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# Scientific Opinion on the safety and efficacy of Aviax 5% (semduramicin sodium) for chickens for fattening

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## Abstract

The feed additive Aviax 5%, containing the active substance semduramicin sodium, an ionophore anticoccidial, is intended to control coccidiosis in chickens for fattening at a dose of 20–25 mg/kg complete feed. Semduramicin sodium is present in Aviax 5% in its mycelial form and is produced by fermentation of *Actinomadura* spp. (ATCC 53664). Semduramicin sodium is active against certain Gram-positive bacteria, while Gram-negative bacteria are resistant; its use as a feed additive is unlikely to increase shedding of *Salmonella*, *Escherichia coli* and *Campylobacter* and to induce resistance and cross-resistance to antimicrobials used of human and animal relevance. In the absence of a tolerance study in chickens for fattening performed according to the current EU standards, the FEEDAP Panel cannot conclude on the safety of Aviax 5%, containing semduramicin mycelium. Semduramicin sodium in the feed is not compatible with the concurrent use of tiamulin. Mycelial semduramicin sodium is not genotoxic. There is no evidence that the mycelial semduramicin is more toxic than the crystalline by oral administration. The acceptable daily intake (ADI) of 0.00125 mg/kg set for the crystalline semduramicin is applicable to mycelial semduramicin. The use of semduramicin sodium is safe for the consumer provided a withdrawal time of 24 h is respected. No conclusions can be made on the irritancy of Aviax 5% to skin and eye and on the potential for dermal and respiratory sensitisation. Model calculations on inhalation exposure of persons handling the additive indicate a serious risk. Aviax 5% used in feed for chickens for fattening up to 25 mg/kg complete feed does not pose a risk for the terrestrial compartment. A risk for the aquatic compartment and for groundwater pollution cannot be excluded. Aviax 5% at a minimum dose of 20 mg/kg feed has the potential to effectively control coccidiosis in chickens for fattening.



**Keywords:** coccidiostats and histomonostats, semduramicin sodium, chickens for fattening, safety, efficacy

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## Summary

Following a request from the European Commission, the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) was asked to deliver a scientific opinion on the safety and efficacy of Aviax 5% (semduramicin sodium) for chickens for fattening.

The feed additive Aviax 5%, containing the active substance semduramicin sodium, an ionophore anticoccidial, is intended to control coccidiosis in chickens for fattening at a dose of 20–25 mg/kg complete feed. Semduramicin sodium is present in Aviax 5% in its mycelial form and is produced by fermentation of *Actinomadura* spp. (ATCC 53664).

The approaches used for taxonomical identification consistently place the strain ATCC 53664 in the genus *Actinomadura* but cannot unequivocally assign to a valid taxonomic species of this genus, suggesting that the strain belongs to a new taxonomic unit within the genus *Actinomadura*. The FEEDAP Panel cannot conclude on the absence of genetic determinants for antimicrobial resistance in *Actinomadura* spp. ATCC 53664.

In the absence of a tolerance study in chickens for fattening performed according to the current European Union (EU) standards, the FEEDAP Panel cannot conclude on the safety of Aviax 5%, containing semduramicin mycelium, for chickens for fattening.

Semduramicin sodium is active against certain Gram-positive bacteria, while Gram-negative bacteria are resistant. The use of semduramicin sodium as a feed additive is unlikely to increase shedding of *Salmonella*, *Escherichia coli* and *Campylobacter* and to induce resistance and cross-resistance to antimicrobials used of human and animal relevance.

Semduramicin sodium in the feed is not compatible with the concurrent use of tiamulin, although the interaction is less severe than that observed with other polyether coccidiostats affecting only performance parameters.

Semduramicin is significantly absorbed, extensively metabolised and mainly excreted through the bile and faeces. Unchanged semduramicin is by far (45%) the main residue in the liver, each of the other 19 metabolites representing less than 10% of the total radioactivity in this tissue. Unchanged semduramicin represents about 16% of total radioactivity in the excreta, metabolite F 24% and the other polar metabolites less than 10% each. Metabolic pathways are similar in the rat, the dog and chicken. Semduramicin is the marker residue.

Mycelial semduramicin sodium is not genotoxic. There is no evidence that the mycelial semduramicin is more toxic than the crystalline by oral administration. An acceptable daily intake (ADI) of 0.00125 mg/kg is set for the crystalline semduramicin based on a no observed adverse effect level (NOAEL) of 0.125 mg/kg body weight (bw) per day, established from a 2-year toxicity study in rats for decreased serum protein and sodium concentrations and applying an uncertainty factor of 100. The ADI set for the crystalline semduramicin is applicable to mycelial semduramicin.

The use of semduramicin sodium at a dietary concentration of 25 mg/kg complete feed for chickens for fattening is safe for the consumer provided a withdrawal time of 24 h is respected. The maximum residue limits (MRLs) for the different tissues are considered necessary. However, no proposal for MRLs could be made due to lack of data for marker residue concentrations after 24 h withdrawal and limited sensitivity of the analytical method.

The FEEDAP Panel cannot conclude on the irritancy of Aviax 5% to skin and eye and on the potential for dermal and respiratory sensitisation. Model calculations on inhalation exposure of persons handling the additive indicate a serious risk.

The use of semduramicin sodium from Aviax 5% in feed for chickens for fattening up to 25 mg/kg complete feed does not pose a risk for the terrestrial compartment. However, a risk for the aquatic compartment and for groundwater pollution cannot be excluded.

Aviax 5% at a minimum dose of 20 mg/kg feed has the potential to effectively control coccidiosis in chickens for fattening.

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# 1. Introduction

## 1.1. Background and Terms of Reference

Regulation (EC) No 1831/2003[1] establishes the rules governing the Community authorisation of additives for use in animal nutrition. In particular, Article 10(2) of that Regulation also specifies that for existing products within the meaning of Article 10(1), an application shall be submitted in accordance with Article 7, at the latest 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. 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.

The European Commission received two requests from Phibro Animal Health SA[2] for the authorisation of semduramicin sodium, when used as a feed additive for chickens for fattening (category: coccidiostats and histomonostats), the first one for modification of the authorisation followed by a second one for the re-evaluation. Both requests concerned Aviax 5% containing the active substance semduramicin sodium in its mycelial form (i.e. not isolated after the fermentation process).

According to Article 7(1) of Regulation (EC) No 1831/2003, the Commission forwarded the two applications to the European Food Safety Authority (EFSA) as 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 dossiers in support of the applications. According to Article 8 of that Regulation, 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. The particulars and documents in support of the applications were considered valid by EFSA as of 14 August 2014 and 23 June 2016.

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 Aviax 5% (semduramicin sodium), when used under the proposed conditions of use (see Section 3.1.4).

## 1.2. Additional information

Semduramicin sodium is a coccidiostat intended for the control of coccidiosis caused by *Eimeria* spp. in chickens for fattening. The additive Aviax 5% containing the crystalline semduramicin sodium is authorised for use in chickens for fattening.[3] The mycelial form of the active substance is currently not authorised in the European Union (EU).

The Scientific Committee on Animal Nutrition (SCAN) issued an opinion on the use of semduramicin sodium in feedingstuffs for chickens for fattening (European Commission, 2002).

# 2. Data and methodologies

## 2.1. Data

The present assessment is based on data submitted by the applicant in the form of two technical dossiers[4] in support of the authorisation requests for the use of Aviax 5% (semduramicin sodium) as a feed additive. The technical dossiers were prepared following the provisions of Articles 10 and 13 of Regulation (EC) No 1831/2003 and the applicable EFSA guidance documents.

[1] Regulation (EC) No 1831/2003 of the European Parliament and of the Council of 22 September 2003 on additives for use in animal nutrition. OJ L 268, 18.10.2003, p. 29.
[2] Phibro Anianl Health s.a., Chemin du Stocquoy 3, 1300, Wavre, Belgium.
[3] Commission Regulation (EC) No 1443/2006 of 29 September 2006 concerning the permanent authorisations of certain additives in feedingstuffs and an authorisation for 10 years for a coccidiostat. OJ L 271, 30.9.2006, p. 12.
[4] FEED dossier reference: FAD-2014-0009, FAD-2015-0037; References to the annexes of the technical dossier indicate numbering as presented in the dossier of the application FAD-2015-0037.

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 to deliver the present output.

EFSA has verified the EURL reports as they relates to the methods used for the control of the active substance in animal feed. The Executive Summary of the EURL reports can be found in Annex A and Annex B.

## 2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of Aviax 5% is in line with the principles laid down in Regulation (EC) No 429/2008[5] and the relevant guidance documents: Guidance for the preparation of dossiers for coccidiostats and histomonostats (EFSA FEEDAP Panel, 2011a), Technical guidance: Tolerance and efficacy studies in target animals (EFSA FEEDAP Panel, 2011b), Technical Guidance for assessing the safety of feed additives for the environment (EFSA, 2008a), Guidance for establishing the safety of additives for the consumer (EFSA FEEDAP Panel, 2012a) and Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012b), Technical Guidance: Microbial Studies (EFSA, 2008b).

# 3. Assessment

The present opinion assesses the safety and efficacy of the feed additive Aviax 5% containing the active substance semduramicin sodium in its mycelial form.

## 3.1. Characterisation

### 3.1.1. Characterisation of the active substance

Semduramicin sodium is produced by fermentation from a culture of *Actinomadura* spp., ATCC 53664. [redacted]

Semduramicin sodium (CAS number: 119068-77-8, sodium {(2*R*,3*S*,4*S*,5*R*,6*S*)-2,4-dihydroxy-6-[(1*R*)-1-{(2*S*,5*R*,7*S*,8*R*,9*S*)-9-hydroxy-2-[(2*S*,2′*R*,3′*S*,5*R*,5′*R*)-5′-[(2*S*,3*S*,5*R*,6*S*)-6-hydroxy-3,5,6-trimethyloxan-2-yl]-3′-{[(2*S*,5*S*,6*R*)-5-methoxy-6-methyloxan-2-yl]oxy}-2-methyl[2,2′-bioxolan]-5-yl]-2,8-dimethyl-1,6-dioxaspiro[4.5]decan-7-yl}ethyl]-5-methoxy-3-methyloxan-2-yl}acetate) is a monocarboxylic acid polyether ionophore ($C_{45}H_{75}O_{16}Na$; molecular weight 894.5). The structural formula is given in Figure 1.

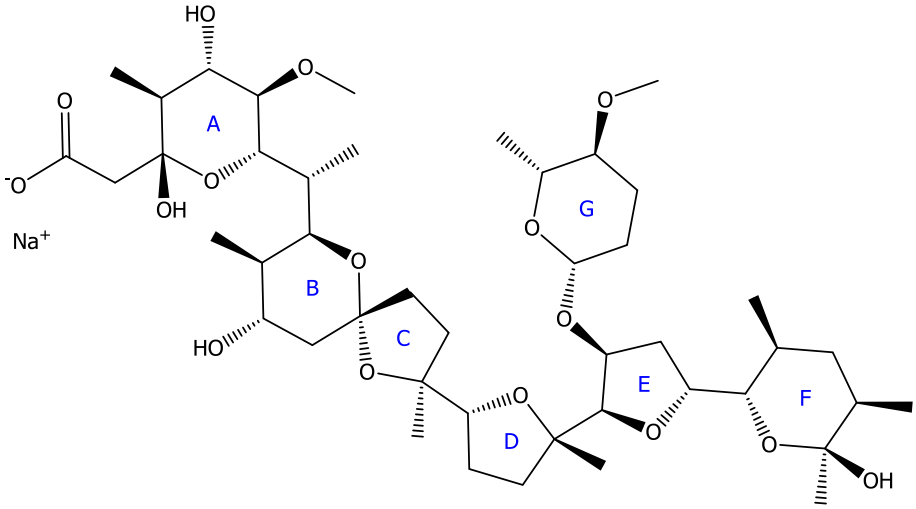


**Figure 1:** Structural formula of semduramicin sodium

[5] Commission Regulation (EC) No 429/2008 of 25 April 2008 on detailed rules for the implementation of Regulation (EC) No 1831/2003 of the European Parliament and of the Council as regards the preparation and the presentation of applications and the assessment and the authorisation of feed additives. OJ L 133, 22.5.2008, p. 1.

The mycelium bulk is specified to contain ≥ 150 g semduramicin sodium/kg.[6] Analytical data of 17 batches confirmed the specification with a mean of 197 mg and a range of 173–218 mg semduramicin sodium/kg.

Seven major semduramicin-related compounds derived from the biosynthetic pathway were identified (semduramicin epimer, semduramicin aglycon (without G ring), desmethyl semduramicin (A ring), desmethyl semduramicin (G ring), hydroxyl semduramicin (F ring), desmethoxyl semduramicin (A ring) and descarboxyl semduramicin, see Appendix A). Specifications were given for any individual semduramicin-related compound with < 3% and for the sum ≤ 7%. Analysis of 17 batches resulted in mean (maximum) concentrations of 0.5% (1.8%) descarboxyl semduramicin, 0.6 (1.3%) epimer and 1.1 (2.5%) hydroxyl semduramicin, being the three main impurities. One batch did not meet the specifications since it exceeded the 3% limit for another unidentified semduramicin-related compound.

The crude nutrient composition of the mycelial bulk was analysed in 17 batches with 4.4% moisture, 12.7% crude fat, 15.1% crude protein and 1.9% crude fibre. Ash contents measured in three of the 17 batches were 39%, 27% and 27%. Identification (by inductively coupled plasma (ICP) analysis) showed 21–27% Si and 5–6% Al, present as $SiO_2$ (40–57%) and $Al_2O_3$ (10–12%).

#### 3.1.1.1. Characterisation of the production organism

The active substance semduramicin sodium is produced by fermentation of a strain of *Actinomadura* spp. The strain, not genetically modified, was originally classified as *Actinomadura roseorufa,* a bacterial name without standing in nomenclature, and deposited in the American Type Culture Collection under the number ATCC 53664.[7]

To achieve the taxonomical identification of the production strain, the 16S rRNA gene sequence was analysed [redacted]

The approaches used for taxonomical identification consistently place the strain ATCC 53664 in the genus *Actinomadura* but cannot unequivocally assign to a valid taxonomic species of this genus, suggesting that the strain belongs to a new taxonomic unit within the genus *Actinomadura*.

Data on antimicrobial susceptibility of the production strain were not provided. Consequently, the FEEDAP Panel cannot conclude on the absence of genetic determinants for antimicrobial resistance in *Actinomadura* spp. ATCC 53664.

The genetic stability was demonstrated [redacted]

The absence of antimicrobial compounds relevant to the use of antibiotics in humans or animals, other than the semduramicin sodium in the mycelial product, was assessed comparing the minimum inhibitory concentrations (MIC) of three batches of the fermentation product with three batches of crystalline semduramicin sodium (94%).[9] The batches were tested against 36 strains of aerobic and anaerobic species of both Gram-positive and Gram-negative bacteria.[10] The MIC values were determined using a twofold broth dilution in appropriate media for the different bacterial species. Semduramicin sodium showed an antimicrobial activity in a concentration range of 2–32 mg/L against all the tested Gram-positive bacterial species, as typical of ionophoric coccidiostats. Differently, all the Gram-negative species were resistant to this ionophore, with MIC values higher than 64 mg/L.

Since no differences in the inhibitory spectrum and in the MIC values were observed between the pure and mycelial form for any of the strains tested, the product is considered to be free of antimicrobial activity, other than semduramicin sodium.

[6] Technical dossier/Section II/Annex II.10.
[7] Technical dossier/Supplementary information December 2016.
[redacted]
[9] Technical dossier/Section III/Annex III.25.
[10] *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Campylobacter jejunii* ATCC 33560, *Staphylococcus aureus* ATCC 29213 and *Streptococcus uberis* ATCC 19436) and 30 strains of animal origin belonging to *E. coli, Campylobacter jejunii* and *Salmonella* Enterica species.

### 3.1.2. Characterisation of the additive

Aviax 5%, a light tan free-flowing powder, contains 5% semduramicin sodium (specification 4.75 to 5.25%). The constituents are the mycelium (about 30%), mineral oil (3–5%), sodium carbonate (4%), sodium aluminosilicate (2%) and soybean mill run (about 60%).

Batch-to-batch consistency was demonstrated by the analysis of 10 industrial batches complying with the specifications and showing a mean semduramicin sodium concentration of 5.04% (range 4.79–5.17%).

Three other batches (5.03%, 5.08% and 5.09% semduramicin sodium) were the basis for all further characterisation.[11]

Mean concentrations of < 0.05 mg As/kg, 0.05 mg Cd/kg, < 0.36 mg Pb/kg and < 0.05 mg Hg/kg were measured. Values for dioxins (polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/F)) ranged between 0.020 and 0.027 ng WHO-PCDD/F-TEQ/kg, and the sum of dioxins and dioxin-like polychlorinated biphenyls (DL-PCBs) was between 0.027 and 0.034 ng WHO-PCDD/F-DL-PCB-TEQ/kg. Aflatoxin $B_1$ was < 2 μg/kg and ochratoxin A < 0.5 μg/kg. Data on microbial contamination were given for total aerobic count ≤ 2,100, total anaerobic plate count ≤ 110, *Escherichia coli* < 10, aerobe spore formers ≤ 90, anaerobic spore formers < 10, moulds ≤ 160 and yeasts < 10 CFU/g.[12]

The absence of viable cells of the production strain was demonstrated for three batches of the semduramicin mycelial bulk.[13]

Bulk density was reported to be 592 kg/$m^3$. Particle size distribution was measured by laser diffraction.[14] The average values (v/v) were: 8.8% of particles < 10 μm, 26.7% < 50 μm and 35.1% < 100 μm.

A Stauber–Heubach test indicated an average dusting potential of 2.03 g/$m^3$ (1.89–2.24 g/$m^3$).[15] Laser diffraction identified 38.2% of dust particles with a diameter < 10 μm and 99.9% < 50 μm. The dust contained 10.2% semduramicin sodium.[16]

### 3.1.3. Stability and homogeneity

#### 3.1.3.1. Shelf life of the additive

The shelf-life studies showed that semduramicin content remained unchanged during 3 years under at 25°C and 60% relative humidity (RH)) and during 6 months at 40°C and 75% RH.[17]

Shelf life of the fermentation bulk was also studied under comparable conditions. No losses of the initial semduramicin content (18.9%) were observed.

#### 3.1.3.2. Stability in premixtures and feedingstuffs

Three vitamin–mineral premixtures, two of them containing choline chloride (80,000 mg/kg), were prepared to contain 2,500 mg semduramicin sodium/kg.[18] These premixtures were used in the preparation of a complete feed for chicken for fattening (incorporation rate 1%). Half of the complete feed was pelleted at 70–80°C. All samples were stored at 25°C, 60% RH and at 40°C, 75% RH, the premixtures for 6 months and the complete feed for 3 months.

The analytical semduramicin concentration of the premixtures (2,451 mg/kg) complied with intended value. Losses during 6 months at 25°C, 60% RH were 13%, at 40°C, 75% RH were 22%. The addition of choline chloride to the premixture did not influence stability.

The complete feed met the intended semduramicin concentration with 25.1 mg/kg. Losses during 3 months storage at 25°C, 60% RH were 7%, at 40°C, 75% RH were 16%. Semduramicin sodium was stable during pelleting (recovery 96%). In the pelleted feed, losses during 3 months storage were 6% at 25°C and 60% RH, and 10% at 40°C and 75% RH.

[11] Technical dossier/Section II/Annex II.28.
[12] Technical dossier/Section II/Annex II.3.
[13] Technical dossier/Section II/Annex II.4.
[14] Technical dossier/Section II/Annex II.7.
[15] Technical dossier/Section II/Annex II.5.
[16] Technical dossier/Section II/Annex II.8 and 9.
[17] Technical dossier/Section II/Annex II.29.
[18] Technical dossier/Section II/Annex II.31 and II.32.

#### 3.1.3.3. Homogeneity in premixtures and feedingstuffs

The capacity of Aviax 5% to distribute homogeneously in premixtures and feedingstuffs was demonstrated by the analysis of 10 subsamples each of the above described (Section 3.1.3.2) premixtures and complete feeds.[19] The coefficients of variation ranged from 2.8% to 3.7% for the premixtures and from 4.1% to 4.9% for the complete feeds.

### 3.1.4. Conditions of use

Aviax 5% (semduramicin sodium) is intended to be used for prevention of coccidiosis in chickens for fattening at a concentration of semduramicin sodium in complete feed of 20–25 mg/kg. A withdrawal time of 1 day is proposed.

## 3.2. Safety

### 3.2.1. Absorption, distribution, metabolism, excretion and residues

#### 3.2.1.1. Absorption, distribution, metabolism and excretion

In the former assessment of semduramicin sodium, absorption, distribution, metabolism and excretion (ADME) in chicken the SCAN (European Commission, 2002) concluded that: (i) semduramicin is significantly absorbed (amount not established), extensively metabolised and mainly excreted through the bile and faeces, (ii) unchanged semduramicin is by far (45%) the main residue in the liver (after 6-h withdrawal), each of the other 19 metabolites representing less than 10% of the total radioactivity in this tissue, (iii) three major metabolites, more polar than the parent compound, have been identified in the droppings as two monohydroxy-semduramicins (*O*-desmethyl (G-ring) semduramicin (metabolite E) and *O*-desmethyl (A-ring) semduramicin (metabolite F)) and a compound corresponding to the F-ring opening of metabolite F (metabolite C); of the excreted amounts in excreta (only 50% extractable), unchanged semduramicin represents about 16%, (8% of the total radioactivity) metabolite F 24% and the other polar metabolites less than 10% each; metabolite F is by far the major metabolite identified in the bile, (iv) comparative metabolism performed in the rat and the dog indicated similar qualitative metabolic pathways to the chicken, (v) semduramicin is the marker residue. The FEEDAP Panel retains the same conclusions for the current assessment.

#### 3.2.1.2. Residues

*Total residues*

A pilot study was designed to establish: (i) the feeding time necessary to reach a plateau level of semduramicin and metabolites in chicken plasma, (ii) total residues in tissues at metabolic steady state.[20] Chickens (22-days-old, 3 males and 3 females per time point) were administered $^{14}$C-semduramicin sodium at a dose of 25 mg/kg feed for 14 consecutive days. Blood was sampled along the exposure period and tissues were sampled at slaughter for radioactivity measurement. Total radioactivity in plasma reached a plateau after 5 days. Total radioactivity in tissues was measured at metabolic steady state, results are reported in Table 1.

In a second total residue study,[21] groups of chickens (3 male and 3 female) were administered 27 mg $^{14}$C-semduramicin/kg feed for 5 consecutive days, then slaughtered after 6, 12 and 24 h withdrawal of the supplemented feed. Total radioactivity of tissues was determined and results are reported in Table 1.

[19] Technical dossier/Section II/Annex II.33.
[20] Technical dossier/Section III/Annex III.28.
[21] Technical dossier/Section III/Annex III.29.

**Table 1:** Semduramicin total residues in chicken tissues (expressed as mg equivalent semduramicin/kg tissue)

| Withdrawal time (h) | Liver | Kidney | Muscle | Skin/fat |
|---|---|---|---|---|
| **0**(1) | 1.972 ± 0.319(3) (2.61) | 0.367 ± 0.049 (0.465) | 0.082 ± 0.010 (0.103) | 0.270 ± 0.032 (0.334) |
| **6**(2) | 0.853 ± 0.410 (1.673) | 0.094 ± 0.041 (0.176) | 0.016 ± 0.006 (0.017) | 0.064 ± 0.019 (0.102) |
| **12**(2) | 0.258 ± 0.101 (0.460) | 0.026 ± 0.007 (0.040) | 0.005 ± 0.002 (0.009) | 0.028 ± 0.011 (0.050) |
| **24**(2) | 0.104 ± 0.034 (0.172) | 0.013 ± 0.005 (0.023) | 0.001 ± 0.001 (0.003) | 0.019 ± 0.009 (0.037) |

(1): Measured in chickens (3 males and 3 females) administered 25 mg $^{14}$C-semduramicin/kg feed for 14 consecutive days.[20]
(2): Measured in chickens (3 males and 3 females) administered 27 mg $^{14}$C-semduramicin/kg feed for 5 consecutive days.[21]
(3): Total residue concentration (mg/kg) ± standard deviation (total residue concentration plus 2 standard deviations).

In the same study, the ratios marker to total residues were calculated. As all marker residue values were below the limit of quantification (LOQ), calculation considered values measured between the LOQs and corresponding limits of detection (LODs). Therefore, only approximate ratios were established to 0.17 for the liver, 0.32 for the kidney, 0.14 for the muscle and 1 for the skin/fat after a 12-h withdrawal.

*Marker residue*

A study of semduramicin residues in chicken tissues from animals fed crystalline semduramicin sodium for 35 days was submitted.[22] Groups of eight chickens (4 males and 4 females) were administered a feed containing 25 mg semduramicin sodium/kg. The animals were slaughtered after 6, 12 and 24 h. Semduramicin was determined using a high-performance liquid chromatography–tandem mass spectrometry (HPLC-MS/MS) method with LOQs of 0.150, 0.050, 0.015 and 0.150 mg/kg for liver, kidney, muscle and skin/fat, respectively. No further details of the experimental protocol were given. Results indicate that after 6 h withdrawal semduramicin amounted 0.278 ± 0.076 mg/kg in the liver, 0.072 ± 0.020 in the kidney, 0.027 ± 0.009 in the muscle and < 150 mg/kg in the skin/fat. After 12 h withdrawal, all values were below the respective LOQs; the approximate average values measured below the LOQs were 0.082 in the liver, 0.025 in the kidney, 0.008 in the muscle and 0.040 mg/kg in the skin/fat.

### 3.2.2. Safety for the target species

#### 3.2.2.1. Tolerance studies

For the current assessment, the applicant provided the same studies in chicken for fattening which were already submitted for the previous assessment by the SCAN (European Commission, 2002), performed a literature search for the period between 2005 and 2015 and submitted a company report on adverse events.

The SCAN assessed the safety of crystalline semduramicin in 2002 following Directive 87/153/EEC[23] and concluded that 'semduramicin was well tolerated by broiler chickens at the maximum dose level of 25 ppm' (European Commission, 2002). The same studies assessed in the SCAN opinion have been reassessed for the current evaluation considering the principles established by Commission Regulation (EC) No 429/2008[24] and the Technical Guidance on Tolerance and efficacy studies in target animals (EFSA FEEDAP Panel, 2011b). None of the studies submitted could be used for the assessment because of lack or limited information. In particular, an exploratory study, in which only performance was measured, could not be considered for the assessment owing to its short duration (21 days), the lack of haematology, blood biochemistry and pathology.[25] A floor pen study (duration of 44 days) in which performance, haematology, feathering and pathology were measured could not be considered also owing to the lack of blood biochemistry and to insufficient haematology (limited to red blood cell count,

[22] Technical dossier/Section III/Annex III.30.
[23] Council Directive 87/153/EEC of 16 February 1987 fixing guidelines for the assessment of additives in animal nutrition. OJ L 64, 7.3.1987, p. 64.
[24] OJ L 133, 22.5.2008, p. 1.
[25] Technical dossier/Section III/Annex III.2.

haemoglobin, haematocrit and prothrombin time).[26] In another floor pen study with duration of 49 days (including a 5-day withdrawal), performance was measured and necropsy of dead birds was made.[27] This study could not be considered due to the lack of haematology, blood biochemistry and pathology. In three battery studies[28] and other two floor pen studies,[29] no semduramicin overdose (> 25 mg/kg feed) was tested. Finally, in another floor pen study, only one level of semduramicin was applied, performance and litter moisture served as endpoints.[30]

The applicant performed a literature search for the time between 2005 and 2015.[31] None of the studies identified could contribute to a new or better knowledge on the safety of semduramicin for chickens for fattening.

[redacted]

Considering the weaknesses of the studies shortly referenced above (non-compliance with the requirements of Commission Regulation (EC) No 429/2008[24]), the FEEDAP Panel was not in a position to assess the safety of semduramicin for chickens for fattening. The applicant was requested to submit a tolerance study with semduramicin mycelium taking into account the recent FEEDAP Guidance on Tolerance and efficacy studies in target animals (EFSA FEEDAP Panel, 2011b).

Upon request of the FEEDAP Panel, an additional tolerance study (dated 1996) and a US regulatory document (Original new animal drug application Aviax II) were submitted.[8]

The newly submitted study had a duration of 42 days and was performed with mycelial semduramicin at use level and two overdose (50 and 75 mg semduramicin sodium/kg). Bird's performance and necropsy of dead birds were evaluated. This study, conducted in 1996, did also not meet the current EU requirements which include haematology, blood biochemistry and pathological examination at the end of the study. The information contained in the US regulatory document was not considered relevant for the purpose of the assessment.

Consequently, the FEEDAP Panel cannot conclude on the safety of Aviax 5%, containing semduramicin mycelium, for chickens for fattening.

#### 3.2.2.2. Interactions

In 2002, the SCAN concluded that:

> 'It is recognised that in general ionophore polyethers are non-compatible with concurrent tiamulin. Indeed, concurrent medication of birds receiving 25 ppm semduramicin in the feed with tiamulin in water (250 ppm) for 3 days resulted in a modification of performance data i.e. depression on body weight gain and deterioration on feed efficiency. Other relevant data as haematology, clinical chemistry, gross pathology and histopathology from treated animals with this combination were not available. Semduramicin in the feed is not compatible with the concurrent use of tiamulin'.

One additional study, not evaluated by the SCAN in 2002, was submitted for the current assessment.[33]

In this study, designed as a growth trial with a supplementation of 25 mg semduramicin/kg feed for 35 days, it was demonstrated that the simultaneous administration of semduramicin and tiamulin at 250 mg/L water for drinking (day 15–19 of the trial) reduced water and feed intake, resulting in a temporary growth depression and impaired feed efficiency in the third week of the trial. These effects were not seen in the chickens receiving only semduramicin or tiamulin indicating a possible interaction between semduramicin and tiamulin; however, these effects were limited to the period of the simultaneous administration, indicating a short-term effect.

The applicant performed a literature search covering the period between 2005 and 2015.[31] The only paper of interest regarding the interaction is the one by Islam et al. (2009), in which the various findings of the last 15 years concerning interactions of ionophores with commonly used drugs in

[26] Technical dossier/Section III/Annex III.3.
[27] Technical dossier/Section III/Annex III.4.
[28] Technical dossier/Section III/Annex III.5, Annex III.6 and Annex III.7.
[29] Technical dossier/Section III/Annex III.8 and Annex III.9.
[30] Technical dossier/Section III/Annex III.10.
[31] CAB Abstracts was searched on Web of Knowledge, a Thomson Reuters product, covering the period 2005 to June 2015. The search term used was 'Semduramicin' with no restrictions.
[32] [redacted]
[33] Technical dossier/Section III/Annex III.13.

poultry medicines are discussed. The author concluded that, in contrast with most other ionophores (monensin, salinomycin and narasin), only a mild interaction exists between tiamulin and semduramicin with the possible effects being a temporary depression of feed and water intake and weight gain.

The FEEDAP Panel reiterates the SCAN conclusion that 25 mg semduramicin sodium/kg feed is not compatible with the concurrent use of tiamulin.

#### 3.2.2.3. Microbial studies

The applicant provided the same studies that were assessed by the SCAN in 2002 (European Commission, 2002). The FEEDAP Panel re-assessed the studies and confirmed the SCAN conclusions that semduramicin sodium is active against certain Gram-positive bacteria, while Gram-negative bacteria are resistant. This pattern was also confirmed in the study[9] in which the mycelial product and crystalline semduramicin sodium were tested against 36 strains of aerobic and anaerobic species of both Gram-positive and Gram-negative bacteria (see Section 3.1.1.1).[10]

In 2002, it was concluded that the use of semduramicin sodium as a feed additive is unlikely to increase shedding of *Salmonella*, *E. coli* and *Campylobacter* and to induce resistance and cross-resistance to antimicrobials used of human and animal relevance. The same conclusions are retained for the current assessment.

#### 3.2.2.4. Conclusions on the safety for the target species

In the absence of a tolerance study in chickens for fattening performed according to the current EU standards, the FEEDAP Panel cannot conclude on the safety of Aviax 5%, containing semduramicin mycelium, for chickens for fattening.

Semduramicin in the feed is not compatible with the concurrent use of tiamulin, although the interaction is less severe than observed with other polyether coccidiostats affecting only performance parameters.

Semduramicin sodium is active against certain Gram-positive bacteria, while Gram-negative bacteria are resistant. The use of semduramicin sodium as a feed additive is unlikely to increase shedding of *Salmonella*, *E. coli* and *Campylobacter* and to induce resistance and cross-resistance to antimicrobials used of human and animal relevance.

### 3.2.3. Safety for the consumer

#### 3.2.3.1. Genotoxicity of mycelial semduramicin sodium

Mycelial semduramicin sodium was tested for the induction of reverse mutations in *Salmonella* Typhimurium tester strains TA 1535, TA 1537, TA 98 and TA 100.[34] The experimental protocol was in line with Organisation for Economic Co-operation and Development (OECD) guideline 471, version 1983. The test item was dissolved in dimethylsulfoxide (DMSO) and tested in either the presence or absence of the metabolic activation system using liver S9 fraction from Aroclor 1254-induced rats at five concentration levels up to 5 mg/plate. Insoluble test material was seen at concentrations $\geq$ 0.05 mg/plate. No toxicity was seen at concentrations up to 5 mg/plate. No indication of mutagenic activity was found in any experimental condition, while a significantly increased number of revertant colonies was observed in the positive controls.

The same mycelial product was tested in an *in vivo* cytogenetic study to determine the potential of the test compound to induce chromosome damage in mice.[35] The experimental protocol was compatible with OECD guideline 475, version 1984. Five male and five female mice per sacrifice time received a single administration of 6 mg test material/kg by oral gavage and were sacrificed 6, 24 or 48 h after dosing. The positive control, mitomycin C, and the vehicle control (0.5% methylcellulose) were administered to five male and five female mice as a single intraperitoneal injection or orally by gavage, respectively, and the animals were sacrificed 24 h post-dosing.

All animals survived until the scheduled sacrifice. Two of 15 females administered with the test material appeared lethargic and unresponsive approximately 4 h after dosing. All other animals appeared normal throughout the study. The mitotic indices of both male and female mice sacrificed 6 h after treatment were approximately 50% suppressed in comparison with the control group, demonstrating the exposure of the target cells to the test item. At 24 h, mitotic indices were

[34] Technical dossier/Section III/Annex III.53.
[35] Technical dossier/Section III/Annex III.54.

comparable to the concurrent controls, while at 48 h a slight reduction in the mitotic index was observed only in males.

Clastogenicity was assessed by microscopic evaluation of metaphase bone marrow cells of each animal for the incidence and types of structural chromosome damage at each sacrifice time. The test item did not cause chromosomal aberrations in the bone marrow cells of treated mice while the positive control performed as expected.

#### 3.2.3.2. Toxicological studies with semduramicin sodium mycelial

In a dose-range finding study, mycelial semduramicin containing 1.26% semduramicin, was administered in feed to groups of 10 Long-Evans Crl:(LE)BR rats of each sex at nominal doses of 0, 0.5, 1.0 and 2.0 mg semduramicin/kg body weight (bw) per day for 36–39 consecutive days.[36] The dosages achieved were 0, 0.47, 1.01 and 2.01 mg/kg bw and day for males and 0, 0.54, 1.04 and 1.92 mg semduramicin/kg bw per day for females. The study complied with Good Laboratory Practices (GLP). Blood and urine were collected for analysis prior to the start of treatment and on days 16–19 and 37–40 of the study. All rats were killed 1 day after the end of treatment, and gross pathology and histopathology were evaluated for all animals. Liver samples from all rats for each group/sex were combined and analysed for semduramicin. The high-dose females ate less and gained less bodyweight than control females, resulting in a 10% decrement in final mean body weight compared to controls. There were no treatment-related effects on haematology, blood biochemistry, urinalysis, ophthalmoscopy, organ weights, gross pathology or histopathology. Analysis of semduramicin in pooled liver samples indicated a sex difference with higher concentrations in females. The no observed effect level (NOEL) for the study was 1.04 mg semduramicin/kg bw per day, based on reduced feed intake and bodyweight gain in females.

In a GLP-compliant study (1990), mycelial semduramicin was prepared in a 0.1% drug/lactose preblend and administered in the diet of Long-Evans Crl:(LE)BR rats, 20 males and 15 females per dose level, to provide nominal doses of 0.5, 1.0, and 2.0 mg semduramicin/kg bw per day for 99–102 consecutive days.[37] A group of control rats received the same amount of lactose as the high-dose group. The dosages achieved were 0, 0.47, 0.95 and 1.92 mg semduramicin/kg bw per day for males and 0, 0.49, 0.96 and 1.93 mg semduramicin/kg bw per day for females. The protocol of the study was consistent with OECD 408. The rats were observed daily for clinical signs. Body weights and food consumption were recorded weekly. Other measurements and observations included ophthalmological examinations (weeks 2 and 14), serum chemistry and haematology (weeks 1, 5 and 14), and urinalysis measurements (weeks -1, 5 and 15). At the end of the dosing period, surviving rats were killed and necropsied. The male rats from this study were used to mate with female rats in a Modified Segment I Reproduction Toxicity Study and were killed in order to assess the effects of the mycelial material on fertility, reproduction, gestation, lactation and early postnatal development, as well as the parameters observed for the 3-month toxicity study. Results of the sperm count and motility assessment were reported in the reproduction study.[38] Approximately 3 weeks (dose weeks 6–8) were allowed for mating. Food consumption for male rats was not determined while mating was in progress. No treatment-related clinical signs were observed. One male animal in the 1.0 mg/kg bw per day group died on day-19, but the death was unrelated to treatment. All remaining animals survived the treatment period. The mean feed intake was decreased in all groups of females during the final week of the study, with the decrease being slightly greater at the top dose. There was a dose-related decrease in body weight gain in females (but not in males), with body weight gains over the duration of the study being 142, 131, 121 and 59 g for the 0, 0.5, 1.0 and 2.0 mg/kg bw per day groups, respectively. No statistical analysis of these results was presented. Serum biochemistry showed statistically significant decreases in 5'-nucleotidase in females given 1.0 or 2.0 mg/kg bw per day and in males given 2.0 mg/kg bw per day; sorbitol dehydrogenase activity was increased in 1.0 mg/kg bw per day females and in 2.0 mg/kg bw per day males at weeks 5 but not at week 14; increases in glucose concentration were seen at week 5 in males given 1.0 or 2.0 mg/kg bw per day but a decrease was seen at week 14 in females given 1.0 mg/kg bw per day. No changes in ophthalmology, haematology or urinalysis were seen. In the high-dose females, there was a statistically significant lower mean absolute liver weight, and statistically significantly higher mean relative weights of the liver, kidney, heart and brain. There were no differences in the relative weights of the organs

[36] Technical dossier/Section III/Annex III.55.
[37] Technical dossier/Section III/Annex III.56.
[38] Technical dossier/Section III/Annex III.59.

compared to brain weight. No treatment-related lesions were detected during gross examination and there was no histological evidence of toxicity in any dose group. The organ weight changes seen in the high-dose females with no associated pathology were considered to be due to the relatively low mean body weights of this group. A no observed adverse effect level (NOAEL) could not be determined for this study, since dose-related effects were seen for feed intake and body weight.

In a GLP-compliant dose-range finding study (1991), mycelial semduramicin sodium was administered in feed to Beagle dogs (two dogs/sex per dose level) at doses to provide 0, 0.5, 1.0 and 2.0 mg semduramicin/kg bw per day for 36 consecutive days.[39] The semduramicin concentration of the mycelial material was 1.26%. Samples of blood and urine were collected prior to treatment and on days 11, 25 and 36 of treatment. Ophthalmoscopic examinations were performed before the start of dosing and close to the end of the study. Electrocardiogram measurements were made prior to dosing, on day 3, and near the end of the study. Indirect measurements of systolic blood pressure were made during the study on days 0, 15 and 29 (using the mean of at least three measurements) using a compression cuff at the base of the tail. All animals were killed after 37 days of treatment and post-mortem examination was performed. The major organs were weighed and examined microscopically. One male in the mid-dose group and one in the top-dose group ate much less than controls. No other treatment-related changes were detected at the low and mid doses, but a series of changes were seen at the top dose: loss of body weight in all dogs (with one female having 12.6% body weight loss); tremors and muscular incoordination in one male and both females; sporadically elevated activities of plasma creatine phosphokinase (CPK) in both females, alanine aminotransferase (ALT) in one male and one female; and aspartate aminotransferase (AST) in one male. The male not showing effects was the one not eating the full dose. Microscopic evidence of muscle alteration that was described as 'a disparity in the size of muscle fibers, with increased prominence of the sarcolemma in the altered fibers' was seen in one female. Organ weights, electrocardiogram results and systolic blood pressure were not affected by treatment. The NOAEL was 1.0 mg semduramicin/kg bw per day, based on the various adverse effects seen at 2.0 mg/kg bw per day.

In a GLP-compliant study (1994), groups of male and female Beagle dogs (three dogs/sex per dose level) were fed for 91 days a mycelial form of semduramicin in a preblend with lactose (0.94% semduramicin) that was mixed with commercial canned dog food to achieve dose levels of 0, 0.25, 0.5 and 1.0 mg semduramicin/kg bw per day.[40] The control group received the same diet as the high-dose animals, but without the test material. Dogs were assessed daily for clinical signs of toxicity and body weights were recorded weekly. Ophthalmoscopy and measurement of vital signs (heart and respiratory rates and rectal temperature) were performed on each dog before dosing and three times during the study. Electrocardiogram measurements and indirect measurements of systolic blood pressure (means of three measurements at each time point) were made were made prior to dosing and on days 9, 43 and 79 of the study. Blood and urine were collected for analysis prior to dosing and two times (days 34–38 and 85–89) during the treatment period. At the conclusion of the dosing period, all animals were killed and necropsied (day 92). Major organs were weighed, and a comprehensive set of tissues was collected and processed for microscopic examination. There was no evidence of toxicity at any of the dose levels during this study. There were no apparent clinical signs of toxicity, and no treatment-related effects on serum biochemistry, haematology and urinalysis, although a single high-dose female had noticeably increased serum AST activity at both measurements during treatment. Rectal temperature, blood pressure, electrocardiogram and ophthalmoscopic examinations were not affected by treatment. There were no treatment-related lesions identified during gross or microscopic examination in any of the treated animals. Based on the results of this investigation, 1.0 mg semduramicin/kg bw per day was considered to be the NOAEL in Beagle dogs treated for 3 months.

In a GLP-compliant reproductive toxicity study,[38] mycelial semduramicin was prepared in a 0.1% drug/lactose pre-blend and administered in the diet of Long-Evans (Crl:LE®BR VAF/PLUS®) female rats, 20 per dose level, to provide nominal doses of 0.5, 1.0, and 2.0 mg semduramicin/kg bw per day. The animals were treated for 14 days prior to mating (with male rats from the 3-month toxicity study described above[40]) and during mating, gestation and lactation. Treatment ended on post-partum day 21. A group of control rats received the same amount of lactose as the high-dose group. The rats were observed daily for clinical signs. Body weights were recorded weekly prior to mating, daily throughout gestation and on days 1, 4, 7, 10, 14, and 21 of lactation. After mating was complete, the males were sacrificed, and semen analysis was performed in addition to the standard necropsy in

[39] Technical dossier/Section III/Annex III.57.
[40] Technical dossier/Section III/Annex III.58.

order to assess the effects on fertility, reproduction, gestation, lactation and early postnatal development. Food consumption was not determined during mating. The $F_1$ pups from each litter were evaluated for a variety of postnatal developmental landmarks (surface righting reflex, air righting, visual cliff avoidance, functional observation battery, motor activity, auditory function, appearance of incisors, eye opening, ophthalmoscopy, vaginal opening and preputial separation). At post-partum day 21, two $F_1$ female offspring per litter were selected for mating to produce an $F_2$-generation, whilst the $F_0$ dams and the rest of the $F_1$ animals were killed and examined. There were no treatment-related mortalities in this study. Mean body weight and body weight gain were unaffected by treatment in the 0.5 and 1.0 mg/kg bw per day dose groups of the $F_0$ generation, but body weight and body weight gain in the 2.0 mg/kg dose group females were significantly decreased from gestational day 3 until the end of the study. Mean pup weights of the 0.5 and 1.0 mg/kg bw per day dose groups were unaffected by treatment. $F_1$ pups in the 2.0 mg/kg group had significantly lower body weights beginning on postnatal day 1 and continued to weigh less through the post-weaning observation period. There were no significant differences in the reproductive parameters (copulation rate, pregnancy rate, size of litters, sex ratio of pups, external and internal abnormalities, microscopic appearance of uteri, number of implantation sites) among the dose groups. Sensory function, motor activity, and functional observational battery assessments of $F_1$ pups showed no treatment-related adverse effects. Male reproductive parameters, sperm count and % total motility were unaffected by treatment. The NOAEL for fertility, reproduction and neonatal outcome in this study is 1.0 mg semduramicin/kg bw per day based on the decreased body weights and food consumption in the $F_0$ animals and the decreased body weight of the $F_1$ pups at 2.0 mg/kg bw per day.

#### 3.2.3.3. Toxicological studies with semduramicin sodium crystalline

The studies using crystalline semduramicin were previously assessed by SCAN (European Commission, 2002). Generally, a similar range of studies (dog 1-month, 3-month and 12-month studies and rat 1-month, 3-month and 24-month) was considered as those described above for the mycelial product. The effects seen in these studies were more marked than those seen with the mycelial product at similar doses. There is no evidence that the mycelial semduramicin is more toxic than the crystalline by oral administration.

#### 3.2.3.4. Conclusions on the toxicology

The previous acceptable daily intake (ADI) of 0.00125 mg/kg bw, based on a NOAEL of 0.125 mg/kg bw per day was derived from a 24-month rat study with the crystalline semduramicin product for decreased serum protein and sodium concentrations in rats (European Commission, 2002).

Positive inotropic effects (an effect seen with some ionophoric coccidiostats) have been previously accepted by the FEEDAP Panel as being relevant to consumer risk assessment, particularly when occurring at doses lower than identified as a toxicological NOAEL (EFSA, 2005). It should be noted that a NOEL for induction of positive inotropy by oral doses of semduramicin was not identified in any of the studies, although electrocardiogram results for dogs showed no adverse effects at up to 4 mg/kg bw per day. On the other hand, some dogs given the highest dose (1 mg/kg bw per day) in the 12-month dog study had increased systolic blood pressure when measured near the end of the treatment period.

Since these values are above the lowest NOAEL previously identified of 0.125 mg/kg bw per day, there is no reason to consider acute cardiovascular effects in the risk assessment. In conclusion, considering the above and the absence of a comparable 24-month study with the mycelial product the previous lowest NOAEL (0.125 mg/kg bw per day) and ADI (0.00125 mg/kg bw) are retained for the mycelial product.

#### 3.2.3.5. Assessment of consumer safety

Taking a conservative approach, the FEEDAP Panel considered that the whole semduramicin-derived residues represent a risk which is at the most equal to an equivalent quantity of semduramicin. The exposure of the consumer to semduramicin-related total residues present in tissues has been calculated according to daily food consumption values of animal products set in Regulation (EC) No 429/2008,[24] using maximised residue concentrations (average plus 2SD, 95% confidence limit) (See Table 1). Compliance with the ADI (0.075 mg/60-kg person per day) was calculated also (Table 2).

**Table 2:** Consumer exposure to semduramicin total residues in tissues of chickens for fattening after different withdrawal times and compliance with the ADI

| | Liver | Kidney | Muscle | Skin/fat | Sum | % ADI |
|---|---|---|---|---|---|---|
| **TRC(1) (mg/kg) + 2SD (0 hr)** | 2.61 | 0.465 | 0.103 | 0.334 | | |
| **DITR(2) (mg/day) (0 hr)** | 0.261 | 0.005 | 0.031 | 0.030 | 0.327 | 436 |
| **TRC (mg/kg) + 2SD (6 h)** | 1.673 | 0.176 | 0.027 | 0.102 | | |
| **DITR (mg/day) (6 h)** | 0.167 | 0.002 | 0.008 | 0.009 | 0.186 | 248 |
| **TRC (mg/kg) + 2SD (12 h)** | 0.460 | 0.040 | 0.009 | 0.050 | | |
| **DITR (mg/day) (12 h)** | 0.046 | 0 | 0.003 | 0.005 | 0.054 | 72 |
| **TRC (mg/kg) + 2SD (24 h)** | 0.172 | 0.023 | 0.003 | 0.037 | | |
| **DITR (mg/day) (24 h)** | 0.017 | 0 | 0.001 | 0.003 | 0.021 | 28 |

(1): Total residue concentration.
(2): Daily intake of total residues.

Residues in all analysed tissues rapidly declined with increasing withdrawal time. After 12-hour withdrawal consumer intake would be below the ADI (72%).

### Proposal for a withdrawal time

Total residue data support the proposal of the applicant for a 1-day withdrawal period when chickens for fattening are fed a complete feed containing 25 mg semduramicin sodium/kg.

### Proposal of the maximum residue limits

The maximum residue limits (MRLs) are usually not lower than three times the LOQ of the method used. The applicant followed this principle and proposed the MRLs reported in Table 3.

**Table 3:** Consumer exposure to semduramicin total residues corresponding to MRLs in tissues of chickens for fattening after 12-h withdrawal and compliance with the ADI

| | Liver | Kidney | Muscle | Skin/fat | Sum | % ADI |
|---|---|---|---|---|---|---|
| **LOQ(1) (mg/kg)** | 0.150 | 0.050 | 0.015 | 0.150 | | |
| **MRL(2) (mg/kg)** | 0.450 | 0.150 | 0.045 | 0.450 | | |
| **RMTR(3)** | 0.17 | 0.32 | 0.14 | 1 | | |
| **DITRMRL(4) (mg/day)** | 0.260 | 0.005 | 0.096 | 0.041 | 0.402 | 536 |

(1): Limit of quantification of the method proposed by the applicant.
(2): MRLs proposed by the applicant.
(3): Ratio marker to total residues.
(4): Dietary intake of total residues calculated from the MRLs.

The MRLs proposed by the applicant do not ensure consumer safety since they would allow consumer intake at about five times the ADI. Even taking the LOQs as MRLs would exceed the ADI by approximately two times (173%). Considering the approximate (between LODs and LOQs) average semduramicin concentrations measured after 12 h withdrawal, consumer exposure calculated using the corresponding ratios marker to total residues (RMTR) would amount to 0.070 mg which is very close to the ADI. Consequently, a minimum of 24 h withdrawal should be considered and residue concentrations should be measured using a more sensitive analytical method.

### 3.2.3.6. Conclusion on consumer safety

Based on a health-based guidance value (ADI) of 0.00125 mg/kg bw, the use of semduramicin sodium at a dietary concentration of 25 mg/kg complete feed for chickens for fattening is safe for consumers provided a withdrawal time of 24 h is respected. Semduramicin is the marker substance. For control purposes, MRLs for the different tissues are considered necessary. However, no proposal for MRLs could be made due to lack of data for marker residue concentrations after 24 h withdrawal and limited sensitivity of the analytical method.

### 3.2.4. Safety for the user

No data on skin and eyes irritancy and skin sensitisation were provided with Aviax 5% containing mycelial semduramicin sodium. Studies performed with the crystalline form showed that semduramicin was slightly irritating to the eye and to abraded skin in rabbits,[41] but was not irritating to intact rabbit skin and was not a dermal sensitiser in guinea pigs.[42]

In the absence of data, the FEEDAP Panel cannot conclude on the irritancy of Aviax 5% to skin and eye and on the potential for dermal and respiratory sensitisation.

The SCAN (European Commission, 2002) referred to an inhalation toxicity study in rats with crystalline semduramicin in which a $LC_{50}$ of 67 mg/$m^3$ was identified. The same study was submitted for the current application.[43] A model calculation (see FEEDAP Technical guidance on user safety; EFSA FEEDAP Panel, 2012b) using the data submitted for dusting potential and semduramicin concentration in the dust, identified that workers in a premixture factory handling the Aviax 5% may be exposed to a concentration of 229 mg semduramicin/$m^3$ resulting in an inhalation exposure of 32 mg per person during a 8-h working day (see Appendix B). These figures do not consider the alveolar fraction. A comparison of the estimated exposure of workers with the $LC_{50}$ indicates a serious risk to health from exposure to dust generated by use of this product.

#### 3.2.4.1. Conclusions on user safety

The FEEDAP Panel cannot conclude on the irritancy of Aviax 5% to skin and eye and on the potential for dermal and respiratory sensitisation.

Model calculations on inhalation exposure of persons handling the additive indicate a serious risk.

### 3.2.5. Safety for the environment

The active substance is not a physiological/natural substance of established safety for the environment. Consequently, according to Regulation (EC) No 429/2008[24] the Phase I assessment has to be continued to determine the predicted environmental concentration.

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

#### 3.2.5.1. Phase I

**Physicochemical properties of semduramicin**

The physicochemical properties of semduramicin sodium are summarised in Table 4.

**Table 4:** Physicochemical properties of semduramicin sodium

| Property | Value | Unit |
|---|---|---|
| Molecular weight | 895 | |
| Octanol/water partition coefficient (log $K_{ow}$)(1) | 4.49 at pH 4<br>2.63 at pH 7<br>2.21 at pH 9 | – |
| Solubility at 20°C(1) | Milli-Ro water 1.39<br>pH 4 buffer 0.163<br>pH 7 buffer 1.24<br>pH 9 buffer 1.02 | g/L |
| Dissociation constant pKa(2) | 5.39 | – |
| Vapour pressure(3) | $6.67 \times E^{-28}$ | Pa |

(1): Technical dossier/Section III/Annex III.66.
(2): Technical dossier/Section III/Annex III.65.
(3): Calculated using the estimation software Epi-Suite.

[41] Technical dossier/Section III/Annex III.36.
[42] Technical dossier/Section III/Annex III.38.
[43] Technical dossier/Section III/Annex III.37.

## Fate and behaviour

*Fate in soil*

Adsorption

Two GLP studies were conducted in which the sorption characteristics of [$^{14}$C]-semduramicin sodium were investigated in soil.

The first study was performed using three soils, in accordance with Food and Drug Administration (FDA) and OECD guidelines which were in force at the time the study was conducted.[44] Adsorption and desorption distribution coefficients ($K_{oc}$) of [$^{14}$C]-semduramicin sodium in 0.01 M aqueous calcium chloride were determined in silty clay loam, silty loam and sandy loam soils. The results indicated that semduramicin sodium had some affinity for each of the soil types investigated. The adsorption distribution coefficients, expressed in terms of soil organic carbon ($K_{oc}$), were 1,800, 1,400 and 150 for silty clay loam, silty loam and sandy loam, respectively. The corresponding desorption $K_{oc}$ values were 1,100, 840 and 79, respectively.

A new study was conducted with two further soils, in accordance with current OECD guideline requirements.[45] In this study, the adsorption and desorption characteristics of [$^{14}$C]-semduramicin sodium were determined in clay loam and silty clay soils. The adsorption distribution coefficients, expressed in terms of soil organic carbon ($K_{oc}$), were 49 and 23 mL/g for clay loam and silty clay, respectively. The corresponding desorption $K_{oc}$ values were 54 and 27 mL/g, respectively.

The five soil types used in the studies represented a range of characteristics found in typical agricultural soils that define the adsorption capacity of a soil, and broadly corresponded with OECD 106 recommendations. The geometric mean $K_{oc}$ value for adsorption, across the five soils, was 212 (Table 5).

The results indicated that adsorption was partially reversible. Under the classification scheme proposed by McCall et al.,[46] semduramicin sodium would be classified as having medium mobility, based on the geometric mean values. The Freundlich adsorption and desorption isotherms indicated that the solution concentration of semduramicin sodium did not influence its adsorption to soil.

**Table 5:** Adsorption coefficient corrected for soil organic carbon content ($K_{oc}$) of semduramicin in different soils

| Soil | $K_{oc}$ (mL/g) | pH | % organic carbon | %Sand | %Clay |
|---|---|---|---|---|---|
| Silty clay loam(1) | 1,800 | 7.7 | 1.47 | 22 | 28 |
| Silty loam(1) | 1,400 | 6.2 | 0.76 | 16 | 26 |
| Sandy loam(1) | 150 | 5.6 | 3.06 | 53.2 | 9.2 |
| Clay loam(2) | 49 | 7.3 | 6.1 | 36 | 34 |
| Silty clay(2) | 23 | 7.6 | 1.8 | 16 | 43 |
| Arithmetic mean | 684 | | | | |
| Geometric mean | 212 | | | | |

(1): Technical dossier/Section III/Annex III.67.
(2): Technical dossier/Section III/Annex III.68.

The sorption of an anionic ionophore like semduramicin is dependent on a number of factors. The pKa of 5.39 indicates that above pH 5.39 the molecule occurs as a carboxylate anion. Normalising the sorption for the amount of organic carbon according to the EFSA guidance (EFSA, 2008a) is not appropriate since semduramicin might also sorb to other soil components. As an ionophore, it can also interact with cations like sodium or calcium during a sorption experiment. Therefore, as a reasonable worse-case estimate, the lowest $K_{oc}$ of 23 mL/g is used for further calculations.

Degradation

The degradation of [$^{14}$C]-semduramicin sodium was investigated in a GLP-compliant study, in accordance with OECD Guideline 307.[47] The rate of degradation and transformation pathway was evaluated in neutral sandy loam soil treated with [$^{14}$C]-semduramicin sodium at an application rate of

[44] Technical dossier/Section III/Annex III.67.
[45] Technical dossier/Section III/Annex III.68.
[46] Technical dossier/Section III/Annex III.72.
[47] Technical dossier/Section III/Annex III.71.

1.4 mg/kg soil (dry weight); an elevated treatment rate was utilised in order to facilitate detection, approximating to 14 mg/kg soil (dry weight). The rate of transformation was determined in three additional soil types (acidic sandy loam, silty loam and clay loam) at an application rate of 1.4 mg/kg soil. The samples were incubated in the dark at 20°C up to 120 days except for the silty loam soil which was incubated up to 176 days. The $^{14}CO_2$ evolution after 120 days varied between soils from 4% till 48%. Chromatographic analysis showed that semduramicin degraded to numerous minor unidentified compounds.

The $DT_{50}$ and $DT_{90}$ values for the parent compound were estimated using a single first-order kinetic model (CAKE, Version 1.4). The $DT_{50}$ values in neutral sandy loam, acidic sandy loam, silty loam and clay loam soils were reported as 74, 79, 66 and 47 days, respectively; the geometric mean was 66 days (Table 6). The corresponding $DT_{90}$ values were 244, 262, 289 and 156 days (geometric mean = 220 days).

**Table 6:** Half-life ($DT_{50}$ and $DT_{90}$) of semduramicin in different soils at 20 and 12°C

| Soil | $DT_{50}$ at 12°C (days) | $DT_{50}$ at 20°C (days) | $DT_{90}$ at 12°C (days) | $DT_{90}$ at 20°C (days) |
|---|---|---|---|---|
| Neutral sandy loam | 158 | 74 | 519 | 244 |
| Acidic sandy loam | 168 | 79 | 558 | 262 |
| Silt loam | 140 | 66 | 466 | 219 |
| Clay loam | 100 | 47 | 332 | 156 |
| Arithmetic mean | 140 | 66 | 469 | 220 |
| Geometric mean | 138 | 65 | 460 | 216 |

When the soil $DT_{50}$ and $DT_{90}$ is adjusted to an incubation temperature of 12°C using the Arrhenius equation,[48] the geometric mean of $DT_{50}$ and $DT_{90}$ is 138 days and 460 days, respectively. These values are used for further calculations.

*Fate in water*

The hydrolytic stability of semduramicin sodium was determined in a GLP-compliant study, in accordance with FAD Guideline 3.09.[49] Solutions of semduramicin sodium were buffered at pH 5, 6, 7, 8 and 9 at 25°C, and at regular intervals, samples were removed for analysis of parent drug. The corresponding half-life values were reported as 11, 36, 90, 115 and 77 days, respectively, and at environmentally relevant pH, semduramicin sodium is considered to be hydrolytically stable.

*Conclusion on fate and behaviour*

A $K_{oc}$ of 23 mL/g and a $DT_{50}$ for transformation of semduramicin of 138 days at 12°C were used for further calculations. Semduramicin sodium is considered to be hydrolytically stable.

## Predicted environmental concentrations

The PECs were calculated according to the FEEDAP technical guidance for assessing the safety of feed additives for the environment (EFSA, 2008a).

The input values used for initial PEC calculations were: 25 mg semduramicin sodium/kg broiler feed, molecular weight (MW) = 895, vapour pressure (VP (Pa)) = 6.67e-28, solubility (SOL) = 1,240 mg/L, $DT_{50}$ 138 days (at 12°C) and $K_{oc}$ = 23 L/kg. The calculated values are given in Table 7.

**Table 7:** Initial predicted environmental concentrations (PECs) of semduramicin in soil (μg/kg), groundwater (μg/L), surface water (μg/L) and sediment (μg/kg dry weight)

| Compartment | PEC |
|---|---|
| Soil | 130 |
| Ground water | 248 |
| Surface water | 83 |
| Sediment | 227 |

[48] The temperature correction was performed according to the scientific opinion of the Panel on Plant Protection Products and their Residues on a request from EFSA related to the default Q10 value used to describe the temperature effect on transformation rates of pesticides in soil (EFSA, 2007).

[49] Technical dossier/Section III/Annex III.73.

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

#### 3.2.5.2. Phase II

**Exposure assessment**

PECs calculation refined in Phase II

No data were provided for the refinement of the PEC based on degradation in manure. The following refinements were done.

*Refinement based on metabolism*

A study was performed to determine the bioactivity against coccidia of semduramicin and metabolites in chickens excreta.[50] Chickens (7–8 birds/sex) were administered for 7 days a feed supplemented with 25 mg semduramicin/kg. Excreta were collected from day 4 to day 7, homogenised and frozen until analysis. Samples from male excreta at day 6 were pooled and extracted (solvent). Total residues in the solvent and residual aqueous phase represented 75 and 21%, respectively. The solvent extract was analysed by HPLC, fractions were collected and equally split for radioactivity measurement and bioassay. After transfer to culture plates containing viable coccidia (*Eimeria tenella*) and an incubation period, the inhibition of the parasites was measured using an enzyme-linked immunosorbent assay (ELISA) endpoint of an antigen–antibody complex. Validation of the procedure showed that 96% of the radioactivity injected was recovered in the HPLC fractions, whereas the bioactivity assay showed a large but acceptable reproducibility for semduramicin amounts > 0.1 μg, corresponding to a background value of 28% inhibition of coccidia. The aqueous extract and the non-extractable radioactivity were devoid of bioactivity above the background level. A comparison of total residues and corresponding bioactivity in the fractionated extracts was carried out; about 11% (a calculated value of 15% in the solvent extract corrected for the proportion injected and the aqueous extract) of the total residues associated to semduramicin (about 5%) and one metabolite (6%) exhibiting close polarity harboured the whole bioactivity. Most the radioactivity was excreted with higher retention times that corresponds to metabolites of greater polarity (e.g. other mono- and multi-hydroxylated semduramicins) devoid (below the background) of anticoccidial activity and, very likely, of ionophoric activity.

The biological activity of ionophores depends on their ability to form complexes of different strength with cations and to penetrate and move across lipid bilayers of cells to exchange cations. Ionophore complexes exhibit a polar interior insuring the linkage to cations and a non-polar highly hydrophobic exterior which allows their free movement across lipid bilayers (EFSA FEEDAP Panel, 2017). Any modification of the polarity of the exterior part, e.g. resulting from its metabolic hydroxylation, impairs the movement across lipid-barriers and inhibits the ionophoric activity. This was retained recently for salinomycin sodium (EFSA FEEDAP Panel, 2017), where 20% of the ionophoric activity of the parent compounds was attributed to all derived metabolites. Based on the bioassay study results, the FEEDAP Panel reasonably assumes that the same proportion could be applied to semduramicin. The data available for the current assessment give different proportions of unchanged semduramicin in the excreta, either about 4%[50] or 8% (see Section 3.2.1.1). Taking a conservative approach, the residues harbouring ionophoric activity would represent 16% (8% each for semduramicin and its bioactive metabolite) plus 84 × 0.2 = 33% of the total residues. For the refinement of the PECs, considering the conservative approach taken, the dose of semduramicin can be refined based on 33% of the residual ionophoric activity of semduramicin and its active metabolite. Based on the above assumption, a dose of 25 × 0.33 = 8.25 mg/kg feed is used for further refinement calculation.

*Refinement of PECs for persistent compounds*

According to EFSA guidance (EFSA, 2008a), if a high persistence in soil is anticipated ($DT_{90}$ > 1 year), the potential for residues to accumulate in soil should be considered. This is the case for semduramicin, having a $DT_{90}$ at 12°C > 1 year.

The input values used for the refined PEC calculations based on metabolism and persistence were: 8.25 mg semduramicin sodium/kg broiler feed, MW = 895, (Pa) = 6.67e-28, SOL = 1,240 mg/L, $DT_{50}$ 138 days (at 12°C) and $K_{oc}$ = 23 L/kg. The calculated values are given in Table 8.

[50] Technical dossier/Section III/Annex III.70.

**Table 8:** Predicted Environmental Concentrations of semduramicin sodium in soil (μg/kg), groundwater (μg/L), surface water (μg/L) and sediment (μg/kg) refined for metabolism and persistent compounds.

| Compartment | PEC |
|---|---|
| Soil | 51 |
| Ground water | 97 |
| Surface water | 32 |
| Sediment | 89 |

*$PEC_{groudwater}$ refined with FOCUS*

The leaching of semduramicin to groundwater was calculated using FOCUS PEARL 3.1.2 according to the EFSA guidance (EFSA, 2008a). The input values were the same as in Table 8. The dose 8.25 mg semduramicin sodium/kg broiler feed gives an application rate of 0.13 kg/ha. The $K_{oc}$ of 23 L/kg was recalculated giving a $K_{om}$ of 13.5 and a Freundlich exponent of 0.9 was assumed. The application was annual incorporation with a depth of 20 cm. The average concentration of semduramicin closest to the 80th percentile at 1 m depth is reported in Table 9.

**Table 9:** Predicted environmental concentration in groundwater in leachate at 100 cm depth (μg/L) following the use of semduramicin sodium in chickens for fattening

| Application rate (kg/ha) | FOCUS scenario | $PEC_{gw}$ Winter cereals (μg/L) |
|---|---|---|
| 0.13 | Jokioinen | 13 |
| 0.13 | Piacenza | 7 |

FOCUS: FOrum for Co-ordination of *pesticide* models and their USe.

The FEEDAP Panel notes that using the selected sorption coefficient of 23 L/kg and the refinement based on metabolism the resulting concentrations of $PEC_{gw}$ are well above the value of 0.1 μg/L identified by the EU as quality standard.[51]

*Conclusions on PECs used for calculation*

The following values are used for the assessment: a $PEC_{soil}$ of 51 μg/kg, a $PEC_{surface\ water}$ of 32 μg/L and a $PEC_{sediment}$ of 89 μg/kg.

## Ecotoxicity studies

Toxicity to terrestrial compartment

*Effects on plants*

The effect of semduramicin sodium on terrestrial plants was investigated in a GLP-compliant study, in accordance with OECD 208.[52] The study was conducted using loamy sand soil and six plant species: soybean, ryegrass, oilseed rape, maize, tomato and radish. The concentration of semduramicin sodium was measured in each of the treatment solutions and the results demonstrated that the actual concentrations were comparable with nominal. As such, nominal concentrations were used for statistical analysis. Effect concentrations ($EC_{50}$ and $EC_{10}$) and no observed effect concentrations (NOEC) for emergence, shoot height and shoot weight are reported in Table 10. The lowest $EC_{10}$ has been selected for the assessment.

[51] Directive 2006/118/EC of the European Parliament and of the Council of 12 December 2006 on the protection of groundwater against pollution and deterioration. OJ L 372, 27.12.2006, p. 19.
[52] Technical dossier/Section III/Annex III.75.

**Table 10:** Semduramicin ecotoxicological effects data ($EC_{50}$, $EC_{10}$ and NOEC) for terrestrial plants (mg/kg dry soil)

| Endpoints | Species | $EC_{50}$ (mg/kg dry soil) | NOEC (mg/kg dry soil) | $EC_{10}$ (mg/kg dry soil) |
|---|---|---|---|---|
| Emergence | Soybean | 11.3 | 3.7 | / |
| | Ryegrass | > 33.3 | 33.3 | >33.3 |
| | Oilseed Rape | 6.4 | 3.7 | / |
| | Maize | 94.1 | 33.3 | / |
| | Tomato | > 33.3 | 11.1 | 12.3 |
| | Radish | 6.5 | 3.7 | / |
| Shoot Height | Soybean | > 33.3 | 33.3 | 18.5 |
| | Ryegrass | > 33.3 | 11.1 | 16.5 |
| | Oilseed Rape | > 11.1 | 11.1 | > 11.1 |
| | Maize | 56.9 | 11.1 | 7.4 |
| | Tomato | > 33.3 | 3.7 | 3.6 |
| | Radish | > 3.7 | 3.7 | > 3.7 |
| Shoot Weight | Soybean | 28.3 | 33.3 | 14.5 |
| | Ryegrass | 24.6 | 11.1 | 1.3 |
| | Oilseed Rape | > 11.1 | 11.1 | 5.8 |
| | Maize | > 11.1 | 11.1 | 5.8 |
| | Tomato | 9.1 | 3.7 | 1.3 |
| | Radish | > 3.7 | 3.7 | 1.6 |

*Effect on earthworms*

The subacute toxicity of semduramicin sodium to earthworms was investigated in a GLP-compliant study, in accordance with FDA TAD 4.12 Guideline.[53] Although some differences between this test guideline and OECD 207 exist, the study can be used to assess the toxicity of semduramicin sodium to earthworms. Earthworms (*Eisenia foetida foetida*) were exposed to semduramicin sodium in soil over a 28-day exposure period, at concentrations of 0, 0.1, 1, 10, 100 and 1,000 mg/kg dry weight of soil. Exposure concentrations were measured and nominal and actual values were comparable. Mortality was evaluated at 7, 14 and 28 days during the study; mortality was < 10% in the control group, and thus the test was considered valid. At 7 days, 100% mortality was noted at 1,000 mg/kg. In the remaining test concentrations, no statistically significant effects on mortality or growth were evident in comparison with the control group.

It was not possible to calculate the median lethal concentration ($LC_{50}$), due to the absence of effects at other concentrations. The acute NOEC for mortality and growth was established as 100 mg/kg.

*Nitrogen Transformation*

The effect of semduramicin sodium on soil nitrogen transformations was investigated in a GLP-compliant study, in accordance with OECD Guideline 216.[54] Exposure concentrations were based on initial PEC calculations which assumed that all feed is medicated in a given year. This introduces a further margin of safety with regards to any effect which semduramicin sodium may have on nitrogen transformations in soil. The study was conducted at semduramicin base equivalent[55] concentrations of 130 μg/kg dry soil (initial PEC) and 1,300 μg/kg dry soil (10x initial PEC). Since the actual and nominal concentrations were comparable, nominal concentrations were used for statistical analysis. At 28 days after treatment, concentrations of nitrate in treated soil at 130 and 1,300 μg/kg soil deviated from the control by less than 25% demonstrating that incorporation of semduramicin sodium into soil at worst-case $PEC_{soil}$ concentrations had no prolonged effect on soil nitrogen transformations.

[53] Technical dossier/Section III/Annex III.76.
[54] Technical dossier/Section III/Annex III.74.
[55] The applicant used base equivalent as a synonymous of active ingredient semduramicin sodium.

Toxicity to aquatic organisms

*Effect on algae*

The applicant provided a test on cyanobacterial species (see 'Additional information aquatic toxicity')[54] and not on green plankton algae. The $EC_{50}$ for growth established in the test in cyanobacterial species is 66 mg semduramicin sodium/L. The extrapolation of the effect from cyanobacterial species to algae is not possible. Moreover, the FEEDAP Panel noted that information from public literature[56] indicates that green algae are more sensitive to semduramicin than cyanobacteria. In particular, in a public release summary of the Australian registration authority a toxicity test with semduramicin sodium on the freshwater green alga, *Selenastrum capricornutum* (*Pseudokirchneriella subcapitata*) is reported with a NOAEL value of 10 mg/L corresponding to a NOEC of 10 mg/L. This non-GLP test could be used for the assessment as it provides basic information on the growth rate of tested species in the control.

*Effect on crustaceans*

The acute toxicity of semduramicin sodium to *Daphnia magna*, under static conditions, was investigated in a GLP-compliant study, in accordance with ASTM Standard E729-80, which was in force at the time the study was conducted.[57] Daphnids were exposed over a 48-h period to nominal semduramicin sodium concentrations of 6.5, 11, 18, 30 and 50 mg/L. A corresponding control was also included in the study. Exposure concentrations were measured by HPLC and were maintained over the 48-h exposure period; results were expressed with respect to mean measured concentrations. The test was considered valid since no mortality was observed in the control group and dissolved oxygen concentrations were > 3 mg/L. Under the conditions described above, the $EC_{50}$ value for semduramicin sodium was reported to be 38 mg/L.

*Effect on fish*

The acute toxicity of semduramicin sodium to rainbow trout (*Salmo gairdneri*) was investigated under static conditions in a GLP-compliant study in accordance with ASTM Standard E729-80, which was in force at the time the study was conducted.[58] Fish were exposed over a 96-h period to semduramicin sodium concentrations of 6.4, 11, 18, 30 and 50 mg/L (mean measured concentrations). A corresponding control was included in the study. Exposure concentrations were measured by HPLC and were maintained over the 96-h test period; results were based on mean measured concentrations. Dissolved oxygen concentrations were noted to be decreased during the first 24 h of exposure and aeration was subsequently introduced into the test vessels, which yielded air saturation values of > 60% for the remainder of the exposure. No mortality was recorded in the control group. Under the conditions described above, the $LC_{50}$ value for semduramicin sodium was established to be 32 mg/L.

*Additional information on aquatic toxicity*

The growth inhibition of algae exposed to semduramicin sodium was studied under static conditions in a GLP-compliant study in accordance with OECD Guideline 201.[54] *Anabaena floaquae* (cyanobacterial species) was exposed to semduramicin sodium concentrations of 4, 8, 18, 41 and 90 mg/L (initial measured concentrations) over a 72-h period. A corresponding control was also included in the study. Exposure concentrations were measured by HPLC and were maintained over the 72-h test period; results were based on initial measured concentrations. OECD validity criteria were met for the study, with the exception of the coefficient of variation (CV) for daily growth rates in the 0–24 h segment. The CV for daily growth rate between parallels was slightly higher (38.6%) than the value expected in the test guideline (35%) and this was thought to be due to low growth in the first 24 h period and chain formation, making it difficult to count a representative sample. After 24 h, growth increased and it was possible to take a representative sample for counting; CVs were less than 35%. This minor deviation was not considered to have affected the results and thus the test was considered valid. Under the described above, the 72 h $EC_{50}$ for growth was established as 66 mg/L.

The acute toxicity of semduramicin sodium to bluegill (*Lepomis macrochirus*), under static conditions, was investigated in a GLP-compliant study in accordance with ASTM Standard E729-80, which was in force at the time the study was conducted.[58] Fish were exposed to semduramicin sodium

[56] https://apvma.gov.au/sites/default/files/publication/14151-prs-semduramicin.pdf
[57] Technical dossier/Section III/Annex III.77.
[58] Technical dossier/Section III/Annex III.79.

concentrations of 7.6, 13, 22, 37, 62 and 100 mg/L (mean measured concentrations) over a 96 h period. A corresponding control was also included in the study. Exposure concentrations were measured by HPLC and were maintained over the 96-h test period; results were based on mean measured concentrations. Dissolved oxygen concentrations were noted to have decreased after 24 h of exposure and aeration was subsequently introduced into the test vessels from 48 h onwards; this resulted in an increase in air saturation values. One fish was died in the control group (5%) and, although air saturation values were lower than the target, the conditions were suitable for the experiment; the test was considered valid. Under the conditions described above, the $LC_{50}$ value for semduramicin sodium was established as 38 mg/L, equivalent to 37 mg/L.

*Effect on sediment dwelling organisms*

The toxicity of semduramicin sodium to chironomids was investigated in a GLP-compliant study, in accordance with OECD Guideline 218, using sediment which had been fortified with $^{14}$C-semduramicin sodium.[58] *Chironomus riparius* larvae were exposed to $^{14}$C-semduramicin sediment concentrations of 42.11, 81.09, 145.27, 264.80 and 516.16 mg/kg (dry weight basis), over a 28-day period, under static conditions. The number and sex of emerged adult chironomids were recorded daily during the exposure period. The distribution of radioactivity in the test system at Day 0 and Day 28 was determined by measuring levels of radioactivity in the sediment, interstitial and overlying water.

At Day 0, exposure concentrations ranged from 86% to 93% of the nominal values. However, by the end of the exposure period, the recovery of radioactivity had declined to 65–79% of nominal. Given that a good dose-response curve was obtained, it is considered that the results of the study adequately represent the toxicological response of chironomids to semduramicin sodium. The Day 0 mean measured test concentrations have been used for statistical analysis.

OECD guideline validity criteria for the test were met and emergence levels in the control and solvent control vessels were 105% and 103%, respectively.

The mean percentage emergence (emergence rate) and the time of emergence (development rate) data were analysed statistically.

The $EC_{50}$ for emergence rate was estimated as 215 mg semduramicin sodium/kg dry weight and the corresponding $EC_{10}$ was 102 mg semduramicin sodium/kg dry weight. Development rate was only inhibited at 516 mg/kg and it was not therefore possible to calculate an $EC_{50}$ value. The $EC_{50}$ for development rate is concluded to lie between 265 and 516 mg semduramicin sodium/kg dry weight.

Conclusions on the ecotoxic effect on soil, water and sediment

The applicant submitted studies which followed OECD guidelines (or other appropriate standardised methods) as proposed in the technical guidance for assessing the safety of feed additives for the environment (EFSA, 2008a). A test on green algae was not submitted; data from a public release summary were taken instead. Tests are valid and the 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 micro-organisms, earthworms and plants. Risk for terrestrial compartment was evaluated based on plant study resulting in the lowest $EC_{10}$ of 1.3 mg/kg. For the aquatic compartment, data are available for algae, aquatic invertebrates and fish. The lowest toxicity value of 10 mg/L for the aquatic compartment was found in a study on the effect on algae.

Ecotoxicological data for sediment-dwelling invertebrate *C. riparius* were provided for the sediment compartment resulting in an $EC_{10}$ of 102 mg/kg.

In the SCAN opinion (European Commission, 2002), several NOECs (or alternative parameters) were identified. The NOECs were for algae (growth: 10 mg/L), daphnids (reproduction: 0.33 mg/L), earthworms (biomass: 100 mg/kg), rainbow trout (96-h $LC_{50}$: 32 mg/L), plants (soybean germination: 0.32 mg/kg), soil microorganisms (100 mg/kg).

The FEEDAP Panel notes that the SCAN used a lower NOEC for plant toxicity (0.32 mg/kg) than that available for the current assessment (1.3 mg/kg). However, consequences of these differences are uncertain since the original data were not available.

### Risk characterisation (PEC/PNEC ratio)

The risk characterisation ratios for terrestrial, freshwater and sediment compartments are reported in the tables below (Tables 11, 12, 13).

**Table 11:** Risk characterisation (PEC/PNEC ratio) of semduramicin for terrestrial compartment

| Taxa | $PEC_{soil}$ (µg/kg) | $NOEC/EC_{10}$ (mg/kg) | AF | PNEC (µg/kg) | PEC/PNEC |
|---|---|---|---|---|---|
| **Earthworm**(1) | 51 | 100 | 1,000 | 100 | 0.51 |
| **Plants**(2) | | 1.3 | 10 | 130 | 0.34 |

AF: assessment factor.
(1): PNEC derived from acute NOEC.
(2): PNEC derived from $EC_{10}$.

**Table 12:** Risk characterisation (PEC/PNEC ratio) of semduramicin for freshwater compartment

| Taxa | $PEC_{surfacewater}$ (µg/L) | $NOEC/EC_{50}/LC_{50}$ (mg/L) | AF | PNEC (µg/L) | PEC/PNEC |
|---|---|---|---|---|---|
| **Algae**(1) *Selenastrum capricornutum (Pseudokirchneriella subcapitata)* | 32 | 10 | 1,000 | 10 | |
| **Aquatic invertebrates**(2) *Daphnia magna* | | 38 | | | 3.2 |
| **Fish**(3) *Brachydanio rerio* | | 32 | | | |

AF: assessment factor.
(1): PNEC derived from NOEC.
(2): PNEC derived from $EC_{50}$.
(3): PNEC derived from $LC_{50}$.

**Table 13:** Risk characterisation (PEC/PNEC ratio) of semduramicin for sediment

| Taxa | $PEC_{sediment}$ (µg/kg) | $EC_{10}$ (mg/kg) | AF | PNEC (µg/kg) | PEC/PNEC |
|---|---|---|---|---|---|
| **Sediment-dwelling invertebrates**(1) *Chironomus riparius* | 89 | 102 | 10 | 10,200 | 0.01 |

AF: assessment factor.
(1): PNEC derived from $EC_{10}$.

**Bioaccumulation and secondary poisoning**

Since semduramicin is partly metabolised in poultry and the log $K_{ow}$ of semduramicin is estimated as 2.63 at pH 7, the bioaccumulation and the risk for secondary poisoning is considered to be low.

#### 3.2.5.3. Conclusions on safety for the environment

The use of semduramicin from Aviax 5% in feed for chickens for fattening up to 25 mg/kg complete feed does not pose a risk for the terrestrial compartment. However, a risk for the aquatic compartment and for groundwater pollution cannot be excluded.

## 3.3. Efficacy

Efficacy data for coccidiostats should derive at least from two types of target animal experiments: (a) natural/artificial infection to simulate use conditions (e.g. floor pen studies with poultry), at least one of the locations should be in the EU, (b) actual use conditions in field trials, all should be done in the EU within the last five years. Anticoccidial sensitivity tests (AST) could replace field trials provided they follow the criteria mentioned in the relevant guidance document on coccidiostats and histomonostats (EFSA FEEDAP Panel, 2011a).[59]

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

### 3.3.1. Floor pen studies

Three floor pen studies, conducted in 2014, were submitted.[60] In each trial, day-old chickens (male Cobb 500 in trial 1; male and female Ross 308 in trials 2 and 3) were penned and distributed into four treatment groups: an uninfected untreated control (UUC) group, an infected untreated control (IUC) group and two infected treated groups receiving feed containing 20 mg (IT20) and 25 mg (IT25) semduramicin sodium from Aviax 5%/kg feed; dosage was analytically confirmed (see Table 14). The duration of the studies was 35/40/36 days; the experimental diets were fed from start until day 30/35/36 in trials 1, 2 and 3, respectively. All birds in the pen of infected groups were inoculated with recent field isolates of pathogenic *Eimeria* species (see Table 14). 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. Intestinal lesions were scored on three birds per pen using the method of Johnson and Reid (1970) (0 = no lesion, 1 = very mild, 2 = mild, 3 = moderate and 4 = severe).

The experimental unit for the statistical analyses was the pen regarding body weight, feed to gain ratio and oocysts number, individual bird data were used for lesion scores (except trial 2). In trial 1, all data were tested for normal distribution by the Shapiro–Wilk test, normally distributed data were further analysed by analysis of variance (ANOVA) followed by Bonferroni test for group differences, not normally distributed data were analysed by the Kruskal–Wallis test followed by Mann–Whitney U test. In trial 2, all data were examined by one-way ANOVA followed by the Newman–Keuls test for group differences. In trial 3, the zootechnical data including oocyst excretion were evaluated by ANOVA, lesion scores by the Kruskal–Wallis test.

**Table 14:** Experimental design of floor pen studies using Aviax 5%

| Trial | Replicates per treatment (Birds per replicate) | Inoculum characteristics | | | | Feed analysis (mg/kg feed)(1) semduramicin sodium |
|---|---|---|---|---|---|---|
| | | Month/Year and country of isolation | Intended dose per bird | | Day and mode of inoculation | |
| **1** | 8 (40) | 04/2014 Germany | 10,500 | *E. acervulina* | Day 14 via feed | 21.7/23.9/19.6 29/27/24.2 |
| | | | 56,000 | *E. tenella* | | |
| | | | 3,500 | *E. maxima* | | |
| | | 02/2014 Spain | 10,000 | *E. necatrix* | Day 23 via feed | |
| **2** | 16 (27–31) | 02/2012 Spain | 150,000 | *E. acervulina* | Day 15 via feed | 18.8/18.1/17.8 and 16.6 22.6/23.5/20.7 and 21.2 |
| | | | 40,000 | *E. tenella* | | |
| | | | 50,000 | *E. maxima* | | |
| | | | 22,000 | *E. necatrix* | | |
| **3** | 12 (25) | 04/2014 Netherlands | 40,000 | *E. acervulina* | Day 14 via syringe | 18.6/18.5/19.5 24.4/22.8/23.3 |
| | | | 25,000 | *E. tenella* | | |
| | | | 4,000 | *E. maxima* | | |
| | | 02/2012Spain | 10,000 | *E. necatrix* | Day 28 via syringe | |

(1): In trial 1, birds received starter diet from day 0 to 12, grower diet from day 12 to 22 and finisher diet from day 22 to 30; in trial 2, birds received starter diet from day 0 to 15, grower diet from day 16 to 21 and finisher diet from day 22 to 35; in trial 3, birds received starter diet from day 1 to 10, grower diet from day 10 to 29 and finisher diet from day 29 to day 36.

Overall mortality (including culled birds) was high in trial 1 (9.2% due to a failure in temperature regulation) and low in trials 2 and 3 (4.8 and 6.6%, respectively). Coccidiosis-related mortality seen in the IUC groups (4.4%, 8.3% and 13.3%, in the three trials respectively) indicated the pathogenic effect of the oocyst inoculation by both administration routes (via feed in trial 1 and 2 and via syringe in trial 3). Coccidiosis-related mortality was reduced numerically[61] in the IT20 and IT25 groups (see Table 17).

[60] FAD-2015-0037; Trial 1: Technical dossier/Section IV/Annex IV.5. Trial 2: Technical dossier/Section IV/Annex IV.1. and Trial 3: Technical dossier/Section IV/Annex IV.7.
[61] Statistical analysis was made on overall mortality.

Table 15 shows the intestinal lesion scores. A significant reduction of lesion scores was seen in birds of both IT groups compared to the IUC groups in trial 1 at day 20 in caeca, in trial 2 at day 21 in the upper and middle parts of duodenum and in caeca, at day 32 in the middle part of duodenum, and in caeca, in trial 3 at day 20 in caeca and at day 35 in the middle part of the duodenum.

**Table 15:** *Eimeria* infection related mean intestinal lesion scores in floor pen trials

| **Trial 1** | **6 days post-inoculation** | | | |
|---|---|---|---|---|
| | **Duodenum (*E. acervulina*)** | **Caecum (*E. tenella*)** | **Jejunum (*E. maxima*)** | ***E. necatrix*** |
| UUC | 0[b] | 0 | 0 | 0 |
| IUC | 0.96[a] | 1.96[a] | 0 | 1.29 |
| IT20 | 0.88[a] | 0.13[b] | 0 | 0 |
| IT25 | 0.54[a] | 0.04[b] | 0 | 0 |
| **Trial 2** | **6 days post-inoculation** | | | |
| | **Upper (*E. acervulina*)** | **Middle (*E. maxima or necatrix*)** | **Caecum (*E. tenella*)** | |
| UUC | 0 | 0 | 0 | |
| IUC | 1.9[a] | 1.7[a] | 2.1[a] | |
| IT20 | 0.2[b] | 0.2[b] | 0.4[b] | |
| IT25 | 0.1[b] | 0[c] | 0.1[c] | |
| **Trial 2** | **16 days post-inoculation** | | | |
| | **Upper (*E. acervulina*)** | **Middle (*E. maxima or necatrix*)** | **Caecum (*E. tenella*)** | |
| UUC | 0 | 0.1 | 0.1 | |
| IUC | 0 | 1.9[a] | 0.6[a] | |
| IT20 | 0 | 0.1[b] | 0.1[b] | |
| IT25 | 0 | 0.2[b] | 0.1[b] | |
| **Trial 3** | **6 days post-inoculation** | | | |
| | ***E. acervulina*** | ***E. maxima*** | ***E. tenella*** | ***E. necatrix*(1)** |
| UUC | 0 | 0 | 0 | 0 |
| IUC | 1.5 | 1.6 | 3.1[a] | 2.4[a] |
| IT20 | 1.9 | 1.4 | 2.7[b] | 0.8[b] |
| IT25 | 1.8 | 1.6 | 2.5[b] | 0.7[b] |

(1): Seven days post-inoculation.
a,b,c: means in a column in a study are significantly different ($p \leq 0.05$).

Results of oocyst excretion are presented in Table 16. Oocyst excretion was significantly reduced in the IT groups compared to the IUC group on day 20 in trial 1 and on day 23 and 25 in trial 2.

**Table 16:** Total number of *Eimeria* oocysts per gram of excreta in floor pen trials

| | **Day 20** | **Day 23** | **Day 25** | **Day 27** | **Day 30** | **Day 35** | **Day 36** |
|---|---|---|---|---|---|---|---|
| **Trial 1** | | | | | | | |
| UUC | 0[c] | 0 | 0 | 0 | 0 | – | – |
| IUC | 170,194[a] | 86,857 | 92,832 | 3,671 | 1,503 | – | – |
| IT20 | 60,945[b] | 14,296 | 46,663 | 1,678 | 150 | – | – |
| IT25 | 49,098[b] | 15,062 | 48,905 | 2,949 | 0 | – | – |
| **Trial 2**(1) | | | | | | | |
| UUC | – | nd | nd | – | – | – | – |
| IUC | – | 4.15 | 3.64 | – | – | – | – |
| IT20 | – | 2.52* | 2.02* | – | – | – | – |
| IT25 | – | 2.07* | nd* | – | – | – | – |

| | Day 20 | Day 23 | Day 25 | Day 27 | Day 30 | Day 35 | Day 36 |
|---|---|---|---|---|---|---|---|
| **Trial 3** | | | | | | | |
| UUC | 0 | 0 | – | – | – | 0 | 0 |
| IUC | 740,000 | 270,000 | – | – | – | 9,400 | 5,700 |
| IT20 | 660,000 | 290,000 | – | – | – | 2,200 | 5,400 |
| IT25 | 650,000 | 320,000 | – | – | – | 3,000 | 3,700 |

–: not determined; nd: not detected.
*: IT means with * are significantly different from the IUC group means ($p \leq 0.05$).
(1): The applicant reported the $\log_{10}$ OPG values.
a,b,c: means in a column in a study are significantly different ($p \leq 0.05$).

Table 17 summarises the results concerning mortality and zootechnical endpoints. In trial 1, body weight and feed to gain ratio were significantly better in the IT25 group compared with IUC group. In trial 2, feed intake, body weight, weight gain and feed to gain ratio were significantly better in both IT groups compared with IUC group. In trial 3, feed to gain ratios were significantly improved in both IT groups compared with IUC group.

**Table 17:** Performance data and mortality in floor pen trials with Aviax 5%(1)

| | Feed Intake(2) | Body weight (kg) | Weight Gain(3) (g) | Feed to gain ratio | Mortality(4)n |
|---|---|---|---|---|---|
| **Trial 1** | | | | | |
| UUC | 108.3 | 1.464$^{a}$ | – | 2.14$^{b}$ | 0 |
| IUC | 101.2 | 1.136$^{c}$ | – | 2.69$^{a}$ | 14/320 |
| IT20 | 99.6 | 1.219$^{bc}$ | – | 2.41$^{a}$ | 0 |
| IT25 | 98.6 | 1.253$^{b}$ | – | 1.96$^{b}$ | 1/320 |
| **Trial 2** | | | | | |
| UUC | 101.2$^{a}$ | 2.398$^{a}$ | 67.3$^{a}$ | 1.56$^{b}$ | 0 |
| IUC | 90.7$^{b}$ | 2.083$^{c}$ | 58.2$^{c}$ | 1.65$^{a}$ | 40/482 |
| IT20 | 100.1$^{a}$ | 2.367$^{a}$ | 66.4$^{a}$ | 1.56$^{b}$ | 0 |
| IT25 | 98.1$^{a}$ | 2.321$^{b}$ | 65.1$^{b}$ | 1.55$^{b}$ | 0 |
| **Trial 3** | | | | | |
| UUC | 3,410$^{c}$ | – | 2,313 | 1.48$^{a}$ | 0 |
| IUC | 3,032$^{a}$ | – | 1,942 | 1.56$^{c}$ | 40/300 |
| IT20 | 3,134$^{b}$ | – | 2,098 | 1.50$^{ab}$ | 2/300 |
| IT25 | 3,116$^{ab}$ | – | 2,068 | 1.51$^{b}$ | 1/300 |

–: not reported;
(1): Results refer to the period 0-35 days for trial 1 and 2 and 0-36 days for trial 3.
(2): In trial 1, results are reported as the average feed consumption per pen (kg); in trial 2 as cumulative average daily feed intake per bird (g); in trial 3 as cumulative feed intake per bird (g).
(3): Results in trial 2 are reported as daily weight gain, results in trial 3 are cumulative weight gain.
(4): Coccidiosis related mortality.
a,b,c: means in a column in a study are significantly different ($p \leq 0.05$).

### 3.3.2. Anticoccidial Sensitivity Tests

Four ASTs, performed in 2014, were submitted.[62] In each test, day-old male birds were randomly allocated to the experimental groups (see Table 18). All birds in all groups were inoculated with recent field isolates of pathogenic *Eimeria* species (no untreated uninfected control was used). In AST-1, four different inoculates were used containing a single *Eimeria* strain (*E. acervulina* or *E. maxima* or *E. tenella* or *E. necatrix*); accordingly, there were four infected untreated groups (IUC-*ac*, IUC-*max*, IUC-*ten* and IUC-*nec*), four infected treated groups receiving feed containing 20 mg semduramicin (IT20-*ac*, IT20-*max*, IT20-*ten* and IT20-*nec*) and four infected treated groups receiving feed containing 25 mg semduramicin (IT25-*ac*, IT25-*max*, IT25-*ten* and IT25-*nec*). In AST-2, two different inoculates were used containing two *Eimeria* species each (*E. maxima* and *E. tenella* or *E. acervulina* and *E. nectarix*); accordingly, there were two infected untreated groups (IUC-*max-ten* and IUC-*nec-*

[62] AST 1: Technical dossier/Section IV/Annex IV.6. AST 2: Annex IV.2. AST 3: Annex IV.3. AST 4: Annex IV.4.

*ac*), two infected treated groups receiving feed containing 20 mg semduramicin (IT20-*max-ten* and IT20-*nec-ac*) and two infected treated groups receiving feed containing 25 mg semduramicin (IT25-*max-ten* and IT25-*nec-ac*). In AST-3, one inoculum was used containing three *Eimeria* species (*E. acervulina*, *E. maxima* and *E. tenella*) and the treatment groups were three (IUC, IT20 and IT25). In AST-4, the three experimental groups (IUC, IT20 and IT25) were inoculated with only *E. necatrix* (Table 18). Animal health and mortality were monitored. Feed intake and body weight of the animals were measured, feed to gain ratio was calculated. Samples of excreta were analysed for oocyst excretion. Intestinal lesions were scored following the Johnson and Reid (1970) scoring system on three (AST-1 and AST-2) or five birds (AST-3 and AST-4) per replicate.

Statistical analysis used the pen as experimental unit for body weight, feed intake, feed to gain ratio, mortality and oocyst excretion. Lesion scores were assessed on an individual basis. All analyses included the control group with another ionophore coccidiostat. In AST-1, all data except lesion score were analysed by ANOVA, lesion score by the Kruskal–Wallis test. In AST-2, one-way ANOVA was applied to all endpoints. When analysis of variances was significant, the difference between treated groups was assessed by the Dunnett test or by the least significant difference (LSD) test. In AST- 3 and 4, data were tested for normal distribution by Shapiro-Wilk test. Normally distributed data were further analysed by ANOVA and Bonferroni test, not normally distributed data by Kruskal–Wallis and Mann–Whitney U test. Mortality was analysed by Pearson's chi-square test.

**Table 18:** Overview of anticoccidial sensitivity tests performed with Aviax 5%(1)

| AST | Replicates per treatment (Birds per replicate) | Breed of chickens for fattening | Inoculum characteristics | | | | | Period of the study (day of life) | Feed analysis (mg/kg feed) semduramicin sodium |
|---|---|---|---|---|---|---|---|---|---|
| | | | Month/Year and country of isolation | Intended dose per bird | | Groups receiving the inoculum | Day and mode of inoculation | | |
| **1** | 4 (16) | SPF GD Animal Healths, NL | 02-2014 The NL | $72.5 \times 10^3$ | *E. acervulina* | IUC-*ac* IT20-*ac* IT25-*ac* | Day 15 via syringe | 13-21 | 20.4 24.0 |
| | | | 02-2014 The NL | $8.5 \times 10^3$ | *E. maxima* | IUC-*max* IT20-*max* IT25-*max* | | | |
| | | | 03-2014 The NL | $40.0 \times 10^3$ | *E. tenella* | IUC-*ten* IT20-*ten* IT25-*ten* | | | |
| | | | Spain | $15.0 \times 10^3$ | *E. necatrix* | IUC-*nec* IT20-*nec* IT25-*nec* | | | |
| **2** | 6 (8) | Ross 308 | 02-2012 Spain | 50,000 | *E. maxima* | IUC-*max-ten* IT20-*max-ten* IT25-*max-ten* | Day 15 via gavage | 9-28 | 19.4-19.7 23.5-25.0 |
| | | | 02-2012 Spain | 10,000 | *E. tenella* | | | | |
| | | | 02-2012 Spain | 10,000 | *E. necatrix* | IUC-*nec-ac* IT20-*nec-ac* IT25-*nec-ac* | | | |
| | | | 02-2012 Spain | 150,000 | *E. acervulina* | | | | |
| **3** | 6 (20) | Cobb 500 | 04-2012 Germany | 56,000 | *E. tenella* | IUC, IT20, IT25 | Day 14 via feed | 10-21 | 21.5 26.6 |
| | | | | 10,500 | *E. acervulina* | | | | |
| | | | | 3,500 | *E. maxima* | | | | |
| **4** | 6 (20) | Cobb 500 | 03-2013 Germany | 10,000 | *E. necatrix* | IUC, IT20, IT25 | Day 14 via feed | 10-21 | 20.4 24.4 |

(1): In all ASTs, in addition to Aviax 5%, another ionophore coccidiostat was tested. Those results were not considered.

Coccidiosis-related mortality was not reported in AST-1 and AST-2, it was low in AST-3 (3/120 for IUC) and not observed in AST-4.

Results of intestinal lesion scores are presented in Table 19 and 20. A significant reduction of intestinal lesion scores (*E. necatrix*) by the Aviax treatment (IT20-*ac*, IT25-*ac* compared to IUC-*ac*) was observed in AST-1. In AST-4, the *E. necatrix* related lesion score was also significantly reduced in both IT groups.

In AST-2, the statistical analysis was performed on the lesion score of different intestinal sections. The lesion scores in the IT groups infected with *E. maxima*/*E. tenella* or *E. necatrix*/*E. acervulina* were found significantly lower than in the corresponding IUC group.

In AST-3, the lesion scores in the duodenum of both IT groups were significantly lower than in the IUC group. Significantly lower lesion scores in jejunum and caecum were seen only in the IT25 group, but not in the IT20 group.

**Table 19:** Species specific mean lesion scores in AST-1 and AST-4 at day 21

| **AST-1** | |
|---|---|
| | ***E. acervulina*** |
| IUC-*ac* | 1.13 |
| IT20-*ac* | 1.20 |
| IT25-*ac* | 1.56 |
| | ***E. maxima*** |
| IUC-*max* | 2.63 |
| IT20-*max* | 1.56 |
| IT25-*max* | 2.44 |
| | ***E. tenella*** |
| IUC-*ten* | 3.13 |
| IT20-*ten* | 2.94 |
| IT25-*ten* | 2.63 |
| | ***E. necatrix*** |
| IUC-*nec* | 1.73[a] |
| IT20-*nec* | 0.06[b] |
| IT25-*nec* | 0.00[b] |
| **AST-4** | |
| | ***E. necatrix*** |
| IUC | 0.77[a] |
| IT20 | 0[b] |
| IT25 | 0[b] |

a,b,c: means in a column in a study are significantly different ($p \leq 0.05$).

**Table 20:** Mean lesion scores in different intestinal sections in AST-2 and AST-3 (mixed infection) at day 21

| | Intestinal sections examined | | |
|---|---|---|---|
| | **Upper** | **Middle** | **Caeca** |
| **AST-2** | | | |
| IUC-*max-ten* | – | 2.2[a] | 2.3[a] |
| IT20-*max-ten* | – | 0.0[b] | 0.2[b] |
| IT25-*max-ten* | – | 0.1[b] | 0.3[b] |
| IUC-*nec-ac* | 2.1[a] | 2.1[a] | – |
| IT20-*nec-ac* | 0.3[b] | 0.2[b] | – |
| IT25-*nec-ac* | 0.1[b] | 0.1[b] | – |
| **AST-3** | | | |
| | **Duodenum** | **Jejunum** | **Caecum** |
| IUC | 1.07[a] | 0.17[a] | 0.17[a] |
| IT20 | 0.17[b] | 0.03[ab] | 0.03[ab] |
| IT25 | 0.23[b] | 0[b] | 0[b] |

–: not detected.
a,b,c: means in a column in a study are significantly different ($p \leq 0.05$).

Table 21 shows the results of oocyst excretion. About 1 week after inoculation, the results of oocyst counts indicated successful *Eimeria* infection. OPG was significantly reduced by the Aviax treatment in AST-2 and AST-4 but not in AST-1 and AST-3. Whereas both IT groups (IT20 and IT25) showed a lower OPG than the IUC group in AST-4 and in AST-2 using *E. maxima*/*E. tenella* as inoculum, a significant reduction of OPG in AST-2 using *E. necatrix*/*E. acervulina* was seen only for IT25.

**Table 21:** Results of oocyst counts per gram (OPG) in ASTs

| **AST-1** | |
|---|---|
| | **Day 21** |
| IUC-*ac* | $4.5 \times 10^6$ |
| IT20-*ac* | $5.3 \times 10^6$ |
| IT25-*ac* | $4.1 \times 10^6$ |
| IUC-*max* | $2.2 \times 10^4$ |
| IT20-*max* | $1.3 \times 10^4$ |
| IT25-*max* | $1.6 \times 10^4$ |
| IUC-*ten* | $3.7 \times 10^4$ |
| IT20-*ten* | $2.1 \times 10^4$ |
| IT25-*ten* | $0.9 \times 10^4$ |
| IUC-*nec* | $3.4 \times 10^3$ |
| IT20-*nec* | $0.1 \times 10^3$ |
| IT25-*nec* | $0.8 \times 10^3$ |

| **AST-2** | | | |
|---|---|---|---|
| | **Day 20** | **Day 22** | **Day 24** |
| IUC-*max-ten* | 631[a] | 154,882[a] | 28,840[a] |
| IT20-*max-ten* | 79[b] | 19,498[b] | 1,230[b] |
| IT25-*max-ten* | nd[b] | 9,333[ab] | 589[b] |
| IUC-*nec-ac* | 81,283[a] | 33,884[a] | 12,023[b] |
| IT20-*nec-ac* | 6,918[ab] | 19,953[ab] | 1,738[ab] |
| IT25-*nec-ac* | 3,020[b] | 490[b] | 245[b] |

| AST-3 | | | |
|---|---|---|---|
| | **Day 19** | **Day 20** | **Day 21** |
| IUC | 5,438 | 854,712 | 1,025,861 |
| IT20 | 10,184 | 317,478 | 530,362 |
| IT25 | 1,086 | 323,215 | 668,126 |
| **AST-4** | | | |
| | **Day 19** | **Day 20** | **Day 21** |
| IUC | 53,578[a] | 67,923[a] | 25,168[a] |
| IT20 | 13[b] | 87[b] | 24[b] |
| IT25 | 24[b] | 25[b] | 50[b] |

nd: not detected.
a,b,c: means in a column in a study are significantly different ($p \leq 0.05$).

Body weight, daily weight gain and feed to gain ratio were not influenced by the coccidiostatic treatment in AST-1 and AST-3. In AST-4, feed to gain ratio was significantly better in IT25 (1.27) than in IUC (1.57) and IT20 (1.55). In AST-2, body weight gain and feed to gain ratio in the one-week period after infection was significantly better in both IT-groups than in the IUC group for both inoculums (*E. tenella*/*E. maxima* and *E. necatrix*/*E. acervulina*) (Table 22).

**Table 22:** Zootechnical parameters of AST-2 (Days 15–21)

| | **Feed Intake (g)** | **Body weight gain (g)** | **Feed to gain ratio** |
|---|---|---|---|
| **AST-2** | | | |
| IUC-*max-ten* | 468 | 273[b] | 1.72[a] |
| IT20-*max-ten* | 499 | 372[a] | 1.34[b] |
| IT25-*max-ten* | 501 | 370[a] | 1.35[b] |
| IUC-*nec-ac* | 448[b] | 264[b] | 1.79[a] |
| IT20-*nec-ac* | 507[a] | 358[a] | 1.42[b] |
| IT25-*nec-ac* | 482[ab] | 350[a] | 1.38[b] |

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

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

One publication was provided which reported sensory characteristics of broiler meat after use of semduramicin in feed (Islam et al., 2007). Semduramicin did not influence the sensory characteristics of broiler meat.

### Conclusion on efficacy

The potential of the lowest proposed dose of semduramicin, 20 mg/kg complete feed to prevent coccidiosis in chickens for fattening was shown in three floor pen studies with artificial infection by significant improvements of intestinal lesion score and in two by a reduction of oocyst excretion. Among the zootechnical data supporting the coccidiosis controlling effect of Aviax 5%, feed to gain ratio was significantly improved by 25 mg semduramicin/kg in all three floor pen studies but only in one by 20 mg/kg.

Evidence for the anticoccidial activity of 20 mg semduramicin/kg feed was also provided by four ASTs, where the lesion score was significantly lower than in the untreated group. The same dose also significantly reduced oocyst excretion in two ASTs.

It is concluded that semduramicin sodium from Aviax 5% at a minimum dose of 20 mg/kg feed has the potential to effectively control coccidiosis in chickens for fattening.

## 3.4. Post-market monitoring

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

## 4. Conclusions

The approaches used for taxonomical identification consistently place the strain ATCC 53664 in the genus *Actinomadura* but cannot unequivocally assign to a valid taxonomic species of this genus, suggesting that the strain belongs to a new taxonomic unit within the genus *Actinomadura*. The FEEDAP Panel cannot conclude on the absence of genetic determinants for antimicrobial resistance in *Actinomadura* spp. ATCC 53664.

In the absence of a tolerance study in chickens for fattening performed according to the current EU standards, the FEEDAP Panel cannot conclude on the safety of Aviax 5%, containing semduramicin mycelium, for chickens for fattening.

Semduramicin sodium is active against certain Gram-positive bacteria, while Gram-negative bacteria are resistant. The use of semduramicin sodium as feed additive is unlikely to increase shedding of *Salmonella*, *E. coli* and *Campylobacter* and to induce resistance and cross resistance to antimicrobials used of human and animal relevance.

Semduramicin sodium in the feed is not compatible with the concurrent use of tiamulin, although the interaction is less severe than that observed with other polyether coccidiostats affecting only performance parameters.

Semduramicin sodium is significantly absorbed, extensively metabolised and mainly excreted through the bile and faeces. Unchanged semduramicin is by far (45%) the main residue in the liver, each of the other 19 metabolites representing less than 10% of the total radioactivity in this tissue. Unchanged semduramicin represents about 16% of total radioactivity in the excreta, metabolite F 24% and the other polar metabolites less than 10% each. Metabolic pathways are similar in the rat, the dog and chicken. Semduramicin is the marker residue.

Mycelial semduramicin sodium is not genotoxic. There is no evidence that the mycelial semduramicin sodium is more toxic than the crystalline by oral administration. An ADI of 0.00125 mg/kg is set for the crystalline semduramicin sodium based on a NOAEL of 0.125 mg/kg bw per day, established from a 2-year toxicity study in rats for decreased serum protein and sodium concentrations and applying an uncertainty factor of 100. The ADI set for the crystalline semduramicin is applicable to mycelial semduramicin.

The use of semduramicin sodium at a dietary concentration of 25 mg/kg complete feed for chickens for fattening is safe for the consumer provided a withdrawal time of 24 h is respected. The MRLs for the different tissues are considered necessary. However, no proposal for the MRLs could be made due to lack of data for marker residue concentrations after 24 h withdrawal and limited sensitivity of the analytical method.

The FEEDAP Panel cannot conclude on the irritancy of Aviax 5% to skin and eye and on the potential for dermal and respiratory sensitisation. Model calculations on inhalation exposure of persons handling the additive indicate a serious risk.

The use of semduramicin sodium from Aviax 5% in feed for chickens for fattening up to 25 mg/kg complete feed does not pose a risk for the terrestrial compartment. However, a risk for the aquatic compartment and for groundwater pollution cannot be excluded.

Aviax 5% at a minimum dose of 20 mg/kg feed has the potential to effectively control coccidiosis in chickens for fattening.

## 5. Recommendations

The simultaneous use of semduramicin and tiamulin should be avoided.

## Documentation provided to EFSA

1) Semduramicin sodium (Aviax 5%) for chickens for fattening. February 2014 and October 2015. Phibro Animal Health S.A.
2) Semduramicin sodium (Aviax 5%) for chickens for fattening. Supplementary information. December 2014. Phibro Animal Health S.A.
3) Semduramicin sodium (Aviax 5%) for chickens for fattening. Supplementary information. October 2015. Phibro Animal Health S.A. Not complete.
4) Semduramicin sodium (Aviax 5%) for chickens for fattening. Supplementary information. December 2016. Phibro Animal Health S.A.

5) Semduramicin sodium (Aviax 5%) for chickens for fattening. Supplementary information. June 2017. Phibro Animal Health S.A.
6) Evaluation reports of the European Union Reference Laboratory for Feed Additives on the Methods(s) of Analysis for semduramicin sodium.
7) Comments from Member States.

## Chronology

| Date | Event |
|---|---|
| 13/02/2014 | Dossier receveid by EFSA (FAD-2014-0009) |
| 31/03/2014 | Reception mandate from the European Commission (FAD-2014-0009) |
| 14/08/2014 | Application validated by EFSA – Start of the scientific assessment (FAD-2014-0009) |
| 30/10/2014 | Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. *Issues: characterisation, safety for the consumer and efficacy* (FAD-2014-0009) |
| 14/11/2014 | Comments received from Member States (FAD-2014-0009) |
| 11/11/2014 | Reception of the Evaluation report of the European Union Reference Laboratory for Feed Additives (FAD-2014-0009) |
| 09/12/2014 | Reception of supplementary information from the applicant - Scientific assessment re-started (FAD-2014-0009) |
| 08/06/2015 | Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended *Issues: Characterisation of the production strain* (FAD-2014-0009) |
| 02/10/2015 | Reception of supplementary information from the applicant - The information was considered not complete. Applicant was informed via email dated 12/11/2015. The scientific assessment remained suspended (FAD-2014-0009) |
| 16/10/2015 | Dossier received by EFSA (FAD-2015-0037) |
| 23/10/2015 | Reception mandate from the European Commission (FAD-2015-0037) |
| 23/06/2016 | Application validated by EFSA – Start of the scientific assessment (FAD-2015-0037) |
| 27/06/2016 | Applicant informed that the two applications will be assessed in one single opinion (FAD-2014-0009 and FAD-2015-0037) |
| 23/09/2016 | Comments received from Member States (FAD-2015-0037) |
| 14/10/2016 | Reception of the Evaluation report of the European Union Reference Laboratory for Feed Additives (FAD-2015-0037) |
| 19/12/2016 | Reception of supplementary information from the applicant - Scientific assessment re-started |
| 24/03/2017 | Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended *Issues: Safety for the target species* |
| 19/06/2017 | Reception of supplementary information from the applicant - Scientific assessment re-started |
| 14/06/2018 | Opinion adopted by the FEEDAP Panel. End of the Scientific assessment |

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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
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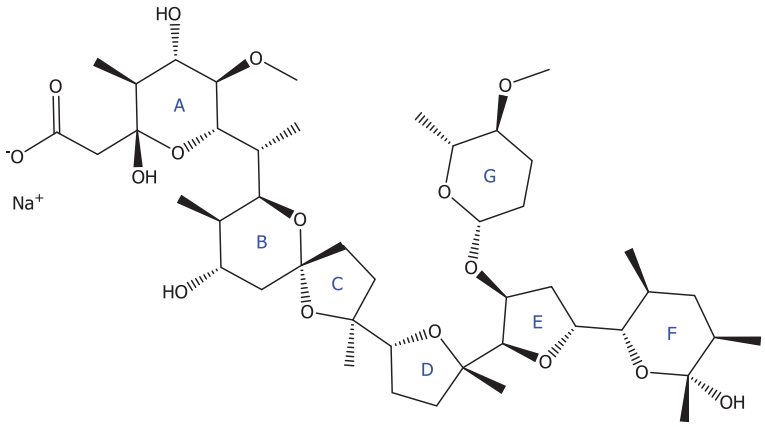
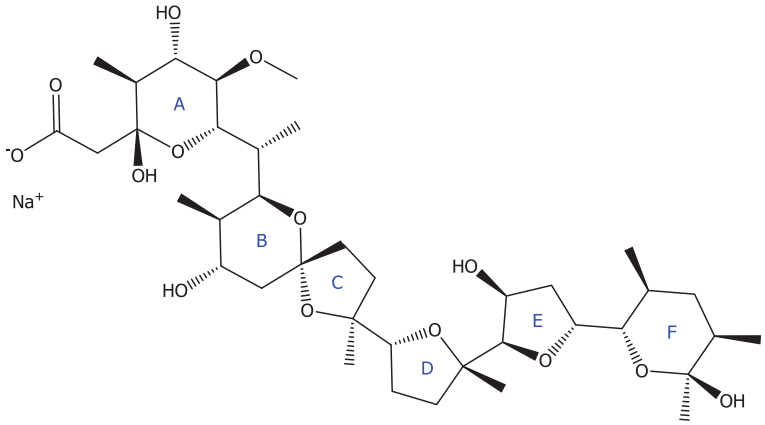
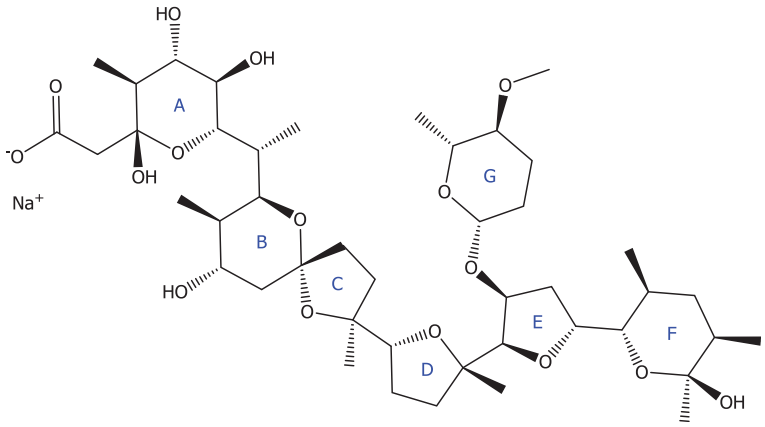
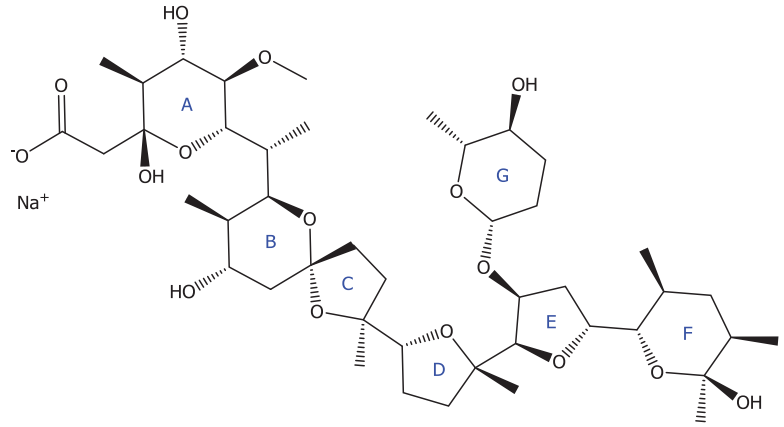
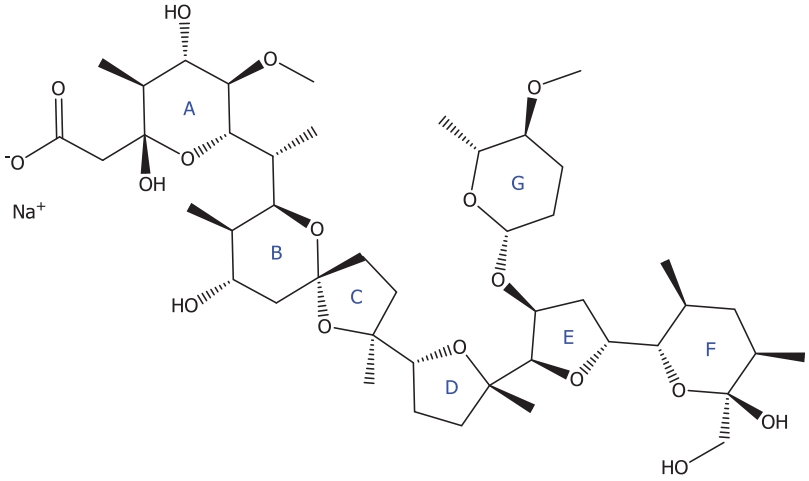
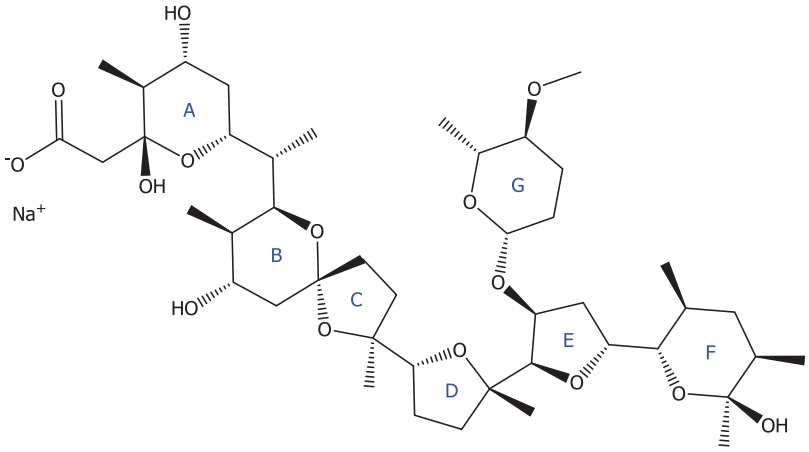
## Abbreviations

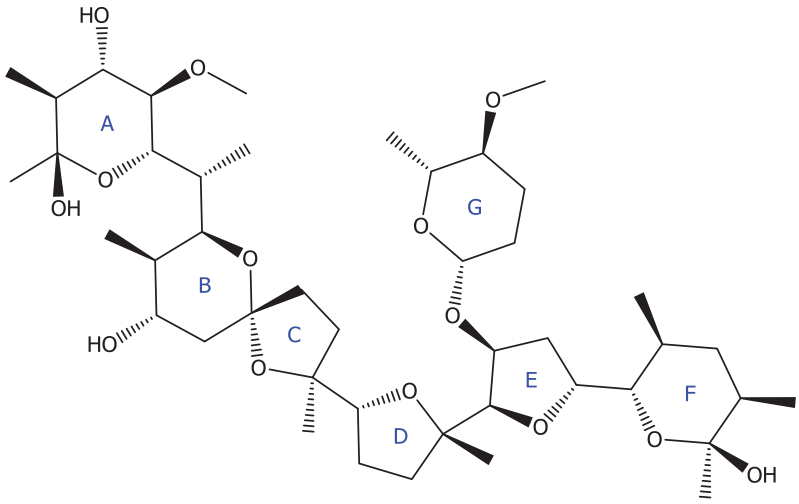
| | |
|---|---|
| ADI | acceptable daily intake |
| ADME | absorption, distribution, metabolism and excretion |
| AF | assessment factor |
| ATCC | American Type Culture Collection |
| bw | body weight |
| CAS | Chemical Abstracts Service |
| CFU | colony forming unit |
| CV | coefficient of variation |
| DITR | daily intake of total residues |
| DITRMRL | dietary intake of total residues calculated from the MRLs |
| DL-PCBs | dioxin-like polychlorinated biphenyls |
| $DT_{50}$ | Disappearance Time 50 (the time within which the concentration of the test substance is reduced by 50%) |
| $DT_{90}$ | Disappearance Time 90 (the time within which the concentration of the test substance is reduced by 90%) |
| $EC_{50}$ | median effective concentration |
| $ErC_{10}$ | median effective concentration which results in a 10% reduction in growth rate |
| $ErC_{50}$ | median effective concentration which results in a 50% reduction in growth rate |
| EURL | European Union Reference Laboratory |
| FDA | Food and Drug Administration |
| FEEDAP | EFSA Panel on Additives and Products or Substances used in Animal Feed |
| FOCUS | FOrum for Co-ordination of pesticide models and their USe |
| GLP | Good Laboratory Practices |
| $K_{oc}$ | adsorption or desorption coefficient corrected for soil organic carbon content |
| $LC_{50}$ | median lethal concentration |
| log $K_{ow}$ | octanol/water partition coefficient |
| LOD | limit of detection |

| | |
|---|---|
| LOQ | limit of quantification |
| LSD | least significant difference |
| MIC | minimum inhibitory concentration |
| MW | molecular weight |
| MRL | maximum residue limit |
| NOAEL | no observed adverse effect level |
| NOEC | no observed effect concentration |
| NOEL | no observed effect level |
| OECD | Organisation for Economic Co-operation and Development |
| OPG | oocyst counts per gram |
| PCDD/F | polychlorinated dibenzo-*p*-dioxins and dibenzofurans |
| PEC | predicted environmental concentration |
| $pK_a$ | Dissociation constant |
| PNEC | predicted no effect concentration |
| RH | relative humidity |
| RMTR | ratio marker to total residues |
| SCAN | Scientific Committee on Animal Nutrition |
| SD | standard deviation |
| SOL | solubility |
| TRC | total residue concentration |
| VP | vapour pressure |
| WHO | World Health Organization |

# Appendix A – Fermentation related impurities in mycelial semduramicin sodium

| | |
|---|---|
|  | epimer |
|  | aglycone |
|  | 'A' ring desmethyl semduramicin sodium |

| | |
|---|---|
|  | 'G' ring desmethyl semduramicin sodium |
|  | hydroxysemduramicin sodium |
|  | 'A' ring desmethoxyl semduramicin sodium |

| | |
|---|---|
|  | descarboxyl semduramicin |

## Appendix B – Estimation of user exposure to semduramicin sodium from the additive Aviax 5%, including consideration of using a filter mask FF P2 or FF P3 as a preventative measure

| Calculation | Identifier | Description | Amount | Source |
|---|---|---|---|---|
| | *a* | Semduramicin concentration in dust (mg/kg) | 10 2000 | Technical dossier |
| | *b* | Dusting potential ($g/m^3$) | 2.245 | Technical dossier |
| $a \times b$ | *c* | Semduramicin in the air ($mg/m^3$) | 228.99 | |
| | | | | |
| | *d* | No of premixture batches made/working day | 10 | EFSA Guidance on user safety (EFSA FEEDAP Panel, 2012b) |
| | *e* | Time of exposure (s) per production of one batch | 20 | EFSA Guidance on user safety (EFSA FEEDAP Panel, 2012b) |
| $d \times e$ | *f* | Total duration of daily exposure/worker (s) | 200 | |
| | *g* | Uncertainty factor | 2 | EFSA Guidance on user safety (EFSA FEEDAP Panel, 2012b) |
| $f \times g$ | *h* | Refined total duration of daily exposure/ worker (s) | 400 | |
| *h*/3 600 | *i* | Refined total duration of daily exposure (h) | 0.11 | |
| | *j* | Inhaled air ($m^3$) per hour | 1.25 | EFSA Guidance on user safety (EFSA FEEDAP Panel, 2012b) |
| $j/8 \times i$ | *k* | Inhaled air during exposure ($m^3$) | 0.14 | |
| $c \times k$ | *l* | Semduramicin (mg) inhaled during exposure per eight-hour working day | 32 | |
| | | | | |
| *l*/10 | | Semduramicin inhaled (mg) per eight-hour working day reduced by filter mask FF P2 (reduction factor 10) | 3 | |
| *l*/20 | | Semduramicin inhaled (mg) per eight-hour working day reduced by filter mask FF P3 (reduction factor 20) | 1.6 | |

# Annex A – Executive Summary of the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Method(s) of Analysis for semduramicin sodium[63]

*Aviax 5%*, is a *feed additive* currently authorized for chickens for fattening by Commission Regulation (EC) No 1443/2006 belonging to the group "Coccidiostats and other medicinal substances" listed in Chapter I of Annex B of Directive 70/524/EEC. In the current application a modification of the existing authorisation under article 13(3) of Regulation (EC) No 1831/2003 is requested. *Aviax 5%* consists of 5% (w/w) *semduramicin,* 3-5% (w/w) mineral oil as dust control agent, 4% (w/w) sodium carbonate as stabilising agent, 2% (w/w) sodium alumino silicate as anticaking and soybean mill run as carrier. The *feed additive* is intended to be incorporated through *premixtures* or directly into *feedingstuffs*. The Applicant proposed a concentration of *semduramicin* in *feedingstuffs* ranging from 20 to 25 mg/kg.

For the quantification of *semduramicin* the Applicant submitted two single-laboratory validated methods based on High Performance Liquid Chromatography (HPLC) with: refractive index detection (RI) for the *feed additive*; and post-column derivatisation coupled to spectrophotometric detection (PCD-UV-Vis) for *premixes* and *feedingstuffs.* The EURL identified instead the ring-trial validated method EN 16158 for the quantification of *semduramicin* in *feedingstuffs*, based on reverse-phase HPLC using mass spectrometry detection (MS) or PCD-UV-Vis. This method was slightly adapted to quantify *semduramicin* in *premixtures* and in the *feed additive* (*Aviax 5%*) with similar method performance characteristics to the original EN 16158 method.

Based on the experimental evidence available the EURL recommends for official control the HPLC-PCD-UV-Vis and/or the HPLC-MS method for the quantification of *semduramicin* in the *feed additive, 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.

[63] The full report is available on the EURL website: https://ec.europa.eu/jrc/sites/default/files/finrep-fad-2014-0009-aviax.pdf

# Annex B – Executive Summary of the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Method(s) of Analysis for semduramicin sodium[64]

*Aviax® 5%* is a feed additive - belonging to the "Coccidiostats and other medicinal substances" group listed in Directive 70/524/EEC - currently authorized for chickens for fattening by Commission Regulation (EC) No 1443/2006. In the current application authorisation is sought under articles 10(2) and 13(3) of the Regulation (EC) No 1831/2003. *Aviax® 5%* consists of 5% (w/w) *semduramicin* as active substance, mineral oil as dust control agent, sodium carbonate as stabilising agent, sodium alumino silicate as anticaking agent and soybean mill run as carrier. The feed additive is intended to be incorporated through *premixtures* or directly into *feedingstuffs*. The Applicant proposed a content of *semduramicin* in *feedingstuffs* ranging from 20 to 25 mg/kg. Furthermore the Applicant proposed MRLs for *semduramicin* in chicken tissues of 45 μg/kg in muscle, 150 μg/kg in kidney and 450 μg/kg in liver and skin/fat *tissues*.

For the quantification of *semduramicin* in the feed additive the Applicant submitted a single-laboratory validated and further verified method based on High Performance Liquid Chromatography coupled to refractive index detection (HPLC-RI).

For the quantification of *semduramicin* in *premixes* and *feedingstuffs* the Applicant submitted another single-laboratory validated method based on normal phase HPLC with post-column derivatisation coupled to spectrophotometric detection (HPLC-PCD-UV-Vis). The EURL identified instead the ring-trial validated method EN 16158, based on reversed-phase HPLC-MS or HPLC-PCD-UV-Vis. This method was slightly adapted to quantify *semduramicin* in the product (*Aviax® 5%*) and *premixtures*, resulting in performance characteristics similar to those reported for the CEN method. Based on the experimental evidence available the EURL recommends for official control the HPLC-PCD-UV-Vis or the HPLC-MS methods for the quantification of *semduramicin* in the feed additive, *premixtures* and *feedingstuffs*.

For the quantification of *semduramicin* in chicken *tissues* the Applicant submitted a single laboratory validated and further verified method based on reversed-phase HPLC coupled to a triple quadrupole mass spectrometer in electrospray ionisation mode using matrix matched standards (HPLC-MS/MS). Based on the performance characteristics provided, the EURL recommends for official control the method proposed by the Applicant or any equivalent analytical methods complying with the requirements set by Commission Decision 2002/657/EC to enforce the *semduramicin* MRLs in the relevant *tissues*.

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

[64] The full report is available on the EURL website: https://ec.europa.eu/jrc/sites/jrcsh/files/finirep_fad-2015-0037_aviax.pdf