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Safety and efficacy of Elancoban® G200 (monensin sodium) for chickens for fattening, chickens reared for laying and turkeys

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Abstract

The feed additive Elancoban® G200, containing the active substance monensin sodium, an ionophore anticoccidial, is intended to control coccidiosis in chickens for fattening, chickens reared for laying and turkeys. The FEEDAP Panel cannot conclude on the safety of the additive for the target species, consumer, user and environment with regard to the safety of the production strain. The following conclusions apply to monensin sodium included in the additive. Based on the available data set, the FEEDAP Panel cannot conclude on the safety of Elancoban® G200 for chickens for fattening. Monensin sodium is safe for turkeys for fattening with a margin of safety of 1.5. Monensin sodium is not genotoxic and not carcinogenic. The pharmacological no observed adverse effect level (NOAEL) of 0.345 mg monensin sodium/kg body weight (bw) per day was identified in dog. The acceptable daily intake (ADI) derived from this NOAEL is 0.003 mg monensin sodium/kg bw applying an uncertainty factor of 100. Elancoban® G200 is safe for the consumer. The existing maximum residue limits (MRLs) ensure consumer safety, provided that the withdrawal period of 1 day is respected. Elancoban® G200 is very irritant for the eye, but it is not a skin irritant. Elancoban® G200 should be regarded as a potential skin and respiratory sensitiser. Inhalation exposure is considered a risk to persons handling the additive. Elancoban® G200 does not pose a risk for the terrestrial compartment, the aquatic compartment and the sediment. The bioaccumulation potential of monensin in the environment is low. Monensin sodium from Elancoban® G200 has the potential to effectively control coccidiosis in chickens for fattening and chickens reared for laying. The FEEDAP Panel cannot conclude on the efficacy Elancoban® G200 as a coccidiostat for turkeys for fattening.

Keywords: coccidiostats, monensin sodium, chickens for fattening, chickens reared for laying, turkeys, safety and efficacy

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Summary

Following a request from the European Commission, the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) was asked to deliver a scientific opinion on the coccidiostat Elancoban® G200 containing monensin sodium as active substance produced by fermentation.

Limited data on the taxonomic identification of the production strain did not allow the proper identification of the strain as *Streptomyces cinnamonensis*. The FEEDAP Panel cannot conclude on the absence of genetic determinants for antimicrobial resistance in the production strain, on the presence of viable cells/spores of the production strain/DNA in the final product. Therefore, the FEEDAP Panel cannot conclude on the safety of the additive for the target species, consumer, user and environment with regard to the safety of the production strain.

The following conclusions apply to monensin sodium included in the additive.

Based on the available data set, the FEEDAP Panel cannot conclude on the safety of the highest applied dietary concentration of monensin (125 mg/kg) for chickens for fattening and to derive a margin of safety. Monensin sodium is safe for turkeys for fattening at inclusion level of 100 mg/kg complete feed, with a margin of safety of 1.5. Gram-positive bacteria are susceptible to monensin. Monensin sodium is not considered to be involved in cross-resistance to other antibiotics. The use of monensin as coccidiostat in chickens did not affect the colonisation or shedding of *Salmonella* in the gastrointestinal tract. The simultaneous use of Elancoban® G200 and certain antibiotic drugs (e.g. tiamulin) is contraindicated.

Monensin sodium is not genotoxic and, based on the results of chronic carcinogenicity studies performed in rats and mice, is not carcinogenic. The lowest no observed adverse effect level (NOAEL) identified from all the toxicological studies was 1.1 mg/kg body weight (bw) per day based on a 2-year chronic toxicity/carcinogenicity assay in rat. The pharmacological NOAEL of 0.345 mg monensin sodium/kg bw per day identified in a dog for acute pharmacological effects on the cardiovascular system has been considered as an appropriate basis for the acute health-based guidance value of 0.003 mg monensin sodium/kg bw per day already established by the FEEDAP Panel in its former opinion applying an uncertainty factor of 100.

The chronic exposure to monensin residues resulting from the use of monensin sodium as a feed additive in chickens would amount to up to 10% of the acceptable daily intake (ADI) (toddlers). The combined chronic exposure to monensin residues resulting from use of monensin as a feed additive in chickens and as a veterinary medicine in bovine would reach up to 48% of the ADI. Acute exposures due to the consumption of poultry tissues were found below the ADI for all age classes. These conclusions are extrapolated to chickens reared for laying and turkeys for fattening. Based on this, the FEEDAP Panel considers that Elancoban® G200 containing monensin sodium is safe for the consumer.

The existing maximum residue limits (MRLs) for poultry tissues ensure consumer safety (acute and chronic exposure; all age classes) provided that the withdrawal period of 1 day is respected.

Elancoban® G200 is very irritant for the eye, but it is not a skin irritant. However, systemic toxicity may occur following skin exposure. Elancoban® G200 should be regarded as a potential skin and respiratory sensitiser. Inhalation exposure is considered a risk to persons handling the additive.

The use of monensin sodium from Elancoban® G200 in complete feed for chickens for fattening does not pose a risk for the terrestrial compartment, the aquatic compartment and the sediment. The bioaccumulation potential of monensin in the environment is low. These conclusions are extended to chickens reared for laying and turkeys.

Monensin sodium from Elancoban® G200 has the potential to effectively control coccidiosis at the minimum applied dose of 100 mg/kg complete feed in chickens for fattening and chickens reared for laying. In turkeys for fattening, the efficacy of 60 mg monensin sodium/kg complete feed from Elancoban® G200 was demonstrated in three floor pen studies and in two anticoccidial sensitivity tests (ASTs), a third AST failing to show anticoccidial efficacy. The FEEDAP Panel is therefore not in the position to conclude on the efficacy of monensin sodium from Elancoban® G200 as a coccidiostat for turkeys for fattening.

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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 Eli Lilly and Company Ltd[2] for re-evaluation of the product Elancoban® G200 (monensin sodium), when used as a feed additive for chickens for fattening, chickens reared for laying 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 13 June 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 Elancoban® G200 (monensin sodium), when used under the proposed conditions of use (see Section 3.1.4).

1.2. Additional information

The additive Elancoban® G200 (monensin sodium) has been authorised for 10 years for use in chickens for fattening, chickens reared for laying and turkeys (authorisation until 30 July 2014).[3] The authorisation has been amended as regards the introduction of a maximum residue limit[4] (MRL) for monensin sodium and the modification of the withdrawal period.[5] The MRLs in force for all the target species are 25 μg monensin sodium/kg of wet skin + fat and 8 μg monensin sodium/kg of wet liver, kidney and muscle. The withdrawal period is 1 day.

The product Coxidin® containing the same active substance (monensin sodium) has been authorised for use in chickens for fattening, turkeys (authorisation until 10 June 2021)[6] and chickens reared for laying (authorisation until 9 March 2022).[7]

The European Food Safety Authority (EFSA) issued an opinion on the re-evaluation of the coccidiostat Elancoban® (monensin sodium) for chickens for fattening, chickens reared for laying and turkeys (EFSA, 2004) followed by the opinions on the MRLs for monensin sodium for chickens and turkeys for fattening (EFSA, 2006a) and on the withdrawal period for Elancoban® for chickens for fattening, chickens reared for laying and turkeys for fattening (EFSA, 2008a). In addition, an opinion on the safety and efficacy of Elancoban® (monensin sodium) as a feed additive for calves for rearing and cattle for fattening was issued (EFSA, 2006b).

[1] Regulation (EC) No 1831/2003 of the European Parliament and of the Council of 22 September 2003 on additives for use in animal nutrition. OJ L 268, 18.10.2003, p. 29.
[2] On 15/6/2018, EFSA was informed by the applicant that the applicant company changed to Elanco GmbH (Heinz-Lohmann-Str. 4, 27472 Cuxhaven Germany).
[3] Commission Regulation (EC) No 1356/2004 of 26 July 2004 concerning the authorisation for 10 years of the additive 'Elancoban' in feedingstuffs, belonging to the group of coccidiostats and other medicinal substances. OJ L 251, 27.7.2004, p. 6.
[4] Commission Regulation (EC) No 180/2007 of 5 February 2007 amending Regulation (EC) No 1356/2004 as regards the conditions for authorisation of the feed additive Elancoban, belonging to the group of coccidiostats and other medicinal substances. OJ L 31, 6.2.2007, p. 4.
[5] Commission Regulation (EC) No 1096/2008 of 6 November 2008 amending Regulation (EC) No 1356/2004 as regards the conditions for authorisation of the feed additive Elancoban, belonging to the group of coccidiostats and other medicinal substances. OJ L 298, 7.11.2008, p. 5.
[6] Commission Implementing Regulation (EU) No 495/2011 of 20 May 2011 amending Regulation (EC) No 109/2007 as regards the composition of the feed additive monensin sodium. OJ L 134, 21.5.2011, p. 6.
[7] Commission Implementing Regulation (EU) No 140/2012 of 17 February 2012 concerning the authorisation of monensin sodium as a feed additive for chickens reared for laying. OJ L 47, 18.2.2012, p. 18.

The same active substance present in different products were also evaluated by the FEEDAP Panel; seven opinions were issued on the product Coxidin® (EFSA, 2005, 2006a,c, 2007a, 2008b, EFSA FEEDAP Panel, 2011a,b, 2013) and two opinions on the product Monimax® (monensin sodium and nicarbazin) (EFSA FEEDAP Panel, 2017a, 2018a).

The Committee for Medicinal Products for Veterinary Use (CVMP) of the European Medicine Agency (EMA) issued two reports on monensin when used in cattle, including dairy cows (EMA-CVMP, 2007 and (EMA-CVMP, 2013). The Joint FAO/WHO Expert Committee on Food Additives (JECFA) evaluated monensin sodium for its use as a veterinary drug (JECFA, 2008, 2012).

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[8] in support of the authorisation request for the use of Elancoban® G200 (monensin sodium) 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 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 the marker residue in tissues. The Executive Summary of the EURL report can be found in Annex A.[9]

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of Elancoban® G200 (monensin sodium) is in line with the principles laid down in Regulation (EC) No 429/2008[10] and the relevant guidance documents: Guidance for the preparation of dossiers for coccidiostats and histomonostats (EFSA FEEDAP Panel, 2011c), Technical guidance: Tolerance and efficacy studies in target animals (EFSA FEEDAP Panel, 2011d), Technical Guidance for assessing the safety of feed additives for the environment (EFSA, 2008c), Guidance for the preparation of dossiers for the re-evaluation of certain additives already authorised under Directive 70/524/EEC (EFSA, 2008d), Guidance for establishing the safety of additives for the consumer (EFSA FEEDAP Panel, 2012a), Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012b), Technical Guidance: Microbial Studies (EFSA, 2008e) and Technical Guidance: Extrapolation of data from major species to minor species regarding the assessment of additives for use in animal nutrition (EFSA, 2008f).

3. Assessment

The present opinion assesses the safety and efficacy of the coccidiostat Elancoban® G200 containing monensin sodium as active substance when used as a feed additive in chickens for fattening, chickens reared for laying and turkeys for fattening.

3.1. Characterisation

3.1.1. Characterisation of the active substance

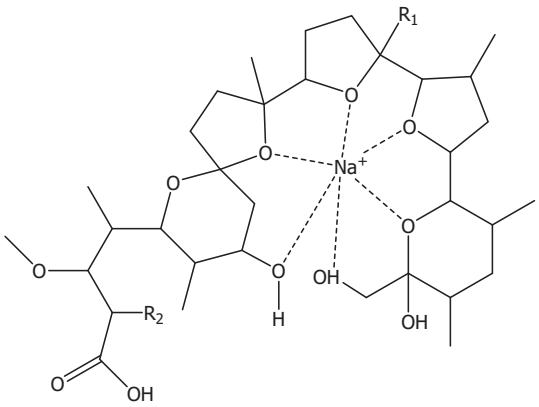
Monensin sodium is a polyether ionophore produced by fermentation by a culture of *Streptomyces* spp.[11] The manufacturing process is fully described in the technical dossier. No changes in the manufacturing process have been introduced since the FEEDAP Panel made the first assessment in 2004 (EFSA, 2004). [redacted] The composition of monensin granulated is given as follows: monensin activity (23%), pelleting aid clay (1%), mycelial solids (36.2%) and rice hulls (39.8%). Monensin granulated is specified to contain ≥ 140 mg monensin

[8] FEED dossier reference: FAD-2013-0037.
[9] The full report is available on the EURL website: https://ec.europa.eu/jrc/sites/default/files/finrep-fad-2013-0037-elancoban.pdf
[10] Commission Regulation (EC) No 429/2008 of 25 April 2008 on detailed rules for the implementation of Regulation (EC) No 1831/2003 of the European Parliament and of the Council as regards the preparation and the presentation of applications and the assessment and the authorisation of feed additives. OJ L 133, 22.5.2008, p. 1.
[11] Technical dossier/Section II/Annex II.8 and II.9.

activity/g. [redacted]

Monensin sodium (CAS number 22373-78-0) consists of the main chemical form monensin A sodium (sodium 4-(2-(2-ethyl-5′-(6-hydroxy-6-(hydroxymethyl)-3,5-dimethyltetrahydro-2*H*-pyran-2-yl)-3′-methyloctahydro-[2,2′-bifuran]-5-yl)-9-hydroxy-2,8-dimethyl-1,6-dioxaspiro[4.5]decan-7-yl)-3-methoxy-2-methylpentanoate, $C_{36}H_{61}NaO_{11}$, molecular weight 692.86), monensin B sodium (sodium 4-(9-hydroxy-2-(5′-(6-hydroxy-6-(hydroxymethyl)-3,5-dimethyltetrahydro-2*H*-pyran-2-yl)-2,3′-dimethyloctahydro-[2,2′-bifuran]-5-yl)-2,8-dimethyl-1,6-dioxaspiro[4.5]decan-7-yl)-3-methoxy-2-methylpentanoate, $C_{35}H_{59}NaO_{11}$, molecular weight 678.84) and monensin C sodium (sodium 2-ethyl-4-(2-(2-ethyl-5′-(6-hydroxy-6-(hydroxymethyl)-3,5-dimethyltetrahydro-2*H*-pyran-2-yl)-3′-methyloctahydro-[2,2′-bifuran]-5-yl)-9-hydroxy-2,8-dimethyl-1,6-dioxaspiro[4.5]decan-7-yl)-3-methoxypentanoate, $C_{37}H_{63}NaO_{11}$, molecular weight 706.89).

The structural formula of monensin sodium is given in Figure 1.



Forms	R_1	R_2
Monensin A sodium	C_2H_5	H
Monensin B sodium	CH_3	H
Monensin C sodium	C_2H_5	CH_3

Figure 1: Structural formula of monensin sodium

The specifications for the composition of monensin are the following: not less than 90% of the monensin activity must be monensin A, not less than 95% of the monensin activity must be monensin A + monensin B. [redacted]

The concentration of monensin sodium is expressed as monensin activity which includes the relative biopotency in terms of 'monensin activity' of the different monensin variants. The relative biopotency is based on the microbiological responses against *Enterococcus faecium* and was determined as 1.000, 0.280, 1.560 and 1.484 for variants A, B, C and D,[13] respectively. Chemical composition of monensin sodium granulated was determined by high-performance liquid chromatography (HPLC) analysis and resulted in 94.67%, 3.98%, 0.26% and 0.57% for variants A, B, C and D, respectively. Monensin activity was determined multiplying the chemical concentrations by the relative biopotency responses resulting in biopotencies of 946.7, 11.1, 4.4 and 8.5 μg/mg for variants A, B, C and D, respectively. Calculating the percentage of biopotency contributions (97.6, 1.1, 0.4 and 0.9%) the results are similar to the chemical compositions.

Pure monensin sodium is a white crystalline solid having a melting point of 267–269°C. Its solubility in water is 4.8 mg/L at pH 7 and 8.9 mg/L at pH 9. It is very soluble in ethyl acetate, acetone, chloroform and dimethyl sulfoxide, slightly soluble in hexane and petroleum ether.

[redacted]

[13] Chemical formula and the molecular structure for variant D was not reported by the applicant.

3.1.1.1. Characterisation of the production organism

The active substance monensin sodium is produced by fermentation with a non-genetically modified strain of *Streptomyces* spp. The producing strain was originally identified as *Streptomyces cinnamonensis* and it is deposited in the American Type Culture Collections with the accession number ATCC 15413.

[redacted] the assignation of the production strain to *S. cinnamonensis* species cannot be confirmed. More detailed data on the taxonomic identification were not provided.

Data on antimicrobial susceptibility of the production strain were not provided. Consequently, the FEEDAP Panel cannot conclude on the absence of genetic determinants encoding antimicrobial resistances in the *Streptomyces* spp. under assessment (Guidance on the characterisation of microorganisms used as feed additives or as production organisms, EFSA FEEDAP Panel, 2018b).

The absence of antimicrobial compounds relevant to the use of antibiotics in humans or animals, other than the monensin sodium in the mycelial product, was assessed[15] comparing the minimum inhibitory concentrations (MIC) of three batches of the fermentation product with three batches of pure monensin [redacted] the product is considered free of antimicrobial activity other than monensin.

A literature review[17] has been carried out to assess the information available on the potential of *S. cinnamonensis* to produce secondary metabolites.[18] Since a conclusive identification of the production strain as *S. cinnamonensis* has not been provided, these data were not considered relevant for the current assessment.

3.1.2. Characterisation of the additive

[redacted] The final additive is produced by mixing monensin granulated with 10–25 g antidusting oil/kg additive and rice hulls or granular limestone which is used to ensure monensin content of Elancoban® G200 within the limit of specification. Elancoban® G200 contains by specification 185–215 g monensin activity/kg. [redacted] The concentrations of heavy metals, arsenic and dioxins were considered of no concern.

The presence of viable cells of the production strain was investigated [redacted]

[redacted]

[15] Technical dossier/Supplementary information January 2018/Annex 36.

[redacted]

[17] Databases searched: Agriculture Online Access/STN, Biosis Previews/STN, CAB Abstracts/STN, Chemical Abstracts Plus/STN, Derwent Biotechnology Resource/STN, Derwent Drug Backfile/STN, Derwent Drug File/STN, Embase/STN, Embase Alert/STN, Food Science and Technology Abstracts/STN, Foodline Science/STN, International Pharmaceutical Abstracts/STN, Medline/STN, Pascal/STN, ProQuest Science & Technology/STN, Science Citation Index/STN; Period: 2005–2015.

[18] Technical dossier/Supplementary information January 2018/Annex 37.

[redacted]

[redacted] the presence of viable cells/spores cannot be excluded.

The FEEDAP Panel recognises that the manufacturing process, [redacted] will probably eliminate all vegetative cells and reduce the number of spores of the production strain in the monensin product.

The applicant did not provide evidence of the absence of the DNA of the production strain. Therefore, uncertainty remains on the presence of viable cells of the production organism and/or its DNA, including the possible presence of genes encoding antibiotic resistances, in the final product.

[redacted] Particle size distribution measured by laser diffraction in three batches of the additive indicated [redacted] The dusting potential [redacted] The particle size distribution of the dust [redacted] The monensin content of the dust was similar to that of the additive [redacted]

3.1.3. Stability and homogeneity

3.1.3.1. Shelf-life of the additive

Six batches of Elancoban® G200 were stored at 25°C for 24 months. Monensin activity measured at the beginning and at the end of the study showed recoveries in the range of 97.8–100.6% (loss on drying was constant). Other three batches were put on stability test at 30°C/65% relative humidity (RH) for 24 months and at 40°C/75% RH for 12 months with recoveries of 94.9–97.1 and 93.4–96%, respectively, at the end of the studies. In both cases, the water content as indicated by loss on drying increased in the first 3 months of the experiment, and then remained constant (~ 6% and ~ 7%, respectively). The packaging material was not reported.[28]

3.1.3.2. Stability in premixtures and feedingstuffs

Three batches of Elancoban® G200 were incorporated in a vitamin/mineral premixture (with choline chloride) for chickens for fattening containing 5 g monensin sodium/kg premixture.[29] The samples were stored at 25°C/60% RH for 6 months and at 40°C/60% RH for 3 months in permeable paper bags. Water content as indicated by loss on drying increased in the first month of storage and then remained constant (5.6% at 25°C and 4.8% at 40°C). The average recovery rate was determined to be 90%.

A similar study design was applied using a complete diet for chickens for fattening[30] (100 and 125 mg monensin sodium/kg feed) and for turkeys for fattening (60 and 100 mg monensin sodium/kg feed).[31] Mash and pelleted samples (pelleting temperature 76–77°C) were stored at 25°C/60% RH for up to 3 months and at 40°C for up to 1 month in permeable paper bags. Concentrations of monensin sodium measured at the end of the storage periods showed recoveries of 92% and 96% for chicken mash feed and 90% and 88% turkey mash feed, respectively for the standard and the accelerated conditions of the stability test. Moisture content of the samples was constant (loss on drying was

[redacted]

[28] Technical dossier/Section II and Supplementary information July 2014.
[29] Technical dossier/Section II/Annex II.15 and II.16.
[30] Technical dossier/Section II/Annex II.17.
[31] Technical dossier/Section II/Annex II.18.

between 10% and 11%). The same measurements were performed with pelleted feed; the results were similar to those obtained with mash feed, thus it was concluded that pelleting did not affect stability.

3.1.3.3. Homogeneity

The capacity of monensin sodium to homogeneously distribute was studied in 10 subsamples of the premixtures,[32] mash feed for chickens for fattening[30] and mash feed for turkeys[31] described above. The coefficients of variation (CVs) for premixture (three batches) were 3.4, 3.8 and 4.5%. Regarding chicken mash feed, two concentrations were tested (100 and 125 mg monensin sodium/kg feed). CVs for the lower dose were 6.3, 9.0 and 5.0% and for the higher dose were 7.1, 6.2 and 6.1% (thee batches each dose). Regarding turkey mash feed, two concentrations were tested (60 and 100 mg monensin sodium/kg feed). CVs for the lower dose were 10.4, 10.0 and 8.2% and for the higher dose were 7.2, 9.4 and 7.1% (thee batches each dose).

3.1.4. Conditions of use

Elancoban® G200 is intended to be used for the prevention of coccidiosis in chickens for fattening at a concentration of 100–125 mg monensin/kg complete feed, in chickens reared for laying at a concentration of 100–120 mg monensin/kg complete feed up to a maximum of 16 weeks of age and in turkeys at a concentration of 60–100 mg monensin/kg complete feed up to a maximum of 16 weeks of age. The withdrawal period in chickens and turkeys is 1 day.

3.2. Safety

3.2.1. Safety of the production strain

Uncertainties remain on the identification and characterisation of the production strain, including the presence of antimicrobial resistance genes. Moreover, the data provided does not allow to exclude the presence of viable cells/spores of the production strain in the additive. In addition, no data has been submitted on the presence of DNA of the production strain in the final additive. Therefore, considering all the above, the Panel cannot conclude on the safety of the additive with regard to the production strain.

3.2.2. Absorption, distribution, metabolism and excretion

No new absorption, distribution, metabolism and excretion (ADME) studies have been provided by the applicant. The data submitted concerning the metabolic fate of monensin sodium in the chicken, turkeys and rat derive from studies already assessed by the FEEDAP Panel in previous opinions on Elancoban® G200 (EFSA, 2004, 2008a). The conclusions can be summarised as follows: (i) monensin sodium is absorbed to a limited extent and this fraction is eliminated largely through bile. It is metabolised extensively by the chicken and turkey; its metabolic fate is similar in these two species and also in the rat, (ii) a great number of metabolites has been isolated from the excreta and tissues, of which eight major metabolites have been identified, each representing less than 10% of the whole residues, (iii) the metabolites result from either single or combined demethylation, decarboxylation and hydroxylation of the molecule, (iv) unchanged monensin in chicken and turkey excreta amounts to less than 20% and 8% of the whole monensin derived metabolites, respectively, (v) monensin represents less than 5% of tissue residues; however, it is considered as the marker residue by default, and (vi) selected monensin hydroxylated metabolites showed reduced biological activity compared to monensin; consequently, the FEEDAP Panel concluded that, taking a weight of evidence approach, monensin-derived residues of toxicological relevance determined after a 1-day withdrawal period probably represent, as a conservative estimate, not more than 50% of total residues.

3.2.3. Residues

The applicant submitted the total and marker residue studies in chickens for fattening previously assessed by the FEEDAP Panel (EFSA, 2004, 2006a, 2008a). The applicant submitted also one new residue study of monensin in turkey tissues and a new residue study in the eggs of chicken reared for laying.

The conclusions of the former FEEDAP Panel assessments and the analysis of the additional studies are reported below.

[32] Technical dossier/Section II/Annex II.15.

Total residues

Monensin sodium total residues were measured in a study carried out in chickens for fattening administered 125 mg [^{14}C]-monensin sodium/kg feed for 6 days (EFSA, 2004). A similar study was performed in turkeys administered 110 mg [^{14}C]-monensin sodium/kg feed for 5 days (EFSA, 2004, 2006a). The results are reported on Table 1.

Table 1: Total residues (expressed as mg monensin equivalent/kg) in tissues of chickens for fattening administered 125 mg [^{14}C]-monensin sodium/kg feed for 6 days and of turkeys for fattening administered 110 mg monensin sodium/kg feed for 5 days

Withdrawal time	Liver	Kidney	Muscle	Skin/fat
Chicken				
0-day	0.935 ± 0.201(1) (1.377)(2)	0.197 ± 0.043 (0.283)	0.059 ± 0.016 (0.091)	0.287 ± 0.138 (0.563)
1-day	0.468 ± 0.075 (0.618)	0.142 ± 0.024 (0.190)	0.047 ± 0.009 (0.065)	0.171 ± 0.065 (0.301)
Turkey				
0.25-day	0.909 ± 0.184 (1.277)	0.159 ± 0.031 (0.221)	< 0.03	0.121 ± 0.025(3) (0.171)

(1): Average ± SD.
(2): Average + 2 SD.
(3): Values calculated from skin and fat data, using the proportion one-third skin and two-third fat.

Total residues measured after 6-h withdrawal in turkey liver and kidney were close to those measured in chicken corresponding tissues at steady state, lower in the muscle and skin/fat. The FEEDAP Panel considered that the physiological proximity of chicken and turkey, two species belonging to the Galliformes, added to the similar qualitative metabolic fate of monensin in both species, justify the use of the chicken data when assessing consumer exposure to total residue (see Section 3.2.5). This supports the view that chickens offer a worst-case situation, also considering that the administered dose is lower in turkeys.

Marker residue

Monensin sodium concentration in chicken and turkey tissues was measured in studies already assessed previously by the FEEDAP Panel (EFSA, 2004, 2006a, 2008a).

In two publications which were not available for the previous assessments (Henri et al., 2009, 2012), monensin sodium concentration in chicken and turkey tissues were measured. Six animals per time point were slaughtered after 0, 2, 4, 6, 8, 10, 12, 14, 24 and 72 h. Liver, muscle and fat were sampled and analyzed for monensin content using a validated liquid chromatography with tandem mass spectrometry (LC–MS/MS) method (Table 2). The relevant results are reported in Table 2.

Table 2: Monensin residues in tissues (mg/kg) of chickens administered 125 mg monensin sodium/kg feed for 33 days and of turkeys administered 100 mg monensin sodium/kg feed for 56 days

Withdrawal time (h)	Liver	Muscle	Fat
Chicken			
0	0.022(1) (6)(2)	0.009 (5)	0.066 (6)
6	0.002 (2)	0.003 (1)	0.017 (6)
12	< LOQ(3)	< LOQ	0.007 (6)
Turkey			
0	0.006 (6)	0.007 (4)	0.055 (6)
6	0.005 (2)	0.007 (2)	0.117 (6)
12	< LOQ	< LOQ	0.005 (2)

(1): Average + 2 SD.
(2): Number of samples analysed.
(3): Limit of quantification (LOQ) of 0.001 monensin/kg liver and 0.0025 mg/kg muscle and fat.

Residual monensin values in liver, muscle and fat, for withdrawal times comprised between zero and one day (Table 2) indicates that all monensin concentrations were below the MRLs after 6 h withdrawal in chicken and 12 h in the turkey. However, due to the limitations in the data set (absence of data for kidneys, the measurement of monensin residues in fat instead of skin/fat and the reduced number of animals tested after 6 h withdrawal in liver and muscle, and after 12 h in the fat) the withdrawal time of 1 day should be maintained.

A study of monensin residues in the first eggs laid by chickens reared for laying has been submitted.[33] Fifty-one-day-old Isa-Brown pullets were kept in a single pen for 16 weeks and fed a complete feed (starter feed until day 43 then grower feed until day 112) supplemented with 0.625 mg Elancoban® G200/kg corresponding to 125 mg monensin sodium/kg (analysed, average of three samples each: 127 and 131 mg/kg, respectively). The pullets were then distributed in individual cages and fed an unsupplemented feed (absence of monensin confirmed). The first two eggs were laid on d-129, then all eggs were collected individually until 10 eggs for at least 10 hens were obtained. Individual egg contents were homogenised and kept at −20°C until analysis. Monensin residues were determined using a validated LC–MS/MS method with a limit of detection (LOD) of 0.06 μg/kg. Monensin content of all eggs was below the LOD.

3.2.4. Safety for the target species

For chickens for fattening the applicant re-submitted the same four tolerance studies which were already provided for the first assessment (EFSA, 2004). Since the current assessment is based on Regulation (EC) No 429/2008, the studies were re-assessed with reference to the EFSA Technical guidance: Tolerance and efficacy studies in target animals (EFSA FEEDAP Panel, 2011d).

For turkeys for fattening, the applicant re-submitted the three tolerance studies already provided and assessed in the 2004 opinion. Since all of them were far from current standards, the applicant was requested to provide a recent tolerance study in turkeys in accordance with Reg (EC) No 429/2008.

3.2.4.1. Tolerance studies in chickens for fattening

The design of the submitted four studies in chickens for fattening is summarised in Table 3.[34] Three of them compared also the toxicity of different monensin sources (e.g. mycelial or crystalline monensin). Since these studies did not show a consistent or relevant effect of the monensin source, data obtained are reported and assessed in the current opinion as mean values irrespective of the monensin source, focusing only on the dietary monensin level. Although male and female birds (1 day old) were used in all studies and sex was considered in statistics as a variable, mean values are used since interactions between monensin treatment and sex were not consistently (rather accidentally) observed and not considered relevant due to the low number of replicates.

Table 3: Design of the tolerance studies in chickens for fattening

Study	Year of the study	Total no animals replicates/treatment (birds/replicate)	Breed sex duration (days)	Monensin sources	Concentrations tested (mg monensin/kg)	Typical feed composition
1	1966	1,600 2 M and 2 F(1) (50)	Arbor Acre Male/Female 56	Crude, crystalline	0 147 442 737	Maize, soybean meal(2)
2	1971	1,600 2 M and 2 F(3) (100)	Breed not given Male/Female 56	Commercial additive	0 121 363 606	Maize, soybean meal(4)
3	1969/70	672 2 M and 2 F(1) (12)	Breed not given Male/Female 56	Mycelial, crystalline	0 121 363 605	Maize, soybean meal(4)

[33] Technical dossier/Supplementary information January 2018/Annex 47.
[34] Study 1: Technical dossier/Section III/Annex III.18. Study 2: Annex III.19. Study 3: Annex III.16 and Study 4: Annex III.17.

Study	Year of the study	Total no animals replicates/treatment (birds/replicate)	Breed sex duration (days)	Monensin sources	Concentrations tested (mg monensin/kg)	Typical feed composition
4	1973/74	384 2 M and 2 F(1) (12)	Hubbard × White Mountain Cross Male/Female 56	Mycelial, crystalline	0 121 363 605	Maize, soybean meal(5)

(1): 4 M and 4 F for the control group.
(2): Starter: 23.0% crude protein (CP), 0.44% methionine (Met), 13.1 MJ metabolisable energy (ME)/kg; finisher: 20.7% CP, 0.35% Met, 13.5 MJ ME/kg.
(3): A fifth replicate with 50 M + 50 F was not considered in the statistics, total number of birds is without these replicates.
(4): Starter: 24.4% CP, 0.57% Met, 12.7 MJ ME/kg; finisher: 24.3% CP, 0.52% Met, 13.2 MJ ME/kg.
(5): Starter: 24.0% CP, 0.53% Met, 13.1 MJ ME/kg; finisher: 21.1% CP, 0.64% Met, 13.7 MJ ME/kg.

No statistical methods were reported for study 1.[35] Treatment effects in study 2 were analysed for the four pens with either 100 males or 100 females by ANOVA. Group differences were examined by the Dunnett's t-test.[36] Statistical evaluation of study 3 was based on Duncan's multiple range test.[37] All data of study 4 were statistically analysed by ANOVA. Group comparisons were made by Dunnett's two-tailed test.[38]

Zootechnical performance was measured in all studies. An overview (final results only) is given in Table 4. In study 1,[39] all levels of monensin caused lower weight gain than those of the control birds. Higher monensin levels showed poorer feed conversion with the highest level resulting in increased mortality. In study 2,[40] body weight gain, feed consumption, and feed to gain ratio were significantly lower at the 363 and 606 mg/kg levels compared to the non-treated controls and the 121 mg monensin/kg group. The 605 mg/kg level of monensin showed significant and negative effects on all parameters compared to the 363 mg/kg level and significantly increased mortality compared to the other treatments. In study 3,[41] a significantly reduced feed consumption was seen at the 363 and 605 mg/kg levels compared to control and 121 mg/kg feed which resulted in a significant reduction of body weight gain. Feed to gain ratio was significantly worse only at the highest monensin level. In study 4,[42] the intermediate (363 mg/kg) and the high (605 mg/kg) monensin levels significantly depressed feed intake, weight gain and feed to gain ratio. The increase in mortality at the high monensin level (33% compared to 2–7%) is, albeit not significant – considered relevant.

Table 4: Effect of monensin sodium on the performance of chickens for fattening in tolerance studies

Study	Treatments	Feed intake (g)	Body weight gain (g)	Feed to gain ratio	Mortality and culling (%)
1(1)	0	–	1,436	2.12	3.0
	147	–	1,366	2.07	3.9
	442	–	435	3.42	4.0
	737	–	130	6.63	32.7
2	0	3,268^{a}	1,462^{b}	2.24^{a}	2.9^{a}
	121	3,183^{a}	1,491^{a}	2.14^{a}	3.5^{a}
	363	1,881^{b}	632^{c}	2.98^{b}	5.1^{a}
	606	1,190^{c}	241^{d}	4.96^{c}	19.0^{b}

[35] Technical dossier/Section III/Annex III.155.
[36] Technical dossier/Section III/Annex III.156.
[37] Technical dossier/Section III/Annex III.163.
[38] Technical dossier/Section III/Annex III.162.
[39] Technical dossier/Section III/Annex III.18.
[40] Technical dossier/Section III/Annex III.19.
[41] Technical dossier/Section III/Annex III.16.
[42] Technical dossier/Section III/Annex III.17.

Study	Treatments	Feed intake (g)	Body weight gain (g)	Feed to gain ratio	Mortality and culling (%)
3	0	3,445[a]	1,693[a]	2.04[b]	2.1
	121	3,405[a]	1,691[a]	2.01[b]	3.2
	363	2,182[b]	991[b]	2.20[b]	2.6
	605	995[c]	341[c]	2.92[a]	11.5
4(2)	0	3,593	1,665	2.18	2.1
	121	3,578	1,683	2.14	3.2
	363	1,640*	612*	2.68*	7.3
	605	990*	269*	3.74*	33.4

a, b, c: Means within a column/trial with different superscript letters are significantly different ($p \leq 0.05$).
(1): Statistical results not presented.
(2): Group comparisons against the control values, differences ($p < 0.05$) are indicated by *.

In studies 2, 3 and 4, five birds/replicate each were selected after 4 and 8 weeks to determine organ weight, for haematology (haemoglobin, haematocrit), blood chemistry (glucose, total protein (TP), alkaline phosphatase (AP), aspartate aminotransferase (AST), in study 4 without AP but with Na and K) and pathological (including histological) examination (weight of heart, liver and kidney). The results summarised below refer to the 8-week data.

In studies 2, 3 and 4, the absolute organ weights were significantly reduced at the intermediate and the high monensin levels, the relative weights for liver and kidney at the high level only in study 2 and 4; and the intermediate and the high level in study 3. No differences were found for the haematological endpoints in studies 2 and 4, but a significant elevation of haemoglobin at the low level in study 3. Concerning blood chemistry, no differences were seen in study 2. In study 3, a significant decrease for AP and AST values was noted for the use level but not at higher doses, thus not considered relevant. Treatment effects were seen in study 4 for TP and AST, the increase reached significance for the high-dose group only. For study 4, slight degeneration and regeneration of skeletal muscle of the abdominal wall and legs were reported in several chickens treated with 363 and 605 mg monensin/kg feed.

None of the studies showed full compliance with the requirements of Regulation (EC) No 429/2008. The design of all studies failed to show or to allow estimating a margin of safety for the use level. Three- and fivefold overdoses were not tolerated, both depressing feed intake and body weight gain, the fivefold overdose increasing mortality. Study 1 reported only zootechnical parameters. Haematological examinations of studies 2, 3 and 4 were limited to haemoglobin and haematocrit, those for blood chemistry to glucose, total protein and AST, AP was determined in two studies, Na and K in one. The FEEDAP Panel also notes that the final 8-week body weight data in the control groups of the four studies (1,436–1,693 g/bird) are considerably below those which are reached under practical conditions for the modern chicken breeds. Due to the above limitations, the FEEDAP Panel is not in a position to conclude on the safety of the highest proposed dietary concentration of monensin (125 mg/kg) for chickens for fattening and to derive a margin of safety.

3.2.4.2. Tolerance study in turkeys for fattening

Since the studies assessed in 2004 could not be considered sufficient according to Reg (EC) No 429/2008, the applicant provided a new tolerance study with Elancoban® G200.[43]

The study was carried out in male/female turkeys for fattening (Hybrid Converter). From a total of 440 one-day-old birds, 20 were taken for blood sampling shortly after arrival and 360 turkeys (+ 60 spare animals) were distributed into 60 pens of 6 animals (+ 1 spare animal) and allocated to 5 dietary treatments (12 replicates per treatment (6 pens of males and 6 pens of females). After 1 week, the spare birds not required to replace dead birds were removed. The treatment consisted of diets supplemented with 0, 100 (use level), 150 (1.5× use level), 200 (2× use level) and 250 mg monensin sodium/kg (2.5× use level), respectively. The intended monensin concentrations were analytically confirmed except for the intended 250 mg monensin sodium/kg (analysed: 221 mg/kg starter, and 213 mg/kg grower). Both diet types consisted mainly of soybean meal, corn and wheat, the starter diet fed for the first 21 days contained 28.2% CP, 1.132% meth+cyst, and 11.9 MJ apparent metabolisable

[43] Technical dossier/Supplementary information January 2018/Annex 46.

energy (AMEn)/kg; the grower diet 24% CP, 0.938% meth+cyst, and 12.4 MJ AMEn/kg. The diets were offered in mash form and on ad libitum basis for a total period of 42 days.

Mortality and health status were checked twice daily, and dead animals were necropsied. Animals were weighed on days 1, 8, 15, 22, 29, 36 and 43, feed intake was registered per pen throughout the study period and feed to gain ratio calculated for the corresponding intervals. Blood samples were obtained from 1 animal per pen on days 22 and 43 for haematology and blood biochemistry.[44] At the end of the experiment, one bird per pen was selected for necropsy. Liver, kidneys and heart were weighed and samples of heart, skeletal muscle, intestine (jejunum, caecum and colon), kidneys, and liver were taken. No histopathology was performed as there were no significant gross lesions observed on any organs.

An ANOVA was done with the performance data (pen basis) with treatment and sex as factor. Group means were compared with Dunnett test. Mortality was analysed using the Kruskal–Wallis method. The significance level was set at 0.05.

Mortality including culling was high in the first week with 8%, no specific lesions were observed. According to the statistics, mortality was treatment but not dose related. However, a careful review of the individual data by the FEEDAP Panel indicated that the losses are pen related, since in four pens (2 pens from the group 2×, 1 pen from the group 2.5× and 1 pen from the control group) all seven turkeys died (82% of total losses). Only two turkeys died after the first week until study completion, one in the control group with chronic nephritis, another in the group 2× because of cardiac decompensation.

No differences were observed for the total and weekly feed intake, except in weeks 3 and 4 where the high-dose group showed a lower feed intake than the groups with 100 and 150 mg monensin sodium/kg. Final body weight was not statistically different between the groups (overall mean 2,069 g). It should be noted that the body weight was low compared to the standard values given by the breeder company (2.94 kg for males and 2.47 kg for female turkeys). Significant differences were observed for the body weight after 2, 3, 4 and 5 weeks, in all cases the high-dose groups showed a significant growth depression compared to the unsupplemented group and the group with the monensin use level. The applicant also calculated the ratio body weight to feed intake. The groups with 200 and 250 mg monensin sodium/kg showed a significantly inferior ratio of body weight to feed than the control group. No significant differences between treatments were observed in water intake.

Results on haematology and clinical biochemistry of the different groups were not different with one exception represented by creatine kinase (CK) at day 22; CK values of the two higher monensin concentrations (1,957 and 5,818 for 200 and 250 mg monensin sodium/kg, respectively) appeared higher than those for the other groups (861, 814 and 834 for the groups with 0, 100 and 150 mg/kg, respectively). CK increase is often considered as a marker of tissue damage (i.e. of muscles and kidneys. However, significant treatment differences could only be identified (Holm–Sidak method) for females (250 mg/kg higher than all other groups). This effect was not seen at the end of the study.

No differences between the treatment groups were identified by necropsy. Also, the relative organ weights of heart, kidneys and liver were not different. It is noted that the test for liver weight had only a power of 0.101 (for α 0.05), the desired power is 0.8. Therefore, differences even when existing were less likely to be detected. Relative liver weight for the control group was 0.0172, 0.0177, 0.0176, 0.0181 and 0.0187, for the groups with 100, 150, 200 and 250 mg monensin/kg, respectively.

Based on the tolerance study it can be concluded that 100 mg monensin sodium/kg complete feed is safe for turkeys for fattening with a margin of safety of about 1.5.

3.2.4.3. Interactions

In 2004, the FEEDAP Panel concluded that: 'It is well known from the literature that severe interactions between the ionophore coccidiostats and the antibiotic tiamulin but also other antibiotics (mainly macrolides) may occur'. Further toxic interactions with polyethers (mainly monensin) became known for sulfonamides (Frigg et al., 1983), chloramphenicol (Dorn et al., 1983; Broz and Frigg, 1987), sulfachlorpyrazine (Braunius, 1986), erythromycin, oleandomycin and furazolidone (Anadon and Reeve-Johnson, 1999).

In summary, the simultaneous use of Elancoban® and certain antibacterial drugs (e.g. tiamulin) is contra-indicated.

[44] Haematology: haematocrit, haemoglobin, heterophils, basophils, eosinophils, lymphocytes, monocytes, white blood cells. Serum biochemistry: sodium, potassium, chloride, calcium, phosphate, magnesium, total protein, albumin, globulin, glucose, uric acid, cholesterol, triglycerides, bilirubin, amylase, aspartate aminotransferase (AST), glutamate dehydrogenase (GLDH), alkaline phosphatase (AP) and creatine kinase (CK).

3.2.4.4. Literature review on tolerance of chickens and turkeys to monensin and drug interactions including pharmacovigilance data

The applicant performed a literature search[45] on the tolerance of chickens and turkeys to monensin sodium.[46] The search included the terms 'monensin AND chicken OR turkey OR layer OR broiler OR poultry' 'monensin AND coccidiosis' 'monensin AND toxicity AND chicken OR poultry' 'monensin AND safety AND chicken OR poultry' 'monensin AND tolerance' 'monensin AND drug interaction'.

Six publications were identified by the applicant as relevant to the tolerance in chickens (Appendix A). No such study in turkeys was found.

The FEEDAP Panel considered only one of these studies with chickens, since in the other studies monensin was administered at 100 mg/kg feed or below, which is less than the highest proposed use level (125 mg/kg).

Zavala et al. (2011) published a case report, in which peracute onset of disease was described in a 42-week-old broiler breeder flock when feed with 638–740 mg monensin/kg was erroneously administered. During the first 10 days post-ingestion, mortality reached 13.7% in hens and 70.9% in roosters. Hen per day production decreased in the same period from 67% to 3%. Twenty-one days after removal of the suspect feed, mortality rate returned to normal in both genders, albeit feed consumption and egg production remained extremely low.

Concerning interactions of monensin with other drugs, the applicant identified six studies as potentially relevant. One publication (Islam et al., 2009) is a review reporting interaction of tiamulin with different ionophore antibiotics in poultry. Another publication (Szűcs et al., 2004) reports findings in the rat on the tiamulin–monensin interaction. The study demonstrated a dual behaviour of tiamulin: induction and inhibition of CYP3A in rat liver and in the case of inhibition the metabolism of monensin could be compromised. Two other publications (Chapman et al., 2004; Sims et al., 2002) are efficacy studies in turkeys. These four publications did not examine interactions other than of tiamulin with monensin, and were not considered.

Sureshkumar et al. (2004) studied the interaction of monensin with enrofloxacin (a fluoroquinolone) in chickens for fattening. Day-old chickens were treated with monensin (100 mg monensin/kg feed) for 41 days and with enrofloxacin (10 mg enrofloxacin/kg bw per os for three consecutive days at days 33, 34 and 35) alone and in combination. Enrofloxacin inhibited in a reversible competitive type manner CYP450 enzymes. Co-administration of enrofloxacin with monensin produced increased CK, AST and ALT serum enzymes suggesting heart and liver injury. This suggests that the co-administration could result in adverse interactions.

Crespo et al. (2008) analysed the vitamin E and monensin concentrations in serum and liver of turkeys with and without the knock-down syndrome. Skeletal muscles and the myocardium were examined by histopathology. From their findings, the authors suggested that monensin could induce lower vitamin E concentration in the liver; but the study did not demonstrate if low vitamin E concentration predisposes birds to knock-down syndrome.

The FEEDAP Panel concludes that findings published between 2004 and 2014 did not add new knowledge to the tolerance or the safety of monensin in target species. The combined administration of enrofloxacin and monensin appears to produce adverse effects. The simultaneous administration of fluoroquinolones and monensin seems to be contraindicated.

The applicant provided a review of pharmacovigilance case reports, looking specifically at tolerance of target animals along with interactions with other drugs.[46] The review returned twelve cases in the target species (chicken, turkey) and three cases in non-target animal species. Two of the cases in a non-target animal species were related to accidental exposure to feed and one case to use in a non-target species (pheasant).

In the target species, 3 out of 12 cases were related to tolerance of target animals. In one case (with high mortality), the dose fed to chickens reared for laying was 638 and 351 mg monensin/kg, in another case 465 mg/kg. A third case referred to turkeys which stopped eating and showed signs of paralysis, the monensin concentration in feed was 153 mg/kg, about double of the labelled dose (80 mg). From the nine remaining cases, two refer to concomitant feeding of monensin and tiamulin and confirm the well-known interaction. Three cases involved sulfonamides (trimethoprim, sulfaquinoxalin) mostly followed by antibiotics which were fed concomitantly with Elancoban®, one case involved neomycin and

[45] Databases searched: ScienceDirect, PubMed: British National Library of Medicine, Google Scholar. Period: 2000–2014.
[46] Technical dossier/Supplementary information May 2015.

three reports involved conazoles. In all of these cases there were confounding factors or insufficient data to draw a conclusion that interaction occurred between these compounds and Elancoban®.

The FEEDAP Panel concludes that the pharmacovigilance report of the applicant did not identify new facts which should be considered in assessing the tolerance of target species to monensin sodium or potential drug interactions with monensin sodium.

3.2.4.5. Microbial studies

For the current assessment, the applicant resubmitted the same studies already assessed in the FEEDAP opinion adopted in 2004 (EFSA, 2004) and some new information.

In 2004, the FEEDAP Panel concluded that: 'Gram-positive bacteria present in the digestive tract of poultry and other livestock are generally susceptible to monensin. While laboratory studies have shown that Gram-positive strains can develop resistance to monensin, there was no evidence to suggest that exposure of Gram-positive bacteria results in the development of cross-resistance to other antibiotics used for therapy in human and veterinary medicine'. 'The use of monensin as a coccidiostat in chickens did not affect the colonisation or shedding of *Salmonella* in the gastro-intestinal tract'.

For the current assessment, the applicant submitted a microbiological expert report[47] on the use of Elancoban® G200 in poultry and the development of resistance to monensin and cross-resistance to antibiotics used in human medicine. As part of that report, a literature review has been conducted (Appendix A).[48] The report concludes that the use of monensin did not promote appearance or selection of monensin-resistance in susceptible bacteria suggesting that the cross-resistance to monensin and antibiotics of human and veterinary importance is unlikely. Moreover, as no dedicated genes to monensin resistance are involved, co-transfer of these and genetic determinants conferring antibiotic resistance is not possible.

New experimental evidence on the development of antibiotic resistance in *Enterococcus* sp. was submitted. *E. faecium* and *Enterococcus faecalis* were isolated from faecal samples of treated and untreated groups collected during two of the three efficacy studies (one in chickens for fattening[49] and one in turkeys[50]) described in 3.3.[51] Increases in MIC values of monensin to *E. faecalis* (687 isolates) or *E. faecium* (596 isolates), isolated up to day 43 of the treatment, were never observed.[52] One *E. faecium* strain having a MIC to monensin $>$ 64 μg/mL was identified in the chickens study at day 42 of the treatment, while 7 isolates of this species (two from day 42 and five from day 43) with a MIC $>$ 64 μg/mL were recovered from the corresponding untreated control group, which suggests that presence of high-resistant strains was not treatment related.

In the light of the new studies, the FEEDAP Panel reiterates the conclusion that 'there is no evidence to suggest that exposure of Gram-positive bacteria to monensin results in the development of cross-resistance to other antibiotics used for therapy in human and veterinary medicine'. In the absence of new data, the FEEDAP Panel reiterates its previous conclusion that 'The use of monensin as coccidiostat in chickens did not affect the colonisation or shedding of *Salmonella* in the gastro-intestinal tract'.

3.2.4.6. Conclusions on the safety for the target species

Based on the available data set, the FEEDAP Panel cannot conclude on the safety of the highest applied dietary concentration of monensin (125 mg/kg) for chickens for fattening and to derive a margin of safety. This conclusion is extended to chickens reared for laying.

Monensin sodium is safe for turkeys for fattening at inclusion level of 100 mg/kg complete feed, with a margin of safety of about 1.5.

Gram-positive bacteria are susceptible to monensin. Monensin sodium is not considered to be involved in cross-resistance to other antibiotics. The use of monensin as coccidiostat in chickens did not affect the colonisation or shedding of *Salmonella* in the gastrointestinal tract.

The simultaneous use of Elancoban® G200 and certain antibiotic drugs (e.g. tiamulin) is contraindicated.

[47] Technical dossier/Supplementary information May 2015/MICROBIOLOGICAL EXPERT REPORT_FINAL.
[48] A PubMed search was conducted on Monday 18th August 2014 using the terms 'Monensin' 'Elancoban' 'ionophores' 'resistant' 'cross-resistance' 'tolerance' 'adaptation' and 'resistance'.
[49] Technical dossier/Section IV/Annex IV.4
[50] Technical dossier/Section IV/Annex IV.13. and IV.14.
[51] Technical dossier/Section III/Annex 60.
[52] Technical dossier/Section III/Annex 61 and 62.

Considering the uncertainties on the identity and characterisation of the production strain and the lack of demonstration of the absence of the production strain and its DNA on the final product, the FEEDAP Panel cannot conclude on the safety of the additive for the target species.

3.2.5. Toxicological studies

In 2004, the FEEDAP Panel assessed the toxicity of monensin sodium (EFSA, 2004). For the current application, the same data assessed in 2004 was submitted with the addition of some studies not available at the time of the previous assessment and a literature search covering the period 2004–2014.

The FEEDAP Panel assessed all the toxicological studies submitted including those evaluated for the previous application (EFSA, 2004) and those re-submitted in the current application.

The FEEDAP Panel noted that the studies reported were performed according to standards appropriate to the time, but in some cases, they were not in accordance either with Good laboratory practice (GLP) or with previous and current OECD guidelines. However, the quality of the studies was considered sufficient for the assessment. An overview of the available studies and the main conclusions are given below.

3.2.5.1. Genotoxicity

All the *in vivo* and *in vitro* genotoxicity studies submitted for the current assessment were already evaluated by the FEEDAP Panel in 2004 (EFSA, 2004). All the studies were performed with crystalline monensin sodium (93.8% purity) and were GLP-compliant and gave negative results.[53]

3.2.5.2. Subchronic toxicity studies in rats, mice, and dogs

The applicant submitted a total of eight subchronic studies in rats, one in mice and two in dogs. In a set of five rat studies the subchronic toxicity of mycelial and crystalline monensin sodium was compared. The FEEDAP Panel re-assessed the old studies and evaluated the studies not considered before.

Two studies in rats,[54] one in mice[55] and the two studies in dogs[56] were already assessed (EFSA, 2004): the lowest no observed adverse effect level (NOAEL) from the rat studies was based on reduction of body weight gain at a dietary level of 50 mg monensin/kg which was calculated to be equivalent to 3 mg/kg body weight (bw) per day; a NOAEL in mice could not be derived from the available study because growth rate was affected in all the groups tested (the lowest dietary concentration tested was 37.5 mg/kg, corresponding to 7.5 mg/kg bw per day); the lowest observed effect level (LOEL) from the dog studies was 5 mg/kg bw per day, based on liver toxicity together with more generalised toxicity at higher doses (15 and 50 mg/kg bw per day) and slight effects on body weight at 5 mg/kg bw per day in males only).

In an additional rat study, groups of Wistar rats (25 of each sex (control) and 15 of each sex (treated)) were maintained for three months on diets containing mycelial monensin at dietary concentrations of 0, 25, 50, 80, and 125 mg monensin/kg.[57] The dietary concentrations provided average daily doses of 0, 0.9–2.4, 1.8–4.6, 3.0–7.7 or 4.5–12 mg monensin/kg bw per day for male and 0, 1.3–2.6, 2.8–5.8, 4.0–12.8 or 10.0–20.2 mg monensin/kg bw per day for female rats. All rats survived the study with no observable physical signs of toxicity. Decreases in body weight, weight gain, and/or food consumption were observed for males at 125 mg/kg and for females in the 50, 80 and 125 mg/kg groups. No effects of treatment were reported in either sex at the lowest dietary concentration of 25 mg monensin/kg. Based on the body weight findings in females, the lowest dietary level of 25 mg/kg, which provided approximately 2 mg monensin/kg bw per day was considered to be the NOAEL in this study.

Four parallel 3-month studies, not previously reported, were conducted to compare the subchronic toxicity of crystalline and mycelial monensin. In these studies, male and female rats (15/sex per group) were fed diets containing 0, 50, 200 or 400 mg monensin/kg as either crystalline,[58] or one of three forms of mycelial monensin: drum-dried mycelial,[59] azeotrope mycelial[60] or flash-dried mycelial.[61]

[53] Technical dossier/Section III/References 93, 94, 95.
[54] Technical dossier/Section III/References 96 and 97.
[55] Technical dossier/Section III/Reference 104.
[56] Technical dossier/Section III/References 105 and 106.
[57] Technical dossier/Section III/Reference 98.
[58] Technical dossier/Section III/Reference 100.
[59] Technical dossier/Section III/Reference 101.
[60] Technical dossier/Section III/Reference 102.
[61] Technical dossier/Section III/Reference 103.

Monensin dietary concentrations of 50, 200 and 400 mg/kg provided 3.7, 15.5 and 31.2 mg monensin/kg bw per day (crystalline); 3.7, 15.4 and 30 mg monensin/kg bw per day (drum-dried mycelial; 4.0, 16.1 and 30.9 mg monensin/kg bw per day (azeotrope mycelial); and 3.7, 14.8 and 29.4 mg monensin/kg bw per day (flash-dried mycelial). Evidence of toxicity (mortality; decreases in body weight gain, food consumption and efficiency of food utilisation; and a low incidence of focal degeneration and interstitial inflammation of muscle) was similar for the various forms of monensin at the two highest doses. The lowest dietary concentration of 50 mg/kg was determined to be the NOAEL in all studies, equivalent to 3.7 mg monensin/kg bw per day for crystalline monensin and 3.7, 4.0 and 3.7 mg monensin/kg bw per day for the three mycelial monensin studies, respectively.

Another study directly compared the effects of feeding crystalline monensin and mycelial monensin to rats (15/sex per group) administered at identical dietary concentrations of 0, 50, 200 or 400 mg/kg.[62] Crystalline and mycelial monensin produced similar effects with substantial growth retardation in both sexes that received either 200 or 400 mg/kg being most severe at 400 mg/kg. At the lowest dietary concentration tested (50 mg/kg) female rats had a transient and very slight retardation of growth during the first 2–4 weeks of the study and male rats were not affected. There were no other differences that could be considered to be consistently associated with treatment. The lowest dietary concentration tested (50 mg/kg), equivalent to approximately 4.5 mg monensin/kg bw per day was considered to be the NOAEL.

3.2.5.3. Chronic oral toxicity studies

The applicant submitted two 2-year chronic toxicity/carcinogenicity studies in rats and one in mice. A 1-year chronic toxicity study was performed in dogs. Both mycelial and crystalline monensin sodium were tested in these studies.

All the submitted studies were previously assessed (EFSA, 2004): the NOAEL in rats for mycelial monensin was reported to be 1.40 mg/kg bw per day for male rats, based on reduced bodyweight gain at the dose 2.18 mg/kg bw per day[63]; the NOAEL for crystalline monensin was 1.14 mg/kg bw per day for male rats, also based on reduced bodyweight gain at 2.57 mg/kg bw per day.[64] The NOAEL for mycelial monensin in mice was 1.2 mg/kg bw per day based on reduced body weight gain and decreased leukocyte counts at 3.1 mg/kg bw per day.[65] The NOAEL for mycelial monensin in the 1-year study in dogs was reported to be 2.5 mg/kg bw per day, based on anorexia, hypoactivity and weakness at 5 and 7.5 mg/kg bw per day and lower mean body weight gain at 7.5 mg/kg bw per day.[66]

The results of chronic toxicity/carcinogenicity studies performed in rats and mice with either the mycelial or the crystalline form suggest that monensin sodium is not carcinogenic.

3.2.5.4. Reproduction toxicity including teratogenicity

The applicant submitted two-three-generation studies in rats (one with mycelial and the other one with crystalline monensin sodium) and a rabbit teratology study. Both the three-generation studies included investigations of developmental toxicity.

All the submitted studies were previously assessed (EFSA, 2004): no treatment-related adverse effects were seen in rats treated with crystalline monensin sodium[67] and the NOAEL for this study was established to be 2.5 mg/kg bw per day, the highest dose tested. The study performed with mycelial monensin sodium[68] gave a lowest observed adverse effect level (LOAEL) of 3.3 mg/kg bw per day, based on maternal toxicity (reduced bodyweight). There was no indication of any embryotoxicity and teratogenicity at the doses used for either crystalline and mycelial monensin. There was no indication of fetotoxicity with the crystalline form of monensin sodium and slight evidence, suggesting fetotoxicity with the mycelial product, was unconvincing. As no treatment-related adverse effects were seen in the teratogenicity study in rabbits,[69] the NOAEL for this study was concluded to be the highest dose level, 0.76 mg/kg per day.

The FEEDAP Panel re-assessed all the submitted studies and reiterates its previous assessment.

[62] Technical dossier/Section III/Reference 99.
[63] Technical dossier/Section III/Reference 109.
[64] Technical dossier/Section III/Reference 110.
[65] Technical dossier/Section III/Reference 111.
[66] Technical dossier/Section III/Reference 107.
[67] Technical dossier/Section III/Reference 112.
[68] Technical dossier/Section III/Reference 113.
[69] Technical dossier/Section III/Reference 114.

3.2.5.5. Safety pharmacology studies

A series of general pharmacology studies were conducted to determine if the ionophore coccidiostat monensin sodium had any undesirable pharmacological properties. In most of these studies, crystalline monensin sodium was used.

A study assessing general behaviour, coordinating activity of skeletal muscle, anti-electroshock seizures and analgesic effects was performed in mice orally administered monensin sodium at 0, 10 or 30 mg/kg.[70] At 30 mg/kg monensin sodium induced in the animals a slightly sedative condition, decrease sensitivity to tactile stimulation, and a slight depression of muscular coordination. The NOAEL was 10 mg monensin sodium/kg.

A study assessing spinal reflex, electroencephalogram and circulatory, respiratory, and autonomic effects was performed in cats orally administered monensin sodium at 30 mg/kg.[70] No effects were seen at the dose tested.

A study assessing charcoal meal transit was performed in mouse orally administered monensin sodium at 0, 10 or 30 mg/kg.[70] Charcoal meal transit was significantly reduced at 30 mg/kg. The NOAEL was set at 10 mg/kg.

The effect of monensin on contractions induced by acetylcholine, histamine and barium chloride ($BaCl_2$) was evaluated in isolated guinea pig ileal preparations.[70] No effects were seen at the concentration of 1×10^{-5} g/mL.

Carrageenan-induced oedema was compared in rats administered orally monensin sodium at 0 or 30 mg/kg feed.[70] No effects were seen at the dose tested.

Cardiac effects

The applicant submitted one study performed in conscious dogs administered monensin sodium orally or intravenously.[71] The study was assessed previously (EFSA, 2004) and it was concluded that the acute pharmacological NOAEL for increased coronary blood flow and mean blood pressure was 0.345 mg/kg bw after oral administration. In the same study the effects of the intravenous administration of monensin sodium were also evaluated but were considered less relevant to the current assessment. A further study also assessed the cardiac and respiratory effects after intravenous administration to anaesthetised pigs and dogs.[72] The administration route of the study was not relevant and consequently, the results of the study were not considered in this assessment.

Horse toxicity

The applicant submitted the same studies in horses that were already evaluated by the FEEDAP Panel in 2004 (EFSA, 2004).[73] Based on these studies, the FEEDAP Panel concluded that monensin in doses proposed for feed supplementation in chickens is toxic to horses. The same conclusions are retained.

3.2.5.6. Literature search

The applicant performed a literature search[45] on the toxicology of monensin sodium.[46] The search included the terms 'monensin AND acute toxicity', 'monensin AND repeat dose toxicity', 'monensin AND carcinogenicity', 'monensin AND reproductive toxicity', 'monensin AND developmental toxicity', 'monensin AND genotoxicity OR mutagenicity', 'monensin and environmental safety'. The outcome of the literature review (Appendix A) did not identify new data requiring consideration in the current opinion.

3.2.5.7. Conclusions

The FEEDAP Panel concludes that monensin sodium is not genotoxic and, based on the results of carcinogenicity studies performed in rats and mice, is not carcinogenic. The Panel considers that the form of monensin (crystalline or mycelial) is very unlikely to influence these outcomes.

From a total of eight subchronic studies in rats assessing the effects of crystalline and mycelial monensin sodium, it is concluded that crystalline and mycelial monensin produced similar subchronic effects. The lowest subchronic NOAEL is 2 mg/kg bw per day in female rats. A NOAEL in mice could not be derived. The LOAEL in dog was 5 mg/kg bw per day.

[70] Technical dossier/Section III/Reference 115.
[71] Technical dossier/Section III/Reference 116.
[72] Technical dossier/Section III/Reference 117.
[73] Technical dossier/Section III/References 124, 125, 127, 127.

No indication of embryotoxicity, fetotoxicity or teratogenicity was found at the doses tested in rats (2.5 and 8 mg/kg per day for crystalline and mycelial monensin, respectively) and rabbits (0.76 mg/kg per day).

The lowest NOAEL identified from all the toxicological studies was 1.1 mg/kg bw per day based on a 2-year chronic toxicity/carcinogenicity assay in rat. However, a NOAEL of 0.345 mg monensin sodium/kg bw per day was identified in the dog for effects on coronary artery flow and mean blood pressure after oral administration. Potential inotropic effects were considered, but no relevant effects on electrocardiogram results were seen in dogs at doses well above the lowest NOAEL of 0.345 mg monensin sodium/kg bw per day. Consequently, it was considered that a specific investigation of inotropic effects was not necessary for the risk assessment for monensin sodium.

The outcome of the literature review did not identify new data requiring consideration in the current opinion.

Overall, the FEEDAP Panel concludes that the pharmacological NOAEL of 0.345 mg monensin sodium/kg bw per day identified in the dog for acute pharmacological effects on the cardiovascular system can be considered as an appropriate basis for the acute health-based guidance value of 0.003 mg monensin sodium/kg bw per day already established by the FEEDAP Panel in its former opinion (EFSA, 2004) applying an uncertainty factor of 100. The FEEDAP Panel will use this reference value to assess acute exposure of the consumers to monensin residues.

Although the guidance value established above is based on acute effects, such effects occur at a significantly lower dose than the lowest NOAEL for chronic effects (1.1 mg/kg bw per day based on absence of reduction in body weight gain in male rats in a 2-year chronic toxicity/carcinogenicity assay). In consequence, it is considered that the same guidance value, although more conservative, would be equally appropriate for chronic risk assessment. This is supported by the ADI of 0.003 mg/kg bw derived from toxicological studies in an assessment of the same substance and from a developmental study in rabbits for maternal toxicity (EFSA, 2005).

3.2.6. Assessment of consumer safety

The chronic exposure of consumers to monensin residues in chicken tissues is calculated (Table 6) following the methodology described in the Guidance on the safety of feed additives for consumers (EFSA FEEDAP Panel, 2017b) and using the Feed Additives Consumer Exposure (FACE) calculator available on EFSA's website (for further details see Appendix B). The residue data, originating from residue studies (see Section 3.2.2), were inserted in the calculator and are summarised in Table 5.

Table 5: Total residues (expressed as mg monensin equivalent/kg) in tissues of chickens for fattening administered 125 mg [^{14}C]-monensin sodium/kg feed for 6 days(1)

	Liver	Kidney	Muscle(2)	Skin/fat(2)
TRC + 2SD(1)	0.618	0.190	0.065	0.301

TRC: total residue concentration; SD: standard deviation.
(1): See Section 3.2.2 on residue studies.
(2): The residue concentration in muscle and skin/fat will be applied to the intake of meat at the following proportions: 90% muscle and 10% skin/fat (EFSA FEEDAP Panel, 2017b). This corresponds to 0.0886 mg/kg.

Table 6: Chronic dietary exposure of consumers to monensin total residues based on residue data in chicken tissues - Summary statistics across European dietary surveys

Population class	Number of surveys	Highest exposure estimate (mg/kg bw per day)	Refined highest exposure estimate (mg/kg bw per day)(1)	% ADI(2)
Infants	6	0.0006	0.0003	10%
Toddlers	10	0.0007	0.0004	12%
Other children	18	0.0006	0.0003	10%
Adolescents	17	0.0004	0.0002	7%
Adults	17	0.0003	0.0002	5%
Elderly	14	0.0002	0.0001	3%
Very elderly	12	0.0002	0.0001	3%

(1): Considering only 50% of the total residue are of toxicological relevance.
(2): ADI: 0.003 mg/kg bw.

The results showed that the highest chronic exposure was for the age class 'toddlers' with 0.0007 mg/kg bw per day. Considering the toxicological relevance of the residues (50% of total residues) this exposure represents 12% of the ADI. The other age classes were between 3% and 10% of the ADI (for detailed results per age class, country and survey see Appendix B, Table B.1).

An acute exposure assessment is considered necessary taking into account acute pharmacological effects seen in toxicological studies. The FACE model provides results of acute dietary exposure for each single tissue for all age classes. The table reported in Appendix B (Table B.2) indicates that the highest exposure can be found of the age class 'other children' consuming liver (0.0034 mg/kg bw per day corresponding to 100% of the ADI), however, this value would represent 50% of the ADI when considering the toxicological relevance of the residues.

The FEEDAP Panel noted that these results, obtained with residue data in chicken tissues, can be considered as a worst-case scenario and extrapolated to consumer exposure to monensin residues in chickens reared for laying and turkeys (Section 3.2.2).

The FEEDAP Panel noted that monensin sodium is authorised in the EU also as veterinary medicine for bovines which may result in exposure of consumers to monensin residues via bovine tissues and milk of dairy cows.[74] The CVMP of the EMA assessed in 2013 a residue study performed in dairy cattle (intraruminal administration with controlled release capsule, delivering approximately 335 mg monensin/day for 95 days). Tissue samples of liver, kidney, muscle and fat were collected from 10 animals 14 days after administration of the Controlled release capsule (EMA-CVMP, 2013). The residue data from this study (highest values for each tissue/product as a worst-case scenario) have been used by the FEEDAP Panel to assess the combined consumer exposure resulting from the use of monensin as a feed additive for poultry and as veterinary medicine for bovine. Table 7 reports the marker residue values measured in the above-mentioned study (maximum values) and the calculated total residues applying the ratios marker to total residues reported by the CVMP.

Table 7: Monensin residues (mg/kg) in bovine tissues and milk after its use as veterinary medicine(1)

	Liver	Kidney	Muscle(3)	Fat(3)	Milk
Marker residue measured(2)	0.0263	0.00145	0.00084	0.00532	0.00048
RMTR(1)	0.05	0.05	0.05	0.05	0.027
Calculated total residues	0.526	0.029	0.017	0.106	0.018

RMTR: ratio marker to total residues.
(1): EMA-CVMP (2013).
(2): Highest value reported by CVMP.
(3): The residue concentration in muscle and skin/fat will be applied to the intake of meat at the following proportions: 80% muscle and 20% skin/fat (EFSA FEEDAP Panel, 2017b). This corresponds to 0.0348 mg/kg.

The combined chronic exposure of consumers to monensin residues originating from the consumption of chicken and bovine tissues and milk is reported in Table 8. The results showed that the highest chronic exposure was for the age class 'other children' with 0.003 mg/kg bw per day. Considering the toxicological relevance of the residues (50% of total residues) this exposure represents 50% of the ADI. The other age classes were between 12 and 43% of the ADI (for detailed results per age class, country and survey, see Appendix B, Table B.3).

[74] COMMISSION REGULATION (EU) No 37/2010 of 22 December 2009 on pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin OJ L 15, 20.1.2010, p. 1. amended by Commission Implementing Regulation (EU) No 59/2013, OJ L 21, 24.1.2013, p. 21.

Table 8: Chronic dietary exposure of consumers to monensin total residues based on residue data in chicken + bovine tissues/products - Summary statistics across European dietary surveys

Population class	Number of surveys	Highest exposure estimate (mg/kg bw per day)	Refined highest exposure estimate (mg/kg bw per day)(1)	% ADI(2)
Infants	6	0.0026	0.0013	43%
Toddlers	10	0.0025	0.0013	42%
Other children	18	0.0030	0.0015	50%
Adolescents	17	0.0013	0.0007	22%
Adults	17	0.0007	0.0004	12%
Elderly	14	0.0006	0.0003	10%
Very elderly	12	0.0007	0.0004	12%

(1): Considering only 50% of the total residue are of toxicological relevance.
(2): ADI: 0.003 mg/kg bw.

The FEEDAP Panel noted that the highest contribution to exposure in all age classes comes from milk consumption (55% (adults) – 93% (infants)).

MRLs for monensin are in force for poultry tissues.[75] The chronic exposure calculation following the Guidance on the safety of feed additives for consumers (EFSA FEEDAP Panel, 2017b) has been also performed calculating the total residue data derived from MRLs in poultry tissues (see Table 9 for input data and Table 10 for the results).

Table 9: Monensin total residues calculated from MRL values(1) of poultry tissues applying the ratios marker to total residue (RMTR) for each tissue/product (mg/kg)

	Liver	Kidney	Muscle(3)	Skin/fat(3)
MRLs (mg/kg wet tissue)	0.008	0.008	0.008	0.025
RMTR(2)	0.020	0.020	0.020	0.171
TR_{MRL}	**0.4**	**0.4**	**0.4**	**0.146**

(1): Reg. (EC) No 180/2007.
(2): EFSA (2008a).
(3): Considering that the calculated total residue concentration in muscle is higher than in skin/fat, this value will be applied to the intake of meat as worst-case scenario.

Table 10: Chronic dietary exposure of consumers to monensin total residues derived from MRLs in poultry tissues - Summary statistics across European dietary surveys

Population class	Number of surveys	Highest exposure estimate (mg/kg bw per day)	Refined highest exposure estimate (mg/kg bw per day)(1)	% of ADI(2)
Infants	6	0.0026	0.0013	43%
Toddlers	10	0.0031	0.0016	50%
Other children	18	0.0026	0.0013	43%
Adolescents	17	0.0017	0.0009	28%
Adults	17	0.0009	0.0005	15%
Elderly	14	0.0008	0.0004	13%
Very elderly	12	0.0008	0.0004	13%

(1): Considering only 50% of the total residue are of toxicological relevance.
(2): ADI: 0.003 mg/kg bw.

The highest exposure would be for the age class 'toddlers' with 0.0031 mg/kg bw per day representing 50% of the ADI after considering the toxicologically relevant residues (for detailed results per age class, country and survey, see Appendix B, Table B.4).

Acute exposure calculation has been also performed based on total residue calculated from MRLs in poultry tissues in force. The results showed that the highest exposure is due to the consumption of meat in the age class 'other children' (0.0057 mg/kg bw per day) (Appendix B, Table B.5). This value

[75] Reg. (EC) No 180/2007.

corresponds to 191% of the ADI, but it is 95% of the ADI when considering the toxicological relevance of the residues.

3.2.6.1. Conclusions on the assessment of consumer safety

The chronic exposure to monensin residues resulting from the use of monensin sodium as a feed additive in chickens would amount to up to 10% of the ADI (toddlers). The combined chronic exposure to monensin residues resulting from use of monensin as a feed additive in chicken and as a veterinary medicine in bovine would reach up to 48% of the ADI. Acute exposure due to the consumption of poultry tissues were found below the ADI for all age classes (up to 50%). These conclusions are extrapolated to chickens reared for laying and turkeys for fattening. Based on this, the FEEDAP Panel considers that Elancoban® G200 containing monensin sodium is safe for the consumer of tissues obtained from chickens for fattening or reared for laying and turkeys fed the additive under the proposed conditions of use.

The existing MRLs for poultry tissues ensure consumer safety (acute and chronic exposure; all age classes) provided that the withdrawal period of 1 day is respected.

Considering the uncertainties on the identity and characterisation of the production strain and the lack of demonstration of the absence of the production strain and its DNA on the final product, the FEEDAP Panel cannot conclude on the safety of the additive for the consumers.

3.2.7. Safety for the user

3.2.7.1. Effects on eyes and skin

No new data have been submitted by the applicant.

The same studies as had been assessed in the former opinion (EFSA, 2004) were re-submitted by the applicant and were re-assessed by the FEEDAP Panel.[76]

The FEEDAP Panel concluded that both mycelial monensin and Elancoban® are 'very irritant for the eye. Neither mycelial monensin nor Elancoban® cause skin irritancy but systemic toxicity may occur following skin exposure. Elancoban® is a weak sensitizer by skin exposure and should be regarded as a potential skin sensitizer in humans'. 'The observations of effects in exposed workers in the feed industry confirm that monensin can cause irritancy to eyes and contact dermatitis'.

3.2.7.2. Effects on the respiratory system

No new data have been submitted by the applicant.

The same studies assessed in the former opinion (EFSA, 2004) were re-submitted by the applicant and re-assessed by the FEEDAP Panel.[77] The FEEDAP Panel considered all the studies submitted and concluded that only one rat study[78] and one in dogs[79] were suitable for assessment of user safety. It was concluded that 'inhalation exposure of the dog and the rat to a high concentration of a respirable fraction of dust from Elancoban® caused systemic adverse effects on the heart', Although the repeat-concentration study in dogs (90 days with 5 days/week of 6-h exposure/day) showed a number of limitations in design (e.g. low number of animals), the lowest no observed effect concentration (NOEC) (0.015 mg monensin/m^3) is retained for the assessment.

Considering that Elancoban® should be regarded as a potential skin sensitiser in humans, its potential for respiratory sensitisation cannot be excluded.

3.2.7.3. Inhalation exposure

The FEEDAP Panel reviewed the information on the physical properties of the additive and on the inhalation toxicity of monensin.

The potential exposure of users by handling the additive to inhaled monensin was calculated according to the Technical Guidance on User safety (EFSA FEEDAP Panel, 2012b) and reported in Appendix C. From the dusting potential and monensin content of the dust, the monensin concentration in the inhaled air could be calculated as 36 mg/m^3, resulting in inhalation exposure of 5 mg monensin from Elancoban® G200 per person during an 8-h working day. Exposure to monensin by the respirable fraction (< 10 μm) of the dust only (25–31%) would be about 1.6 mg. Comparing the monensin

[76] Technical dossier/Section III/Annexes 88, 89, 143, 144, 147, 149.
[77] Technical dossier/Section III/Annexes 88, 134, 135, 136, 137, 138, 139, 140, 141.
[78] Technical dossier/Section III/Annexes 88.
[79] Technical dossier/Section III/Annexes 139.

concentration in the inhaled air of 36 mg/m^3 and the NOEC in dogs of 0.015 mg/m^3, the inhalation exposure of users handling Elancoban® G200 is considered a risk.

3.2.7.4. Conclusions on the safety for the user

The FEEDAP Panel concludes that both mycelial monensin and Elancoban® are very irritant for the eye. Neither mycelial monensin nor Elancoban® cause skin irritancy but systemic toxicity may occur following skin exposure.

Elancoban® should be regarded as a potential skin and respiratory sensitiser.

On the basis of the available information, inhalation exposure is considered a risk to persons handling the additive.

Considering the uncertainties on the identity and characterisation of the production strain and the lack of demonstration of the absence of the production strain and its DNA on the final product, the FEEDAP Panel cannot conclude on the safety of the additive for the users.

3.2.8. Safety for the environment

The active ingredient under assessment is not a physiological/natural substance of established safety for the environment. The additive is also not intended for companion animals only. Consequently, according to Regulation (EC) No 429/2008, the Phase I assessment has to be continued to determine the predicted environmental concentration (PEC), according to the proposed conditions of use in chickens for fattening. In Phase I and Phase II, initially a total residues approach will be taken, meaning that the PECs will be calculated, based on the assumption that the additive is excreted 100% as parent compound.

The FEEDAP Panel evaluated the new studies provided in the dossier and re-assessed the studies already considered in its previous opinions (EFSA, 2004). The applicant performed a literature search[80] on the environmental safety of monensin sodium.[81] The full search strategy has been provided in the dossier. The outcome of the literature review (97 literature references were identified from a total of 1248 references) is reported in Appendix A and the relevant papers used for the assessment are quoted in the text below.

3.2.8.1. Phase I

Physicochemical properties

The physicochemical properties of monensin sodium are summarised in Table 11.

Table 11: Physicochemical properties of monensin sodium

Property	Value	Unit
Octanol/water partition coefficient(1) (log K_{ow} 25°C)	4.24 (pH 5) 2.75–2.87 (pH 7) 3.79 (pH 9)	–
Water solubility(2) (20°C)	109.0 (Milli-Ro water) 4.809 (pH 7) 8.912 (pH 9)	mg/L
Dissociation constant(3) (pKa)	6.6	–
Vapour pressure(4) (VP)	3×10^{-28}	Pa

(1): Determined by the shake flask method, FDA environmental technical assistance section 3.02 March 1987. Technical dossier/Supplementary information May 2015/Reference 9.
(2): Determined in accordance with OECD 105. At pH 4 monensin sodium degraded after 24 h incubation at approximately 30°C. Technical dossier/Supplementary information May 2015/Reference 7.
(3): Merck Index, 2013. Technical dossier/Supplementary information May 2015/Reference 8.
(4): EPI Suite, 2015.

[80] Technical dossier/Supplementary information January 2018/Reference 48.
[81] Agricola, BIOSIS Previews, CAB Abstracts, Chemical Abstracts Plus, Elsevier Biobase, Embase, Food Science and Technology Abstracts, IPA, Medline, ProQuest Science & Technology, Registry, SciSearch, Toxcenter; Period: 2000–2016.

Fate and behaviour

Fate in soil

Adsorption/desorption in soil

A study[82] on adsorption/desorption (K_{oc}) was conducted in accordance with OECD 106 on five soils. Soil suspensions in 0.01 M $CaCl_2$ were dosed with monensin and equilibrated for 2, 4, 6, 24 and 48 h. Concentration of the test substance in the aqueous phase was determined using liquid scintillation counting (LSC). Equilibrium (quasi-equilibrium) was attained after approximately 24 h of equilibration mixing by a shaker or 0.5 h mixing by a vortexer.

For desorption data, the solid phase was re-suspended in 0.01 M $CaCl_2$, and the concentrations of monensin in both the aqueous phase and the soil solids determined at quasi-equilibrium. The results of the adsorption of monensin sodium in different soils are presented in Table 12.

Table 12: Adsorption of monensin sodium in different soils

Soil	pH	%OC	K_{oc} (mL/g)	1/n	K_{om} (mL/g)
Clay loam (TB-PF)	7.2	5.0	408	1.06	237
Sandy clay loam (MSF-PF)	6.2	1.9	555	0.97	322
Clay loam (Du-Loam)	5.2	4.1	396	1.07	230
Loamy sand (Roger Myron)	5.7	1.3	439	1.04	255
Clay (Montana Clay)	7.7	0.7	524	0.95	304
Geometric mean			460		267

%OC: % of organic carbon; K_{oc}: adsorption coefficient corrected for soil organic carbon content; $K_{om} = K_{oc}/1.724$.

The monensin distribution coefficients for the studied soils in the adsorption stage ranged from 3.7 to 20.4 mL/g and in the desorption stage from 5.6 to 26.8 mL/g. The organic carbon normalised distribution coefficients ranged from 396 to 555 mL/g at the adsorption stage of the test. The geometric mean value of 460 mL/g for K_{oc} will be considered the reference input data for PEC calculations.

Degradation in soil

The applicant submitted the same study that was already assessed by the FEEDAP Panel in 2004 (EFSA, 2004). In the context of the present assessment, the same study was re-evaluated as follow. The degradation of [^{14}C]-monensin under aerobic conditions was assessed in three different soils, sandy loam, silty loam and clay loam soils (pH 7.3–7.5) at an application rate of 1.5 mg a.i./kg soil at 20 ± 2 °C for up to 84 days.[83] This GLP study was conducted in accordance with the procedures outlined in SETAC document 'Procedures for assessing the Environmental Fate and Ecotoxicity of Pesticides (Lynch, 1995). The FEEDAP Panel noted that at the time of the initiation of the study, OECD 307 had not been adopted and the procedures followed in this study were the recognised standard at that time and are one of the procedures used for the preparation of OECD 307.

Radioactivity levels in all soil types declined over the duration of the study (84 days) which overlapped with an increase in $^{14}CO_2$ evolution. At the end of the study, $^{14}CO_2$ accounted for 43–81% of applied radioactivity, demonstrating significant mineralisation of monensin. Monensin degraded to a number of unidentified components, one of which increased at various intervals during incubation, up to 36% in clay loam, but declined to less than 2% at study termination. Only 1–2% of applied radioactivity was characterised as monensin at the end of the study, in all the three soils tested.

Degradation of monensin in all soils was rapid with DT_{50} values, recalculated according single first-order (SFO) kinetics of 4, 10 and 6 days for sandy loam, silt loam and clay loam, respectively. The corresponding DT_{90} values ranges are 13, 32 and 19 days for the same soil types. This indicates that monensin would not be persistent, nor accumulate in the soil. Since just three soils are available, the worst-case value of 10 days is considered the most appropriate for further evaluation. This value, normalised to 12°C using the Arrhenius equation,[84] corresponds to a DT_{50} of 21 days.

[82] Technical dossier/Supplementary information May 2015/Reference 6.
[83] Technical dossier/Supplementary information May 2015/Reference 10.
[84] 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, 2007b).

Literature review

In addition to the above, the FEEDAP Panel reviewed the outcome of the literature review performed by the applicant regarding the sorption, degradation and environmental risk of monensin sodium.

The sorption characteristics of monensin in soil are reported in two studies (El Sayed and Prasher 2014, Sassman and Lee, 2007). Sassman and Lee (2007) used a range of eight different soils, from United States and Korea, and reported that the sorption coefficients (log K_{oc}) ranged from 2.1 to 3.8. The results derived from the OECD 106 study performed by the applicant are comprised in a shorter range, (log K_{oc} ranges from 2.6 to 2.7); however, the OECD 106 study is performed on European soils and both adsorption and desorption values were reported. The sorption value reported in the El Sayed and Prasher (2014) is related to the interaction with a non-ionic surfactant, Brij35, added to water used to irrigate soils. While the relative data within this study provide valuable information, the single soil sorption value is considered of limited use to assess monensin used as a feed additive. The sorption of monensin was found to decrease with increasing levels of surfactant suggesting that, where water for irrigation contains surfactants, monensin could move more rapidly to greater depths in the soil and potentially into groundwater. In contrast, other studies suggest that monensin applied through the use of fertilisers is unlikely to reach groundwater due to the adsorption to particulate matter and the rapid degradation in soil. Carlson and Mabury (2006) did not detect monensin below a soil depth of 25 cm despite a surface concentration up to 1,465 μg/kg.

For degradation in soil, there is 'typical range' from 2 to 13 days (El Sayed and Prasher, 2014; Carlson and Mabury, 2006; Sassman and Lee, 2007; Donoho, 1984; Yoshida et al., 2013; EFSA, 2004, 2005), but a single study falls outside of this with a range from 3 to 36 weeks. (Zizek et al., 2011). Explanations for the variability include differences in the organic carbon content, pH, soil type, temperature and moisture content. The majority of data reported are dissipation DT_{50} rather than degradation DT_{50}.

In conclusion, data that are available from literature are generally not considered to be the most relevant data to be used in evaluating the potential concentration of monensin in environment nor its adsorption potential. The FEEDAP Panel considered more appropriate to use the values for adsorption coefficient from the study submitted by the applicant. The same approach has been taken regarding the degradation. A half-life of 10 days in soil from the laboratory studies has been retained in the current assessment. This value is obtained from a radiolabelled degradation study using three soil types and while there is some suggestion that laboratory studies give a higher half-life value than field plots, it is considered that the radiolabelled study provides a degradation rate rather than disappearance.

Conclusion on fate and behaviour

A K_{oc} of 460 L/kg and a DT_{50} of 21 days at 12°C will be used for the assessment.

Predicted environmental concentrations (PECs)

The calculated PEC initial values are given in Table 13. The highest dose recommended for chicken for fattening (125 mg monensin/kg feed) was considered, since this value provides the worst-case exposure, which covers also chickens reared for laying and turkey.

Table 13: Initial predicted environmental concentrations (PECs) of monensin sodium, in soil (μg/kg), groundwater (μg/L), surface water (μg/L) and sediment (μg/kg dry weight)

Input	Value
Dose (mg monensin/kg feed)	125
Molecular weight	692.86
Vapour pressure (Pa)	3×10^{-28}
Solubility (mg/L)	4,809
K_{oc} (L/kg)	460
DT_{50} in soil at 12°C (days)	21
Output	
PEC_{soil}	649
$PEC_{ground\ water}$	70
$PEC_{surface\ water}$	23
$PEC_{sediment}$	647

K_{oc}: adsorption or desorption coefficient corrected for soil organic carbon content; DT_{50}: disappearance time 50 (the time within which the concentration of the test substance is reduced by 50%.

The Phase I PEC trigger values are exceeded; therefore a Phase II assessment is considered necessary.

3.2.8.2. Phase II

Exposure assessment

PECs calculation refined in Phase II

PEC_{soil} refined for metabolism and degradation in manure

In 2004, the FEEDAP Panel considered that the ionophoric activity of monensin sodium and its metabolites in chicken excreta would not exceed in total 20% of the orally administered dose (EFSA, 2004). In the absence of new data, for the current assessment the FEEDAP Panel used the same approach and the dose used to calculate the PEC_{soil} refined based on metabolism was $125 \times 0.2 = 25$ mg/kg feed.

Further refinement of the initial PEC might be made based on the degradation in excreta. A wide variation is reported for the half-life for both litter/manure and soil. The half-life reported in fresh manure/litter generally falls within the ranges from 4 to 63 days, (Arikan et al., 2016; Dolliver et al., 2008; EFSA, 2004). Due the variability across Europe, a DT_{50} of 63 days may be considered appropriate to refine PEC_{soil}. Refinement of the PEC is based on a storage time equal to the period of the cycle for chickens for fattening (41 days), that is to say to introduce a degradation factor in PEC calculation:

$$PEC_{soil\ refined} = PEC_{soil} \times \exp(-kT_{st}/2)$$

where,

$k = \ln 2/DT_{50\ manure}$

T_{st} = length of time manure is stored.

The refined PEC_{soil}, $PEC_{groundwater}$, $PEC_{surface\ water}$ and $PEC_{sediment}$ based on metabolism and degradation in excreta are reported in Table 14.

Table 14: Refined predicted environmental concentrations (PECs) of monensin sodium, in soil (μg/kg), groundwater (μg/L), surface water (μg/L) and sediment (μg/kg dry weight)

Input	Value
Dose (mg/kg feed)	25
Molecular weight	692.86
Vapour Pressure (Pa) (at 25°C)	3×10^{-28}
Solubility (mg/L)	4,809
K_{oc} (L/kg)	460
DT_{50} in soil at 12°C (days)	21
Output	
PEC_{soil}	104
$PEC_{groundwater}$	11
$PEC_{surface\ water}$	3.7
$PEC_{sediment}$	103

K_{oc}: adsorption or desorption coefficient corrected for soil organic carbon content; DT_{50}: disappearance time 50 (the time within which the concentration of the test substance.

When the $PEC_{groundwater}$ is set equal to the concentration in pore water, based on a worst-case assumption (the total residue approach), the monensin concentration exceeds the trigger value of 0.1 μg/L identified by the EU as quality standard.[85]

$PEC_{groundwater}$ refined with FOCUS

Leaching of monensin to groundwater was simulated using the FOCUS recommended leaching model PEARL (FOCUS Version 4.4.4) (EFSA, 2008c). The calculated groundwater concentrations for the scenarios Jokioinen and Piacenza were below 0.001 μg/L. No concern for groundwater is expected from the use on monensin sodium in chickens and turkeys.

[85] Directive 2006/118/EC of the European Parliament and of the Council of 12 December 2006 on the protection of groundwater against pollution and deterioration. OJ L 372, 27.12.2006, p. 19.

Conclusions on PEC used for calculation

The following values are used for the assessment: PEC_{soil} of 104 μg/kg, $PEC_{surface\ water}$ of 3.7 μg/L and $PEC_{sediment}$ 103 μg/kg dry weight.

Ecotoxicity studies

Toxicity to soil organisms

Effects on plants

Effect of monensin sodium on the emergence and growth of seedlings of winter oat (*Avena sativa*), radish (*Raphanus sativus*) and mung bean (*Phaseolus aureus*) was studied in accordance with OECD TG 208[86] in sandy loam soil mixed with horticultural grade sand at measured exposure concentration of 0.31, 4.35 and 35.97 mg/kg (nominal levels of 0.3, 3.0 and 30 mg/kg). The test period was 14 days after at least 50% emergence in controls. This study was already considered in EFSA opinion in 2004 (EFSA, 2004) and re-evaluated in the current assessment.

A lowest NOEC of 4.35 mg/kg was determined for three species of plant, for both emergence and growth (fresh weight). However, the emergence of winter outs was not affected even at the highest tested concentration. The median lethal concentration (LC_{50}) values for emergence and the median effective concentration (EC_{50}) values for growth are summarised in Table 15. Due to the limited number of concentrations tested, the study failed to provide emergence LC_{50} value for winter outs (best estimate greater than 35.97 mg/kg) as well as the growth EC_{50} value (best estimate greater than 4.347 mg/kg) for radish.

Table 15: Monensin ecotoxicological effects data for terrestrial plants (mg monensin/kg)

Plant species	Emergence		Growth	
	LC_{50} (mg/kg)	**NOEC (mg/kg)**	**EC_{50} (mg/kg)**	**NOEC (mg/kg)**
Winter oats	> 35.97	> 35.97	12.9	4.35
Radish	9.8	4.35	> 4.35	4.35
Mung bean	24.1	4.35	32.9	4.35

LC_{50}: median lethal concentration; NOEC: no observed effect concentration; EC_{50}: median effective concentration.

Radish appeared to be the most sensitive species. At the highest tested concentration (36 mg/kg), no plants emerged. The other species showed a significant reduction in growth at this concentration.

In a newly performed study,[87] the effects of monensin on emergence and growth for seven species of plants were evaluated. The species tested were Oat (*Avena sativa*), Radish (*Raphanus sativus*, the most sensitive species from the previous study), mung bean (*Phaseolus aureus*), oilseed Rape (*Brassica napus*), perennial ryegrass (*Lolium perenne*), turnip (*Brassica rapa*) and wheat (*Triticum aestivum*). Nominal concentrations tested were 0, 0.5, 1, 2, 4, 8 and 16 mg/kg for perennial ryegrass; 0, 1, 2, 4, 8, 16 and 32 mg/kg for oats and wheat; and 0, 2, 4, 8, 16 and 32 mg/kg for the other plant species. The results are summarised in Table 16.

Table 16: Monensin ecotoxicological effects data for terrestrial plants (mg/kg)

Plant species	Emergence		Growth	
	LC_{50} (mg/kg)	**NOEC (mg/kg)**	**EC_{50} (mg/kg)**	**NOEC (mg/kg)**
Oats	> 32	≥ 32	20.3	8.0
Radish	26.8	8.0	11.9	8.0
Mung Bean	12.1	4.0	18.4	8.0
Oil seed rape	> 32	16.0	14.6	8.0
Perennial ryegrass	> 16	≥ 16	7.2	2.0
Turnip	> 24.1	16.0	8.4	4.0
Wheat	> 32	≥ 32	16.2	4.0

LC_{50}: median lethal concentration; NOEC: no observed effect concentration; EC_{50}: median effective concentration.

[86] Technical dossier/Supplementary information May 2015/Reference 11.
[87] Technical dossier/Supplementary information May 2015/Reference 12.

The most sensitive plant species was Perennial Ryegrass with an EC_{50} for growth of 7.2 mg/kg and a NOEC for growth of 2.0 mg/kg. However, it should be noted that either emergence LC_{50} or NOEC for this species could not be estimated, as the emergence was not significantly affected even at the highest tested concentration (16 mg/kg). For crops likely to be grown on cultivated soils receiving poultry excreta the most sensitive species was mung bean with an LC_{50} for emergence of 12.1 mg/kg and a NOEC for emergence of 4.0 mg/kg. However, it should be noted that the exposure duration in test with mung beans lasted considerably longer (28 days after 50% emergence of control plants vs. 14 days after 50% emergence in controls in tests with any other plants).

Taken together, the lowest endpoint was growth NOEC of 2.0 mg/kg for perennial ryegrass.

Effect on earthworms

The acute toxicity of monensin (nominal concentrations of 50, 125, 250, 500 and 1,000 mg/kg) to the earthworm *Eisenia foetida* was already assessed in the 2004 FEEDAP opinion (EFSA, 2004).[88] The study was conducted in accordance with OECD 207 and indicated that 7- and 14-day EC_{50} was 690.3 and 264.2 mg/kg, respectively. At 14 days, the mortality values showed a nonlinear response and hence the 14-day EC_{50} should be treated with caution.

A newly performed study, performed in accordance with OECD 222[89] was submitted to evaluate the chronic effect of monensin to the earthworm *E. foetida*. The survival and reproduction were assessed upon exposure to nominal concentrations of 13, 25, 50, 100 and 200 mg/kg monensin factor A for 28- and 56-day exposure. The 28-day LC_{50} was determined as 76 mg/kg, and the NOEC for both survival and weight change was 50 mg/kg. For reproduction the 56-day EC_{50} was 70 mg/kg, and the 56-day NOEC was 50 mg/kg.

Recent studies reported in the literature, on the impact of monensin on the reproductive capacity of earthworms and Isopods have shown that while Isopods are relatively unaffected by monensin in the soil or the feed (Zizek et al., 2011; Zidar and Zizek, 2012), earthworms are more sensitive. The NOEC and EC_{50} for earthworm reproduction were reported to be 3.5 and 12.7 mg/kg soil, equivalent to nominal concentrations of 5 and 16.5 mg/kg soil (Zizek et al., 2011). There is no apparent reason for the 10-fold difference in the NOEC reported in the literature and those provided in the assessment provided by the applicant. Both studies are reported to be in accordance with OECD 222. It is noted that in the paper of Zizek et al. (2011) a soil degradation value considerably different to other reports (25, 28, 29, 30 and 36 weeks in comparison to 2–13 days) is reported. Therefore, it is considered appropriate to retain the applicant own data for the risk evaluation.

Taken together, the lowest *E. foetida* endpoint was 28-day survival/56-day reproduction NOEC of 50 mg/kg.

Effects on soil microorganisms

The potential effects of monensin on the rate of microbial respiration and on the nitrification and nitrogen-mineralisation capacity of soil microflora under aerobic conditions were investigated in a study conducted to OECD guidelines 216 and 217.[90] The study was already assessed in the 2014 FEEDAP opinion and it was concluded that the NOEC for effect on soil respiration and soil nitrification is established at > 15 mg/k soil. The same conclusions are retained for the current assessment.

Toxicity to aquatic organisms

Effect on algae

A static toxicity test (OECD 201) was conducted to evaluate the effects of monensin on the green alga *Selenastrum capricornutum*. The study was already assessed in 2004 (EFSA, 2004) and re-evaluated in the context of the current opinion; the same conclusions are retained for the current assessment.[91] The 72-h E_rC_{50} (median effective concentration which results in 50% reduction in growth rate) values for area under the curve and average specific growth rate were 0.98 mg/L and 4.33 mg/L, respectively. The NOEC's were 0.055 and 0.32 mg/L for area under the curve and average specific growth rate, respectively. The E_rC_{50} of 4.33 mg/L has been used for aquatic risk assessments.

[88] Technical dossier/Supplementary information May 2015/Reference 13.
[89] Technical dossier/Supplementary information May 2015/Reference 14.
[90] Technical dossier/Supplementary information May 2015/Reference 15.
[91] Technical dossier/Supplementary information May 2015/Reference 18.

Effect on crustaceans

The toxicity of monensin to *Daphnia magna* has been investigated in a 48-h static test.[92] The results of the study were already mentioned in 2004 (EFSA, 2004) but the original report was not available for the evaluation. While not conducted to current OECD guidelines, it is a GLP study and the data are considered acceptable. First-instar of *D. magna* (i.e. ≤ 24 h old) were exposed to assayed concentrations of 0.0 (control), 2.6, 4.2, 5.6, 7.1, 10.8, 14.4 and 18.1 mg/L of monensin for 48 h. The monensin sodium exposure levels remained relatively stable over the test period. Test solutions temperature averaged 20°C and had the following water quality characteristics: average dissolved oxygen 8.1 mg/L; pH averaged 8.5 and ranged from 8.2 to 8.6, total hardness 120 mg/L as $CaCO_3$; total alkalinity 135 mg/L as $CaCO_3$; and conductivity 250 μmhos/cm. No physical signs of toxicity were observed in *Daphnia* populations exposed to monensin concentrations ≤ 4.2 mg/L. Concentration related immobilisation frequencies of 7% to 100% occurred at monensin concentrations ≥ 5.6 mg/L. The NOEC was 4.2 mg/L and the 48-h EC_{50} was 10.7 mg/L.

Effect on fish

The toxicity of monensin to fish has been investigated in a 96-h static test.[93] The results of the study were already mentioned in 2004 (EFSA, 2004) but the original report was not available for the evaluation. While this study is not conducted to current OECD guidelines, it is a GLP study and the data are considered to be acceptable. Juvenile rainbow trout were exposed to test solutions with assayed monensin concentrations of 0 (control), 0.7, 1.12, 1.48, 4.3, 5.2, 6.6, 8.2, 10.6, 12.5 and 15.7 mg/L. The monensin sodium exposure levels remained relatively stable over the test period. The water quality characteristics were as follows: pH 8.0–8.4; dissolved oxygen 10.4 mg/L; temperature 25°C; total hardness 120 mg/L as $CaCO_3$; total alkalinity 145 mg/L as $CaCO_3$; and conductivity 240 μmhos/cm. No mortality or signs of sublethal toxicity were observed at monensin concentrations of ≤ 0.7 mg/L. Concentration related signs of toxicity ranging from hypoactivity to death occurred at monensin concentrations ≥ 1.12 mg/L. Mortality frequencies of 10% to 100% occurred at concentrations of 6.6 up to 15.7 mg/L. The acute NOEC of monensin was 0.7 mg/L and the 96-h LC_{50} was 9 mg/L.

Effect on sediment dwelling organisms

No data on sediment were submitted. A log K_{oc} or log K_{ow} ≥ 3 for an organic chemical is used as a trigger value for sediment effect assessment (ECHA, 2008). The predicted no effect concentration in sediment ($PNEC_{sed}$) calculated based on equilibrium partitioning is 105.8 μg/kg for monensin sodium. Calculation was performed with a K_{oc} of 460 L/kg and a $PNEC_{surfacewater}$ of 4.3 μg/L for monensin sodium.

Conclusions on the ecotoxic effect of monensin sodium on soil, water and sediment

For the terrestrial compartment, data are available for plants, earthworms and microorganisms. The results of newly conducted study with earthworms are considered valid and reliable, so the risk characterisation is based on chronic reproduction NOEC value for earthworms of 50 mg/kg and appropriate assessment factor of 10 (EFSA, 2008c). The newly submitted plant study was conducted with seven plant species (including the most sensitive tested in the previous one). The results are considered valid and reliable. The NOEC of the most sensitive endpoint (2.0 mg/kg) out of seven species tested is used for risk characterisation with the appropriate assessment factor of 10 (EFSA, 2008c).

For the aquatic compartment, data are available for algae, aquatic invertebrates, and fish. The ErC_{50} used in the assessment is 4.3 mg/L for algae, respectively. The 48-h EC_{50} for immobilisation of daphnids was determined to be 10.7 mg monensin sodium/L and the 96-h LC_{50} for fish was 9.8 mg monensin sodium/L.

Ecotoxicological data for sediment-dwelling invertebrates were not provided for the sediment compartment. The PNEC for sediment, calculated based on equilibrium partitioning, is 105.8 μg/kg for monensin sodium.

Risk characterisation (PEC/PNEC ratio)

The risk characterisation ratios for terrestrial and aquatic compartment are reported in Tables 17 and 18. The risk characterisation for sediment is reported in Table 19.

[92] Technical dossier/Supplementary information May 2015/Reference 16.
[93] Technical dossier/Supplementary information May 2015/Reference 17.

Table 17: Risk characterisation of monensin (PEC/PNEC ratio) for terrestrial compartment

Taxa	PEC_{soil} (µg/kg)	NOEC (mg/kg)	AF	PNEC (µg/kg)	PEC/PNEC
Earthworm	104	50	10	5,000	0.02
Plants		2.0	10	200	0.52

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

Table 18: Risk characterisation (PEC/PNEC ratio) for aquatic compartment

Taxa	$PEC_{surfacewater}$ (µg/L)	72-h E_rC_{50}/48-EC_{50}/96-h LC_{50} (mg/L)	AF	PNEC (µg/L)	PEC/PNEC
Algae *Selenastrum capricornutum*	3.7	4.3(1)	1000	4.30	0.86
Aquatic invertebrates *Daphnia magna*		10.7(2)	1000		
Fish *Salmo gairdneri*		9.0(3)	1000		

AF: assessment factor (1,000 for acute studies and 100 for chronic); PEC: predicted environmental concentration; PNEC: predicted no effect concentration; E_rC_{50}: median effective concentration; EC_{50}: median effective concentration; LC_{50}: median lethal concentration.
(1): 72-h E_rC_{50}.
(2): 48-h EC_{50}.
(3): 96-h LC_{50}.

Table 19: Risk characterisation (PEC/PNEC ratio) for sediment

Taxa	$PEC_{sediment}$ (µg/kg dry weight)	NOEC (mg/kg)	AF	PNEC (µg/kg)	PEC/PNEC
EqP	103	–	–	105.8	0.97

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

Bioaccumulation and risk for secondary poisoning

No data on bioaccumulation on monensin sodium were submitted. The highest log K_{OW} of 4.24 was reported at pH 5, log K_{OW} at pH 7 was reported at range 2.75–2.87 and 3.79 at pH 9. There is evidence that monensin is degraded in the animal body (see Section 3.2.2), therefore, bioaccumulation and risk for secondary poisoning is unlikely.

3.2.8.3. Conclusions on safety for the environment

The use of monensin sodium from Elancoban® G200 in complete feed for chickens for fattening does not pose a risk for the terrestrial compartment, aquatic compartment and sediment. The bioaccumulation potential of monensin in the environment is low. These conclusions are extended to chickens reared for laying and turkeys.

Moreover, considering the uncertainties on the identity and characterisation of the production strain and the lack of demonstration of the absence of the production strain and its DNA on the final product, the FEEDAP Panel cannot conclude on the safety of the additive for the environment.

3.3. Efficacy

For coccidiostats under re-evaluation, efficacy data should derive from two types of target animal experiments: (a) natural/artificial infection to simulate use conditions (e.g., floor pen studies with poultry), at least one of the locations should be in the EU, (b) actual use conditions in field trials, all should be done in the EU within the last 5 years. Anticoccidial sensitivity tests (ASTs) could replace

field trials provided they follow the criteria mentioned in the relevant guidance document on coccidiostats and histomonostats (EFSA FEEDAP Panel, 2011a).[94]

3.3.1. Efficacy in chickens for fattening

The applicant submitted three floor pens studies, three ASTs performed with recent field isolates and two ASTs performed with laboratory strains. These last two studies were not considered for the demonstration of efficacy because the laboratory strains do not represent field conditions (EFSA FEEDAP Panel, 2011a).

3.3.1.1. Floor pen studies in chickens for fattening

Three floor pen trials in chickens for fattening, conducted in 2011–2012, were submitted.[95] In trial 3, two parallel experiments with different inoculates were performed.[96] In each trial, 1-day-old chickens were penned and distributed into three treatments, an uninfected untreated control group (UUC), an infected untreated control group (IUC) and an infected treated group (IT). The IT group received feed containing 100 mg monensin sodium/kg feed, the lowest dose applied. The intended dietary monensin concentration was analytically confirmed (see Table 20). In the infected groups, all birds were inoculated with recent field isolates of pathogenic *Eimeria* species. Animal health and mortality were monitored daily. 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 on three birds per pen in trial 1, and one bird per pen in trial 2 and on four birds per pen in trial 3, following the method of Johnson and Reid (1970) (0 = no lesion, 1 = very mild, 2 = mild, 3 = moderate and 4 = severe).

Table 20: Experimental design of floor pen studies with chickens for fattening using Elancoban® G200

Trial	Replicates per treatment (birds[(1)] per replicate)	Inoculum characteristics			Feed analysis monensin sodium (mg/kg feed)[(2)]
		Year and country of isolation	Intended dose (number of oocysts) and strain per bird		Day and mode of inoculation
1	12 (41–42)	2012 Spain	100,000	*E. acervulina*	Day 14 via feed	98.0/96.7/95.9
			10,000	*E. tenella*		
			50,000	*E. maxima*		
2	12 (12)	2010 UK	33,991	*E. acervulina*	Day 16 orally via syringe	104.0/83.3
			25,349	*E. tenella/necatrix*		
			2,916	*E. maxima*		
			15,432	*E. mitis*		
			714	*E. praecox/brunetti*		

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

[95] Trial 1: Technical dossier/Section IV/Annex IV.2. Trial 2: Technical dossier/Section IV/Annex IV.3. and Trial 3: Technical dossier/Section IV/Annex IV.4.

[96] Trials 3a and 3b were conducted in the same research institute, at the same time, with the same feed, and used the same UUC group. Taking into account that the inoculum with sporulated oocysts is the most critical factor in this type of studies and that the UUC group (common control) is used only to verify the growth of the animals under farming conditions, 3a and 3b can be considered as separate studies in the assessment of the coccidiostatic efficacy of the additive against *Eimeria* infection.

Trial	Replicates per treatment (birds[1] per replicate)	Inoculum characteristics			Day and mode of inoculation	Feed analysis monensin sodium (mg/kg feed)[2]
		Year and country of isolation	Intended dose (number of oocysts) and strain per bird			
3a	8 (20)	2011 The Netherlands	104,000	*E. acervulina*	Day 14 orally via syringe	82.7/101.0
			30,000	*E. tenella*		
			86,000	*E. maxima*		
			12,000	*E. praecox/necatrix*		
			4,000	*E. mitis*		
3b	8 (20)	2011 Belgium	77,000	*E. acervulina*		
			12,000	*E. tenella*		
			20,000	*E. maxima*		
			3,000	*E. praecox/necatrix*		
			2,000	*E. mitis*		

(1): Male Ross 308 in trial 1, female Ross cobs in trial 2, breed and gender not reported for trial 3.
(2): The experimental diets were fed for 41 days in trial 1 and for 42 days in trials 2 and 3. In trial 1, birds received starter diet from day 0 to 14, grower diet from day 14 to 29 and finisher diet from day 29 to 41. In trial 2, birds received starter diet from day 0 to 10, grower diet from day 10 until study completion. In trial 3, birds received starter diet from day 0 to 14 and grower diet from day 14 to 42.

In trial 1, oocyst counts and lesions scores were analysed by non-parametric Kruskal-Wallis test. Performance parameters were analysed by a randomised block design using the pen as experimental unit. Differences between the IUC and the IT groups were compared with post hoc tests (least significant difference (LSD) test. In trial 2, Wilcoxon–Mann–Whitney test was used to analyse oocyst count and lesions scores. Performance parameters were analysed by a t-test (IT vs IUC). In trial 3, an ANOVA was performed with the data and IUC and IT were compared with post hoc tests (not reported). Level of significance was set at a p value ≤ 0.05.

Mortality (Table 21) in the IT groups of all trials was lower than in the IUC groups; however, the difference reached significance only in trial 3a. In trial 3a, nine birds were culled and eight birds were found dead. The dead birds were subjected to post-mortem examination; four of them, all belonging to IUC, showed signs of coccidiosis. In trial 3b, 10 birds were culled and four birds were found dead and taken for post-mortem examination; two of them, all belonging to IUC, showed signs of coccidiosis.

Table 21: Mortality (number of dead animals) registered in the floor pen trials[1]

	Trial 1	Trial 2	Trial 3a	Trial 3b
UUC	16 (2)	18 (0)	5	5
IUC	10 (5)	20 (6)	11	6
IT	7 (0)	7 (5)	1*	3

Mean values with * are significantly different from IUC ($p \leq 0.05$).
(1): Results of trials 1 and 2 refer to total mortality in the post-inoculation period; results of trials 3a and 3b refer to the whole study duration. In brackets coccidiosis-related mortalities are indicated.

Table 22 shows the results of intestinal lesion scoring. A significant reduction of the lesion scores was observed in the IT group compared to IUC in trial 1 (upper and middle intestine) and in trial 2 (upper intestine). Lesion scores in trial 3 were comparable in the IT and IUC groups.

Table 22: *Eimeria* infection related intestinal lesion scores in different intestinal sections 6 days post-inoculation(1)

	Upper	Middle	Lower	Caecal	Total
Trial 1					
UUC	0	0	–	0	–
IUC	1.9	1.4	–	2.1	–
IT	1.3*	0.6*	–	1.8	–
Trial 2					
UUC	0	0	–	0	1
IUC	2.3	2.4	–	1.3	2.4
IT	1.3*	2.5	–	1.1	2.1
Trial 3a					
UUC	0.7	0.4	0.1	0.1	–
IUC	1.2	0.4	0.1	1.0	–
IT	1.0	0.6	0.2	0.7	–
Trial 3b					
UUC	0.7	0.4	0.1	0.1	–
IUC	1.0	0.4	0.2	1.6	–
IT	1.0	0.7	0.1	1.1	–

– : not reported or not measured.
*: IT mean significantly different from IUC mean ($p \leq 0.05$).
(1): Lesions in the upper intestine were probably due to *E. acervulina*, in the middle intestine to *E. maxima* and in the caecal intestine to *E. tenella*.

Oocyst excretion on day 23 (9 days post-inoculation) was significantly reduced in the IT group compared to the IUC group for all three different *Eimeria* species in trial 1 (See Appendix D). In trial 2, species-specific results were not reported. Total oocyst counts measured on days 22, 24, 27, 29 and 31 showed numerically lower counts in the IT group compared to the IUC group. Significant differences were seen only on the last day of the trial (IT 592 vs. IUC 39,841). In trial 3a, oocyst excretion for *E. maxima* was significantly lower in IT compared to IUC at all time points (days 20, 22 and 28). In trial 3b, oocyst excretion per gram faeces (OPG) was significantly reduced by the treatment on day 22 for *E. mitis*, however, it is noted that the excretion of the same *Eimeria* species on day 20 was significantly higher in the IT group compared to the IUC group.

Table 23 summarises the results concerning the zootechnical endpoints. In all four experiments, weight gain of the IT birds was significantly higher compared to the IUC birds and reached the level of the UUC groups in trials 1 and 2. In trial 3, increased feed intake of the IT groups resulted in higher body weight gain than in the UUC groups with significant improvement of the feed to gain ratio.

Table 23: Performance parameters of chickens for fattening in floor pen trials

	Feed intake(1) (g)	Final body weight (g)	Weight gain(2) (g)	Feed to gain ratio(3)
Trial 1				
UUC	126	3,057	73	1.72
IUC	126	2,924	70	1.79
IT	125	3,064*	73*	1.70*
Trial 2				
UUC	3,262	2,557	2,046	1.59
IUC	3,132	2,427	1,886	1.66
IT	3,448*	2,593*	2,064*	1.67
Trial 3a				
UUC	3,348	–	1,538	2.18
IUC	3,690	–	1,684	2.19
IT	3,783	–	1,836*	2.06*

	Feed intake(1) (g)	Final body weight (g)	Weight gain(2) (g)	Feed to gain ratio(3)
Trial 3b				
UUC	3,348	–	1,538	2.18
IUC	3,365	–	1,551	2.18
IT	3,535	–	1,719*	2.06*

– : not reported.
*: IT mean significantly different from IUC mean ($p \leq 0.05$).
(1): Mean results of trial 1 refer to daily feed intake per bird during the whole study duration; those of trial 2 refer to total feed intake per bird in the period after inoculation (days 16–42); those of trial 3 refer to total feed intake per bird during the whole study duration.
(2): Mean results of trial 1 refer to daily weight gain per bird considering the whole study duration; those of trial 2 refer to the total weight gain per bird in the period after inoculation (days 16–42); those of trials 3a and 3b refer to total weight gain per bird during the whole study duration.
(3): Results of trials 1 and 3 refer to the feed to gain ratio calculated for the whole study duration; results of trial 2 refer to the ratios calculated for the period 16–42 days.

3.3.1.2. Anticoccidial sensitivity tests in chickens for fattening

Three ASTs performed in 2012 were submitted.[97] Recent field isolates of *Eimeria* species were used for inoculation. The birds were randomly allocated to the groups (UUC, IUC, IT). The IT group received feed containing 100 mg monensin sodium/kg feed, the lowest dose applied. Three other anticoccidial additives were also tested in all studies. The experimental design is described in Table 24. The intended dietary monensin concentration was analytically confirmed (Table 24). 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).

The statistical tests were two-sided; the level of significance was set at a p value ≤ 0.05. In AST-1 and AST-2, performance parameters and oocyst counts (log_{10} transformed) were compared by t-test (IT vs IUC). Intestinal lesions scores were analysed by Fisher's Exact test. In AST-3, intestinal lesion scores and mortality were analysed by Kruskal–Wallis test. Performance parameters and oocyst counts (log_{10} transformed) were analysed by ANOVA. Least squares means were compared by LSD test.

Table 24: Experimental design of ASTs with chickens for fattening using Elancoban® G200

Trial	Replicates per treatment (birds(1) per replicate)	Inoculum characteristics			Day of inoculation	Anticoccidial treatment(2) (days of life)	Feed analysis monensin sodium (mg/kg feed)
		Month/year and country of isolation	Intended dose (number of oocysts) per bird and strain				
1	4 (5)	03/2012 UK	296,375	*E. acervulina*	14	7–21	97.7
			23,399	*E. maxima*			
			31,198	*E. tenella*			
			31,198	*E. praecox*			
			7,799	*E. mitis*			
2	4 (5)	12/2011 France	218,548	*E. acervulina*	14	7–21	95.0
			29,802	*E. maxima*			
			21,855	*E. tenella*			
			11,722	*E. praecox*			
			3,974	*E. mitis*			

[97] AST-1: Technical dossier/Section IV/Annex IV.21. AST-2: Technical dossier/Section IV/Annex IV.22. AST-3: Technical dossier/Section IV/Annex IV.20.

Trial	Replicates per treatment (birds[1] per replicate)	Inoculum characteristics				Anticoccidial treatment[2] (days of life)	Feed analysis monensin sodium (mg/kg feed)
		Month/year and country of isolation	Intended dose (number of oocysts) per bird and strain		Day of inoculation		
3	4 (8)	03/2012 Spain	100,000	*E. acervulina*	15	8–21[3]	90.1
			25,000	*E. maxima*			
			25,000	*E. brunetti*			
			10,000	*E. tenella*			
			10,000	*E. necatrix*			

(1): Female Ross 308 in AST-1 and AST-2; Cobb 500 in AST-3, sex not indicated.
(2): Birds in the IT group were fed a basal diet supplemented with Elancoban® G200. Animals in the control groups UUC and IUC received the same basal diet without inclusion of the coccidiostat.
(3): Performance and intestinal lesions were assessed on day 21, oocyst excretion was measured on day 24.

There were no mortalities in AST-1, and one IUC bird died in AST-2. Increased mortality due to coccidiosis was seen only in IUC of AST-3; 12 birds died which is significantly different to the IT group in which no mortality occurred.

Table 25 summarises the results of the ASTs. Significantly lower OPG value in the IT group, showing the effect of the coccidiostatic treatment, was seen only in AST-3. A reduction of lesion scores by treatment (IT) was observed in all tests; however, significance was reached only in AST-1 (upper intestine) and AST-3 (mid and lower intestine).

In AST-2, bodyweight gain and feed intake were significantly better in the IT than in the IUC group over the 7 days post-inoculation (feed to gain ratio not reported for this period). In the same period, in AST-3, results of weight gain and feed to gain ratio were significantly better in IT than in the IUC group.

Table 25: Results of anticoccidial sensitivity tests with chickens for fattening

Group	Final body weight (g)	Feed intake (g)	Average body weight gain (g)	Feed to gain ratio	Total log_{10}OPG			Intestinal lesion scores			
								Upper	Mid	Low	Caeca
	D21	D14–21	D14–21	D14–21	D19	D20	D21	D21			
AST-1											
UUC[1]	700	2,518	355	–	0	0	0	0	0	0	0
IUC	530	2,073	194	–	6.52	6.51	5.64	2.6	1.1	0	0.7
IT	544	2,070	213	–	6.60	6.42	5.72	1.4*	0.9	0	0.1
AST-2											
UUC[1]	473	2,433	214	–	0	0	0	0	0	0	–
IUC	491	2,015	187	–	6.12	6.65	6.49	2.2	0.9	2.7	–
IT	628	2,480*	276*	–	6.13	6.49	6.31	2.3	0.8	2.0	–
	D21	D15–21	D15–21	D15–21	D21–24			D21			
AST-3											
UUC	538	–	253*	1.29*	0	0	0	0	0		
IUC	370	–	86	2.46	5.07	2.6	1.9	2.1	2.8		
IT	409	–	125*	1.56*	4.71*	2.3	1.3*	0.5*	2.6		

–: Not reported.
*: IT mean/UUC mean significantly different from IUC mean ($p \leq 0.05$).
(1): The cages of the UUC group were kept in another building than those of IUC and IT groups. The zootechnical data of UUC group are therefore not directly comparable to IUC and IT.

3.3.1.3. Synopsis of efficacy studies in chickens for fattening

The synopsis is based on three floor pen studies and three ASTs made with the lowest recommended dietary concentration of the coccidiostat Elancoban® G200 (100 mg monensin sodium/kg feed).

Mortality in the floor pen studies did not indicate a coccidiostatic effect since mortality rates were not significantly different and appeared even not to be influenced by *Eimeria* inoculation (mean UUC 6.3%, mean IUC 7.1%). In the ASTs, mortality was very low in two trials; however, it was affected by oocyst inoculation in a third trial (AST-3) and significantly reduced by monensin treatment.

Lesion scores in the upper, middle and lower intestine of *Eimeria* inoculated birds were numerically reduced by monensin sodium in all three floor pen studies and ASTs. However, this reduction reached significance only in two floor pen studies (in trial 1 for the upper and middle intestine and in trial 2 in the upper intestine) and in two ASTs (in AST-1 for the upper intestine and in AST-3 also for the middle and low intestine).

Oocyst excretion of all tested four *Eimeria* species was significantly reduced by Elancoban® G200 in one floor pen study (trial 1) and for *Eimeria maxima* in another study (trial 3a). A third study (trial 2) showed only numerical reductions of oocyst excretion (except on the last day of the trial where significance was reached). In AST-1 and AST-2, *Eimeria* oocyst excretion on days 3 to 7 post-inoculation was not affected by the Elancoban® treatment, whereas it was significantly reduced in AST-3.

Body weight gain of birds in the floor pen studies was significantly higher for the Elancoban® G200-treated groups compared to the infected non treated birds. These results are considered as further evidence of the coccidiostatic efficacy supported by the results of the specific endpoints.

In summary, the anticoccidial efficacy of 100 mg monensin sodium/kg feed from Elancoban® G200 is demonstrated in three floor pen studies (in floor pen study 1 by the endpoints lesion scores and oocyst excretion, in floor pen study 2 by reduced oocyst excretion and improved body weight, in floor pen study 3 by reduced oocyst excretion) and in two ASTs (in AST-1 by lesion scores and in AST-3 by reduced mortality, lesion scores and oocyst excretion). A third AST (AST-2) failed to demonstrate any significant improvement of the coccidiosis related endpoints.

3.3.2. Efficacy in chickens reared for laying

The applicant submitted four floor pens studies with the duration of 112 days and three floor pen studies with the duration of 23, 28 and 28 days. The FEEDAP Panel did not consider these last three studies as they did not meet the requirement for the minimum duration of efficacy studies in chickens reared for laying (EFSA FEEDAP Panel, 2011b). The applicant submitted four ASTs performed with recent field isolates and one AST performed with laboratory strains. This last study was not considered for the demonstration of efficacy because the laboratory strains do not represent field conditions (EFSA FEEDAP Panel, 2011a).

3.3.2.1. Floor pen studies in chickens reared for laying

Four floor pen studies in chickens reared for laying, conducted in 2012, were submitted.[98] Two of the trials were conducted in the same institute and using the same feed but with different inocula and control group (UUC); therefore, they will be reported here as trials 3a and 3b.[96] In each trial, replacement pullets (Hyline brown) were penned and distributed into the treatments (in trial 1, IUC and IT groups; in trials 2 and 3, UUC, IUC and IT groups). The IT group received feed containing 100 mg monensin sodium/kg feed, the lowest dose applied. The experimental design is summarised in Table 26. The intended dietary monensin concentrations were analytically confirmed (see Table 26). The experimental diets were fed for 112 days. In the infected groups, all birds were inoculated with recent field isolates of pathogenic *Eimeria* species. Animal health and mortality were monitored daily. 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 on three birds per pen in trial 1, on two birds per pen in trials 3a and 3b and on one bird per pen in trial 2, following the method of Johnson and Reid (1970) (0 = no lesion, 1 = very mild, 2 = mild, 3 = moderate and 4 = severe).

[98] Trial 1: Technical dossier/Section IV/Annex IV.5. Trial 2: Technical dossier/Section IV/Annex IV.7. Trial 3: Technical dossier/Section IV/Annex IV.9. and Trial 4: Technical dossier/Section IV/Annex IV.11.

In trial 1, an ANOVA was performed with the performance data and oocyst counts (after $\log_{10}$ transformation). Lesions scores were analysed by Kruskal-Wallis test and mortality was analysed by Chi squared test. In all tests IUC was compared with IT. In trial 2, Wilcoxon–Mann–Whitney test was used to analyse oocyst count and lesions scores. Performance parameters were analysed by a t-test (IUC vs IT). In trials 3a and 3b, an ANOVA was performed with the data and IUC and IT were compared with post hoc tests (not reported). Level of significance was set at a p value ≤ 0.05.

Table 26: Experimental design of floor pen studies with chickens reared for laying using Elancoban® G200

Trial	Replicates per treatment (birds per replicate)	Inoculum characteristics				Feed analysis monensin sodium (mg/kg feed)(1)
		Year and country of isolation	Intended dose (number of oocysts) and strain per bird		Day and mode of inoculation	
1	10 (30)	2012 Spain	100,000	*E. acervulina*	Day 14, via feed	84.4–95.1
			10,000	*E. tenella*		
			55,000	*E. maxima*		
			50,000	*E. brunetti*		
			10,000	*E. necatrix*		
2	12 (12)	2012 UK	341	*E. acervulina*	Day 15, by oral gavage	73.9–114.0
			5,000	*E. tenella/necatrix*		
			114	*E. praecox/brunetti*		
			170	*E. mitis*		
3a	10 (15)	2012 The Netherlands	70,800	*E. acervulina*	Day 14, orally via syringe	96.3–98.3
			14,000	*E. tenella*		
			8,800	*E. maxima*		
			10,400	*E. brunetti*		
			4,800	*E. praecox/necatrix*		
			12,000	*E. mitis*		
3b	10 (15)	2012 Belgium	62.400	*E. acervulina*	Day 14, orally via syringe	96.3–98.3
			25.600	*E. tenella*		
			25.600	*E. maxima*		
			8.800	*E. brunetti*		
			1,600	*E. praecox/necatrix*		
			8,000	*E. mitis*		

(1): In trial 1, birds received starter diet from day 0 to 42, grower diet from day 42 to 84 and developer diet from day 84 to 112. In trial 2, birds received starter diet from day 0 to 36 followed by grower diet until study completion. In trials 3 and 4, birds received starter diet from day 0 to 56 followed by grower diet until study completion.

Mortality is reported in Table 27. In trial 1, inoculation with *Eimeria* oocyst resulted in 38% losses. This mortality rate was reduced by the treatment to 9% which is considered as a clear evidence of the coccidiostatic efficacy of monensin sodium. Mortality of trials 2, 3a and 3b was very low also in the IUC group and could therefore not contribute in supporting monensin efficacy.

Table 27: Mortality (number of dead animals) post-inoculation in floor pen trials(1)

	Trial 1	Trial 2	Trial 3a	Trial 3b
UUC	–	1	1	0
IUC	115 (114)	0	3	0
IT	29* (28*)	1(1)	0	0

Mean values with * are significantly different from IUC ($p \leq 0.05$).
(1): In brackets coccidiosis-related mortalities are indicated.

Table 28 provides information on the lesion scores in different intestinal sections. Lesion scores were significantly reduced by the treatment in trial 1 in the lower intestine and in trial 3a in the caecum (Table 28). No other consistent changes were observed.

Table 28: *Eimeria* infection related intestinal lesion scores in different intestinal sections 7 days post-infection (5 days post-infection trial 2)

	Upper	Middle	Low	Caecal
Trial 1				
IUC	1.4	1.3	1.4	2.4
IT	1.5	1.1	0.9*	2.1
Trial 2				
UUC	0	0	–	0
IUC	0.9	0.3	–	1.4
IT	0.8	0.3	–	2.1
Trial 3a				
UUC	0.1	0.7	0.4	0.2
IUC	0.5	0.7	0.3	0.6
IT	0.4	0.9	0.4	0.1*
Trial 3b				
UUC	0.2	0.6	0.4	0.2
IUC	0.2	0.7	0.4	1.1
IT	0.3	0.7	0.4	1.0

–: not reported.
*: IT mean significantly different from IUC mean ($p \leq 0.05$).

In trial 1, mean OPG was significantly reduced by the treatment at days 8 (log_{10}OPG 4.67 vs. 5.08) and 11 (log_{10}OPG 3.74 vs. 4.65) after inoculation. In trial 2, OPG was measured on days 19, 21, 23, 26, 28, 30, 33, 35 and in biweekly intervals until day 77. Among these 11 control points, only at days 28 and 30, 13 and 15 days after inoculation, a significant reduction by the treatment could be seen (log_{10}OPG 5.17 vs. 5.60 and 5.38 vs. 5.57, respectively). Although 62 days after inoculation the difference in OPGs was significant, total OPG of IT (log_{10}OPG 1.98) and IUC (log_{10}OPG 2.80) was low, particularly when compared to the UUC group (log_{10}OPG 4.48). In trials 3a and 3b, OPG was measured on days 21, 28 and 70 (7, 14 and 56 days post-inoculation). *Eimeria* oocysts were counted for *E. acervulina*, *tenella*, *maxima*, *brunetti*, *nectarix/praecox* and *mitis*. The sum was also considered. Although for all *Eimeria* species at all three timepoints of trial 3a a numerical reduction of oocyst excretion by the treatment became evident (with the exception of days 28 and 70 for *E. necatrix/praecox*) none of these differences (including the sum of all *Eimeria* species) reached significance. In trial 3b, total OPG 7 days after inoculation (log_{10}OPG IUC 5.69, IT 5.47) was higher compared to study 3a (log_{10}OPG IUC 5.37, IT 5.04). The only significant differences in OPG observed in trial 3b were found 7 days after inoculation for *E. tenella* (log_{10}OPG IT 3.79 vs IUC 4.74) and *E. necatrix/praecox* (log_{10}OPG IT 2.08 vs IUC 3.52).

Table 29 summarises the results concerning zootechnical endpoints. No statistically significant differences were seen in any of the parameters in the four experiments except for the feed to gain ratio in trial 3b showing improvement in the IT birds compared to IUC birds.

Table 29: Performance parameters of chickens reared for laying in floor pen trials

	Feed intake(1) (g)	Final body weight (g)	Weight gain(2) (g)	Feed to gain ratio(3)
Trial 1				
IUC	45	1,316	11	3.95
IT	44	1,309	11	3.90
Trial 2				
UUC	7,573	1,645	1,605	–
IUC	7,481	1,570	1,529	–
IT	7,514	1,558	1,517	–
Trial 3a				
UUC	5,017	–	1,477	3.40
IUC	5,092	–	1,501	3.39
IT	5,057	–	1,498	3.38
Trial 3b				
UUC	5,295	–	1,520	3.48
IUC	5,352	–	1,531	3.50
IT	5,192	–	1,546	3.36*

– : not reported.
*: IT mean significantly different from IUC mean ($p \leq 0.05$).
(1): Mean results of trial 1 refer to daily feed intake per bird; those of trials 2, 3a and 3b to total feed intake per bird during the whole study duration.
(2): Mean results of trial 1, refer to daily weight gain per bird considering the whole study duration; those of trials 2, 3a and 3b refer to the total weight gain per bird during the whole study duration.
(3): Results refer to the feed to gain ratio calculated for the whole study duration.

3.3.2.2. Anticoccidial sensitivity tests in chickens reared for laying

Four ASTs performed in 2012–2013 were submitted.[99] Recent field isolates of *Eimeria* species were used for inoculation. The birds were randomly allocated to the groups UUC, IUC and IT, the latter receiving feed supplemented with Elancoban® at an intended concentration of 100 mg monensin sodium/kg feed, the lowest dose applied. One other anticoccidial additive was also tested in all studies. The experimental design is described in Table 30. The intended dietary monensin concentration was analytically confirmed (Table 30). 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).

The statistical tests were two-sided; the level of significance was set at a p value ≤ 0.05. For pair-wise comparisons, the reference group was the IUC. Performance parameters and oocyst counts (natural log) were analysed by ANOVA and pair-wise comparisons were done by LSD test. Intestinal lesions were analysed by a non-parametric trend test (Jonckheere-Terpstra). In AST-2, intestinal lesion scores and mortality were analysed by Kruskal–Wallis test. Performance parameters and oocyst counts (ln or $\log_{10}$ transformed) were analysed by ANOVA. Least squares means were compared by LSD test. In AST-3 and AST-4, performance parameters and oocyst counts ($\log_{10}$ transformed) were compared by t-test (IT vs IUC). Intestinal lesions scores were analysed by Fisher's Exact test.

[99] AST-1: Technical dossier/Section IV/Annex IV.23. AST-2: Technical dossier/Section IV/Annex IV.24. AST-3: Technical dossier/Section IV/Annex IV.25. and AST-4: Technical dossier/Section IV/Annex IV.26.

Table 30: Experimental design of ASTs with chickens reared for laying using Elancoban® G200

AST	Replicates per treatment (birds(1) per replicate)	Inoculum characteristics				Anticoccidial treatment(2) days	Feed analysis monensin sodium (mg/kg feed)
		Month/Year and country of isolation	Intended dose (number of oocyst) per bird and strain		Day of inoculation		
1	6 (5)	2012 Belgium	16,400	*E. acervulina*	31	25–37	90
			8,600	*E. tenella*			
			11,800	*E. maxima*			
			1,600	*E. brunetti*			
			200	*E. mitis*			
			1,400	*E. necatrix*			
2	6 (8)	2012 Spain	100,000	*E. acervulina*	15	8–21(3)	94.7
			25,000	*E. maxima*			
			25,000	*E. brunetti*			
			10,000	*E. tenella*			
			10,000	*E. necatrix*			
3	4 (5)	2012 UK	201,457	*E. acervulina*	14	7–21	96.8
			21,207	*E. maxima*			
			21,207	*E. tenella*			
			15,905	*E. mitis*			
			5,302	*E. praecox*			
4	4 (5)	2012 France	211,200	*E. acervulina*	14	7–21	98.3
			24,000	*E. maxima*			
			19,968	*E. tenella*			
			18,048	*E. brunetti*			
			6,144	*E. necatrix*			
			< 10,000	*E. praecox*			
			< 10,000	*E. mitis*			

(1): ISA brown layer in AST-1, AST-3 and AST-4. Hyline brown in AST-2.
(2): Birds in the IT group were fed a basal diet supplemented with Elancoban® G200. Animals in the control groups UUC and IUC received the same basal diet without inclusion of the coccidiostat.
(3): Performance and intestinal lesions were assessed on day 21, oocyst excretion was measured on day 24.

There were no mortalities in AST-1, AST-3 and AST-4. In AST-2, three IUC birds died due to coccidiosis and none in the IT group. Tables 31 and 32 summarise the results of the ASTs.

Significantly lower OPG value in the IT group, showing the effect of the coccidiostatic treatment, was seen in AST-1 and AST-2. A reduction of lesion scores by treatment (IT) was observed in all tests.

In AST-1, bodyweight gain was significantly better in the IT than in the IUC group over the seven days post-inoculation. In the same period, in AST-2, results of weight gain and feed to gain ratio were significantly better in IT than in the IUC group.

Table 31: Results of anticoccidial sensitivity tests with chickens for fattening: AST-1

Group	Body weight (g)	Feed intake (g/day)	Weight gain (g/day)	Feed to gain ratio	Total lnOPG	Intestinal lesion scores(1)			
						Acer	*Max*	*Ten*	Total
	D37	D31–37	D31–37	D31–37	D37	D37			
AST-1									
UUC	349*	41.4	12.0*	3.47*	0*	0.2	0.3	0	0.4
IUC	330	44.1	9.1	4.95	14.0	1.5	1.8	1.3	4.7
IT	340	49.2	11.3*	4.35	13.2*	0.6*	1.8	1.6	3.9

(1): *Eimeria* species are *Acervulina* (*acer*), *Tenella* (*ten*), *Maxima* (*max*).
*: IT mean/UUC mean significantly different from IUC mean ($p \leq 0.05$).

Table 32: Results of anticoccidial sensitivity tests with chickens for fattening:; AST-2, AST-3 and AST-4

Group	Final body weight (g)	Feed intake (g)	Weight gain (g)	Feed to gain ratio	Total log_{10}OPG			Intestinal lesion scores			
								Upper	*Mid*	*Low*	*Caeca*
	D21	D15–21	D15–21	D15–21	D21–24			D21			
AST-2											
UUC	170	–	61.6*	2.29*	0			0	0	0	0
IUC	124	–	16.0	3.65	5.34			2.6	1.8	2.0	2.7
IT	154	–	45.3*	2.67*	4.77*			2.3*	1.2*	0.6*	2.4*
	D21	D14–21	D14–21	D14–21	D19	D20	D21	D21			
AST-3											
UUC(1)	220	1,410	85.6	–	0	0	0	0	0	–	0
IUC	189	808	59.0	–	6.72	5.70	6.37	1.8	1.4	–	0.25
IT	178	793	53.4	–	6.40	6.35	6.23	1.3	0.6*	–	0.6
AST-4											
UUC(1)	219	1,810	87.8	–	0	0	0	0	0	0	0
IUC	199	975	75.3	–	6.03	7.05	6.22	2.5	0.8	0.8	2.1
IT	203	905	75.1	–	5.93	6.99	6.48	1.4*	1.1	0.9	2.3

–: not reported.
*: IT mean/UUC mean significantly different from IUC mean ($p \leq 0.05$).
(1): The cages of the UUC group were kept in another building than those of IUC and IT groups. The zootechnical data of UUC group are therefore not directly comparable to IUC and IT.

3.3.2.3. Synopsis of efficacy studies in chickens reared for laying

Monensin sodium significantly reduced coccidiosis related mortality in floor pen study 1. Intestinal lesion scores were significantly reduced by the treatment in the lower intestine of trial 1 and in the caecum in trial 3a. The treatment significantly reduced oocyst excretion measured as total oocyst in the period up to approximately 2 weeks after inoculation in trials 1 and 2; in trial 3b, where total oocyst excretion was not significantly affected by the treatment, a significant reduction was found for two *Eimeria* species (*E. tenella*, *E. necatrix/praecox*) due to the monensin treatment.

In two of four ASTs, oocyst excretion was significantly reduced in the monensin-treated groups. In all four ASTs, the intestinal lesion scores were lower in the IT roups compared to the IUC groups. In AST-1, the total intestinal lesions scores were significantly reduced; in AST-2, significantly lower lesion scores were observed in all intestinal segments assessed. In AST-3 and AST-4, significant reduction of the intestinal lesions was seen in the middle and upper intestine, respectively.

In summary, the results of all submitted studies (four floor pen studies and four ASTs) are considered indicative for the coccidiostatic potential of 100 mg monensin sodium from Elancoban® G200/kg complete feed for chickens reared for laying.

3.3.3. Efficacy in turkeys for fattening

The applicant submitted four floor pens studies, three ASTs performed with recent field isolates and one AST performed with laboratory strains. This last study was not considered for the demonstration of efficacy because the laboratory strains do not represent field conditions (EFSA FEEDAP Panel, 2011a). A field study was also submitted.

3.3.3.1. Floor pen studies in turkeys for fattening

Four floor pen studies in turkeys for fattening, conducted in 2012–2013, were submitted.[100] Two trials were conducted in the same institute and using the same feed but different inocula and control

[100] Trial 1: Technical dossier/Section IV/Annex IV.12. Trial 2a: Technical dossier/Section IV/Annex IV.13. Trial 2b: Technical dossier/Section IV/Annex IV.14. Trial 3: Technical dossier/Section IV/Annex IV.15.

group (UUC); therefore, they will be reported here as trials 2a and 2b.[101] In each study, turkeys were penned and distributed into the treatment groups (UUC, IUC, IT). The IT group received feed containing 60 mg monensin sodium/kg feed, the lowest dose applied. The experimental design is summarised in Table 33. The intended dietary monensin concentration was analytically confirmed (see Table 33). In the infected groups, all birds were inoculated with recent field isolates of pathogenic *Eimeria* species. Animal health and ortality were monitored daily. Feed intake and body weight of the animals were measured, feed to gain ratio was calculated. Samples of excreta were analysed for oocyst excretion. Faecal consistency was examined in trial 3.

Table 33: Experimental design of floor pen studies with turkeys using Elancoban® G200

Trial	Replicates per treatment (Birds(1) per replicate)	Inoculum characteristics				Feed analysis monensin sodium (mg/kg feed)(2)
		Year and country of isolation	Intended dose (number of oocysts) and strain per bird		Day and mode of inoculation	
1	8 (29)	2012 France	60,000	*E. meleagrimitis*	Day 14 via feed	58.2/61.4/58.0
			111,600	*E. gallopavonis* + *E. adenoeides*		
			70,000	*E. meleagridis*		
2a	10 (15)	2011 Germany	104,000	*E. meleagrimitis*	Day 14 orally via syringe	58.2/55.9/55.6/ 58.9
			30,000	*E. dispersa*		
			86,000	*E. adenoeides*		
2b	10 (15)	2011 Belgium	77,000	*E. meleagrimitis*		
			12,000	*E. dispersa*		
			20,000	*E. adenoeides*		
3	12 (12)	2011 France	80,274	*E. meleagrimitis*	Day 15 orally via syringe	52/29.1-49.2/44.8-55.0
			8,386	*E. dispersa*		
			31,151	*E. adenoeides*		

(1): Female BUT9 in trial 1, male and female Wirral White in trial 3, breed and gender not reported for trial 2.
(2): The experimental diets were fed for 84, in trial 1 and for 112 days in trials 2 and 3. In trial 1, birds received starter diet from week 0 to 4, grower diet from week 4 to 8 and finisher diet until study completion. In trial 2, birds received starter diet from day 0 to 28, rearer diet from day 28 to 56, grower diet from day 56 to 84 and finisher diet from day 84 until 112. In trial 3, birds received starter, grower 1 and grower 2 diets.

In trial 1, an ANOVA was performed with the performance data. Oocyst counts were analysed by Kruskal-Wallis test and mortality was analysed by chi-squared test. In all tests, IUC was compared with IT using LSD test. In trials 2a and 2b, an ANOVA was performed with the data and IUC and IT were compared with post hoc tests (not reported). In trial 3, Wilcoxon–Mann–Whitney test was used to analyse oocyst counts. Performance parameters were analysed by a t-test (IUC vs IT). Level of significance was set at a p value ≤ 0.05 for two-sided tests.

In trial 1, 64 birds died during the 2 weeks following inoculation (days 14–26), all belonging to IUC and confirmed by post-mortem examination to be coccidiosis-related (about 27% loss). Statistical analysis confirmed this difference as significant. Five more birds died in the following grower period (days 26–56), one from UUC and 4 from IUC, all confirmed coccidiosis related. Mortality in trials 2a and 2b was low (18 out of 450 in 2a and 16 out of 450 in 2b). No significant differences were seen between the groups. Dead birds were not necropsied/examined for coccidiosis. In trial 3, losses in the critical period two weeks after inoculation were low (between 1 and 2 per group). Not all birds, which died or were euthanised after inoculation, were sent for post-mortem analysis or were necropsied. Mortality rate could not be used as an endpoint for the assessment of the monensin efficacy in trial 3.

In trial 1, oocyst excretion was counted on days 25, 56 and 84 (11, 42 and 70 days post-inoculation). Distinction was made between big oocysts (*E. gallopavonis, E. meleagridis, E. adenoides*) and small oocysts (*E. meleagrimitis*). No statistically significant difference in OPGs was found between

[101] Trials 2a and 2b were conducted in the same research institute, at the same time, with the same feed, and used the same UUC group. Taking into account that the inoculum with sporulated oocysts is the most critical factor in this type of studies and that the UUC group (common control) is used only to verify the growth of the animals under farming conditions, 3a and 3b can be considered as separate studies in the assessment of the coccidiostatic efficacy of the additive against *Eimeria* infection.

IT and IUC at any days examined. In trials 2a and 2b, total oocyst excretion values on days 21, 28 and 70 (7, 14 and 56 days post-inoculation) did not show significant differences between the IUC and IT groups. Concerning individual OPG counts, OPGs of *E. dispersa* in trial 2a and of *E. adenoides* in trial 2b were significantly higher on day 21 in IUC birds compared to IT birds (log_{10}OPG 4.68 vs 3.30 and 2.73 vs below detection limit, respectively). No other significant differences were observed in any other individual OPG counts. In trial 3, OPG was measured on days 19, 21, 23, 26, 28, 30, 33, 35 and in biweekly intervals until day 77. The data showed an inconsistent pattern concerning the difference between IUC and IT. Only OPG on day 18 post-inoculation (day 33) was significantly lower in the IT group (log_{10}OPG 2.49) than in the IUC group (log_{10}OPG 3.30). However, the low number of excreted oocyst indicates a low infection level.

In contrast to chicken, the intestinal lesion score is not considered a sensitive indicator for the efficacy of a coccidiostat in turkeys. The faecal score should be used instead (Guidance on the assessment of the efficacy of feed additives; FEEDAP Panel, 2018). Faecal score was measured in trial 3 on days 15, 19, 20, 21, 22 with scores ranging from 1 (normal) to 4 (runny mucous-like consistency).[102] Four days after inoculation, a mean faecal score of 1.0 was observed for all experimental groups. On days 5, 6 and 7 post-inoculation, faecal scores for the UUC groups were between 1.17 and 1.33; in the IT group between 1.0 and 1.6; the IUC groups showing constantly higher scores of 1.75. The difference between IUC and IT (1.75 vs 1.0) reached near significance ($p = 0.056$) on day 7 post-infection.

Table 34 summarises the results concerning zootechnical endpoints.

After completion of the 84-day experimental period of trial 1, average daily feed intake and daily weight gain were significantly higher in the IT group compared to the IUC group. This is probably a result of the significant increase in feed intake and average daily gain during 6 weeks after inoculation, the more sensitive period of the study. Feed to gain ratio was significantly improved by the treatment only in the two weeks immediately after inoculation. Cumulative feed intake and weight gain of the 112-day experimental period of studies 2a and 2b were not significantly different, however, there was a transient significant positive effect compared to IUC on weight gain and feed to gain ratio in the first two weeks after inoculation of trial 2a and on weight gain and feed intake in trial 2b in the same time period.

No significant differences in total body weight gain were seen in trial 3, cumulative data on feed intake were not statistically assessed. For earlier experimental periods (7–43 days) significant differences between IUC and IT became apparent for males and females, demonstrating a partial compensation of the growth depression caused by the *Eimeria* infection. A comparable effect on feed to gain ratio was only seen in the first week after inoculation. No consistent differences in the feed to gain ratio between IT and IUC were observed in the following periods.

Table 34: Performance data of turkeys in floor pen trials

	Feed intake(1) (g)	Final body weight (g)	Weight gain(2)	Feed to gain ratio
Trial 1				
UUC	175.3	6,831	80.7	2.17
IUC	157.3	6,537	77.2	2.04
IT	166.7*	6,747*	79.7*	2.09
Trial 2a				
UUC	34,981	–	15,567	2.25
IUC	35,074	–	15,431	2.27
IT	35,181	–	15,591	2.26
Trial 2b				
UUC	34,981	–	15,567	2.25
IUC	35,110	–	15,382	2.28
IT	34,932	–	15,608	2.24
Trial 3(3)				
UUC	19,732/25,565	7,204/10,248	7,143/10,190	–

[102] Score 1= Normal, Score 2- Any In pen showing mucous-like consistency, faeces still well formed Score 3 Some showing mucous-like consistency, some well formed, some runny. Score 4 – all showing runny mucous-like consistency.

	Feed intake(1) (g)	Final body weight (g)	Weight gain(2)	Feed to gain ratio
IUC	19,460/24,323	7,030/9,855	6,971/9,795	–
IT	20,066/24,454	7,269/10,060	7,209/10,000	–

–: not reported.
*: IT is significantly different from IUC ($p \leq 0.05$).
(1): Mean results in trial 1 refer to daily feed intake per bird; those of trial 2 and 3 refer to total feed intake per bird during the whole study duration.
(2): Mean results in trial 1 refer to daily gain per bird, results of trial 2a and 2b are given as total gain per bird.
(3): Female/male.

3.3.3.2. Anticoccidial sensitivity tests in turkeys for fattening

Three ASTs performed in 2012–2013 were submitted.[103] Recent field isolates of Eimeria species were used for inoculation. BIG-6 turkeys were randomly allocated to the groups UUC, IUC and IT, the latter receiving feed supplemented with Elancoban® G200 at an intended concentration of 60 mg monensin sodium/kg feed, the lowest dose applied. One other anticoccidial additive was also tested in all studies. The experimental design is described in Table 35. The intended dietary monensin concentration was analytically confirmed (Table 35). 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. ntestinal lesions were examined in AST-2 and AST-3, but only a descriptive summary was provided (not individual scores).

The statistical tests were two-sided; the level of significance was set at a p value ≤ 0.05. For pair-wise comparisons, the reference group was the IUC. In AST-1, mortality was analysed by Kruskal–Wallis test. Performance parameters and oocyst counts (log_{10} transformed) were analysed by ANOVA. Least squares means were compared by LSD test. In AST-2 and AST-3, performance parameters and oocyst counts (log_{10} transformed) were compared by t-test (IT vs IUC).

Table 35: Experimental design of ASTs with turkeys for fattening using Elancoban® G200

AST	Replicates per treatment (birds per replicate)	Inoculum characteristics			Day of inoculation	Anticoccidial treatment(1)	Feed analysis monensin sodium (mg/kg feed)
		Year and country of isolation	Intended dose (number of oocysts) per bird and strain				
1	6 (8)	– France	45,000	*E. meleagrimitis*	15	8–21(2)	59.3
			52,200	*E. gallopavonis*			
			27,200	*E. adenoeides*			
			122,200	*E. meleagridis*			
2	4 (5)	2012 UK	21,100	*E. meleagrimitis*	14	7–21	59.0
			9,591	*E. dispersa*			
			7,673	*E. adenoeides*			
3	4 (5)	2012 France	80,720	*E. meleagrimitis*	14	7–21	54.2
			46,898	*E. meleagridis*			
			104,432	*E. adenoeides*			

–: not reported.
(1): Birds in the IT group were fed a basal diet supplemented with Elancoban® G200. Animals in the control groups UUC and IUC received the same basal diet without inclusion of the coccidiostat.
(2): Performance and intestinal lesions were assessed on day 21, oocyst excretion was measured on day 24.

In AST-1, a total of 18 birds died between 5 and 8 days post-infection, all in the IUC group (mortality rate 37.5%) Clinical coccidiosis was confirmed in all these 18 birds. In AST-3, a total of 13 birds died, all in the IUC group (mortality rate 65%). Post-mortem analysis was foreseen but not reported. No coccidiosis-related mortality was reported in AST-2.

In ASTs 2 and 3, the intestinal content was assessed particularly considering its consistency and presence of parasites in smears. In AST-2, no *Eimeria* were seen in the intestinal content of the UUC group. In contrast, few *E. meleagrimitis, E. dispersa* and *E. adenoeides* were seen in the intestinal

[103] AST-1: Technical dossier/Section IV/Annex IV.28. AST-2: Technical dossier/Section IV/Annex IV.29. AST-3: Technical dossier/Section IV/Annex IV.30.

content of the IUC group and only a few *Eimeria dispersa* were found in the mid-intestine of the IT group. Consistency of intestine content was normal in UUC, in contrast it was mucoid and watery in IUC and IT. Some mild diarrhoea was reported in three of four replicates of the IUC group on day 4 post-inoculation. In AST-3, the consistency of the intestinal content of the UUC group was reported as normal whereas in the IUC group the consistency was described as mucoid and 'cheesy cores' in caeca were reported. The content of duodenum and caeca in the IT group appeared to be watery. No parasites were detected in the smears of the UUC and the IT group whereas for the IUC group numerous *Eimeria meleagrimitis, meleagridis and adenoeides* oocysts were found.

Table 36 summarises the performance parameters and OPGs. In AST-1, the samples used for OPG determination were collected on day 24, however, they contained droppings from day 21 until day 24. A significantly smaller OPG value was found in the IT group compared to the IUC group. In AST-2, in days 19, 20 and 21 oocysts were found in the droppings of both the IUC and IT groups. The differences between the groups were not significant at any time point (and did not show a consistent trend). In AST-3, oocyst excretion could first be observed on day 19 in the IUC group but not in the IT group. On day 20 OPGs were highest but very similar in both groups. No significant differences between IUC and IT were detected on days 20 and 21.

In all three ASTs, bodyweight gain in the sensitive period (of 7 days) after inoculation was significantly better in the IT group than in the IUC group as also feed to gain ratio in AST-1 (which was not reported for AST-2 and 3 for the sensitive period).

Table 36: Results of anticoccidial sensitivity tests in turkeys for fattening

Group	Body weight (g)	Feed intake (g)	Weight gain (g)	Feed to gain ratio	Total log_{10}OPG		
	D21	D15–21	D15–21	D15–21	D21–24		
AST 1							
UUC	489	–	231*	1.62*	0		
IUC	322	–	64	3.12	5.29		
IT	393	–	135*	1.93*	4.91*		
AST 2					**D19**	**D20**	**D21**
UUC(1)	537	2,031	259	–	0	0	0
IUC	359	1,376	76	–	4.47	6.24	5.78
IT	378	1,429	101*	–	4.64	6.35	5.77
AST 3					**D19**	**D20**	**D21**
UUC(1)	577	2,013	273	–	0	0	0
IUC	340	1,050	49	–	4.99	6.15	5.82
IT	423	1,465*	123*	–	0*	5.94	5.69

–: not reported.
*: IT mean significantly different from IUC mean ($p \leq 0.05$).
(1): The cages of the UUC group were kept in another building than those of the IUC and IT groups. The zootechnical data of the UUC group are therefore not directly comparable to IUC and IT.

3.3.3.3. Field trial

The applicant provided a field trial from 2012 carried out under controlled field conditions.[104] Two treatments were considered, complete feed either supplemented with 60 mg monensin sodium from Elancoban® G200 or with another chemically synthesised coccidiostat. The trial was carried out in two different poultry houses, each containing one of the two experimental treatments. Both groups were treated with antibiotics on day 33 for 4 days and on day 60 for 5 days. For these reasons, the trial could not be considered further.

Synopsis of efficacy studies in turkeys for fattening

In floor pen study 1, the absence of coccidiosis-related mortality was observed in the IT group during the first six weeks after artificial infection which per se introduced a high mortality. In studies 2a and 2b, the only coccidiosis related direct positive effect of the monensin treatment was a reduction of excretion of one *Eimeria* species (of a total of three) in each study part 7 days after

[104] Technical dossier/Section IV/Annex IV.27.

inoculation. With respect to these findings, the significant compensation of growth depression in the IUC group by the treatment in the first two weeks after inoculation is considered a coccidiosis related effect. A turkey-specific endpoint for coccidiosis control is faecal consistency. In trial 3, faecal score was significantly improved (higher faecal consistency) by monensin sodium 7 days post-inoculation.

The absence of coccidiosis-related mortality in the presence of high-mortality rates of the IUC groups in two ASTs (AST-1 and AST-3) indicates the severe challenge by the inoculation and the effectiveness of the treatment in reducing mortality. In AST-1, also oocyst excretion was significantly reduced by the treatment. No parasites could be found in the intestinal content of duodenum and caeca in the IT group of AST-3 whereas in the UUC group numerous *Eimeria* oocysts were found. None of the coccidiostatic endpoints in AST-2 supported the evidence of efficacy of monensin sodium.

3.3.4. Conclusions on efficacy for the target species

In chickens for fattening, the efficacy of 100 mg monensin sodium/kg complete feed from Elancoban® G200 was demonstrated in three floor pen studies and in two ASTs, a third AST failing to show anticoccidial efficacy. In chickens reared for laying, the efficacy of 100 mg monensin sodium/kg complete feed from Elancoban® G200 was demonstrated in four floor pen studies and in four ASTs.

Considering the total number of studies showing efficacy in chickens for fattening and in chickens reared for laying and that the same *Eimeria* species are prevalent in these two species, the FEEDAP Panel concludes that monensin sodium from Elancoban® G200 has the potential to effectively control coccidiosis at the minimum applied dose of 100 mg/kg complete feed in chickens for fattening and chickens reared for laying.

In turkeys for fattening, the efficacy of 60 mg monensin sodium/kg complete feed from Elancoban® G200 was demonstrated in three floor pen studies and in two ASTs, a third AST failing to show anticoccidial efficacy. The FEEDAP Panel is therefore not in the position to conclude on the efficacy of monensin sodium from Elancoban® G200 as a coccidiostat for turkeys for fattening.

3.4. Post-market monitoring

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

4. Conclusions

Elancoban® G200 contains the active substance monensin sodium which is produced by fermentation. Limited data on the taxonomic identification of the production strain does not allow the proper identification of the strain as *S. cinnamonensis*. The FEEDAP Panel cannot conclude on the absence of genetic determinants for antimicrobial resistance in the production strain, on the presence of viable cells/spores of the production strain/DNA in the final product. Therefore, the FEEDAP Panel cannot conclude on the safety of the additive for the target species, consumer, user and environment with regard to the safety of the production strain.

The following conclusions apply to monensin sodium included in the additive.

Based on the available data set, the FEEDAP Panel cannot conclude on the safety of the highest applied dietary concentration of monensin (125 mg/kg) for chickens for fattening and to derive a margin of safety. Monensin sodium is safe for turkeys for fattening at inclusion level of 100 mg/kg complete feed, with a margin of safety of 1.5. Gram-positive bacteria are susceptible to monensin. Monensin sodium is not considered to be involved in cross-resistance to other antibiotics. The use of monensin as a coccidiostat in chickens did not affect the colonisation or shedding of *Salmonella* in the gastrointestinal tract. The simultaneous use of Elancoban® G200 and certain antibiotic drugs (e.g. tiamulin) is contraindicated.

Monensin sodium is not genotoxic and, based on the results of chronic carcinogenicity studies performed in rats and mice, is not carcinogenic. The lowest NOAEL identified from all the toxicological studies was 1.1 mg/kg bw per day based on a 2-year chronic toxicity/carcinogenicity assay in rat. The pharmacological NOAEL of 0.345 mg monensin sodium/kg bw per day identified in dog for acute pharmacological effects on the cardiovascular system has been considered as an appropriate basis for the acute health-based guidance value of 0.003 mg monensin sodium/kg bw per day already established by the FEEDAP Panel in its former opinion applying an uncertainty factor of 100.

The chronic exposure to monensin residues resulting from the use of monensin sodium as a feed additive in chickens would amount to up to 10% of the ADI (toddlers). The combined chronic exposure to monensin residues resulting from use of monensin as a feed additive in chicken and as a veterinary medicine in bovine would reach up to 48% of the ADI. Acute exposures due to the consumption of poultry tissues were found below the ADI for all age classes. These conclusions are extrapolated to chickens reared for laying and turkeys for fattening. Based on this, the FEEDAP Panel considers that Elancoban® G200 containing monensin sodium is safe for the consumer.

The existing MRLs for poultry tissues ensure consumer safety (acute and chronic exposure; all age classes) provided that the withdrawal period of 1 day is respected.

Elancoban® G200 is very irritant for the eye, but it is not a skin irritant. However, systemic toxicity may occur following skin exposure. Elancoban® G200 should be regarded as a potential skin and respiratory sensitiser. Inhalation exposure is considered a risk to persons handling the additive.

The use of monensin sodium from Elancoban® G200 in complete feed for chickens for fattening does not pose a risk for the terrestrial compartment, aquatic compartment and for sediment. The bioaccumulation potential of monensin in the environment is low. These conclusions are extended to chickens reared for laying and turkeys.

Monensin sodium from Elancoban® G200 has the potential to effectively control coccidiosis at the minimum applied dose of 100 mg/kg complete feed in chickens for fattening and chickens reared for laying. In turkeys for fattening, the efficacy of 60 mg monensin sodium/kg complete feed from Elancoban® G200 was demonstrated in three floor pen studies and in two ASTs, a third AST failing to show anticoccidial efficacy. The FEEDAP Panel is therefore not in the position to conclude on the efficacy of monensin sodium from Elancoban® G200 as a coccidiostat for turkeys for fattening.

5. Remark

Monensin sodium is toxic to horses at doses proposed for feed supplementation in poultry.

Documentation provided to EFSA and Chronology

Date	Event
29/07/2013	Dossier received by EFSA; Elancoban® G200 for chickens for fattening, chickens reared for laying and turkeys for fattening submitted by Eli Lilly and Company Ltd.
05/09/2013	Reception mandate from the European Commission
13/06/2014	Application validated by EFSA – Start of the scientific assessment
01/07/2014	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*
10/07/2014	Reception of supplementary information from the applicant - Scientific assessment re-started
24/07/2014	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. *Issues: safety for target species, safety for the consumer, safety for the user and environment*
13/09/2014	Comments received from Member States
12/09/2014	Reception of the Evaluation report of the European Union Reference Laboratory for Feed Additives
23/10/2014	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Addendum. *Issues: safety for the environment*
26/05/2015	Reception of supplementary information from the applicant - Scientific assessment re-started
25/06/2015	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended *Issues: characterisation of the additive and of the production strain, efficacy*
17/05/2016	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Addendum. *Issues: safety for the target species, for the consumer and for the environment*
17/01/2018	Reception of supplementary information from the applicant - Scientific assessment re-started
07/10/2019	Opinion adopted by the FEEDAP Panel. End of the Scientific assessment
13/11/2019	Opinion withdrawn by the FEEDAP Panel. Amended opinion adopted by the FEEDAP Panel

References

Anadon A and Reeve-Johnson L, 1999. Macrolide antibiotics, drug interactions and microsomal enzymes: implications for veterinary medicine. Research in Veterinary Science, 66, 197–203.

Arikan O, Mulbry W and Rice C, 2016. The effect of composting on the persistence of four ionophores in dairy manure and poultry litter. Waste Management, 54, 110–117.

Braunius WW, 1986. Monensin/sulfachlorpyrazine poisoning in turkeys. Tijdschrift voor Diergeneeskunde, 111, 676–678.

Broz J and Frigg M, 1987. Incompatibility between lasalocid and chloramphenicol in broiler chicks after a long-term simultaneous administration. Veterinary Research Communications, 11, 159–172.

Carlson JC and Mabury SA, 2006. Dissipation kinetics and mobility of chortetracyclines, tylosionand monensin in an agricultural soil in Northumberland County, Ontario, Canada. Environmental Toxicology and Chemistry, 15, 1–10.

Donoho AL, 1984. Biochemical studies on the fate of Monensin in animals and in the environment. Journal of Animal Sciences, 58, 1528–1539.

Dorn P, Weber R, Weikel J and Wessling E, 1983. Intoxikation durch gleichzeitige Verabreichung von Chloramphenicol und Monensin bei Puten. Prakt. Tierarzt, 64, 240–243.

ECHA (European Chemicals Agency), 2008. Guidance on information requirements and chemical safety assessment Chapter R.10: Characterisation of dose [concentration]-response for environment. Available online: https://echa.europa.eu/documents/10162/13632/information_requirements_r10_en.pdf

EFSA (European Food Safety Authority), 2004. Opinion of the Scientific Panel on Additives and Products or Substances used in Animal Feed on the request of the Commission on the reevaluation of coccidiostat Elancoban in accordance with article 9G of Council Directive 70/524/EEC. EFSA Journal 2004;2(3):42, 61 pp. https://doi.org/10.2903/j.efsa.2004.42

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). EFSA Journal 2005;3(11):283, 53 pp. https://doi.org/10.2903/j.efsa.2005.283

EFSA (European Food Safety Authority), 2006a. Opinion of the Panel on additives and products or substances used in animal feed (FEEDAP) on the Maximum Residue Limit for monensin sodium for chickens and turkeys for fattening. EFSA Journal 2006;4(11):413, 13 pp. https://doi.org/10.2903/j.efsa.2006.413

EFSA (European Food Safety Authority), 2006b. Opinion of the Panel on additives and products or substances used in animal feed (FEEDAP) on the safety and efficacy of the coccidiostat Elancoban® (monensin sodium) as a feed additive for calves for rearing and cattle for fattening in accordance with Regulation (EC) No 1831/2003. EFSA Journal 2006;4(10):387, 33 pp. https://doi.org/10.2903/j.efsa.2006.387

EFSA (European Food Safety Authority), 2006c. Opinion of the Panel on additives and products or substances used in animal feed (FEEDAP) on the safety of Coxidin® (monensin sodium). EFSA Journal 2006;4(7):381, 10 pp. https://doi.org/10.2903/j.efsa.2006.381

EFSA (European Food Safety Authority), 2007a. Scientific Opinion of the Panel on Additives and Products or Substances used in Animal Feed on the efficacy of Coxidin® 25% (monensin sodium) as a feed additive for turkeys. EFSA Journal 2007;6(7):545, 13 pp. https://doi.org/10.2903/j.efsa.2007.545

EFSA (European Food Safety Authority), 2007b. 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 Journal 2007;5(12):622, 32 pp. https://doi.org/10.2903/j.efsa.2007.622

EFSA (European Food Safety Authority), 2008a. Scientific Opinion of the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) on a request from the European Commission on the withdrawal period for Elancoban® for chickens for fattening, chickens reared for laying and turkeys for fattening. EFSA Journal 2008;6(7):730, 16 pp. https://doi.org/10.2903/j.efsa.2008.730

EFSA (European Food Safety Authority), 2008b. Scientific Opinion of the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) on a request from the European Commission on the withdrawal period for Coxidin® for chickens and turkeys for fattening and the re-examination of the provisional Maximum Residue Limit. EFSA Journal 2008;6(7):731, 14 pp. https://doi.org/10.2903/j.efsa.2008.731

EFSA (European Food Safety Authority), 2008c. Technical Guidance of the Scientific Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) for assessing the safety of feed additives for the environment. EFSA Journal 2008;6(10):842, 28 pp. https://doi.org/10.2903/j.efsa.2008.842

EFSA (European Food Safety Authority), 2008d. Guidance of the Scientific Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) for the preparation of dossiers for the re-evaluation of certain additives already authorised under Directive 70/524/EEC. EFSA Journal 2008;6(9):779, 9 pp. https://doi.org/10.2903/j.efsa.2008.779

EFSA (European Food Safety Authority), 2008e. Technical Guidance: microbial Studies. EFSA Journal 2008;6(10):836, 3 pp. https://doi.org/10.2903/j.efsa.2008.836

EFSA (European Food Safety Authority), 2008f. Technical Guidance: extrapolation of data from major species to minor species regarding the assessment of additives for use in animal nutrition. EFSA Journal 2008;6(9):803, 5 pp. https://doi.org/10.2903/j.efsa.2008.803

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2011a. Scientific Opinion on the modification of the authorisation of the feed additive Coxidin® (monensin sodium) for chickens and turkeys for fattening. EFSA Journal 2011;9(2):2009, 12 pp. https://doi.org/10.2903/j.efsa.2011.2009

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2011b. Scientific Opinion on the safety and efficacy of Coxidin® (monensin sodium) as a feed additive for chickens reared for laying. EFSA Journal 2011;9(12):2442, 15 pp. https://doi.org/10.2903/j.efsa.2011.2442

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2011c. Guidance for the preparation of dossiers for coccidiostats and histomonostats. EFSA Journal 2011;9(5):2174, 12 pp. https://doi.org/10.2903/j.efsa.2011.2174

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2011d. Technical guidance: tolerance and efficacy studies in target animals. EFSA Journal 2011;9(5):2175, 15 pp. https://doi.org/10.2903/j.efsa.2011.2175

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2012a. Guidance for establishing the safety of additives for the consumer. EFSA Journal 2012;10(1):2537, 12 pp. https://doi.org/10.2903/j.efsa.2012.2537

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2012b. Guidance on studies concerning the safety of use of the additive for users/workers. EFSA Journal 2012;10(1):2539, 5 pp. https://doi.org/10.2903/j.efsa.2012.2539

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2013. Scientific Opinion on the modification of the withdrawal period for Coxidin® (monensin sodium) for chickens for fattening and chickens reared for laying. EFSA Journal 2013;11(1):3045, 2 pp. https://doi.org/10.2903/j.efsa.2013.3045

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Rychen G, Aquilina G, Azimonti G, Bampidis V, Bastos ML, Bories G, Chesson A, Cocconcelli PS, Flachowsky G, Kolar B, Kouba M, López-Alonso M, López Puente S, Mantovani A, Mayo B, Ramos F, Saarela M, Villa RE, Wallace RJ, Wester P, Brantom P, Halle I, van Beelen P, Holczknecht O, Vettori MV and Gropp J, 2017a. Scientific Opinion on the safety and efficacy of Monimax® (monensin sodium and nicarbazin) for turkeys for fattening. EFSA Journal 2017;15(12):5094, 49 pp. https://doi.org/10.2903/j.efsa.2017.5094

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Rychen G, Aquilina G, Azimonti G, Bampidis V, Bastos ML, Bories G, Chesson A, Cocconcelli PS, Flachowsky G, Gropp J, Kolar B, Kouba M, López-Alonso M, López Puente S, Mantovani A, Mayo B, Ramos F, Saarela M, Villa RE, Wallace RJ, Wester P, Anguita M, Dujardin B, Galobart J and Innocenti ML, 2017b. Guidance on the assessment of the safety of feed additives for the consumer. EFSA Journal 2017;15(10):5022, 17 pp. https://doi.org/10.2903/j.efsa.2017.5022

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Bampidis V, Azimonti G, Bastos ML, Christensen H, Dusemund B, Kouba M, Kos Durjava M, López-Alonso M, López Puente S, Marcon F, Mayo B, Pechová A, Petkova M, Ramos F, Sanz Y, Villa RE, Woutersen R, Aquilina G, Bories G, Brantom P, Cocconcelli PS, Halle I, Kolar B, van Beelen P, Wester P, Holczknecht O, Vettori MV and Gropp J, 2018a. Scientific Opinion on the safety and efficacy of Monimax® (monensin sodium and nicarbazin) for chickens for fattening and chickens reared for laying. EFSA Journal 2018;16(11):5459, 34 pp. https://doi.org/10.2903/j.efsa.2018.5459

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Rychen G, Aquilina G, Azimonti G, Bampidis V, Bastos ML, Bories G, Chesson A, Cocconcelli PS, Flachowsky G, Gropp J, Kolar B, Kouba M, López-Alonso M, López Puente S, Mantovani A, Mayo B, Ramos F, Saarela M, Villa RE, Wallace RJ, Wester P, Glandorf B, Herman L, Kärenlampi S, Aguilera J, Anguita M, Brozzi R and Galobart J, 2018b. Guidance on the characterisation of microorganisms used as feed additives or as production organisms. EFSA Journal 2018;16(3):5206, 24 pp. https://doi.org/10.2903/j.efsa.2018.5206

EMA-CVMP, 2007. COMMITTEE FOR MEDICINAL PRODUCTS FOR VETERINARY USE, MONENSIN (Cattle, including dairy cows) SUMMARY REPORT EMEA/CVMP/185123/2007-Final May 2007.

EMA-CVMP, 2013. European public MRL assessment report (EPMAR) Monensin (modification of MRLs) 4 February 2013 EMA/CVMP/78198/2012.

Frigg M, Broz J and Weber G, 1983. Compatibility studies of ionophore anticoccidials with various antibiotics and chemotherapeutics. Archiv fur Geflügelkunde, 47, 213–220.

Henri J, Burel C, Sanders P and Laurentie M, 2009. Bioavailability, distribution and depletion of monensin in chickens. Journal of Veterinary Pharmacology and Therapeutics, 32, 451–456.

Henri J, Maurice R, Postollec G, Dubreil-Cheneau E, Roudaut B, Laurentie M and Sanders P, 2012. Comparison of the oral bioavailability and tissue disposition of monensin and salinomycin in chickens and turkeys. Journal of Veterinary Pharmacology and Therapeutics, 35, 73–81.

JECFA (Joint FAO/WHO Expert Committee on Food Additives), 2008. FAO JECFA Monographs 6(2009), TRS 954-JECFA 70.

JECFA (Joint FAO/WHO Expert Committee on Food Additives), 2012. TRS 969/JECFA 75 and FNP 12/12-JECFA 75.
Johnson J and Reid WM, 1970. Anticoccidial drugs: lesion scoring techniques in battery and floor pen experiments with chickens. Experimental Parasitology, 28, 30–36.
Lynch MR, 1995. Procedures for assessing the environmental fate and ecotoxicity of pesticides. Society of Environmental Toxicology and Chemistry - Europe (SETAC-Europe), Brussels.
Sassman SA and Lee LS, 2007. Sorption and degradation in soils of veterinary ionophore antibiotics: Monensin and Lasalocid. Environmental Toxicology and Chemistry, 26, 1614–1621.
Yoshida N, Castro MJL and Cirelli AF, 2013. Effect of feedlot design and management on the fate and distribution of monensin. Chemistry in Ecology, 29, 379–390.
Zidar P and Zizek S, 2012. The impact of coccidiostats monensin and lasalocid on Cd and Pb uptake in the isopod Porcellio scaber. Applied Soil Ecology, 55, 36–43.
Zizek S, Hrženjak R, Kalcher GT, Šrimpf K, Šemrov N and Zidar P, 2011. Does monensin in chicken manure from poultry farms pose a threat to soil invertebrates? Chemosphere, 83, 517–523.

Abbreviations

ADI	acceptable daily intake
AF	assessment factor
ANOVA	analysis of variance
AP	alkaline phosphatase
AST	aspartate aminotransferase/anticoccidial sensitivity tests
bw	body weight
CAS	Chemical Abstracts Service
CFU	colony forming unit
CK	creatine kinase
CV	coefficient of variation
CVMP	Committee for Medicinal Products for Veterinary Use
DM	dry matter
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
ErC_{50}	median effective concentration which results in 50% reduction in growth rate
EMA	European Medicines Agency
EURL	European Union Reference Laboratory
FACE	Feed Additives Consumer Exposure
FOCUS	FOrum for Co-ordination of pesticide fate models and their USe
GLDH	glutamate dehydrogenase
GLP	Good Laboratory Practice
HPLC	high-performance liquid chromatography
HRP	highest reliable percentile
JECFA	The Joint FAO/WHO Expert Committee on Food Additives
Kd	soil adsorption coefficient
Koc	adsorption or desorption coefficient corrected for soil organic carbon content
LC_{50}	median lethal concentration
LC–MS/MS	liquid chromatography with tandem mass spectrometry
LOAEL	lowest observed adverse effect level
LOEL	lowest observed effect level
LOD	limit of detection
LOQ	limit of quantification
log K_{ow}	logarithm of octanol-water partition coefficient
LSC	liquid scintillation counting
LSD	least significant difference
ME	metabolisable energy
MIC	minimum inhibitory concentration
MRL	maximum residue limit
NOAEL	no observed adverse effect level
NOEC	no observed effect concentration

OC	organic carbon
OECD	Organisation for Economic Co-operation and Development
OPG	oocysts per gram of excreta
pKa	dissociation constant
PEC	predicted environmental concentration
PNEC	predicted no effect concentration
RAC	raw agricultural commodities
RH	relative humidity
RMTR	ratio marker to total residue
SFO	single first-order
TP	total protein
TRC	total residue concentration
VP	vapour pressure

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

Tolerance

Rambozzi L, Renna M, Cornale P, Perona G, Malfatto V, Mimosi A, 2012. Effect of the granulometric characteristics of monensin sodium on controlling experimental coccidiosis in broiler chickens. Revista Brasileira de Parasitologia Veterinária, 21, 60–64.

Zavala G, Anderson DA, Davis JF, Dufour-Zavala L, 2011. Acute monensin toxicosis in broiler breeder chickens. Avian Diseases, 55, 516–521.

Hume ME, Clemente-Hernández S, Oviedo-Rondón EO, 2006. Effects of feed additives and mixed Eimeria species infection on intestinal microbial ecology of broilers. Poultry Science, 85, 2106–11.

Vissiennon T, Kröger H, Köhler T, Kliche R, 2000. Effect of avilamycin, tylosin and ionophore anticoccidials on Clostridium perfringens enterotoxaemia in chickens. Berl Munch Tierarztl Wochenschr, 113, 9–13.

Guban J, Korver DR, Allison GE, Tannock GW, 2006. Relationship of dietary antimicrobial drug administration with broiler performance, decreased population levels of Lactobacillus salivarius, and reduced bile salt deconjugation in the ileum of broiler chickens. Poultry Science, 85, 2186–2194.

Jiang YW, Sims MD, Conway DP, 2005. The efficacy of TAMUS 2032 in preventing a natural outbreak of colibacillosis in broilerchickens in floor pens. Poultry Science, 84, 1857–1859.

Interaction with other drugs

Sureshkumar V, Venkateswaran KV, Jayasundar S, 2004. Interaction between enrofloxacin and monensin in broiler chickens. Veterinary and Human Toxicology, 46, 242–245.

Crespo R, Shivaprasad HL, Sommer F, Puschner B, 2008. Interaction of ionophore and vitamin E in knockdown syndrome of turkeys. Journal of Veterinary Diagnostic Investigation, 20, 472–476.

Islam KMS, Klein U, Burch DGS, 2009. The activity and compatibility of the antibiotic tiamulin with other drugs in poultry medicine—A review. Oxford Journal of Science and Mathmatical Poultry Science, 88, 2353–2359.

Szűcs G, Tamási V, Laczay P and Monostory K, 2004. Biochemical background of toxic interaction between tiamulin and monensin. Chemico-Biological Intercation, 147, 151–161.

Chapman HD, Matsler PL and Chapman ME. 2004. Control of Coccidiosis in Turkeys with Diclazuril and Monensin: effects upon performance and development of immunity to Eimeria Species. AAP Journals, 48, 631–634.

Sims MD and Hooge DM, 2002. Efficacy of nitarsone, monensin or nitarsone followed by monensin, in female Turkeys in litter pens and exposed to coccidial (25 days) and cochlosomal (28 days) inoculations. International Journal of Poultry Sciences, 1, 164–166.

Resistance and cross-resistance

Aarestrup FM, Bager F, Jensen NE, Madsen M, Meyling A and Wegener HC, 1998. Surveillance of antimicrobial resistance in bacteria isolated from food animals to antimicrobial growth promoters and related therapeutic agents in Denmark. APMIS, 106, 606–622.

Bowen RE, 1973. Studies designed to determine the effect of passing bacteria forty times in media containing monensin on their resistance to monensin and thirteen other antimicrobials. Elanco Internal Pooled Study Reports 460-G107-203.

Butaye P, Devriese LA and Haesebrouck F, 2001. Differences in antibiotic resistance patterns of Enterococcus faecalis and Enterococcus faecium strains isolated from farm and pet animals. Antimicrobial Agents and Chemotherapy, 45, 1374–1378.

Callaway TR and Russell JB, 1999. Selection of a highly monensin-resistant Prevotella bryantii subpopulation with altered outer membrane characteristics. Applied and Environmental Microbiology, 65, 4753–4759.

Callaway TR, Erdington TS, Rychlik JL, Genovese KJ, Poole TL, Yung YS, Blachoff KM, Anderson RC and Nisbet DJ. 2003. Ionophores: their use as ruminant growth promotants and impact on food safety. Current Issues in Intestinal Microbiology, 4, 43–51.

Chen M and Wolin MJ, 1979. Effect of monensin and lasalocid-sodium on the growth of methanogenic and rumen saccharolytic bacteria. Applied and Environmental Microbiology, 38, 72–77. in bacteria from food animals, food and humans in Denmark. http://www.dfvf.dk

Dawson KA and Boling JA, 1987. Effects of potassium ion concentrations on the antimicrobial activities of ionophores against ruminal anaerobes. Applied and Environmental Microbiology, 53, 2363–2367.
Devriese LA, Daube G, Hommez G and Haesebrouck F, 1993. In vitro susceptibility of Clostridium perfringens isolated from farm animals to growth-enhancing antibiotics. Journal of Applied Bacteriology, 75, 55–57.
Edrington TS, Callway TR, Varey PD, Jug YS, Bischoff KM, Elder RO, Anderson RC, Kutter E, Brabban AD and Nisbet NJ, 2003. Effects of the antibiotic ionophores monensin, lasalocid, laidlomycin propionate and bambermycin on Salmonella and E. coli O157:H7 in vitro. Journal of Applied Microbiology, 94, 207–213.
Houlihan AJ and Russell JB, 2003. The susceptibility of ionophore-resistant Clostridium aminophilum F to other antibiotics. Journal of Antimicrobial Chemotherapy, 52, 623–628.
Johansson A, Greko C, Engström BE and Karlsson M, 2004. Antimicrobial susceptibility of Swedish, Norwegian and Danish isolates of Clostridium perfringens from poultry, and distribution of tetracycline resistance genes. Veterinary Microbiology, 99, 251–257.
Martel A, Devriese LA, Cauwerts K, De Gussem K, Decostere A and Haesebrouck F, 2004. Susceptibility of Clostridium perfringens strains from broiler chickens to antibiotics and anticoccidials. Avian Pathology, 33, 3–7.
McConville M, Gate J and Shryock TR, 2003. Lack of selection of monensin-resistant bacteria. 13th European Congress of Clinical Microbiology and Infectious Disease.
Morehead MC and Dawson KA, 1992. Some growth and metabolic characteristics of monensin-sensitive and monensin-resistant strains of Prevotella (Bacteroides) ruminicola. Applied and Environmental Microbiology, 58, 1617–1623.
Russell JB and Houlihan JB, 2003. Ionophore resistance of ruminal bacteria and its potential impact on human health. FEMS Microbiology Reviews, 27, 65–74.
Russell JB and Strobel HJ, 1989. Effect of ionophores on ruminal fermentation. Applied and Environmental Microbiology, 55, 1–6.
Rychlik JL and Russell JB, 2002. The adaptation and resistance of Clostridium aminophilum F to the butyrivibriocin-like substance of Butyrivibrio fibrisolvens JL5 and monensin. FEMS Microbiology Letters, 209, 93–98.
Simjee S, Heffron A-L, Pridmore A and Shryock TR, 2012. Reversible monensin adaptation in Enterococcus faecium, Enterococcus faecalis and Clostridium perfringens of cattle origin: potential impact on human food safety. Journal of Antimicrobial Chemotherapy, 67, 2388–2395.
Smith II CK, Galloway RB and White SL, 1981. Effect of ionophores on survival, penetration and development of Eimeria tenella sporozoites in vitro. Journal of Parasitology, 67, 511–516.
Smith II CK and Galloway RB, 1983. Influence of monensin on cation influx and glycolysis of Eimeria tenella sporozoites in vitro. Journal of Parasitology, 69, 666–670.
Watkins KL, Shryock TR, Dearth RN and Saif YM, 1997. In-vitro antimicrobial susceptibility of Clostridium perfringens from commercial turkey and broiler chicken origin. Veterinary Microbiology, 54, 195–200.

Toxicology

De Souza Spinosa H, Nicolau AA, Maruo VM, Bernardi MM, 1999. Effects of monensin feeding during development on female rats and their offspring. Neurotoxicology and Teratology, 21, 467–470.
Novilla MN, 2011. Ionophores. Reproductive and Developmental toxicology. Academic Press, 373–384.
Dacasto M, Ceppa L, Cornaglia E, Valenza F, Carletti M, Bosio A, Bosia S, Ugazio G, Nebbia C, 1999. Effects of the ionophore antibiotic monensin on hepatic biotransformations and target organ morphology in rats. Pharmacological Research, 39, 5–10.
Singh M, Kalla NR, Sanyal SN, 2014. Testicular effects of monensin, a golgi interfering agent in male rats. Drug Chemical Toxiology, 37, 384–390.

Environmental safety

Zidar P, Kos M, Vogel-Mikus K, van Elteren JT, Debeljak M, Zizek S, 2016. Impact of ionophore monensin on performance and Cu uptake in earthworm Eisenia andrei exposed to copper-contaminated soil. Chemosphere, 161, 119–126.
Couperus NP, Pagsuyoin SA [Reprint Author], Bragg LM, Servos MR, 2016. Occurrence, distribution, and sources of antimicrobials in a mixed-use watershed. Science of the Total Environment, 541, 1581–1591.

Bartikova H, Skalova L, Stuchlikova L, Vokral I, Vanek T, Podlipna R, 2015. Xenobiotic-metabolizing enzymes in plants and their role in uptake and biotransformation of veterinary drugs in the environment. Drug Metabolism Reviews, 47, 374–387.
Konopka M, Henry HAL, Topp E (correspondence) - Marti R, Topp E (correspondence), 2015. Multi-year and short-term responses of soil ammonia-oxidizing prokaryotes to zinc bacitracin, monensin, and ivermectin, singly or in combination. Environmental Toxicology and Chemistry, 34, 618–625.
Fairbairn DJ, Karpuzcu ME, Arnold WA, Barber BL, Kaufenberg EF, Koskinen WC, Novak PJ, Rice PJ, Swackhamer DL, 2015. Sediment-water distribution of contaminants of emerging concern in a mixed use watershed. Science of the Total Environment, 505, 896–904.
Winkworth CL, Salis RK, Matthaei CD, 2015. Interactive multiple-stressor effects of the antibiotic monensin, cattle effluent and light on stream periphyton. Freshwater Biology, 2410–2423.
Brown D, Bartelt-Hunt SL (correspondence) S, Daniel Hunt GA, 2015. Persistence of pharmaceuticals in effluent-dominated surface waters. Journal of Environmental Quality, 44, 299–304.
Chen H, Liu S, Xu X-R, Zhou G-J, Liu S-S, Yue W-Z, Sun K-F, Ying G-G, 2015. Antibiotics in the coastal environment of the Hailing Bay region, South China Sea: spatial distribution, source analysis and ecological risks. Marine Pollution Bulletin, 95, 365–373.
Bak SA, Bjorklund E, 2014. Antibiotics. Occurrence of Ionophores in the Danish Environment, 3, 564–571.
Zizek S, Gombac M, Svara T, Pogacnik M, 2014. Monensin - a review of factors influencing its presence in the environment and recommendations for safe storage and use of monensin-contaminated manure. Slovenian Veterinary Research, 51, 53–63.
Sun P, Cabrera ML, Huang C-H, Pavlostathis SG, 2014. Biodegradation of veterinary ionophore antibiotics in broiler litter and soil microcosms. Environmental Science & Technology, 48, 2724–2731.
El Sayed EM, Prasher SO, 2014. Fate and transport of monensin in the presence of nonionic surfactant Brij35 in soil. Science of the Total Environment, 490, 629–638.
Sun P, Barmaz D, Cabrera ML, Pavlostathis SG, Huang C-H, 2013. Detection and quantification of ionophore antibiotics in runoff, soil and poultry litter. Journal of Chromatography, 1312, 10–17.
Bak SA, Hansen M, Pedersen KM, Halling-Sorensen B, Bjoerklund E, 2013. Quantification of four ionophores in soil, sediment and manure using pressurised liquid extraction. Journal of Chromatography, 1307, 27–33.
Yoshida N, Cirelli AF, Castro MJL, 2013. Effect of feedlot design and management on the fate and distribution of monensin. Chemistry in Ecology, 29, 379–390.
Kang DH, Gupta S, Rosen C, Fritz V, Singh A, Chander Y, Murray H, Rohwer C, 2013. Antibiotic Uptake by Vegetable Crops from Manure-Applied Soils. Journal of Agricultural and Food Chemistry, 61, 9992–10001.
Yuan Q, Snow DD, Bartelt-Hunt SL, 2013. Potential water quality impacts originating from land burial of cattle carcasses. Science of the Total Environment, 456–457, 246–253.
Netthisinghe AMP [Reprint Author], Cook KL, Rice C, Gilfillen RA, Sistani KR, 2013. Soil Nutrients, Bacteria Populations, and Veterinary Pharmaceuticals across a Backgrounding Beef Feedlot. Journal of Environmental Quality, 42, 532–544.
Hafner SC, Parikh SJ, 2013. Sorption of the veterinary antibiotic monensin to soil minerals. Abstracts of Papers, 245th ACS National Meeting & Exposition, New Orleans, LA, United States, April 7-11, 2013, (2013) pp. GEOC-70. CODEN: 69QTVP.
Ravva SV [Reprint Author], Sarreal CZ, Mandrell RE, 2013. Altered Protozoan and Bacterial Communities and Survival of Escherichia coli O157:H7 in Monensin-Treated Wastewater from a Dairy Lagoon. PLoS One, 8, Article No.: e54782.
Zizek S, Zidar P, Vidrih M, Dobeic M, Pintaric S, Pogacnik M, Kobal S, 2013. Coccidiostats in poultry manure - a potential threat to beneficial soil invertebrates. Fourth International Scientific Symposium "Agrosym 2013", Jahorina, Bosnia and Herzegovina, 3-6 October, 2013. Book of Proceedings (2013), pp. 994–999.
Zidar P, Kos M, Zizek S, 2013. Coccidiostats in poultry manure - a potential impact on metal accumulation in beneficial soil invertebrates. Fourth International Scientific Symposium "Agrosym 2013", Jahorina, Bosnia and Herzegovina, 3-6 October, 2013. Book of Proceedings (2013), pp. 926–931.
Zhou L-J, Ying G-G, Liu S, Zhao J-L, Yang B, Chen Z-F, Lai H-J, 2013. Occurrence and fate of eleven classes of antibiotics in two typical wastewater treatment plants in South China. Science of the Total Environment, 452–453, 365–376.

Kim S-C, Chung DY, Kim KH, Lee JH, Kim HK, Yang JE, Ok YS, Almarwei YAO, 2012. Concentration and environmental loading of veterinary antibiotics in agricultural irrigation ditches. Korean Journal of Soil Science and Fertilizer, 45, 867–876.

Merchant LE, Rempel H, Forge T, Kannangara T, Bittman S, Delaquis P, Topp E, Ziebell KA, Diarra MS, 2012. Characterization of antibiotic-resistant and potentially pathogenic Escherichia coli from soil fertilized with litter of broiler chickens fed antimicrobial-supplemented diets. Canadian Journal of Microbiology, 58, 1084–1098.

Herrero P, Borrull F, Pocurull E, Marce RM, 2012. Novel amide polar-embedded reversed-phase column for the fast liquid chromatography-tandem mass spectrometry method to determine polyether ionophores in environmental waters. Journal of Chromatography A, 1263, 7–13.

Sun P, Hong y, Pavlostathis SG, Huang C-H, 2012. Hydrolytic and photolytic transformation of ionophore antibiotics in the environment. Abstracts of Papers, 243rd ACS National Meeting & Exposition, San Diego, CA, United States, March 25-29, 2012 (2012), ENVR-192 Publisher: American Chemical Society, Washington, D. C. CODEN: 69PCV3.

Broekaert N, Daeseleire E, Delezie E, Vandecasteele B, de Beer T, van Poucke C, 2012. Can the Use of Coccidiostats in Poultry Breeding Lead to Residues in Vegetables? An Experimental Study. Journal of Agricultural and Food Chemistry), 60, 12411–12418.

Sun P, Doydora S, Cabrera M, Thompson A, Huang C-H, Pavlostathis S, 2012. Biotransformation of ionophore veterinary antibiotics in poultry litter-water-soil system. Abstracts of Papers, 243rd ACS National Meeting & Exposition, San Diego, CA, United States, March 25-29, 2012, (2012) pp. ENVR-214. CODEN: 69PCV3.

Simjee S, Heffron A-L, Pridmore A, Shryock TR, 2012. Reversible monensin adaptation in Enterococcus faecium, Enterococcus faecalis and Clostridium perfringens of cattle origin: potential impact on human food safety. Journal of Antimicrobial Chemotherapy, 67, 2388–2395.

Zidar P, Zizek S, 2012. The impact of coccidiostats monensin and lasalocid on Cd and Pb uptake in the isopod Porcellio scaber. Applied Soil Ecology, 55, 36–43.

Kim SC, Yang JE, Ok YS, Jung DY, Carlson K, 2012. Degradation kinetics of three veterinary antibiotics in composted and stockpiled manure. Korean Journal of Soil Science and Fertilizer, 45, 43–50.

Broekaert N, Daeseleire E, Delezie E, Vandecasteele B, van Poucke C, van Poucke C Editor(s): Schilt R, 2012. Is there a necessity to establish maximum residue limits for coccidiostats in vegetables? An experimental study. Residues of veterinary drugs in food. Proceedings of the EuroResidue VII Conference, Egmond aan Zee, The Netherlands, 14-16 May, 2012. Volume 1, 2 and 3 (2012), pp. 77–82, 7 refs.

Iglesias A, Nebot C [Reprint Author], Miranda JM, Vazquez BI, Cepeda A, 2012. Detection and quantitative analysis of 21 veterinary drugs in river water using high-pressure liquid chromatography coupled to tandem mass spectrometry. Environmental Science and Pollution Research International, 19, 3235–3249.

Toth JD, Feng Y, Dou Z, 2011. Veterinary antibiotics at environmentally relevant concentrations inhibit soil iron reduction and nitrification. Soil Biology & Biochemistry, 43, 2470–2472.

Kim SH, Fan M, Prasher SO, Patel RM, Hussain SA, 2011. Fate and transport of atrazine in a sandy soil in the presence of antibiotics in poultry manures. Agricultural Water Management, 98, 653–660.

Doydora S, Thompson A, Cabrera M, 2011. Sorption of monensin on poultry litter-amended pasture soil and mineral sorbents. Abstracts of Papers, 241st ACS National Meeting & Exposition, Anaheim, CA, United States, March 27-31, 2011 (2011), ENVR-276.

Wang Y, Zhang X-Y, Diao X-P, Yang B, 2011. Effect of Monensin Exposure on DNA Damage in Earthworm (Eisenia fetida). Journal of Agro-Environment Science [J. Agro-Environ. Sci.], 30, 849–854.

Sun P, Pavlostathis SG, Cabrera ML, Thompson A, Huang C-H, 2011. Quantification and transformation evaluation for ionophore antibiotics in water, soil, and poultry litter. Abstracts of Papers, 241st ACS National Meeting & Exposition, Anaheim, CA, United States, March 27-31, 2011 (2011), ENVR-6 Publisher: American Chemical Society, Washington, D. C. CODEN: 69NZUR.

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2011. Scientific opinion on the safety and efficacy of Coxidin® (monensin sodium) as a feed additive for chickens reared for laying. EFSA Journal 2011;9(12):2442 p., 20 refs. ISSN: 1831-4732 Published by: European Food Safety Authority, Parma.

Parrott J, Tabe S, Pileggi V, Feisthauer N, Kleywegt S, Schroeder J, Seto P, Backus S, 2011. Fathead minnow lifecycle exposure to three Canadian municipal wastewater effluents. Canadian Technical Report of Fisheries and Aquatic Sciences, 2949, 92–93.

Watanabe N, Bergamaschi BA, Loftin KA, Meyer MT, Harter T, 2010. Use and Environmental Occurrence of Antibiotics in Freestall Dairy Farms with Manured Forage Fields. Environmental Science & Technology, 44, 6591–6600.
Ding Y, Chiou CT, Li H, 2010. Selected Veterinary Pharmaceuticals in Agricultural Water and Soil from Land Application of Animal. Journal of Environmental Quality [J. Environ. Qual.], 39, 1211–1217.
Swan K and Spongberg A, 2010. Sorption Characteristics of Monensin, Lasalocid, and Ivermectin to Soil Clay Minerals. 20100411 Conference: 2010 Joint Meeting of the North-Central and South-Central Sections of Geological Society of America, Branson, Missouri, 11 Apr 2010 - 13 Apr 2010.
Yoshida N, Castro MJL, Fernandez Cirelli A, 2010. Degradation of monensin on soils: influence of organic matter and water content. Chemistry and Ecology, 26, 27–33.
Song W, Ding Y, Chiou C, Li H, 2010. Selected Veterinary Pharmaceuticals in Agricultural Water and Soil from Land Application of Animal Manure. Journal of Environmental Quality, 39, 1211–1217.
Hansen M, Krogh KA, Bjorklund E, Brandt A, Halling-Sorensen B, 2009. Environmental risk assessment of ionophores. Trends in Analytical Chemistry, 28, Sp. Iss. SI, 534–542.
Snow DD, Bartelt-Hunt SL, Devivo S, Saunders S, Cassada DA, 2009. Detection, occurrence, and fate of emerging contaminants in agricultural environments. Water Environment Research, 81, 941–958.
Jensen J, Diao X, Hansen AD, 2009. Single- and Two-species Tests to Study Effects of the Anthelmintics Ivermectin and Morantel and the Coccidiostatic Monensin on Soil Invertebrates. Environmental Toxicology and Chemistry, 28, 316–323.
Furtula V, Huang L, Chambers PA, 2009. Determination of veterinary pharmaceuticals in poultry litter and soil by methanol extraction and liquid chromatography-tandem mass spectrometry. Journal of Environmental Science and Health Part B Pesticides Food Contaminants and Agricultural Wastes, 44, 717–723.
Lee S-S, Kim S-C, Yang J-E, Ok Y-S, 2010. Seasonal monitoring of residual antibiotics in soil, water, and sediment adjacent to a cattle manure composting facility. Korean Journal of Soil Science and Fertilizer, 43, 612–618.
Wang Y, Diao X-P, Zhang X-Y, 2010. Ecotoxicological Effects of Monensin Pollution on Earthworm (Eisenia fetida). Journal of Agro-Environment Science [J. Agro-Environ. Sci.], 29, 1091–1097.
Ritter L, Nichol K, Kirby G, Cerniglia C, Bulder IA, Anadon A, 2009. Monensin. WHO Food Additives Series, (2009) Vol. 61, No. Toxicological Evaluation of Certain Veterinary Drug Residues in Food, pp. 93–132.
Watanabe N [Reprint Author], Harter TH, Bergamaschi BA, 2008. Environmental occurrence and shallow ground water detection of the antibiotic monensin from dairy farms. Journal of Environmental Quality, 37(Suppl. S), S78–S85.
Watanabe N, Harter T, Bergamaschi B, 2008. Environmental Fate of Monensin in Dairy Farms. 20081005 Conference: 2008 Joint Annual Meeting of the Geological Society of America (GSA), Soil Science Society of America (SSSA), American Society of Agronomy (ASA), Crop Science Society of America (CSSA), and the Gulf Coast Association of Geological Societies with the Gulf Coast Section of SEPM (GCAGS), Houston, Texas (USA), 5 Oct 2008 - 9 Oct 2008 Sponsor(s): The Geological Society of America Soil Science Society of America American Society of Agronomy Crop Science Society of America.
Kools SAE, Boxall ABA, Moltmann JF, Bryning G, Koschorreck J, Knacker T, 2008. A ranking of European veterinary medicines based on environmental risks. Integrated Environmental Assessment and Management, 4, 399–408.
Kim Y, Jung J, Kim M, Park J, Boxall ABA, Choi K, 2008. Prioritizing veterinary pharmaceuticals for aquatic environment in Korea. Environmental Toxicology and Pharmacology, 26, 167–176.
Dolliver H, Gupta S, Noll S, 2008. Antibiotic Degradation during Manure Composting. Journal of Environmental Quality, 37, 1245–1253.
McGregor EB, 2008. Microcosm assessment of the effects of monensin, 10:2 saturated fluorotelomer carboxylic acid, and atrazine on aquatic macrophytes and responses of individuals versus assemblages. Masters Abstracts International, 46, 191.
Dolliver HAS, Gupta SC, 2008. Antibiotic Losses from Unprotected Manure Stockpiles. Journal of Environmental Quality, 37, 1238–1244.
Dolliver H, Gupta S, 2008. Antibiotic Losses in Leaching and Surface Runoff from Manure-Amended Agricultural Land. Journal of Environmental Quality, 37, 1227–1237.

Perkins AN, Meyerhoff RD, Kelly A, Teeter JS, Banks SD, 2007. Environmental risk assessment of tylosin, narasin, and monensin. Abstracts of Papers, 233rd ACS National Meeting, Chicago, IL, United States, March 25–29, 2007 (2007), AGRO-044 Publisher: American Chemical Society, Washington, D. C. CODEN: 69JAUY.

Perkins AN [Reprint Author], Meyerhoff RD, Kelly A, Teeter JS, Banks SD, 2007. AGRO 44-Environmental risk assessment of tylosin, narasin, and monensin. Abstracts of Papers American Chemical Society, (MAR 25 2007) Vol. 233, pp. 316. Meeting Info.: 233rd National Meeting of the American-Chemical-Society. Chicago, IL, USA. March 25–29, 2007. Amer Chem Soc. CODEN: ACSRAL. ISSN: 0065-7727.

Perkins AN, Meyerhoff RD, Kelly A, Teeter JS, Banks SD, 2007. Environmental Risk Assessment of Tylosin, Narasin, and Monensin. 20070325 Conference: 233rd National Meeting and Exposition of the American Chemical Society, Chicago, Illinois (USA), 25 Mar 2007–29 Mar 2007 Sponsor(s): American Chemical Society (ACS).

McGregor EB, Solomon KR, Hanson ML, 2007. Monensin Is Not Toxic to Aquatic Macrophytes at Environmentally Relevant Concentrations. Archives of environmental contamination and toxicology, pp. 541–551. ISSN: 0090-4341 Published by: New York: Springer-Verlag Source Note: 2007 Nov., v. 53, no. 4.

Cha J, 2007. Occurrence and biodegradation of antibiotic compounds in the aquatic environment. Dissertation Abstracts International. Vol. 68, no. 8. 2007 Dissertation Number: AAI3279497 ISSN: 0419-4217 Published by: University Microfilms International, P.O. Box 1764, Ann Arbor, MI, 48106, USA.

Seo Y-H, Choi J-K, Kim S-K, Min H-K, Jung Y-S, 2007. Prioritizing environmental risks of veterinary antibiotics based on the use and the potential to reach environment. Korean Journal of Soil Science and Fertilizer, 40, 43–50.

McGregor EB, Hanson ML, Solomon KR, 2007.

Books

Estimation of environmentally relevant chemical properties of veterinary ionophore antibiotics. Environmental science and pollution research international (2016), Volume 23, Number 18, pp. 18353-18361 ISSN: 0944-1344 Published by: Springer Berlin Heidelberg Source Note: 2016 Sept., v. 23, no. 18.

The effect of composting on the persistence of four ionophores in dairy manure and poultry litter. Waste Management (2016), Volume 54, pp. 110–117. ISSN: 0956-053X Source Note: 2016 Aug., v. 54.

Studies on uptake and distribution of antibiotics in red cabbage. Journal fur Verbraucherschutz und Lebensmittelsicherheit = (2016), Volume 11, Number 1, pp. 61–69. ISSN: 1661-5751 Published by: Springer International Publishing Source Note: 2016 Mar., v. 11, no. 1.

WHO. W. H. O. Tech. Rep. Ser., 2012. Evaluation of Certain Veterinary Drug Residues in Food. Publisher: WORLD HEALTH ORGANIZATION, DISTRIBUTION and SALES SERVICE, 1211 27 GENEVA, SWITZERLAND. Series: WHO Technical Report Series.

FAO JECFA Monographs, 2012. ix + 177 p., No. 12. Residue evaluation of certain veterinary drugs. Joint FAO/WHO Expert Committee on Food Additives. 75th meeting 2011. ISSN: 1817-7077.

FAO/WHO Food Standards Programme, 2012. v + 57 p. Report of the twentieth session of the Codex Committee on Residues of Veterinary Drugs in Foods.

WHO Food Additives Series, 2009. v + 240 p., No. 61, many refs. ISSN: 0300-0923. Toxicological evaluation of certain veterinary drug residues in food.

Appendix B – Calculation of consumer exposure with FACE model

Methodology

As described in the Guidance on the safety of feed additives for consumers (EFSA FEEDAP Panel, 2017b), consumption data of edible tissues and products as derived from the EFSA Comprehensive European Food Consumption Database (Comprehensive Database) will be used to assess exposure to residues from the use of feed additives in different EU countries, age classes[105] and special population groups. For each EU country and age class, only the latest survey available in the Comprehensive Database will be used.

While the residue data reported for feed additives refer to organs and tissues (raw agricultural commodities. RAC), the Comprehensive Database includes consumption data for foods as consumed. In order to match those consumption data with the available residue data for feed additives, the consumption data reported in the Comprehensive Database have been converted into RAC equivalents. For assessing the exposure to coccidiostats from their use in (non-reproductive) poultry, the following list of commodities is considered: meat, fat, liver, other offals (including kidney).

Depending on the nature of the health-based guidance derived, either a chronic or acute exposure assessment may be required.

For chronic exposure assessments, the total relevant residues will be combined for each individual with the average daily consumptions of the corresponding food commodities, and the resulting exposures per food will be summed in order to obtain total chronic exposure at individual level (standardised by using the individual body weight). The mean and the higher percentile (usually the 95th percentile) of the individual exposures will be subsequently calculated for each dietary survey (country) and each age class separately.

As opposed to the chronic exposure assessments, acute exposure calculation will be carried out for each RAC value separately. The higher percentile (usually the 95th percentile) exposures based on the consuming days only will be calculated for each food commodity, dietary survey and age class separately.

Detailed results on chronic exposure calculation

Table B.1: Chronic dietary exposure per population class, country and survey of consumers (mg/kg bw per day) to monensin total residues based on residue data in chicken tissues

Population class	Survey's country	Number of subjects	HRP(1)	HRP description
Infants	Bulgaria	523	0.0006023004	95th
Infants	Germany	142	0.0001132686	95th
Infants	Denmark	799	0.0001313668	95th
Infants	Finland	427	0.0001964025	95th
Infants	United Kingdom	1,251	0.0002695864	95th
Infants	Italy	9	0.0000000000	50th
Toddlers	Belgium	36	0.0002896951	90th
Toddlers	Bulgaria	428	0.0007189578	95th
Toddlers	Germany	348	0.0001884209	95th
Toddlers	Denmark	917	0.0001457581	95th
Toddlers	Spain	17	0.0002839744	75th
Toddlers	Finland	500	0.0003137353	95th
Toddlers	United Kingdom	1,314	0.0003008401	95th
Toddlers	United Kingdom	185	0.0003054779	95th
Toddlers	Italy	36	0.0002587523	90th
Toddlers	Netherlands	322	0.0003178539	95th
Other children	Austria	128	0.0002509460	95th

[105] Infants: < 12 months old, toddlers: ≥ 12 months to < 36 months old, other children: ≥ 36 months to < 10 years old, adolescents: ≥ 10 years to < 18 years old, adults: ≥ 18 years to < 65 years old, elderly: ≥ 65 years to < 75 years old, and very elderly: ≥ 75 years old.

Population class	Survey's country	Number of subjects	HRP(1)	HRP description
Other children	Belgium	625	0.0003465484	95th
Other children	Bulgaria	433	0.0006309453	95th
Other children	Czech Republic	389	0.0005365447	95th
Other children	Germany	293	0.0002035676	95th
Other children	Germany	835	0.0001974921	95th
Other children	Denmark	298	0.0001629345	95th
Other children	Spain	399	0.0003689514	95th
Other children	Spain	156	0.0005163667	95th
Other children	Finland	750	0.0002756368	95th
Other children	France	482	0.0002542903	95th
Other children	United Kingdom	651	0.0002696993	95th
Other children	Greece	838	0.0002639457	95th
Other children	Italy	193	0.0002781187	95th
Other children	Latvia	187	0.0002996973	95th
Other children	Netherlands	957	0.0002318732	95th
Other children	Netherlands	447	0.0002919776	95th
Other children	Sweden	1,473	0.0002187615	95th
Adolescents	Austria	237	0.0001733979	95th
Adolescents	Belgium	576	0.0001586059	95th
Adolescents	Cyprus	303	0.0001676798	95th
Adolescents	Czech Republic	298	0.0004063538	95th
Adolescents	Germany	393	0.0001516495	95th
Adolescents	Germany	1,011	0.0001244170	95th
Adolescents	Denmark	377	0.0001274236	95th
Adolescents	Spain	651	0.0002142808	95th
Adolescents	Spain	209	0.0002875639	95th
Adolescents	Spain	86	0.0002348923	95th
Adolescents	Finland	306	0.0001607405	95th
Adolescents	France	973	0.0001679927	95th
Adolescents	United Kingdom	666	0.0001976671	95th
Adolescents	Italy	247	0.0001235962	95th
Adolescents	Latvia	453	0.0001826405	95th
Adolescents	Netherlands	1,142	0.0002188828	95th
Adolescents	Sweden	1,018	0.0001647145	95th
Adults	Austria	308	0.0001985704	95th
Adults	Belgium	1,292	0.0001596687	95th
Adults	Czech Republic	1,666	0.0001984561	95th
Adults	Germany	10,419	0.0001233192	95th
Adults	Denmark	1,739	0.0000830659	95th
Adults	Spain	981	0.0001999179	95th
Adults	Spain	410	0.0001996245	95th
Adults	Finland	1,295	0.0001584927	95th
Adults	France	2,276	0.0001466797	95th
Adults	United Kingdom	1,265	0.0001458976	95th
Adults	Hungary	1,074	0.0002468397	95th
Adults	Ireland	1,274	0.0001969244	95th
Adults	Italy	2,313	0.0001083925	95th
Adults	Latvia	1,271	0.0001643512	95th
Adults	Netherlands	2,055	0.0001846426	95th
Adults	Romania	1,254	0.0003039818	95th

Population class	Survey's country	Number of subjects	HRP(1)	HRP description
Adults	Sweden	1,430	0.0001681158	95th
Elderly	Austria	67	0.0001749422	95th
Elderly	Belgium	511	0.0001351120	95th
Elderly	Germany	2,006	0.0000962208	95th
Elderly	Denmark	274	0.0000671801	95th
Elderly	Finland	413	0.0001271367	95th
Elderly	France	264	0.0001142862	95th
Elderly	United Kingdom	166	0.0001249615	95th
Elderly	Hungary	206	0.0002054980	95th
Elderly	Ireland	149	0.0001631647	95th
Elderly	Italy	289	0.0001253259	95th
Elderly	Netherlands	173	0.0001368953	95th
Elderly	Netherlands	289	0.0001163296	95th
Elderly	Romania	83	0.0002125537	95th
Elderly	Sweden	295	0.0001565474	95th
Very elderly	Austria	25	0.0000417360	75th
Very elderly	Belgium	704	0.0001434023	95th
Very elderly	Germany	490	0.0001051597	95th
Very elderly	Denmark	12	0.0000350785	75th
Very elderly	France	84	0.0001164876	95th
Very elderly	United Kingdom	139	0.0000901233	95th
Very elderly	Hungary	80	0.0001577334	95th
Very elderly	Ireland	77	0.0001629198	95th
Very elderly	Italy	228	0.0001079636	95th
Very elderly	Netherlands	450	0.0001152571	95th
Very elderly	Romania	45	0.0001994053	90th
Very elderly	Sweden	72	0.0001195411	95th

(1): HRP: highest reliable percentile, i.e. the highest percentile that is considered statistically robust for combinations of dietary survey, age class and possibly raw primary commodity, considering that a minimum of 5, 12, 30 and 61 observations are respectively required to derive 50th, 75th and 90th and 95th percentile estimates. Estimates with less than 5 observations were not included in this table.

Table B.2: Acute dietary exposure of consumers (mg/kg bw per day) to monensin total residues based on residue data in chicken – Summary statistics across European dietary surveys

Raw primary commodity	Population class	Number of surveys	Maximum HRP(1)
Birds liver	Other children	7	0.0033990000
Birds liver	Adults	12	0.0029957288
Birds liver	Adolescents	4	0.0016854545
Birds meat	Other children	20	0.0012669800
Birds meat	Infants	5	0.0010565963
Birds meat	Toddlers	11	0.0009887022
Birds liver	Infants	2	0.0009270000
Birds meat	Adolescents	20	0.0007771852
Birds liver	Elderly	6	0.0007357143
Birds meat	Adults	23	0.0005679430
Birds meat	Elderly	16	0.0004825660
Birds meat	Very elderly	14	0.0004692519
Birds liver	Toddlers	1	0.0004635000
Birds offals and slaughtering products (other than liver)	Adults	3	0.0004453125

Raw primary commodity	Population class	Number of surveys	Maximum HRP(1)
Birds liver	Very elderly	4	0.0002990323
Birds fat tissue	Adolescents	2	0.0002748261
Birds fat tissue	Other children	4	0.0002661474
Birds fat tissue	Toddlers	1	0.0002247467
Birds fat tissue	Adults	8	0.0001980147
Birds fat tissue	Very elderly	3	0.0001023400
Birds fat tissue	Elderly	4	0.0000752500

(1): Maximum of the highest reliable percentile values across European dietary surveys.

Table B.3: Chronic combined exposure of consumers per population class, country and survey of consumers (mg/kg bw per day) to monensin total residues based on residue data in chicken and bovine tissues and milk

Population class	Survey's country	Number of subjects	HRP(1)	HRP description
Infants	Bulgaria	523	0.0025795276	95th
Infants	Germany	142	0.0014547696	95th
Infants	Denmark	799	0.0020668941	95th
Infants	Finland	427	0.0015294067	95th
Infants	United Kingdom	1,251	0.0012772728	95th
Infants	Italy	9	0.0006203849	50th
Toddlers	Belgium	36	0.0019626350	90th
Toddlers	Bulgaria	428	0.0023722484	95th
Toddlers	Germany	348	0.0020802463	95th
Toddlers	Denmark	917	0.0022701722	95th
Toddlers	Spain	17	0.0016183958	75th
Toddlers	Finland	500	0.0024682670	95th
Toddlers	United Kingdom	1,314	0.0020698643	95th
Toddlers	United Kingdom	185	0.0019356257	95th
Toddlers	Italy	36	0.0016299638	90th
Toddlers	Netherlands	322	0.0019377226	95th
Other children	Austria	128	0.0029663681	95th
Other children	Belgium	625	0.0019696607	95th
Other children	Bulgaria	433	0.0020019580	95th
Other children	Czech Republic	389	0.0020093355	95th
Other children	Germany	293	0.0016126611	95th
Other children	Germany	835	0.0012521815	95th
Other children	Denmark	298	0.0017740835	95th
Other children	Spain	399	0.0014008329	95th
Other children	Spain	156	0.0016667807	95th
Other children	Finland	750	0.0018880110	95th
Other children	France	482	0.0017635718	95th
Other children	United Kingdom	651	0.0013924741	95th
Other children	Greece	838	0.0016647713	95th
Other children	Italy	193	0.0014725156	95th
Other children	Latvia	187	0.0014360723	95th
Other children	Netherlands	957	0.0015537739	95th
Other children	Netherlands	447	0.0013142318	95th
Other children	Sweden	1,473	0.0015842171	95th
Adolescents	Austria	237	0.0008900732	95th
Adolescents	Belgium	576	0.0006819726	95th

Population class	Survey's country	Number of subjects	HRP(1)	HRP description
Adolescents	Cyprus	303	0.0006060521	95th
Adolescents	Czech Republic	298	0.0013220976	95th
Adolescents	Germany	393	0.0009387438	95th
Adolescents	Germany	1,011	0.0006868897	95th
Adolescents	Denmark	377	0.0008316968	95th
Adolescents	Spain	651	0.0008075476	95th
Adolescents	Spain	209	0.0009650033	95th
Adolescents	Spain	86	0.0007418586	95th
Adolescents	Finland	306	0.0009103862	95th
Adolescents	France	973	0.0009490053	95th
Adolescents	United Kingdom	666	0.0006904988	95th
Adolescents	Italy	247	0.0008230436	95th
Adolescents	Latvia	453	0.0009474830	95th
Adolescents	Netherlands	1,142	0.0008921214	95th
Adolescents	Sweden	1,018	0.0009512468	95th
Adults	Austria	308	0.0007121166	95th
Adults	Belgium	1,292	0.0006386811	95th
Adults	Czech Republic	1,666	0.0007309850	95th
Adults	Germany	10,419	0.0006345642	95th
Adults	Denmark	1,739	0.0005774042	95th
Adults	Spain	981	0.0006644088	95th
Adults	Spain	410	0.0006479132	95th
Adults	Finland	1,295	0.0007399569	95th
Adults	France	2,276	0.0006614246	95th
Adults	United Kingdom	1,265	0.0004972914	95th
Adults	Hungary	1,074	0.0007046084	95th
Adults	Ireland	1,274	0.0005702101	95th
Adults	Italy	2,313	0.0005247628	95th
Adults	Latvia	1,271	0.0006511800	95th
Adults	Netherlands	2,055	0.0006546694	95th
Adults	Romania	1,254	0.0006732205	95th
Adults	Sweden	1,430	0.0005954770	95th
Elderly	Austria	67	0.0006494093	95th
Elderly	Belgium	511	0.0006419146	95th
Elderly	Germany	2,006	0.0005983576	95th
Elderly	Denmark	274	0.0005629677	95th
Elderly	Finland	413	0.0006286208	95th
Elderly	France	264	0.0005978217	95th
Elderly	United Kingdom	166	0.0005293803	95th
Elderly	Hungary	206	0.0005981138	95th
Elderly	Ireland	149	0.0006029148	95th
Elderly	Italy	289	0.0004540141	95th
Elderly	Netherlands	173	0.0005613266	95th
Elderly	Netherlands	289	0.0005486190	95th
Elderly	Romania	83	0.0005302204	95th
Elderly	Sweden	295	0.0006285113	95th
Very elderly	Austria	25	0.0004458340	75th
Very elderly	Belgium	704	0.0007087520	95th
Very elderly	Germany	490	0.0006093196	95th
Very elderly	Denmark	12	0.0003850268	75th

Population class	Survey's country	Number of subjects	HRP(1)	HRP description
Very elderly	France	84	0.0005832936	95th
Very elderly	United Kingdom	139	0.0005898587	95th
Very elderly	Hungary	80	0.0006274331	95th
Very elderly	Ireland	77	0.0005430224	95th
Very elderly	Italy	228	0.0004586513	95th
Very elderly	Netherlands	450	0.0005456926	95th
Very elderly	Romania	45	0.0005814443	90th
Very elderly	Sweden	72	0.0006775374	95th

(1): HRP: highest reliable percentile, i.e. the highest percentile that is considered statistically robust for combinations of dietary survey, age class and possibly raw primary commodity, considering that a minimum of 5, 12, 30 and 61 observations are respectively required to derive 50th, 75th and 90th and 95th percentile estimates. Estimates with less than 5 observations were not included in this table.

Table B.4: Chronic dietary exposure per population class, country and survey (mg/kg bw per day) of consumers exposure of consumers to monensin total residues derived from poultry MRLs

Population class	Survey's country	Number of subjects	HRP(1)	HRP description
Infants	Bulgaria	523	0.0026412521	95th
Infants	Germany	142	0.0004815856	95th
Infants	Denmark	799	0.0005930780	95th
Infants	Finland	427	0.0008866932	95th
Infants	United Kingdom	1,251	0.0011695825	95th
Infants	Italy	9	0.0000000000	50th
Toddlers	Belgium	36	0.0013078788	90th
Toddlers	Bulgaria	428	0.0030840855	95th
Toddlers	Germany	348	0.0008506587	95th
Toddlers	Denmark	917	0.0006580501	95th
Toddlers	Spain	17	0.0012820513	75th
Toddlers	Finland	500	0.0014164124	95th
Toddlers	United Kingdom	1,314	0.0013486905	95th
Toddlers	United Kingdom	185	0.0013791325	95th
Toddlers	Italy	36	0.0011681818	90th
Toddlers	Netherlands	322	0.0014350065	95th
Other children	Austria	128	0.0011329392	95th
Other children	Belgium	625	0.0015122316	95th
Other children	Bulgaria	433	0.0025659761	95th
Other children	Czech Republic	389	0.0024154589	95th
Other children	Germany	293	0.0008832389	95th
Other children	Germany	835	0.0008916123	95th
Other children	Denmark	298	0.0007355960	95th
Other children	Spain	399	0.0016656946	95th
Other children	Spain	156	0.0023312266	95th
Other children	Finland	750	0.0011949041	95th
Other children	France	482	0.0010114065	95th
Other children	United Kingdom	651	0.0011924485	95th
Other children	Greece	838	0.0011916283	95th
Other children	Italy	193	0.0012556148	95th
Other children	Latvia	187	0.0013530353	95th
Other children	Netherlands	957	0.0010468318	95th

Population class	Survey's country	Number of subjects	HRP(1)	HRP description
Other children	Netherlands	447	0.0013181835	95th
Other children	Sweden	1,473	0.0009876366	95th
Adolescents	Austria	237	0.0007828349	95th
Adolescents	Belgium	576	0.0007146809	95th
Adolescents	Cyprus	303	0.0007570196	95th
Adolescents	Czech Republic	298	0.0017332514	95th
Adolescents	Germany	393	0.0006846480	95th
Adolescents	Germany	1,011	0.0005606271	95th
Adolescents	Denmark	377	0.0005752760	95th
Adolescents	Spain	651	0.0009674077	95th
Adolescents	Spain	209	0.0012982568	95th
Adolescents	Spain	86	0.0010604620	95th
Adolescents	Finland	306	0.0007256906	95th
Adolescents	France	973	0.0006501045	95th
Adolescents	United Kingdom	666	0.0008924024	95th
Adolescents	Italy	247	0.0005579962	95th
Adolescents	Latvia	453	0.0008245619	95th
Adolescents	Netherlands	1,142	0.0009881844	95th
Adolescents	Sweden	1,018	0.0007436319	95th
Adults	Austria	308	0.0008964804	95th
Adults	Belgium	1,292	0.0006750652	95th
Adults	Czech Republic	1,666	0.0008783021	95th
Adults	Germany	10,419	0.0005502277	95th
Adults	Denmark	1,739	0.0003750154	95th
Adults	Spain	981	0.0009014085	95th
Adults	Spain	410	0.0009012392	95th
Adults	Finland	1,295	0.0007101270	95th
Adults	France	2,276	0.0005436905	95th
Adults	United Kingdom	1,265	0.0006586801	95th
Adults	Hungary	1,074	0.0008071707	95th
Adults	Ireland	1,274	0.0008829231	95th
Adults	Italy	2,313	0.0004731626	95th
Adults	Latvia	1,271	0.0007368243	95th
Adults	Netherlands	2,055	0.0008100476	95th
Adults	Romania	1,254	0.0009388828	95th
Adults	Sweden	1,430	0.0007589879	95th
Elderly	Austria	67	0.0007126895	95th
Elderly	Belgium	511	0.0005483946	95th
Elderly	Germany	2,006	0.0004233469	95th
Elderly	Denmark	274	0.0003032962	95th
Elderly	Finland	413	0.0005739806	95th
Elderly	France	264	0.0004450013	95th
Elderly	United Kingdom	166	0.0005254240	95th
Elderly	Hungary	206	0.0006154615	95th
Elderly	Ireland	149	0.0007217702	95th
Elderly	Italy	289	0.0004585927	95th
Elderly	Netherlands	173	0.0006180373	95th
Elderly	Netherlands	289	0.0005251902	95th
Elderly	Romania	83	0.0008284211	95th
Elderly	Sweden	295	0.0007067604	95th

Population class	Survey's country	Number of subjects	HRP(1)	HRP description
Very elderly	Austria	25	0.0001884245	75th
Very elderly	Belgium	704	0.0006294046	95th
Very elderly	Germany	490	0.0004577186	95th
Very elderly	Denmark	12	0.0001583679	75th
Very elderly	France	84	0.0005259034	95th
Very elderly	United Kingdom	139	0.0004068770	95th
Very elderly	Hungary	80	0.0005908067	95th
Very elderly	Ireland	77	0.0007355296	95th
Very elderly	Italy	228	0.0004749960	95th
Very elderly	Netherlands	450	0.0005047293	95th
Very elderly	Romania	45	0.0008302886	90th
Very elderly	Sweden	72	0.0005396889	95th

(1): HRP: highest reliable percentile, i.e. the highest percentile that is considered statistically robust for combinations of dietary survey, age class and possibly raw primary commodity, considering that a minimum of 5, 12, 30 and 61 observations are respectively required to derive 50th, 75th and 90th and 95th percentile estimates. Estimates with less than 5 observations were not included in this table.

Table B.5: Acute dietary exposure of consumers (mg/kg bw per day) to monensin total residues derived from poultry MRLs – Summary statistics across European dietary surveys

Raw primary commodity	Population class	Number of surveys	Maximum HRP(1)
Birds meat	Other children	20	0.0057200000
Birds meat	Infants	5	0.0047701863
Birds meat	Toddlers	11	0.0044636667
Birds meat	Adolescents	20	0.0035087368
Birds meat	Adults	22	0.0025640769
Birds liver	Other children	7	0.0022000000
Birds meat	Elderly	16	0.0021786275
Birds meat	Very elderly	14	0.0021185185
Birds liver	Adults	12	0.0019389831
Birds liver	Adolescents	4	0.0011804878
Birds offals and slaughtering products (other than liver)	Adults	3	0.0009375000
Birds liver	Infants	2	0.0006000000
Birds liver	Elderly	6	0.0004761905
Birds liver	Toddlers	1	0.0003000000
Birds liver	Very elderly	4	0.0001935484
Birds fat tissue	Adolescents	2	0.0001333043
Birds fat tissue	Other children	4	0.0001290947
Birds fat tissue	Toddlers	1	0.0001090133
Birds fat tissue	Adults	8	0.0000960470
Birds fat tissue	Very elderly	3	0.0000496400
Birds fat tissue	Elderly	4	0.0000365000

(1): Maximum of the highest reliable percentile values across European dietary surveys.

Appendix C – Estimation of user exposure to monensin sodium from the additive Elancoban® G200, including consideration of using a filter mask FF P2 or FF P3 as a preventative measure

Calculation	Identifier	Description	Amount	Source
	a	Monensin in the dust (mg/g)	[redacted]	Technical dossier
	b	Dusting potential (g/m^3)	[redacted]	Technical dossier
a × *b*	*c*	monensin in the air (mg/m^3)	36.1	
	d	No of premixture batches prepared/working day	10	EFSA Guidance on user safety (EFSA FEEDAP Panel, 2012b)
	e	Time of exposure per production of one batch (s)	20	EFSA Guidance on user safety (EFSA FEEDAP Panel, 2012b)
d × *e*	*f*	Total duration of daily exposure/worker (s)	200	
	g	Uncertainty factor	2	EFSA Guidance on user safety (EFSA FEEDAP Panel, 2012b)
f × *g*	*h*	Refined total duration of daily exposure/worker (s)	400	
h/3 600	*i*	Refined total duration of daily exposure (h)	0.11	
	j	Inhaled air per hour (m^3)	1.25	EFSA Guidance on user safety (EFSA FEEDAP Panel, 2012b)
j × *i*	*k*	Inhaled air during exposure (m^3)	0.14	
c × *k*	*l*	Monensin inhaled during exposure per eight-hour working day (mg)	5.0	
	m	Particles below 10 μm in the dust (%) generated during the Stauber-Heubach measurement	31	Technical dossier
l × *m*/100	*n*	Monensin inhaled per eight-hour working day (mg) reduced by respirable fraction	1.6	
n/10	*o*	Monensin inhaled per eight-hour working day (mg) reduced by filter mask FF P2 (reduction factor 10)	0.16	
n/20	*p*	Monensin inhaled per eight-hour working day (mg) reduced by filter mask FF P3 (reduction factor 20)	0.08	

Appendix D – Total number of *Eimeria* oocysts per gram of faeces (OPG) in floor pen trials with chickens for fattening(1)

	Day 23 log_{10}opg			
	E. acervulina	***E. maxima***	***E. tenella***	***Total***
Trial 1				
UUC	nd	nd	nd	nd
IUC	4.38	4.07	4.29	4.75
IT	3.74*	3.52*	3.72*	4.16*

	Geometric mean of faecal oocyst counts at different days						
	22	**24**	**27**	**29**	**31**	**36**	**42**
Trial 2							
UUC	0	0	1	0	0	2	12
IUC	16,682,947	12,338,450	3,377,339	740,500	100,091	10,987	39,841
IT	8,553,853	5,187,342	322,431	557,880	131,755	43,038	592*

	OPG × 10^3						
	***E. acer*(2)**	***E. ten***	***E. max***	***E. bru***	***E. nec/prae***	***E. mit***	**Total**
Trial 3a							
	Day 20						
UUC	302	8.1	0	0	0.3	3.2	313
IUC	420	12.0	51.6	0	6.9	27.1	517
IT	384	6.5	3.0*	0	27.2*	48.8	469
	Day 22						
UUC	79	0	0.3	0	0	6.6	86
IUC	136	14.5	18.7	0	28.6	25.9	223
IT	74	6.5	1.0*	0	8.3	11.7	101
	Day 28						
UUC	111	7.9	0	0	23.8	22.1	165
IUC	26	2.67	1.47	0	5.2	5.8	41
IT	64	0.53	0.15*	0	6.9	5.6	78

	OPG × 10^3						
	E. acer	***E. ten***	***E. max***	***E. bru***	***E. nec/prae***	***E. mit***	**Total**
Trial 3b							
	Day 20						
UUC	302	8.1	0	0	0.3	3.2	313
IUC	677	8.2	0	0	6.0	19.5	710
IT	1,268	3.2	0	0	39.8*	93.0*	1404
	Day 22						
UUC	79	0	0.3	0	0	6.6	86
IUC	576	188	19.2	0	564	134	1481
IT	475	50	21.8	0	205	40*	792
	Day 28						
UUC	111	7.9	0	0	23.8	22.1	165
IUC	34	1.3	10.3	0	4.3	3.4	53
IT	111*	1.47	12.2	0.25	10.3	9.5	145*

(1): IT with * is significantly different from control (IUC).
(2): *Eimeria* species are *Acervulina* (*acer*), *Tenella* (*ten*), *Maxima* (*max*), *Brunetti* (*bru*), *Necatrix*/*Praecox* (*nec*/*prae*), *Mitis* (*mit*).

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

Elancoban®G200 is a feed additive currently authorized for turkeys, chickens for fattening and reared for laying by Commission Regulation (EC) No 1356/2004 belonging to the "Coccidiostats and other medicinal substances" group listed in Directive 70/524/EEC. The authorisation was further amended by Commission Regulation (EC) No 1096/2008. In the current application an authorisation according to article 10 (2) of Regulation (EC) No 1831/2003 is requested. Elancoban®G200 consists of granular monensin (active substance) containing 20% (w/w) monensin, anti-dusting oil, and rice hulls or limestone granular as base material. It is intended to be preferably incorporated into feedingstuffs through premixtures. The Applicant proposed a concentration of monensin in feedingstuffs ranging from 60 to 125 mg/kg, depending on the target species.

Furthermore the Applicant suggested the following Maximum Residue Limits (MRLs) in tissues of turkeys and chickens for fattening and reared for laying: 8 μg/kg in liver, muscle and kidney and 25 μg/kg in skin/fat tissues, as already established by Commission Regulation (EC) No 1096/2008.

For the quantification of monensin in the feed additive, premixes and feedingstuffs the Applicant submitted the AOAC ring-trial validated methods (AOAC 997.04) based on High Performance Liquid Chromatography with post-column derivatisation coupled to spectrophotometric detection (HPLC-PCD-UV-Vis.). In addition, the EURL identified another multi-analyte ring-trial validated method (EN ISO 14183) using a similar experimental protocol. Based on the performance characteristics available the EURL recommends for official control both methods for the quantification of monensin in the feed additive, premixtures and/or feedingstuffs.

For the quantification of monensin in chicken and turkey tissues the Applicant submitted the ring trial validated method (AOAC 2011.24) based on Reversed Phase High Performance Liquid Chromatography coupled to a triple quadrupole mass spectrometer (RP-HPLC-MS/MS) in electrospray ionisation mode using matrix matched standards. Based on the performance characteristics available the EURL recommends for official control this AOAC ring-trial validated method or any equivalent methods complying with the requirements set by Commission Decision 2002/657/EC, to enforce the monensin MRLs in the relevant tissues.

Further testing or validation of the methods to be performed through the consortium of National Reference Laboratories as specified by Article 1.