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Safety and efficacy of Robenz® 66G (robenidine hydrochloride) for chickens for fattening and turkeys for fattening

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Abstract

Following a request from 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 Robenz® 66G (robenidine hydrochloride (HCl)) when used as a feed additive for chickens for fattening and turkeys for fattening. The coccidiostat Robenz® 66G is considered safe for chickens for fattening at the highest proposed level of 36 mg robenidine HCl/kg complete feed with a margin of safety of approximately 2.5. This conclusion is extrapolated to turkeys for fattening. Robenidine HCl is active against Gram-positive but not against Gram-negative bacteria. It is not expected that the use of robenidine HCl as a feed additive would induce resistance or cross-resistance to antimicrobials used in human and animal therapy. The use of robenidine HCl from Robenz® 66G at the highest proposed level of 36 mg/kg complete feed in chickens and turkeys for fattening is considered safe for the consumer. The existing maximum residues limits for both avian species are confirmed. Robenidine HCl is not a skin or eye irritant and not a skin sensitiser. The risk via inhalation is considered negligible. The use of robenidine HCl from Robenz® in feed for chickens for fattening and turkeys for fattening up to 36 mg/kg complete feed does not pose a risk to either the terrestrial or the aquatic compartment. A risk for bioaccumulation cannot be excluded. The risk for secondary poisoning is not likely to occur. The FEEDAP Panel concludes that 36 mg robenidine HCl/kg complete feed from Robenz® 66G has the potential to effectively control coccidiosis of chickens for fattening under field conditions but cannot conclude on the efficacy of robenidine HCl in turkeys for fattening. The existing 5-day withdrawal period to avoid off-flavours in edible tissues should be maintained.

Keywords: Robenz® 66G, robenidine hydrochloride, coccidiostats, chickens for fattening, turkeys for fattening, safety, efficacy

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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 Zoetis Belgium SA[2] for re-evaluation of the product Robenz® 66G, robenidine hydrochloride, when used as a feed additive for chickens for fattening and turkeys 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 18 August 2014.

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 Robenz® 66G (robenidine hydrochloride), when used under the proposed conditions of use (see Section 3.1.5).

1.2. Additional information

The additive Robenz® 66G was authorised in 2004 with the name Cycostat® 66G for use in chickens for fattening and in turkeys for 10 years. The following maximum residue limits (MRL) are in force for chickens for fattening (μg robenidine hydrochloride/kg wet tissue: 800 for liver, 350 for kidney, 200 for muscle and 1,300 for skin fat) and for turkeys (μg robenidine hydrochloride/kg wet tissue: 400 for liver, 200 for kidney, 200 for muscle and 400 for skin/fat).[3] Robenz® 66G is also authorised as a feed additive for rabbits for fattening and rabbits for breeding until 20 June 2021.[4] The withdrawal period is 5 days.

The Scientific Committee on Animal Nutrition (SCAN) issued an opinion on the use of robenidine in feedingstuffs for rabbits (European Commission, 1982) and on the extension of the use of robenidine in feedingstuffs for rabbits for breeding purposes (European Commission, 1995). In 2004, EFSA issued two opinions on the re-evaluation of Cycostat® 66G in accordance with article 9G of Council Directive 70/524/EEC (EFSA, 2004a,b). In 2008, EFSA issued an opinion on MRLs and withdrawal period for Cycostat® 66G for chickens and turkeys for fattening (EFSA, 2008a). In 2011, EFSA issued an opinion on the re-evaluation of Cycostat® 66G for rabbits for fattening and breeding in accordance with Regulation (EC) No 1831/2003 (EFSA FEEDAP Panel, 2011a).

[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] Zoetis Belgium SA, Rue Laid Burniat 1, 1348, Louvain-la-Neuve, Belgium.

[3] Commission Regulation (EC) No 1800/2004 of 15 October 2004 concerning the authorisation for 10 years of the additive Cycostat 66G in feedingstuffs, belonging to the group of coccidiostats and other medicinal substances. OJ L 317, 16.10.2004, p. 37. Amended by Commission Regulation (EC) No 101/2009 of 3 February 2009, OJ L 34, 4.2.2009, p. 5. (as regards MRLs); amended by Commission Regulation (EC) No 214/2009 of 18 March 2009, OJ L 73, 19.3.2009. p. 12. (as regards the trade name); amended by Commission Implementing Regulation (EU) No 118/2012 of 10 February 2012, OJ L 38 11.2.2012 p. 36. (as regards the holder of authorisation); amended by Commission Implementing Regulation (EU) No 1014/2013 of 22 October 2013, OJ L 281, 23.10.2013. p. 1. (as regards the holder of authorisation).

[4] Commission Implementing Regulation (EU) No 532/2011 of 31 May 2011 concerning the authorisation of robenidine hydrochloride as a feed additive for rabbits for breeding and rabbits for fattening (holder of authorisation Alpharma Belgium BVBA) and amending Regulations (EC) No 2430/1999 and (EC) No 1800/2004. OJ L 147, 1.6.2011. p. 7.

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[5] in support of the authorisation request for the use of Robenz® 66G (robenidine hydrochloride) as a feed additive.

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 and, 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.[6]

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of Robenz® 66G (robenidine hydrochloride) is in line with the principles laid down in Regulation (EC) No 429/2008[7] and the relevant guidance documents: Guidance for the preparation of dossiers for coccidiostats and histomonostats (EFSA FEEDAP Panel, 2011b), Technical guidance: Tolerance and efficacy studies in target animals (EFSA FEEDAP Panel, 2011c), Guidance on the assessment of the safety of feed additives for the target species (EFSA FEEDAP Panel, 2017a), Technical Guidance for assessing the safety of feed additives for the environment (EFSA, 2008b), Guidance for the preparation of dossiers for the re-evaluation of certain additives already authorised under Directive 70/524/EEC (EFSA, 2008c), Guidance for establishing the safety of additives for the consumer (EFSA FEEDAP Panel, 2012a), Guidance on the assessment of the safety of feed additives for the consumer (EFSA FEEDAP Panel, 2017b), Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012b) and Technical Guidance: Microbial Studies (EFSA, 2008d).

3. Assessment

The current opinion is aimed at assessing the safety and efficacy of the coccidiostat Robenz® 66G (robenidine hydrochloride) when used as a feed additive for chickens for fattening and turkeys for fattening.

3.1. Characterisation

3.1.1. Characterisation of the additive

Robenz® 66G contains the active substance robenidine hydrochloride (robenidine HCl) (6.6%), calcium sulfate dihydrate (89.4%) and calcium lignosulfonate (4%). Analysis of five batches of Robenz® 66G indicated product consistency; mean robenidine HCl content was 6.7% (range: 6.60–6.90%).[8]

Three batches of Robenz® 66G were analysed for content of fluorine, arsenic, heavy metals, dioxins, dioxin-like polychlorinated biphenyls (DL-PCBs) and non-DL PCBs and microbiological impurities.[9] All values were low and of no safety concern (fluorine: 167–204 mg/kg, arsenic: 0.35–1.20 mg/kg, lead: 0.28–0.62 mg/kg, cadmium: 0.01 mg/kg, mercury: < 0.005 mg/kg; dioxins (polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/F)): 0.17–0.69 ng WHO-PCDD/F-TEQ/kg, the sum of dioxins and DL-PCBs: 0.28–0.83 ng WHO-PCDD/F-PCB-TEQ/kg, non-DL PCBs: 0.002 mg/kg); *Salmonella*: absent in 25 g, Enterobacteriaceae: < 10 CFU/g, moulds: 300 CFU/g, yeasts: < 100 CFU/g, *E. coli*: < 10 CFU/g, aerobic plate count: < 10 CFU/g coliforms: < 10 CFU/g and *Staphylococcus aureus* < 10 CFU/g).

[5] FEED dossier reference: FAD-2013-0051.
[6] The full report is available on the EURL website: https://ec.europa.eu/jrc/en/eurl/feed-additives/evaluation-reports/fad-2013-0051
[7] OJ L 133, 22.5.2008, p. 1.
[8] Technical dossier/Section II/Annex II.1.3.2.
[9] Technical dossier/Section II/Annex II.1.4.1.3, Annex II.1.4.1.4 and Annex II.1.4.1.5.

Robenz® 66G is a greyish coloured free-flowing granular preparation with a bulk density of 0.85 kg/L and a tapped density of 0.97 kg/L.[10] Sieve analysis of three batches showed that >99% of the product consists of particles between 150 and 850 μm.[10] Only a minor fraction passes a 150-μm mesh (≤ 0.40%). The dusting potential, measured in three batches (Stauber-Heubach), was calculated to be 0.14 g/m^3.[11]

3.1.2. Characterisation of the active substance

Robenidine hydrochloride (*N*1,*N*3-bis[(*p*-chlorobenzilidene)amino] guanidine hydrochloride; CAS number: 25875-50-7) is a chemically synthesised substance. A minimum purity of 97% is specified by the applicant. Its molecular formula is $C_{15}H_{13}Cl_2N_5 \cdot HCl$; the molecular weight is 370.7 g/mol. Its structural formula is given in Figure 1.

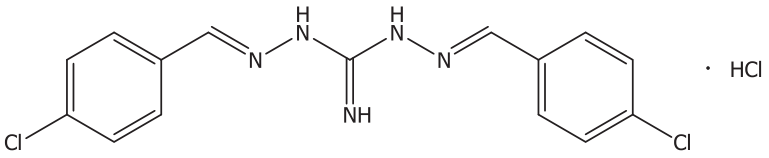

Figure 1: Structural formula of robenidine hydrochloride

Robenidine HCl is a white-yellowish crystalline powder with a melting point of 288.4°C.[12] Two major impurities associated to the synthetic process have been identified as *N*,*N*′,*N*″-tris[(*p*-chlorobenzylidene) amino]guanidine (TRIS) and bis-(4-chlorobenzylidene)hydrazine (AZIN). The structural formula of these compounds is given in Appendix A.

The applicant provided the following specifications: purity > 97%, TRIS ≤ 0.5%, AZIN ≤ 0.5%, any individual unknown impurity ≤ 0.2% and sum of unknown impurities ≤ 1.0%. The analytical data were in compliance with these specifications: analysis of three batches of robenidine HCl showed a mean robenidine HCl concentration of 99.8%. Water content (loss on drying) was between 0.10% and 0.25%. The results for the impurities were: TRIS < 0.01%, AZIN < 0.02%, any individual unknown impurity < 0.2% and the sum of unknown impurities < 0.2%.[13] Additional data was provided on the analysis of the impurities showing the same levels of AZIN (< 0.01%) and TRIS (0.02%); limit of detection (LOD) and limit if quantification (LOQ) were provided.[14]

[redacted]

3.1.3. Manufacturing process

[redacted]

[10] Technical dossier/Section II/Annex II.1.5.1.
[11] Technical dossier/Section II/Annex II.1.5.3.
[12] Technical dossier/Supplementary information April 2015/Annex 1.5.
[13] Technical dossier/Section II/Annex II.1.3.4.
[14] Technical dossier/Supplementary information April 2015/Annex 1.3; AZIN LOD: 0.01%, LOQ: 0.03%; TRIS LOD: 0.02%, LOQ: 0.06%.

[redacted]

3.1.4. Stability and homogeneity

3.1.4.1. Shelf-life of the additive

Three batches of the additive were stored at 25°C/60% relative humidity (RH) for up to 36 months and at 40°C/75% RH for six months.[10] No significant loss of robenidine HCl was observed after 36 months at 25°C/60% RH. Recovery of robenidine HCl at 40°C/75% RH was > 97% after three months and 93% after six months.

3.1.4.2. Stability of the additive used in premixtures and feedingstuffs

The stability of Robenz® 66G in vitamin/mineral premixtures was determined in premixtures for chickens for fattening (containing choline chloride) and for turkeys.[20] Robenidine HCl inclusion level was 6.6 g/kg. The premixtures were kept at 25°C/60% RH for up to 12 months and at 40°C/75% RH for 3 months. Recovery of robenidine HCl after 12 months at 25°C was > 96%; after 3 months at 40°C was > 90%.

In a second study, the stability of the additive incorporated into turkey complete feed at a level of 33 mg robenidine HCl/kg diet following pelleting at 80°C was studied.[21] Pelleting did not influence the robenidine HCl content. The pelleted feed was kept at 25°C for 3 months; the recovery was between 75 and 88%.

3.1.4.3. Homogeneity

Premixtures used for stability testing were analysed for homogeneity.[20] The coefficient of variation (CV) of seven samples was 6.8% in the premixture for chickens for fattening and 4.7% in the premixture for turkeys. Samples of mash turkey feed showed CVs from seven samples of 14.0–14.6%.[21]

In an additional study, homogeneity was studied in a premixture and in a feed for chickens for fattening, mash and pelleted, (8 samples each) at target concentrations of 10 g robenidine HCl/kg and 33 mg robenidine HCl/kg, respectively.[22] The CV in the premixture was 2.5%, in the mash feed 14.6% and in the pelleted feed 6.0%.

3.1.5. Conditions of use

Robenz® 66G is a feed additive for the prevention of coccidiosis in chickens for fattening and turkeys for fattening. The recommended feed inclusion level is 30 to 36 mg robenidine HCl/kg complete feed, with a 5-day withdrawal period.

3.2. Safety

3.2.1. Absorption, distribution, metabolism, excretion and residues

3.2.1.1. Absorption, distribution, metabolism, and excretion

No new studies have been submitted. The applicant made reference to the studies on the metabolic fate of robenidine HCl in chicken, turkeys and laboratory animals already assessed by the FEEDAP Panel in its previous opinions (EFSA 2004a, 2008a). The FEEDAP Panel considered that the data from those studies are still relevant for the current assessment and the same conclusions can be retained:

1) Robenidine HCl is absorbed at a limited extent and excreted rapidly by the chicken and turkey
2) Robenidine main metabolic pathways in chicken and turkey involve the hydrolysis of the semi-carbazide bonds of the molecule, followed by the oxidation of the resulting p-chlorobenzaldehyde to *p*-chlorobenzoic acid. Conjugation of *p*-chlorobenzoic acid with lysine or ornithine and formation of mixed conjugates with exogenous benzoic acid and hydroxybenzoic acid occur. In the chicken, the cyclisation of the aminoguanidine moeity of robenidine leads to a triazole derivative. No significant gender difference was observed

[20] Technical dossier/Section II/Annex II.4.1.2.
[21] Technical dossier/Section II/Annex II.4.1.3.
[22] Technical dossier/Section II/Annex II.4.2.1.

3) Unchanged robenidine is the major compound (40–80%) excreted by the chicken and turkey, all the metabolites accounting each less than 10% of the whole robenidine-related compounds excreted
4) Robenidine represents also the major identified residue (marker residue) in chicken and turkey, metabolites accounting for less than 10% each. The liver is the target tissue
5) The metabolic fate of robenidine in the rat is qualitatively very similar to that in the chicken and turkey, the only difference relating to the nature of the amino acids involved in the conjugation of *p*-chlorobenzoic acid. Moreover, very small quantities of the triazole cyclic metabolite identified in the chicken have been found in the fat of the rat.

A literature review made by the applicant[23] did not identify any relevant paper for the current assessment (Appendix B).

3.2.1.2. Residue studies

No new study has been submitted. The applicant made reference to the total and marker residue studies in chickens and turkeys which were evaluated by the FEEDAP Panel in 2008 (EFSA, 2008a). The FEEDAP Panel considers that the data from those studies are still valid for the current assessment. An overview of the relevant data is given below.

Robenidine-derived total residues in chicken and turkey tissues were evaluated in studies following a similar protocol in which birds (three males and three females) were administered 36 mg radiolabelled robenidine HCl/kg feed until study state and slaughtered at 0-, 1- and 3-day withdrawal times (Table 1).

Table 1: Kinetics of robenidine-derived total residues in tissues of chickens (average of three males and three females ± standard deviation) and turkeys (average of three males and three females ± standard deviation) administered 36 mg radiolabelled robenidine HCl/kg feed until steady state and slaughtered at different withdrawal times (expressed as mg robenidine equivalents/kg wet tissue)

	Withdrawal (days)	Liver	Kidney	Skin/fat	Muscle
Chicken	0	2.516 ± 0.732	0.885 ± 0.251	1.372 ± 0.364	0.169 ± 0.043
	1	1.569 ± 0.468	0.540 ± 0.131	0.821 ± 0.349	0.070 ± 0.025
	3	0.633 ± 0.137	0.161 ± 0.024	0.211 ± 0.042	0.007 ± 0.004
Turkeys	0	1.610 ± 0.332	0.570 ± 0.052	0.829 ± 0.126	0.065 ± 0.012
	1	0.814 ± 0.282	0.279 ± 0.099	0.389 ± 0.214	0.023 ± 0.013
	3	0.498 ± 0.082	0.145 ± 0.024	0.250 ± 0.072	0.010 ± 0.003

Total residues in all relevant tissues are higher in chicken compared to turkey. Considering the given physiological proximity of chicken and turkey and the similar qualitative metabolic fate of robenidine in both species, the assessment for turkeys can rely on the chicken data (EFSA, 2008a).

In the same study, robenidine residues were also determined in chicken and turkey tissues (Table 2).

Table 2: Kinetics of robenidine residues in chicken and turkey tissues (average of three males and three females) administered 36 mg radiolabelled robenidine HCl/kg feed until steady state and slaughtered at different withdrawal times (expressed as mg robenidine/kg wet tissue)

	Withdrawal (days)	Liver	Kidney	Muscle	Skin/fat
Chicken	0(1)	0.507	0.229	0.060	0.823
	1(2)	0.232	0.134	< LOQ	0.420
	3(2)	< LOQ	< LOQ	< LOQ	< LOQ
Turkeys	0(1)	0.261	0.030	0.006	0.257
	1(2)	0.131	< LOQ	< LOQ	0.197

(1): Determined by radio-HPLC; average values from pooled samples (males and females separately).
(2): Determined by HPLC in individual samples; average values, LOQ = 0.1 mg/kg for all tissues.

[23] Technical dossier/Supplementary information April 2015. Databases searched: Medline, Embase, Biosis, CAB Abstracts, Derwent Veterinary Drug File, Agricola, Pascal, Toxcenter, SciSearch, Chemical Abstracts, Dissertation Abstracts and ProQuest SciTech Collection. Period: 2004–2015.

Marker residue concentrations in all tissues were higher in the chicken compared to turkey after 0- and 1-day withdrawal.

3.2.2. Safety for the target species

3.2.2.1. Tolerance study in chickens for fattening

A total of 600 one-day old Ross 708 chickens (300 males and 300 females) were randomised into five treatment groups which were fed diets containing 0, 33 (1× the recommended level), 49.5 (1.5×), 66 (2×) and 82.5 (2.5×) mg robenidine HCl/kg complete feed (analytically confirmed), respectively, for 35 days.[24] Group size was 120 birds per treatment (6 replicates for each gender with 10 birds each). The basal diet consisted mainly of maize and methionine supplemented soybean meal; the starter formulation was calculated to contain 21.7% crude protein (CP), 3.8% crude fat (CF) and 0.59% methionine; the grower formulation 20.0% CP, 4.1% CF and 0.55% methionine. The starter was fed as crumbles for 21 days, the grower as pellets until the end of the study. The birds had *ad libitum* access to the feed. Clinical examination was performed twice daily and measurements of individual body weight and feed consumption were performed weekly. Blood samples were taken for haematology[25] and clinical biochemistry[26] from one animal per pen on day 35. The same animals were killed, necropsied and tissue samples[27] were collected. Histopathology was performed on the organs collected from the control and the high-dose group (and on any abnormal tissues from other animals). Statistical evaluation was done by a general linear mixed model (GLMM) with the fixed effects of treatment, sex, and interaction treatment × sex. The pen was considered the statistical unit; differences were considered significant at a level of $p < 0.1$ (two-sided).

A total of six birds died during the course of the study. The main results are summarised in Table 3.

Table 3: Main results of a 35-day tolerance study in chickens for fattening with Robenz® 66G

Robenidine HCl (mg/kg feed)	Average feed intake (g/bird per day)	Final bw (g)	Feed to gain ratio	Mortality (n)(1)
0	79	1899	1.46	2
33	77	1874	1.45	2
49.5	79	1888	1.46	0
66	79	1893	1.45	1
82.5	77	1867	1.45	3

(1): Including culls.

There were no significant differences in final body weight, average daily gain, or feed intake between treatments or at any time point during the course of the study. There were no significant differences observed in feed to gain ratio for the overall period.

There were no biologically relevant treatment-related effects during hematologic evaluation. The only significant differences found in haematology were males of the use level group had a significantly higher monocyte value (1.17 × 103/μL) than males of all other groups (0.27–0.70 × 103/μL). Males at the 1.5× level group had a significantly lower monocyte value (0.27) compared to the 2.5× level group (0.7 × 103/μL). There were no treatment-related findings for clinical chemistry parameters except serum calcium (slightly decreased levels in males of groups 1× and 1.5× compared to controls) and serum phosphorus (slightly increased in combined male and female birds of groups 1× and 2× compared to controls). These findings are not considered treatment-related because they were not observed in higher dose groups, and had no associated morphologic changes.

[24] Technical dossier/Supplementary information June 2016.

[25] Red blood cell count (RBC), haematocrit, mean corpuscular haemoglobin (MCH), red cell distribution width (RDW), thrombocyte check, heterophils, lympocytes, haemoglobin, mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC), white blood cell count (WBC) and differentials, eosinophils, basophils, monocytes.

[26] Aspartate aminotransferase (AST), alkaline phosphatase, creatine kinase, total protein, total cholesterol, blood urea nitrogen (BUN), glucose, calcium, phosphorus, sodium, potassium, chloride, uric acid, alanine aminotransferase (ALT), lactate dehydrogenase (LDH), gamma-glutamyl transpetidase (GGT), magnesium.

[27] The following samples were collected, weighed, and placed into 10% buffered neutral formalin: liver; heart; spleen; kidneys; and bursa of Fabricius. The following samples were collected and placed into formalin without being weighed: crop; gizzard; small intestine (duodenum with pancreas, jejunum and ileum); caecum; proventriculus; and skeletal muscle.

There were no treatment-related effects on organ weights. Kidney weights (absolute and relative to body weight) were statistically increased over control birds for females in groups 1× and 1.5×, and for males in group 1×. These changes were not considered treatment-related because there were no microscopical changes associated.

There were no treatment-related findings during gross pathology including microscopy. Gross pathology and microscopic findings were considered incidental, of the nature commonly observed in this breed and age of chicken and/or were of similar incidence in the control and treated animals.

Conclusions on the tolerance study in chickens for fattening

The tolerance study in chickens for fattening did not identify significant differences of the treated groups to the untreated control group for the performance parameters, for haematology, blood biochemistry and gross pathology and microscopy. Small, however, significant differences, in plasma calcium and phosphorus, as well as in kidney weight, were not treatment related. The FEEDAP Panel concludes therefore that the minimum use level (33 mg robenidine HCl/kg feed) is safe for chickens for fattening with a margin of safety of 2.5. However, the highest applied robenidine concentration is 36 mg/kg feed. Considering study design with the use of 1.5-, 2- and 2.5-fold of the use level and the observed margin of safety, the Panel does not see any concern to extend its conclusion on the safety of robenidine HCl for chickens for fattening to 36 mg/kg complete feed.

3.2.2.2. Tolerance study in turkeys for fattening

As the margin of safety of Robenz® 66G in chickens for fattening is approximately 2.5, the FEEDAP Panel considers that the conclusion reached in the tolerance study in chickens for fattening can be extrapolated to turkeys for fattening at the same dose (EFSA FEEDAP Panel, 2017a). Supporting evidence of this conclusion is provided by a tolerance study in turkeys for fattening. The study, which showed several limitations, is described below.

A total of 80 one-day-old BUT 10 turkeys (40 males and 40 females) were randomised into four treatment groups which were fed diets containing 0, 36 (1× the maximum recommended level), 108 (3×) and 180 (5×) mg robenidine HCl/kg complete feed (analytically confirmed), respectively, for 56 days.[28] Group size was 20 birds per treatment (2 replicates for each gender with 5 birds each). The basal diet consisted mainly of wheat, maize and extracted soya; no information on the CP, CF and methionine content was provided. Starter and grower diets were offered as pellets for *ad libitum* access, the starter for the first 2 weeks, followed by the grower diet until study completion. Clinical examination, measurements of individual body weight, feed and water consumption were performed weekly. Cumulative data for feed intake were not submitted, feed to gain ratio was not calculated. Blood samples were taken for haematology[29] and clinical biochemistry[30] on two animals per pen on day 56. The same animals were killed, necropsied and organ samples[31] were collected. Histopathology was performed on the tissues collected from the control and the high-dose group (and on any abnormal tissues from other animals).

Statistical evaluation was done not considering the pen as the experimental unit. It was done by a GLMM with fixed effects of treatment, sex, and interaction treatment × sex. Differences were considered significant at a level of $p < 0.1$ (two-sided).

During the study, no mortalities or treatment-related clinical signs were seen as well as no significant differences in final body weight of turkeys were observed (4,673, 4,514, 4,488 and 4,743 g in the four experimental groups, respectively). Cumulative data for feed intake were not submitted; feed to gain ratio was not calculated.

Leucocyte, heterophile lymphocyte counts were significantly lower in the high-dose robenidine group compared to the control group (12.4 vs 6.7, 6.18 vs 3.54 and 5.54 vs 2.81 × 10^9, respectively).

[28] Technical dossier/Section III/Annex III.1.1.

[29] Red blood cell count (RBC), haemoglobin concentration, haematocrit, mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular haemoglobin (MCH), total leucocyte count (WBC) monocyte count, heterophil count, eosinophil count, basophils count, lymphocyte count.

[30] Sodium, potassium, chloride, calcium, phosphate, magnesium, total protein, albumin, globulin, glucose, uric acid, cholesterol, amylase, alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), alkaline phosphatase (ALP), creatine kinase, glutamate dehydrogenase (GLDH).

[31] The following samples were collected, weighed and placed into 10% buffered neutral formalin: liver, heart, spleen, kidneys; the following samples were collected and placed into formalin without being weighed: thyroid, adrenal glands, pancreas, spinal cord, eyes, lung, trachea, skeletal muscle, brain, skin, bone and marrow, bursa of Fabricius and thymus, gall bladder, proventriculus, ventriculus, duodenum, jejunum, ileum, colon, caecum; testes and epididymis, entire length of oviducts, uterus, ovaries.

Another small significant difference was found for aspartate aminotransferase (AST) for the high-dose robenidine group (5×) compared to the control group, (259 vs 298 IU/L).

No significant differences between the organ weights relative to body weight were observed, as well as no specific macroscopic/microscopic findings were noted.

Overall, the study design (insufficient number of replicates per treatment and gender, pen not used as experimental unit) and reporting (no data for cumulative feed intake/bird and feed to gain ratio) do not allow the FEEDAP Panel to derive any conclusion on the safety of robenidine HCl in turkeys from zootechnical data. However, it is noted that haematology indicated treatment-related effects of the fivefold overdose (significant reduction of leucocyte, heterophile and lymphocyte counts). No other relevant and treatment-related significant adverse effects were seen in clinical biochemistry, necropsy and histopathology. No adverse findings were described for the robendine HCl use level and its threefold concentration.

Conclusions on the tolerance study in turkeys for fattening

As the margin of safety of Robenz® 66G in chickens for fattening is approximately 2.5, the FEEDAP Panel considers that the conclusion reached in the tolerance study in chickens for fattening can be extrapolated to turkeys for fattening at the same dose. Therefore, the FEEDAP Panel concludes that Robenz® 66G is safe for turkeys for fattening at 36 mg/kg complete feed.

3.2.2.3. Interactions

In its opinion in 2011 (EFSA FEEDAP Panel, 2011a), the FEEDAP Panel concluded that 'No interactions or incompatibilities with feed materials, carriers, other approved additives or veterinary drugs have been recorded or reported'.

No relevant papers were found in the target species by the literature search performed by the applicant (Appendix B).[23]

In line with former conclusions, no interactions or incompatibilities with feed materials, carriers, other approved additives or veterinary drugs are expected when Robenz® 66G is used as a feed additive for chickens and turkeys for fattening.

3.2.2.4. Microbial studies

The microbiological safety of robenidine HCl was already assessed by the FEEDAP Panel in 2004 and 2011 (EFSA, 2004a,b, EFSA FEEDAP Panel, 2011a). For the current assessment, the applicant made reference to the studies previously assessed and performed a literature review covering the period 2004–2015; a total of five publications were found; none of them was considered relevant for the current assessment.[23]

In the absence of new data the FEEDAP Panel reiterates its previous conclusion:

> 'Robenidine is active against Gram-positive but not against Gram-negative bacteria. Gram positive bacteria are susceptible at concentrations relevant for the *in vivo* situation, considering the proposed dose range'.

The literature review did not identify publications regarding the effect of robenidine HCl on the development of resistance *in vitro* and *in vivo* and on cross-resistance to other antimicrobials. Moreover, considering also the mode of action, it is not expected that the use of robenidine HCl as a feed additive would induce resistance or cross-resistance to antimicrobials used in human and animal therapy.

The literature review did not report studies on the effect of robenidine on the increase of shedding or colonisation of enteropathogens. However, it cannot be excluded that the use of robenidine HCl as a feed additive may increase shedding of enteropathogenes.

3.2.2.5. Conclusions on the safety for the target species

The FEEDAP Panel concludes that the use of Robenz® 66G at the concentration of 36 mg robenidine HCl/kg feed is safe for chickens for fattening with a margin of safety of approximately 2.5. This conclusion is extrapolated to turkeys for fattening.

No interactions or incompatibilities are expected when Robenz® 66G is used as a feed additive for chickens and turkeys for fattening.

Robenidine is active against Gram-positive but not against Gram-negative bacteria. It is not expected that the use of robenidine HCl as a feed additive would induce resistance or cross-resistance

to antimicrobials used in human and animal therapy; it cannot be excluded that the use of robenidine HCl as a feed additive may increase shedding of enteropathogenes.

3.2.3. Safety for the consumer

3.2.3.1. Toxicological studies

The toxicological profile of robenidine HCl was already assessed by the FEEDAP Panel in 2004 and 2011 (EFSA, 2004a,b; EFSA FEEDAP Panel, 2011a). For the current assessment, the applicant made reference to the toxicological studies previously assessed and performed a literature review covering the last 10 years (until 2015).[23]

The FEEDAP Panel re-assessed the toxicological studies available in the context of previous submissions (EFSA, 2004a,b; EFSA FEEDAP Panel, 2011a). The main results can be summarised as follows.

Genotoxicity of robenidine HCl was tested in studies performed according to the relevant OECD guidances. It gave negative results in a bacterial reverse mutation assay, was not clastogenic in cultured mammalian cells and did not induce micronuclei in the bone marrow of treated mice. Therefore, robenidine HCl can be considered as not genotoxic.

Subchronic studies (90 days) were performed in rats, mice and dogs. In the rat study (performed in 1968), no substance-related changes were observed and a no observed adverse effect level (NOAEL) of 13.5 mg/kg body weight (bw) per day, based on the highest dose in males, has been identified. A NOAEL of 14 mg/kg bw per day in mice was proposed (study performed in 1968) based on renal changes (focal nephritis) seen at the dose of 28 mg/kg bw per day (EFSA, 2004a,b; EFSA FEEDAP Panel, 2011a). A re-evaluation of the 90-day dog study (performed in 1968) showed that the original robenidine HCl concentrations were given in mg/kg feed. The lowest no observed effect level (NOEL) in this study was recalculated to be 19 mg/kg bw per day derived from the data on the increase in relative liver weight observed at the dose of 34 mg/kg bw per day (EFSA FEEDAP Panel, 2011a). The recalculated NOAEL resulted to be about 2.5 times higher than the value the FEEDAP Panel calculated in 2004 (EFSA, 2004a,b). In a 90-day Good Laboratory Practice (GLP)-compliant rat study performed according to OECD 408, no treatment-related changes were observed at the mid-dose of 37 mg/kg bw per day and this dose was considered as the NOAEL of the study (EFSA FEEDAP Panel, 2011a).

Two chronic studies were conducted in rats and dogs. In the rat study, no tumour developments or preneoplastic lesions were observed; in the dog study, tumour developments were not recorded. The Panel noted that the studies had some limitation in their design. However, the lack of genotoxic potential considered alongside the absence of any findings of pre-carcinogenic lesions in toxicological studies supports the conclusion that robenidine HCl is not carcinogenic (EFSA, 2004a,b; EFSA FEEDAP Panel, 2011a).

No negative maternal effects or effects on litters by the treatment were seen at any stage of a reproduction study in rabbits; no treatment-related teratogenic effects or influence on reproduction were observed at any stage of a two-generation study in rats on females, or on litters and fetuses; the maternal and fetal NOAEL in a developmental toxicity study in rabbits was 20 mg robenidine HCl/kg bw per day.

In its opinion in 2011, the FEEDAP Panel noted that the NOAELs of the available 90-day studies are in a narrow range: 13.5 and 37 mg robenidine HCl/kg bw per day in the rat, 14 mg/kg bw per day in mice and 19 mg/kg bw per day in dogs. In the same opinion, the Panel evaluated a tolerance study in rabbits performed in line with the requirements of Regulation (EC) No 429/2008[7] and concluded that the study was adequate to derive a NOAEL, considering also that study duration (84 days) was comparable with the 90-day studies in rodents and the dog. The NOAEL of 11 mg robenidine HCl/kg bw per day was taken from the study based on the absence of reproductive effects.

Considering that the literature review performed by the applicant covering the last 10 years (until 2015)[32] did not identify any new data that would require modification of the previous assessment (Appendix B), the lowest NOAEL of 11 mg robenidine HCl/kg bw per day from the tolerance study in rabbits is still considered adequate for the establishment of a health-based guidance value for the assessment of consumer safety.

[32] Technical dossier/Supplementary information April 2015. Databases searched: Medline, Embase, Biosis, CAB Abstracts, Derwent Veterinary Drug File, Agricola, Pascal, Toxcenter, SciSearch, Chemical Abstracts, Dissertation Abstracts and ProQuest SciTech Collection.

3.2.3.2. Assessment of consumer safety

In its former assessment of robenidine HCl (EFSA FEEDAP Panel, 2011a), the FEEDAP Panel proposed a health-based guidance value (acceptable daily intake (ADI)) of 0.11 mg robenidine HCl/kg bw, equivalent to 6.6 mg/day for a 60-kg adult, based on the lowest NOAEL of 11 mg robenidine HCl/kg bw day derived from a rabbit tolerance study, applying a uncertainty factor of 100. The Panel noted that the uncertainty factor used for the derivation of the ADI in 2011 needs to be updated to 200, according to the principles set in the Guidance on selected default values to be used by the EFSA Scientific Committee, Scientific Panels and Units in the absence of actual measured data (EFSA Scientific Committee, 2012). The recalculated ADI used for the assessment of consumer safety is therefore 0.055 mg robenidine HCl/kg bw (corresponding to 3.3 mg/day for a 60-kg adult).

Daily exposure of consumer to robenidine total residues resulting from the consumption of chicken tissues was recalculated according to the daily food consumption values of animal products set in Regulation (EC) No 429/2008[7], using the data given above at 0-day withdrawal (see Section 3.2.1.2). The results are reported in Table 4.

Table 4: Consumer theoretical exposure to robenidine total residue concentrations (TRCs) in chickens administered Robenz® 66G at a dose corresponding to 36 mg robenidine/kg feed at 0-day withdrawal

	Liver	Kidney	Muscle	Skin/fat	Sum
TRC + 2SD(1)	3.980	1.387	0.285	2.100	
Consumption (g/day)(2)	100	10	300	90	500
DITR (mg/day)(3)	0.398	0.014	0.086	0.189	
% ADI	12	0.4	2	6	20.4

(1): TRC: total residue concentration (average) + 2 standard deviations.
(2): Based on the food basket of Regulation (EC) No 429/2008.
(3): Dietary intake calculated from total residues.

The exposure represents 20% of the health-based guidance value. The contribution of total robenidine residues in edible tissues of chicken to 20% of the ADI is based on the food basket of the Regulation (EC) No 429/2008. Applying instead European food consumption data from EFSA's Comprehensive European Food Consumption Database (see Guidance on the assessment of the safety of feed additives for the consumer (EFSA FEEDAP Panel, 2017b)), this contribution would account only to about 6% of the ADI. As far as total residues in turkey tissues at 0-day withdrawal are lower than in the chicken, the results of the calculation made for chicken tissues would also apply to turkey residues.

Therefore, the FEEDAP Panel reiterates its previous conclusions that no risk is expected from the consumption of tissues from chickens and turkeys fed robenidine HCl at the maximum recommended level without withdrawal period.

Commission Regulation (EC) No 101/2009[33] established the following MRLs (on a wet tissue basis) for chickens for fattening: 800 μg robenidine hydrochloride/kg liver, 350 μg/kg kidney, 200 μg/kg muscle and 1,300 μg/kg skin fat. Dietary intake calculated from these MRLs would represent 64% of the ADI. The same Regulation established the following MRLs for turkeys: 400 μg robenidine hydrochloride/kg liver, 200 μg/kg kidney, 200 μg/kg muscle and 400 μg/kg skin fat. Dietary intake calculated from these MRLs would represent 32% of the ADI.

In Commission Regulation (EC) No 101/2009[33], a 5-day withdrawal period is specified to avoid off-flavours in edible tissues from poultry treated with robenidine HCl at 36 mg/kg complete feed.

In the absence of new data, the same withdrawal period should be maintained (see Section 3.3.3.).

3.2.3.3. Conclusions on safety for the consumer

The use of robenidine HCl from Robenz® 66G at the highest proposed level of 36 mg/kg complete feed in chickens for fattening and turkeys for fattening is considered safe for the consumer.

[33] OJ L 34, 4.2.2009, p. 5.

3.2.4. Safety for the user

No new data have been submitted by the applicant.

The same studies, assessed in former opinions (EFSA, 2004a, 2008a), were re-submitted by the applicant and re-assessed by the FEEDAP Panel.

In a 4-hour acute inhalation study in rats conducted in 1999 according to GLPs with the active substance robenidine HCl, the FEEDAP Panel noted that the inhalation LC_{50} was > 5.2 mg/L (EFSA, 2004a).

Skin and eye irritation studies were conducted in 1968 with the active substance robenidine HCl and with a formulation containing 10% of robenidine HCl. A sensitisation study was performed with robenidine HCl according to OECD Guideline 406 and GLP. The FEEDAP Panel could confirm that the studies provided evidence that robenidine HCl is not a skin or eye irritant and not a skin sensitiser.

The FEEDAP Panel reviewed the information on the physical properties of the additive. Robenz® 66G is a granular product with only a minor fraction of particles smaller than 150 μm (≤ 0.40%) and with a low dusting potential (0.14 g/m^3); therefore, exposure of users/workers handling Robenz® 66G would be very low.

The literature search provided by the applicant covering the last 10 years (until 2015) did not reveal any new data relevant to user safety (Appendix B).[23]

The FEEDAP Panel reiterates its previous conclusions that robenidine HCl is not a skin or eye irritant and not a skin sensitiser. Based on the low acute inhalation toxicity and low exposure, the risk via inhalation is considered negligible.

3.2.5. Safety for the environment

The active substance is not a physiological/natural substance of established safety for the environment. Consequently, according to Regulation (EC) No 429/2008[7] the Phase I assessment has to be continued to determine the predicted environmental concentration (PEC).

In Phase I, a total residues approach will be taken meaning that the predicted environmental concentrations will be calculated based on the assumption that the additive is excreted 100% as parent compound.

The applicant submitted the same studies assessed by the FEEDAP Panel in its opinion on the safety and efficacy of Cycostat® 66G (robenidine HCl) for rabbits for breeding and fattening (EFSA FEEDAP Panel, 2011a) and, upon request, performed a literature review covering the last 10 years (until 2015).[34]

3.2.5.1. Phase I

The physico-chemical properties of robenidine HCl are summarised in Table 5.[35] The vapour pressure of robenidine HCl was not provided.

Table 5: Physico-chemical properties of robenidine HCl

Property	Value	Unit
Molecular weight	370.7	G/mol
Octanol/water partition coefficient (log K_{ow}) by HPLC method (OECD 117)	3.3	–
Octanol/water partition coefficient (log K_{ow}) by Shake flask method (pH = 7) (OECD 107)	4.7(1)	
Solubility at 20°C (pH around 3.5)	118	mg/L
Dissociation constant pKa	3.4	–
Vapour pressure	Not provided	Pa

(1): Based on the solubility of robenidine HCl in buffer solution at pH 7 and in octanol, a log K_{ow} of 2.9 and 4.7 could be determined. However, as the recovery of the item in the test system was below 90%, the latest value has been taken as a reasonable worst-case estimate.

Robenidine HCl is protonated at acidic pH and not charged at pH 7. The physico-chemical properties of neutral form (robenidine) are relevant for the environmental risk assessment. The FEEDAP Panel noted that the solubility of the neutral molecule was not provided. The solubility of the

[34] Technical dossier/Supplementary information April 2015. Period: 2004–2015.
[35] Technical dossier/Section III/Annex 4.12.

neutral form of robenidine was estimated using EPI Suite to be 4.877 mg/L and the vapour pressure was estimated to be 1.22 E-06 Pa. These values were used for the calculation of the PECs since the applicant did not provide relevant data.

The FEEDAP Panel noted that the log K_{ow} for robenidine HCl has been determined according to both OECD guideline 117 (high-performance liquid chromatography (HPLC) method) and 107 (shake flask method), (EFSA FEEDAP Panel 2011a). Based on the HPLC method, the log K_{ow} of robenidine HCl is 3.3. The shake flask method was performed at pH 2.3, 7 and 9.8. At pH 2.3, a log K_{ow} value of 2.5 was determined. At pH 7 and 9, difficulties arose due to the low solubility and maintenance of the pH at the desired level. Based on the solubility of robenidine HCl in the buffer solution at pH 7 and in the octanol, a log K_{ow} of 2.9 and 4.7 could be determined. However, as the recovery of the item in the test system was below 90%, the latest value has been taken as a reasonable worst case estimate for the assessment of secondary poisoning.

Fate and behaviour

Fate in soil

Degradation in soil

The degradation of robenidine HCl was already assessed by the FEEDAP Panel in its opinion in 2011 (EFSA FEEDAP Panel, 2011a) as follow: 'A study on the transformation of robenidine HCl performed in three types of soil gave DT_{50} values for robenidine HCl of 12 and six days for sandy loam (pH 6.5, 1.4% organic carbon, 17.2% clay) and clay loam (pH 7.2, 1.5% organic carbon, 31.7% clay) and 162 days for loamy sand (pH 3.5, 1.1% organic carbon, 3.4% clay). The extraction efficiency was low in particular for loamy sand: 13%. The fast degradation in sandy loam and clay loam was mainly based on a formation of unknown component B and accounted for a maximum of 24% of the applied radioactivity after 32 days in extracts of sandy loam. It was postulated that this compound was formed by re-arrangement of the parent compound, tentatively named 3-amino-4-(p-chlorobenzylideneamino)-5-(p-chlorophenyl)-4H-1,2,4-triazole (using the naming tool in ACD/Chemsketch, see Figure 2). The DT_{50} values for component B were estimated to be 138 and 97 (average 118 days) days for sandy loam and clay loam, respectively.

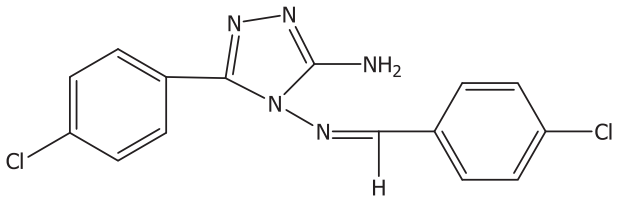

Figure 2: Proposed structure of the major degradation product formed in soil

The reason for the difference in degradation rate observed between loamy sand and the other two soil types might be the form in which the substance is present. The pH of loamy sand is low at which the compound will be protonated which hamper the re-arrangement of the parent compound. In the two soils the compound will be present in the non-ionized form. As a pH of 3.5 is not considered to be relevant for agricultural soils, the DT_{50} value measured in loamy sand is not considered in this risk assessment'.

The FEEDAP Panel agreed to use the same DT_{50} of 118 days at 20°C. This value corresponds to 251 days when it is adjusted to an incubation temperature of 12°C using the Arrhenius equation (DT_{90} > 1 year).[36]

Adsorption/desorption in soil

The adsorption of robenidine HCl was already assessed by the FEEDAP Panel in its opinion published in 2011 (EFSA FEEDAP Panel, 2011a).

The FEEDAP Panel re-assessed the studies available and noted that the applicant claimed that they could not perform a sorption study according to OECD 106 due to the rapid transformation of robenidine HCl; this might have been caused by the low pH of a solution of robenidine HCl in 10 mM

[36] 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).

$CaCl_2$. The applicant performed also an HPLC study according to OECD 121 at pH 2.5 and 6 (dichlorodiphenyltrichloroethane (DDT) being the reference standard). Since the pKa of robenidine is 3.4, it can be expected that the cationic form is present at pH 2.5 and the neutral form at pH 6. At pH 6, HPLC analysis resulted in the elution of two components indicating that robenidine was partly converted into a degradation product. An attempt was made to identify this product by repeating the sorption study under dark and daylight conditions. It appears that the peak of the degradation product was only observed under daylight conditions, suggesting that the formation of the unknown peak is a photolytic reaction. The transformation of robenidine HCl by photolysis is confirmed by Hansen et al. (2009). Several attempts were made to elucidate the structure of the second peak, but these were unsuccessful. It was demonstrated that in a methanol and ethanol buffer solution, the second peak occurred as well. Such a degradation process was previously observed and the resulting product was characterised as 3-amino-4-(*p*-chlorobenzylideneamino)-5-(*p*-chlorophenyl)-4*H*-1,2,4-triazole (EFSA FEEDAP Panel, 2011a), which is the same structure as proposed for the major metabolite observed in the soil transformation study. Although the precise identity is still not confirmed, it is considered very likely that the major metabolite formed in soil and the second peak observed in the sorption study are identical compounds and have the same adsorption behaviour.

Both the parent robenidine and the degradation product eluted slower than the reference compound DDT. Therefore, the assumption of the applicant that the sorption of robenidine HCl was stronger than that of DDT (K_{oc} = 426,580) was followed by the Panel.

Fate in water

The rate of hydrolysis of robenidine HCl was already assessed by the FEEDAP Panel in its opinion published in 2011 (EFSA FEEDAP Panel, 2011a) and it was noted that the rate of hydrolysis was tested at pH 4, 7 and 9.16. The FEEDAP Panel reiterates its previous conclusions that robenidine HCl is hydrolytically stable at pH 7 and 9, but shows hydrolysis at more acidic conditions (pH 4).

Conclusion on fate and behaviour

The K_{oc} of 426,580 L/kg is used for the further risk assessment.

Taking a conservative approach, the FEEDAP Panel selected a DT_{50} of 251 days for robenidine HCl derived from a transformation product.

Predicted environmental concentrations

PECs were calculated according to the FEEDAP technical guidance for assessing the safety of feed additives for the environment (EFSA, 2008b) and are given in Table 6.

Table 6: Initial predicted environmental concentrations (PECs) of robenidine HCl in soil (μg/kg), groundwater (μg/L), surface water (μg/L) and sediment (μg/kg dry weight)

Input	**Value**	
Dose (mg/kg feed)	36	
Molecular weight (g/mol)	370.7	
Vapour Pressure (Pa)	1E-06	
Solubility (mg/L)	4.877	
K_{oc} (L/kg)	426,580	
Output	**Chickens for fattening**	**Turkeys for fattening**
PEC_{soil}	187	167
$PEC_{groundwater}$	0.025	0.022
$PEC_{surfacewater}$	0.008	0.007
$PEC_{sediment}$	177	158

The Phase I PEC trigger values were exceeded. Therefore, a Phase II assessment is considered necessary.

3.2.5.2. Phase II

Exposure assessment

PECs calculation refined in Phase II

Studies on the excretion of robenidine HCl and its metabolites in the environment were assessed by the FEEDAP Panel in 2011 (EFSA FEEDAP Panel, 2011a). The results of the studies indicated that in the chicken, unchanged robenidine amounted to 44% (male) and 34% (female) of the radioactivity excreted; up to nine metabolites were separated but not identified, each amounting to less than 10%. In the turkey, robenidine represented 52% (male) and 44% (female) of the radioactivity excreted while eight metabolites (not identified) represented less than 10%, each. Based on the lack of data on the toxicological potential of robenidine metabolites on the environment, the impact of the metabolites cannot be discarded from the assessment. A prudent approach was retained considering the whole robenidine-derived metabolites in excreta as toxicologically relevant.

According to EFSA guidance (EFSA, 2008b), if a high persistence in soil is anticipated (DT_{90} > 1 year), the potential for residues to accumulate in soil should be considered. This is the case for robenidine HCl. The input values used for the refined PEC calculations based on persistence were the same indicated in Table 6; a DT_{50} 251 days (at 12°C) was used. The calculated values are given in Table 7.

Table 7: Predicted environmental concentrations of robenidine HCl in soil (μg/kg), groundwater (μg/L), surface water (μg/L) and sediment (μg/kg dry weight) refined for persistent compounds

Compartment	Chickens for fattening	Turkeys
PEC_{soil}	294	263
$PEC_{groundwater}$	0.039	0.035
$PEC_{surfacewater}$	0.013	0.012
$PEC_{sediment}$	278	248

Conclusions on PECs used for assessment

The following values are used for the assessment in chickens for fattening and turkey (worst case approach): a PEC_{soil} of 294 μg/kg, a $PEC_{surface\ water}$ of 0.013 μg/L and a $PEC_{sediment}$ of 278 μg/kg dry weight. No concern is expected for groundwater.

Ecotoxicity studies

Effects on plants

The effect of robenidine HCl on plants was already assessed by the FEEDAP Panel in its opinion published in 2011 (EFSA FEEDAP Panel, 2011a) as follows: 'Effect of robenidine hydrochloride on the emergence and growth of seedlings of wheat (*Triticum aestivum*), radish (*Raphanus sativus*) and mung bean (*Phaseolus aereus*) was studied in sandy loam soil (pH 5.6, organic carbon 0.4%, organic matter 0.7%) according to OECD guideline 208. Emergence and growth rate of all three plants species showed no reduction at the maximum concentration tested (EC_{50} and NOEC > 100 mg/kg)'.

The FEEDAP Panel re-assessed the study and reiterates its previous conclusions.

Effect on earthworms

The acute effect of robenidine HCl in earthworms was already assessed by the FEEDAP Panel in its opinion published in 2011 (EFSA FEEDAP Panel, 2011a) as follows: 'The acute toxicity to earthworms is determined in a 14-day test. No mortality was observed at the maximum concentration tested (LC_{50} > 1,000 mg/kg). A significant difference in mean weight change was reported at all doses. In fact, the control animals showed a higher reduction in body weight than the treated animals. After 14 days the mean body weight of all treated animals differed not more than 5% from the control animals. The pH in the soil was 6 ± 0.5, therefore, values should refer to the non-ionised compound'.

The FEEDAP Panel re-assessed the study and reiterates its previous conclusions.

Nitrogen Transformation

The effects of robenidine HCl on soil microorganisms were already assessed by the FEEDAP Panel in its opinion published in 2011 (EFSA FEEDAP Panel, 2011a) as follows: 'Effects on soil micro-organisms

were studied on nitrification and nitrogen mineralization in sandy loam soil (pH 6.5, organic carbon 1.3%, total nitrogen 1526 mg/kg, 40% maximum water holding capacity) at concentrations of 0.36 and 1.8 mg/kg. After 28 days no effects were observed at the maximum concentration tested (NOEC > 1.8 mg/kg). No information on the toxicity of degradation compound B is provided. Expecting that the degradation pathway observed in the soil biodegradation study also takes place in the soils used to investigate the toxicity to earthworms, plants and micro-organisms, it is assumed that the test organisms are also exposed to this compound'.

The FEEDAP Panel re-assessed the study and reiterates its previous conclusions.

Toxicity to aquatic organisms

The acute toxicity of robenidine to algae, daphnids and fish were already assessed by the FEEDAP Panel in its opinion published in 2011 (EFSA FEEDAP Panel, 2011a). In 2011, the FEEDAP Panel noted that: 'Acute toxicity to algae, daphnids and fish has been determined according to the current OECD standards. Due to the relatively low solubility of robenidine hydrochloride in water, difficulties were encountered during the preparation of test concentrations in all three acute toxicity studies conducted with aquatic organisms. Furthermore, the test item tended to adsorb onto surfaces of materials used in the studies. Therefore, supersaturated suspensions of the test item were prepared and filtrated, and the filtrate plus dilutions thereof were used in the studies. Concentrations of robenidine hydrochloride were measured prior to test start, at defined time points during the study and at the end of the test. Results were reported as overall mean measured concentrations (average over all measurements per test concentration)'.

The FEEDAP Panel re-assessed the studies and for the current assessment will follow the same approach.

Effects on algae

The effect of robenidine HCl on the green algal species *Scenedesmus subspicatus* was investigated in a 72-h GLP study following the OECD 201 guidelines. During the test, the concentration of the test item declined; therefore, the average measured values of 0.009, 0.011, 0.026 and 0.069 mg/L were considered for the calculation of the effect of the tested substance on algae. The test meets the validity criteria resulting in inhibitory effect on the growth rate at 72-h E_rC_{50} of 0.067 mg/L and NOEC of 0.026 mg/L.

Effects on crustaceans

The acute toxicity of robenidine HCl on the crustacean species *Daphnia magna* was investigated in a 48-h static test according to the OECD Guideline 202 (1984). The 10-fold dilution series were prepared (1:2.2–1:22). The results on acute effect of the robenidine HCl were related to the total mean measured test substance concentrations (calculated as the average over all measurements per test concentration) which were 0.037 mg/L (dilution 1:2.2) and 0.082 mg/L (undiluted filtrate). The acute effect concentration of the robenidine HCl was determined as 48-h EC_{50} with the value of 0.061 mg/L.

Effects on fish

The acute toxicity of robenidine HCl on zebra fish *Brachydanio rerio* was investigated in a study following OECD Guideline 203 (1992). In the 96-h, test the 10–fold series were prepared (1:2.2–1:22). Results are related to the total mean measured test item concentrations (calculated as the average over all measurements per test concentration) which were 0.019 mg/L (dilution 1:4.6), 0.067 mg/L (dilution 1:2.2) and 0.18 mg/L (undiluted filtrate). The acute effect concentration of the robenidine HCl was determined as 96-h EC_{50} with the value of 0.036 mg/L.

The data showed that robenidine HCl is highly toxic to aquatic organisms. The following EC_{50} values have been determined (Table 8).

Table 8: Acute toxic effects of robenidine HCl to aquatic organisms

Species	EC_{50} values (µg/L)
Scenedesmus subspicatus	67 (based on growth rate)(1)
Daphnia magna	61(2)
Brachydanio rerio	36(3)

(1): 72-h E_rC_{50}.
(2): 48-h EC_{50}.
(3): 96-h EC_{50}.

As the pH of all three studies was between 7.7 and 8.0, robenidine HCl was present in the non-ionised form.

Effect on sediment dwelling organisms

The toxicity of robenidine HCl to *Chironomus riparius* was already assessed by the FEEDAP Panel in its opinion published in 2011 (EFSA FEEDAP Panel, 2011a) as follows: 'The toxicity of robenidine hydrochloride to *Chironomus riparius* was investigated using spiked sediment in accordance to OECD 218. First instar larvae were exposed to concentrations ranging from 6.25 to 100 mg/kg dry sediment. The pH of the overlying water varied between 7.7 and 8.3. The test item was almost completely found in the sediment, confirming the strong adsorption of robenidine hydrochloride to sediment particles at environmentally relevant pH. The NOEC was 50 mg/kg based on both emergence and development rate'.

The FEEDAP Panel re-assessed the study and reiterates its previous conclusions.

Conclusions on the ecotoxic effect on soil, water and sediment

For the terrestrial compartment, data are available for plants, earthworms and microorganisms. Acute toxic concentrations resulted in 1,000 and 100 mg/kg of dry soil for earthworms and plants, respectively. For the aquatic compartment, data are available for algae, aquatic invertebrates and fish. The lowest toxicity value of 96-h EC_{50} of 36 µg/L for the aquatic compartment was found in a study on the effect on fish. Nevertheless, considering the very low solubility of the substance at neutral pH, the values obtained are uncertain.

Ecotoxicological data for sediment-dwelling invertebrate *Chironomus riparius* is provided for the sediment compartment resulting in NOEC of 50 mg/kg.

Risk characterisation (PEC/PNEC ratio)

Robenidine HCl emissions into the environment are higher when it is used in the feed for chicken for fattening than in turkeys for fattening. Therefore, the environmental risk assessment is based on use of this active ingredient in chickens. The risk characterisation ratios for terrestrial, freshwater and sediment compartments when the manure is incorporated into the soil are reported in the tables below (Tables 9, 10 and 11).

Table 9: Risk characterisation (PEC/PNEC ratio) of robenidine HCl for terrestrial compartment

Taxa	PEC_{soil} (µg/kg)	LC_{50}/EC_{50}/NOEC (mg/kg)	AF	PNEC (µg/kg)	PEC/PNEC
Earthworm	294	1,000(1)	1,000	1,000	0.3
Plants		100(2)	100	1,000	0.3

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

Table 10: Risk characterisation (PEC/PNEC ratio) of robenidine HCl for freshwater compartment

Taxa	$PEC_{surfacewater}$ (µg/L)	72-h E_rC_{50}/48-h EC_{50}/96-h EC_{50} (µg/L)	AF	PNEC (µg/L)	PEC/ PNEC
Algae(1) *Pseudokirchneriella subcapitata*	0.013	67(1)	1,000	36	0.0004
Aquatic invertebrates *Daphnia magna* acute		61(2)			
Fish *Brachydanio rerio*		36(3)			

AF: assessment factor.
(1): 72-h E_rC_{50}.
(2): 48-h EC_{50}.
(3): 96-h EC_{50}.

Considering the very low PEC/PNEC ratio (four orders of magnitude < 1), even if there are uncertainties in the results of the acute toxicity test, the FEEDAP Panel considers that there is an acceptable risk for surface water.

Table 11: Risk characterisation (PEC/PNEC ratio) of robenidine HCl for sediment

Taxa	$PEC_{sediment}$ (µg/kg)	NOEC (mg/kg)	AF	PNEC (µg/kg)	PEC/PNEC
Sediment-dwelling invertebrates *Chironomus riparius*	278	50	10	5,000	0.06

AF: assessment factor.

Bioaccumulation and secondary poisoning

Based on log K_{ow} of 4.7, robenidine HCl has a potential for bioaccumulation. In order to assess the risk for secondary poisoning, the method according the EU Technical Guidance Document for new and existing substances has been considered (EMA, 2016). Using the lowest NOEC for birds of 225 mg/kg feed, adopted by the FEEDAP Panel in 2004 (EFSA, 2004a), the PNEC oral is 7.5 mg/kg. This value is higher than the estimated concentration in the worms and fish of 0.051 and 0.051 mg/kg, respectively, which are based on PECs presented in Table 7. Hence, a risk for secondary poisoning for worm/fish-eating birds and mammals is not likely to occur.

3.2.5.3. Conclusions on safety for the environment

The use of robenidine HCl from Robenz® in feed for chickens for fattening and turkeys for fattening up to 36 mg/kg complete feed does not pose a risk to either the terrestrial or the aquatic compartment. No concern is expected for groundwater. A risk for bioaccumulation cannot be excluded. The risk for secondary poisoning is not likely to occur.

The literature search provided by the applicant on robenidine HCl 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 five years. Anticoccidial sensitivity tests (ASTs) could replace

field trials provided they follow the criteria mentioned in the guidance document on coccidiostats and histomonostats (EFSA FEEDAP Panel, 2011b).[37]

3.3.1. Efficacy in chickens for fattening

3.3.1.1. Floor pen studies

Three floor pen studies were submitted.[38] One-day-old birds (male Ross 308, male Ross PM3 and male/female of a slowly growing strain ISA JA 757 in trials 1, 2 and 3, respectively) were penned and distributed into treatment groups as indicated in Table 12. Treated groups received feed with an intended concentration of 33, 30 and 30 mg robenidine HCl/kg feed in trials 1, 2 and 3, respectively. The target concentrations were analytically confirmed in trials 2 and 3, while in trial 1 only 84% of the intended concentration could be measured (see Table 12). Infected groups were inoculated with field isolates of pathogenic *Eimeria* species (see Table 13 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 birds per pen following the method of Johnson and Reid (1970) (0 = no lesion, 1 = very mild, 2 = mild, 3 = moderate and 4 = severe).

The pen was the experimental unit for statistical purposes. In trial 1, all parameters were analysed by multifactorial analysis of variance (ANOVA), group means were compared by least significant difference (LSD) test. In trial 2, data were statistically analysed by one-way ANOVA; group differences for the zootechnical endpoints were examined by Tukey test and for lesion scores by Newman–Keuls test. Statistical analysis in trial 3 was done by GLMM. Mortality, oocyst counts per gram (OPG) and lesion scores were analysed for normal distribution by the Kolmogorov–Smirnov test and as data showed to be not normally distributed, the parameters were subjected to the Kruskal–Wallis test and the Mann–Whitney U-test.

Table 12: Experimental design of floor pen studies performed with Robenz® 66G

Trial	Study duration(1) (days)	Replicates per treatment (birds per replicate)	Treatment groups	Intended concentration of robenidine HCl (mg/kg feed)	Analysed robenidine HCl(2) (mg/kg feed)
1	41	8 (40)	Uninfected untreated control group (UUC)	–	–
			Infected untreated control group (IUC)	–	–
			Infected treated group (IT)	33	27.3/28
2	36	8 (90)	Infected untreated control group (IUC)	–	–
			Infected treated group (IT)	30	31/32
3	42	8 (30)	Infected untreated control group (IUC)	–	–
			Infected treated group (IT)	30	31.4/27.4

(1): Supplemented feed was administered until day 36 in trial 1 and until day 31 in trial 2.
(2): The two values correspond to the two different diets administered in the first and second half of the study.

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

[38] Trial 1: Technical dossier/Section IV/Annex IV.3.1; Trial 2: Technical dossier/Section IV/Annex IV.3.2; Trial 3: Technical dossier/Section IV/Annex IV.3.3.

Table 13: Summary of inoculation in floor pen studies performed with Robenz® 66G

Trial	Start date	Inoculum characteristics			
		Month/year and country of isolation	Intended dose per bird		Day and mode of inoculation
1	4/2007	Belgium	217,000	*E. acervulina*	Day 15 via feed
			24,800	*E. tenella*	
			8,300	*E. maxima*	
2	5/2011	6/2009 Ireland	250,000	*E. acervulina*	Day 17 via feed
			28,450	*E. maxima*	
3	5/2011	2/2010 The Netherlands	2.2×10^5	*E. acervulina*	Day 13 via feed
			1.5×10^4	*E. maxima*	
			2.0×10^4	*E. tenella*	

Mortality after inoculation in trial 1 was low (between 2 and 7 birds per group) and in no case coccidiosis related. In trial 2, coccidiosis-related mortality was found in the week following inoculation (1 in IUC and 3 in IT), but the difference was not significant. In trial 3, overall mortality was very low (6 from 480) and coccidiosis-related mortality amounted to 1 bird only in the IUC group.

In trial 1, the *Eimeria*-specific lesion scores (due to *E. acervulina, E. maxima* and *E. tenella*) about 1 week after inoculation were significantly reduced by the treatment compared to IUC. No significant effect was seen 1 week later (Table 14).

Table 14: *Eimeria* species-specific lesion scores in trial 1

	E. acervulina		***E. maxima***		***E. tenella***		**Total**	
	Day 22	**Day 29**	**Day 22**	**Day 29**	**Day 22**	**Day 29**	**Day 22**	**Day 29**
UUC	0.07^a	0.80	0.30^a	0.45	0.05^a	0.20	0.42^a	1.45
IUC	1.40^c	0.42	0.92^b	0.27	1.45^b	0.10	3.77^b	0.80
IT	0.62^b	0.62	0.30^a	0.42	0.27^a	0.15	1.20^a	1.20

a,b,c,: Means in a column with different superscript are significantly different ($p \leq 0.05$).

In trial 2, lesion scores in the duodenum and jejunum–ileum were significantly reduced by the treatment one week after inoculation but not in the subsequent week (Table 15).

Table 15: Mean lesion scores in different intestinal regions in trial 2

	Duodenum		**Jejunum-ileum**		**Caecum**	
	Day 24	**Day 31**	**Day 24**	**Day 31**	**Day 24**	**Day 31**
IUC	2.59	0.88	1.64	1.50	0	0.48
IT	1.95*	0.65	1.15*	1.35	0	0.30

Mean values with * are significantly different from IUC ($p \leq 0.05$).

In trial 3, inoculation with pathogenic *Eimeria* species resulted 6 and 8 days after inoculation in intestinal lesion scores between 0.5 and 2.0 for the different intestinal regions of the IUC group (Table 16). No lesions were found in the IT group.

Table 16: Mean lesion scores in different intestinal regions in trial 3

	Upper intestine		**Middle intestine**		**Caecum**	
	Day 19	**Day 21**	**Day 19**	**Day 21**	**Day 19**	**Day 21**
IUC	1.0	1.0	0.5	1.0	2.0	1.0
IT	0*	0*	0*	0*	0*	0*

Mean values with * are significantly different from IUC ($p \leq 0.001$).

A reduction of oocyst excretion by the treatment with robenidine HCl was seen in all trials. It was significant in trial 1 one week after inoculation but not later (2 and 3 weeks after inoculation). It was also significant in trial 2 one week after inoculation (and three weeks after inoculation), OPG values 11 and 14 days after inoculation were not different between IUC and IT. In trial 3, oocyst excretion was significantly reduced by the treatment at several days after inoculation (Table 17).

Table 17: Total number of *Eimeria* oocysts per gram of excreta (OPG) in floor pen studies

Trial 1	Day 22	Day 29	Day 36					
UUC	412[a]	12,975	4,375					
IUC	78,812[b]	26,225	2,612					
IT	38,812[ab]	23,610	1,587					
Trial 2	Day 24	Day 24 *E. maxima*	Day 28	Day 28 *E. maxima*	Day 31	Day 31 *E. maxima*	Day 36	Day 36 *E. maxima*
IUC	904,350	10,350	136,650	2,000	3,825	25	8,750	138
IT	367,700*	12,200	120,550	550*	4,038	50	40,900*	763
Trial 3	Day 18	Day 21	Day 24	Day 27	Day 30	Day 33	Day 36	Day 42
IUC	23,250	76,025	70,700	125,250	4,200	1,800	50	0
IT	425*	10,550*	3,050*	33,500	325*	0*	0	0

a,b,c: Means in a column with different superscript are significantly different ($p \leq 0.05$).
Mean values with * are significantly different from IUC ($p \leq 0.05$).

The use of robenidine HCl resulted also in a statistical improvement of feed intake and body weight/gain in trials 1 and 2 and of feed to gain ratio in trial 1. No such effect was seen in trial 3 (Table 18). The performance of the birds was in all trials in compliance with the performance objectives of the breeds.

Table 18: Performance data of chickens in floor pen trials with Robenz® 66G

	Feed Intake (g/day)	**Final body weight (g)**	**Weight Gain(1) (g)**	**Feed to gain ratio**
Trial 1				
UUC	106.4[a]	2,748[a]	66.1[a]	1.61[ab]
IUC	102.9[b]	2,633[b]	63.3[b]	1.63[b]
IT	105.6[a]	2,773[a]	66.7[a]	1.58[a]
Trial 2				
IUC	84.2	1,983	–	1.55
IT	87.1*	2,053*	–	1.55
Trial 3				
IUC	–	–	987	3.83
IT	–	–	1,021	3.61

–: not reported.
a,b,c: Means in a column within a trial with different superscript are significantly different ($p \leq 0.05$).
Mean values with * are significantly different from IUC ($p \leq 0.05$).
(1): Results of trial 1 refer to daily gain; results of trial 3 refer to total body weight gain.

3.3.1.2. Field trials

One field trial was submitted in which 8,000 one-day-old female Ross 308 chickens for fattening were randomly allocated to two different rooms with 4,000 birds each and fed the experimental diet for 37 days followed by a withdrawal period of 5 days.[39] The two diets were supplemented with either robenidine HCl from Robenz® 66G (target concentration 33 mg/kg; analysed concentration 45.8, 39.8 and 37.1 mg/kg feed in starter, grower and finisher feed, respectively) or an ionophore coccidiostat (target concentration 7 mg/kg).

Overall mortality was high; with 10.4% in the robenidine group and 12.2% in the ionophore group. At the end of the study, body weight of the robenidine group was significantly higher than that of the ionophore group (2,458 vs. 2,308 g) with a tendency (not significant) for a better feed to gain ratio (1.79 vs. 2.00).

[39] Technical dossier/Section IV/Annex IV.3.7.

Faecal samples from each group were collected on days 14, 21, 28, 35 and 42. No oocysts were found in any of the examined samples. Consequently no intestinal lesions related to coccidiosis were found when examined on six birds per group on days 21 and 28.

Since no *Eimeria* infection could be shown, the field trial could not be accepted for the demonstration of efficacy of robenidine HCl.

3.3.1.3. Anticoccidial sensitivity tests

Four anticoccidial tests were submitted. One of the ASTs could not be used for the demonstration of efficacy since the infection level was too low as indicated by the very low intestinal lesion scores.[40] The other three studies are described below.[41]

The tests were made with the groups UUC, IUC and IT, the latter receiving feed supplemented with intended concentrations of 36, 33 and 36 mg robenidine HCl/kg feed in AST-1, AST-2 and AST-3, respectively. The analysed values were 36, 35 and 37.2 mg robenidine HCl/kg feed.

Male Ross PM3 chickens were used in AST-1 and AST-2 and male and female Ross 308 in AST-3. The 1-day-old birds were fed the basal diet until day 13 and then randomly allocated to the groups with the supplemented diets. Group size was 18 chickens (3 replicates with 6 birds) in AST-1 and AST-2, and 120 chickens (10 replicates with 12 birds) in AST-3. Birds were artificially infected with sporulated oocysts from field isolates on day 15 in AST-1 and AST-2 and on day 13 in AST-3.[42] Endpoints were: mortality, oocyst excretion, intestinal lesions (lesion score scale ranging from 0 to 4 was applied according to the method of Johnson and Reid, 1970), body weight gain, feed consumption and feed to gain ratio. The observational period was 1 week after infection.

Statistical analysis was based on ANOVA using general linear models (GLM) procedure. In AST-1 and AST-2, intestinal lesion scores were analysed by Kruskal–Wallis test and the differences between groups were assessed using the Newman–Keuls test.

There was no coccidiosis-related mortality in any of the studies.

The results of the similar studies AST-1 and AST-2 are summarised in Table 19. The data show that 36 mg (analysed) robenidine HCl/kg complete feed reduced the *E. maxima*-related lesion score significantly but not that related to *E. acervulina* in AST-1. In AST-2, the *E. acervulina*-related lesion score was significantly reduced by 35 mg (analysed) robenidine HCl/kg feed but not that related to *E. maxima* and *E. tenella*. The total oocyst excretion was not positively affected by the treatment. The excretion of *E. maxima* oocysts was significantly lower in the robenidine groups of both ASTs. The infection significantly affected the parameters of body weight and feed to gain ratio (only in AST-2), the treatment with robenidine HCl resulted in a partial compensation.

Table 19: Results of AST-1 and AST-2

	Tr. group	Feed intake (g/day)	Body weight (g)	Weight gain (g)	Feed to gain ratio	Mean lesion scores			OE × $10^{6(1)}$	
						E. acervulina	*E. maxima*	*E. tenella*	Total	*E. maxima*
		D13–22	D22	D13–22	D13–22	D22			D20–22	
AST-1	UUC	104.2	1,040.6^{a}	66.0^{a}	1.59^{b}	0.0^{b}	0.0^{c}	–	0^{c}	0^{b}
	IUC	87.8	817.5^{c}	39.5^{b}	2.24^{a}	3.7^{a}	2.8^{a}	–	210^{b}	8^{a}
	IT	101.1	919.4^{b}	52.5^{b}	1.93^{a}	3.2^{a}	1.1^{b}	–	385^{a}	0^{b}
AST-2	UUC	90.3	1,035^{a}	65.9^{a}	1.37^{c}	0.0^{c}	0.0^{b}	0.0^{b}	0^{b}	0^{c}
	IUC	76.8	780.7^{c}	37.2^{c}	2.07^{a}	3.2^{a}	2.1^{a}	3.3^{a}	646^{a}	6.2^{a}
	IT	86.1	876.5^{b}	49.2^{b}	1.76^{b}	1.9^{b}	1.4^{a}	2.3^{a}	475^{a}	0.4^{b}

a,b,c: Means in columns within a study with different superscript are significantly different ($p \leq 0.05$).

(1): OE: oocyst excretion/bird and day. Figures were calculated from the droppings sampled per cage in 24 hrs and divided by the number of birds in the respective cage.

[40] Technical dossier/Supplementary information February 2017/Annex 1.

[41] AST-1: Technical dossier/Section IV/Annex IV.2.1. (2009) AST-2: Technical dossier/Section IV/Annex IV.2.2. (2011) AST-3: Technical dossier/Supplementary information August 2017/Annex 1. (2017).

[42] AST 1: Polish field isolate (2009); estimated dosage per bird: 335,000 sporulated oocysts (288,750 *E. acervulina*, 46,250 *E. maxima*); AST 2: Belgian field isolate (2010); estimated dose per bird: 273,100 sporulated oocysts (245,000 *E. acervulina* + 2,500 *E. maxima* + *25,600 E. tenella)*; AST-3: Belgian field isolate (2017); estimated dose per bird was 220,000 sporulated oocysts (138,000 *E. acervulina*, 60,000 *E. tenella*, 4,000 *E. maxima* and 18,000 *E. mitis*).

The results of AST-3 with 37 mg (analysed) robenidine HCl/kg feed are summarised in Tables 20 and 21. Only *E. tenella*-related lesion scores were significantly reduced by the treatment, whereas *E. tenella* specific oocyst excretion was only numerically lower than in IUC. A significant reduction of OPG was observed with *E. maxima* oocysts. The zootechnical parameters were significantly reduced by the infection in AST-3 and the reduction was compensated by the treatment.

Table 20: Results of coccidiosis-related endpoints in AST-3

Tr. group	Mean lesion scores			OPG				
	E. acer	*E. max*	*E. ten*	Total	*E. acer*	*E. max*	*E. ten*	*E. mit*
	D20			D13–20				
UUC	0.6^{c}	0.2^{b}	0.1^{b}	10,019^{b}	7,822^{b}	3^{c}	31^{b}	50^{b}
IUC	1.4^{b}	0.7^{a}	1.0^{a}	364,547^{a}	202,870^{a}	61,909^{a}	22,344^{a}	7,512^{a}
IT	1.9^{a}	0.5^{a}	0.1^{b}	315,676^{a}	282,394^{a}	5,369^{b}	2,523^{a}	8,092^{a}

E. acer: *Eimeria acervulina*; *E. maxima*: *Eimeria maxima*; *E. ten*: *Eimeria tenella*; *E. mit*: *Eimeria mitis*.
a,b,c: Means in columns within a study with different superscript are significantly different ($p \leq 0.05$).

Table 21: Results of performance in AST-3

Tr. group	Feed intake (g/day)	Weight gain (g)	Feed to gain ratio
UUC	91.7^{a}	68.1^{a}	1.35^{c}
IUC	76.0^{c}	47.3^{c}	1.61^{a}
IT	84.1^{b}	57.4^{b}	1.47^{b}

a,b,c: Means in columns within a study with different superscript are significantly different ($p \leq 0.05$).

Conclusions of efficacy in chickens for fattening

In the three floor pen studies made with 33, 30 and 30 mg robenidine HCl/kg feed, a significant reduction of oocyst excretion and of intestinal lesion scores was seen in the treated groups in all studies. The three ASTs made with 36, 33 and 36 mg robenidine HCl/kg feed showed some improvements of intestinal lesion scores and oocyst excretion. The intestinal lesion score was reduced in AST-1 against *E. maxima*, in AST-2 against *E. acervulina* and in AST-3 against *E. acervulina* and *tenella;* in all ASTs *E. maxima*, *E. acervulina* and *E. tenella* were examined. In AST-1 and AST-2, total oocyst excretion was reduced, in AST-3 only that of *E. maxima* (of a total of four *Eimeria* species examined). These findings together allow the FEEDAP Panel to conclude on a potential of the additive to control coccidiosis under field conditions. However, considering the robenidine HCl concentrations applied in the six studies, a coccidiostatic effect of robenidine in chickens for fattening was demonstrated at the inclusion level of 36 mg/kg complete feed.

3.3.2. Efficacy in turkeys for fattening

3.3.2.1. Floor pen studies

Three floor pen studies in turkeys for fattening, conducted in 2012, were submitted.[43] The studies followed the same design. One-day-old birds (BUT5, Aviagen Big 6 and BUT male turkeys for fattening in studies 1, 2 and 3, respectively) were penned and distributed into four treatment groups: an UUC group, an IUC group, an uninfected treated (UT) group and IT group. The IT group received feed at an intended concentration of 30 mg robenidine HCl/kg feed (see Table 22). Group size was 60 turkeys (6 replicates with 10 birds). Infected groups were inoculated on day 14 with recent field isolates of pathogenic *Eimeria* species via a syringe.[44] Diets were fed in three or four phases to all treatment groups as detailed in Table 22. Animal health and mortality were monitored daily. Feed intake and body

[43] Trial 1: Technical dossier/Section IV/Annex IV.3.4; Trial 2: Technical dossier/Section IV/Annex IV.3.5; Trial 3: Technical dossier/Section IV/Annex IV.3.6.
[44] Trial 1: Belgium field isolate (07/2011); estimated dosage per bird: 117,000 *E. meleagrimitis*, 48,000 *E. adenoeides*, 4,000 *E. dispersa*; Trial 2: German field isolate (08/2011); estimated dose per bird: 8,300 *E. meleagrimitis*, 1,400 *E. adenoeides*, 300 *E. dispersa*; Trial 3: French field isolate (07/2011); estimated dose per bird was 18,000 *E. meleagrimitis*, 9,340 *E. adenoeides*, 1,840 *E. dispersa*.

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 one bird per pen on days 21, 28 and 35 following the method of Johnson and Reid (1970) (0 = no lesion, 1 = very mild, 2 = mild, 3 = moderate and 4 = severe). Trials lasted 112 days followed by 5-day withdrawal period.

Table 22: Experimental diets fed to turkeys for fattening in the floor pen studies

Trial	Diet(1)		
	Type	Period (days)	Feed analysis (mg/kg feed) robenidine HCl
1	Starter	0–28	22.9
	Grower 1	28–56	30
	Grower 2	56–84	24.8
	Finisher	84–112	20.3
2	Starter	0–28	27
	Grower 1	28–78	28
	Grower 2	78–112	33/34
3	Starter	0–28	30
	Grower 1	28–56	24.4
	Grower 2	56–84	27
	Finisher	84–112	27

(1): Birds in the UT and IT groups were fed a basal diet supplemented with Robenz® 66G. Animals in the UUC and IUC groups received the same basal diet without inclusion of the coccidiostat.

Statistical analyses were conducted at the 0.05 level of significance using two-sided tests. Body weight and oocyst counts were analysed with a general linear repeated measures mixed model. Feed consumption, feed to gain ratio and mortality were analysed with GLMMs. Intestinal lesion scores were analysed using Fisher's Exact test.

Mortality after inoculation in trial 1 was low and there were no statistically significant differences among the treatments (Table 23). No information was provided on the cause of the deaths. In trials 2 and 3 mortality was high. In trial 2, no explanation was provided for the high mortality reaching in an average of the UUC and UT groups about 18%. For this reason, this trial could not be further considered for the evaluation of efficacy. In trial 3, the high mortality rates were due to a bacterial infection at the beginning of the study. All birds received antibiotic treatment from day 5 to day 10. Due to the high mortality rate and the concurrent medication, this trial could not be further considered for the evaluation of efficacy.

In trial 1, there were no significant differences between groups IUC and IT in the percentage of birds with abnormal lesion scores (i.e. lesion scores other than 0) seen in the lower small intestine and in caecum on days 21, 28 and 35.

A reduction of oocyst excretion by the treatment with robenidine HCl was seen in trial 1. It became significant 3 weeks after inoculation (day 35) (Table 23).

Table 23: Total number of *Eimeria* oocysts per gram of excreta (OPG) in floor pen trial 1

Trial 1	Day 21	Day 28	Day 35	Day 42	Day 49	Day 56	Day 63	Day 70
UUC	0	0	3	149	1,532	1,424	164	22
IUC	10,539	3,182	809	358	6	9	2	2
UT	0	0	0	12	1	8,203	127	20
IT	5,453	1,659	116*	4*	0*	10	2	3

*: IT significantly different from IUC mean ($p \leq 0.05$).

Performance parameters were not significantly affected by the treatment (mean body weight 12.3 kg, mean feed to gain ratio 2.33).

3.3.2.2. Anticoccidial sensitivity tests

Three ASTs with a similar experimental design, performed in 2013, were submitted.[45] Each test was made with the groups UUC, IUC and IT. The IT group received feed supplemented with Robenz® 66G at an intended concentration of 36 mg robenidine HCl/kg feed (analysed concentrations: 40, 34 and 38 mg robenidine HCl/kg feed in the three tests, respectively). In each test, one-day-old male turkeys for fattening (Big 6 is AST-1 and AST-2, Converter in AST-3) were randomly allocated to the experimental groups on day 10 (AST-1 and AST-2) and 12 (AST-3). Group size was 30 birds (5 replicates with 6 birds). Birds were artificially infected on study day 14 (AST-1 and AST-2) or 16 (AST-3) with sporulated oocysts from field isolates.[46] The experiment lasted for a total of 21 days (AST-1 and AST-2) or 23 days (AST-3). Animal health and mortality were monitored. Feed intake and body weight of the animals were measured, feed to gain ratio was calculated. Samples of excreta were analysed for oocyst excretion. Intestinal lesions were scored following the method of Johnson and Reid (1970) (0 = no lesion, 1 = very mild, 2 = mild, 3 = moderate and 4 = severe).

Body weight and oocyst counts were analysed with a general linear repeated measured mixed model with fixed effects (treatment, time point and treatment by time point) and random effects (block, and block by treatment). Feed consumption, feed to gain ratio and lesions scores were analysed with a GLMM with the fixed effect of treatment and the random effect of block. All hypothesis tests were conducted at the 0.05 level of significance using two-sided tests.

Table 24 summarises the results of the three ASTs. In AST-1 and AST-3, no mortality occurred. In AST-2, two birds died in the robenidine HCl treated group; one on the day before infection and one on the day of infection.

Table 24: Results of anticoccidial sensitivity tests in turkeys

	Tr. group	Daily Feed Intake (g)	Body weight (g)	Daily Weight Gain (g)	Feed to gain ratio	Total OPG	Lesion scores(1)	
							Lower small intestine	Caecum
		D14–20	D20	D14–20	D14–20	D20	D20	
AST-1	UUC	55^{a}	553^{a}	36^{ab}	1.51^{a}	0^{b}	93.3^{a}	90.9^{a}
	IUC	48^{b}	550^{a}	33^{a}	1.47^{a}	236,157^{a}	33.3^{b}	9.4^{b}
	IT	51^{ab}	575^{a}	39^{b}	1.30^{b}	232,819^{a}	86.7^{a}	84.3^{a}
AST-2	UUC	69^{a}	448^{a}	30^{a}	2.31^{a}	0^{b}	100^{a}	100^{a}
	IUC	68^{a}	385^{b}	21^{b}	3.27^{b}	113,585^{a}	52.5^{b}	93.8^{a}
	IT	74^{a}	458^{a}	28^{a}	2.63^{ab}	142,134^{a}	46.6^{b}	97.2^{a}
		D16–22	D22	D16–22	D16–22	D22	D22	
AST-3	UUC	52^{a}	507^{a}	33^{a}	1.56^{a}	0^{b}	43.3^{a}	83.3^{a}
	IUC	49^{ab}	469^{b}	28^{b}	1.78^{b}	238,143^{a}	43.3^{a}	50.0^{a}
	IT	47^{b}	477^{ab}	29^{b}	1.61^{a}	212,687^{a}	30.0^{a}	63.4^{a}

a,b,c: Means in a column within a study with different superscript are significantly different ($p \leq 0.05$).
(1): Percentage of birds without lesions (lesion score = 0).

Comparing the percentage of birds with 0 lesion scores (in small intestine and caecum) in the different treatment groups, significant differences were found between IUC and IT group only in AST-1. OPGs were not affected by the coccidiostatic treatment. Higher body weight, higher daily weight gain and improved feed to gain ratio were seen in all studies for the IT groups compared to IUC groups. The difference was significant in AST-1 for daily weight gain and feed to gain ratio, in AST-2 for body weight and daily weight gain and in AST-3 for feed to gain ratio.

[45] AST-1: Technical dossier/Section IV/Annex IV.2.3. AST-2: Annex IV.2.4. AST-3: Annex IV.2.5.
[46] AST-1: German field isolate (06/2011), estimated dosage per bird: 53,600 *E. meleagrimitis*, 3,200 *E. dispersa,* 6,800 *E. adenoeides*; AST-2: Polish field isolate (Oct/2012), estimated dose per bird: 18,750 *E. meleagrimitis*, 250 *E. dispersa,* 6,000 *E. adenoeides;* and AST-3: UK field isolate (Jan/2013), estimated dosage per bird: 61,920 *E. meleagrimitis*, 2,160 *E. dispersa* 5,280 *E. adenoeides*.

Conclusions of efficacy in turkeys for fattening

The potential of 30 mg robenidine HCl/kg complete feed for turkeys to be efficacious as coccidiostat was shown in one floor pen study only. Two other floor pen studies could not be assessed due to high not coccidiosis-related mortality. Since conclusions on the efficacy of a coccidiostat require three floor pen studies, no final conclusion can be drawn.

In summary, only one AST showed a significant coccidiostatic effect in turkeys, and that at the high-dose proposed (36 mg robenidine HCl/kg feed).

3.3.3. Studies on the quality of the animal products where this is not the effect claimed

The applicant made reference to the previous opinion of the FEEDAP Panel in which the effect of robenidine HCl on the flavour of broiler meat and tissues was assessed (EFSA, 2008a). The Panel noted that off-flavours were observed in edible tissues of chickens for fattening until 3 days after withdrawal. In the absence of data on the impact of robenidine on the sensory properties of turkey products, the FEEDAP Panel considered that chickens and turkeys should be similarly regarded.

3.3.4. Conclusions on efficacy for the target species

Based on the results of three floor pen studies with 30 mg robenidine HCl/kg feed and three ASTs with 36 mg robenidine HCl/kg feed, the FEEDAP Panel concludes that 36 mg robenidine HCl/kg complete feed has the potential to effectively control coccidiosis of chickens for fattening under field conditions.

Since in turkeys for fattening, only one of the three submitted floor pen studies could be assessed and only one of the three submitted ASTs indicated anticoccidial activity of robenidine HCl, the FEEDAP Panel cannot conclude on the efficacy of the additive in turkeys for fattening.

The existing 5-day withdrawal period to avoid off-flavours in edible tissues should be maintained in both chickens for fattening and turkeys for fattening.

3.4. Post-market monitoring

Field monitoring of *Eimeria* spp. resistance in chickens for fattening and turkeys for fattening to robenidine HCl should be undertaken, preferably during the latter part of the period of authorisation.

4. Conclusions

The FEEDAP Panel concludes that the use of Robenz® 66G at the concentration of 36 mg robenidine HCl/kg feed is safe for chickens for fattening with a margin of safety of approximately 2.5. This conclusion is extrapolated to turkeys for fattening.

No interactions or incompatibilities are expected when robenidine HCl is used as a feed additive for chickens and turkeys for fattening.

Robenidine is active against Gram-positive but not against Gram-negative bacteria. It is not expected that the use of robenidine HCl as a feed additive would induce resistance or cross-resistance to antimicrobials used in human and animal therapy.

The use of robenidine HCl from Robenz® 66G at the highest proposed level of 36 mg/kg complete feed in chickens and turkeys for fattening is considered safe for the consumer. The existing MRLs for both avian species are confirmed.

Robenidine HCl is not a skin or eye irritant and not a skin sensitiser. Based on the low acute inhalation toxicity and low exposure, the risk via inhalation is considered negligible.

The use of robenidine HCl from Robenz® 66G in feed for chickens for fattening and turkeys for fattening up to 36 mg/kg complete feed does not pose a risk to either the terrestrial or the aquatic compartment. A risk for bioaccumulation cannot be excluded. The risk for secondary poisoning is not likely to occur.

The FEEDAP Panel concludes that 36 mg robenidine HCl/kg complete feed from Robenz® 66G has the potential to effectively control coccidiosis of chicken for fattening under field conditions.

The FEEDAP Panel cannot conclude on the efficacy of robenidine HCl in turkeys for fattening.

The existing 5-day withdrawal period to avoid off-flavours in edible tissues should be maintained in both chickens for fattening and turkeys for fattening.

Documentation provided to EFSA

1) Robenz® 66G for chickens for fattening and turkeys. October 2013. Submitted by Zoetis Belgium SA.
2) Robenz® 66G for chickens for fattening and turkeys. Supplementary information. April 2015. Submitted by Zoetis Belgium SA.
3) Robenz® 66G for chickens for fattening and turkeys. Supplementary information. June 2016. Submitted by Zoetis Belgium SA.
4) Robenz® 66G for chickens for fattening and turkeys. Supplementary information. February 2017. Submitted by Zoetis Belgium SA.
5) Robenz® 66G for chickens for fattening and turkeys. Supplementary information. August 2017. Submitted by Zoetis Belgium SA.
6) Evaluation report of the European Union Reference Laboratory for Feed Additives on the Methods of Analysis for Robenz® 66G.
7) Comments from Member States.

Chronology

Date	Event
30/10/2013	Dossier received by EFSA
29/11/2013	Reception mandate from the European Commission
18/8/2014	Application validated by EFSA – Start of the scientific assessment
24/10/2014	Reception of the Evaluation report of the European Union Reference Laboratory for Feed Additives
30/10/2014	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. *Issues: characterisation, safety for target species, safety for the consumer, safety for the environment and efficacy*
18/11/2014	Comments received from Member States
4/5/2015	Reception of supplementary information from the applicant - Scientific assessment re-started
29/6/2015	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended *Issues: Safety for the target species*
1/6/2016	Reception of supplementary information from the applicant - Scientific assessment re-started
18/7/2016	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended *Issues: Safety for the target species, safety for the environment and efficacy*
14/2/2017	Reception of supplementary information from the applicant - Scientific assessment re-started
27/2/2017	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended *Issues: Efficacy*
30/8/2017	Reception of supplementary information from the applicant - Scientific assessment re-started
24/1/2019	Opinion adopted by the FEEDAP Panel. End of the Scientific assessment

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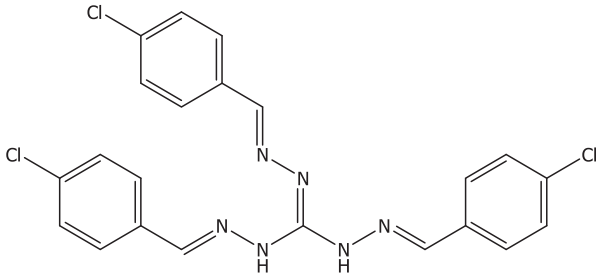
Abbreviations

ADI	acceptable daily intake
ADME	absorption, distribution, metabolism and excretion
AF	assessment factor
ANOVA	analysis of variance
CFU	colony forming unit
CV	coefficient of variation
DITR	Dietary intake of total residues
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%)

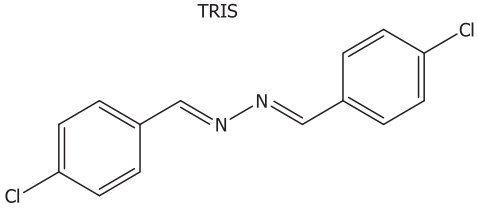
EC_{50}	median effective concentration
E_rC_{50}	median effective concentration which results in a 50% reduction in growth rate
EURL	European Union Reference Laboratory
FEEDAP	EFSA Panel on Additives and Products or Substances used in Animal Feed
GLM	general linear models
GLMM	general linear mixed model
GLP	Good Laboratory Practice
HPLC	high-performance liquid chromatography
K_{oc}	adsorption or desorption coefficient corrected for soil organic carbon content
LC_{50}	median lethal concentration
LOD	limit if detection
log K_{ow}	octanol/water partition coefficient
LOQ	limit of quantification
LSD	least significant difference
MRL	maximum residue limit
NOEC	no observed effect concentration
NOAEL	no observed adverse effect level
NOEL	no observed effect level
OECD	Organisation for Economic Co-operation and Development
PEC	predicted environmental concentration
PNEC	predicted no effect concentration
SCAN	Scientific Committee on Animal Nutrition
SD	standard deviation
TRC	total residue concentration

Appendix A – Molecular formula of impurities in robenidine HCl



N,*N*',2-tris((*E*)-4-chlorobenzylidene)hydrazine-1-carbohydrazonhydrazide
Chemical Formula: $C_{22}H_{17}Cl_3N_6$
Molecular Weight: 471.77

TRIS



1,2-bis((*E*)-4-chlorobenzylidene)hydrazine
Chemical Formula: $C_{14}H_{10}Cl_2N_2$
Molecular Weight: 277.15

AZIN

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

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Annex A – Executive Summary of the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Methods of Analysis for Robenz® 66G

Robenz® 66G is a feed additive initially authorized for rabbits for breading and chickens, rabbits and turkeys for fattening as Cycostat 66G by Commission Regulation (EC) No 1800/2004, belonging to the group "Coccidiostats and other medicinal substances" listed in Chapter I of Annex B of Directive 70/524/EEC. This regulation has been modified by Commission Regulations (EC) No 214/2009 and No 1014/2013. In the current application an authorisation of an existing product under article 10 (2) of the Regulation (EC) No 1831/2003 is requested. Robenz® 66G consists of 6.6% (w/w) of robenidine hydrochloride (active substance), calcium lignosulphonate as binder and calcium sulphate dihydrate as diluent/carrier. The Applicant proposed a concentration of robenidine in feedingstuffs ranging from 30 to 36 mg/kg. Furthermore the Applicant suggested Maximum Residue Limits (MRLs) in wet tissues ranging from 200 to 1300 μg/kg for chicken for fattening and from 200 to 400 μg/kg for turkeys for fattening, as already established by Commission Regulation (EC) No 214/2009.

For the quantification of robenidine in feedingstuffs the Applicant submitted the ring-trial validated Community method based on High Performance Liquid Chromatography coupled to Ultraviolet detection (HPLC-UV). Furthermore the Applicant applied the Community method with minor experimental modifications to the feed additive (Robenz® 66G) and premixtures and obtained similar method performance characteristics. Based on the provided performance characteristics the EURL recommends for official control the HPLC-UV method for the quantification of robenidine in the feed additive, premixtures and feedingstuffs.

For the quantification of robenidine in tissues (chicken kidney and turkey skin/fat) the Applicant submitted a method based on reverse phase High Performance Liquid Chromatography coupled to a triple quadrupole mass spectrometer (RP-HPLC-MS/MS) in electrospray ionisation mode using matrix-matched standards. This method was developed and validated according to Commission Decision 2002/657/EC by the European Union Reference Laboratory for Pharmacologically Active Substances (BVL). The satisfactory results provided by the Applicant for kidney and skin/fat demonstrate the applicability - and therefore extension of scope - of the BVL method to these two additional tissues. Based on the performance characteristics presented the EURL recommends for official control the RP-HPLC-MS/MS method - or any equivalent methods complying with the requirements set by Commission Decision 2002/657/EC - for the determination of robenidine in chicken and turkey 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.