Safety and efficacy of Sacox® microGranulate (salinomycin sodium) for rabbits for fattening

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Abstract

Sacox® microGranulates, containing salinomycin sodium (SAL-Na), for chickens for fattening and chickens reared for laying have been recently re-evaluated by Panel on Additives and Products or Substances used in Animal Feed. Following an urgent request from the European Commission, the safety and efficacy of the product when fed to rabbits for fattening was assessed based on the available data submitted by the applicant at the beginning of the assessment. SAL-Na is largely absorbed and metabolised. Metabolites have a reduced ionophoric activity. SAL is the marker residue. SAL-Na is not genotoxic and not a carcinogen. A no observed adverse effect level (NOAEL) of 0.5 mg/kg body weight (bw) per day is derived from a study in dogs. Only data on feed intake and body weight were available to conclude on the safety of SAL for rabbits. Levels of 35 mg SAL/kg feed and higher were not tolerated by growing rabbits. The Panel considers the available data indicate that the additive is tolerated by rabbits for fattening up to 25 mg/kg. The safety of SAL in rabbits for fattening needs to be established by a tolerance study compliant with the current standards. Adverse effects on breeding does cannot be excluded. The simultaneous use of SAL-Na with certain medicinal substances (e.g. tiamulin and valnemulin) and bentonite is contraindicated. Consumer exposure to residues of toxicological concern complies with the acceptable daily intake (ADI) of 0.005 mg/kg bw, after 1-day withdrawal. A provisional maximum residue limit (MRL) of 0.01 mg/kg liver would ensure consumer safety. A 5-day withdrawal period as proposed by the applicant is supported. SAL-Na in feed for rabbits will not pose a risk for the aquatic environment. A risk for the terrestrial ecosystem is considered unlikely. Efficacy and effective dose of SAL-Na under present farming conditions could not be established.

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Keywords: Coccidiostat, Sacox®, salinomycin sodium, rabbits for fattening, safety, efficacy

Requestor: European Commission
Question number: EFSA-Q-2017-00661
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Acknowledgements: The EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed) wishes to thank the following for the support provided to this scientific output: Jaume Galobart, Gloria Lopez-Galvez and Paola Manini.

Suggested citation: EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Rychen G, Aquilina G, Azimonti G, Bampidis V, Bastos ML, Bories G, Chesson A, Cocconcelli PS, Flachowsky G, Kolar B, Kouba M, López-Alonso M, López Puente S, Mantovani A, Mayo B, Ramos F, Saarela M, Villa RE, Wallace RJ, Wester P, Brantom P, Halle I, van Beelen P, Holczknecht O, Vettori MV and Gropp J, 2018. Scientific Opinion on the safety and efficacy of Sacox® microGranulate (salinomycin sodium) for rabbits for fattening. EFSA Journal 2018;16(3):5209, 19 pp. https://doi.org/10.2903/j.efsa.2018.5209

ISSN: 1831-4732

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

Following an urgent request from European Commission, the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) was asked to deliver a scientific opinion on the safety and efficacy of salinomycin sodium (SAL-Na) from Sacox® microGranulate when fed to rabbits for fattening. Due to the type of the request (Article 15 of Regulation (EC) No 1831/2003 'Urgent authorisation'), the FEEDAP Panel made the assessment without asking supplementary information from the applicant.

SAL-Na is largely absorbed in the rabbit intestine and extensively metabolised. The metabolic fate of SAL is common to rabbit and laboratory animal (rat). Unchanged SAL represents a very small fraction of the numerous metabolites, predominantly a monohydroxylated compound, which have been identified in tissues and excreta. SAL-related metabolites have a reduced ionophoric activity when compared to SAL. Liver is the target tissue. SAL can be used as the marker residue by default.

SAL does not induce gene mutations in vitro and it is not genotoxic in vivo. SAL is not a carcinogen. The findings in reproduction toxicity studies do generally not lead to concern, however a no observed adverse effect level (NOAEL) of 0.63 mg/kg body weight (bw) per day identified in developmental studies and based on rabbit embryo-foetal toxicity may be of relevance for breeding target species. A NOAEL of 0.5 mg/kg bw per day is derived from a cardiovascular study in dogs (pharmacological NOAEL) as well as from a 12-month dog study (toxicological NOAEL). This value is further supported by the NOAEL from the recent 90-day study in rats. An acceptable daily intake (ADI) of 0.005 mg/kg bw, applying a safety factor of 100, is confirmed.

Only data on feed intake and body weight were available to conclude on the safety of SAL for rabbits. Levels of 35 mg SAL/kg feed and higher were not tolerated by growing rabbits. A reduction in feed intake was considered as the initial adverse effect even at doses < 35 mg/kg; its compensation over time remains uncertain. The Panel considers the available data indicate that the additive is tolerated by rabbits for fattening up to 25 mg/kg. The safety of SAL in rabbits for fattening needs therefore to be established by a tolerance study compliant with the current standards. Adverse effects on breeding does, when sharing feed with rabbits for fattening containing the SAL-Na doses proposed, cannot be excluded. The simultaneous use of SAL-Na with certain medicinal substances (e.g. tiamulin and valnemulin) and bentonite is contraindicated.

SAL-Na is active against certain Gram-positive bacteria, while Gram-negative species are resistant. The use of SAL-Na as a feed additive at the proposed dietary concentration is unlikely to increase shedding of *Salmonella*, *Escherichia coli* and *Campylobacter* and to induce resistance and cross-resistance to antimicrobials important in human and animal therapy.

Consumer exposure to SAL related residues of toxicological concern from rabbit edible tissues complies with the ADI after 1-day withdrawal. The FEEDAP Panel considers that a provisional maximum residue limit (MRL) of 0.01 mg/kg liver would ensure consumer safety. However, considering the identified uncertainties, the Panel supports the proposal of the applicant of a 5-day withdrawal period.

SAL-Na from Sacox® microGranulates is not irritant to skin and eyes, it is considered a potential dermal sensitiser and a likely respiratory sensitiser. Exposure to SAL-Na from dust is likely. Since no data on the chronic inhalation toxicity of SAL are available, a risk by inhalation for persons handling the additive cannot be excluded.

The use of the SAL-Na in feed for rabbits for fattening up to the highest proposed dose will not pose a risk for aquatic environment. Although the predicted environmental concentration/predicted no effect concentration (PEC/PNEC) ratio for plants slightly exceeds the threshold value, a risk for the terrestrial ecosystem is considered unlikely due to metabolism and the expected degradation of SAL in the environment.

The SCAN assessment as well as the study from 2008 found that SAL-Na had the potential to effectively control intestinal coccidiosis in rabbits at a dietary concentration of 20 mg/kg. In the absence of recent data, efficacy and effective dose under present farming conditions could not be established.

In order to allow a final conclusion, the FEEDAP Panel made some remarks on the safety for the target species and for the consumer and on the efficacy.
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1. Introduction

1.1. Background and Terms of Reference

Regulation (EC) No 1831/2003\(^1\) establishes the rules governing the Community authorisation of additives for use in animal nutrition. In particular, Article 15 establishes that in specific cases where urgent authorisation is needed to ensure the protection of animal welfare, the Commission may provisionally authorise the use of an additive for a maximum period of 5 years.

The European Commission received a request from Huvepharma N.V.\(^2\) for the urgent authorisation of the product Sacox® microGranulate (salinomycin sodium), when used as a feed additive for rabbits for fattening (category: coccidostats and histomonostats).

During the discussion in the Standing Committee on Plants, Animals, Food and Feed, Section-Animal Nutrition, from 17 to 19 July 2017, the Member States accepted the application under Article 15 of Regulation 1831/2003.

According to Article 7(1) of Regulation (EC) No 1831/2003, on 11 September 2017 the Commission forwarded the applications to the European Food Safety Authority (EFSA) as an application under Article 15 (urgent authorisation) and asked EFSA to deliver an opinion by 28 February 2018.

In order to comply with the deadline established by the EC, this time limit was not extended and no supplementary information was requested from the applicant.

EFSA received directly from the applicant the technical dossiers in support of the applications. The particulars and documents in support of the applications were considered valid by EFSA as of 4 October 2017.

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 Sacox® microGranulate (salinomycin sodium), when used under the proposed conditions of use (see Section 3.1).

1.2. Additional information

Sacox® 120 (salinomycin sodium) for rabbits for fattening is currently not authorised in the European Union (EU). It had been previously authorised in accordance with Council Directive 70/524/EEC for 10 years until 31 May 2011.\(^3\)

The SCAN issued an opinion on the use of salinomycin sodium in feedingstuffs for rabbits in 1992 (European Commission, 1992). Recently, the product has been re-evaluated under Regulation (EC) No. 1831/2003 for chickens for fattening and chickens reared for laying (EFSA FEEDAP Panel, 2017).

2. Data and methodologies

2.1. Data

In order to comply with the deadline to deliver the opinion under Article 15 (urgent authorisation), no supplementary information was requested from the applicant. The FEEDAP Panel used the data submitted by the applicant for the urgent assessment of Sacox® 120 microGranulate and Sacox® 200 microGranulate (salinomycin sodium) as a feed additive for rabbits for fattening,\(^4\) together with data from other sources, such as previous risk assessments by EFSA or other expert bodies, peer-reviewed scientific papers and other scientific reports to deliver the present output.

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of Sacox® microGranulate (salinomycin sodium) is in line with the principles laid down in Regulation (EC) No 429/2008 and the relevant guidance documents: Guidance for the preparation of dossiers for

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\(^1\) 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.

\(^2\) Huvepharma NV, Uitbreidingstraat 80, 2600 Antwerp, Belgium.

\(^3\) Commission Regulation (EC) No 937/2001 of 11 May 2001 concerning the authorisation of new additives uses, new additives preparation, the prolongation of provisional authorisation and the 10 year authorisation of an additive in feedingstuffs. OJ L 130, 12.5.2001, p. 25.

\(^4\) FEED dossier references: FAD-2016-0044.
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), Technical Guidance: Microbial Studies (EFSA, 2008b), Guidance for establishing the safety of additives for the consumer (EFSA FEEDAP Panel, 2012a), Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012b) and Guidance on the assessment of bacterial susceptibility to antimicrobials of human and veterinary importance (EFSA FEEDAP Panel, 2012c).

3. Assessment

According to Article 15 of Regulation (EC) No 1831/2003 (‘Urgent authorisation’): ‘In specific cases where urgent action is needed to ensure the protection of animal welfare, the Commission may, in accordance with the procedure referred to in Article 22(2), provisionally authorise the use of an additive for a maximum period of 5 years’.

The European Commission forwarded an urgent request to the FEEDAP Panel to assess the safety and efficacy of salinomycin sodium from Sacox® microGranulate when fed to rabbits for fattening.

Due to the type of the request, the FEEDAP Panel made the assessment without asking supplementary information from the applicant.

Sacox® microGranulate, containing the ionophore salinomycin sodium (SAL-Na) as active substance, has been assessed by the FEEDAP Panel in 2016 for use in chickens for fattening and reared for laying (EFSA FEEDAP Panel, 2017).

3.1. Characterisation

The additives Sacox® 120 microGranulate and Sacox® 200 microGranulate contain 12% and 20% of SAL-Na as an active substance, respectively. SAL-Na is produced by Streptomyces azureus (DSM 32267) during a controlled fermentation and is not isolated at the end of the fermentation process. The final product Sacox® microGranulate is obtained by mixing the fermentation broth with calcium carbonate (diluent) and silicon dioxide (flowability enhancer) and granulation of the resulting suspension in a fluid-bed drying equipment.

The identity of the additive, characterisation of the active substance, manufacturing process and technological properties of the additive have been recently reviewed by the FEEDAP Panel (EFSA FEEDAP Panel, 2017).

Sacox® 120 microGranulate and Sacox® 200 microGranulate, is a feed additive for the prevention of coccidiosis in rabbits for fattening. The SAL-Na dose range is 20–25 mg/kg complete feed. The applicant proposes a 5-day withdrawal period.

3.2. Safety

3.2.1. Metabolic and residue studies

Eight studies concerning the absorption, distribution, metabolism and excretion (ADME) of SAL-Na in rabbit, chicken, pig, dog and rat, and six residue studies in the rabbit have been submitted. Most were performed in the 1980s and 1990s following protocols not in compliance with the present requirements in terms of number of animals, dosage, identification and quantitation of residues in tissues. Only those relevant for the assessment and offering reliable data were retained and are described below.²

3.2.1.1. Absorption, distribution, metabolism and excretion

In a first study, the metabolic balance of SAL was established in six rabbits (three males and three females) administered a complete feed supplemented with 20 mg SAL from Sacox®/kg for 15 days then all along the experiment; at day-0 of the experiment a single dose (about 0.4 mg) of ¹⁴C-SAL labelled on C1, C11 and C27, incorporated in the feed, was administered. Urine and faeces were collected for 8 consecutive days. Animals were sacrificed at day-8, liver and digestive tract were removed and the whole carcass processed for total radioactivity measurement. A similar protocol was applied to rabbits holding a collar to prevent coprophagy. A prolonged and similar linear excretion of radioactivity in the faeces was observed over the 8-day period in rabbits with and without coprophagy.

² List the studies not considered.
In 'normal' rabbits (without the collar), 7.8–8.3% (males and females) of the administered dose was recovered in the urine and 81.0–82.8% in the faeces; the corresponding values for rabbits with collars were 3.8–4.1% and 92.8–94.6%, respectively; no sex-related difference was observed.6

A study of the kinetics of residues in male rabbit tissues also gave similar results with 9.2% and 79.8% of the total radioactivity administered excreted in the urine and faeces, respectively.7 In another study where male rabbits were administered by gavage a daily dose of 2.8 mg \(^{14}\text{C}-\text{SAL}\) (corresponding to about 25 mg/kg feed) for 7 days, unchanged SAL was shown to represent 1.0–2.7% (average 1.8% for six rabbits) of total residues in the whole faeces collected over the 7-day period.8

In the first study, 12 male rabbits were accustomed for 8 days then fed all along the experiment a complete feed supplemented with 20 mg SAL/kg. At day-0 of the experiment and for 15 consecutive days, they received an additional quantity (0.75 mg) of \(^{14}\text{C}-\text{SAL}\) incorporated into the feed. Rabbits were killed (one per time point) after 1, 3, 5, 9, 12 and 15 days. The remaining animals received the complete feed only and were killed (one per time point) after 1, 2, 3, 5, 8 and 12 days. Organs, tissues and gallbladder were removed and total radioactivity measured. The first phase of the experiment indicated that a steady state was reached after 1-day administration of the label in all tissues and organs; liver was the target tissue followed by the kidney, much lower amounts of radioactivity were measured in the fat and the muscle; significant amounts of radioactivity were present in the bile. The depletion phase showed a slow decrease of total radioactivity; however, the limited number of animals did not allow a quantification of the phenomenon. The third part of the same study assessed the biliary excretion of SAL. One rabbit with a cannulation of the bile duct was administered a single dose of 0.25 mg \(^{14}\text{C}-\text{SAL}\). The bile was collected every 15 min during 6 h and total radioactivity determined. A metabolic profile was obtained by high-performance liquid chromatography (HPLC) and fraction collection from the bile excreted during the first 2 h. A similar protocol was applied to one rat. In the rabbit, a peak of excretion was observed after 1 h and 46.3% of the dose administered was excreted in 6 h; the metabolic profiles were qualitatively similar in the rabbit and the rat, indicating the absence of unchanged SAL and the presence of major peaks corresponding to individual or groups of metabolites.

In a complementary study, the pattern of liver metabolites of the same rabbits was analysed along the withdrawal period, using a radio-HPLC technique. At steady state (0-day withdrawal) four metabolites (A, B, C and D by decreasing order of polarity) were separated amounting 10.5%, 6.1%, 74.5% and 8.9% of total residues (2.3 mg equivalent SAL/kg liver); SAL (retention time between C and D) was absent. After 1 day, metabolites A and C represented 7.5% and 92.5% of 2.2 mg equivalent SAL/kg liver. Only metabolite C remained in the liver after 3 days and for longer withdrawal periods.9 A similar study performed with the rat gave the same pattern of metabolites in the liver; rat hepatocytes were incubated with SAL to produce metabolite C in sufficient quantity to allow its structural analysis by liquid chromatography–mass spectrometry (LC–MS). This metabolite was identified as a monohydroxy-SAL; due to the limited amount of purified metabolite available a nuclear magnetic resonance (NMR) analysis was not possible and the position of the hydroxylation was not completely established (on one of the carbons C4, C5, C40, C41 or C42).9

3.2.1.2. Residues

**Total residues**

A good laboratory practice (GLP) kinetic study of SAL total residues in rabbit tissues was submitted.7 New Zealand male rabbits (24 animals, 1.9–2.4 kg body weight (bw)) were administered by gavage for 7 consecutive days \(^{14}\text{C}-\text{SAL}\) a dose equivalent to 25 mg/kg feed. Six animals were slaughtered per time point corresponding to 1, 2, 3 and 4 days withdrawal; organs and tissues sampled and total radioactivity measured with a limit of quantification (LOQ) of 0.011 mg/kg. The results are presented in Table 1.

This study confirms that liver is the target tissue and that the depletion is slow in liver and kidneys.

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6 Technical dossier/Section III/Reference 13.
7 Technical dossier/Section III/Reference 21.
8 Technical dossier/Section III/Reference 15.
9 Technical dossier/Section III/Reference 14.
The major residue identified and measured in the liver after 1-day withdrawal (93% of total residues), a monohydroxy-SAL, once fully identified, should be considered as the marker residue. The practicality of this marker relies on its availability as a reference compound for the analytical procedure to be developed. In the meantime, the approach followed for the other ionophores should be retained, considering by default the parent substance (SAL) as the marker.

SAL was determined in the pivotal study of total residues described above, but only in the liver and using a non-validated HPLC method with limited sensitivity (LOQ = 0.04 mg/kg). After 1-day withdrawal, SAL residues were < LOQ.

In another study already described, groups of six male rabbits were sacrificed after 1, 2, 3 and 4 days withdrawal and livers were sampled for total residue measurement and SAL determination by thin-layer chromatography and radioluminography detection, a method not validated for quantitative purposes (no LOQ given) and of limited accuracy (the authors emphasised that only approximate values were given). After 1-day withdrawal, SAL residues represented an average of 0.5% (0.3–0.7%) of total radioactivity in the liver.

Two studies of the kinetics of the parent compound in rabbit tissues have been performed in animals (four per group in the first one, five in the second) that received a feed supplemented with 20 mg SAL/kg for 42 days. Groups of rabbits were slaughtered after 0, 12, 24, 48 and 72 h. SAL residues were determined using a non-validated microbiological method (by agar diffusion), known as sensitive but with limited accuracy, with a LOQ of 0.01 mg/kg (about 10 times higher than the modern LC–MS approach). In the first study, residues after 1-day withdrawal amounted to < LOQ (n = 2)-0.02 (n = 3) mg SAL/kg in the liver, < LOQ (n = 3)-0.02 (n = 1) in the kidney, < LOQ in the muscle and 0.01–0.02 in the fat; after 2-day withdrawal, SAL concentration was < LOQ in all tissues. In the second study, SAL concentration was < LOQ in all tissues after 1-day withdrawal.

3.2.2. Toxicology studies

The toxicity of SAL was assessed by the FEEDAP Panel in 2004 (EFSA, 2004) and it was concluded that:

Based on the data provided, SAL does not induce gene mutations in vitro and it is not genotoxic in mouse bone marrow studies in vivo.

The SAL biomass was not carcinogenic in studies on mouse and rats.

Reproduction studies (one-two-generation study with Sacox® 120 and three developmental studies, two with Sacox® 120 and one with SAL biomass) did not indicate concern, the no observed adverse effect level (NOAEL) being 1.1 mg SAL/kg bw per day in the two generation study (based on
decreased pup weight in the F1A-generation) and 0.63 mg/kg bw per day as the lowest NOAEL in developmental studies, (based on rabbit embryo-fetal toxicity).

The lowest no observed effect level (NOEL) for SAL-Na from Sacox® 120 microGranulate was 0.5 mg/kg bw per day, identified from the results of a 12-month dog study. An acceptable daily intake (ADI) of 0.005 mg/kg bw, applying a safety factor of 100, was derived.

The toxicity of SAL was re-evaluated by the FEEDAP Panel in 2016 (EFSA FEEDAP Panel, 2017) considering also the results of new studies made available in the meanwhile. It was concluded that:

After consideration of the data previously submitted in 2004 and the studies conducted more recently, the FEEDAP Panel concludes that a NOAEL of 0.5 mg/kg bw per day could be derived from a cardiovascular study in dogs (pharmacological NOAEL) as well as from a 12-month dog study (toxicological NOAEL). This is further supported by the NOAEL from the recent 90-day study in rats (0.6 mg/kg bw per day).

The above conclusions are considered valid for the current assessment, and confirm the ADI of 0.005 mg/kg bw.

3.2.3. Safety for the target species

The applicant submitted the same tolerance studies previously assessed by the SCAN (European Commission, 1992). For the current assessment, no new tolerance studies were provided. In the absence of recent information the FEEDAP Panel re-evaluated four studies made between 1978 and 1983 already assessed by the SCAN. The FEEDAP Panel noted that the available data set is not line with the requirements of the FEEDAP technical guidance on tolerance and efficacy studies in target animals (EFSA FEEDAP Panel, 2011b).

3.2.3.1. Tolerance studies

In the first study, the zootchnical performance of rabbits receiving feed supplemented with 0, 50, 75, 100 and 125 mg SAL/kg for 4 weeks was investigated. Each group consisted of eight rabbits (both sexes) with a body weight at start of about 1,200 g. Body weight gain was reduced by 3%, 16% and 29% in the groups with 75, 100 and 125 mg SAL/kg, feed intake by 6%, 15% and 21%, respectively. The reductions were significant only for the two highest SAL doses. In the view of the FEEDAP Panel, there was a dose-dependent depression of feed intake and body weight gain starting at 75 mg SAL/kg feed. No other endpoints were investigated in the study.\(^{11}\)

In a second study, three groups of rabbits of different ages were fed diets supplemented with 0, 35 or 50 mg SAL/kg for 5–6, 2 and 2 weeks, respectively. The first group consisted of 80 five-week-old animals with a mean body weight of about 900 g, the second group of 60 eight-week-old animals, with a mean body weight of about 1,500 g and the third group of 48 eleven-week-old animals, with a mean body weight of about 2,400 g. Body weight gain, feed intake, histology of liver, kidneys and heart were assessed; several haematology\(^{12}\) and clinical biochemistry\(^{13}\) parameters were analysed. Depression of feed intake and body weight gain became apparent already after 2 weeks of feeding the groups of 5 weeks of age at 35 mg SAL/kg. The increase of creatinphosphokinase (CPK) in 17% of the animals given 50 mg SAL/kg above the mean plus two times the standard deviation. No other differences in haematology or clinical biochemistry were seen. The FEEDAP Panel notes that the lowest dose tested, 35 mg SAL/kg, was not tolerated.\(^{14}\)

In a third study, a total of 120 five-week-old rabbits were fed diets supplemented with 0, 25, 50 and 75 mg SAL/kg for 6 weeks. Group size was four replicates with six animals each. Only performance parameters were monitored. Daily feed intake, average daily gain and feed to gain ratio for the untreated control were 150 g, 38 g and 3.91. The corresponding data for the group with 25 mg SAL-Na/kg were 147 g, 38 g and 3.92. The group with 50 and 75 mg SAL-Na/kg did not reach the level of the control group.\(^{15}\)

11 Technical dossier/Section III/Reference 3.
12 Haemoglobin, packed cell volume, erythrocytes, leucocytes.
13 Electrolytes, glucose, total protein, urea, lipids, creatinphosphokinase (CPK), creatinine.
14 Technical dossier/Section III/Reference 4.
15 Technical dossier/Section III/Reference 5.
In a fourth study (divided into three protocols), the action of SAL in rabbits infected with five different _Eimeria_ sp. was investigated.\(^{16}\)

Protocol 1 determined the lowest concentration of SAL effective against _Eimeria flavescens_ and _Eimeria intestinalis_ and the highest concentration not affecting the performance of rabbits. A total of 144 coccidia-free rabbits with an age of 30-42 days were allocated to 18 treatments (six feed types (with different SAL levels) x three controls (untreated uninfected, untreated and infected with two _Eimeria_ species)), group size was four cages with two animals each. Study duration was 5 weeks (body weight gain and feed intake reported for 26 days). The experimental diets were given 4 days before _Eimeria_ inoculation. The intended SAL concentrations were 0, 12, 20, 30, 50 and 80 mg SAL/kg (confirmed by analyses). Feed intake in the period reported was 138, 141, 140, and 121 g/day for uninfected groups with 0, 12, 20 and 30 mg SAL/kg feed, the corresponding daily gain was 37, 37, 36, and 31 g/day. Feed intake and body weight gain was severely depressed in the untreated controls by infection with the two _Eimeria_ species, SAL-Na restored daily feed intake in the _E. flavescens_ infected rabbits to the level of the uninfected group, whereas feed intake did not fully reach the level of the infected control (in an average of the three groups 4% less).

Protocol 2 was a dose-range finding study with _E. flavescens, E. intestinalis, Eimeria irresidua_ and _Eimera magna_. A total of 216 rabbits (72 coccidia-free and 144 conventionally reared) with an age of 31 days was allocated to 18 treatments (four feed types (different SAL levels) x four _Eimeria_ sp. inoculation) plus control (two feed types), group size was six cages with two animals each. Study duration was 5 weeks (2 weeks before and 3 weeks after inoculation). The experimental diets were given 7 days before inoculation. The intended SAL concentrations were 0, 20, 30 and 50 mg SAL/kg (confirmed by analyses). Data on feed intake and body weight gain were incompletely reported: the data of a short interim period (day 4 to day 14) were given in a table for the untreated and the high dose group only. No differences between these two groups were found in daily gain (34 g/day) and feed consumption 102 g). In the average of the four infected groups, 20 and 30 mg SAL-Na/kg feed improved the weight gain (from 29 to 35 g/day) and feed consumption (from 94 to 104 g/day).

Protocol 3 was designed to confirm the most essential results of the preceding studies on coccidiosis related to _E. flavescens_ and _E. intestinalis_ and to examine the effect of the additive on bile duct coccidiosis by _Eimeria stiedai_. A total of 104 coccidia-free rabbits with an age of 30 days was allocated to 13 treatments (four feed types (different SAL levels) x three _Eimeria_ sp. ( _E. flavescens, E. intestinalis_ and _E. stiedai_)) plus an uninfected untreated control group; group size was four cages with two animals each. Study duration was 6 weeks (2 weeks before and 4 weeks after inoculation). The experimental diets were given 4 days before inoculation. The intended SAL concentrations were 0, 20, 30 and 50 mg SAL/kg (confirmed by analyses). No data on feed intake and body weight gain for the periods before and after inoculation were reported for the uninfected treated groups.

**Conclusions**

None of the studies is compliant with current standards of a tolerance study and the requirements of the FEEDAP technical guidance on tolerance and efficacy studies in target animals (EFSA FEEDAP Panel, 2011b). Only data on feed intake and body weight were available to conclude on the safety of SAL for rabbits. Levels of 35 mg SAL/kg feed and higher were not tolerated by growing rabbits. A reduction in feed intake was considered as the initial adverse effect even at doses < 35 mg/kg; its compensation over time remains uncertain. The Panel considers the available data indicate that the additive is tolerated by rabbits for fattening up to 25 mg/kg.

Effects of SAL-Na on haematology, clinical blood chemistry and pathology in rabbits were not investigated at the SAL-Na levels proposed for use. Therefore, the safety of SAL-Na from Sacox® in rabbits for fattening needs to be established by a tolerance study compliant with the current standards.

The FEEDAP Panel further noted that the available data on reproductive toxicity of SAL-Na in rabbits (see Section 3.2.2) indicate embryo–fetal toxicity with a NOAEL of 0.63 mg/kg bw per day (EFSA, 2004). Thus, effects on breeding does when sharing feed with rabbits for fattening containing the SAL-Na doses proposed cannot be excluded.

**3.2.3.2. Interactions**

Interactions between ionophores and other drugs (tiamulin, sulfonamides, chloramphenicol, erythromycin, oleandomycin and furazolidone) were already described for poultry in an earlier FEEDAP opinion (EFSA, 2004); the Panel concluded that:

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\(^{16}\) Technical dossier/Section III/Reference 6.
Incompatibilities or interactions with feedingstuffs, carriers, other approved additives are not to be expected given the known history of salinomycin. It could also be shown that SAL-Na from Sacox is fully compatible with some veterinary drugs. On the other hand it is well known from the literature that severe interactions between the ionophore coccidiostats and the diterpene-antibiotic tiamulin as well as other antibiotic substances (mainly macrolides) may occur. Therefore the simultaneous use of Sacox and certain antibiotic drugs (e.g. tiamulin) is contra-indicated.

More recent literature reviewed by the EFSA FEEDAP Panel in 2017 confirmed the above interactions in poultry (EFSA FEEDAP Panel, 2017). Considering the proximity of the metabolic fate of SAL in rabbits and laboratory rodents (see Section 3.2.1.1) the same interaction is also expected to exist in rabbits. This is confirmed by available public information on valnemulin (a pleuromutilin derivative, like tiamulin) when used in rabbits.

The FEEDAP Panel extends its former conclusion on interactions from the simultaneous use of SAL and certain antibiotics (e.g. tiamulin and valnemulin) in chickens to the use of SAL in rabbits.

Bentonite (0.5%) decreased the anticoccidial effects of low levels of monensin (55 mg/kg) and salinomycin (22 mg/kg) in chicken but not when the coccidiostats were used at the recommended levels (Gray et al., 1998). Considering the salinomycin concentration in feed for rabbit (20–25 mg/kg feed) and the likely mode of action of bentonite in the intestine of chicken, any bentonite supplementation of rabbit feed would interact with salinomycin reducing its anticoccidial effect.

3.2.3.3. Microbial studies

The FEEDAP Panel concluded in its opinion on Sacox® for chickens for fattening and chicken reared for laying (EFSA FEEDAP Panel, 2017) that ‘SAL is active against certain Gram-positive bacteria, while Gram-negative bacteria are resistant. The use of SAL-Na 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’.

The above conclusions are considered valid for the assessment of the use of Sacox® in rabbits.

3.2.3.4. Conclusions on safety for the target species

Only data on feed intake and body weight were available to conclude on the safety of SAL for rabbits. Levels of 35 mg SAL/kg feed and higher were not tolerated by growing rabbits. A reduction in feed intake was considered as the initial adverse effect even at doses < 35 mg/kg; its compensation over time remains uncertain. The Panel considers the available data indicate that the additive is tolerated by rabbits for fattening up to 25 mg/kg. The safety of SAL in rabbits for fattening needs therefore to be established by a tolerance study compliant with the current standards.

Available data on reproductive toxicity in rabbits indicate foetal toxicity with a NOAEL of 0.63 mg/kg bw per day. Thus, adverse effects on breeding does, when sharing feed with rabbits for fattening containing the SAL-Na doses proposed, cannot be excluded.

The simultaneous use of SAL-Na with certain medicinal substances (e.g. tiamulin and valnemulin) and bentonite is contraindicated.

SAL-Na is active against certain Gram-positive bacteria, while Gram-negative species are resistant. The use of SAL-Na as a feed additive at the proposed concentration is unlikely to increase shedding of Salmonella, E. coli and Campylobacter and to induce resistance and cross-resistance to antimicrobials important in human and animal therapy.

3.2.4. Assessment of consumer safety

As SAL main metabolic pathway is common to rabbit and laboratory animals (see Section 3.2.1), the FEEDAP Panel considers that the residues to which the consumer would be exposed have been tested through the toxicological studies. Taking a conservative approach, the FEEDAP Panel considers that the whole SAL derived residues represent a risk which is at the most equal to an equivalent quantity of SAL.

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17 Enroflaxacine, CTC-HCl, sulfadimidine-Na, colistine, erythromycine (EFSA, 2004).
18 https://vetxed.com/en/e/Econor/5/#43_12__SPECIAL_WARNING_S: Valnemulin has been shown to interact with ionophores such as monensin, salinomycin and narasin and may result in signs indistinguishable from an ionophore toxicosis. Pigs and rabbits should not receive products containing monensin, salinomycin or narasin, during or at least 5 days before or after treatment with valnemulin. Severe growth depression, ataxia, paralysis or death may result.
The exposure of the consumer to SAL-related residues was calculated according to daily food consumption values of animal products set in Regulation (EC) No 429/2008 and the calculated maximised (average plus 2 standard deviations (SD), 95% confidence limit) total residues measured in different tissues and at different withdrawal times (Table 2).

Table 2: Consumer exposure to SAL total residues (total residue concentrations (TRCs)) in rabbit tissues

| TRC + 2SD (mg/kg)         | Liver | Kidney | Muscle | Fat | Sum   | % ADI(b) |
|---------------------------|-------|--------|--------|-----|-------|----------|
| 1-day withdrawal          | 7.610 | 0.854  | < LOQ  | 0.035 |       |          |
| 2-day withdrawal          | 6.957 | 0.779  | < LOQ  | 0.039 |       |          |
| 3-day withdrawal          | 2.866 | 0.226  | < LOQ  | 0.007 |       |          |
| DITR(a) (mg/day)          |       |        |        |      |       |          |
| 1-day withdrawal          | 0.761 | 0.009  | --     | 0.002 | 0.772 | 257      |
| 2-day withdrawal          | 0.696 | 0.008  | --     | 0.002 | 0.706 | 235      |
| 3-day withdrawal          | 0.273 | 0.002  | --     | --   | 0.275 | 92       |

ADI: acceptable daily intake; LOQ: limit of quantification.
(a): Daily intake of total residue (mg/person per day).
(b): ADI: 0.005 mg/kg bw per day.

The results indicate that consumer exposure to the whole SAL residues present in rabbit tissues after three-day withdrawal complies with the ADI (92%).

In its former assessment of SAL-Na from Sacox® for chickens for fattening the FEEDAP Panel considered that SAL metabolites (mono-, di-, tri- and tetra-hydroxy derivatives) have a lesser binding capacity to cations than the parent compound and therefore a lesser ionophoric activity (about 20% of the parent compound for liver metabolites) which represents the property of toxicological concern (EFSA FEEDAP Panel, 2017). As far as the metabolic biotransformations are similar in the chicken and rabbit and that the liver of treated rabbits contains by far the highest residues at all withdrawal times (mainly under the form of a monohydroxy metabolite), it can be assumed that only 20% of these residues would be of toxicological concern. Consequently, the consumer exposure (daily intake of total residues (DITR)) to residues of concern after 1-day withdrawal would be 20% of 0.772 mg, i.e. 0.154 mg, complying with the ADI (51%).

3.2.4.1. Proposal for maximum limits of residues (MRLs)

Liver is the target tissue in the rabbit. After 1-day withdrawal, 91% of consumer exposure to SAL residues relates to the consumption of liver. Therefore, only a MRL for liver is worth consideration.

Due to the uncertainties related to: (i) the reduced number of animals tested (four and five in two studies, respectively, instead of six normally required), the lower SAL supplementation of feeds (20 instead of 25 mg/kg) and the limitations of the analytical method prevailing in the marker residue study, (ii) the fact that the marker residue determined at the same time as total residues was measured only in liver using a method with limited accuracy, no reliable ratio SAL/total residues could be calculated. Therefore, no MRL can be established according to EFSA Guidance for the evaluation of consumer safety (EFSA FEEDAP Panel, 2012a).

However, it appears that residues are very low in all tissues and practically below or around the LOQ (0.011 mg SAL/kg tissue) after 1-day withdrawal. Consequently, a provisional MRL of 0.01 mg SAL/kg liver could be retained that would ensure that the withdrawal period of 1-day is respected.

Considering the approximate ratio marker residue vs total residues (RMTR) of 0.005 established in the liver after 1-day withdrawal, the consumer exposure to the provisional MRL (DITRMRL) in liver would amount about 0.01 (MRL)/0.005 (RMTR) × 0.1 (kg liver consumed per day) = 0.2 mg/person per day representing 67% of the ADI.

The validity of the proposed MRL for SAL in rabbit liver could also be derived from another calculation based on the following findings: (i) the residue after 1-day withdrawal does not exceed 0.01 mg SAL/kg liver, (ii) the maximum expected total salinomycin related residue after 1-day withdrawal would be 7.61 mg/kg (Table 2), (iii) the toxicological potency of this residue amounts to 20% of that of SAL, (iv) the RMTR would then be 0.01 (maximum SAL residue)/1.522 (total toxicologically relevant SAL related liver residue) = 0.0066. A SAL content of 0.01 mg/kg liver would correspond to 0.01 (MRL)/0.0066 (RMTR) × 0.1 (kg liver consumed per day) = 0.15 mg total residues of toxicological concern/person per day (representing 50% of the ADI).

Both model calculations confirm the validity of the proposed MRL (however provisional) for liver to ensure consumer safety.
3.2.4.2. Proposal for a withdrawal period

Residue studies would indicate that consumer safety is ensured after 1-day withdrawal. However, considering the uncertainties related to the exposure estimate, the questionable validity of the marker compound selected and the limitations of the analytical methods applied, the FEEDAP Panel proposes to maintain a 5-day withdrawal time, as proposed by the applicant.

3.2.4.3. Conclusions on safety for the consumer

The metabolic fate of SAL is common to rabbit and laboratory animal (rat); the ADI derived from studies with laboratory animals can be applied in the assessment of the consumer safety of food from rabbits fed SAL. Consumer exposure to residues of toxicological concern was below the ADI after 1-day withdrawal. The FEEDAP Panel considers that a provisional MRL of 0.01 mg/kg liver would ensure consumer safety. However, considering the identified uncertainties, the Panel supports the proposal of the applicant of a 5-day withdrawal period.

3.2.5. Safety for the user

The safety for the user of SAL was assessed by the FEEDAP Panel in 2004 and 2016 (EFSA, 2004 and EFSA FEEDAP Panel, 2017). The FEEDAP Panel considered that the use of the additive in rabbits will not introduce concerns not already considered in the previous assessments and reiterated the same conclusions for the current assessment:

Salinomycin from Sacox® 120 microGranulate is not irritant to skin and eyes. The additive is considered a potential dermal sensitiser and a likely respiratory sensitiser. These conclusions are considered also valid for the Sacox® 200 microGranulate.

The LC50 for acute inhalation toxicity is > 1.2 mg SAL/L. The potential exposure of users by handling the additive to inhaled SAL was calculated. The 8 h exposure to SAL from inhalation would be about 0.6 mg from Sacox® 120 and 2.2 mg for Sacox® 200 microGranulate. These figures do not consider the alveolar fraction. Data for particle < 10 μm in the dust were only available for Sacox® 200 microGranulate, allowing a reduction of the critical exposure to 44% of the total SAL (about 1 mg). Since no data on the chronic inhalation toxicity of SAL were available, a risk from inhalation toxicity cannot be excluded.

3.2.6. Safety for the environment

The active ingredient is not a physiological/natural substance of established safety for the environment. Consequently, according to Regulation (EC) No 429/2008, the Phase I assessment has to be continued to determine the predicted environmental concentration (PEC), according to the proposed conditions of use in chickens for fattening.

3.2.6.1. Phase I

Physical-chemical properties of salinomycin sodium

The physical-chemical properties of SAL-Na are summarised in Table 3.

The low vapour pressure indicates that the substance will not volatilise to any great extent. SAL is a carboxylic acid which is converted to the anionic form below pH 6.4. At low pH, the neutral form is insoluble but the anion is soluble. High solubility (at high pH) is generally associated with a low sorption. Low sorption leads to a higher risk of the pollution of groundwater and aquatic ecosystems.

Table 3: Physical-chemical properties of SAL-Na

| Property                                | Value               | Unit   |
|-----------------------------------------|---------------------|--------|
| Octanol/water partition coefficient (log Kow 25°C)(a) | 5.12 (pH 7.4)       | –      |
| Water solubility (20°C)(b)              | < 5 (pH 4)          | mg/L   |
|                                         | 622.3 (pH 7)        |        |
|                                         | 1371.2 (pH 9)       |        |
| Vapour pressure(b)                      | < 5 × 10^-4 (25°C)  | Pa     |
| Dissociation constant(b)                | 6.4 (20°C)          | –      |

(a): EFSA FEEDAP Panel (2017).
(b): Technical dossiers/Supplementary information July 2015/Reference 31.
Studies assessing SAL-Na adsorption/desorption and biodegradation in soil were assessed by the FEEDAP Panel in its opinion on the safety and efficacy of Sacox® for poultry (EFSA FEEDAP Panel, 2017); the same conclusions on the fate and behaviour of SAL-Na can be retained for the current assessment: ‘The FEEDAP Panel considers a K\text{oc} value of 77 L/kg for SAL-Na. An average DT\text{50} of 31 days (at 12°C) was taken for further use’.

**Predicted environmental concentrations (PECs)**

Based on the proposed use of 25 mg SAL/kg feed for rabbits for fattening the calculated PEC\text{soil} and PEC\text{groundwater} are 348 µg/kg and 236 µg/L, respectively. Both values exceed the trigger values of 10 µg/kg and 0.1 µg/L as indicated in the FEEDAP Technical guidance for assessing the safety of feed additives for the environment (EFSA, 2008a). Therefore, the environmental risk assessment of SAL requires a Phase II environmental risk assessment.

### 3.2.6.2. Phase II

**Exposure assessment**

In its opinion on the safety and efficacy of Sacox® for poultry (EFSA FEEDAP Panel, 2017), the FEEDAP Panel noted that: ‘SAL-Na added to chickens feed is not completely mineralised to carbon dioxide and water and therefore degradation products are present in the manure. There is a large number of metabolites which are not well characterised. Therefore, a detailed environmental risk assessment of each of these degradation products is not feasible. However, SAL plus its metabolites in chicken manure may represent up to 20% of the ionophoric activity’. Considering the similarity of the metabolism of SAL in chicken and rabbits, the same assumption can be applied to rabbits.

**Characterisation of residues in manure**

In its opinion on the safety and efficacy of Sacox® for poultry (EFSA FEEDAP Panel, 2017) the FEEDAP Panel assumed that ‘the SAL-derived ionophoric activity in manure would not exceed 20% of the SAL administered dose’. As far as the metabolic fate of SAL in the chicken is similar to that in the rabbit (1% excreted as unchanged SAL, the rest as hydroxylated metabolites), the FEEDAP Panel is of the opinion that the above assumption can be made for the SAL-derived ionophoric activity in rabbit manure, and for further calculation, it is assumed that the SAL-derived ionophoric activity in rabbit manure would not exceed 20% of the SAL administered dose.

**PECs calculation refined in Phase II**

Assuming that the ionophoric activity of SAL and its metabolites in excreta would not exceed in total 20% of the orally administered dose, the refined dose used for PEC calculations was 5 mg/kg feed. The PEC\text{soil}, PEC\text{surface water} and PEC\text{sediment} are reported in Table 4.

**Table 4:** Predicted environmental concentrations (PECs) of SAL in soil, groundwater, surface water and sediment

| Input                                      | Value                      |
|--------------------------------------------|----------------------------|
| Dose (mg SAL/kg feed)                      | 25 * 20% = 5              |
| MW SAL-Na                                  | 772.99                     |
| VP (Pa)                                    | 5 * 10⁻⁵                  |
| Solubility (mg/L)                          | 622                        |
| Log K\text{ow} (pH 7.4)                    | 5.12                       |
| K\text{oc} (L/kg)                          | 77                         |
| DT\text{50} at 12 °C (days)                | 31                         |

| Output                                     |                             |
|--------------------------------------------|-----------------------------|
| PEC\text{soil} (µg/kg)                     | 70                          |
| PEC\text{groundwater} (µg/L)               | 47                          |
| PEC\text{surface water} (µg/L)             | 16                          |
| PEC\text{sediment} (µg/L)                  | 86                          |

MW: molecular weight; VP: vapour pressure; K\text{oc}: organic carbon sorption constant; DT\text{50}: degradation half-time; PEC: predicted environmental concentration.
The FEEDAP Panel noted that the PEC values do not refer to SAL but consider the relative ionophoric activity of SAL plus its metabolites. The assumption that the $K_{oc}$ for SAL is the same as the one of metabolites is made. These metabolites can be more mobile than SAL which would cause higher PEC surface water than shown in the table above.

Since in the exposure route from soil via groundwater to surface water there is also ample time for further mineralisation of the initial degradation products of SAL, the FEEDAP Panel notes that the trigger value of 0.1 $\mu$g/L will not be exceeded in groundwater. This conclusion is supported with the FOCUS groundwater exposure calculation on the parent compound that results in very low predicted concentration of SAL in groundwater ($1.8 \times 10^{-5}$ $\mu$g/L).

**Effect assessment**

The effects of SAL-Na on the aquatic, terrestrial and sediment compartments were studied in a number of ecotoxicity studies already assessed in the FEEDAP opinion on the safety and efficacy of Sacox® for poultry (EFSA FEEDAP Panel, 2017). The same conclusions can be retained for the current assessment:

‘For the terrestrial compartment, data are available for micro-organisms, earthworms and plants. Based on the lowest $E(L)C_{50}$ of 4.51 mg/kg for plants, PNEC that is used in the risk assessment is 45.1 $\mu$g SAL/kg, applying an assessment factor (AF) of 100.

For the aquatic compartment, data are available for algae and cyanobacteria, aquatic invertebrates and fish. Based on the lowest $E(L,r)C_{50}$ of 6.98 mg SAL/L for fish, the PNEC used in the risk assessment is 69.8 $\mu$g/L, applying an AF of 100.

Ecotoxicological data for sediment-dwelling invertebrates are provided for the sediment compartment. The calculated PNEC for the risk assessment is 660 $\mu$g/kg, applying an AF of 10 to the NOEC of 6.6 mg/kg’.

**Risk characterisation**

The risk characterisation ratios for terrestrial, freshwater and sediment compartments are reported in Tables 5, 6 and 7, respectively. While for the aquatic and sediment compartment the predicted environmental concentration/predicted no effect concentration (PEC/PNEC) ratios are all $< 1$, the PEC/PNEC ratio for plants is exceeding the value of 1 (1.5), indicating that a risk for the terrestrial compartment cannot be completely excluded.

**Table 5: Risk characterisation (PEC/PNEC ratio) for terrestrial compartment**

| Taxa               | $\text{PEC}_{\text{soil}}$ ($\mu$g/kg) | $E(L)C_{50}/\text{NOEC}$ (mg/kg) | AF  | PNEC ($\mu$g/kg) | PEC/PNEC |
|--------------------|---------------------------------------|----------------------------------|-----|------------------|----------|
| Earthworm          | 70                                    | 103/–                            | 100 | 1030             | 0.06     |
| Plants             | 4.51/–                                | 100                              | 45.1| 1.5              |          |

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

**Table 6: Risk characterisation (PEC/PNEC ratio) for freshwater compartment**

| Taxa                        | $\text{PEC}_{\text{surface water}}$ ($\mu$g/kg) | $E(L,r)C_{50}/\text{NOEC}$ (mg/L) | AF  | PNEC $\mu$g/L | PEC/PNEC |
|-----------------------------|-----------------------------------------------|----------------------------------|-----|----------------|----------|
| Algae Scenedesmus subspicatus | 16                                           | 28.4/6.25                        | 100 | 284$^{(a)}$    | 0.06     |
| Cyanobacteria Anabaena flos-aquae |                                    | 25.5/–                           | 100 | 255            | 0.06     |
| Aquatic invertebrates Daphnia magna |                                    | > 12.33/–                         | 100 | /              | /        |
| Fish Danio rerio            | 6.98/–                                        | 100                              | 69.8| 0.23           |          |

PEC: predicted environmental concentration; PNEC: predicted no effect concentration; AF: assessment factor; $E(L,r)C_{50}$: lowest effective concentration (growth rate).

(a): Based on the amount of ecotoxicity data on aquatic species and sediment, an AF of 100 is selected.

19 Value obtained with FOCUS calculations with the following parameters (200 g/ha, calculated from 20% of 25 as the dose; $K_{oc} = 77$, $DT_{50} = 31$ days at 12°C for the Chateaudun scenario with winter cereals. In the model, SAL was incorporated into the soil.
Bioaccumulation

The FEEDAP Panel noted that the high octanol/water partition coefficient (log Kow of 5.12) of SAL-Na allows bioconcentration in environmental food chains. Considering that SAL is extensively metabolised in the chicken and that there is similarity of the metabolism of SAL in chickens and rabbits, the risk for bioaccumulation is considered low.

3.2.6.3. Conclusion on environmental risk assessment

The use of the SAL-Na in feed for rabbits for fattening up to the highest proposed dose will not pose a risk for aquatic environment. Although the PEC/PNEC ratio for plants slightly exceeds the threshold value, a risk for the terrestrial ecosystem is considered unlikely due to the metabolism and the expected degradation of SAL in the environment.

3.3. Efficacy

In 1992, the SCAN concluded that ‘a salinomycin dosage of 20–25 mg/kg complete feedingstuffs is effective in protecting rabbits from coccidiosis and in improving alimentary efficiency of infected animals even under conditions of more severe experimental infections than occurring under natural conditions. Dosages < 18 mg/kg of feedingstuffs are not proved as sufficiently effective to prevent liver coccidiosis’ (European Commission, 1992).

The studies currently submitted were performed more than 10 years ago, with the exception of a cage study (performed in 2008, see Section 3.3.1). The available data set is not line with the requirements of the FEEDAP Guidance on coccidiostats and histomonostats (EFSA FEEDAP Panel, 2011a).

3.3.1. Cage study with artificially infected rabbits

A total of 180 weaned rabbits (28-days-old, from 36 litters with 5 weanlings each, mixed sex) were distributed to five treatment groups: an uninfected untreated group (UUC), an infected untreated group (IUC), an infected treated (IT) group using SAL-Na at 20 mg/kg and two other IT groups using two other chemically synthesised coccidiostats.20 Group size was 12 replicates (cages) with three animals per cage. Study duration was 28 days. On day 0, all rabbits of the infected groups received a mixed inoculum (a total of 50,000 sporulated oocysts from *E. magna*, *E. media* and *E. perforans*) by oral gavage. Supplemented feed was provided 3 days before inoculation until study completion. A preventive treatment with zinc-bacitracin via water for drinking was administered during the first 14 days of the trial to control enteropathic problems.

Animal health and mortality were monitored daily. Feed intake and body weight of the animals were measured, feed to gain ratio was calculated. Number of oocysts excreted per gram faeces (OPG, pooled samples) was determined on day 7, 11, 15, 21 and 28.

The data was statistically analysed by factorial analysis of variance (ANOVA).

A strong increase of OPG could be observed in the IUC group at days 7 and 11 indicating successful infection. From day 15 onwards, OPG values returned to normal, at the level of the UUC group. Treatment with SAL-Na resulted in a marked decrease of OPG compared to IUC, at day 7 by more than 2 logarithms and at day 11 by more than 3 logarithms. *E. magna* was the dominant species among the excreted oocytes of the IUC group during the entire study. Species differentiation could not be made in the IT group due the low number of oocysts.

Table 8 summarises the zootechnical results. No mortality occurred in the UUC and IUC groups. Two rabbits died in the IT group; however, the deaths were not coccidiosis related. Due to inoculation, a highly significant effect on feed intake occurred in the 2 weeks following the infection. In the first

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20 Technical dossier/Section IV/Ref. 9.
week after inoculation, IUC rabbits showed a 40% lower feed intake than the UUC rabbits. For the overall period, rabbits of the IT group had a feed intake comparable to that of the UUC rabbits. In total, there was no significant difference between the UUC and the IT groups for feed intake, body weight gain and feed to gain ratio, whereas the IUC group showed always significantly inferior values to UUC (except feed to gain ratio for the entire feeding period) and IT.

Table 8: Results of feed intake, body weight gain and feed to gain ratio

| Feed Intake (g) | Weight gain (g) | Feed to gain ratio |
|----------------|----------------|-------------------|
| D 0–7 | D -3–28 | D 0–7 | D -3–28 | D 0–7 | D -3–28 |
| UUC | 705a | 3,725a | 349a | 1,428a | 2.02a | 2.61a |
| IUC | 413b | 3,258b | 136b | 1,194b | 4.05b | 2.65b |
| IT | 700a | 3,735a | 364a | 1,486a | 1.92a | 2.51a |

UUC: uninfected untreated group; IUC: infected untreated group; IT: infected treated.
a,b,c: Means within a column within a study with different superscript are significantly different (p ≤ 0.05).

3.3.2. Conclusion on the efficacy of salinomycin sodium in rabbits

The SCAN assessment as well as the study from 2008 found that SAL-Na had the potential to effectively control intestinal coccidiosis in rabbits at a dietary concentration of 20 mg/kg. In the absence of recent data, efficacy and effective dose under present farming conditions could not be established. No conclusion on the effect of SAL on the prevention of bile duct coccidiosis (by E. stiedai) is possible due to lack of data.

3.4. Post-market monitoring

The FEEDAP Panel considers that there is no need for specific requirements for a post-market monitoring plan other than those established in the Feed Hygiene Regulation21 and Good Manufacturing Practice.

Field monitoring of Eimeria spp. resistance to SAL-Na in rabbits should be undertaken, preferably during the latter part of the period of authorisation.

4. Conclusions

Following an urgent request from European Commission, the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) was asked to deliver a scientific opinion on the safety and efficacy of SAL-Na from Sacox® microGranulate when fed to rabbits for fattening. Due to the type of the request (Article 15 of Regulation (EC) No 1831/2003 ‘Urgent authorisation’), the FEEDAP Panel made the assessment without asking supplementary information from the applicant.

SAL-Na is largely absorbed in the rabbit intestine and extensively metabolised. The metabolic fate of SAL is common to rabbit and laboratory animal (rat). Unchanged SAL represents a very small fraction of the numerous metabolites, predominantly a monohydroxylated compound, which have been identified in tissues and excreta. SAL-related metabolites have a reduced ionophoric activity when compared to SAL. Liver is the target tissue. SAL can be used as the marker residue by default.

SAL does not induce gene mutations in vitro and it is not genotoxic in vivo. SAL is not a carcinogen. The findings in reproduction toxicity studies do generally not lead to concern, however a NOAEL of 0.63 mg/kg bw per day identified in developmental studies and based on rabbit embryo-foetal toxicity may be of relevance for breeding target species. A NOAEL of 0.5 mg/kg bw per day is derived from a cardiovascular study in dogs (pharmacological NOAEL) as well as from a 12-month dog study (toxicological NOAEL). This value is further supported by the NOAEL from the recent 90-day study in rats. An ADI of 0.005 mg/kg bw, applying a safety factor of 100, is confirmed.

Only data on feed intake and body weight were available to conclude on the safety of SAL for rabbits. Levels of 35 mg SAL/kg feed and higher were not tolerated by growing rabbits. A reduction in feed intake was considered as the initial adverse effect even at doses < 35 mg/kg; its compensation over time remains uncertain. The Panel considers the available data indicate that the additive is tolerated by rabbits for fattening up to 25 mg/kg. The safety of SAL in rabbits for fattening needs

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21 Regulation (EC) No 183/2005 of the European Parliament and of the Council of 12 January 2005 laying down requirements for feed hygiene. OJ L 35, 8.2.2005, p. 1.
therefore to be established by a tolerance study compliant with the current standards. Adverse effects on breeding does, when sharing feed with rabbits for fattening containing the SAL-Na doses proposed, cannot be excluded. The simultaneous use of SAL-Na with certain medicinal substances (e.g. tiamulin and valnemulin) and bentonite is contraindicated.

SAL-Na is active against certain Gram-positive bacteria, while Gram-negative species are resistant. The use of SAL-Na as a feed additive at the proposed dietary concentration is unlikely to increase shedding of Salmonella, E. coli and Campylobacter and to induce resistance and cross-resistance to antimicrobials important in human and animal therapy.

Consumer exposure to SAL related residues of toxicological concern from rabbit edible tissues complies with the ADI after 1-day withdrawal. The FEEDAP Panel considers that a provisional MRL of 0.01 mg/kg liver would ensure consumer safety. However, considering the identified uncertainties, the Panel supports the proposal of the applicant of a 5-day withdrawal period.

SAL-Na from Sacox® microGranulates is not irritant to skin and eyes, it is considered a potential dermal sensitiser and a likely respiratory sensitiser. Exposure to SAL-Na from dust is likely. Since no data on the chronic inhalation toxicity of SAL are available, a risk by inhalation for persons handling the additive cannot be excluded.

The use of the SAL-Na in feed for rabbits for fattening up to the highest proposed dose will not pose a risk for aquatic environment. Although the PEC/PNEC ratio for plants slightly exceeds the threshold value, a risk for the terrestrial ecosystem is considered unlikely due to metabolism and the expected degradation of SAL in the environment.

The SCAN assessment as well as the study from 2008 found that SAL-Na had the potential to effectively control intestinal coccidiosis in rabbits at a dietary concentration of 20 mg/kg. In the absence of recent data, efficacy and effective dose under present farming conditions could not be established.

5. Recommendations

The FEEDAP Panel proposes two warning statements under Other provisions:

- Feed containing SAL should be given to rabbits for fattening only, avoid exposure of does.
- The simultaneous use of Sacox® and certain antibiotic drugs (e.g. tiamulin and valnemulin) and bentonite is contraindicated in rabbits.

6. Remarks

For a final assessment, the following additional data are required:

- A tolerance study in rabbits for fattening following the applicable EFSA guidance document; considering the NOAEL of a developmental study and feeding practices, where does and the weanlings receive the same feed until weaning, the tolerance study should also include does;
- A total and marker residue study; to reduce the uncertainties, the FEEDAP Panel would recommend the use of the monohydroxy metabolite as the marker substance. If SAL had to be considered as a marker substance by default, residue studies should be performed with the multianalyte method established by Commission Decision 2002/657;
- A complete package of recent/valid efficacy studies in rabbits for fattening, also considering bile duct coccidirosis.

Documentation provided to EFSA

1) Sacox® microGranulate for rabbits for fattening. July 2016. Submitted by Huvepharma N. V.
2) Comments from Member States.

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Abbreviations

| Abbreviation | Description |
|--------------|-------------|
| ADI          | acceptable daily intake |
| ADME         | absorption, distribution, metabolism and excretion |
| AF           | assessment factor |
| ANOVA        | analysis of variance |
| bw           | body weight |
| CPK          | creatinphosphokinase |
| DITR         | daily intake of total residue |
| DT<sub>50</sub> | degradation half-time |
| E(L)C<sub>50</sub> | lowest effective concentration |
| E(L,r)C<sub>50</sub> | lowest effective concentration (growth rate) |
| FEEDAP       | EFSA Panel on Additives and Products or Substances used in Animal Feed |
| GLP          | good laboratory practice |
| HPLC         | high-performance liquid chromatography |
| IT           | infected treated |
| IUC          | infected untreated group |
| K<sub>oc</sub> | organic carbon sorption constant |
| K<sub>ow</sub> | octanol/water partition coefficient |
| LC<sub>50</sub> | lethal concentration, median |
| LC-MS        | liquid chromatography-mass spectrometry |
| LOQ          | limit of quantification |
| MRL          | maximum residue limit |
| MW           | molecular weight |
| NMR          | nuclear magnetic resonance |
| NOAEL        | no observed adverse effect level |
| NOEC         | no observed effect concentration |
| NOEL         | no observed effect level |
| OPG          | oocysts excreted per gram |
| PEC          | predicted environmental concentration |
| PNEC         | predicted no effect concentration |
| RMTR         | ratio marker residue vs total residues |
| SAL-Na       | salinomycin sodium |
| SCAN         | Scientific Committee on Animal Nutrition |
| SD           | standard deviations |
| TRC          | total residue concentration |
| UUC          | uninfected untreated group |
| VP           | vapour pressure |