at the time the study was conducted. The FEEDAP Panel noted that although there are some minor experimental differences compared with the current OECD guideline design, the results from the study are adequate to assess any impact which decoquinate might have on the emergence and growth of terrestrial plants. The study was conducted using a sandy loam soil and three plant species: wheat, mustard and mung beans. The LC_{50} for emergence and the EC_{50} for growth were higher than the highest concentration tested (100 mg/kg dry weight soil) for each of the species evaluated.

Effect on earthworms

The acute toxicity of decoquinate to *Eisenia foetida andrei* was tested at a limit nominal rate of 1,000 mg/kg soil in a 14 days study at 20°C.[42] An artificial soil was used composed of 70% sand, 20%

[40] The temperature correction was performed according to the scientific opinion of the Panel on Plant Protection Products and their Residues on a request from EFSA related to the default Q10 value used to describe the temperature effect on transformation rates of pesticides in soil (EFSA, 2007).
[41] Technical dossier/Section III/Annex III_4_1_Reference 19.
[42] Technical dossier/Section III/Annex III_4_1_Reference 20.

kaolinite clay and 10% sphagnum moss peat. No mortality or abnormal behaviour was observed. Though, the weight was significantly reduced by 12%, indicating that decoquinate can have an effect on the weight loss at the concentrations of 1,000 mg/kg.

The effect of decoquinate on the reproductive performance of earthworms was investigated in a 56 day GLP-compliant study,[43] based on a draft OECD guidance which was available at the time the study was conducted.[44] The FEEDAP Panel noted that some minor differences exist between this study and the current OECD guideline 222 reproduction study test design, but these are not considered to impact on the results obtained. Earthworms were exposed to decoquinate in artificial soil over a 28-day exposure period, at nominal concentrations equivalent to 62.5, 125, 250, 500 and 1,000 mg/kg dry soil. The nominal concentrations were not verified by chemical analysis. Adult earthworms were removed from test soil and assessed for mortality, weight change and sublethal effects after 28 days of exposure. Soil was replaced in the test container and juveniles were allowed to grow for a further 28 days, after which time they were removed from the soil, counted and sub-lethal effects assessed. No significant adult mortality was noted during the test, nor effects on adult body weight. The FEEDAP Panel noted that during the test, clean manure was added to feed the worms. This is not representable for the situation in the field, where coccidiostats are added via the manure. Reproduction was reduced at 250 mg/kg, but no such effect was evident in the higher decoquinate treatments (500 and 1,000 mg/kg). The no observed effect concentration (NOEC) for reproduction is 1,000 mg/kg.

Effects on soil microorganisms

The effect of decoquinate on soil nitrogen transformations was investigated in a GLP-compliant study (OECD guideline 216).[45] The possible effects of decoquinate on soil microflora under aerobic conditions were studied upon the short-term respiration and nitrogen transformation. The test substance was incorporated into the sandy loam soil at concentrations of 1.839 and 9.195 mg/kg of decoquinate.[46] At the day 28 after the treatment, concentrations of nitrate in decoquinate-treated soil at the concentrations tested deviated from the control by less than 25%. No prolonged effects could be expected when using decoquinate as additive for chickens for fattening.

Toxicity to aquatic organisms

Effects on algae

The effect of decoquinate to the algal species *Raphidocelis subcapitata* (former names: *Selenastrum capricornutum, Pseudokirchneriella subcapitata*) was determined at a limit nominal concentration of 100 mg/L following the OECD guidelines 201.[47] At the beginning of the test (after filtration), the solubility of the decoquinate was analysed in six parallel samples resulting the highest concentration of 0.234 mg/L, which is far above the reported water solubility. After 72 h, the concentration of the tested substance was below limit of quantification (LOQ) of 0.039 mg/L. Undissolved material was present in the test system. After 72 h, no significant effect on growth rate could be estimated leading to the conclusion that the NOEC is equal to the highest obtained soluble concentration of decoquinate in the test (0.234 mg/L). However, the applicant claims, that the solubility of the decoquinate in the test was overestimated. The recalculation of solubility of tested substance was provided as a time-weighted mean resulting in highest solubility concentration of 0.073 mg/L and the NOEC value of 0.073 mg/L (73 μg/L).[48]

Effects on crustaceans

The acute toxicity of decoquinate to *Daphnia magna*, under semi-static conditions, was investigated following the OECD guideline 202.[49] As decoquinate was shown not to be toxic in the range finding phase of the study, the definitive phase was conducted as a limit test. Due to the very limited aqueous solubility of decoquinate, only very low concentrations of decoquinate could be assessed for toxicity. A saturated solution was prepared by ultra-sonication of excess decoquinate in daphnid medium

[43] Technical dossier/Section III/Annex III_4_1_Reference 21.
[44] OECD Draft Guideline Earthworm Reproduction Test (2000), BBA Guideline Part VI 2-2 (1994) and ISO Guideline 11268-2 (1998) Part 2.
[45] Technical dossier/Section III/Annex III_4_1_Reference 18.
[46] The values taken for the studies were considered as PEC and 5*PEC by the applicant at the time when conducting the study.
[47] Technical dossier/Section III/Annex III_4_1_Reference 24.
[48] Technical dossier/Section III/Annex III_4_1_Reference 23.
[49] Technical dossier/Section III/Annex III_4_1_Reference 22.

(nominally 100 mg/L), prior to the addition of the test organisms. Daphnids were exposed over a 48 h exposure period, under semi-static conditions (24 h replacements). The aqueous soluble fraction (prepared following filtration) was determined at intervals during the study by HPLC. However, the measured concentration of decoquinate was noted to decline between medium changes and as such, the EC_{50} and NOEC values were calculated in terms of maximum achievable solubility. These results were subsequently recalculated in terms of time-weighted means[48] and these values have been used for risk assessment purposes. Under the conditions of the study, the EC_{50} value for decoquinate was reported as > 0.034 mg/L, based on the time-weighted mean.

The effect of [^{14}C]-decoquinate on the reproductive capacity of *D. magna* was investigated following the OECD guideline 211 in a GLP-compliant study.[50] The study was conducted over a 21-day exposure period, under semi-static conditions with test solutions renewed three times per week. A range finding test indicated that the exposure concentrations were nominally 6, 10, 17, 29 and 50 μg/L of decoquinate, in addition to untreated and solvent control groups. No precipitation of [^{14}C]-decoquinate was observed in any of the test vessels during the study. Analysis of test samples from the definitive test in freshly prepared solutions and on days 3, 5, 7, 10, 12, 14, 17, 19 and 21 in expired solutions indicated that concentrations of [^{14}C]-decoquinate were not maintained within ±20% of the initial measured values. The corresponding geometric mean measured concentrations of [^{14}C]-decoquinate were 4.33, 7.57, 14.47, 22.84 and 35.25 μg/L. As such, the results of the reproduction test were based on the geometric mean measured concentration of [^{14}C]-decoquinate.

During the reproduction test, one (10%) immobile adult *D. magna* was recorded in the 4.33 μg/L treatment group. Offspring from the adult which died during the test were omitted from the calculation of total number of offspring and mean number of offspring per test animal. First offspring were observed in treatment groups, in the control, and in the solvent control on the day 8 and on the day 9 of the test.

During the reproduction test, test solution quality parameters were examined and found within the required ranges and validity criteria for a valid test were also met.

The lack of significant adult immobilisation or reduction in offspring production indicated that exposure to [^{14}C]-decoquinate over a 21-day period had no effect on *D. magna* survival and reproduction at any of the tested concentrations. The 21-day EC_{50} for [^{14}C]-decoquinate for reproduction in *D. magna* was therefore concluded to be > 35.25 μg/L and the 21-day NOEC was determined as 35.25 μg/L, based on geometric mean measured concentrations.

Effects on fish

The acute toxicity of decoquinate to rainbow trout *Oncorhynchus mykiss* was determined under semi-static conditions at a nominal concentration of 100 mg/L decoquinate according to OECD guideline 203.[51] Test solutions were ultrasonicated for 10 minutes to achieve the maximum soluble concentration. Test solutions were replaced after 24 h. The test solutions appeared opaque and off-white colour with small quantities at the surface. Excess undissolved material was retained in the test system. At the start (after filtration), a concentration was measured of 0.040 mg/L decoquinate. After 24 h, the concentration was below the limit of detection (LOD) (0.008 mg/L). After 96 h, no mortality was observed. The EC_{50} was ≥ 16 μg/L (time weighted average).[48]

Effect on sediment dwelling organisms

The toxicity of decoquinate to chironomids was investigated in a GLP-compliant study, using sediment which had been fortified with [^{14}C]-decoquinate.[52] The test design was based on draft guidance which was available at the time study was initiated.[53] The FEEDAP Panel noted that some minor differences exist between this study and the current OECD guideline 233 on spiked sediment toxicity test design, but these are not considered to have an impact on the results obtained from this study.

A preliminary range finding phase was conducted with non-radiolabelled decoquinate and no effects were evident at the highest sediment concentration which was tested (1,000 mg/kg dry weight). The applicant noted that, although conventionally, 1,000 mg/kg would have been selected as the exposure concentration for the definitive limit test, nominal concentration equivalent to 10 times the preliminary

[50] Technical dossier/Supplementary information September 2016/Annex 1.
[51] Technical dossier/Section III/Annex III_4_1_Reference 25.
[52] Technical dossier/Section III/Annex III_4_1_Reference 26.
[53] OECD Draft Guideline: Chironomid Toxicity Test using Spiked Sediment (2002) and BBA Final Draft Guideline: Effect of Plant Protection Products on the Development of Sediment Dwelling Larvae of Chironomus riparius in a Water Sediment System (1995).

PEC (18 mg/kg) would provide the required LOD for aqueous samples, while also providing adequate data on the toxicity of decoquinate to chironomids.

Chironomus riparius larvae were exposed to [^{14}C]-decoquinate, adsorbed to quartz sand as a carrier, at an actual concentration of 16.3 mg/kg sediment (dry weight basis), over a 28-day period, under static conditions. The number and sex of emerged adult chironomids were recorded daily during the exposure period. The distribution of radioactivity in the test system at day 0, 7 and 28 was determined by measuring levels of radioactivity in the sediment, interstitial and overlying water. Mass balance was demonstrated to be quantitative.

No significant differences were observed between the emergence ratio and development rate of the solvent control (chloroform) and decoquinate treatment. Statistically, 16.3 mg/kg was confirmed as the NOEC; however, the results of the range finding phase indicated that the NOEC was 1,000 mg/kg. For the risk assessment, the NOEC for decoquinate has been conservatively assigned as 16.3 mg/kg (dry weight basis), thus providing a considerable margin of safety.

Conclusions on the ecotoxic effect on soil, water and sediment

The applicant submitted studies which followed OECD guidelines (or other appropriate standardised methods) as proposed in the technical guidance for assessing the safety of feed additives for the environment (EFSA, 2008a). Tests, with the exception of test on earthworm reproduction, are valid and the test results can be accepted and used for determination of predicted no effect concentrations (PNECs) and to establish the safe values for exposed environmental compartments.

For the terrestrial compartment, data are available for microorganisms, earthworms and plants. Risk for terrestrial compartment was evaluated based on the acute effect in earthworm at the limit test of 1,000 mg/kg soil applying an assessment factor (AF) of 1,000 (Table 3). For the aquatic compartment, data are available for algae, aquatic invertebrates and fish. The lowest toxicity value for the aquatic compartment was found in a study on effect on reproduction of daphnids. A safety factor of 50 was used to derive the PNEC for aquatic organism (Table 4).

Ecotoxicological data for sediment-dwelling invertebrates are provided for the sediment compartment. The calculated PNEC for the risk assessment is 1,630 μg/kg decoquinate, applying an AF of 10 to the NOEC of 16.3 mg/kg (Table 5).

Risk characterisation (PEC/PNEC ratio)

The risk characterisation ratios for terrestrial, freshwater and sediment compartments are reported in Tables 3, 4 and 5, respectively.

Table 3: Risk characterisation (PEC/PNEC ratio) of decoquinate for terrestrial compartment

Taxa	PEC_{soil} (μg/kg)	EC_{50}/LC_{50}/NOEC (mg/kg)	AF	PNEC (μg/kg)	PEC/PNEC
Earthworm	365	1,000(1)	1,000	1,000	0.4
Plants		> 100(2)	100	> 1,000	0.4

AF: assessment factor.
(1): NOEC.
(2): EC_{50} and LC_{50}.

Table 4: Risk characterisation (PEC/PNEC ratio) of decoquinate for freshwater compartment

Taxa	$PEC_{surfacewater}$ (μg/L)	EC_{50}/LC_{50}/NOEC (μg/L)	AF	PNEC (μg/L)	PEC/PNEC
Algae *Raphidocelis subcapitata*	0.017	73(1)	50	1.5	0.01
Aquatic invertebrates *Daphnia magna*		35(1)	50	0.7	0.02
Fish *Brachydanio rerio*		≥ 16(2)	50	> 0.16	0.11

AF: assessment factor.
(1): NOEC.
(2): EC_{50} and LC_{50}.

Table 5: Risk characterisation (PEC/PNEC ratio) of decoquinate for sediment

Taxa	$PEC_{sediment}$ (µg/kg)	NOEC (mg/kg)	AF	PNEC (µg/kg)	PEC/PNEC
Sediment-dwelling invertebrates *Chironomus riparius*	342	16.3	10	1,630	0.21

AF: assessment factor.

Bioaccumulation of decoquinate

A GLP-compliant study based on the OECD guideline 317 was conducted on [^{14}C]-decoquinate and a non-radiolabelled decoquinate spiked onto the artificial soil.[54] Earthworms were exposed to the test substance at mean measured concentration of 10.24 mg/kg dry soil during the 21 days of uptake period. The uptake was followed by the 21 days of elimination phase. Coefficient of variation of measured concentrations of decoquinate in the series of samples did not exceed 15% at any sampling day. Earthworms and soil samples from the test trials were regularly sampled throughout the course of the study and analysed for the content of decoquinate, the mortality, weight change and behaviour. The concentration of decoquinate (or its equivalents) was constant during the uptake phase, ranging from 9.7 to 10.8 mg/kg soil (dry weight). Limited uptake of decoquinate (or its equivalents) was evident in earthworms during the uptake phase and concentrations ranged from 0.20 to 0.50 µg equivalents/g tissue wet weight basis. The calculated bioaccumulation factor (BAF) was the highest at day 2 (BAF of 0.27 calculated on wet weight basis); after day 14, the BAF was lowered to 0.11–0.15 wet weight. The plateau was reached in a few hours after the beginning of exposure. During the elimination phase, concentrations of decoquinate equivalents quickly declined and within 5 h (first sampling interval in the elimination phase) were below the LOD (0.05 µg equivalents/g tissue wet weight basis). At the end of the 21-day exposure period, a BAF of about 0.03 (wet weight) was established.

3.2.4.3. Conclusions on safety for the environment

The PEC/PNEC ratios for terrestrial, aquatic compartment and sediment are below 1, indicating that decoquinate used in chickens for fattening up to the highest proposed dose, does not pose a risk for these compartments; as well, no risk is expected neither for secondary poisoning nor for groundwater contamination.

The literature search provided by the applicant on decoquinate did not reveal any new data relevant to the safety for the environment.

3.3. Efficacy

For coccidiostats under re-evaluation, efficacy data should derive from two types of target animal experiments: a) natural/artificial infection to simulate use conditions (e.g., floor pen studies with poultry), at least one of the locations should be in the EU, b) actual use conditions in field trials, all should be done in the EU within the last 5 years. Anticoccidial sensitivity tests (AST) could replace field trials provided they meet the criteria mentioned in the relevant guidance document on coccidiostats and histomonostats (EFSA FEEDAP Panel, 2011a).[55]

3.3.1. Floor pen studies

Three floor pen studies in chickens for fattening were submitted.[56] Birds were penned and distributed into treatment groups as indicated in Table 6.[57] Treated groups received feed containing 20 mg decoquinate/kg feed; dosage was analytically confirmed. Infected groups were inoculated with

[54] Technical dossier/Section III/Annex III_4_2_5_1.

[55] The FEEDAP Panel stated in its guidance for the preparation of dossiers for coccidiostats and histomonostats (EFSA FEEDAP Panel, 2011a) that studies with artificial infection would be preferred over field trials due to their inherent weaknesses. These short term studies should use field strains of *Eimeria*, recently confirmed as pathogenic/resistant by a sensitivity test or recognised problems in the poultry operation (confirmed by veterinary certificate). The *Eimeria* field strains should ideally undergo one, but in any case not more than two passage(s) before use in such trials.

[56] Trial 1: Technical dossier/Section IV/Annex IV.2.4. Trial 2: Technical dossier/Section IV/Annex IV.2.5. and Trial 3: Technical dossier/Section IV/Annex IV.2.6.

[57] Trial 1: male and female chickens, strain not indicated Trial 2: male chickens Ross 308 Trial 3: male chickens Cobb 500.

recent field isolates of pathogenic *Eimeria* species (see Table 7 for details). Animal health and mortality were monitored daily. Feed intake and body weight of the animals were measured throughout the study, feed to gain ratio was calculated. Samples of excreta were analysed for oocyst excretion and intestinal lesions were scored on five animals per pen following the method of Johnson and Reid (1970) (0 = no lesion, 1 = very mild, 2 = mild, 3 = moderate and 4 = severe).

In trial 1, mortality data was statistically analysed by chi-square test, lesion scores by Mann–Whitney U-test and all other parameters by analysis of variance (ANOVA). In trials 2 and 3, data were statistically analysed using a general linear mixed model. Pair-wise treatment comparisons were made if the general analysis indicated significance. In all trials, the hypothesis tests were conducted at the 0.05 level of significance using two-sided tests.

Table 6: Experimental design of floor pen studies performed with Deccox®

Trial	Study Duration (days)	Replicates per treatment (birds per replicate)	Test animal	Treatment groups	Analysed decoquinate (mg/kg feed)(2)
1	35(1)	8 (30)	Male and female chickens Strain not indicated	Infected untreated control group (IUC)	–
				Infected treated group (IT)	17.9
2	35	12 (30)	Male chickens Ross 308	Uninfected untreated control group (UUC)	–
				Infected untreated control group (IUC)	–
				Uninfected treated group (UT)	16.3/18.8
				Infected treated group (IT)	16.3/18.8
3	35	12 (30)	Male chickens Cobb 500	Uninfected untreated control group (UUC)	–
				Infected untreated control group (IUC)	–
				Uninfected treated group (UT)	20/23.6/23.7
				Infected treated group (IT)	20/23.6/23.7

(1): Starting with 8-day-old chicken.
(2): In trial 1, starter feed was administered throughout the study. In trial 2, birds received starter diet from day 0 to 21, grower diet from day 21 to 35. In trial 3, birds received starter diet from day 0 to 14, grower diet from day 14 to 28 and finisher diet from 28 to 35.

Table 7: Summary of inoculation in floor pen studies performed with Deccox®

Trial	Start Date	Inoculum characteristics			
		Month/Year and country of isolation	Intended dose per bird (oocysts)		Day and mode of inoculation
1	6/2011	3/2010 Spain	1.2×10^5	*E. acervulina*	Day 15 via gavage
			2.5×10^4	*E. praecox*	
			1.3×10^4	*E. tenella*	
			1.2×10^4	*E. maxima*	
2	6/2012	9/2010 The Netherlands	5.3×10^4	*E. acervulina*	Day 14 via gavage
			1.1×10^4	*E. tenella*	
			1.1×10^4	*E. maxima*	
			2.6×10^3	*E. mitis*	
			4.0×10^2	*E. necatrix/E. praecox*	
3	6/2012	4/2012 Germany	1.5×10^3	*E. acervulina*	Day 14 via gavage
			5.0×10^2	*E. maxima*	
			8.0×10^3	*E. tenella*	

Coccidiosis-related endpoints in floor pen trials are summarised in Tables 8, 9, 10 and 11; performance data and mortality are summarised in Table 12.

Table 8: Total number of *Eimeria* oocysts per gram of excreta (OPG) in floor pen studies

Trial 1	Day 22	Day 24	Day 29	Day 36	Day 43		
IUC	2,500,000	300,000	36,000	8,100	6,000		
IT	89,000*	640,000	85,000	5,900	6,600		
Trial 2	Day 17	Day 21	Day 23	Day 28	Day 30	Day 32	Day 35
UUC	0	6	1	261	130	114	688
IUC	2	115,410	17,113	9,791	27,734	11,851	10,492
UT	1	6	9	1,771	255	120	69
IT	0	129,093	7,502	2,664	3,231*	6,708	631*
Trial 3	Day 17	Day 21	Day 23	Day 28	Day 30	Day 32	Day 35
UUC	0	0	0	0	0	0	0
IUC	0	60,975	64,985	1,490	794	213	37
UT	0	0	0	0	0	1	0
IT	0	174,943	65,321	466	230	230	87

IT group mean with * is significantly different from IUC group mean ($p \leq 0.05$).

Table 9: Mean lesion scores in floor pen trial 1

Day	***E. acervulina***			***E. tenella***			***E. maxima***		
	22	**29**	**43**	**22**	**29**	**43**	**22**	**29**	**43**
IUC	2.03	0.20	0.10	3.65	0.15	0.05	0.63	0.23	0
IT	0.60*	1.05*	0	1.55*	0.33	0.03	0.40	0.08	0

IT group mean with * is significantly different from IUC group mean ($p \leq 0.05$).

Table 10: Mean lesion scores in floor pen trial 2

Day	**Duodenum and upper small intestine**		**Lower small intestine**		**Caecum**	
	21	**28**	**21**	**28**	**21**	**28**
UUC	2.4	2.9	1.7	1.9	1.6	1.8
IUC	2.6	3.1	1.8	1.9	1.9	2.2
UTC	2.4	3.0	1.6	1.9	1.6	1.7
IT	2.7	3.0	1.7	1.9	1.6	1.7

No statistical analysis was performed on the mean values.

Table 11: Mean lesion scores in floor pen trial 3

Day	**Duodenum and upper small intestine**		**Lower small intestine**		**Caecum**	
	21	**28**	**21**	**28**	**21**	**28**
UUC	0	0	0	0	0	0
IUC	0.5	1.3	0	0.7	0	0.3
UTC	0	0	0	0	0	0
IT	1.4	0.4	0.1	0.2	0.2	0

No statistical analysis was performed on the mean values.

Table 12: Performance data and mortality of chickens in floor pen trials with Deccox®

	Feed Intake(1) (g)	Body weight (g)	Weight Gain(2) (g)	Feed to gain ratio(3)	Mortality n (%)
Trial 1					
IUC	39,550	998	887	2.0	17 (7.1)
IT	45,800*	1,074*	963*	2.0	1 (0.4)*
Trial 2					
UUC	92.5	2.267	64	1.44	14 (3.9)
IUC	91.6	2.228	63	1.47	17 (4.7)
UTC	92.1	2.274	64	1.43	17 (4.7)
IT	90.3	2.225	62	1.46	14 (3.9)
Trial 3					
UUC	98.6	2.204	62	1.60	32 (8.9)
IUC	95.7	2.059	58	1.66	52 (14.4)
UTC	97.9	2.225	62	1.58	42 (11.7)
IT	91.5*	2.014	56	1.64	58 (16.1)

IT group mean with * is significantly different from IUC group mean ($p \leq 0.05$).
(1): Results of trials 2 and 3 refer to daily feed intake, results of trial 1 refer to total feed intake (day 8–43) per pen since daily feed intake was not reported.
(2): Results of trials 2 and 3 refer to daily gain, results of trial 1 refer to total body weight gain (day 8–43) per bird since daily weight gain was not reported.
(3): In trial 1, no information was provided on how the feed to gain ratio was calculated.

In trial 1, decoquinate at 20 mg/kg feed reduced significantly coccidiosis-related mortality. Highest OPG (oocysts per gram of excreta) values were found 7 days after inoculation; decoquinate reduced significantly OPG compared to the untreated group (Table 8). No significant differences were found at the following examination days. No *E. maxima* oocysts were found in the excreta of the treated group 7 and 9 days after inoculation compared to the untreated group (1.6×10^4 and 2.5×10^3, respectively). The *E. maxima* oocyst excretion remained at a lower level, although not significant, for the rest of the observation period.

Lesions were separately recorded for three *Eimeria* species of the inoculum. Mean lesion scores for *E. acervulina* and *E. tenella* species were significantly lower 7 days after inoculation in the treated group. *E. acervulina* lesion score 14 days after inoculation was significantly higher in the treated group. There was a tendency for lower lesion scores 28 days after inoculation in the treated group (Table 9).

Cumulative feed intake and final body weight were significantly higher in IT group than in IUC group. Feed to gain ratios were not significantly influenced by the treatment. Body weight gains were significantly higher in IT group than in IUC group (Table 12).

In trial 2, mortality/culls did not differ significantly among groups (overall mean 4.3%). Higher OPG values from day 7 after inoculation until the end of the study were characteristic for the infected groups. The OPG values reached a peak 7 days after inoculation with no differences between IUC and IT groups. All later observations found OPG values at a level of about 10–25% of the peak OPG. Significantly reduced OPGs by the treatment were found 16 and 21 days after inoculation (Table 8).

The presence of blood in faeces was first observed in 8 pens of the IUC group 14 days after inoculation followed by 7 and 9 pen observations 1 and 2 days later, respectively. In contrast to that, bloody faeces were found in the IT group only in 4 and 1 pens 14 and 16 days after inoculation, respectively.

Lesions were recorded 7 and 14 days after inoculation for the duodenum and upper small intestine, lower small intestine and caecum. The mean values were at the same level for all four groups (Table 10). Considering the frequency of the single lesion scores, the only significant difference in favour of the treatment was found for lesion score 3 in the caecum on both observation days.

The 35-day body weight and feed to gain ratio of all groups were nearly the same (overall mean 2,249 g and 1.45) without differences between the IUC and IT groups (Table 12).

Trial 3 showed high mortality already in the uninfected groups (mean 10.3%). Infection resulted in an increase of mortality (15.3%) without a positive effect of the treatment. No significant differences

in the OPG values were found on any of the observation days between the untreated and the treated groups and within the uninfected and the infected groups, respectively (Table 8).

No lesions were found for the uninfected groups 7 and 14 days after inoculation (Table 11). Mean lesion scores of the IT group showed lower values compared to the IUC group only 14 days after inoculation. Although not significant, the frequency of lesion scores in the IUC group decreased for score 0 from day 7 to 14 after inoculation and increased for the scores 1, 2 and 3. The IT group showed an inverse picture, increase in score 0 observations and a decrease in scores 1, 2 and 3.

The final body weight of the uninfected groups and that of the infected groups were 2,215 g and 2,036 g respectively. Feed to gain ratio showed relations similar to those seen for body weight (Table 12).

3.3.2. Anticoccidial sensitivity tests

Three ASTs were submitted.[58] The three tests followed a similar design. Each test was made with the groups UUC, IUC and IT the latter receiving feed supplemented with 30 mg decoquinate/kg feed from Deccox® (analysed values 29, 31 and 30 mg decoquinate/kg feed in ASTs 1, 2 and 3, respectively). Other anticoccidial additives were tested (five in ASTs 1 and 3, six in AST 2) and the statistical analysis referred to all groups. The day-old birds (Ross PM3) were randomly allocated to the groups; group size was 18 chickens (3 replicates with 6 birds).

Supplemented feed was administered from day 13. Birds were artificially infected on day 15 with sporulated oocysts from field isolates.[59] The tests lasted 22 days in total. Endpoints were: body weight, body weight gain, feed consumption, feed to gain ratio and at the end of the study OPG and intestinal lesions caused by *E. acervulina* (and *E. maxima* in AST 3 and *E. tenella* in ASTs 1 and 3). The lesion score scale ranging from 0 to 4 was applied (Johnson and Reid, 1970).

Statistical analysis was performed by one-way ANOVA, group differences were tested by Newman–Keuls procedure. To approach normal distribution mortality data were transformed by square root and OPG by natural logarithm. Results are summarised in Table 13.

Table 13: Summary of anticoccidial sensitivity tests performed with Deccox®

AST	Tr. group	Feed Intake (g/day)	Body weight (g)	Weight Gain (g)	Feed to gain	Mortality (n)	Mean lesion scores(1)			OPG × 10^6
							E. acervulina	*E. maxima*	*E. tenella*	Total
		D13–22	D22	D13–22	D13–22	Total	D21			D20–22
1	UUC	100.4	951.0^{a}	559.3^{a}	1.62^{b}	0	0.0^{b}	na	0.0^{c}	0^{c}
	IUC	83.9	735.4^{b}	343.4^{b}	2.23^{a}	1 (5.6%)	3.0^{a}	na	3.3^{a}	902^{a}
	IT	98.0	947.9^{a}	555.1^{a}	1.59^{a}	0	0.4^{b}	na	1.9^{b}	2.5^{b}
2	UUC	100.8	1017.8^{a}	596.4^{a}	1.52^{b}	0	0.0^{c}	na	na	0^{c}
	IUC	94.9	857.4^{b}	434.0^{b}	1.97^{a}	0	3.4^{a}	na	na	1102^{a}
	IT	104.9	1046.6^{a}	625.0^{a}	1.58^{b}	0	0.7^{b}	na	na	5^{b}
3	UUC	90.3	1035.6^{a}	610.4^{a}	1.37^{c}	0	0.0^{b}	0.0^{b}	0.0^{b}	0
	IUC	76.8	780.7^{c}	353.8^{c}	2.07^{a}	0	3.2^{a}	2.1^{a}	3.3^{a}	646^{a}
	IT	79.8	886.5^{b}	456.0^{b}	1.66^{b}	0	0.7^{b}	1.9^{a}	0.2^{b}	27^{b}

Means in columns within a study with different superscript are significantly different ($p \leq 0.05$). na: not applicable.
(1): 0 = no lesion, 1 = very mild, 2 = mild, 3 = moderate and 4 = severe.

In all three studies, final body weight (body weight gain) and feed to gain ratio of the infected group was significantly improved by the addition of 30 mg decoquinate/kg feed compared to the untreated group. Significantly higher OPG values were found in the infected untreated group compared to the uninfected group. Decoquinate significantly reduced total OPG compared to the IUC group near to the level of the UUC group. The mean score of intestinal lesions caused by *E. acervulina* was significantly lower and near to control level when the infected birds received decoquinate. Scores of

[58] AST 1: Technical dossier/Section IV/Annex IV.2.1. AST 2: Annex IV.2.2. AST 3: Annex IV.2.3.

[59] AST 1: German field isolate (2008); estimated dosage per bird: 251,105 sporulated oocysts (225,550 *E. acervulina*, 25,555); AST 2: Poland field isolate (2010); estimated dose per bird: 271,100 *E. acervulina* sporulated oocysts; and AST 3: Belgium field isolate (2010); estimated dose per bird was 273,100 sporulated oocysts (245,000 *E. acervulina*, 2,500 *E. maxima*, 25,600 *E. tenella*).

lesions caused by *E. tenella* (ASTs 1 and 3) were also significantly reduced by decoquinate. No significant effect was seen in the lesion score related to *E. maxima* infection.

3.3.3. Conclusions on efficacy

Among the three floor pen trials submitted, only one (trial 1) provides evidence of the anticoccidial efficacy of 20 mg decoquinate/kg feed based on endpoints relevant for coccidiostatic efficacy; however, the study design and reporting showed some weaknesses. Anticoccidial effects by decoquinate could be seen in trial 2 in reduced lesion scores in the caecum and less pens with blood in the faeces. The lack of a clear demonstration of anticoccidial efficacy in this trial maybe due to an insufficient pathogenicity of the *Eimeria* spp. inoculum. The only positive effect of decoquinate seen in the third study (trial 3) consists of a decrease in the severity of intestinal lesions with time. In summary, the anticoccidial efficacy of 20 mg decoquinate/kg complete feed for chickens for fattening could not be demonstrated by floor pen trials.

The anticoccidial sensitivity tests demonstrated the efficacy of 30 mg decoquinate/kg feed by an improvement of all endpoints related to this kind of test.

The three floor pens studies could not convincingly demonstrate the efficacy of decoquinate whereas the three anticoccidial sensitivity tests did. Since an assessment of the anticoccidial potential of an active substance has to be based on the positive outcome of three studies of each study type, the potential of decoquinate to prevent coccidiosis in chickens for fattening at the lowest applied dose of 20 mg/kg complete feed cannot be established.

3.4. Post-market monitoring

Field monitoring of *Eimeria spp*. resistance to decoquinate should be undertaken, preferably during the latter part of the period of authorisation.

4. Conclusions

Decoquinate from Deccox® is safe for chicken for fattening at the highest proposed concentration in complete feed of 40 mg/kg. The margin of safety is between 5 and 10.

No practically relevant interactions with other additives or veterinary drugs exist except with bentonite. Decoquinate does not have antibacterial action.

Decoquinate is not genotoxic and is not carcinogenic. Deccox® is safe for the consumer under the proposed conditions of use. No withdrawal period is required to ensure consumer safety. No MRLs are considered necessary.

The inhalation risk for user is considered negligible since inhalation toxicity and exposure are very low. Deccox® is not an irritant to skin and eyes and has no sensitisation potential.

The PEC/PNEC ratios for terrestrial, aquatic compartment and sediment are below 1, indicating that decoquinate used in chickens for fattening up to the highest proposed dose, does not pose a risk for these compartments; as well, no risk is expected neither for secondary poisoning nor for groundwater contamination.

Due to insufficient evidence, the potential of decoquinate to prevent coccidiosis in chickens for fattening cannot be established.

Documentation provided to EFSA

1) Deccox® 150G (decoquinate) for chickens for fattening. July 2013. Submitted by Pfizer Ltd..
2) Deccox® 150G (decoquinate) for chickens for fattening. June 2014. Supplementary information. Submitted by Zoetis Belgium SA.
3) Deccox® 150G (decoquinate) for chickens for fattening. April 2015. Supplementary information. Submitted by Zoetis Belgium SA.
4) Deccox® 150G (decoquinate) for chickens for fattening. September 2016. Supplementary information. Submitted by Zoetis Belgium SA.
5) Evaluation report of the European Union Reference Laboratory for Feed Additives on the Methods of Analysis for decoquinate.
6) Comments from Member States.

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Abbreviations

ADI	acceptable daily intake
AF	assessment factor
AST	anticoccidial sensitivity tests
BAF	bioaccumulation factor
bw	body weight

CVMP	Committee for Medicinal Products for Veterinary Use
DT_{50}	disappearance time 50 (the time within which the concentration of the test substance is reduced by 50%)
DT_{90}	disappearance time 90 (the time within which the concentration of the test substance is reduced by 90%)
EC_{50}	median effective concentration
EMA	European Medicines Agency
EURL	European Union Reference Laboratory
FEEDAP	EFSA Panel on Additives and Products or Substances used in Animal Feed
GLP	Good Laboratory Practice
K_{oc}	adsorption or desorption coefficient corrected for soil organic carbon content
LC_{50}	median lethal concentration
LD_{50}	median lethal dose
LOD	limit of detection
log K_{ow}	octanol/water partition coefficient
LOQ	limit of quantification
MIC	minimum inhibitory concentrations
MRL	maximum residue limit
NOEC	no observed effect concentration
NOEL	no observed effect level
OECD	Organisation for Economic Co-operation and Development
OPG	oocysts per gram of excreta
PEC	predicted environmental concentration
pKa	dissociation constant
PNEC	predicted no effect concentration

Appendix A – List of references retrieved from the literature search provided by the applicant

Tolerance

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Annex A – Executive Summary of the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Method(s) of Analysis for Deccox®

Deccox® is a feed additive currently authorised in chickens for fattening by Commission Regulation (EC) No 1289/2004 belonging to the group 'Coccidiostats and other medicinal substances' listed in Chapter I of Annex B of Directive 70/524/EEC. In the current application an authorisation of an existing product under article 10 (2) of the Regulation (EC) No 1831/2003 is requested. *Deccox*® consists of 6% *decoquinate*, 0.6% colloidal silica, 2.85% soya-bean oil on a wheat middlings carrier. The *Deccox*® active substance is *decoquinate*, a quinoline coccidiostat, with a minimum purity of 98%. *Deccox*® is a buff-coloured coarse powder formulation to be incorporated in *feedingstuffs* through *premixtures*. The Applicant suggested a concentration of *decoquinate* in *feedingstuffs* ranging from 20–40 mg/kg.

For the determination of decoquinate in the *feed additive*, *premixtures* and *feedingstuffs*, the Applicant submitted the ring-trial validated CEN standard method (EN 16162:2012) based on reversed-phase high performance liquid chromatography coupled to fluorescence detection (RP-HPLC-FL). The experimental evidence provided, allows the EURL to recommend for official control this CEN standard method for the determination of *decoquinate* in the *feed additive*, in *premixtures* and *feedingstuffs*.

For the determination of *decoquinate residues* in *tissues*, the Applicant submitted a single laboratory validated and further verified method, based on reversed-phase high-performance liquid chromatography coupled to a triple quadrupole mass spectrometer in electrospray ionisation mode (RP-HPLC–MS/MS) using matrix matched standards. The following performance characteristics are reported: precision (repeatability and/or intermediate precision) ranging from 0.9 to 7.2% and a recovery rate ranging from 95 to 110%. Based on the performance characteristics presented, the EURL recommends for official control the RP-HPLC–MS/MS method proposed by the Applicant to enforce the *decoquinate* MRLs in the relevant *tissues*.

Further testing or validation of the methods to be performed through the consortium of National Reference Laboratories as specified by Article 10 (Commission Regulation (EC) No 378/2005) is not considered necessary.