Assessment of the safety and efficacy of monensin sodium (Coxidin®) as a feed additive for chickens and turkeys for fattening, laying and breeding

Reference number RP140-141-142-284

Regulated Products Risk Assessment Unit

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Regulated Product Dossier Assessment

Assessment finalised: 18/08/2023

Summary

Four applications were submitted to the Food Standards Agency between January 2021 and February 2021 from Huvepharma NV ("the applicant") for the authorisation of an additive (Coxidin ®), under the category of 'coccidiostats and histomonostats', two using wheat bran as a carrier and two using calcium carbonate as a carrier. The additive is proposed to be used at doses of 100-125 mg/kg in chickens and 60-100 mg/kg in turkeys.

To support the Food Standards Agency (FSA) and Food Standards Scotland (FSS) in evaluating the dossier, the Animal Feed and Feed Additives Joint Expert Group (AFFAJEG) and the Advisory Committee on Animal Feedingstuffs (ACAF) were asked to review the dossier and the supplementary information from the applicant.

The AFFAJEG evaluated the identity and characterisation of the additive and concluded that the additive showed incompatibility with tiamulin, erythromycin, oleandomycin and furazolidone.

The AFFAJEG concluded that the additive can be considered safe for the target species at the proposed dose of 100-125 mg/kg in chickens and 60-100 mg/kg in turkeys, although a margin of safety could not be established. There is evidence of safety for consumers at the 6-hour withdrawal mark prior to slaughter. There is no evidence of safety for consumers at the 3-hour withdrawal mark. The additive should be considered irritant to the eyes and highly toxic by inhalation. It was concluded that the additive poses an acceptable risk to the environment.

Based on data presented in six efficacy trials, which showed reduction of lesions and higher weight gain for the treated groups, the AFFAJEG concluded that the product can be considered efficacious for reducing the impact of coccidia species in chickens and turkeys when used at the proposed doses.

The views of AFFAJEG and ACAF have been taken into account in the safety assessment which represents the opinion of the FSA and FSS.

1. Introduction

The FSA and FSS have undertaken a risk assessment for a feed additive (Coxidin ®, Huvepharma NV., Uitbreidingstraat 80, 2600 Antwerp, Belgium) under regulation (EC) No 1831/2003[1] under the category of 'coccidiostats and histomonostats'. Four applications were received for authorisation, two using wheat bran as a carrier and two using calcium carbonate as a carrier:

Carrier calcium carbonate

- A renewal of authorisation in chickens for fattening, turkeys and chickens reared for laying.
- An extension of use in turkeys reared for breeding.

Carrier wheat bran

- A renewal of authorisation in chickens and turkeys for fattening.
- An extension of use in chickens reared for laying and turkeys reared for breeding.

The four applications were evaluated together, as they are formulated with the same concentration of the active substance (monensin sodium) and shared the safety and efficacy data. To support the safety assessment by FSA and FSS, the AFFAJEG and the ACAF provided advice to the FSA and FSS outlined in this document.

The dossier was evaluated on behalf of the FSA and FSS by the AFFAJEG. In line with Article 8 of 1831/2003, the assessment has considered whether the feed additive complies with the conditions laid down in Article 5, including: safety considerations for human, animal and environmental health; efficacy of the additive for its intended effect; potential impairment of the distinctive features of animal products This, and the guidance put in place by EFSA for the evaluation of feed additive applications, has formed the basis and structure for the assessment.

With thanks to the members of the AFFAJEG and ACAF during the course of the assessment, who were: Professor John Wallace, Professor Nicholas Jonsson, Martin Briggs, Dr. Katrina Campbell, Susan MacDonald, Professor Matthew Fisher, Christine McAlinden, Dr. Donald Morrison, Derek Renshaw, Dr. Michael Salter, Dr. Adam Smith, Dr. Helen Warren and Dr. Nick Wheelhouse.

The dossier was evaluated by the AFFAJEG at their June 2021, February 2022 and April 2022 meetings. Further information was provided by the applicant in May 2021 and January 2022, responding to queries by the FSA. The conclusions by the AFFAJEG were reviewed and approved by the ACAF at their October 2022 meeting.

This document outlines the discussion and conclusions of the AFFAJEG's assessment on the safety and efficacy of monensin sodium as a feed additive.

2. Assessment

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

The additive's active substance is monensin sodium, produced from *Streptomyces cinnamonensis*, at a concentration of 250 g/kg, and using either wheat bran or calcium carbonate, as well as perlite, acting as carriers. The applicant provided data from six batches supporting the identification values outlined below (Table 1):

Table 1: Identity table monensin sodium

Composition	
Monensin sodium	250 g/kg
Perlite	150-200 g/kg
Calcium carbonate / Wheat bran	Up to 1.0 kg
Appearance	
Light beige to brown powder	
Chemical-physical specifications	
Loss on drying %	Not more than 8.0
Coxidin® + Wheat bran carrier: Dusting potential Particle size distribution	95 – 100 mg/m³ <90 µm (~19%); 90 – 710 µm (~79%); >710 µm (~2%)
Coxidin® + Calcium carbonate carrier: Dusting potential Particle size distribution	920 – 980 mg/m³ <90 µm (31.8%); 90 – 710 µm (66.5%); >710 µm (1.7%)
Impurities	
Salmonella, CFU/25 g	Absent
Arsenic, mg/kg (at 12% moisture)	Not more than 2.0
Lead, mg/kg (at 12% moisture)	Not more than 10.0
Cadmium, mg/kg (at 12% moisture)	Not more than 1.0
Aflatoxin B_1, mg/kg (at 12% moisture)	Not more than 0.02

The Group noted that the production strain *Streptomyces cinnamonensis* 28682 was correctly characterised. A question was raised regarding the inadequacy of the pelleting stability trials presented in the original application. Upon request of the FSA, the applicant provided new stability tests under stress conditions of up to 86°C for 30 seconds. The AFFAJEG concluded this showed the stability of the additive for the majority of poultry feeds, except for turkeys reared for breeding, for which a *Salmonella* kill-step of 86°C for 6 minutes is common practice in Great Britain.

The AFFAJEG concluded, based on the information presented in the dossier, that the additive is not compatible with the antibiotics tiamulin, erythromycin, oleandomycin and furazolidone. Erythromycin and tylosin were shown to be compatible if used for 7 and 10

days, respectively if the monensin concentration is of 120 mg/kg, but not when used for 14 and 20 days if the monensin concentration is of 360 mg/kg.

The application requested the reduction of the withdrawal period of the additive from 1 to 0 days for its use in chickens for fattening and laying. The AFFAJEG concluded that this reduction in the withdrawal period was not fully supported by information provided (see Safety evaluation). The conditions of use of the additive are described in Table 2:

Table 2: Proposed mode of use of monensin sodium as described in the application

Proposed mode of use in animal nutrition				
Additive		Monensin sodium		
CAS No		22373-78-0		
Category(-ies) of additive		Coccidiostats and histomonostats		
Description				
Composition, description				
Preparation of monensin sodium, perlite and calcium carbonate/wheat bran				
Trade name (if appropriate)		Coxidin®		
Name of the holder of authorisation (if appropriate)		--		
Conditions of use				
Species or category of animal	**Min-max Age**	**Min. content**	**Max. content**	**Withdrawal period**
		Ppm of monensin in complete feedingstuffs		
Chickens	Up to 16 weeks	100	125	1 day
Turkeys	Up to 16 weeks	60	100	1 day

2.1.1. Conclusions on Section II

The AFFAJEG concluded that the additive is stable after pelleting at temperatures of 86ºC for 30 seconds. Stability at 86ºC for 6 minutes was not demonstrated. The additive showed incompatibility with tiamulin, erythromycin, oleandomycin and furazolidone.

No further concerns were raised for Section II of the dossier.

2.2. Section III: Safety

Upon the request by the FSA, the applicant provided an updated literature review to support the evidence of safety of the additive, covering the period from 2011 to 2020. The AFFAJEG evaluated the new literature review and accepted the conclusions presented that no new safety concerns for the target species, consumers, users and environment had been reported.

2.2.1. Safety for the target species

No new data were provided for the renewal of authorisation or the new uses proposed in the applications. The FEEDAP Panel concluded in their 2005 opinion[2] that "tolerance tests showed that Coxidin® is safe for the target animals at the highest recommended level (125 mg/kg for chicken, 100 mg/kg for turkey). However, there was a small margin of safety (of less than twice the maximum recommended dose)". Based on these conclusions and the evaluation of the literature review, the AFFAJEG concluded that the additive can be considered safe for the target species at the proposed dose of 100-125 mg/kg in chickens and 60-100 mg/kg in turkeys.

2.2.2. Safety for the consumer

The application proposed a reduction in the withdrawal period of the additive from 1 day to 0 days. The AFFAJEG evaluated the information presented in the dossier supporting the claim, and determined it was not sufficient to conclude on the safety of the withdrawal period reduction. Upon request of the FSA, the applicant provided a new consumer exposure calculation based on a 6-hour withdrawal period to account for collection of birds, slaughter time and fasting period to reduce carcass contamination. Based on the data presented by the applicant, AFFAJEG members concluded that at the 6-hour mark, the residue levels from the recommended use of Coxidin® in chickens were depleted below the maximum residue level (MRL) of 8 µg/kg kidney, liver and muscle. However, at the 3-hour mark, calculation of residue levels in the liver, based on the formula 'Mean + 2xStandard Deviation (SD)' showed maximum levels of 3.30 + (2x3.98) = 11.26 µg/kg, above the MRL of 8 µg/kg established for liver (Table 3). The AFFAJEG concluded there is evidence of safety for the 6-hour withdrawal mark, but not for the 3-hour withdrawal mark.

Table 3: Residue levels for monensin A sodium in broiler chicken tissues

Withdrawal time (hours)	Mean concentration of monensin A sodium (µg/kg) ± standard deviation			
	Muscle	Kidney	Liver	Skin/fat
0	3.37 ± 1.60	8.68 ± 3.80	16.53 ± 5.85	38.70 ± 12.91
1	1.19 ± 0.59	2.35 ± 1.58	5.24 ± 3.25	15.87 ± 6.93
3	0.92 ± 0.51	2.18 ± 1.70	3.30 ± 3.98	11.30 ± 6.09
6	< LOQ	< LOQ	< LOQ	3.02 ± 1.00

2.2.3. Safety for the user

No new data were provided by the applicant, who referred to previous opinions by the EFSA panel in 2005[2] and 2011[3]. The AFFAJEG evaluated the information presented and concluded that the additive should be considered irritant to the eyes and to be highly

toxic by inhalation. It was concluded the additive is not irritant to the skin and it is not a skin sensitiser. The product is likely to form an inhalable dust, particularly the formulation including calcium carbonate as a carrier.

2.2.4. Safety for the environment

A new Environmental Risk Assessment was provided by the applicant following scientific guidance recommendations, showing results that the release of monensin to the environment as a result of the use of Coxidin® at the recommended dose poses an acceptable risk to the environment. The AFFAJEG evaluated the report and agreed with the conclusion presented that the additive can be considered safe for the environment when used at the proposed concentrations in chickens and turkeys.

2.2.5. Conclusions on safety

- The AFFAJEG concluded that the additive can be considered safe for the target species at the proposed dose of 100-125 mg/kg in chickens and 60-100 mg/kg in turkeys.
- No ample margin of safety for the target species could be established.
- There is evidence of safety for consumers at the 6-hour withdrawal mark prior to slaughter. No evidence of safety for consumers at the 3-hour withdrawal mark.
- The additive should be considered irritant to the eyes and highly toxic by inhalation.
- The additive poses an acceptable risk to the environment.

2.3. Section IV: Efficacy

The Group evaluated Section IV of the dossier, containing evidence of efficacy, presented in six different trials: three battery cage studies in chickens and three anticoccidial sensitivity tests in turkeys.

2.3.1. Battery cage studies in chickens

Three battery cage studies with chickens were carried out using three groups: uninfected-untreated control (UUC), infected-untreated control (IUC) and infected-treated with monensin at a concentration of 100 mg/kg.

In Study 1, animals were treated from day 14 until day 23. Results showed a significantly higher body weight and daily weight gain in the treated group when compared to the IUC group. Significantly lower lesion scores were observed for the treatment group compared to the IUC group (Table 4).

Table 4: Results battery cage study 1

Group	DWG D14-21	DWG D14-22	DWG Day 14-23	FCR	Total mean lesion scores (D21, D22, D23)
UUC	70.2	74.5	80.0*	1.55	1.1**
IUC	73.0	71.7	62.3	1.61	2.8
Coxidin®	71.5	75.8	77.1*	1.56	1.4**

*p-value<0.05 as compared to the IUC group; **p-value<0.001 as compared to the IUC group; DWG: daily weight gain; FCR: feed conversion ratio.

In Study 2, animals were treated from day 12 until day 21. No significant improvement in performance was observed in this study, but significantly lower lesion scores were observed compared to the IUC group (Table 5).

Table 5: Results battery cage study 2

Group	DWG D14-21	DFI D14-21	FCR D14-21	Total mean lesion scores (D19, D20, D21)	OPG *E. acervulina* D20
UUC	53.3	105.0	1.50	0.52**	0**
IUC	52.2	106.0	1.53	1.90	646
Coxidin®	51.3	104.7	1.54	1.08*	4**

*p-value<0.05 as compared to the IUC group; **p-value<0.001 as compared to the IUC group; DFI: daily feed intake.

In Study 3, animals were treated from day 12 until day 21. No significant improvement in performance was observed. Significantly lower lesion scores were observed in the treated group compared to the IUC group (Table 6).

Table 6: Results battery cage study 3

Group	DWG D14-21	DFI D14-21	FCR D14-21	Total mean lesion scores D19	Total mean lesion scores D21	Total mean lesion scores (D19, D20, D21)	OPG *E. acervulina* D20
UUC	47.9	101.07	1.59	0.88**	0.12**	0.35**	13**
IUC	45.8	95.29	1.58	4.75	2.62	3.23	734189
Coxidin®	48.3	100.05	1.56	2.62*	1.69*	2.42*	96449

*p-value<0.05 as compared to the IUC group; **p-value<0.001 as compared to the IUC group;

2.3.2. Anticoccidial sensitivity tests in turkeys

Three anticoccidial sensitivity tests with turkeys were carried out using three groups: uninfected-untreated control (UUC), infected-untreated control (IUC) and infected-treated (IT) with monensin at a concentration of 60 mg/kg.

In Study 1, animals were treated from day 2 until day 13. There was no significant difference in the number of oocysts excreted in the IT group compared to the IUC group. A significant improvement of 8.8% in weight gain weight was observed between the IT

group and the IUC group. Birds in the IT group had a statistically significantly lower FCR (1.53) compared to the IUC group (1.62).

In Study 2, animals were treated from day 2 until day 13. There was no significant difference in the number of oocysts excreted in the IT group compared to the IUC group. A significant improvement of 7.3% in weight gain weight was observed between the IT group and the IUC group. Birds in the IT group had a non-significantly lower FCR (1.58) compared to the IUC group (1.62).

In Study 3, animals were treated from day 14 until day 22. No significant differences were observed in DFI and FCR amongst the groups included in the study. A significant difference was found in parasitological parameters, including lower lesion scores, number of oocysts excreted, and clinical signs in the IT group compared to the IUC group.

2.3.3. Conclusions on efficacy

The Group concluded that the trials had been conducted following recommended standards and that their conclusions were sufficient to demonstrate efficacy of the additive.

The AFFAJEG concluded that the product can be considered efficacious for reducing the impact of coccidia species in chickens and turkeys when used at the proposed doses.

3. Analytical methods evaluation

Conclusions on the analytical methods are presented here as an extract from the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Method(s) of the Analysis for Coxidin®4:

“For the quantification of monensin in the feed additive, premixtures and feedingstuffs the applicant submitted single-laboratory validated methods based on Reversed Phase High Performance Liquid Chromatography with post-column derivatisation coupled to spectrophotometric detection (RP-HPLC-PCD-UV-Vis). In addition, the EURL identified another ring-trial validated method (EN ISO 14183) using a similar experimental protocol. Based on the performance characteristics available the EURL recommends for official control the EN ISO 14183 method for the quantification of monensin in the feed additive, premixtures and feedingstuffs.

For the quantification of monensin sodium in chicken and turkey tissues the applicant submitted a single-laboratory validated method based on Reversed Phase High Performance Liquid Chromatography coupled to a triple quadrupole mass spectrometer (RP-HPLC-MS/MS) in an electrospray ionisation mode without providing a verification study. The EURL found instead a similar single-laboratory validated and further verified method submitted by the same applicant in the frame of another monensin dossier consisting of a Reversed Phase High Performance Liquid Chromatography coupled to a triple quadrupole mass spectrometer (RP-HPLC-MS/MS). Based on the performance characteristics available, the EURL recommends for official control this single-laboratory

validated and further verified method or any equivalent methods complying with the requirements set by Commission Decision 2002/657/EC, to enforce the monensin sodium MRLs in the relevant tissues."

FSA/FSS accepts the EURL analytical method evaluation reports. FSA/FSS determined the analytical method as appropriate for official controls for this feed additive.

4. Conclusions

The AFFAJEG evaluated the identity and characterisation of the additive and concluded that it is stable at temperatures of 86°C for 30 seconds, although stability at 86°C for 6 minutes was not proven. The additive showed incompatibility with tiamulin, erythromycin, oleandomycin and furazolidone.

The AFFAJEG concluded that the additive can be considered safe for the target species at the proposed dose of 100-125 mg/kg in chickens and 60-100 mg/kg in turkeys, although an ample margin of safety could not be established. There is evidence of safety for consumers at the 6-hour withdrawal mark prior to slaughter. There is no evidence of safety for consumers at the 3-hour withdrawal mark. The additive should be considered irritant to the eyes and highly toxic by inhalation. It was concluded that the additive poses an acceptable risk to the environment.

Based on data presented in six efficacy trials, which showed reduction of lesions and higher weight gain for the treated groups, the AFFAJEG concluded that the product can be considered efficacious for reducing the impact of coccidia species in chickens and turkeys when used at the proposed doses.

FSA/FSS accepts the EURL analytical method evaluation reports. FSA/FSS determined the analytical method as appropriate for official controls for this feed additive.

5. References

1. EC (European Commission), 2003. Regulation No 1831/2993 of the European Parliament and of the Council on additives for use in animal nutrition. Available at https://www.legislation.gov.uk/eur/2003/1831/contents
2. EFSA (European Food Safety Authority), 2005. Opinion of the Scientific Panel on additives and products or substances used in animal feed (FEEDAP) on the evaluation of the coccidiostat COXIDIN® (Monensin Sodium). The EFSA Journal, 283, 1-53. https://doi.org/10.2903/j.efsa.2005.291
3. EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP), 2011. Scientific Opinion on the safety and efficacy of Coxidin® (monensin sodium) as a feed additive for chickens reared for laying. EFSA Journal;9(12):2442. https://doi.org/10.2903/j.efsa.2011.2442
4. EURL-FA (European Reference Laboratory for Feed Additives), 2017. Evaluation Report on the Analytical Methods submitted in connection with the Application for

Authorisation of a Feed Additive according to Regulation (EC) No 1831/2003. Monensin sodium. Available at: https://joint-research-centre.ec.europa.eu/publications/fad-2016-0009_en

6. Abbreviations

ACAF	Advisory Committee on Animal Feedingstuffs
AFFAJEG	Animal Feed and Feed Additives Joint Expert Group
DFI	Daily feed intake
DWG	Daily weight gain
EC	European Commission
EFSA	European Food Safety Authority
EURL	European Union Reference Laboratory
EURL-FA	European Reference laboratory for feed additives
FCR	Feed conversion ratio
FEEDAP	EFSA Panel on Additives and Products or Substances used in Animal Feed
FSA	Food Standards Agency
IT	Infected-treated
IUC	Infected-untreated-control
LOQ	Limit of quantification
MRL	Maximum residue limits
RP-HPLC-MS/MS	Reversed Phase High Performance Liquid Chromatography coupled to a triple quadrupole mass spectrometer
RP-HPLC-PCD-UV-Vis	Reversed Phase High Performance Liquid Chromatography with post-column derivatisation coupled to spectrophotometric detection
UUC	Uninfected-untreated-control

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