SCIENTIFIC OPINION

EFSA Journal

ADOPTED: 16 May 2017

doi: 10.2903/j.efsa.2017.4857

Safety and efficacy of Avatec® 150G (lasalocid A sodium) for chickens for fattening and chickens reared for laying, and modification of the terms of authorisation for chickens for fattening, chickens reared for laying, turkeys for fattening, minor avian species (pheasants, guinea fowl, quails and partridges) except laying birds

EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP), Guido Rychen, Gabriele Aquilina, Giovanna Azimonti, Vasileios Bampidis, Maria de Lourdes Bastos, Georges Bories, Andrew Chesson, Pier Sandro Cocconcelli, Gerhard Flachowsky, Boris Kolar, Maryline Kouba, Marta López-Alonso, Secundino López Puente, Alberto Mantovani, Baltasar Mayo, Fernando Ramos, Maria Saarela, Roberto Edoardo Villa, Robert John Wallace, Pieter Wester, Paul Brantom, Ingrid Halle, Patrick van Beelen, Orsolya Holczknecht, Maria Vittoria Vettori and Jürgen Gropp

Abstract

Avatec® 150G is an anticoccidial feed additive containing 15% of the active ingredient lasalocid A sodium, an ionophore anticoccidial. The tolerance study indicated that oral administration of lasalocid A sodium via feed at doses at and above the targeted lasalocid dose of 125 mg/kg was not tolerated in chickens for fattening. Consequently, no safe dose can be established by the tolerance study submitted. Concurrent administration of lasalocid with tiamulin and certain other medicinal substances should be avoided. Lasalocid sodium has a selective antimicrobial activity against Gram-positive bacterial species while many Enterobacteriaceae are naturally resistant. Induction of resistance and/or cross-resistance was not observed in experimental conditions. Lasalocid A sodium is not genotoxic and not carcinogenic. The newly conducted cardiovascular study in dogs indicated an acute no-observed-adverse-effect level (NOAEL) of 1 mg/kg body weight (bw) per day. Since this NOAEL is above the lowest NOAEL of 0.5 mg/kg bw per day previously identified in a 2-year toxicity study in rats and a developmental study in rabbits, it is concluded that this NOAEL (0.5 mg/kg bw per day) is an appropriate base for establishing an Acceptable Daily Intake of 0.005 mg lasalocid sodium/kg bw. The use of lasalocid A sodium from Avatec® 150G at the highest proposed level of 125 mg/kg complete feed is safe for the consumer. For compliance with the maximum residue limits (MRLs), a withdrawal period of 3 days is necessary. No risk for the user is expected from the use of Avatec® 150G. The use of lasalocid A sodium from Avatec® 150G in feed for chickens for fattening and chickens reared for laying up to the highest proposed dose does not pose a risk for the environment. Insufficient evidence of the anticoccidial efficacy of lasalocid A sodium was provided in chickens for fattening/reared for laying.

© 2017 European Food Safety Authority. *EFSA Journal* published by John Wiley and Sons Ltd on behalf of European Food Safety Authority.

Keywords: Coccidiostat, Avatec, lasalocid A sodium, safety, efficacy, poultry

Requestor: European Commission

Question number: EFSA-Q-2013-00813

Correspondence: feedap@efsa.europa.eu

Panel members: Gabriele Aquilina, Giovanna Azimonti, Vasileios Bampidis, Maria de Lourdes Bastos, Georges Bories, Andrew Chesson, Pier Sandro Cocconcelli, Gerhard Flachowsky, Jürgen Gropp, Boris Kolar, Maryline Kouba, Marta López-Alonso, Secundino López Puente, Alberto Mantovani, Baltasar Mayo, Fernando Ramos, Guido Rychen, Maria Saarela, Roberto Edoardo Villa, Robert John Wallace and Pieter Wester.

Note: This scientific opinion has been amended following the adoption of the decision of the Commission on confidentiality claims submitted by the applicant, in accordance with Article 8(6) and Article 18 of Regulation (EC) No 1831/2003. The modified section is indicated in the text.

Suggested citation: EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP), 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, 2017. Scientific Opinion on the safety and efficacy of Avatec® 150G (lasalocid A sodium) for chickens for fattening and chickens reared for laying, and modification of the terms of authorisation for chickens for fattening, chickens reared for laying, turkeys for fattening, minor avian species (pheasants, guinea fowl, quails and partridges) except laying birds. EFSA Journal 2017;15(8):4857, 32 pp. https://doi.org/10.2903/j.efsa.2017.4857

ISSN: 1831-4732

© 2017 European Food Safety Authority. *EFSA Journal* published by John Wiley and Sons Ltd on behalf of European Food Safety Authority.

This is an open access article under the terms of the Creative Commons Attribution-NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited and no modifications or adaptations are made.

The EFSA Journal is a publication of the European Food Safety Authority, an agency of the European Union.

Summary

Following a request from the European Commission, the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) was asked to deliver a scientific opinion on the safety and efficacy of Avatec® 150G (lasalocid A sodium) for chickens for fattening and chickens reared for laying. The FEEDAP Panel was also requested to assess the compliance with maximum residue limits (MRLs) established by Implementing Regulation (EU) No 1277/2014 for chickens for fattening, chickens reared for laying, turkeys for fattening and other minor avian species, except laying birds. In addition, the proposed reduction of the withdrawal period (from five to two days) is evaluated.

Avatec® 150G is an anticoccidial feed additive containing 15% of the active ingredient lasalocid A sodium, an ionophore anticoccidial. It is indicated for use for the control of coccidiosis in chickens for fattening, chickens reared for laying, turkeys and minor avian species (pheasants, guinea fowl, quails, and partridges other than laying birds).

The tolerance study indicated that oral administration of lasalocid A sodium via feed at doses at and above the targeted lasalocid dose of 125 mg/kg was not tolerated in chickens for fattening. Consequently, no safe dose can be established by the tolerance study submitted. Concurrent administration of lasalocid with tiamulin and certain other medicinal substances should be avoided.

Lasalocid sodium has a selective antimicrobial activity against Gram-positive bacterial species while many Enterobacteriaceae are naturally resistant. Induction of resistance and/or cross-resistance was not observed in experimental conditions.

Lasalocid A sodium is not genotoxic and is not carcinogenic. The newly conducted cardiovascular study in dogs indicated an acute no-observed-adverse-effect level (NOAEL) of 1 mg/kg body weight (bw) per day. Since this NOAEL is above the lowest NOAEL of 0.5 mg/kg bw per day previously identified in a 2-year toxicity study in rats and a developmental study in rabbits, it is concluded that this NOAEL (0.5 mg/kg bw per day) is an appropriate base for establishing an Acceptable Daily Intake of 0.005 mg lasalocid sodium/kg bw.

The use of lasalocid sodium from Avatec® 150G at the highest proposed level of 125 mg/kg complete feed in chickens and turkeys for fattening, in chicken reared for laying up to the 16th week of life and growing pheasants, partridges, quails and guinea fowl is safe for the consumer. For compliance with the MRLs, a withdrawal period of 3 days is necessary.

No risk for the user is expected from the use of Avatec® 150G in poultry nutrition.

The use of lasalocid sodium from Avatec® 150G in feed for chickens for fattening and chickens reared for laying up to the highest proposed dose does not pose a risk for the environment.

Insufficient evidence of the anticoccidial efficacy of lasalocid A sodium was provided in chickens for fattening/reared for laying. Regulation (EC) No 429/2008 requires three floor pen studies and three field studies supporting the control of coccidiosis by the additive. Such an effect could only be shown in two floor pen studies with 75 mg lasalocid A sodium/kg complete feed and three anticoccidial sensitivity tests (ASTs) with 100 mg lasalocid A sodium/kg complete feed.

Table of contents

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 13(3) of that Regulation lays down that if the holder of an authorisation proposes changing the terms of the authorisation by submitting an application to the Commission, accompanied by the relevant data supporting the request for the change, the Authority shall transmit its opinion on the proposal to the Commission and the Member States. 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 1 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 7 years after the entry into force of this Regulation for additives authorised without a time limit or pursuant to Directive 82/471/EEC.

The European Commission received a request from Zoetis Belgium SA[2] for re-evaluation of the product Avatec® 150G (lasalocid A sodium), when used as a feed additive for chickens for fattening and chickens reared for laying and for change of the terms of authorisation (change of the maximum residue limits (MRLs) and withdrawal time) in chickens for fattening, chickens reared for laying, turkeys for fattening and minor avian species (pheasants, guinea fowls, quails and partridges) except laying birds (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) and under Article 13(3) (modification of the authorisation of a feed additive). EFSA received directly from the applicant the technical dossier in support of this application. The particulars and documents in support of the application were considered valid by EFSA as of 18 November 2013.

According to Article 8 of Regulation (EC) No 1831/2003, EFSA, after verifying the particulars and documents submitted by the applicant, shall undertake an assessment in order to determine whether the feed additive complies with the conditions laid down in Article 5. EFSA shall deliver an opinion on the safety for the target animals, consumer, user and the environment and on the efficacy of the product Avatec® 150G (lasalocid A sodium), when used under the proposed conditions of use (see Section 3.1.3).

1.2. Additional information

Avatec® 150G is an anticoccidial feed additive containing 15% of the active ingredient lasalocid A sodium, an ionophore anticoccidial. It is indicated for use for the control of coccidiosis in chickens for fattening, chickens reared for laying, turkeys and minor avian species (pheasants, guinea fowl, quails, and partridges other than laying birds).

The Scientific Committee on Animal Nutrition (SCAN) issued an opinion on the use of this product for turkeys (European Commission, 1991) and chickens for fattening (European Commission, 1982). EFSA issued five opinions on the feed additive Avatec® 150G. Two opinions on the re-evaluation of the product for chickens for fattening and chickens reared for laying in accordance with article 9G of Council Directive 70/524/EEC (EFSA, 2004a,b), one opinion on a new formulation of Avatec® 150G in accordance with Regulation (EC) No 1831/2003 (EFSA, 2005a), one opinion on the re-evaluation of the product for turkeys in accordance with Regulation (EC) No 1831/2003 (EFSA FEEDAP Panel, 2010a) and one opinion on the safety and efficacy in pheasants, partridges, quails and guinea fowl (EFSA FEEDAP Panel, 2011a).

Avatec® 150G is authorised for its use in chickens for fattening and chickens reared for laying until August 2014,[3] in turkeys until October 2020[4] and in pheasants, guinea fowl, quails, and partridges other than laying birds until September 2021.[5] The MRLs referred in the above mentioned regulations

[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] Rue Laid Burniat 1, 1348 Louvain-la-Neuve, Belgium.
[3] Commission Regulation (EC) No 2037/2005 of 14 December 2005 amending the condition for authorisation of a feed additive belonging to the group of coccidiostats. OJ L 328 of 15.12.2005, p. 21.
[4] Commission Regulation (EU) No 874/2010 of 5 October 2010 concerning the authorisation of lasalocid A sodium as a feed additive for turkeys up to 16 weeks (holder of authorisation Alpharma (Belgium) BVBA) and amending Regulation (EC) No 2430/1999. OJ L 263, 6.10.2010, p. 1.
[5] Commission Implementing Regulation (EU) No 900/2011 of 7 September 2011 concerning the authorisation of lasalocid A sodium as a feed additive for pheasants, guinea fowl, quails and partridges other than laying birds (holder of authorisation Alpharma (Belgium) BVBA). OJ L 231, 8.9.2011, p. 15.

were those put in force by Regulation (EU) No 37/2010 on pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin.[6]

During the course of the present assessment, the Committee for Medicinal Products for Veterinary Use (CVMP) of the European Medicines Agency (EMA) adopted an opinion on the establishment of maximum residue limits for lasalocid in poultry (EMA CVMP, 2013). On 1st December 2014, the European Commission adopted the Implementing Regulation (EU) No 1277/2014[7] modifying the MRLs for lasalocid A in poultry as follows: 60 μg/kg (muscle), 300 μg/kg (liver), 300 μg/kg (skin/fat), 150 μg/kg (kidney) and 150 μg/kg (eggs).

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 Avatec® 150G (lasalocid A sodium) as a feed additive. The technical dossier was prepared following the provisions of Article 7 and Article 13 of Regulation (EC) No 1831/2003, Regulation (EC) No 429/2008[9] and the applicable EFSA guidance documents.

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, other scientific reports and experts' elicitation knowledge, to deliver the present output.

EFSA has verified the European Union Reference Laboratory (EURL) report as it relates to the methods used for the control of the active substance in animal feed and marker residue in tissues. The Executive Summary of the EURL report can be found in Annex A.[10]

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of Avatec® 150G (lasalocid A sodium) is in line with the principles laid down in Regulation (EC) No 429/2008 and the relevant guidance documents: Guidance for the preparation of dossiers for coccidiostats and histomonostats (EFSA FEEDAP Panel, 2011b), Technical guidance: Tolerance and efficacy studies in target animals (EFSA FEEDAP Panel, 2011c), Technical Guidance for assessing the safety of feed additives for the environment (EFSA, 2008a), Guidance for the preparation of dossiers for the re-evaluation of certain additives already authorised under Directive 70/524/EEC (EFSA, 2008b), Guidance for establishing the safety of additives for the consumer (EFSA FEEDAP Panel, 2012a), Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012b), Technical Guidance: Microbial Studies (EFSA, 2008c), and Technical Guidance: Extrapolation of data from major species to minor species regarding the assessment of additives for use in animal nutrition (EFSA, 2008d).

3. Assessment

The current opinion is aimed at assessing the safety and efficacy of Avatec® 150G for chickens for fattening and chickens reared for laying and at assessing the compliance with MRLs established by Implementing Regulation (EU) No 1277/2014[7] for chickens for fattening, chickens reared for laying, turkeys for fattening and other minor avian species, except laying birds. In addition, the proposed reduction of the withdrawal period (from 5 to 2 days) is evaluated.

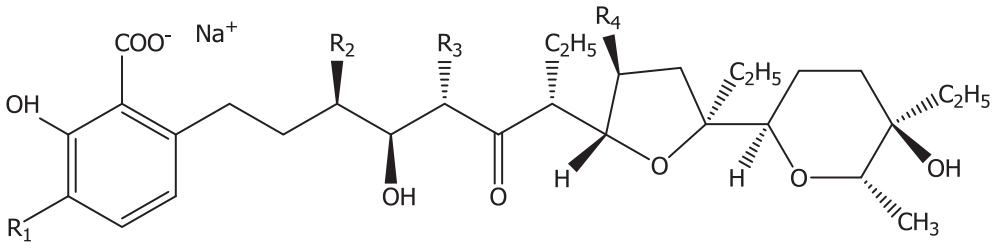
[6] Commission Regulation (EU) No 37/2010 of 22 December 2009 on pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin. OJ L 15, 20.1.2010, p. 1.
[7] Commission Implementing Regulation (EU) No 1277/2014 of 1 December 2014 amending Regulation (EU) No 37/2010, as regards the substance "lasalocid". OJ L 346, 1.12.2014, p. 23.
[8] FEED dossier reference: FAD-2013-0040.
[9] 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.
[10] The full report is available on the EURL website: http://www.pihin.eu/wordpress/FAD/FinRep-FAD-2013-0040.pdf

3.1. Characterisation

3.1.1. Characterisation of the active substance[11]

Lasalocid A sodium is a monocarboxylic polyether ionophore obtained via fermentation of a strain of *Streptomyces*. No changes in the process have been introduced since the FEEDAP Panel made the first assessment in 2004 (EFSA, 2004a,b,c).

Lasalocid A sodium (sodium 6-3*R*, 4*S*,5*S*,7*R*)-7-[2*S*,3*S*,5*S*)-5-ethyl-5-[(2*R*,5*R*,6*S*)-5-ethyl-5-hydroxy-6-methyltetrahydro-2*H*-pyran-2-yl]-tetrahydro-3-methyl-2-furyl]-4-hydroxy-3,5-dimethyl-6-oxononyl]-2,3-cresotate; $C_{34}H_{54}O_8Na$) has the CAS No 25999-20-6. Lasalocid sodium homologues B, C, D and E are also present. The structural formula of lasalocid sodium is given in Figure 1.



	R_1	R_2	R_3	R_4
Lasalocid A	CH_3	CH_3	CH_3	CH_3
Lasalocid B	C_2H_5	CH_3	CH_3	CH_3
Lasalocid C	CH_3	C_2H_5	CH_3	CH_3
Lasalocid D	CH_3	CH_3	C_2H_5	CH_3
Lasalocid E	CH_3	CH_3	CH_3	C_2H_5

Figure 1: Structural formula of lasalocid sodium

Data of five batches of the active substance showed that lasalocid sodium A amounted to 84.7–90.6% and the homologues were between 2.1% and 3.5%, which sum up to a total of 87.3–93.6%.[12]

3.1.1.1. Characterisation of the production organism

The active substance lasalocid A sodium is produced by fermentation of a strain of *Streptomyces*. The strain, not genetically modified, was originally deposited in the American Type Culture Collection (ATCC) as *Streptomyces lasaliensis* ATCC 31180.[13] Recently, it has been deposited at the Leibnitz Institute DSMZ-German Collection of Microorganisms and Cell cultures under the following number: *Streptomyces lasaliensis* PF 2-7 – DSM 32487.[14]

The unique identification of the production strain was achieved by analysing the whole sequence of the 7.7 Mb genome.[15] The strain was originally identified as *Streptomyces lasaliensis* subsp. *lasaliensis* (ATCC 31180); however, this is not a valid taxonomic name with standing in prokaryotic nomenclature.

The approaches used for taxonomical identification consistently place the strain ATCC 31180 in the genus *Streptomyces* but cannot unequivocally assign to a valid taxonomic species of this genus, suggesting that strain ATCC 31180 belongs to a new taxonomic unit within the genus *Streptomyces*.[16]

3.1.2. Characterisation of the additive

The final product Avatec® 150G is obtained by mixing purified lasalocid A sodium extracted from the fermentation medium with lignosulfonate (binder), ferric oxide (colouring agent) and calcium sulfate dihydrate (carrier).

[11] This section has been amended following the provisions of Article 8(6) and Article 18 of Regulation (EC) No 1831/2003.
[12] Technical dossier/Section II/Annex II.1.4.1.7.
[13] Technical dossier/Section II/Annex II.2.1.1.
[14] Letter 4/5/2017 – Certificate of deposition.
[15] Technical dossier/Supplementary information July 2016/Annex 2.12 Conf.
[16] Technical dossier/Supplementary information July 2016 and Spontaneous submission March 2017.

Avatec® 150G is specified to contain 139–161 g lasalocid A sodium, 40 g calcium lignosulfonate, 1 g ferric oxide (Fe_2O_3) per kilogram and calcium sulfate dihydrate (*quantum satis*). Batch to batch consistency was demonstrated by the analysis of five batches.[17] The lasalocid A sodium content ranged from 148 to 152 g/kg Avatec® 150G (mean 150 g/kg).

Data on impurities were provided from three batches.[18,19] Results showed concentrations of arsenic between 0.45 and 0.83 mg/kg, cadmium between < 0.01 and 0.013 mg/kg, lead between 0.26 and 0.52 mg/kg and mercury below 0.005 mg/kg. Values for dioxins (polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/F)) ranged between 0.15 and < 0.17 ng WHO-PCDD/F-TEQ per kg, and the sum of dioxins and dioxin-like polychlorinated biphenyls (DL-PCBs) was between 0.29 and < 49 ng WHO-PCDD/F-DL-PCB-TEQ per kg. Aflatoxin B_1 was < 0.001 mg/kg in all three batches. None of these impurities are of concern.

Data on microbial contamination were given in three batches for total aerobic count (< 10, 150, < 10 CFU/g), coliforms (< 10 CFU/g), Enterobacteriaceae, *Escherichia coli* and *Staphylococcus aureus* (< 10 CFU/g), *Salmonella* (negative/25 g), moulds (< 100, 90, 10 CFU/g) and yeasts (< 100, 20, < 10 CFU/g).

According to the applicant, the product does not contain live organisms from the production strain; however, no evidence was provided.

Ethyl acetate is used as a solvent during the manufacturing process and may be present in the product. Data on residual ethyl acetate from the analysis of three recent batches of the active substance was provided. Results showed that levels of ethyl acetate were between 19 and 29 mg/kg which is below the VICH Class 3 limit of 5,000 mg/kg.

Avatec® 150G is a red-brown free flowing granular preparation with an average bulk density of 930 kg/m^3 and average tapped density of 800 kg/m^3.[20] Three batches were analysed for particle size (sieve analysis). Avatec® 150G consisted of 99.7% and 0.3% particles (w/w) with a diameter < 850 μm and < 150 μm, respectively. The dusting potential (Stauber–Heubach test) of one batch was 370 mg/kg (average of five samples).

3.1.3. Stability and homogeneity

The studies submitted on shelf-life,[21] stability in premixtures and feedingstuffs[22] were already assessed by the FEEDAP Panel in 2005 (EFSA, 2005a) and 2010 (EFSA FEEDAP Panel, 2010a). The FEEDAP Panel reviewed the former studies and considered them acceptable for the current assessment for chickens for fattening and chickens reared for laying. These studies are summarised below.

3.1.3.1. Shelf-life of the additive

Three batches of Avatec® 150G showed no decrease of the initial lasalocid sodium content (150–151 g/kg) after 24 months at 25°C/60% relative humidity (RH), but a slight decrease (about 7%) was detected after 36 months. Data from three batches of the additive stored at 40°C/60% RH showed no change in lasalocid sodium content after 6 months (151–152 g/kg). The data support a shelf-life of 24 months for Avatec® 150G.

3.1.3.2. Stability and homogeneity of the additive in premixtures and feedingstuffs

The stability of Avatec® 150G in premixtures or complete feed was evaluated at 25°C/60% RH. Three different batches of Avatec® 150G were incorporated in broiler mineral/vitamin premixtures containing choline chloride and for 3 months in complete mash feed formulations. Storage stability was assessed at 0, 1, 3 and 6 months (premixtures) or 0, 1, 2 and 3 months (feeds). The broiler premixture gave recoveries of 92% and 110% (after 3 and 6 months storage, respectively). The broiler mash feed gave recoveries of 92% and 100% (after 1 and 6 months storage, respectively). The previous conclusion that Avatec® 150G showed good stability for up to 6 months in broiler premixtures and for 3 months in complete mash feed formulations when stored at 25°C and 60% RH is reiterated (EFSA, 2005a).

[17] Technical dossier/Supplementary information July 2016/Annex 2.1–2.5.
[18] Technical dossier/Section II/Annexes II.1.4.1.3. II.1.4.1.4. and II.1.4.1.5.
[19] Technical dossier/Supplementary information/July 2016 Annex 2.6 and 2.7.
[20] Technical dossier/Section II/Annex II.1.5.1 and II.1.5.3.
[21] Technical dossier/Section II/Annex II.4.1.1.
[22] Technical dossier/Section II/Annex II.4.1.2.

Homogeneity in premixtures and mash feed was also demonstrated in the same broiler premixture and feed used in the stability studies. Ten subsamples were analysed showing in both cases a coefficient of variation below 5%.

3.1.4. Conditions of use

Avatec® 150G is a feed additive for the prevention of coccidiosis for chickens for fattening, chickens reared for laying, turkeys for fattening, minor avian species (pheasants, guinea fowl, quails and partridges) except laying birds at a dose range of 75–125 mg lasalocid A sodium/kg complete feed. Two days withdrawal period is proposed by the applicant.

3.2. Safety

3.2.1. Safety for the target species

3.2.1.1. Tolerance study in chickens for fattening

A total of 600 one-day-old Ross 708 chickens (300 males and 300 females) were randomly allocated to five treatment groups which were fed diets containing 0, 125 (1x maximum proposed level), 187.5 (1.5x), 250 (2x) and 312.5 (2.5x) mg lasalocid sodium/kg feed (analytically confirmed, see Table 1), respectively, for 35 days.[23] Group size was 120 birds per treatment (6 replicates for each gender with 10 birds each). The basal diet consisted mainly of maize and methionine supplemented soybean meal; the starter formulation was calculated to contain 21.7% crude protein (CP), 0.59% methionine (met), and 12.9 MJ metabolisable energy (ME)/kg; the grower formulation 20.0% CP, 0.55% met and 13.1 MJ ME/kg. The starter was fed as crumbles for 21 days, the grower as pellets until the end of the study. The birds had *ad libitum* access to feed and water. Bird health, litter conditions and mortality were recorded daily. Birds were weighed individually on day 0 and by pen on day 21 and day 35. Zootechnical parameters (feed intake, average daily feed intake, feed to gain ratio, weight gain and average daily gain) were calculated. Blood samples were taken for haematology[24] and clinical biochemistry[25] from one animal per pen on day 35. The same animals were necropsied, and organ and tissue samples[26] were collected, and preserved for histology. Organs were weighed and examined for lesions and abnormalities. All birds, which died in the course of the study, were necropsied.

Data were analysed by a general linear mixed model with the fixed effects of treatment, sex and interaction treatment per sex. The pen was considered the statistical unit; differences were considered significant at a level of $p < 0.1$ (two-sided).

The results are summarised in Table 1. Mortality was low for the control group and the groups fed lasalocid at use level and the 1.5 and 2 overdose (about 4% as a mean) and not dose related. Only the high lasalocid dose group (2.5x) showed a higher mortality rate (24%). Tissues of 30 birds (out of 49 dead birds) were microscopically examined. Sixteen had changes consistent with lasalocid toxicity only (degeneration and regeneration of skeletal muscle, myocardial degeneration and regeneration (diagnosed as cardiomyopathy) and neuropathy) and six further birds showed changes of lasalocid toxicity plus evidence of bacterial septicaemia. The report did not allow an assignment of these 22 birds to the experimental groups.

In the overall study period (Table 1), all treated birds performed significantly worse than the control birds. The differences in average daily gain between the lasalocid use level and the control group were small (−4%) however significant. For the groups with the two intermediate (1.5x and 2x) and the high lasalocid (2.5x) levels, a considerable reduction in average daily gain (−28%, −49% and −65%, respectively) and feed intake (average reduction for females and males: −19%, −38% and −54%, respectively) was seen. This resulted in a significant impairment of feed to gain ratio (by

[23] Technical dossier/Supplementary information April 2015/Annexes_1_Safety_for_the_target_species.

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

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

[26] The following samples were collected, weighed, and placed into 10% buffered neutral formalin: liver, heart, spleen, kidneys, and bursa of Fabricius. The following samples were collected and placed into formalin without being weighed: crop, gizzard, small intestine caecum and skeletal muscle.

+12%, +24% and +44%, respectively) for the three groups compared to the control. No significant differences between the use level group and the control group were seen for feed intake of the females and feed to gain ratio for both genders. Feed intake of male birds decreased significantly in the use level group.

Table 1: Least square means of the most relevant parameters from the tolerance study in chickens for fattening with lasalocid A sodium (35 days duration, six replicates per treatment and gender, one bird per replicates for serum values)

		Control	1x	1.5x	2x	2.5x
Concentration of lasalocid A sodium (mg/kg feed) in the diet						
Intended		0	125	187.5	250	312.5
Analysed, starter		Nd	116	176	248	297
Analysed, grower		Nd	120	186	251	304
Mortality(1)	M+F	3	7	4	6	29
Performance parameters						
Final body weight (g)	M+F	1,831^{a}	1,745^{b}	1,349^{c}	942^{d}	678^{e}
Average daily gain (g/bird per day)	M+F	51^{a}	49^{b}	37^{c}	26^{e}	18^{e}
Average feed intake (g/bird per day)	M	76^{a}	71^{b}	60^{c}	42^{d}	30^{e}
	F	80^{a}	76^{a}	66^{b}	54^{c}	41^{d}
Average feed to gain ratio	M	1.50^{a}	1.53^{a}	1.77^{b}	1.95^{c}	2.34^{d}
	F	1.48^{a}	1.46^{a}	1.58^{b}	1.76^{c}	1.96^{d}
Haematology						
Eosinophils ($\times 10^3/\mu L$)	M	0.40	0.39	0.38	0.35	0.71
	F	0.52^{b}	0.98^{a}	1.14^{a}	0.35^{b}	0.18^{b}
Mean corpuscular haemoglobin (pg)	M	42.73^{a}	40.73^{ab}	38.13^{b}	38.35^{b}	38.15^{b}
	F	40.32	41.12	39.92	39.72	38.22
Serum chemistry						
AST (U/L serum)	M+F	184^{a}	243^{b}	250^{b}	245^{b}	235^{b}
Cholesterol (mg/dL serum)	M+F	129^{a}	137^{ab}	147^{bc}	154^{bc}	175^{c}
Calcium (mg/dL serum)	M+F	11.2^{a}	10.7^{ab}	10.2^{b}	10.4^{b}	10.2^{b}

Nd: not detected; AST: aspartate aminotransferase.
Means in the same raw with different superscript are significantly different ($p \leq 0.05$).
(1): n out of 120 per treatment group including culled birds.

No statistically significant differences were observed in the haematological parameters between the different groups with the exception of increase absolute counts of eosinophils in females of the 1.5x, and a reduced mean corpuscular haemoglobin (MCH) in males in all lasalocid overdose groups. The changes, however, were considered not biologically relevant.

Similarly, no statistically significant differences between treatment groups were observed for most clinical chemistry parameters.[27] An increase of aspartate aminotransferase (AST) was observed in all four lasalocid groups (see Table 1) compared to the control group. Significantly lower calcium levels were measured in the 1.5, 2 and 2.5x groups compared to the control group. The differences seen in these two parameters were not dose related. In contrast, total cholesterol increased significantly in a dose-dependent manner in the 1.5, 2 and 2.5x groups compared to the control. Differences seen between the three overdose groups and the control in other parameters (total protein, creatine kinase (CK), gamma-glutamyl transpetidase (GGT) and serum potassium) were not considered biologically relevant due to small magnitude of the effects or effects only in one gender or a lack of a dose-dependent change.

Small differences were observed in the absolute organ weights of bursa of Fabricius, heart, kidneys, liver and spleen of the overdose groups and in bursa of Fabricius and kidneys (reduced weight) of the use level group compared to control birds. However, no differences in the relative weight of these organs were seen with the exception of bursa of Fabricius in which it was significantly reduced in the

[27] AP, ALT, blood urea nitrogen, chloride, glucose, lactate dehydrogenase, magnesium, sodium, phosphorus and uric acid.

use level group compared to the control and the twofold overdose group. In summary, there were no relevant effects of the treatment on necropsy findings.

The literature review made by the applicant for the time period 2004–2015 did not identify new studies which should be considered when assessing the safety for the target species of lasalocid.[28]

3.2.1.2. Interactions

In previous opinions (EFSA, 2004a,b,c; EFSA FEEDAP Panel, 2010a, 2011a), the FEEDAP Panel concluded that:

> 'The data submitted regarding the possible interactions between lasalocid sodium and tiamulin used in chicken husbandry were insufficient. Consequently, the FEEDAP Panel considers that it would be safer to avoid concurrent administration of lasalocid with tiamulin and certain other medicinal substances, and that the current advice should be retained'.

The literature review made by the applicant for the time period 2004–2015 did not identify new studies which should be considered when assessing the interaction of lasalocid with other additives or veterinary drugs.[29] The review also refers to five EFSA FEEDAP opinions on the compatibility of different microbial additives with lasalocid.[30] It should be noted that a review paper (Islam et al., 2009) concluded that tiamulin is compatible with lasalocid, in contrast to most other coccidiostats, but the review is based on publications between 1984 and 1992.

The FEEDAP Panel therefore reiterates its former conclusions.

3.2.1.3. Microbial studies

Lasalocid A sodium and the other homologues, as other polyether ionophores, show antimicrobial activity against Gram-positive bacteria in a range between 0.06 and 4 mg/L.

This was recognised in the FEEDAP Panel's conclusions from 2004 and reiterated in 2010 and 2011 (EFSA, 2004a,b,c; EFSA FEEDAP Panel, 2010a, 2011a):

> 'Lasalocid sodium has a selective antimicrobial activity in a concentration range between 0.06 and 4 mg/L against Gram-positive bacterial species such as *Enterococcus faecium* and *E. faecalis* and *Staphylococcus* spp., while many Enterobacteriaceae are naturally resistant. Inhibitory concentrations for susceptible strains are lower than the dose incorporated in feed and antibacterial concentrations are likely to be attained *in vivo*. Induction of resistance and/or cross-resistance was not observed in experimental conditions. Increased shedding of *Salmonella Typhimurium* in chickens for fattening was shown in two studies.'

A literature review considering papers published in the years 2000–2015 and focusing on the emergence of resistance to lasalocid A sodium, on the cross-resistance to antimicrobial and to the shedding of enteropathogens was made by the applicant.[31] Fifteen papers dealing with lasalocid A sodium were identified and in none of them evidence of insurgence of resistance to this ionophore or cross resistance to antimicrobial was reported. Moreover, the use of lasalocid A sodium in farmed animals does not affect the shedding of *Salmonella* (Edrington et al., 2006), *Campylobacter* (Anderson et al., 2014) and *Escherichia coli* (Edrington et al., 2006).

In conclusion, lasalocid sodium has a selective antimicrobial activity against Gram-positive bacterial species while many Enterobacteriaceae are naturally resistant. Induction of resistance and/or cross-resistance was not observed in experimental conditions. On the basis of recent literature, no shedding of *Salmonella* is expected.

[28] Technical dossier/Supplementary information April 2015/Annex 4_I. Databases: Medline, Embase, Biosis, CAB Abstracts, Derwent Veterinary Drug File, Agricola, Pascal, Toxcenter, SciSearch, Chemical Abstracts, Dissertation Abstracts, ProQuest SciTech Collection; time-span 2000–2015.

[29] Technical dossier/Supplementary information April 2015/Annex 4_III. Databases: Medline, Embase, Biosis, CAB Abstracts, Derwent Veterinary Drug File, Agricola, Pascal, Toxcenter, SciSearch, Chemical Abstracts, Dissertation Abstracts, ProQuest SciTech Collection; time-span 2000–2015.

[30] *Bacillus subtilis* (EFSA, 2009a), *Enterococcus faecium* (EFSA, 2009b), *Clostridium butyricum* (EFSA FEEDAP Panel, 2009), *Bacillus subtilis* (EFSA FEEDAP Panel, 2010b), *Enterococcus faecium* (EFSA FEEDAP Panel, 2011d).

[31] Technical dossier/Supplementary information April 2015/Annex 4_II. Databases: Medline, Embase, Biosis, CAB Abstracts, Derwent Veterinary Drug File, Agricola, Pascal, Toxcenter, SciSearch, Chemical Abstracts, Dissertation Abstracts, ProQuest SciTech Collection; time-span 2000–2015.

3.2.1.4. Conclusions on the safety for the target species

The results of the tolerance study submitted indicate that oral administration of lasalocid A sodium in feed at all the doses tested (the highest recommended dose proposed by the applicant and multiples of that) are not tolerated in chickens for fattening. Consequently, no safe dose of lasalocid A sodium could be established.

Concurrent administration of lasalocid with tiamulin and certain other medicinal substances should be avoided.

Lasalocid sodium has a selective antimicrobial activity against Gram-positive bacterial species while many Enterobacteriaceae are naturally resistant. Induction of resistance and/or cross-resistance was not observed in experimental conditions.

3.2.2. Safety for the consumer

3.2.2.1. Metabolism and residue studies

Metabolism studies

The metabolism studies in chickens and turkeys submitted by the applicant were already assessed by the FEEDAP Panel in its previous opinions (EFSA, 2004a,b,c, EFSA FEEDAP Panel 2010a, 2011a), and are summarised below:

> Lasalocid is extensively absorbed and metabolised mainly by oxidation to mono- and multi-hydroxy metabolites. Excretion occurs essentially through the bile and faeces as unchanged lasalocid (about 10%) and un-resolved peaks (HPLC) corresponding to a great number of metabolites of increasing polarity and likely representing each less than 5% of the whole radioactivity excreted. Lasalocid is the major component (26% for both sexes) in the liver, whereas a major metabolite (15%) has been identified as a monohydroxy-lasalocid (hydroxylation position not established); other metabolites of increasing polarity, representing each less than 10%, have been separated but not identified. Complementary data established the similarity of the metabolic fate in the chicken and turkey, but also the rat where monohydroxy- and dihydroxy-lasalocid were identified in the urine. Unchanged lasalocid A appeared to be the marker residue and the liver the target tissue.

Residue studies

The applicant submitted total and marker residue studies in chickens for fattening, chickens reared for laying, turkeys, pheasants and quails previously assessed by the FEEDAP Panel (EFSA, 2004a,b,c; EFSA FEEDAP Panel, 2010a, 2011a). For the current assessment, the applicant submitted two new marker residue studies in chickens for fattening.

An overview of the total and marker residue studies, relevant for the assessment of the safety for the consumer (see Section 3.2.2.3), is given below.

Total residue studies

When residues in chickens are concerned, the applicant refers to the study of total residues (TR) of lasalocid sodium in chicken tissues already assessed (EFSA, 2004a,b,c). In this study,[32] four groups of six males and six females per time point were administered 125 mg ^{14}C-lasalocid sodium/kg feed for seven consecutive days then slaughtered after 0, 1, 3 and 5 days withdrawal of the supplemented feed. Tissues were sampled and analysed for total residues. The results are presented in Table 2.

Table 2: Kinetics of lasalocid total residues in tissues of chickens (six male and six female birds per time point) administered 125 mg ^{14}C-lasalocid sodium/kg feed for seven consecutive days then slaughtered at different withdrawal periods (mg equivalent lasalocid/kg wet tissue)[(a)]

Withdrawal time (day)	Liver	Kidney	Muscle	Skin/fat
0	1.223 ± 0.469 (2.161)	0.395 ± 0.190 (0.775)	0.080 ± 0.024 (0.128)	0.432 ± 0.363 (1.158)
1	0.838 ± 0.182 (1.202)	0.172 ± 0.051 (0.274)	0.020 ± 0.005 (0.030)	0.112 ± 0.032 (0.176)

[32] Technical dossier/Section III/Annex III 2.1.1.1.

Withdrawal time (day)	Liver	Kidney	Muscle	Skin/fat
3	0.558 ± 0.160 (0.878)	0.097 ± 0.020 (0.137)	0.015 ± 0.005 (0.025)	0.070 ± 0.023 (0.116)
5	0.325 ± 0.123 (0.571)	0.068 ± 0.020 (0.072)	0.012 ± 0.005 (0.022)	0.055 ± 0.018 (0.091)

(a): Mean ± standard deviation (mean + 2 SD).

The data on total residues in turkey tissues following the administration of 125 mg ^{14}C-lasalocid sodium/kg feed for 14 days were already assessed by the FEEDAP Panel in 2010 (EFSA FEEDAP Panel, 2010a). Tissue radioactivity was determined at zero-day withdrawal time in three animals of both sexes. Although the values for kidney, muscle and skin/fat were similar or below those derived in the residue study described below, the liver [^{14}C]-lasalocid derived total residues amounted to 3.38 ± 0.57 mg/kg wet tissue. For withdrawal periods of 1, 2, 3, 4 and 5 days, only data of one male and one female were available. The residues declined in the liver, kidney and skin/fat over 5-day withdrawal times, based on the highest of two values per time points. The highest values at 1-day withdrawal time were for liver 1.73, for kidney 0.21, for muscle < 0.02 and for skin/fat 0.22 mg/kg tissue.

Marker residue studies

A good laboratory practice (GLP) residue study[33] was carried out in 1-day-old chickens (six males and six females per time point) administered 130 mg lasalocid sodium/kg complete feed from Avatec® 150G for 42 days. The animals were slaughtered after 0, 1, 2 and 3 days withdrawal and tissues sampled. Lasalocid sodium concentration in feed was analytically confirmed. Lasalocid analysis in tissues was performed using a validated LC–MS/MS method with a limit of quantification (LOQ) of 0.005 mg/kg (Table 3).

Table 3: Kinetics of lasalocid residues in tissues of chickens (six male and six female birds per time point) administered 130 mg lasalocid/kg feed from Avatec® 150G for 42 days then slaughtered at different withdrawal periods (mg lasalocid/kg wet tissue)[(a)]

Withdrawal time (day)	Liver	Kidney	Muscle	Skin/fat
0	1.840 ± 0.385 (2610)	1.050 ± 0.339 (1.728)	0.447 ± 0.144 (0.735)	1.040 ± 0.282 (1.604)
1	0.244 ± 0.329 (0.902)	0.128 ± 0.194 (0.516)	0.065 ± 0.103 (0.271)	0.106 ± 0.165 (0.436)
2	0.138 ± 0.131 (0.400)	0.061 ± 0.070 (0.201)	0.023 ± 0.023 (0.069)	0.037 ± 0.054 (0.145)
3	0.072 ± 0.056 (0.184)	0.026 ± 0.011 (0.048)	0.008 ± 0.003 (0.014)	0.010 ± 0.004 (0.018)

(a): Mean ± standard deviation (mean + 2 SD).

The applicant submitted another new marker residue study[34] where lasalocid sodium was administered to chickens at a dose of 125 mg/kg feed for 42 days. Lasalocid residues were measured only in two tissues (muscle and skin/fat) and therefore these data were not used for the assessment.

A marker residue study in turkeys was already assessed by the FEEDAP Panel in 2010 (EFSA FEEDAP Panel, 2010a). The study has been conducted to investigate the depletion of residues of lasalocid A in edible tissues (liver, kidney, muscle and skin/fat) following oral administration of 130 mg lasalocid sodium/kg complete feed from Avatec® 150G for 112 consecutive days (16 weeks). Five groups of six turkeys (three males and three females) were slaughtered after withdrawal periods of 0, 3, 5, 7 and 10 days. A validated LC–MS/MS analysis of lasalocid sodium was applied with LOQs of 0.050, 0.025, 0.010 and 0.050 mg/kg for liver, kidney, muscle and skin/fat, respectively, with corresponding limit of detections (LODs) of 0.005, < 0.001, 0.001 and 0.005 mg/kg. Lasalocid residues in liver, kidney, muscle and skin/fat were below the LOQs of the analytical method after a 3-day withdrawal period.

The marker residue studies in pheasants and quails, already assessed by the FEEDAP Panel in 2011 (EFSA FEEDAP Panel, 2011a), have been resubmitted for the current evaluation. In the present

[33] Technical dossier/Section III/Annex III 2.1.2.1
[34] Technical dossier/Section III/Annex III 2.1.2.2

assessment, the FEEDAP Panel reiterates its previous conclusion that these additional studies in pheasants and quails confirm that the conclusions on turkeys can be extrapolated to the minor species pheasants, partridges, quails and guinea fowl.

Residue in eggs

In its former opinion (EFSA, 2004b), a study was assessed in which the influence of lasalocid sodium supplementation (125 mg/kg) to diets of chickens reared for laying during the first 16-weeks of age on the potential presence of residues in the first eggs (after onset of laying) was examined. Lasalocid concentrations in the first eggs (20th week) were below 0.01 mg/kg (LOD of the analytical method) with the exception of two eggs out of 30. These two eggs contained 0.01 and 0.02 mg lasalocid/kg whole egg.

3.2.2.2. Toxicological studies

In previous FEEDAP opinions (EFSA, 2004a,b,c; EFSA FEEDAP Panel, 2010a), it was concluded that:

'Absence of mutagenic effects at the gene level both in bacteria and in mammalian cells was demonstrated. There was no evidence for *in vitro* genotoxicity of lasalocid sodium. The results of chronic oral toxicity/carcinogenicity studies carried out in rats and dogs suggest no evidence of carcinogenicity. Foetotoxicity in rabbits was observed but was related to developmental and maternal toxicity. No specific teratogenicity study was available in rodents. However, relevant data from the rat two-generation study is considered sufficient supporting evidence for the lack of teratogenicity of lasalocid'.

'A lowest NOAEL of 0.5 mg/kg bw per day was established from the 2-year chronic oral toxicity study in rats and maternal toxicity study in rabbits. A toxicological ADI of 0.005 mg/kg bw (or 0.3 mg/60 kg person per day) has been derived applying a safety factor of 100'.

Lasalocid sodium caused positive inotropic effects after single intravenous dosing to dogs (EFSA, 2004b). Since these effects have been previously accepted as being relevant to consumer risk assessment, particularly when occurring at doses lower than identified as a toxicological no-observed-adverse-effect level (NOAEL) (EFSA, 2005b), the applicant was requested to perform an acute study in dogs, by the oral route, investigating these effects to identify whether they would be critical to the risk assessment for this product.

Following the above requests, the applicant submitted an acute study of cardiovascular effects in dogs (inotropic effect).[35] The applicant also performed, upon request, a literature review covering the period 2004–2015.[36] The literature review identified no new data requiring consideration in the latest opinion. Details and results of the requested study is summarised below:

Five male pure-bred beagles were used in the study. Doses of lasalocid sodium were administered orally by capsule, as indicated in Table 4, so that each animal received each dose. The control dose (0 mg/kg) was the carrier gelatine capsule alone.

Table 4: Dosing schedule for cardiovascular study in dogs with lasalocid sodium[(a)]

Animals	Dose (mg/kg bw)				
	Day 1	Day 8	Day 15	Day 22	Day 29
H12899	1.25	0	1	3	0
H12900	4	1	6	NA	NA
H12901	12.5	NA	NA	NA	NA
H12902	0	3	0	1	3
H12903	NA	6	3	0	1

NA: not applicable (no dose was administered to the relevant animal on that day); bw: body weight.
(a): Telemetry data not collected on day 1 and from animal H12903 on day 8; animals H12900 and H12901 were euthanised on days 15 and 2, respectively; data from these animals was not included in the analysis.

[35] Technical dossier/Supplementary information July 2016/Annex 3_1.
[36] Technical dossier/Supplementary information April 2015/Annex 4_IV. Databases: Medline, Embase, Biosis, CAB Abstracts, Derwent Veterinary Drug File, Agricola, Pascal, Toxcenter, SciSearch, Chemical Abstracts, Dissertation Abstracts, ProQuest SciTech Collection; time-span 2000–2015.

Assessment of cardiovascular function was based on qualitative electrocardiogram (ECG) evaluation and quantitative analysis of ECG (PR interval, QRS duration, QT interval and corrected QT (QTc) interval) and haemodynamic parameters (heart rate; systolic, diastolic, mean arterial pressures and arterial pulse pressure). Telemetry ECG, arterial blood pressure and body temperature data were recorded for at least 90 min prior to dosing and continuously through at least 19 h after the dosing. Assessment of general health was based on clinical observations and body weight.

One animal administered 12.5 mg/kg body weight (bw) on day 1 and one animal administered 6 mg/kg bw on day 15 were euthanised on days 2 and 15, respectively, due to test article-related paralysis of hind limbs. The three remaining animals survived until their scheduled transfer back to the stock colony on day 30. Due to the deaths, the original 4 × 4 Latin square dosing design was revised to a modified 4 × 3 crossover design (three animals on days 8, 15, 22 and 29) for telemetry data analysis and reporting.

Administration of ≥ 3 mg/kg lasalocid sodium was associated with vomitus but no abnormal clinical observations were attributed to lasalocid sodium administration at 1 mg/kg bw.

No body weight changes were attributed to administration of lasalocid sodium at any dose used in the study. No qualitative ECG effects or changes in PR interval, QRS duration, QT interval, QTc interval, or body temperature were attributed to lasalocid sodium administration at 1 or 3 mg/kg.

At a dose of 3 mg/kg bw, lasalocid sodium mildly increased heart rate, diastolic pressure and mean arterial pressure and mildly decreased arterial pulse pressure. Heart rate was increased by up to 15 bpm (+18%), while diastolic and mean arterial pressures were increased by up to 11 mm Hg (+15%) and 12 mmHg (+12%), respectively. Arterial pulse pressure was decreased by up to 11 mmHg (−13%). Lasalocid sodium administered at a dose of 1 mg/kg had no effect on any haemodynamic parameter measured in this study.

Although the higher doses used in this study caused effects which led to the loss of two animals from the design, the remaining three dogs showed sufficiently consistent results to conclude that there was evidence of adverse cardiovascular effects at a dose of 3 or 6 mg/kg bw lasalocid sodium but no such effects at the lowest dose tested of 1 mg/kg bw.

Conclusions on toxicology

The newly conducted cardiovascular study in dogs indicates an acute NOAEL for lasalocid A sodium of 1 mg/kg bw per day, with only limited effects seen at 3 mg/kg. Since this NOAEL is above the lowest NOAEL previously identified of 0.5 mg/kg bw per day, observed in a 2-year toxicity study in rats[37] and a developmental study in rabbits,[38] there is no reason to consider cardiovascular effects in the risk assessment. The previously identified lowest NOAEL of 0.5 mg/kg bw per day is concluded to be an appropriate base for establishing an Acceptable Daily Intake (ADI).

3.2.2.3. Assessment of consumer safety

An ADI of 0.005 mg lasalocid sodium/kg bw (or 0.3 mg/60-kg person/day) has been derived applying a safety factor of 100, based on a NOAEL (0.5 mg/kg bw per day) derived from the results of a 2-year oral toxicity study in rats and maternal toxicity in a reproduction study in rabbits.

Consumer exposure to total residues from tissues of chickens (Table 2) and turkeys (EFSA FEEDAP Panel, 2010a) calculated applying the food basket of Regulation 429/2008 indicates compliance with the ADI after 1-day withdrawal. Considering the similarity of dose regimen and physiology with major poultry, this conclusion was extrapolated to pheasants, partridges, quails and guinea fowl for fattening (EFSA FEEDAP Panel, 2011a).

New MRLs for poultry have been recently established following an assessment of EMA CVMP (2013) by Commission Implementing Regulation (EC) No 1277/2014)[39] as follow: 300 μg/kg liver, 150 μg/kg kidney, 60 μg/kg muscle, 300 μg/kg skin/fat and 150 μg/kg eggs.

The results of the marker residue study in chickens for fattening (Table 3) indicate that a 3-day withdrawal period is necessary for the 95th percentile residues (mean plus 2 standard deviations) to decline below the MRLs in all tissues. The residues in liver, kidney and muscle after a 2-day withdrawal period are above the MRLs.

[37] Based on effects on effects (minor changes in haematological profiles, increased liver weight in both sexes, increased adrenal weight in females) seen at dietary concentrations of 1.8 mg/kg or more.
[38] Based on effects seen on pregnancy performance and fetal body weight seen at dietary concentrations of 1 and 2 mg/kg.
[39] OJ L 346, 1.12.2014, p. 23.

Marker residues measured in tissues of turkeys (three males and three females), after administration of lasalocid sodium at a concentration of 130 mg/kg feed for 112 days and following a withdrawal period of three days, were all below the respective LOQs (EFSA FEEDAP Panel, 2010a). These values comply with the current MRLs after 1 and 3-day withdrawal.

Marker residue studies in pheasants and quails allow the conclusion that the marker residues in tissues of minor poultry (pheasants, partridges, quails and guinea fowl for fattening) are below the MRLs after a 3-day withdrawal.

The lasalocid level in the first eggs laid after a 16 week administration of 125 mg lasalocid/kg feed of chickens reared for laying was considerably lower than the MRL.

Conclusions on safety for the consumer

The use of lasalocid sodium from Avatec® 150G at the highest proposed level of 125 mg/kg complete feed in chickens and turkeys for fattening, in chicken reared for laying up to the 16th week of life and growing pheasants, partridges, quails and guinea fowl is safe for the consumer provided that a withdrawal period of three days is respected.

3.2.3. Safety for the user

No new data have been submitted by the applicant.

On the basis of studies with the active substance lasalocid sodium, the FEEDAP Panel concluded in its former opinions (EFSA, 2004a, 2005a) that:

> 'Lasalocid sodium dust has the potential to cause local toxicity to the respiratory tract and lungs and also systemic toxicity to other organs. Acute dermal toxicity was found to be of a low order indicating poor skin absorption and lasalocid sodium did not cause skin irritation or skin sensitisation, but did cause eye irritation'.

In 2005a, the Panel mentioned that the additive:

> 'Avatec® 150G is granulated, which limits exposure via a respiratory or ocular route. The analysis of several batches showed that the mean particle size was around 500 μm and that only 0.1% by weight passes a 106 μm mesh sieve (the lowest tested). The low dusting potential of Avatec® 150G was confirmed by a Stauber-Heubach test (EFSA, 2005a). Based on those data, inhalation studies would be not necessary for Avatec® 150G'.

In 2010, the FEEDAP Panel concluded on the above basis that there is no likely risk to the user/worker handling Avatec® 150G.

The applicant also performed a literature review covering the period 2004–2015.[36] The literature review identified no new data requiring consideration in the latest opinion.

3.2.3.1. Conclusions on safety for the user

No risk for the user is expected from the use of the additive under application.

3.2.4. Safety for the environment

The applicant makes reference to the same studies assessed in EFSA FEEDAP opinions (EFSA FEEDAP Panel, 2010a) with the addition of one new study to evaluate the toxicity of lasalocid to sediment dwelling organisms. The FEEDAP Panel re-assessed the previous studies, the new study and the outcome of a literature search performed by the applicant covering the period 2004–2015.[40]

The active ingredient is not a physiological/natural substance of established safety for the environment. The additive is also not intended for companion animals. Consequently, according to Regulation (EC) No 429/2008[41] the Phase I assessment has to be performed to determine the predicted environmental concentrations (PECs).

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

[40] Technical dossier/Supplementary information April 2015/Annex 4_V. Databases: Medline, Embase, Biosis, CAB Abstracts, Derwent Veterinary Drug File, Agricola, Pascal, Toxcenter, SciSearch, Chemical Abstracts, Dissertation Abstracts, ProQuest SciTech Collection; time-span 2000–2015.

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

3.2.4.1. Phase I

Physicochemical properties

The physicochemical properties of lasalocid sodium are summarised in Table 5.[42] The vapour pressure of lasalocid sodium was not provided.

Table 5: Physicochemical properties of lasalocid A sodium

Property	Value	Unit
Octanol/water partition coefficient (log K_{ow} 25°C)	2.3	–
Water solubility (20°C)	1,060	mg/L
Dissociation constant pKa	5.66	–

Fate and behaviour

Fate in manure

No new data have been provided. The fate in manure was already assessed by the FEEDAP Panel in its opinion published in 2004 (EFSA, 2004b) using a study with ^{14}C lasalocid sodium from 1975 with thin layer chromatography autography. The degradation was studied in freshly spiked faeces at concentrations of 5 and 10 mg/kg. The faeces were stored at 32°C and 85% RH. The degradation showed a rapid initial decrease before the first hour of incubation followed by a slower degradation up to 75% of the original lasalocid after 30 days. Samples that were dried out accidentally did not show lasalocid degradation. According to the technical guidance on the environmental risk assessment (EFSA, 2008a), degradation in chicken manure should be studied under dry aerobic conditions. Therefore, the finding that lasalocid is not degraded in dry chicken manure is used for further calculations.

Fate in soil

Adsorption

The adsorption of [^{14}C]-lasalocid sodium was determined in silty clay loam (pH 6.1, 2.5% organic carbon, 20% clay), sandy loam (pH 7.5, 1.3% organic carbon, 13% clay) and clay loam (pH 5.3, 4.3% organic carbon, 34% clay) according to OECD 106 (EFSA, 2004b and EFSA FEEDAP Panel 2010a). The soil sorption coefficient (K_{oc}) value for silty clay loam, sandy loam and clay loam was 1400, 846 and 1,024, respectively. In all three soil types, the Freundlich constant were similar and in the range of 0.86–0.94.

Sassman and Lee (2007) investigated the adsorption behaviour of lasalocid in eight different soil types, with pH ranging from 4.2 to 7.5, clay percentage from 5 to 41, and organic carbon content from 0.52% to 2.91%. The K_{oc} values were ranging from 732 to 15,700 and were inversely correlated with soil pH.

The mean K_d and K_{oc} values of lasalocid determined in the OECD study described above are 31 and 1,140, respectively and these values are comparable with the lowest K_{oc} values found by Sassman and Lee in soil with neutral pH. The sorption of ionophoric anions like lasalocid is influenced by the pH and probably also by the presence of cations. Therefore, the lowest K_{oc} of 732 was used for the calculation of the PEC.

Degradation

The study to investigate the biodegradation in soil was conducted in 2003 using radiolabelled [^{14}C]-lasalocid (purity 97.5%). The fate and behaviour in soil, was measured using high-performance liquid chromatography and thin-layer chromatography for the identification of the potential metabolites and a CO_2 trap for quantifying mineralisation (EFSA, 2004b; EFSA FEEDAP Panel 2010a). The soils used were sandy loam, clay loam and silty clay loam incubated at 20°C under aerobic conditions. Several metabolites were found, but not identified. Only one metabolite, described as unknown C, was observed at concentrations higher than 10% of the applied radioactivity, with a maximum of 20% in silty clay loam at 32 days after application decreasing to 17% at 120 days. Mineralisation to CO_2 accounted for 23% in sandy loam, 15% in clay loam and 11% in silty loam. Non-extractable residues

[42] Technical dossier/Section II.

accounted for 57% in sandy loam. The reported DT_{50} values (at 20°C) for sandy loam, clay loam and silty clay loam were estimated as 1.8 days, 0.6 days and 14.2 days, respectively.

Sassman and Lee (2007) investigated the degradation of lasalocid in two different soil types: clay loam (pH 7.3, 2.2% organic carbon, 33% clay – Oakville-31) and sandy soil (pH 7.0, 0.8% organic carbon, 11% clay – Drummer-30). In the first soil, the degradation was also investigated in the presence of manure at a rate of 20 mg/kg. The soils were pre-incubated for 72 h, and then lasalocid was added at an application rate of 2.1 mg/kg soil. The soils were incubated at 23°C under aerobic conditions. Sterilised soils were spiked and incubated under the same conditions. Samples were taken and analysed at regular intervals. Lasalocid degraded rapidly in both soils, with half-lives of 3.6, 4.3 and 1.5 days in the Drummer-30, manure-amended Drummer-30 and the Oakville-31 soils, respectively. In contrast, virtually no degradation was observed in the sterile soils, indicating that the mechanism of removal of lasalocid is a biotic process. The DT_{50} values observed are similar to those found in the previous study.

Fate in water

The hydrolysis of lasalocid sodium was determined at different pH values according to OECD 111 (EFSA, 2004b; EFSA FEEDAP Panel 2010a). At 50°C and pH 4 and 7, less than 10% was degraded after five days; at pH 9, about 32% was degraded.

Conclusion on fate and behaviour

The sorption behaviour of an ionophoric anion like lasalocid can be complicated therefore the lowest measured K_{oc} value of 732 will be used the risk assessment.

No sufficiently reliable data were provided to determine conclusions on the biodegradation rate of lasalocid in manure. The DT_{50} of lasalocid in soil is between 0.6 and 14.2 days, with a mean value of 2.4 days. One major unidentified metabolite is formed with an unknown biological activity. The hydrolysis of lasalocid is slow.

Predicted environmental concentrations (PECs)

The methodology for the calculation of the maximum PECs in soil, groundwater, surface water and sediment are described in the technical guidance for assessing the safety of feed additives for the environment (EFSA, 2008a). The input values used were: 125 mg/kg broiler feed, molecular weight 612.77, vapour pressure 1.59E-26 Pa,[43] solubility 1,060 mg/L, DT_{50} 2.4 days (corresponding to 5 days at 12°C)[44] and K_{oc} 732 L/kg. The calculated values are given in Table 6.

Table 6: Initial predicted environmental concentration of lasalocid in soil (μg/kg), groundwater, surface water and sediment (μg/L)

Compartment	PEC
Soil	649
Groundwater	51
Surface water	17
Sediment	634

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

3.2.4.2. Phase II

Exposure assessment

Characterisation of residue in manure

A metabolism study in the rat showed that mono- and dihydroxy-lasalocid were excreted as main metabolites. Metabolism data in chicken showed that parent lasalocid is excreted at about 10% the administered dose with a great number of minor metabolites (each less than 5% the whole [^{14}C]

[43] EPI Suite estimation using the Modified Grain method.

[44] EMA-CVMP recommends in its guideline on the assessment of persistent, bioaccumulative and toxic compounds (EMA CVMP, 2015) to calculate the DT_{50} at a temperature of 12°C, typical for most European countries. If data at 12°C are not available, data obtained at 20°C could be extrapolated using the Arrhenius equation (activation energy: 65.4 kJ/mol according to the EFSA guidance for use in FOCUS (EFSA, 2008)). Consequently, a factor of 2.12 is used to calculate the DT_{50} at 12°C.

activity) of greater polarity that would likely correspond to mono- and multihydroxylated lasalocid (see Section 3.2.2.1). Hydroxylated metabolites of the ionophore antibiotics monensin sodium, salinomycin and narasin have been shown to exert reduced ionophoric activity and therefore much lesser toxicity than the parent compound (Donoho, 1984; Dimenna et al., 1988; Rocha et al., 2014; EFSA FEEDAP Panel, 2017). It can be reasonably assumed that the same would occur with lasalocid hydroxy-metabolites. The ionophoric activity of the metabolites of monensin sodium (EFSA, 2004c) and salinomycin (EFSA FEEDAP Panel, 2017) was considered to represent 20% of the parent compound. The same assumption is made for lasalocid sodium metabolites. Consequently, following a conservative approach, the ionophoric activity of lasalocid-related excreted products (parent compound plus hydroxylated metabolites) would amount to about 30% (unchanged parent compound (10%) + hydroxylated metabolites (90% × 20% = 18%)). In the view of the FEEDAP Panel, the toxicological relevance of lasalocid residues is given by its ionophoric activity.

PEC refinement

Assuming that the ionophoric activity of lasalocid and its metabolites in chicken excreta will not exceed in total 30% of the orally administered dose, the refined dose used for PEC calculations was 37.5 mg/kg feed. The refined PEC_{soil}, $PEC_{groundwater}$, $PEC_{surfacewater}$ and $PEC_{sediment}$ are reported in Table 7.

When the $PEC_{groundwater}$ is set equal to the concentration in pore water based on a worst

Table 7: Refined predicted environmental concentrations of lasalocid in soil (μg/kg), groundwater, surface water and sediment (μg/L)

Compartment	Refined PEC
Soil	195
Groundwater	15
Surface water	5
Sediment	190

calculation (the total residue approach), the concentration exceeds the trigger value of 0.1 μg/L. A simple first step refined exposure assessment is performed using Table 2 from the EFSA guidance on environmental risk assessment (EFSA, 2008a). The refined application rate was calculated to be 0.6 kg/ha. Based on the Okehampton scenario using FOCUS_PEARL (version 3.0), the leaching concentration will be below 0.01 μg/L when the organic matter/water distribution coefficient (K_{om}) is larger than $-5.9 + 9.1\ DT_{50}$ (at 20°C). With a DT_{50} of 2.4 days, the K_{om} should be larger than 16. This corresponds to a K_{oc} of 27 L/kg carbon. The lowest reported K_{oc} of lasalocid is 732, which is much higher than 27 indicating that there is no risk for leaching of lasalocid to groundwater.

Ecotoxicity studies

Toxicity to soil organisms

Effects on plants

The effect of lasalocid sodium on the emergence and growth of seedlings of perennial ryegrass (*Lolium perenne*), radish (*Raphanus sativus*) and mung bean (*Phaseolus aureus*) was studied in sandy loam soil according to OECD guideline 208 (GLP study) (EFSA, 2004b; EFSA FEEDAP Panel, 2010a). The test period was 18 days, representing 14 days after at least 50% emergence. For plant species, the LC_{50} for emergence was greater than the highest concentration tested (100 mg/kg soil dry weight (dw)). The lowest EC_{50} for growth was estimated as 87.8 mg lasalocid/kg for ryegrass. The no observed effect concentration (NOEC) is established at 10 mg lasalocid/kg soil.

Effect on earthworms

The acute toxicity of lasalocid sodium to *Eisenia foetida andrei* was determined in a limit test at a nominal rate of 600 mg/kg dry soil for 14 days at 20°C according to OECD 207 (GLP study) (EFSA, 2004b; EFSA FEEDAP Panel, 2010a). An artificial soil composed of 70% sand, 20% kaolinite clay and 10% sphagnum moss peat was used. All worms were dead after 14 days at the two highest concentrations (300 and 600 mg/kg). The LC_{50} normalised to 5% organic matter is 71.8 mg lasalocid/kg.

In addition, the study on effects of lasalocid sodium on earthworm reproduction was investigated in a GLP study in accordance with OECD guideline 222 (EFSA FEEDAP Panel, 2010a). According to this guideline, the substance is mixed into the soil and the earthworms are fed with clean manure without any toxicants. This is different from the situation in the field where the substance is present in the manure at much higher concentrations than the final concentration in soil. Lasalocid was added to the artificial soil (identical to OECD 207) at concentrations of 0, 5.15, 10.30, 20.60, 41.20 and 82.40 mg/kg. At day 56 of reproduction test, the NOEC was determined to be 41.20 mg/kg.

Avoidance test

An avoidance test on earthworms (*Eisenia andrei*) was published by Žižek and Zidar (2013).[45] The authors compared results of a reproduction test (OECD 222) with the avoidance behaviour as an endpoint.

In comparison to the OECD reproduction test, the avoidance behaviour of earthworm proved to be a more sensitive endpoint. The calculated EC_{50} of 12.3 mg/kg dry soil for avoidance behaviour was five times lower in comparison to earthworm reproduction (EC_{50} of 69.6 mg lasalocid/kg dry soil).

The endpoint avoidance behaviour is not foreseen in the Technical Guidance for assessing the safety of feed additives for the environment (EFSA, 2008a); therefore, it cannot be considered in the assessment of environmental risk of lasalocid. However, it provides useful additional information on the potential impact of lasalocid on terrestrial oligochaetae.

Effects on soil microorganisms

The inhibitory effect of lasalocid sodium on the respiration rate of aerobic wastewater microorganisms of activated sludge was investigated in a 3-h respiration inhibition test (GLP) at a concentration range of 10–1,000 mg/L (OECD 209) (EFSA, 2004b and EFSA FEEDAP Panel 2010a). Only at the highest concentration tested an inhibitory effect found of 27%. Consequently, the EC_{50} was > 1,000 mg/L. Test conditions and validity criteria were within the acceptable limits as set in OECD guidelines.

In a GLP study, the effect of lasalocid sodium on nitrification and respiration was studied in sandy loam at 40% of the maximum water holding capacity according OECD 216 and 217, respectively (EFSA, 2004b and EFSA FEEDAP Panel 2010a). The experiments showed that the prolonged exposure to incorporated lasalocid sodium in soil at 1x PEC (1.039 mg/kg) and 5x PEC (5.195 mg/kg) had no effect on short-term microbial respiration. At 28 days after treatment, concentrations of nitrate in treated soil, at both treatment rates, deviated from the controls by less than 25%. Using this tolerance level, the experiments showed that incorporation of lasalocid sodium in soil at PEC 1x (1.039 mg/kg) and PEC 5x (5.195 mg/kg) had no prolonged effect on soil nitrogen transformations (nitrification and mineralisation).

Toxicity to aquatic organisms

Effect on algae

The acute toxicity of lasalocid sodium to *Scenedesmus subspicatus* was determined at a nominal concentration range of 0.22–10 mg lasalocid/L according to OECD 201 (GLP study) (EFSA, 2004b and EFSA FEEDAP Panel 2010a). The actual concentration range was 86–99% of the nominal values and remained stable until the end of the test. All the other test conditions were within the acceptable limits. The 72-h-ErC_{50} based on growth rate was 3.1 mg lasalocid/L and the ErC_{10} based on growth rate was 1.4 mg lasalocid/L.[46]

Effect on crustaceans

The acute toxicity of lasalocid sodium to the crustacean *Daphnia magna* was determined under static conditions at a nominal concentration range of 1.5–32 mg lasalocid/L according to OECD 202 and in compliance with GLP (EFSA, 2004b and EFSA FEEDAP Panel 2010a). The actual concentration range was 86–89% of the nominal values and remained stable until the end of the test. The 48-h-EC_{50} was 5.4 mg lasalocid/L (95% confidence limit: 4.1–7.1) based on the actual concentration.

[45] Performed according to the method ISO 17512-1:2008(E).
[46] EC_{50} in terms of reduction of growth after exposure for 72 h.

Effect on fish

The GLP acute toxicity of lasalocid sodium to zebra fish *Brachydanio rerio* was determined under static conditions at a nominal concentration range of 0.32–32 mg lasalocid/L according to OECD 203 (EFSA, 2004b and EFSA FEEDAP Panel 2010a). The actual concentration range was 75–87% of the nominal values and remained stable until the end of the test. The 96-h-LC_{50} was 2.5 mg lasalocid/L (95% confidence limit: 1.1–5.5) based on the actual concentration.

Effect on sediment dwelling organisms

The GLP study was performed in accordance with OECD 218 to assess the effects of lasalocid sodium on the sediment-dwelling larvae of the freshwater dipteran *Chironomus riparius*.[47] The test was conducted with nominal dry weight based sediment concentrations containing 3.125, 6.25, 12.5, 25 and 50 mg/kg radiolabelled [^{3}H]-lasalocid sodium. Total radioactivity was monitored and reported as equivalents of [^{3}H]-lasalocid sodium/kg of dry sediment.

The mean sediment concentrations at day 0 were 2.15, 4.07, 9.31, 18.06 and 36.31 mg equivalents/kg while the mean sediment concentrations on day 28 were 1.87, 3.97, 8.19, 16.44 and 28.79 mg lasalocid sodium equivalents/kg. The recovery of radioactivity on day 28 was between 79% and 97%. The measured total radioactivity measured on days 0 and 28 of [^{3}H]-lasalocid sodium equivalents in overlying water and in interstitial water accounted for 2.6–7.8% and 0.2–0.8% of the recovered dose, respectively.

Measured concentrations at day 0 in the sediment were about 65.1–74.5% of the nominal, indicating some losses during sediment preparation. However, given that concentrations were maintained over the exposure period and that a dose–response curve was obtained, it is considered that the initial losses did not impact on the biological effects assessments.

The EC_{10} value for mortality/emergence ratio at 28 days was 8.90 mg lasalocid sodium equivalents/kg sediment (equivalent to 5.34 mg lasalocid sodium equivalents/kg wet weight sediment) and the corresponding EC_{50} value was estimated to be 33.97 mg lasalocid sodium equivalents/kg (equivalent to 20.38 mg lasalocid sodium equivalents/kg wet weight sediment). The EC_{50} value for development rate at 28 days was estimated to be > 36.31 mg lasalocid sodium equivalents/kg, the highest concentration used in this study (equivalent to 21.79 mg lasalocid sodium equivalents/kg wet weight sediment).

Conclusions on the ecotoxic effect on soil, water and sediment

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

For the terrestrial compartment, data are available for microorganisms, earthworms and plants. The lowest L(E)C_{50} is 87.8 mg lasalocid/kg found for plants. This results in a PNEC for the terrestrial environment of 0.88 mg lasalocid/kg, applying a safety factor of 100 (Table 8).

For the aquatic compartment, data are available for algae, aquatic invertebrates and fish. The lowest toxicity value for the aquatic compartment was found for fish: 96-h-LC_{50} was 2.5 mg lasalocid/L. By applying a safety factor of 100, the PNEC for aquatic organism is 25 μg lasalocid/L (Table 9).

Ecotoxicological data for sediment-dwelling invertebrates are provided for the sediment compartment. The calculated PNEC for the risk assessment is 890 μg lasalocid/kg, applying an assessment factor (AF) of 10 to the NOEC of 8.9 mg/kg (Table 10).

Risk characterisation (PEC/PNEC ratio)

The risk characterisation ratios for terrestrial, freshwater and sediment compartments are reported in Tables 8, 9 and 10, respectively.

Table 8: Risk characterisation (PEC/PNEC ratio) for terrestrial compartment

Taxa	PEC_{soil} (μg/kg)	E(L)C_{50}/NOEC (mg/kg)	AF	PNEC (μg/kg)	PEC/PNEC
Earthworm	195	41.2	10	4,120	0.05
Plants		87.8	100	878	0.22

PEC_{soil}: predicted environmental concentrations in soil; E(L)C_{50}: lowest effective concentration; NOEC: no observed effect concentration; AF: assessment factor; PNEC: predicted no effect concentrations.

[47] Technical dossier/Section III/Annex III.4_2_1_1.

Table 9: Risk characterisation (PEC/PNEC ratio) for freshwater compartment

Taxa	$PEC_{surfacewater}$ (µg/L)	$E(L,r)C_{50}$ (mg/L)	AF*	PNEC (µg/kg)	PEC/PNEC
Algae *Selenastrum subspicatus*	5	3.1	100	31	0.16
Aquatic invertebrates *Daphnia magna*		5.4	100	54	0.09
Fish *Brachydanio rerio*		2.5	100	25	0.20

$PEC_{surfacewater}$: predicted environmental concentrations in surface water; $E(L,r)C_{50}$: lowest effective concentration (growth rate); AF: assessment factor; PNEC: predicted no effect concentrations.
*: Based on the amount of ecotoxicity data on aquatic species and sediment, an AF of 100 is selected.

Table 10: Risk characterisation (PEC/PNEC ratio) for sediment

Taxa	$PEC_{sediment}$ (µg/kg)	EC_{10}(mg/kg)	AF	PNEC* (µg/kg)	PEC/PNEC
Sediment-dwelling invertebrates *Chironomus riparius*	190	8.9	10	890	0.21

$PEC_{sediment}$: predicted environmental concentrations in surface water; EC_{10}: effective concentration; AF: assessment factor; PNEC: predicted no effect concentrations.
*: PNEC based on the acute endpoint E(r)C is lower than long term NOEC.

Bioaccumulation

No data on bioaccumulation have been submitted. Since the log K_{ow} < 3, the risk for bioaccumulation is considered to be low.

3.2.4.3. Conclusions on safety for the environment

The use of lasalocid sodium from Avatec® 150G in feed for chickens for fattening and chickens reared for laying up to the highest proposed dose does not pose a risk 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 European Union (EU), (b) actual use conditions in field trials, all should be done in the EU within the last 5 years. Anticoccidial sensitivity tests (AST) could replace field trials provided they follow the criteria mentioned in the relevant guidance document on coccidiostats and histomonostats (EFSA FEEDAP Panel, 2011b).[48]

3.3.1. Floor pen studies

Four floor pen studies were submitted.[49] Trial 1, 2 and 3 were performed with chickens for fattening and trial 4 with replacement pullets. Birds were penned and distributed into treatment groups as indicated in Table 11. Treated groups received feed containing 75 mg lasalocid sodium/kg feed, dosage was analytically confirmed. Infected groups were inoculated with recent field isolates of pathogenic *Eimeria* species (see Table 12 for details). Animal health and mortality were monitored daily. Feed intake and body weight of the animals were measured throughout the study, feed to gain ratio was calculated. Samples of excreta were analysed for oocyst excretion and intestinal lesions were scored on five birds per pen following the method of Johnson and Reid (1970) (0 = no lesion, 1 = very mild, 2 = mild, 3 = moderate and 4 = severe).

[48] The FEEDAP Panel stated in its guidance for the preparation of dossiers for coccidiostats and histomonostats (EFSA FEEDAP Panel, 2011b) that studies with artificial infection would be preferred over field trials due to their inherent weaknesses. These short-term studies should use field strains of *Eimeria*, recently confirmed as pathogenic/resistant by a sensitivity test or recognised problems in the poultry operation (confirmed by veterinary certificate). The *Eimeria* field strains should ideally undergo one, but in any case not more than two passage(s) before use in such trials.
[49] Trial 1: Technical dossier/Section IV/Annex IV.3.1. Trial 2: Technical dossier/Section IV/Annex IV.3.2. Trial 3: Technical dossier/Section IV/Annex IV.3.3. Trial 4: Technical dossier/Section IV/Annex IV.3.4.

The pen was the experimental unit for statistical purposes. In trial 1 and 4, all endpoints were analysed with the general linear mixed model at the 0.05 level of significance using two-sided tests. For oocyst excretion, oocysts per gram of excreta (OPG) values were logarithmically transformed to approach a normal distribution. In trial 2, data were statistically analysed by one-way analysis of variance (ANOVA); group differences for the zootechnical endpoints were examined by Tukey test and for lesion scores by Newman–Keuls test. For oocyst excretion, OPG values were logarithmically transformed to approach a normal distribution. Statistical analysis in trial 3 was done by general linear mixed model. Mortality, OPG and lesion scores were analysed for normal distribution by the Kolmogorov–Smirnov test and as data showed to be not normally distributed, the parameters were subjected to the Kruskal–Wallis test and the Mann–Whitney U-test.

Table 11: Experimental design of floor pen studies performed with Avatec® 150G[(a)]

Trial	Study duration (days)	Birds per pen (replicates per treatment)	Test animal	Treatment groups	Analysed lasalocid Na (mg/kg feed)
1	35	30 (12)	1-day-old Ross 308 male chickens	Uninfected untreated control group (UUC)	–
				Infected untreated control group (IUC)	–
				Uninfected treated control group (UTC)	74–76
				Infected treated group (IT)	74–76
2	36[(b)]	90 (8)	1-day-old Ross PM3 male chickens	Infected untreated control group (IUC)	–
				Infected treated group (IT)	80–82
3	35	30 (8)	1-day-old ISA JA 757 male/female chickens	Infected untreated control group (IUC)	–
				Infected treated group (IT)	72
4	112	30 (8)	1-day-old Hy-line Brown female replacement pullet	Uninfected untreated control group (UUC)	–
				Infected untreated control group (IUC)	–
				Uninfected treated control group (UTC)	70–92
				Infected treated group (IT)	70–92

(a): In Trial 1, 2 and 3, Avatec® 150G and a second coccidiostat were tested.
(b): Supplemented feed was administered until day 31.

Table 12: Summary of inoculation in floor pen studies performed with Avatec® 150G

Trial	Start date	Inoculum characteristics			
		Month/year and country of isolation	Intended dose per bird		Day and mode of inoculation
1	6/2012	09/2010 The Netherlands	5.26×10^4	*Eimeria acervulina*	Day 14 via gavage
			1.12×10^4	*Eimeria tenella*	
			1.14×10^4	*Eimeria maxima*	
			2.60×10^3	*Eimeria mitis*	
			4.00×10^2	*Eimeria necatrix/Eimeria praecox*	
2	05/2011	6/09 Ireland	2.500×10^5	*E. acervulina*	Day 17 via feed
			2.845×10^4	*E. maxima*	
3	05/2011	2/2010 The Netherlands	2.2×10^5	*E. acervulina*	Day 13 via feed
			1.5×10^4	*E. maxima*	
			2.0×10^4	*E. tenella*	
4	08/2012	3/2012 Belgium	1.548×10^5	*E. acervulina*	Day 14 via gavage
			1.920×10^4	*E. maxima*	
			4.520×10^4	*E. tenella*	
			4.000×10^3	*Eimeria brunetti*	
			1.360×10^4	*E. mitis*	
			9.360×10^3	*E. necatrix/E. praecox*	

Performance data and mortality are summarised in Table 13.

Table 13: Performance data and mortality of chickens in floor pen trials with Avatec® 150G

	Feed intake (g/day)	Body weight (g)	Weight Gain(1) (g)	Feed to gain ratio	Mortality n (%)
Trial 1					
UUC	92.5	2,267	64	1.44	14 (3.9)
IUC	91.6	2,228	63	1.47	17 (4.7)
UTC	92.1	2,258	63	1.44	11 (3.1)
IT	91.2	2,232	63	1.47	24 (6.7)
Trial 2					
IUC	84.2[b]	1,983[b]	–	1.55[a]	26 (4.1)
IT	90.1[a]	2,153[a]	–	1.53[b]	26 (4.1)
Trial 3					
IUC	–	–	987[b]	3.83	5 (2.1)
IT	–	–	1,078[a]	3.85	11 (4.6)
Trial 4					
UUC	43.8	1,363	12	3.71	1 (0.4)
IUC	43.5	1,381	12	3.63	1 (0.4)
UTC	48.5	1,371	12	4.08	6 (2.5)
IT	44.5	1,389	12	3.70	2 (0.8)

–: not reported; UUC: uninfected treated control group; IUC: infected untreated control group; UTC: uninfected treated control group; IT: infected treated group.
Means in columns with different superscript are significantly different ($p \leq 0.05$).
(1): Results of trial 1 and 4 refer to daily gain; results of trial 3 refer to total body weight gain.

The only statistically different zootechnical endpoints were seen in trial 2 for feed intake, body weight and feed to gain ratio, and in trial 3 for total weight gain (body weight not given). Lasalocid improved these parameters. In trial 4, with a total duration of 112 days, there was only a transient improvement of body weight by the lasalocid treatment in the first two weeks after inoculation.

Results of oocysts excretion are presented in Table 14.

Table 14: Total number of *Eimeria* oocysts per gram of excreta (OPG) in floor pen studies

Trial 1	Day 17	Day 21	Day 23	Day 28	Day 30	Day 32	Day 35		
UUC	0	6	1	261	130	114	688		
IUC	2	115,410	17,113	9,791	27,734	11,851	10,492		
UTC	0	3	4	64	422	450	49		
IT	0	53,672	10,669	8,064	674*	344*	341*		
Trial 2	Day 17	Day 24	Day 24 *E. maxima*	Day 28	Day 28 *E. maxima*	Day 31	Day 31 *E. maxima*	Day 36	Day 36 *E. maxima*
IUC	0	904,350	10,350	136,650	2,000	3,825	25	8,750	138
IT	0	765,800	800	142,250	650*	3,750	13	5,225	1,075
Trial 3	Day 18	Day 21	Day 24	Day 27	Day 30	Day 33			
IUC	23,250	76,025	70,700	125,250	4,200	1,800			
IT	1,850	55,450	133,750	31,625*	16,550	11,225			
Trial 4	Day 17	Day 21	Day 23	Day 28	Day 30	Day 32	Day 35	Day 42	Day 49
UUC	0	0	3	0	1	0	0	8	0
IUC	17	132,321	60,819	95,825	31,837	15,751	8,713	1,534	1,984
UTC	0	0	5	0	0	0	0	3	1
IT	2	100,524	46,528	172,761	47,279	9,382	8,005	4,922	2,431

UUC: uninfected treated control group; IUC: infected untreated control group; UTC: uninfected treated control group; IT: infected treated group.
Mean values for IT with * are significantly different from IUC ($p \leq 0.05$).

The measurement of *Eimeria* oocyst excretion showed signs of infection in all inoculated groups. Differences between the infected treated and untreated groups were rare and not consistent. The only statistical differences were seen on day 30 in Trial 1, on day 28 in trial 3 for total oocyst excretion and on day 28 in trial 2 for *E. maxima* oocyst excretion; the OPGs were lower in the lasalocid treated groups compared to the infected untreated groups. In trial 4 with chickens reared for laying, there was a tendency for lower OPG in the lasalocid treated group, however, significance was only reached on days 70 and 84.

In trial 1 (Table 15), mean lesion scores were numerically lower in the treated group with respect to the infected untreated control. There were no significant differences between groups when statistics was performed on the percentage of animals presenting normal lesion scores.

Table 15: Mean lesion scores in trial 1

	Duodenum and upper small intestine		Lower small intestine		Caecum	
	21	**28**	**21**	**28**	**21**	**28**
UUC	2.4	2.9	1.7	1.9	1.6	1.8
IUC	2.6	3.1	1.8	1.9	1.9	2.2
UTC	2.4	2.9	1.7	1.8	1.5	1.9
IT	2.6	3.0	1.8	1.7	1.7	1.9

UUC: uninfected treated control group; IUC: infected untreated control group; UTC: uninfected treated control group; IT: infected treated group.
No statistical analysis was performed on the mean values.

In trial 2 (Table 16), on day 24 and 31, infected treated (IT) groups had significantly lower mean values for the lesion scores in the duodenum (*E. acervulina*) and in the jejunum-ileum (*E. maxima*) and on day 24 in the caecum (*E. tenella*).

Table 16: Mean lesion scores in trial 2

	Duodenum (*E. acervulina*)		Jejunum-ileum (*E. maxima*)		Caecum (*E. tenella*)	
	24	**31**	**24**	**31**	**24**	**31**
IUC	2.6	0.9	1.6	1.5	0	0.5
IT	2.1*	0.9	0.4*	1.0*	0	0.1*

IUC: infected untreated control group; IT: infected treated group.
Mean values with * are significantly different from IUC ($p \leq 0.05$).

In trial 3 (Table 17), on day 19, the IT group in caeca (*E. tenella*) showed significantly less lesions compared to the control group.

Table 17: Median in lesion scores in trial 3

	Upper part of the duodenum (*E. acervulina*)			Small intestine about the yolk sac diverticle (*E. maxima*)			Caecum (*E. tenella*)		
	19	**21**	**All**	**19**	**21**	**All**	**19**	**21**	**All**
IUC	1.0	1.0	1.0	0.5	1.0	0	2.0	1.0	1.0
IT	2.0	1.0	1.0	0.0	0	0	1.0*	1.0	0

IUC: infected untreated control group; IT: infected treated group.
Mean values with * are significantly different from IUC ($P \leq 0.001$).

In general, in trial 4 (Table 18), mean lesion scores were numerically lower in the treated groups with respect to the infected untreated control. Statistics was performed on the percentage of animals presenting normal lesion scores. Significant differences were found between the IT group and the infected treated control (IUC) group on day 28 and 35 in the lower small intestine and caecum (*E. brunetti* 8.0% vs 0.3% and 22.0% vs. 6.6%) and on day 28 in caecum (*E. tenella* 25.4% vs 7.7%).

Table 18: Mean lesion scores in trial 4

	Duodenum/upper small intestine (*E. acervulina*)			Duodenum/upper small intestine (*E. necatrix*)			Lower small intestine (*E. maxima*)			Lower small intestine and caecum (*E. brunetti*)			Caecum (*E. tenella*)		
	21	28	35	21	28	35	21	28	35	21	28	35	21	28	35
UUC	2.1	1.5	2.5	1.9	1.5	1.9	1.3	1.1	1.3	1.0	1.0	1.3	0.9	0.9	1.4
IUC	2.5	2.4	2.7	2.1	1.9	1.9	1.5	1.3	1.4	1.3	1.3	1.1	0.9	1.3	1.3
UTC	1.2	2.2	2.6	1.1	2.0	2.1	0.7	1.4	1.3	0.5	1.3	1.1	0.5	1.3	1.2
IT	2.2	2.1	2.5	1.8	1.7	2.1	1.1	1.1	1.3	1.1	1.0	1.9	1.0	1.0	0.9

UUC: uninfected treated control group; IUC: infected untreated control group; UTC: uninfected treated control group; IT: infected treated group.
No statistical analysis was performed on the mean values.

3.3.2. Field trials

The applicant provided two trials carried out under controlled field conditions.[50] Both trials were conducted in 2008 at the same location.

In both trials, two treatments were considered, complete feed either supplemented with 90 mg lasalocid sodium from Avatec® 150G or with another chemically synthesised coccidiostat. The trials were carried out in two different poultry houses, each containing one of the two experimental treatments. In trial 1, the Avatec® 150G group was treated on days 30–32 with antibiotics. In both trials, the other group was treated with two antibiotics in different periods because of intestinal problems indicative for clostridium infection. For these reasons, both trials could not be considered further.

3.3.3. Anticoccidial sensitivity tests (AST)

Three ASTs were submitted.[51] Each test was made with the groups uninfected untreated control (UUC), IUC and IT, the latter receiving feed supplemented with 100 mg lasalocid from Avatec® 150G/kg feed (analysed values 100, 101 and 96 mg lasalocid/kg feed in AST 1, 2 and 3, respectively). Different other anticoccidial additives were also tested (six in AST 1, five in AST 2 and 3). The day-old birds (Ross PM3) were randomly allocated to the groups; group size was 18 chickens (3 replicates with 6 birds). Birds were artificially infected on day 15 with sporulated oocysts from field isolates.[52] ASTs lasted 22 days on total.

Statistical analysis was done by one-way ANOVA, group differences were tested by Newman–Keuls procedure. To approach normal distribution, mortality data were transformed by square root and OPG by natural logarithm.

The results are summarised in Table 19.

Table 19: Summary of anticoccidial sensitivity tests performed with Avatec® 150G

AST	Tr. group	Feed intake (g/day)	Body weight (g)	Weight gain (g)	Feed to gain ratio	Mortality (n)	Mean lesion scores(1)			OPG × 10^6	
							E. acervulina	*E. maxima*	*E. tenella*	Total	*E. maxima*
		D13–22	D22	D13–22	D13–22	Total	D21			D20–22	
1	UUC	99.6	1,051.4[a]	600.4[a]	1.49[a]	0	0.0[b]	0.0[c]	na	0[c]	0[c]
	IUC	89.9	849.9[b]	397.7[b]	1.99[b]	0	2.2[a]	2.9[a]	na	53[b]	18[a]
	IT	99.1	1,013.1[a]	560.9[a]	1.59[a]	0	0.7[b]	1.1[b]	na	147[a]	7[b]

[50] Technical dossier/Section IV/Annex IV.3.5. and IV.3.6.
[51] AST 1: Technical dossier/Section IV/Annex IV.2.1. and IV.2.2. AST 2: Technical dossier/Section IV/Annex IV.3.5. AST 3: Cross-reference letter from Zoetis Belgium SA. 6 March 2017 to FAD-2013-0034 Deccox Technical dossier/Section IV/Annex IV.2.1.
[52] AST 1: Irish field isolate (2009); estimated dosage per bird: 308,750 sporulated oocysts (248,750 *E. acervulina*, 10,000 *E. praecox* and 50,000 *E. maxima*); AST 2: Belgian field isolate (2010); estimated dose per bird: 273,100 sporulated oocysts (245,000 *E. acervulina* + 2,500 *E. maxima* + *25,600 E. tenella);* and AST 3: German field isolate (2008); estimated dose per bird was 251,105 sporulated oocysts (225,550 *E. acervulina* + 25,555 *E. tenella*).

AST	Tr. group	Feed intake (g/day)	Body weight (g)	Weight gain (g)	Feed to gain ratio	Mortality (n)	Mean lesion scores(1)			OPG × 10^6	
							E. acervulina	*E. maxima*	*E. tenella*	Total	*E. maxima*
		D13–22	D22	D13–22	D13–22	Total	D21			D20–22	
2	UUC	90.3	1,035.6^a	610.4^a	1.37^a	0	0.0^b	0.0^b	0.0^b	0^b	0^c
	IUC	76.8	780.7^c	353.8^c	2.07^c	0	3.2^a	2.1^a	3.3^a	646^a	6.2^a
	IT	78.6	860.7^b	435.2^b	1.65^b	0	2.3^a	1.3^a	2.0^a	602^a	0.3^b
3	UUC	100.4	951.0^a	559.3^a	1.62^b	0	0.0^b	na	0.0^c	0^b	na
	IUC	83.9	735.4^b	343.4^b	2.23^a	1	3.0^a	na	3.3^a	902^a	na
	IT	86.2	791.2^b	400.2^b	2.15^a	2	2.3^a	na	1.5^b	410^a	na

UUC: uninfected treated control group; IUC: infected untreated control group; IT: infected treated group.
Means in columns within a study with different superscript are significantly different ($p \leq 0.05$).
(1): Intestinal lesions were scored on three birds per cage following the method of Johnson and Reid (1970) (0 = no lesion, 1 = very mild, 2 = mild, 3 = moderate and 4 = severe).

Although there was no mortality in AST 1 and 2, the zootechnical parameters as well as the mean lesion scores clearly indicate the *Eimeria* controlling effect of 100 mg lasalocid/kg feed. In AST 3, there was no significant difference between the IUC and the IT groups concerning zootechnical parameters. Only the mean lesion score for *E. tenella* was significantly lower in the lasalocid treated group compared to the infected untreated group.

3.3.4. Conclusions

Among the floor pen studies with 75 mg lasalocid/kg complete feed for chickens for fattening, the anticoccidial efficacy of lasalocid was clearly demonstrated on different parameters in one study. Two other studies did not show a modification of endpoints which would allow the Panel to conclude on the protective effect of the additive to coccidiosis. The floor pen study with chickens reared for laying showed a transient protection of birds within the first two weeks after infection, measured by lower lesion scores.

Considering the results of three ASTs, 100 mg lasalocid sodium/kg complete feed for chickens for fattening have the potential to control *Eimeria* infection in chickens for fattening. This conclusion is extended to chickens reared for laying.

Overall, insufficient evidence of the anticoccidial efficacy of lasalocid A sodium was provided in chickens for fattening/reared for laying. Regulation (EC) No 429/2008 requires three floor pen studies and three field studies supporting the control of coccidiosis by the additive. Such an effect could only be shown in two floor pen studies with 75 mg lasalocid A sodium/kg complete feed and three ASTs with 100 mg lasalocid A sodium/kg feed.

3.4. Post-market monitoring

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

4. Conclusions

The tolerance study indicates that oral administration of lasalocid A sodium via feed at doses at and above the targeted lasalocid dose of 125 mg/kg is not tolerated in chickens for fattening. Consequently, no safe dose can be established by the tolerance study submitted. Concurrent administration of lasalocid with tiamulin and certain other medicinal substances should be avoided.

Lasalocid sodium has a selective antimicrobial activity against Gram-positive bacterial species while many Enterobacteriaceae are naturally resistant. Induction of resistance and/or cross-resistance was not observed in experimental conditions.

Lasalocid A sodium is not genotoxic and is not carcinogenic. The newly conducted cardiovascular study in dogs indicates an acute NOAEL of 1 mg/kg bw per day. Since this NOAEL is above the lowest NOAEL of 0.5 mg/kg bw per day previously identified in a 2-year toxicity study in rats and a developmental study in rabbits, it is concluded that this NOAEL (0.5 mg/kg bw per day) is an appropriate base for establishing an ADI of 0.005 mg lasalocid sodium/kg bw.

The use of lasalocid sodium from Avatec® 150G at the highest proposed level of 125 mg/kg complete feed in chickens and turkeys for fattening, in chicken reared for laying up to the 16th week of life and growing pheasants, partridges, quails and guinea fowl is safe for the consumer. For compliance with the MRLs, a withdrawal period of 3 days is necessary.

No risk for the user is expected from the use of Avatec® 150G in poultry nutrition.

The use of lasalocid sodium from Avatec® 150G in feed for chickens for fattening and chickens reared for laying up to the highest proposed dose does not pose a risk for the environment.

Insufficient evidence of the anticoccidial efficacy of lasalocid A sodium was provided in chickens for fattening/reared for laying. Regulation (EC) No 429/2008 requires three floor pen studies and three field studies supporting the control of coccidiosis by the additive. Such an effect could only be shown in two floor pen studies with 75 mg lasalocid A sodium/kg complete feed and three ASTs with 100 mg lasalocid A sodium/kg complete feed.

Documentation provided to EFSA

1) Avatec® 150G (lasalocid A sodium) for chickens for fattening, chickens reared for laying, turkeys for fattening, minor avian species (pheasants, guinea fowl, quails and partridges) except laying birds. August 2013. Submitted by Zoetis Belgium SA.
2) Avatec® 150G (lasalocid A sodium) for chickens for fattening, chickens reared for laying, turkeys for fattening, minor avian species (pheasants, guinea fowl, quails and partridges) except laying birds. Supplementary information. January 2014. Submitted by Zoetis Belgium SA.
3) Avatec® 150G (lasalocid A sodium) for chickens for fattening, chickens reared for laying, turkeys for fattening, minor avian species (pheasants, guinea fowl, quails and partridges) except laying birds. Supplementary information. June 2014. Submitted by Zoetis Belgium SA.
4) Avatec® 150G (lasalocid A sodium) for chickens for fattening, chickens reared for laying, turkeys for fattening, minor avian species (pheasants, guinea fowl, quails and partridges) except laying birds. Supplementary information. April 2015. Submitted by Zoetis Belgium SA.
5) Avatec® 150G (lasalocid A sodium) for chickens for fattening, chickens reared for laying, turkeys for fattening, minor avian species (pheasants, guinea fowl, quails and partridges) except laying birds. Supplementary information. July 2016. Submitted by Zoetis Belgium SA.
6) Avatec® 150G (lasalocid A sodium) for chickens for fattening, chickens reared for laying, turkeys for fattening, minor avian species (pheasants, guinea fowl, quails and partridges) except laying birds. Spontaneous submission. March 2017. Submitted by Zoetis Belgium SA.
7) Evaluation report of the European Union Reference Laboratory for Feed Additives on the Methods(s) of Analysis for lasalocid sodium.
8) Comments from Member States.

References

Anderson RC, Harvey RB, Wickersham TA, MacDonald JC and Ponce CH, 2014. Effect of distillers feedstuffs and lasalocid on Campylobacter carriage in feedlot cattle. Journal of Food Protection, 77, 1968–1975.

Dimenna GP, Lyon FS, Thompson FM, Creegan JA and Wrigt GJ, 1988. Effect of antibiotic combination, dosing period, dose vehicle and assay method on salinomycin residue levels and their ionophoricity in chicken tissues. Journal of Agriculture and Food Chemistry, 37, 668–676.

Donoho AL, 1984. Presented at a symposium on "Monensin in Cattle" held August 10, 1982 at the Joint Annu. Meet. Can-Amer. Soc. Of Anima. Sci. Univ. of Guelph, Guelph, Canada.

Edrington TS, Looper ML, Duke SE, Callaway TR and Genovese KJ, 2006. Effect of ionophore supplementation on the incidence of Escherichia coli O157:H7 and Salmonella and antimicrobial susceptibility of fecal coliforms stocker cattle. Foodborne Pathog Diseases, 3, 284–291.

EFSA (European Food Safety Authority), 2004a. Opinion of the Scientific Panel on additives and products or substances used in animal feed on the re-evaluation of coccidiostat Avatec in accordance with article 9G of Council Directive 70/524/EEC. EFSA Journal 2004;2(5):53, 44 pp. https://doi.org/10.2903/j.efsa.2004.53

EFSA (European Food Safety Authority), 2004b. Update of an opinion of the Scientific Panel on additives and products or substances used in animal feed on the re-evaluation of coccidiostat Avatec in accordance with article 9G of Council Directive 70/524/EEC. EFSA Journal 2004;2(7):77, 45 pp. https://doi.org/10.2903/j.efsa.2004.77

EFSA (European Food Safety Authority), 2004c. Opinion of the Scientific Panel on Additives and Products or Substances used in Animal Feed on the request of the Commission on the re-evaluation 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), 2005a. Opinion of the Scientific Panel on additives and products or substances used in animal feed on the change of terms of the authorisation of "Avatec 15%" as a feed additive, regarding a new formulation (Avatec® 150G) in accordance with Regulation (EC) No 1831/2003. EFSA Journal 2005;3(8):258, 8 pp. https://doi.org/10.2903/j.efsa.2005.258

EFSA (European Food Safety Authority), 2005b. 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), 2008a. 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), 2008b, revised in 2009. 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), 2008c. 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), 2008d. 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 (European Food Safety Authority), 2009a. Safety and efficacy of Miya-Gold®S (Clostridium butyricum) as feed additive for chickens for fattening. EFSA Journal, 2009;7(4):1039, 17 pp. https://doi.org/10.2903/j.efsa.2009.1039

EFSA (European Food Safety Authority), 2009b. Safety and efficacy of Bonvital (Enterococcus faecium) as feed additive for chickens for fattening. EFSA Journal, 2009;7(3):990, 12 pp. https://doi.org/10.2903/j.efsa.2009.990

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2009. Scientific opinion on the safety and efficacy of Bacillus subtilis PB6 (Bacillus subtilis) as a feed additive for chickens for fattening. EFSA Journal 2009;7(9):1314, 14 pp. https://doi.org/10.2903/j.efsa.2009.1314

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2010a. Scientific Opinion on the safety and efficacy of Avatec® 150G (lasalocid A sodium) for turkeys. EFSA Journal 2010; 8(4):1575. 25 pp. https://doi.org/10.2903/j.efsa.2010.1575

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2010b. Scientific Opinion on the compatibility of the microbial product 035 (*Bacillus subtilis*) with lasalocid sodium, maduramycin ammonium, monensin sodium, narasin, salinomycin sodium and semduramycin sodium. EFSA Journal, 2010; 8(3):1552, 7 pp. https://doi.org/10.2903/j.efsa.2010.1552

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2011a. Scientific Opinion on the safety and efficacy of Avatec® 150G (lasalocid A sodium) for pheasants, partridges, quails and guinea-fowl. EFSA Journal 2011;9(4):2116, 18 pp. https://doi.org/10.2903/j.efsa.2010.2116

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2011b. 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), 2011c. 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), 2011d. Scientific opinion on the safety and efficacy of Fecinor and Fecinor plus (Enterococcus faecium) as a feed additive for chickens for fattening. EFSA Journal, 2011; 9(3):2118, 2 pp. https://doi.org/10.2903/j.efsa.2011.2118

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), 2017. Scientific opinion on the safety and efficacy of Sacox® microGranulate (salinomycin sodium) for chickens for fattening and chickens reared for laying. EFSA Journal 2017;15(1): 4670, 40 pp. https://doi.org/10.2903/j.efsa.2017.4670

EMA CVMP (European Medicines Agency Committee for Medicinal Products for Veterinary Use), 2013. Opinion of the Committee for Medicinal Products for Veterinary Use on the establishment of maximum residue limits. Procedure no: EU/12/204/PFZ. Name of the substance: Lasalocid (INN). Adopted on 12 December 2013.

EMA CVMP (European Medicines Agency Committee for Medicinal Products for Veterinary Use) 2015. Guideline on the assessment of persistent, bioaccumulative and toxic (PBT) or very persistent and very bioaccumulative (vPvB) substances in veterinary medicinal products London: European medicines agency, 2015 EMA/CVMP/ERA/52740/2012.

European Commission, 1982, online. Report of the Scientific Committee for animal Nutrition on the use of lasalocid sodium in feedingstuffs for chickens. Available online: https://ec.europa.eu/food/sites/food/files/safety/docs/animal-feed_additives_rules_scan-old_report_antibiotics-17.pdf

European Commission, 1991, online. Report of the Scientific Committee for animal Nutrition on the use of lasalocid sodium in feedingstuffs for turkeys. Available online: https://ec.europa.eu/food/sites/food/files/safety/docs/animal-feed_additives_rules_scan-old_report_antibiotics-19.pdf

Islam KM, Klein U and Burch DG, 2009. The activity and compatibility of the antibiotic tiamulin with other drugs in poultry medicine–a review. Poultry Science, 88, 2353–2359.

Johnson J and Reid WM, 1970. Anticoccidial drugs: lesion scoring techniques in battery and floor pen experimentswith chickens. Experimental Parasitology, 28, 30–36.

Rocha BA, de Oliveira AR, Pazin M, Dorta DJ, Rodrigues AP, Berretta AA, Peti AP, de Moraes LA, Lopes NP, Popisil S, Gates PJ and Assis Md, 2014. Jacobsen catalyst as a cytochrome P450 biomimetic model for the metabolism of monensin A. Biomed Reserach International, 2014. Available online: http://dx.doi.org/10.1155/2014/152102

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.

Žižek S and Zidar P, 2013. Toxicity of the ionophore antibiotic lasalocid to soil-dwelling invertebrates: avoidance tests in comparison to classic sublethal tests. Chemosphere, 92, 570–575.

Abbreviations

ADI	Acceptable Daily Intake
AF	assessment factor
ALT	alanine aminotransferase
ANI	average nucleotide identity
ANOVA	analysis of variance
AP	alkaline phosphatase
AST	anticoccidial sensitivity test/aspartate aminotransferase
ATCC	American Type Culture Collection
BUN	blood urea nitrogen
bw	body weight
CAS	Chemical Abstract Service
CFU	colony forming unit
CK	creatine kinase
CP	crude protein
CVMP	Committee for Medicinal Products for Veterinary Use
DL-PCB	dioxin-like polychlorinated biphenyl
DT50	period required for 50% dissipation (define method of estimation)
Dw	dry weight
EC_{50}	effective concentration
ErC_{50}	effective concentration (growth rate)
ECG	electrocardiogram
EMA	European Medicines Agency
EURL	European Union Reference Laboratory
FEEDAP	EFSA Panel on Additives and Products or Substances used in Animal Feed
FOCUS	Forum for the Co-ordination of Pesticide Fate Models and their Use
GGT	gamma-glutamyl transpetidase
GLP	good laboratory practice
IT	infected treated group
IUC	infected untreated control group
K_{oc}	soil sorption coefficient
K_{om}	organic matter/water distribution coefficient
K_{ow}	octanol/water partition coefficient
LC_{50}	lethal concentration, median
LC–MS/MS	liquid chromatography with tandem mass spectrometry
LDH	lactate dehydrogenase
LOD	limit of detection

LOQ	limit of quantification
MCH	mean corpuscular haemoglobin
MCHC	mean corpuscular haemoglobin concentration
MCV	mean corpuscular volume
ME	metabolisable energy
Met	methionine
MRL	maximum residue limits
NOEC	no observed effect concentration
NOAEL	no-observed-adverse-effect level
OECD	Organisation for Economic Co-operation and Development
OPG	oocysts per gram of excreta
pKa	dissociation constant
PCDD/F	polychlorinated dibenzo-p-dioxins and dibenzofurans
PEC	predicted environmental concentration
$PEC_{groundwater}$	predicted environmental concentrations in groundwater
$PEC_{sediment}$	predicted environmental concentrations in sediment
PEC_{soil}	predicted environmental concentrations in soil
$PEC_{surfacewater}$	predicted environmental concentrations in surface water
PNEC	predicted no effect concentrations
RBC	red blood count
RDW	red cell distribution width
RH	relative humidity
SCAN	Scientific Committee on Animal Nutrition
TR	total residues
UTC	uninfected treated control group
UUC	uninfected untreated control group
WBC	white blood cell count

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

In the current application authorisation is sought for *Avatec® 150G* under articles 10(2) and 13(3) for the category "coccidiostats and histomonostats" of Regulation (EC) No 1831/2003. Authorisation is sought for use for chickens for fattening and reared for laying, turkeys and pheasants, partridges, quails and guinea fowl. This *feed additive* is already authorized in chickens for fattening and reared for laying, in turkeys and in pheasants, partridges, quails and guinea fowl.

Avatec® 150G is a red-brown free flowing granular preparation formulated to contain - 15% *lasalocid A sodium*, 4% calcium lignosulphonate binder, 0.1% ferric oxide colouring agent, and a calcium sulphate dehydrate carrier. *Avatec® 150G* is meant to be incorporated in *feedingstuffs* through *premixtures*. The Applicant suggested a *lasalocid A sodium* content in *feedingstuffs* ranging from 75-125 mg/kg for all the animal species of concern.

MRLs for *lasalocid A* in poultry tissues and eggs are already set by the Commission Regulation (EC) No 37/2010, therefore the corresponding methods of analysis do not need to be evaluated by the EURL.

For the determination of *lasalocid A sodium* in the *feed additive, premixtures* and *feedingstuffs*, the Applicant submitted the European Community method published in Commission Regulation (EC) No 152/2009. In the frame of a previous dossier (cf. FAD-2008-0050) the Applicant demonstrated the applicability of the Community method to the *feed additive*. Furthermore, the Applicant provided additional experimental data obtained analysing the *feed additive*, *premixtures* and *feedingstuffs* containing *Avatec 150G*. Based on the experimental evidence provided, the EURL recommends for official control the European Community method published in Commission Regulation (EC) No 152/2009 based on Reversed Phase High Performance Liquid Chromatography coupled to fluorescence detection (RP-HPLC-FL) for the determination of *lasalocid A sodium* in the *feed additive,* in *premixtures* and *feedingstuffs.*

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