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SCIENTIFIC OPINION

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Safety and efficacy of a feed additive consisting of salinomycin sodium (Sacox®) for rabbits for fattening (Huvepharma N.V.)

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Abstract

Following a request from the European Commission, EFSA was asked to deliver a scientific opinion on the safety and efficacy of the coccidiostat salinomycin sodium (Sacox®) for rabbits for fattening. The EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) concluded that the use of salinomycin sodium (SAL-Na) from Sacox® does not raise safety concerns for the target species, consumers, users and the environment with regard to the production strain. In the absence of adequate tolerance studies, the FEEDAP Panel could not conclude on the safety of SAL-Na from Sacox® for rabbits for fattening. The FEEDAP Panel concluded that the additive is safe for the consumer when it is used at the proposed maximum level of 25 mg SAL-Na/kg complete feed for rabbits and a withdrawal period of 1 day is respected. The following maximum residue limits (MRL) are proposed for the marker residue compound salinomycin (SAL): 0.2 and 0.03 mg SAL/kg for liver and kidney, respectively. The additive is not irritant to skin and eyes but should be considered a potential dermal and respiratory sensitiser. A risk for inhalation toxicity could not be excluded. The use of the SAL-Na from Sacox® in feed for rabbits for fattening up to the highest proposed level will not pose a risk for the terrestrial and aquatic compartment and ground water. The risk of secondary poisoning can be excluded for worm-eating birds and mammals, while it cannot be excluded for fish-eating birds and mammals. The FEEDAP Panel concludes that SAL-Na from Sacox® at the minimum concentration of 20 mg SAL-Na/kg complete feed has the potential to control coccidiosis in rabbits for fattening. Development of resistance to SAL-Na of field *Eimeria* spp. strains isolated from rabbits for fattening should be monitored.

KEYWORDS

coccidiostat, efficacy, rabbits for fattening, Sacox, safety, salinomycin sodium

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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 4(1) of that Regulation lays down that any person seeking authorisation for a feed additive or for a new use of feed additive shall submit an application in accordance with Article 7.

The European Commission received a request from Huvepharma N.V.[2] for the authorisation of the additive consisting of salinomycin sodium (Sacox®), when used as a feed additive for rabbits for fattening (category: coccidiostats and histomonostats; functional group: coccidiostats).

According to Article 7(1) of Regulation (EC) No 1831/2003, the Commission forwarded the application to the European Food Safety Authority (EFSA) as an application under Article 4(1) (authorisation of a feed additive or new use of a feed additive). The dossier was received on 8 April 2020 and the general information and supporting documentation are available at https://open.efsa.europa.eu/questions/EFSA-Q-2020-00282. The particulars and documents in support of the application were considered valid by EFSA as of 23 July 2020.

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 feed additive consisting of salinomycin sodium (Sacox®), when used under the proposed conditions of use (see **Section 3.1.4**).

1.2 | Additional information

Salinomycin sodium (Sacox®) is currently not authorised for rabbits for fattening, but it is authorised for chickens for fattening and chickens reared for laying.[3] The FEEDAP Panel adopted three opinions on the safety and efficacy of this product for chickens for fattening (EFSA, 2004; EFSA FEEDAP Panel, 2017a) and for rabbits for fattening (EFSA FEEDAP Panel, 2018a).

2 | DATA AND METHODOLOGIES

2.1 | Data

The present assessment is based on data submitted by the applicant in the form of a technical dossier[4] in support of the authorisation request for the use of salinomycin sodium (Sacox®) as a feed additive.

The FEEDAP Panel used the data provided by the applicant together with data from other sources, such as previous risk assessments by EFSA or other expert bodies, peer-reviewed scientific papers and other scientific reports to deliver the present output.

The European Union Reference Laboratory (EURL) considered that the conclusions and recommendations reached in the previous assessment regarding the methods used for the control of the salinomycin sodium in animal feed/salinomycin marker residue in tissues are valid and applicable for the current application.[5]

2.2 | Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of salinomycin sodium (Sacox®) is in line with the principles laid down in Regulation (EC) No 429/2008[6] and the relevant guidance documents: Guidance on the assessment of the safety of feed additives for the consumer (EFSA FEEDAP Panel, 2017b), Guidance on the identity, characterisation and conditions of use of feed additives (EFSA FEEDAP Panel, 2017c), Guidance on the assessment of the safety of feed additives for the target species (EFSA FEEDAP Panel, 2017d), Guidance on the assessment of the efficacy of feed additives (EFSA FEEDAP Panel, 2018b), Guidance on the characterisation of microorganisms used as feed additives or as production organisms (EFSA FEEDAP Panel, 2018c), Guidance on the assessment of the safety of feed additives for the environment

[1]Regulation (EC) No 1831/2003 of the European Parliament and of the council of 22 September 2003 on the additives for use in animal nutrition. OJ L 268, 18.10.2003, p. 29.
[2]Huvepharma NV, Uitbreidingstraat 80, 2600 Antwerp, Belgium.
[3]Commission Implementing Regulation (EU) 2017/1914 of 19 October 2017 concerning the authorisation of salinomycin sodium (Sacox 120 microGranulate and Sacox 200 microGranulate) as a feed additive for chickens for fattening and chickens reared for laying and repealing Regulations (EC) No 1852/2003 and (EC) No 1463/2004 (holder of authorisation Huvepharma NV). OJ L 271, 20.10.2017, p. 1.
[4]Dossier reference: FAD-2019-0093.
[5]Evaluation report received on 08/09/2014 and available on the EU Science Hub https://joint-research-centre.ec.europa.eu/eurl-fa-eurl-feed-additives/eurl-fa-authorisation/eurl-fa-evaluation-reports_en.
[6]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.

(EFSA FEEDAP Panel, 2019), EFSA statement on the requirements for whole genome sequence analysis of microorganisms intentionally used in the food chain (EFSA, 2021) and the Guidance on studies concerning the safety of use of the additive for the users (EFSA FEEDAP Panel, 2023).

3 | ASSESSMENT

The additive under assessment Sacox® is intended to be used as a coccidiostat in feed for rabbits for fattening. The active substance is salinomycin (SAL), a monocarboxylic polyether ionophore, in the form of its sodium salt. Accordingly, the studies submitted are based on salinomycin sodium (SAL-Na). However, it should be recognised that SAL-Na in aqueous media dissociates into sodium cation and salinomycin anion. Salinomycin is a week acid (pKa 6.4); the pH of the biological fluid/physiological environment will determine whether SAL is mainly present in its anionic or in its undissociated (acidic) form. Since the biological effect is due to salinomycin, the term SAL will be used when referring to the biological properties of the active substance while SAL-Na is used to describe the chemical form in which the active substance is present in the additive.

3.1 | Characterisation

The additive Sacox®, contains as active substance salinomycin sodium (SAL-Na), ($C_{42}H_{69}NaO_{11}$; molecular weight 773 g/mol; CAS number: [55721-31-8]). The solubility in water (20°C) is 1371.2 mg/L (pH 9), 622.3 mg/L (pH 7) and < 5 mg/L (pH 4); the n-octanol/water partition coefficient (log K_{ow}) is 5.12 (pH 7.4) (EFSA FEEDAP Panel, 2018a).

3.1.1 | Characterisation of the production microorganism

SAL is produced by fermentation with a non-genetically modified strain of *Streptomyces* sp. The FEEDAP Panel evaluated the production strain in its former opinion (EFSA FEEDAP Panel, 2017a). In the current application, the applicant completed the former data set in line with the most recent Guidance on the characterisation of microorganisms used as feed additives or as production organisms (EFSA FEEDAP Panel, 2018c).

The strain was originally identified as *Streptomyces albus* and deposited at the Deutsche Sammlung von Mikroorganismen Zellkulturen with the accession number DSM 32267.[7]

The whole genome sequence (WGS) data were used for the taxonomical identification of the production strain [redacted][8] [redacted][9] [redacted]. Based on the data available, DSM 32267 belongs to the genus *Streptomyces* but cannot be allocated to any species.

The susceptibility of the production strain DSM 32267 to the relevant antibiotics listed in the Guidance on the characterisation of microorganisms used as feed additives or as production organisms (EFSA FEEDAP Panel, 2018c) was tested with an agar disk diffusion method.[10] [redacted]

The WGS data of the strain were interrogated for the presence of antimicrobial resistance (AMR) genes, [redacted][11] [redacted]

The WGS data were interrogated for the presence of genes encoding for virulence factors [redacted][12] [redacted]

The presence of antimicrobial compounds relevant to the use of antibiotics in humans or animals, other than SAL, was assessed by determining the inhibitory activity of three batches of the fermentation broth [redacted] compared to those obtained with one sample of pure SAL-Na [redacted]

[7]Technical dossier/Supplementary information data March 2022/Annex_RTQ_II_2.
[8]Technical dossier/Supplementary information February 2021/Annex_RTQ_II_1_1, Annex_RTQ_II_1_2, Annex_RTQ_II_1_3.
[9]Technical dossier/Supplementary information May 2021/Annex_RTQ_I_a.
[10]Technical dossier/Supplementary information February 2021/Annex_RTQ_II_2_1.
[11]Technical dossier/Supplementary information January 2024/Annex_RTQ_II_2.
[12]Technical dossier/Supplementary information January 2024/Annex_RTQ_II_2.

[redacted][13] [redacted] the product is considered free of antimicrobial activity other than that of SAL.

Based on the data provided, the FEEDAP Panel cannot conclude on the taxonomic identification of the production strain DSM 32267 at species level. [redacted]

3.1.2 | Characterisation of the additive

Salinomycin (SAL) is produced by fermentation [redacted] SAL-Na is not isolated after the fermentation process; thus, it is present in Sacox® in its mycelial form.

The full characterisation of the additive is described in a former opinion on the same additive (EFSA FEEDAP Panel, 2017a). The applicant stated that no modifications in the manufacturing process and composition of the two formulations have been introduced since the last authorisation. New data have been submitted on the batch-to-batch variation and on the impurities.

The additive is available in two formulations, Sacox® 120 microGranulate and Sacox® 200 microGranulate. Sacox® 120 microGranulate is specified to contain 114–132 g SAL-Na/kg while Sacox® 200 microGranulate is specified to contain 190–220 g SAL-Na/kg.

The formulations are composed of the dried fermentation substrate containing SAL-Na (28.5%–42.6% and 77.3%–85.6%, respectively), silicon dioxide (E551)[14] (up to 10% and 15%, respectively) and calcium carbonate (up to 70% and 15%).

The compliance with the specifications was confirmed in the batch-to-batch variation of five batches of each formulation of the additive with an average content of 122.4 g SAL-Na/kg (range: 121–124 g/kg) and 205.8 g SAL-Na/kg (range: 201–212 g/kg), respectively, for the two formulations.[15]

The current authorisation for chickens for fattening and reared for laying[16] set limits for the following related impurities: ≤ 10 mg elaiophylin/kg SAL-Na, ≤ 2 g 17-epi-20-desoxy-salinomycin/kg SAL-Na, ≤ 10 g 20-desoxy-salinomycin/kg SAL-Na, ≤ 10 g 18,19-dihydro salinomycin/kg SAL-Na and 10 g methylated salinomycin/kg SAL-Na. These substances were monitored in three batches of Sacox® 120 microGranulate. Elaiophylin was not detected, 17-epi-20-desoxy-salinomycin amounted to 0.1 g/kg SAL-Na in all three batches, 20-deoxysalinomycin ranged from 2 to 4 g/kg SAL-Na, methylated salinomycin(s) and 18,19-dihydro salinomycin were below 0.1 g/kg SAL-Na.[17] The same impurities were also analysed in three batches of Sacox® 200 microGranulate. Elaiophylin was not detected, 17-epi-20-desoxy-salinomycin ranged from 0.1 to 0.2 g/kg SAL-Na, 20-deoxysalinomycin ranged from 8 to 9 g/kg SAL-Na, methylated salinomycin(s) and 18,19-dihydro salinomycin were below 0.1 g/kg SAL-Na.[18]

Three batches of each formulation were analysed for the presence of chemical impurities and *Salmonella* spp.[19] In the formulation Sacox® 120 microGranulate, results showed concentrations of arsenic between 0.282 and 0.940 mg/kg, cadmium between 0.003 and 0.192 mg/kg, lead between 0.38 and 3.44 mg/kg and mercury below the limit of quantification (LOQ) except in one batch in which the concentration was 0.009 mg/kg.[20] In Sacox® 200 microGranulate, results showed concentrations of arsenic between 0.182 and 0.682 mg/kg, cadmium between 0.026 and 0.109 mg/kg, lead between 0.30 and 0.93 mg/kg and mercury below the LOQ. Levels of aflatoxins B1, B2, G1, G2 were below the LOQs in both formulations.[21] Polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and dioxin-like polychlorinated biphenyls (PCBs) were analysed. In both formulations, the calculated upper bound (UB) concentration was 0.137 ng WHO-PCDD/F TEQ/kg for the sum of dioxins, and 0.269 ng WHO PCCD/F + PCB TEQ/kg for the sum of dioxins and dioxin-like PCBs, the UB for the sum of non-dioxin-like PCBs was 0.003 mg/kg.[22] In both formulations, pesticides were quantified in a multianalyte analysis in two batches and found below their corresponding LOQs. *Salmonella* spp. was absent in 25 g of the additive. The

[13]Technical dossier/Supplementary information February 2021/Annex_RTQ_II_1_5.

[14]Currently under re-evaluation.

[15]Technical dossier/Supplementary information January 2024/Annex_RTQ_II_1_1.

[16]OJ L 271, 20.10.2017, p. 1.

[17]Annex_RTQ_II_1_2 Limit of detection in mg/kg for Elaiophylin was 10.

[18]Annex_RTQ_II_1_3 Limit of detection in mg/kg for Elaiophylin was 10.

[19]Technical dossier/Supplementary information January 2024/Annex_RTQ_II_1_5 Annex_RTQ_II_1_6.

[20]LOQ for mercury: 0.005 mg/kg.

[21]LOQ for aflatoxins: 1 μg/kg.

[22]Upper bound concentrations are calculated on the assumption that all values of the different congeners below the limit of quantification are equal to the limit of quantification. TEQ = toxic equivalency factors for dioxins, furans and dioxin-like PCBs established by the WHO in 2005 (van den Berg et al., 2006).

FEEDAP Panel considers that the microbial contamination and the amounts of the detected impurities do not raise safety concerns.

The presence of viable cells of the production strain was investigated in three independent batches of the active substance (intermediate after the recovery process) analysed in triplicate.[23] [redacted] No colonies were detected after 14 days of incubation at 33°C in the analysed batches.

The presence of DNA of the production strain in both formulations of the product was excluded by polymerase chain reaction (PCR) analysis of 1 g from three batches (in triplicate).[24] [redacted]

Since viable cells and DNA of the production strain *Streptomyces* sp. DSM 32267 were not detected in the product, the presence in its genome of a macrolide resistance gene and of any potential virulence factors encoding gene (see Section 3.1.1) is not considered a risk.

3.1.3 | Physical properties of the additive

Both formulations appear as beige to brown granules. Sieve analysis of the granules showed that 6% and 3% of the particles (w/w) passed through 100 μm sieve (EFSA FEEDAP Panel, 2017a), for Sacox® 120 microGranulate and Sacox® 200 microGranulate, respectively. The applicant submitted[25] (i) particle size analysis data of Sacox® 200 microGranulate using scanning electron microscopy (SEM) and transmission electron microscopy (TEM) techniques as foreseen in the Guidance on technical requirements for regulated food and feed product applications to establish the presence of small particles including nanoparticles (EFSA SC, 2021a) and (ii) a confirmation that the same active substance and same formulation have been used since the early stage of development in studies of target animal safety, dusting potential, local toxicity, residue studies and others. The submitted EM data did not allow the FEEDAP Panel to conclude on the absence of (a fraction of) small particles including nanoparticles due to shortcomings identified in the methodology. In particular, the provided descriptive SEM and TEM analysis demonstrate the presence of particles of variable size. The presented electron micrographs were taken at relatively low magnification (μm size scale bars) making it impossible to evaluate the presence of particles in the nano range (i.e. 1–250 nm). Moreover, the product consists of different components and the applied methodology does not allow to differentiate the particles of each component.

The FEEDAP Panel notes that pure SAL-Na is classified as very slightly soluble[26] in water, while it is soluble in organic solvents to some extent (the log K_{ow} is 5.12 (pH 7.4) (EFSA FEEDAP Panel, 2017a)) and that the test items used in the absorption, distribution, metabolism and excretion (ADME), residue and toxicological studies, including tolerance studies, were representative of the active substance (see Section 3.2; EFSA, FEEDAP Panel, 2018a). Based on the physico-chemical properties (log K_{ow}), ADME and toxicological profile of SAL-Na, it can be concluded that systemic distribution of solid particles is unlikely to occur; therefore, no further characterisation of the fraction of small/nano particles is needed.

No new data on the technological properties of the additive have been submitted. Since there were no changes in the manufacturing process and the composition, the FEEDAP Panel agreed that the data assessed in the former assessment (EFSA FEEDAP Panel, 2017a) and resubmitted in the present application can be considered to support the stability of the additive and its capacity to homogeneously distribute in feed/premixtures.

3.1.4 | Conditions of use

Sacox® is intended to be used in the prevention of coccidiosis in rabbits for fattening at a minimum use level of 20 mg SAL-Na/kg complete feed and a maximum of 25 mg SAL-Na/kg complete feed. A one-day withdrawal period is proposed.

3.2 | Safety

3.2.1 | Safety for the target species

In its former opinion, the FEEDAP Panel assessed the safety of SAL-Na from Sacox® for rabbits for fattening (EFSA FEEDAP Panel, 2018a). The evaluation relied on studies performed between 1978 and 1983 that were not compliant with current standards of a tolerance study. The FEEDAP Panel considered that levels of 35 mg SAL-Na/kg feed and higher were not tolerated by growing rabbits and that the available data provided some indications that the additive might be tolerated by rabbits for fattening up to 25 mg/kg. In addition, it was noted that the available data on reproductive toxicity of SAL-Na in

[23]Technical dossier/Supplementary information data February 2021/Annex_RTQ_II_1_6.

[24]Technical dossier/Supplementary information data March 2022/Annex_RTQ_II_1.

[25]Technical dossier/Supplementary information March 2023/Annex_RTQ_5.

[26]For solubility terms see Table 2 of EFSA SC (2021a).

rabbits indicated embryo–fetal toxicity with a no observed adverse effects level (NOAEL) of 0.63 mg/kg body weight (bw) per day (EFSA, 2004); thus, effects on breeding does when sharing feed with rabbits for fattening could not be excluded. Hence, the FEEDAP Panel recommended that feed containing SAL-Na 'should be given to rabbits for fattening only, avoid exposure of does'.

In the current application, two tolerance studies with Sacox 120® microGranulate in rabbits for fattening were submitted to support the safety of the additive at the maximum proposed level of 25 mg SAL-Na/kg complete feed.[27,28]

None of the studies could be considered acceptable to support the safety of the target species. In the first trial, a very high mortality occurred (average 21%), likely due to *E. coli* infection, which would indicate that animals were not healthy. In the second study, several shortcomings were identified: All animals were treated with bacitracin at the start of the trial for 14 days, no haematological end-points were measured due to problems with the sampling and the control group showed signs of *Eimeria* infection.

The FEEDAP Panel concluded in its former opinion that the simultaneous use of SAL-Na and certain antibiotics (e.g. tiamulin and valnemulin) and bentonite in rabbits is contraindicated (EFSA FEEDAP Panel, 2018a). In the absence of additional information, the same conclusions apply to the present assessment.

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

Conclusions on safety for the target species

In the absence of adequate tolerance studies, the FEEDAP Panel cannot conclude on the safety of Sacox® for rabbits for fattening.

3.2.2 | Safety for the consumer

In the former assessment related to the use of SAL-Na from Sacox® in rabbits (EFSA FEEDAP Panel, 2018a), the FEEDAP Panel confirmed the acceptable daily intake (ADI) of 0.005 mg SAL/kg bw and concluded that the safety of consumers of rabbit edible tissues was ensured applying a 5-day withdrawal period; a provisional maximum residue limit (MRL) of 0.01 mg SAL/kg liver was established. For the present assessment, the applicant submitted new studies which are assessed below. In addition, the applicant proposed a 1-day withdrawal time.

Absorption, distribution, metabolism, excretion and residues

The ADME of the active substance in rabbits can be summarised as follows: (i) SAL is largely absorbed in the rabbit intestine, extensively metabolised and excreted (about 80% in the faeces and 9% in the urine with only 1.8% unchanged SAL), (ii) SAL represents a very small fraction as compared with the numerous metabolites, predominantly a mono-hydroxylated compound, that have been identified in tissues and excreta, (iii) the metabolic fate of SAL is common to rabbit and laboratory animals (rat), (iv) liver is the target tissue, (v) SAL-related metabolites have a reduced ionophoric activity (20%) when compared to SAL (EFSA FEEDAP Panel, 2018a).

In the former assessment, only a provisional MRL of 0.01 mg SAL/kg liver could be established (EFSA FEEDAP Panel, 2018a). This restriction was related to a number of uncertainties identified in the studies, reported as: '(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.' The Panel noted also that the chemical structure of the major mono-hydroxy SAL metabolite in the liver was not fully elucidated, the hydroxylation position being hypothesised to occur at one of the carbons C4, C5, C40, C41 or C42. The applicant submitted new studies to address the uncertainties identified in the former opinion.

Total residues and metabolite identification

A study[29] was submitted on the identification and quantification of total residues in rabbit tissues and excreta following the oral administration of ^{14}C-SAL-Na. Three male and three female New Zealand rabbits (2.5–3.5 kg bw, 9–15 weeks old) were fed for 10 days a complete feed supplemented with 25 mg SAL-Na/kg, including ^{14}C-SAL-Na (3.7 MBq/kg bw, no indication on the labelling position on the molecule, radiochemical purity 95.9%). Twenty-four-hour individual urine and faeces were

[27]Technical dossier/Section III/Annex_III_2.
[28]Technical dossier/Section III/Annex_III_3.
[29]Technical dossier/Supplementary information March 2022/Annex_Additional data_III_1 Page 43.

collected along the experimental period and pooled by sex. The animals were slaughtered 24 h after having access to the supplemented feed, and liver, kidneys, muscle and fat were individually sampled. Total radioactivity in all samples was measured by liquid scintillation counting. Metabolite profiling was performed by high-performance liquid chromatography using an in-line radiodetector (radio-HPLC). Metabolite identification was obtained using radio-high-resolution liquid chromatography hyphened to tandem mass spectrometry (LC–MS/MS). The total residues in tissues are given in Table 1.

TABLE 1 Total residue concentrations in tissues (mg/kg wet tissue) of rabbits fed a complete feed supplemented with 25 mg ^{14}C-labelled SAL-Na/kg for 10 days followed by a 1-day withdrawal period.

	Liver	Kidney	Muscle	Skin/fat
TRC ± SD	3.232 ± 0.923	0.526 ± 0.203	0.007 ± 0.006	0.016 ± 0.006
TRC + 2SD	5.078	0.932	0.019	0.028

Abbreviations: SD, standard deviation; TRC, total residue concentration.

These results confirm that liver is by far the target tissue, in line with the conclusions of the former study (EFSA FEEDAP Panel, 2018a).

The radio-chromatographic profiles of urines from day 9 of the experiment showed three metabolites, U6, U9 and U10, amounting to 10.1%, 15.1% and 12.9% of the total radioactivity, respectively, in the urine of females, with only U9 being detected in urine of males (19%). In the faeces of the same day, all metabolites, including unchanged SAL, represented less than 5% the total residues.

The profiles obtained from liver extracts (extraction yields close to 100%) showed the presence of six compounds with two major ones, L10 and L17, amounting to an average of 28.8% and 11.5% of the whole radioactivity, respectively; the others represented each less than 10%. In kidney extracts (extraction yield ≥ 85%) between four (one female) and 10 (one male) compounds were separated of which only one (K16) amounted to an average of 16.8%, the others representing each less than 10%. The metabolic profiles obtained from pooled muscle and fat samples identified a number of compounds representing each less than 10% of the whole radioactivity.

Attempts were made to identify the compounds separated from the urine and the liver and kidney extracts. The LC–MS/MS analysis of the urine allowed to identify a compound of 260 Da corresponding to U9; no structural information was obtained but a likely hypothesis would suggest it could correspond to a metabolite issued from the breakdown of the SAL molecule (end part containing the carboxylic group). No chemical structure could be attributed to U6 and U10. It was shown that the major compound L10 from the liver corresponded to a mono-hydroxy-SAL and that L17 was a SAL isomer; due to the higher sensitivity of the analytical approach when compared to radio-HPLC, SAL was also identified as a minor compound; no confirmation of the mono-hydroxyl-SAL structure could be obtained. In the kidney, the main metabolite K6 corresponded to an SAL isomer and unchanged SAL was identified also as a minor compound.

The SAL isomer was identified in the labelled SAL used in the study that would correspond to a side fermentation product; however, its abiotic or metabolic origin cannot be ruled out.

Additional investigations were made considering the described hydroxylation reactions mediated by iron-oxo ferryl species (Huang & Groves, 2017), that could be used to predict possible hydroxylation sites of the SAL molecule. In the case of SAL in the liver, the applicant suggested that the iron-oxo group would be brought by the haem group resulting from haemoglobin metabolism and could be bound at several locations. Different mechanistic considerations led to the conclusion that hydroxylation would be more likely to occur on the C30 or C32 positions, less likely at C22 and C26.

An experimental confirmation of these theoretical considerations was provided by an additional in vivo metabolomic study[30] designed to identify unambiguously the main hydroxylated metabolite(s) of SAL by high-resolution mass spectrometry (HRMS). One male and one female rabbit (New Zealand white) were administered by gavage (twice a day) a daily dose of 3 mg SAL-Na from Sacox® 200 microgranulate (equivalent to 25 mg/kg complete feed) for 8 consecutive days. The rabbits were killed, and livers sampled 2 h after the last administration. The identification of SAL and SAL-related oxidised metabolites was based on their retention time and their fragmentation pattern using UHPLC-HRMS/MS detection. A sorting approach was applied to isolate the SAL-derived metabolites from other non-SAL-related compounds. Finally, SAL, an SAL-isomer and eight metabolites (four mono-hydroxy, two di-hydroxy and two tri-hydroxy-SAL) were identified. Quantification of SAL in liver referred to a response curve established with nigericin as internal standard. In the absence of information on their respective response factors, SAL-related compounds (the 8 main metabolites plus SAL-isomer) were semi-quantified. SAL was detected at very low concentrations (mean level of 0.028 mg/kg liver) which corresponds to a mean percentage of 3.2% of the total of all detected compounds. The semi-quantification of the metabolites confirms the prevalence of one of the mono-hydroxy-SAL (XM1) corresponding to metabolite L10 of the former study and of the SAL isomer corresponding to L17. The structural identification of XM1 was further investigated based on (i) UHPLC-HRMS/MS analyses performed in positive and in negative detection modes with MSe and data-dependent acquisition (DDA) modes and (ii) theoretical regioselective ferryl-mediated hydroxylation considerations. Based on literature review and the DDA-HRMS/MS fragments observed, the author concluded that two oxidation sites remained as likely hypothesis and could be proposed: C30 and C32. The author claims the impossibility of synthesising these metabolites at present.

[30]Technical dossier/Supplementary information March 2022/Annex_Additional data_III_1 Page 264.

The FEEDAP Panel notes that a progress has been made on the identification of SAL metabolites as mono-, di- and tri-hydroxy-SAL, the major liver metabolites being a mono-hydroxy compound likely hydroxylated at C30 or C32.

The proportion of SAL metabolites in tissues being much higher than that of unchanged SAL, according to the definition of the marker residue the most abundant of these metabolites should be retained as the marker. However, due to the present unavailability of a standard of this compound, an analytical method for its determination in tissues cannot be proposed. The very sensitive analytical method available for the determination of SAL in tissues allowed to calculate ratios marker to total residue in liver and kidneys after 1-day withdrawal and subsequently to set MRLs for these tissues. Consequently, the FEEDAP Panel considers that parent SAL has to be retained as the marker residue.

Marker residue

Twelve rabbits allocated to three groups of four animals each (2 males and 2 females) were administered orally 20 mg SAL-Na from Sacox® 120 microGranulate/kg feed, the lowest dose proposed (analytically confirmed) during 28 consecutive days, starting after an acclimatisation period of 8 days.[31] The animals were killed after 0-, 1- and 2-day withdrawal of the supplemented feed, and muscle, fat (perirenal, abdominal and subcutaneous), liver and kidneys were sampled. SAL was measured using a fully validated liquid chromatography–tandem mass spectrometry (UHPLC–MS/MS) technique with an LOQ of 0.005 mg/kg fresh tissue. The results are shown in Table 2. Due to the limited number of animals, and the large variation noticed, the individual values are given.

TABLE 2 Marker residue in tissues (mg SAL/kg wet tissue) of rabbits administered a complete feed supplemented with 20 mg SAL-Na/kg for 28 days followed by withdrawal periods of 0, 1 and 2 days.

Withdrawal time (days)	Liver	Kidney	Muscle	Fat
0	0.064	0.011	< 0.005	< 0.005
	0.319	0.066	< 0.005	< 0.005
	0.105	0.025	< 0.005	< 0.005
	0.088	0.014	< 0.005	< 0.005
1	0.127	0.026	< 0.005	< 0.005
	0.162	0.022	< 0.005	< 0.005
	0.009	< 0.005	< 0.005	< 0.005
	0.023	< 0.005	< 0.005	< 0.005
2	0.084	0.014	< 0.005	< 0.005
	0.023	0.006	< 0.005	< 0.005
	< 0.005	< 0.005	< 0.005	< 0.005
	0.009	< 0.005	< 0.005	< 0.005

Note: LOQ = 0.005 mg/kg fresh tissue.

Conclusions on residue studies

The new studies submitted by the applicant confirm the former data concerning total residues of SAL-Na in rabbit tissues, i.e. very low absolute residue levels in muscle and fat (0.4% and 0.6% of total residue concentration in liver, respectively). Marker residue concentrations in these tissues were below the LOQ (0.005 mg/kg). The FEEDAP Panel concludes that the parent SAL has to be retained as the marker residue. The ratios marker to total residues (RTMR) could be calculated at 1-day withdrawal for the liver and kidney only, since marker residue levels in muscle and fat were below the LOQ. The RTMRs calculated by the use of the highest total residue (mean + 2 SD, Table 1) and the highest individual value of the marker residue (Table 2) are 0.032 for liver and 0.028 for kidney.

Toxicological studies

The genotoxicity studies originally assessed in 2004 (EFSA, 2004) have been reassessed by the FEEDAP Panel in the context of the current application following the latest guidance documents (EFSA FEEDAP Panel, 2017b; EFSA SC, 2021b). The FEEDAP Panel considered that the formerly reached conclusions can be reiterated: 'SAL-Na does not induce gene mutations *in vitro* and it is not genotoxic in mouse bone marrow studies in vivo.'

The toxicity of SAL-Na was re-evaluated by the FEEDAP Panel in 2017 (EFSA FEEDAP Panel, 2017a); the lowest NOAEL of 0.5 mg SAL/kg bw per day could be identified from a single dose toxicity cardiovascular study in dogs (pharmacological NOAEL) as well as from a 12-month dog study (toxicological NOAEL). This was further supported by the NOAEL identified

[31]Technical dossier/Supplementary information/March 2023/Annex_RTQ_III_2.

from a 90-day study in rats (0.6 mg/kg bw per day). No new information was made available to reconsider the conclusions on the toxicity of SAL-Na.

Therefore, from the toxicological and pharmacological NOAELs of 0.5 mg SAL/kg and applying an uncertainty factor (UF) of 100, the FEEDAP Panel derives, respectively, an ADI and acute reference dose (ARfD) of 0.005 mg SAL/kg bw.

Consumer exposure and consumer safety assessment

The assessment of consumer safety carried out in 2018, is updated taking into account the new residue data made available.

In its former assessment of SAL-Na from Sacox® for chickens for fattening (EFSA FEEDAP Panel, 2017a), 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. As far as the metabolic biotransformation are similar in the chicken and rabbit, it can be assumed that only 20% of these residues would be of toxicological concern also in the rabbits (EFSA FEEDAP Panel, 2018a).

The chronic exposure of consumers to SAL residues in rabbit tissues was calculated following the methodology described in the Guidance on consumer safety (EFSA FEEDAP Panel, 2017b). The input values of total residues after 1-day withdrawal (shortest period tested) used for the calculation are given in Table 3. The results of the calculation for different population classes are presented in Table 4.

TABLE 3 Input values used to calculate consumer exposure to SAL residues (mg/kg) in rabbit tissues.

	Input values (mg/kg tissue)
Mammals fat tissue	0.028
Mammals liver	5.078
Mammals meat[a]	0.021
Mammals offals and slaughtering products (other than liver)	0.932

[a]The residue concentration in muscle (0.019 mg/kg) and skin/fat (0.028 mg/kg) was used to calculate the intake of meat at the following proportions: 80% muscle and 20% skin/fat (EFSA FEEDAP Panel, 2017b).

TABLE 4 Chronic dietary exposure to SAL total residues based on residue data in rabbit tissues after a 1-day withdrawal period.

Population class	Highest exposure estimate[a] (mg/kg bw per day)	% ADI[b]
Infants	0.0025	50
Toddlers	0.0029	58
Other children	0.0022	44
Adolescents	0.0012	24
Adults	0.0022	45
Elderly	0.0017	33
Very elderly	0.0013	27

[a]Expressed as maximum highest reliable percentile.

[b]ADI = 0.005 mg/kg bw.

The results on the chronic exposure (Table 4) showed that the highest chronic exposure was for the population class 'toddlers' with 0.0029 mg/kg bw per day, corresponding to 58% of the ADI (for detailed results per age class, country and survey see Appendix A, Table A1). Considering that the residues of toxicological concern represent only 20% of the total residues in tissues and organs of the target animals, the exposure to residues of toxicological concern for the population class 'toddlers' would be approximately 12% of the ADI. The Panel notes that these calculations are very conservative as they result from the assumption that all mammal meat and other food products would come from rabbits.

Acute exposure to SAL residues should also be considered taking into account the ARfD of 0.005 mg/kg bw. The Panel notes that the food consumption data used in the Feed Additive Consumer Exposure tool developed by EFSA does not have data on consumption of rabbit liver and kidney to allow an accurate acute exposure assessment. In the absence of such data, the Panel considered it more appropriate to back-calculate the amount of rabbit meat, liver, kidney and fat that would be necessary for a 70-kg consumer to reach an exposure level equal to the ARfD, considering the residues of toxicological concern (Table 5). Considering an approximate weight of 65 g for rabbit liver and 18 g for rabbit kidney,[32] an adult

[32]Reported by Metzger et al. (2006) for rabbits of various genotypes (body weight at slaughter was about 2600 g).

consumer should eat roughly five rabbit livers or 105 kidneys to reach the ARfD while for a toddler the consumption of one liver or 18 kidneys would reach the ARfD. The FEEDAP Panel consequently concludes that the consumption of rabbit edible tissues and organs considered for the acute exposure remains safe for the consumer.

TABLE 5 Amount of rabbit edible tissues to be consumed by adults and toddlers to reach the ARfD.

	Liver	Kidney	Muscle	Fat
Total residue value (mg SAL/kg tissue)	5.078	0.932	0.019	0.028
Residues of toxicological concern[a] (mg SAL/kg tissue)	1.016	0.186	0.004	0.006
ARfD for a person of 70 kg bw (mg SAL/day)	0.35			
ARfD for toddlers of 12 kg bw (mg SAL/day)	0.06			
Amount to reach ARfD for a person of 70 kg bw (kg tissue/day)	0.344	1.882	87.5	58.3
Amount to reach ARfD for a toddler of 12 kg bw (kg tissue/day)	0.059	0.323	15	10

[a]Only 20% of the residues represent a concern.

Proposal for maximum residue limits (MRLs)

The RTMR could be calculated at 1-day withdrawal for the liver and kidney only, since marker residue levels in muscle and fat were below the LOQ.

The RTMRs calculated by the use of the highest total residue (mean + 2 SD, Table 1) and the highest individual value of the marker residue (Table 2) are 0.032 for liver and 0.028 for kidney. Considering the very low absolute residue levels in muscle and fat (0.4% and 0.6% of total residue concentration in liver, respectively) and that the marker residue concentrations in these tissues were below the LOQ (0.005 mg/kg), no MRLs would be proposed for muscle and fat.

The FEEDAP Panel proposed in its 2018 opinion a provisional MRL of 0.01 mg/kg liver wet tissue. Considering the new marker residue study, the FEEDAP Panel considers that the following MRLs should be applied: 0.2 mg SAL/kg liver and 0.03 mg SAL/kg kidney. No MRLs are proposed for muscle or fat.

The FEEDAP Panel agrees to the proposal of the applicant of a withdrawal period of 1-day.

The chronic exposure of consumers to SAL residues in rabbit tissues can be calculated based on the proposed MRLs by deriving total residue concentrations applying the RMTR to the proposed MRLs (Table 6). The input values for the calculation are given in Table 7 and the results for different population categories are presented in Table 8.

TABLE 6 SAL total residues calculated from the proposed MRL for rabbit liver and kidney applying the ratios marker to total residue (RMTR) for each tissue/product (mg/kg).

	Liver	Kidney
MRLs proposed	0.2	0.03
RMTR	0.032	0.028
TR_{MRL}	6.250	1.071

Abbreviations: MRL, maximum residue limit; RMTR, ratio marker to total residues; TR_{MRL}, total residue value calculated based on MRLs.

TABLE 7 Input values used to calculate consumer exposure to SAL residues (mg/kg) calculated from MRLs of liver and kidney in rabbit tissues.

	Input values (mg/kg tissue)
Mammals fat tissue	0.028
Mammals liver	6.250
Mammals meat[a]	0.021
Mammals offals and slaughtering products (other than liver)	1.071

[a]The residue concentration in muscle (0.019 mg/kg) and skin/fat (0.028 mg/kg) will be applied to the intake of meat at the following proportions: 80% muscle and 20% skin/fat (EFSA FEEDAP Panel, 2017b).

TABLE 8 Chronic dietary exposure to SAL total residues derived from MRLs in rabbit tissues.

Population class	Highest exposure estimate[a] (mg/kg bw per day)	% ADI[b]
Infants	0.0031	62
Toddlers	0.0036	72
Other children	0.0027	54
Adolescents	0.0015	30
Adults	0.0027	54
Elderly	0.0020	40
Very elderly	0.0016	32

[a]Expressed as maximum highest reliable percentile.
[b]ADI = 0.005 mg/kg bw.

The results on the chronic exposure (Table 8) showed that the highest chronic exposure was for the population class 'toddlers' with 0.0036 mg/kg bw per day, corresponding to 72% of the ADI (for detailed results per age class, country and survey see Appendix A, Table A2). Considering that the residues of toxicological concern represent only 20% of the total residues in tissues and organs of the target animals, the exposure to residues of toxicological concern would be approximately 14% of the ADI.

Thus, the Panel concludes that these MRLs (0.2 and 0.03 mg SAL/kg for liver and kidney, respectively) ensure consumer safety.

Additional considerations on consumer exposure

Consumer exposure to residues of SAL needs to take into consideration all potential dietary sources. SAL-Na is authorised in the EU for chickens for fattening and chickens reared for laying. MRLs in force for chicken liver, kidney, muscle and skin/fat are 0.150, 0.040, 0.015 and 0.150 mg SAL/kg wet tissue, respectively.

The chronic dietary exposure to SAL residues from all sources (i.e. consumption of chicken and rabbit) can be calculated based on the MRLs: (i) those proposed for rabbit liver and kidney and (ii) the authorised MRLs for chicken tissues. For the purpose of this calculation, total residue data are derived from the respective MRLs, the values are given in Table 7 (rabbit) and Table 9 (chicken). The input values for the calculation are given in Table 10 and the results for different population categories are presented in Table 11.

TABLE 9 SAL total residues calculated from authorised MRL values of chicken tissues applying the ratios marker to total residue (RMTR) for each tissue/product (mg/kg).

	Liver	Kidney	Muscle	Skin/fat
MRLs in force for chicken tissues	0.150	0.040	0.015	0.150
RMTR[a]	0.06	0.15	0.30	0.56
TR_{MRL}	2.500	0.267	0.050	0.268

Abbreviations: MRL, maximum residue limit; RMTR, ratio marker to total residues; TR_{MRL}, total residue value calculated based on MRLs.
[a]EFSA FEEDAP Panel (2017a).

TABLE 10 Input values used to calculate consumer exposure to SAL residues (mg/kg) calculated from proposed MRLs of liver and kidney in rabbit tissues and authorised MRLs in chicken tissues.

	Input values (mg/kg tissue)
Birds fat tissue	0.268
Birds liver	2.500
Birds meat[a]	0.050
Birds offals and slaughtering products (other than liver)	0.267
Mammals fat tissue	0.028
Mammals liver	6.250
Mammals meat[b]	0.021
Mammals offals and slaughtering products (other than liver)	1.071

[a]The residue concentration in muscle and skin/fat will be applied to the intake of bird meat at the following proportions: 90% muscle and 10% skin/fat (EFSA FEEDAP Panel, 2017b).
[b]The residue concentration in muscle and skin/fat will be applied to the intake of mammal meat at the following proportions: 80% muscle and 20% skin/fat (EFSA FEEDAP Panel, 2017b).

TABLE 11 Chronic dietary exposure to SAL total residues based on authorised MRLs in chicken tissues and the proposed MRLs in rabbit tissues.

Population class	Highest exposure estimate[a] (mg/kg bw per day)	% ADI[b]
Infants	0.0031	62
Toddlers	0.0036	72
Other children	0.0027	54
Adolescents	0.0016	32
Adults	0.0028	56
Elderly	0.0025	50
Very elderly	0.0017	34

[a]Expressed as maximum highest reliable percentile.
[b]ADI = 0.005 mg/kg bw.

The results on the chronic exposure of consumers to total SAL residues taking into account all sources (i.e. consumption of chicken and rabbit) showed that the highest chronic exposure was for the population class 'toddlers' with 0.0036 mg/kg bw per day, corresponding to 72% of the ADI (for detailed results per age class, country and survey, see Appendix A, Table A3). Considering that the residues of toxicological concern represent only 20% of the total residues in tissues and organs of the target animals, the exposure to residues of toxicological concern would be approximately 14% of the ADI. Therefore, the Panel concludes that the proposed MRLs for rabbit ensure consumer safety also when considering all other potential SAL sources.

Conclusions of consumer safety assessment

The FEEDAP Panel concludes that SAL-Na from Sacox® is safe for the consumer when it is used at the proposed maximum level of 25 mg SAL-Na/kg complete feed for rabbits and a withdrawal period of 1 day is respected. The following MRLs are proposed: 0.2 and 0.03 mg SAL/kg for liver and kidney, respectively.

3.2.3 | Safety for the user

The safety of both formulations of Sacox® was assessed in the previous opinions of the FEEDAP Panel (EFSA, 2004; EFSA FEEDAP Panel, 2017a) based on skin and eye irritation, skin sensitisation studies (Sacox® 120 microGranulate) and acute inhalation toxicity studies (Sacox® 120 microGranulate and Sacox® 200 microGranulate). The Panel concluded that both formulations of the additive are not irritant to skin and eyes, but should be considered potential dermal and respiratory sensitisers. A risk for inhalation toxicity could not be excluded. No additional information was submitted in the present application, and therefore, the Panel reiterates its previous conclusions.

3.2.4 | Safety for the environment

The environmental safety for SAL-Na from Sacox® when used as feed additive in chickens for fattening and chickens reared for laying was evaluated in 2017 (EFSA, FEEDAP Panel, 2017a) and for rabbits for fattening in 2018 (EFSA FEEDAP Panel, 2018a). The applicant submitted an updated environmental risk assessment in line with the requirements of the FEEDAP guidance to evaluate the safety of the additives for the environment (EFSA FEEDAP Panel, 2019).

3.2.4.1 | *Phase I*

Physico-chemical properties of SAL-Na

The physico-chemical properties of SAL-Na have been reviewed in previous opinions (EFSA FEEDAP Panel, 2017a, 2018a) and are summarised in Table 12.

The low vapour pressure indicates that the substance is unlikely to volatilise. SAL is a weak carboxylic acid; at higher pH (above 6.4) is present in its anionic form which is soluble in water while at low pH, it is predominantly in its undissociated form, which is poorly soluble in water. Higher solubility (at high pH) is generally associated with low sorption. Low sorption leads to a higher risk of the pollution of groundwater and aquatic ecosystems.

TABLE 12 Physico-chemical properties of SAL-Na.

Property	Value	Unit
Octanol/water partition coefficient[a] (log Kow 25°C)	5.12 (pH 7.4)	–
Water solubility[b] (20°C)	< 5 (pH 4) 622.3 (pH 7) 1371.2 (pH 9)	mg/L
Vapour pressure[c]	$< 5 \times 10^{-5}$ (25°C)	Pa
Dissociation constant[d] pKa	6.4 (20°C)	–

[a]OECD Guideline 117, shake-flask method.
[b]OECD Guideline 105.
[c]Gas saturation method.
[d]OECD Guideline 112.

Fate and behaviour

Fate in soil

Adsorption

The study on the characterisation of the adsorption/desorption in three soils as assessed in previous opinions (EFSA, 2004; EFSA FEEDAP Panel, 2017a, 2018a) and performed according to an old version of OECD Guideline 106, is still considered acceptable. Table 13 reports the soil characteristics, the Freundlich coefficient K_f, the adsorption coefficient K_d and the corresponding K_{oc} values.

TABLE 13 Soil sorption coefficients (K_{oc}) of SAL in different studies.

Soil	% OC	% clay	pH	K_f (L/kg)	K_d (L/kg)	K_{oc} (L/kg)
Clay loam	1.6	30.3	7.3	5	2.9	180
Loamy sand	1.8	6.6	4.7	31	5.9	368
Sandy loam	1.6	17.2	6.0	12	23.5	1306

Two literature studies were provided to further address adsorption. The first one, (Ramaswamy et al., 2012), performed on three soils, clearly deviates from the standard experimental approach for the determination of adsorption coefficients. In particular, the use of methanol as the liquid phase could have had significant effects on the sorption coefficients generated; therefore, the data generated on the sorption of SAL cannot be considered reliable for use in the risk assessment. The second one, Hussain and Prasher (2011), performed batch equilibrium studies on two soils establishing sorption isotherms at seven concentrations. This study is closer to standard criteria; nevertheless, considering that the mass balance reported is between 77% and 88%, the study cannot be considered reliable for use in the risk assessment.

Considering the characteristics of SAL and the limited number of soils investigated, the lowest K_{oc} value of 180 L/kg will be considered for further assessment.

Biodegradation in soil

Two studies were made available to investigate the degradation of ^{14}C-SAL-Na in soil following OECD Guideline 307.

One of the studies was already assessed in a previous opinion (EFSA FEEDAP Panel, 2017a). Results indicated that SAL was rapidly degraded into a few components in all four types of soils investigated. Two major metabolites were found at levels below 10%, but they were not identified. Most of the SAL was degraded to $^{14}CO_2$. The half-life (DT_{50}) values calculated according to single first-order kinetics (SFO) for SAL-Na ranged from 8 days up to 19 days.

In the second study,[33] degradation of SAL-Na was investigated in three soils with different properties and the route of degradation in one soil (sandy loam). SAL-Na was applied to the soils at a nominal rate of 4.5 mg/kg dry soil and incubated for up to 131 days at a nominal temperature of 20°C. The mass balance achieved in the sandy loam soil used to determine the degradation route was less than 90% at 120 days of incubation and it was proposed that the low recovery was a result of incomplete trapping of $^{14}CO_2$. Due to the rapid degradation of SAL and insufficient number of measurements, specific DT_{50} and DT_{90} values could not be established for the sandy loam and clay loam soils. A DT_{50} value could only be established for the silty clay loam soil: according to SFO kinetics, a DT_{50} of 16.2 days and a DT_{90} of 53.8 days can be derived.

The DT_{50} and DT_{90} from the five soils at 20°C together with DT_{50} at 12°C and the relative geometric and arithmetic mean are reported in Table 14.

[33]Technical dossier/Supplementary information/March 2023/Annex_RTQ_III_1.

TABLE 14 Soil biodegradation rate of SAL in soils.

Soil	DT_{50} (day) (20°C)	DT_{90} (day) (20°C)	DT_{50} (day) (12°C)
Silty clay loam[a]	16.2	53.8	34.3
Sand[b]	12.9	42.7	27.3
Loamy sand[b]	16.1	53.5	34.1
Sandy loam[b]	7.81	26.0	16.6
Clay[b]	18.7	62.0	39.6
Arithmetic mean	14.3	47.6	30.4
Geometric mean	13.7	45.6	29.1

[a]Technical dossier/Supplementary information/Annex_RTQ_III_1.
[b]EFSA FEEDAP Panel (2017a).

The geometric mean DT_{50} of 29.1 days (at 12°C) will be used for further evaluation.

Conclusion on fate and behaviour

The FEEDAP Panel considers a K_{oc} value of 180 L/kg and a geomean DT_{50} of 29.1 days (at 12°C) as most appropriate input values for environmental exposure assessment.

Predicted environmental concentrations (PECs) – Phase I

The predicted environmental concentrations (PECs) were calculated according to the FEEDAP technical guidance for assessing the safety of feed additives for the environment (EFSA FEEDAP Panel, 2019).

The input values used for initial PEC calculations were 24.29 mg SAL/kg rabbit feed, molecular weight (SAL) = 751 g/mol, vapour pressure $< 5 \times 10^{-5}$ Pa, solubility = 622.3 mg/L, DT_{50} = 29.1 (at 12°C) and K_{oc} = 180 L/kg. The calculated PEC values are reported in Table 15.

TABLE 15 Initial predicted environmental concentrations (PECs) of SAL in soil (μg/kg) and groundwater (μg/L).

Compartment	PEC (μg/kg or μg/L)
Soil	330
Ground water	22

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

3.2.4.2 | *Phase II*

Exposure assessment

PECs calculation refined in Phase II

In its opinion on the safety and efficacy of Sacox® for chickens for fattening and chickens reared for laying (EFSA FEEDAP Panel, 2017a), 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 same assumption can be made for the SAL-derived ionophoric activity in rabbit manure.

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 PEC calculations are reported in Table 16.

TABLE 16 Predicted environmental concentrations (PECs) of SAL in soil, groundwater, surface water and sediment.

	Value
Input	
Dose (mg SAL/kg feed)	24.29
Fraction excreted (%)	20
Molecular weight SAL (g/mol)	751
Vapour pressure (Pa)	5×10^{-5}
Solubility (mg/L)	622
Log K_{ow} (pH 7.4)	5.12
K_{oc} (L/kg)	180
DT50 at 12°C (days)	29.1
Output	
PEC_{soil} (µg/kg)	66.1
$PEC_{groundwater}$ (µg/L)	4.4
$PEC_{surfacewater}$ (µg/L)	1.5
$PEC_{sediment}$ (µg/L)	31.9

PEC_{gw} refinement

According to EFSA guidance (2019), the FOCUS PEARL Metamodel can be used to identify feed additives whose leaching concentration is expected to be below 0.1 µg/L.

The minimum K_{om} (K_{oc}/1.7) of SAL is 104.4 L/kg, and the geometric mean soil DT_{50} is 13.7 days at 20°C. Based on the metamodel, SAL concentration in groundwater is not expected to be higher than the limit value of 0.1 µg/L.

Therefore, no concern for groundwater is expected when the additive is used at the supported conditions in rabbits for fattening.

Ecotoxicity studies

Toxicity to terrestrial compartment

Two studies following OECD guideline 208 were submitted to investigate the effect of SAL on terrestrial plants. Both studies were evaluated in a previous opinion of the FEEDAP Panel (EFSA FEEDAP Panel, 2017a). The studies were re-evaluated for the current assessment and the previous conclusions are confirmed: The first study indicated that *R. sativus* was the most sensitive species for emergence with median effective concentration (EC_{50}) of 2.55 mg of SAL/kg of soil and the second one that the most sensitive chronic endpoint established was no observed effect concentration (NOEC) of 0.52 mg SAL/kg soil for the emergence in *C. sativa*. A species sensitivity distribution (SSD) was performed following EMA (2017). Ecotoxicity data for eight plant species deriving from the reports of the studies meet the minimum requirements for generating an SSD. The available data set contains NOEC and/or EC_{10} values for eight species from six different families (three monocotyledon and five dicotyledon species, one of which is a *Brassica*). The most sensitive endpoint (EC_{10} or NOEC) for each available species instead of recommended eight common endpoints (either EC_{10} or NOEC) was used to construct SSD. Such an approach is considered sufficiently conservative. The SSD was generated using the software package ETX version 2.3 (RIVM, Netherlands). The available data passed the goodness of fit for normality (Anderson–Darling test). As recommended by EMA, the median lower confidence level (LL HC_5) of 185.2 µg SAL/kg can be used for risk characterisation without the assessment factor.

An acute toxicity study following OECD guideline 207 was performed on earthworms (*Eisenia fetida*). The conclusions reached by the FEEDAP Panel in 2017 are still valid for the current evaluation: the 14-day 50% lethal concentration (LC_{50}) was 102.9 mg of SAL/kg of soil dry weight (dw).

In a 28-day study, performed according to the OECD guideline 216, no effect on soil nitrogen transformation were observed at PEC or 10 times the PEC (EFSA FEEDAP Panel, 2017a). The same conclusions are valid for the present assessment.

Toxicity to aquatic organisms

The applicant submitted two studies on primary producers, both evaluated in 2017 by the FEEDAP Panel. In the first one, conducted in line with OECD 201 guideline, the 72 h E_rC_{50} and the 72 h NOEC based on the inhibition of growth of algae species *Desmodesmus subspicatus* (formerly known as *Scenedesmus subspicatus*) were determined to be 3.57 and 0.764 mg/L of SAL, respectively. The second one, conducted in line with OECD 201, was performed on cyanobacteria *Anabaena flos-aquae*. This study indicated that the 72 h E_rC_{50} was 25.6 mg/L and the 72 h NOEC < 1 mg/L. The FEEDAP Panel notes that the submitted test on cyanobacteria *A. flos-aquae* cannot be accepted to extrapolate the effects of SAL on algae or phytoplankton.

Cyanobacteria are prokaryotic primary producers and not related to eukaryotic green algae. The physiological differences between these two groups do not allow extrapolation of test results from one group to another. The E_rC_{50} for algae was almost one magnitude lower that the E_rC_{50} for cyanobacteria (The 72 h E_rC_{50} of 3.57 mg/L vs. 72 h E_rC_{50} of 25.6 mg/L of SAL for algae and cyanobacteria, respectively).

To investigate the effect of SAL on aquatic invertebrates and fish the applicant submitted the same studies, evaluated by the FEEDAP Panel in 2017 (EFSA FEEDAP Panel, 2017a). In those studies, the 48 h EC_{50} for immobilisation of *D. magna* was determined to be > 12.3 mg/L of SAL, the 96 h LC_{50} for *Danio rerio* was determined to be 6.98 mg/L of SAL. The same results are considered adequate for the current evaluation.

Sediment-dwelling invertebrates

A study was performed on larvae of *Chironomus riparius* according to the OECD Guideline 218 to assess chronic effects of SAL on sediment-dwelling organisms. The chironomid larvae were exposed to 0.356, 0.824, 1.65, 3.29, 6.59, 13.17 and 29.93 mg SAL/kg dw of sediment (mean measured concentrations).

The study was valid, the emergence in the controls was ≥ 70% at the end of the test, the emergence of adults in the control vessels occurred between 12 and 28 days, the oxygen concentration was > 60% of the air saturation value. SAL was not stable during the exposure period with mean measured concentrations ranging between 60.6% and 84.2% of nominal start concentrations at the end of the test; therefore, the evaluation of biological endpoints was performed using mean measured concentrations.

The NOEC was determined on both the emergence and development rate as 6.59 mg SAL/kg dw of sediment.

Risk characterisation

The risk characterisation for the terrestrial, aquatic and sediment is summarised in the Tables 17–19.

TABLE 17 Risk characterisation (PEC/PNEC ratio) for terrestrial compartment.

Taxa	PEC_{soil} (μg/kg)	LC_{50}/LL HC_5 (mg/kg)	AF	PNEC (μg/kg)	PEC/PNEC
Earthworm	66.1	103[a]	1000	103	0.64
Plants		0.185[b]	1	185	0.36

Abbreviation: AF, assessment factor.
[a]LC_{50}.
[b]LL HC_5.

TABLE 18 Risk characterisation (PEC/PNEC ratio) for freshwater compartment.

Taxa	$PEC_{surfacewater}$ (μg/L)	E_rC_{50}/EC_{50}/LC_{50} (mg/L)	AF	PNEC (μg/L)	PEC/PNEC
Algae *Desmodesmus subspicatus*	1.5	3.57[a]	1000	3.57	0.42
Aquatic invertebrates *Daphnia magna*		> 12.3[b]			
Fish *Danio rerio*		6.98[c]			

Abbreviation: AF, assessment factor.
[a]72 h E_rC_{50}.
[b]48 h EC_{50}.
[c]96 h LC_{50}.

TABLE 19 Risk characterisation (PEC/PNEC ratio) for sediment.

Taxa	$PEC_{sediment}$ (μg/kg)	NOEC (mg/kg)	AF	PNEC (μg/kg)	PEC/PNEC
Sediment-dwelling invertebrates *Chironomus riparius*	31.9	6.59	100	65.9	0.48

Bioaccumulation and secondary poisoning

Based on the logK_{ow} of 5.12 of SAL-Na, the additive has the potential to bioaccumulate and the risk of secondary poisoning has to be assessed. The applicant submitted an assessment of the risk for secondary poisoning.[34]

[34]Technical dossier/Supplementary information/March 2022/Annex_RTQ_III_1.

To assess the risk of secondary poisoning for worm/fish-eating birds and mammals through the food web chain the method proposed in the relevant Guidance from the European Medicines Agency (EMA) has been considered (EMA, 2016). A $\log K_{ow}$ of 5.12 at pH 7 was used for the assessment. SAL-Na is largely absorbed in the rabbit intestine, extensively metabolised and excreted. 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 PEC for soil, groundwater and surface water was used for the calculation of PEC_{fish} and $PEC_{earthworm}$.

Since there were no bioaccumulation data available for fish and for terrestrial organisms, the FEEDAP Panel assessed secondary poisoning of SAL for aquatic and terrestrial food chain. Using the lowest NOAEL for dog of 0.5 mg SAL/kg bw per day, which was derived from a 1-year dog toxicity study, the NOEC value of 20 mg SAL/kg feed was calculated, using the conversion factor of 40 for dogs. Using an assessment factor of 30, the corresponding $PNEC_{oral}$ was equivalent to 0.67 mg SAL/kg feed. This value is lower than the estimated concentration in fish of 13.5 mg/kg, based on PEC_{sw} of 1.5 µg/L and higher than the estimated concentration in earthworms of 0.15 mg/kg based on PEC_{gw} < 0.1 µg/L and PEC_{soil} of 66.1 µg/kg. The risk of secondary poisoning cannot be excluded for fish-eating birds and mammals and can be excluded for worm-eating birds and mammals. The PEC/PNEC ratios for exposure through the aquatic and terrestrial food chain are given in Table 20.

TABLE 20 The assessment of secondary poisoning for SAL through the aquatic and terrestrial food chains based on 100% of the proposed recommended dose.

PEC_{fish} (mg/kg)	PEC_{worm} (mg/kg)	$PNEC_{oral}$ (mg/kg)	$PEC_{fish}/PNEC_{oral}$	$PEC_{worm}/PNEC_{oral}$
13.5	0.15	0.67	20.2	0.22

Abbreviations: $PEC_{fish\ (oral,\ predator)}$, predicted concentration in fish; $PEC_{earthworm\ (oral,\ predator)}$, predicted concentration in earthworm.

3.2.4.3 | *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 the terrestrial and aquatic compartment and ground water. The risk of secondary poisoning can be excluded for worm-eating birds and mammals, while it cannot be excluded for fish-eating birds and mammals.

3.3 | Efficacy

3.3.1 | Cage studies

A total of 10 cage studies were provided to support the efficacy of SAL-Na in rabbits for fattening with a minimum of 42-day duration covering coccidiostatic endpoints at weekly intervals after inoculation and at study end. Data on performance parameters were also monitored throughout the whole study period. One cage study could not be considered for this assessment due to the following limitations: lack of an uninfected untreated control group (UUC) and lack of information on the origin of the inoculum.[35]

The other nine cage studies[36] were performed in four study sites, using inoculum isolated from different regions of five different countries and following a similar experimental design (Table 21). Weaned rabbits (at least 5-week old) were allocated to three experimental groups: a UUC group, an infected untreated control (IUC) group and an infected treated (IT) group. The IT group received complete feed containing 20 mg SAL-Na/kg from Sacox® 120 microGranulate, confirmed by analysis (Table 22). Water and feed in pelleted form were provided for ad libitum access for 42 or 45 days (see Table 21). Rabbits in the IUC and IT groups were orally inoculated with pathogenic *Eimeria* spp. isolated from rabbit farms (Table 22). The inoculum used in trials 3–9 were tested in virulence-titration studies.

General health was monitored daily or twice daily. Body weight and feed intake were measured, and daily weight gain and feed-to-gain ratio were calculated. Faecal samples were collected and analysed for oocyst excretion; results were given as number of oocysts per gram faeces (OPG). Gut lesion scoring was performed in seven trials (all except trials 1 and 2). Lesions were scored following a three-scale method (0 = no gross lesion, 1 = mild congestion, 2 = severe congestion) on the intestinal tract sections duodenum, jejunum and ileum, caecum and colon.[37] Liver lesions were examined in four trials (2 to 5). Morbidity was given as a percentage of observed coccidiosis-related signs[38] in relation to the total number of observations in the period. Mortality was recorded daily until the end of the study in all the studies and all dead rabbits were necropsied.

[35]Technical dossier/Section IV/Annex_IV_6.

[36]Technical dossier/Section IV/Annex_IV_4 (trial 1); Annex_IV_5 (trial 2); Technical dossier/Supplementary information March 2022/Additional data_IV_1. (trial 3); Additional data_IV_2. (trial 4); Additional data_IV_3. (trial 5); Technical dossier/Supplementary information March 2023/Additional data_IV_1. (trial 6); Additional data_IV_2. (trial 7); Additional data_IV_3. (trial 8); Additional data_IV_4. (trial 9).

[37]Method adapted from Biotechnology, guidelines and techniques on coccidiosis research. Volumes 89–820 of COST (European Co-operation in the Field of Scientific and Technical Research) 1995.

[38]Clinical signs include: diarrhoea without blood, diarrhoea with blood, faeces with mucus, hunched posture, unresponsive/listless, ruffled (ungroomed hair coat).

Data on body weight and daily weight gain were analysed using general linear models (GLM) with treatment as a fixed effect and cage (trials 1 and 2) or individual animal (trials 3–9) as an experimental unit (and cage as a random effect). Data on daily feed intake, feed to gain ratio and log-transformed OPG were analysed using GLM with treatment as a fixed effect. Coccidiosis lesion scores data were also analysed using GLM procedures except trials 1 and 2 where a logistic regression model was used. Mortality was analysed with a binomial logistic regression model except in trials 1 and 2 in which a Cox's proportional model[39] was used. Both UUC and IT groups were compared with IUC group and statistical significance was set at $p \leq 0.05$.

TABLE 21 Experimental design of cage studies performed in rabbits fed Sacox®.

Trial no	Total no of animals (animals/replicate) replicates/treatment	Breed and sex	Basal diet	Duration (days)	Coccidiosis-related endpoints
1	256 (8) 8 (UUC), 12(IUC),12(IT)	Hybrid ♀♂	Sugar beet pulp, sunflower meal, alfalfa meal, barley and wheat middlings	45	Mortality, OPG
2	256 (8) 8 (UUC),12 (IUC),12 (IT)	Hybrid ♀♂	Sugar beet pulp, sunflower meal, alfalfa meal, barley and wheat middlings	45	Mortality, OPG, liver lesions and intestinal content consistency[a]
3,4,5	210 (7) 10	New Zealand white ♀♂	Sugar beet pulp, sunflower meal, alfalfa meal, wheat and wheat middlings	42	Mortality, morbidity, OPG, liver and gut lesions[b]
6,7	360 (10) 8	Hyla Hybrid ♀♂	Commercial diet	42	Morbidity, mortality OPG, gut lesions[b]
8,9	168 (8) 7	New Zealand white ♀♂	Wheat, wheat middlings, alfalfa meal, sugar beet pulp, sunflower meal	42	Morbidity, mortality, OPG, gut lesions[b]

Abbreviation: OPG, number of oocysts per gram faeces.

[a]Scored on one rabbit per cage.

[b]Scored on two rabbits per cage.

TABLE 22 Inoculum composition and analysed SAL-Na concentration in the diet in cage studies performed with rabbits fed Sacox®.

		Inoculum characteristics					
Trial no	Study year	Date and country of isolation	Intended dose (number of oocysts) per bird		Day of inoculation	Age (day) BW (g) at study start	SAL-Na (mg/kg feed)
1	2019	09/2018 Belgium	64,500	*E. vejdovskyi/coecicola/media/stiedai*	4	(39) 1000	20
			13,000	*E. exigua*			
			0	*E. perforans*			
2	2019	07/2018 France	139,000	*E. vejdovskyi/coecicola/media/stiedai*	4	(35) 1060	24
			7000	*E. magna/irresidua*			
			19,000	*E. perforans*			
3	2022	10/2021 Spain	146,300	*E.vejdovskyi/coecicola/media/stiedai*	2	(35) 960	23
			15,200	*E. magna/irresidua*			
			19,000	*E. perforans*			
4	2022	10/2021 Spain	178,560	*E.vejdovskyi/coecicola/media/stiedai*			
			40,320	*E. magna/irresidua*			
5	2022	10/2021 France	71,520	*E.vejdovskyi/coecicola/media/stiedai*			
			50,960	*E. magna/irresidua*			
			3920	*E. perforans*			

(Continues)

[39]Regression method for survival analysis that takes into consideration the population survival rate and the elapse time since the inoculation.

TABLE 22 (Continued)

Trial no	Study year	Inoculum characteristics			Day of inoculation	Age (day) BW (g) at study start	SAL-Na (mg/kg feed)
		Date and country of isolation	Intended dose (number of oocysts) per bird				
6	2022	11/2021 Italy	37,050	*E.vejdovskyi/coecicola/media/stiedai*	3	(35) 1130	24
			64,600	*E. magna/irresidua*			
7	2022	11/2021 Portugal	83,420	*E.vejdovskyi/coecicola/media/stiedai*			
			75,660	*E. magna/irresidua*			
			12,610	*E. perforans*			
8	2022	11/2021 Italy	84,920	*E.vejdovskyi/coecicola/media/stiedai*	2	(35) 904	22
			19,800	*E. magna/irresidua*			
			440	*E. perforans*			
9	2022	11/2021 Italy	33,200	*E.vejdovskyi/coecicola/media/stiedai*			
			51,045	*E. magna/irresidua*			
			2075	*E. perforans*			

Mortality results are reported in Table 23. Total mortality was significantly lower in IT group compared to IUC in all trials except trial 1 (reporting a low mortality level), 5 and 9. Coccidiosis-related mortality and morbidity was significantly lower in the SAL-Na treated group compared to IUC in the five trials in which it was calculated (trials 3, 4, 5, 8 and 9).

TABLE 23 Mortality data in cage studies performed with rabbits fed Sacox®.

Trial no	Groups	Mortality % (*n*)	Coccidiosis-related mortality % (*n*)	Coccidiosis-related morbidity % (*n*)
1	UUC IUC IT	(1) (3) (0)	n.c.	n.c.
2	UUC IUC IT	6.4‡ 14.9 7.3‡	n.c.	n.c.
3	UUC IUC IT	0* (0) 15.7 (11) 2.9* (2)	0* (0) 12.9 (9) 1.4* (1)	0* 8.0 0.4*
4	UUC IUC IT	0* (0) 14.3 (10) 1.4* (1)	0* (0) 10.0 (7) 0* (0)	0* 5.7 0*
5	UUC IUC IT	0* (0) 10.0 (7) 2.9 (2)	0* (0) 10.0 (7) 0* (0)	0* 7.5 0*
6	UUC IUC IT	11.3* 30.0 15.0*	n.c.	n.c.
7	UUC IUC IT	11.3* 41.3 20.0*	n.c.	n.c.
8	UUC IUC IT	10.7* 30.4 1.8*	5.4* 28.6 1.8*	3.1* 15.2 1.0*
9	UUC IUC IT	10.7 21.4 8.9	5.4* 19.6 5.4*	3.1* 17.0 3.1*

Abbreviation: n.c., not calculated.

*Significantly different from IUC ($p \leq 0.05$).

‡Significantly different from IUC ($p \leq 0.1$).

Results of oocyst counts are reported in Table 24. OPG values were significantly lower in IT group compared to IUC in all time points in six trials (trials 1, 3, 5, 6, 8 and 9) in two time points in trial 4, in one time point in trial 7 and showed no differences in trial 2.

TABLE 24 Results of oocyst excretion in cage studies performed with rabbits fed Sacox®.

Trial no	Groups	Total oocyst excretion (OPG)		
		Day 10		Day 45
1	UUC	172,481		148,423
	IUC	545,008		23,690
	IT	5391*		105*
2[a]	UUC	18,100		58,175
	IUC	9517		42,292
	IT	7350		16,799
		Day 8	Day 13	Day 42
3	UUC	335,206*	41,464*	386
	IUC	825,188	2,466,313	3346
	IT	207,264*	61,690*	1*
4	UUC	335,206*	41,464*	386
	IUC	1,248,158	1,612,977	4772
	IT	225,970*	87,199	2*
5	UUC	335,206*	41,464*	386
	IUC	2,674,360	274,514	655
	IT	17,672*	14,586*	1*
		Day 8		Day 13
8	UUC	66,540*		262,982
	IUC	636,601		174,987
	IT	3460*		5980*
9	UUC	66,540*		262,982
	IUC	1,985,308		192,920
	IT	5339*		4658*

		Oocyst excretion specific to *Eimeria* species (OPG)					
		Day 10		Day 14		Day 17	
Trial no	Groups	*E. vejdovskyi* *E. coecicola* *E. media* *E. stiedai*	*E. magna* *E. irresidua*	*E. vejdovskyi* *E. coecicola* *E. media* *E. stiedai*	*E. magna* *E. irresidua*	*E. vejdovskyi* *E. coecicola* *E. media* *E. stiedai*	*E. magna* *E. irresidua*
6	UUC	16,581	4460	8486	4527	7389*	37,931
	IUC	40,018	4200	104,795	35,060	71,170	4841
	IT	3256*	80	4708*	1892*	10,318*	126*
7	UUC	16,581	4460	8486	4527	7389	37,931
	IUC	29,758	3958	26,673	31,084	44,775	16,145
	IT	4038*	76	11,430	2619	42,386	0*

[a]Expressed as area under the curve (AUC) based on results at days 10, 14, 17, 24, 31, 38 and 45.

*Significantly different from IUC ($p \leq 0.05$).

Results of intestinal and liver lesion scores are reported in Table 25. The intestinal lesion scores were significantly lower in IT compared to IUC groups in all intestinal segments and for all observation days in trials 5, 8 and 9, and in at least one of the two observation days in trials 3 and 4. Significantly lower lesion scores were found in IT compared to IUC groups in jejunum-ileum (day 10) and caecum-colon (day 7 and 10) in trial 6 and in duodenum and caecum-colon on day 7 in trial 7. In trial 2, liver lesions were identified but could not be attributed to *E. stiediae*. In trials 3 and 4, no liver lesions caused by *E. stiediae* were detected in rabbits from the IT group while in trial 5, two rabbits out of 20 showed liver lesions due to *E. stiediae*; no statistical analysis was performed relative to the IUC group due to the low number of observations.

TABLE 25 Results of intestinal and liver lesion scoring[a] in cage studies performed with rabbits fed Sacox®.

Trial no	Groups	Intestinal lesions duodenum		Intestinal lesions jejunum-ileum		Intestinal lesions caecum-colon		Liver lesions[b] Day 24 (%)
2	UUC	–		–		–		13
	IUC							25
	IT							29
		Day 6	**Day 10**	**Day 6**	**Day 10**	**Day 6**	**Day 10**	**Day 42 (*n*)**
3	UUC	0.0*	0.25*	0.25*	0.85	0.50*	0.25*	(1/20)
	IUC	1.60	0.70	1.05	0.95	1.05	1.15	(2/15)
	IT	1.05*	0.55	0.55*	1.10	0.15*	0.65*	(0/20)
4	UUC	0.0*	0.25*	0.25*	0.85*	0.50*	0.25*	(1/20)
	IUC	1.75	0.90	0.90	1.40	1.10	1.25	(0/17)
	IT	1.45	0.50*	0.60	0.90*	0.40*	0.40*	(0/20)
5	UUC	0.0*	0.25*	0.25*	0.85*	0.50*	0.25*	(1/20)
	IUC	1.70	0.65	0.80	1.35	1.10	1.25	(3/18)
	IT	0.30*	0.05*	0.20*	0.45*	0.05*	0.20*	(2/20)
		Day 7	**Day 10**	**Day 7**	**Day 10**	**Day 7**	**Day 10**	
6	UUC	0.30*	0.50	0.40	0.75*	0.10*	0.50*	–
	IUC	0.95	0.74	0.75	1.26	1.05	1.53	
	IT	0.60	0.40	0.60	0.50*	0.20*	0.35*	
		Day 7	**Day 14**	**Day 7**	**Day 14**	**Day 7**	**Day 14**	
7	UUC	0.30*	0.39	0.40	0.69	0.1*	0.56	–
	IUC	1.40	0.85	1.1	0.73	1.15	0.5	
	IT	0.55*	0.41	0.45	0.75	0.25*	0.58	
		Day 6	**Day 10**	**Day 6**	**Day 10**	**Day 6**	**Day 10**	
8	UUC	0.0*	0.0*	0.0*	0.13*	0.0*	0.0*	–
	IUC	1.69	0.81	1.19	1.75	1.75	1.44	
	IT	0.13*	0.0*	0.0*	0.25*	0.06*	0.0*	
9	UUC	0.0*	0.0*	0.0*	0.13*	0.0*	0.0*	–
	IUC	1.69	1.0	1.0	1.87	1.75	1.44	
	IT	0.0*	0.13*	0.0*	0.25*	0.0*	0.0*	

Note: -: not measured. 0 = no gross lesion, 1 = mild congestion, 2 = severe congestion.

[a]Intestinal lesions were scored on two rabbits per pen. Liver lesions were scored on one rabbit per pen (trial 2) or on two rabbits per pen (trial 3–5).

[b]The results of liver lesions refer to total lesions in trial 2 and to lesions due to *E. stiediae* in trials 3–5.

In trial 2, abnormal intestinal content was found in 54% of the IUC rabbits while significantly lower values were found in UUC and IT rabbits (7% and 4%, respectively).

Performance results are reported in Table 26. In trials 1–5, rabbits of the IT group showed significantly higher final body weight and average weight gain compared to the rabbits in the IUC group; feed to gain ratio was also significantly improved. Improved performance was also seen in trials 6–9; however, it is noted that the statistical analysis relied on a very low number of animals due to the high mortality and removal of rabbits selected for the necropsy.

TABLE 26 Results of performance parameters in cage studies performed with rabbits fed Sacox®.

Trial no	Groups	Average daily feed intake (g)	Final body weight (g)	Average daily weight gain (g)	Feed to gain ratio
1	UUC	149	2971*	36	4.14
	IUC	148	2862	34	4.40
	IT	150	3176*	41*	3.62*
2	UUC	126*	2843*	36*	3.5*
	IUC	115	2643	30	4.0
	IT	123	2872*	39*	3.2*
3	UUC	114*	2510*	37*	3.22*
	IUC	84	2167	28	5.94
	IT	108*	2425*	35*	3.45*
4	UUC	114*	2510*	37*	3.22*
	IUC	81	2104	28	4.75
	IT	110*	2482*	36*	3.38*

TABLE 26 (Continued)

Trial no	Groups	Average daily feed intake (g)	Final body weight (g)	Average daily weight gain (g)	Feed to gain ratio
5	UUC	114*	2510*	37*	3.22*
	IUC	87	2191	29	4.76
	IT	111*	2514*	37*	3.35*
6	UUC	113	2977*	44*	3.40*
	IUC	89	2308	27	6.72
	IT	134*	2862*	41*	3.90*
7	UUC	113*	2962*	43*	3.38*
	IUC	80	2094	22	11.58
	IT	121*	2794*	40*	4.09*
8	UUC	100*	2335	32*	3.16*
	IUC	81	2240	19	4.34
	IT	106*	2473	38*	2.84*
9	UUC	100	2335	32*	3.16
	IUC	66	1794	13	6.29
	IT	103*	2410*	34*	3.08*

*Significantly different from IUC ($p \leq 0.05$).

3.3.2 | Field trials

The five field trials submitted were conducted in two EU countries. Three of the studies could not be considered for the assessment due to inappropriate design[40] (treatments were not provided simultaneously), due to concomitant treatment with antibiotics[41] (for 22 out of 28-day study duration) and due to a high mortality (15.7 and 17.0%).[42]

In the two studies remaining, described below, diets were supplemented with Sacox® 120 microGranulate at a concentration of 20 mg SAL-Na/kg complete feed or with an alternative chemically synthesised coccidiostat (control group).

One of the trials[43] had a duration of 4 weeks, followed by a 1-week withdrawal period. The age of weaned rabbits at the start was 40 days; body weight 1138 g. Group size was 16 pens with 30 rabbits each (a total of 480 rabbits). Endpoints measured were body weight, feed intake and faecal oocyst count. Mortality was low, but somewhat higher in the control group (8.3% vs. 5.0%) mainly due to enteropathy. Body weight at the end of additive administration was not different (2336 vs. 2317 g (control)), but after withdrawal, the SAL-Na group showed a significantly higher body weight relative to the control (2744 vs. 2654 g). Cumulative data for feed intake (measured for 2 pens together) and feed to gain ratio were calculated only for the entire period including withdrawal (149 g/day and 3.45 for the SAL-Na group, 154 g/day and 3.84 for the control group) and were not significantly different. Oocyst excretion at the end of additive administration was significantly lower in the SAL-Na group compared to the control; however after withdrawal, a significantly higher excretion was seen in the SAL-Na group. Overall, the SAL-Na group showed a similar zootechnical performance as the group with the control coccidiostat; SAL-Na was at least as effective in suppressing oocyst excretion as the control coccidiostat. However, the FEEDAP Panel notes that intestinal lesions were not examined and that inconsistent values were reported regarding the number of rabbits per pen and the results of body weight and growth rate in the SAL-Na group. Therefore, the results of this study should be considered with caution.

In a second trial,[44] after weaning the rabbits at approximately 35 days of age, kits were moved to two houses where they stayed in groups in the parks until slaughter at the age of approximately 70 days. Rabbits were allocated at random to one of the two departments (with 20 parks) at the moment of weaning (total number of animals for SAL-Na group 5264, for control 3160). Body weight was measured on three rabbits of 20 parks each. Only eight parks were used for faeces sampling. No differences in weight gain were observed, feed intake was not reported. Overall mortality in SAL-Na rabbits was 5.1% compared to 10.9% in the control group. Total OPG was significantly lower in SAL-Na rabbit faeces (Ln OPG 5.4) compared to control rabbit faeces (Ln OPG 11.4). A non-inferiority test indicated that SAL-Na was not inferior to the control coccidiostat.

Synopsis on efficacy studies

A total of nine independent cage studies were considered for this assessment performed between 2019 and 2023 using inoculum from recent field isolates collected in rabbit farms in Belgium, Spain, France, Portugal and Italy.

SAL-Na showed efficacy in reducing coccidiosis-related mortality in five trials. Oocyst excretion was also reduced due to SAL-Na treatment in eight trials. Intestinal lesions showed significantly lower scores in SAL-Na-treated rabbits in all seven trials in which this endpoint was examined.

[40]Technical dossier/Section IV/Annex_IV_3.

[41]Technical dossier/Supplementary information February 2021/Additional data_IV_1.

[42]Technical dossier/Supplementary information February 2021/Additional data_IV_3.

[43]Technical dossier/Section IV/Annex_IV_2.

[44]Technical dossier/Supplementary information February 2021/Additional data_IV_2.

The results of seven studies (trials 3–9) provided evidence that 20 mg SAL-Na/mg complete feed have the potential to control coccidiosis in rabbits for fattening during the productive cycle of the animals while in another study (trial 1) evidence of efficacy was seen based only on one coccidiostatic endpoint (oocyst excretion). The above results allow to conclude on the sensitivity of the *Eimeria* species used in the inocula to the coccidiostat Sacox®.

The results of two field trials support the conclusions reached based on the results of the cage studies.

Conclusions on efficacy

The FEEDAP Panel concludes that the feed additive Sacox® at the minimum concentration of 20 mg SAL-Na/kg complete feed has the potential to control coccidiosis in rabbits for fattening.

3.4 | Post-market monitoring

Development of resistance to SAL-Na of field *Eimeria* spp. strains isolated from rabbits for fattening should be monitored.

4 | CONCLUSIONS

The FEEDAP Panel concludes with regard to the production strain, that the use of SAL-Na from Sacox® does not raise safety concerns for the target species, consumers, users and the environment.

In the absence of adequate tolerance studies, the FEEDAP Panel cannot conclude on the safety of Sacox® for rabbits for fattening.

The FEEDAP Panel concludes that SAL-Na from Sacox® is safe for the consumer when it is used at the proposed maximum level of 25 mg SAL-Na/kg complete feed for rabbits and a withdrawal period of 1 day is respected. The parent compound salinomycin has to be retained as the marker residue; the following MRLs are proposed: 0.2 and 0.03 mg SAL/kg for liver and kidney, respectively.

The additive is not irritant to skin and eyes but should be considered a potential dermal and respiratory sensitiser. A risk for inhalation toxicity could not 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 the terrestrial and aquatic compartment and ground water. The risk of secondary poisoning can be excluded for worm-eating birds and mammals, while it cannot be excluded for fish-eating birds and mammals.

The FEEDAP Panel concludes that Sacox® at the minimum concentration of 20 mg SAL-Na/kg complete feed has the potential to control coccidiosis in rabbits for fattening. Development of resistance to SAL-Na of field *Eimeria* spp. strains isolated from rabbits for fattening should be monitored.

ABBREVIATIONS

ADI	acceptable daily intake
BW	body weight
CAS	Chemical Abstracts Service
CFU	colony-forming unit
CV	coefficient of variation
DM	dry matter
EMA	European Medicines Agency
EURL	European Union Reference Laboratory
FEEDAP	EFSA Scientific Panel on Additives and Products or Substances used in Animal Feed
GC–MS	gas chromatography–mass spectrometry
IUPAC	International Union of Pure and Applied Chemistry
JECFA	The Joint FAO/WHO Expert Committee on Food Additives
LOD	limit of detection
Log Kow	logarithm of octanol–water partition coefficient
LOQ	limit of quantification
MIC	minimum inhibitory concentration
MRL	maximum residue limit
MW	molecular weight
NOAEL	no observed adverse effect level
OECD	Organisation for Economic Co-operation and Development
RH	relative humidity
UF	uncertainty factor
WHO	World Health Organisation

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CONFLICT OF INTEREST

If you wish to access the declaration of interests of any expert contributing to an EFSA scientific assessment, please contact interestmanagement@efsa.europa.eu.

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APPENDIX A

Detailed results on chronic exposure calculation

TABLE A1 Chronic dietary exposure per population class, country and survey of consumers (mg/kg bw per day) to salinomycin total residues based on residue data in rabbit tissues.

Population class	Survey's country	Number of subjects	HRP value	HRP description
Infants	Bulgaria	523	0.0001262611	95th
Infants	Germany	142	0.0009573451	95th
Infants	Denmark	799	0.0024970227	95th
Infants	Finland	427	0.0000851573	95th
Infants	Italy	9	0.0000003055	50th
Infants	United Kingdom	1251	0.0000888628	95th
Toddlers	Belgium	36	0.0008398053	90th
Toddlers	Bulgaria	428	0.0010779337	95th
Toddlers	Germany	348	0.0011416976	95th
Toddlers	Denmark	917	0.0028302213	95th
Toddlers	Spain	17	0.0002735276	75th
Toddlers	Finland	500	0.0001996392	95th
Toddlers	Italy	36	0.0001441326	90th
Toddlers	Netherlands	322	0.0029168299	95th
Toddlers	United Kingdom	1314	0.0011724084	95th
Toddlers	United Kingdom	185	0.0025419698	95th
Other children	Austria	128	0.0017112551	95th
Other children	Belgium	625	0.0017391705	95th
Other children	Bulgaria	433	0.0014626340	95th
Other children	Germany	293	0.0008601125	95th
Other children	Germany	835	0.0013510094	95th
Other children	Denmark	298	0.0015147223	95th
Other children	Spain	399	0.0014016556	95th
Other children	Spain	156	0.0015723566	95th
Other children	Finland	750	0.0019308307	95th
Other children	France	482	0.0010778418	95th
Other children	Greece	838	0.0001590980	95th
Other children	Italy	193	0.0001796078	95th
Other children	Latvia	187	0.0015514796	95th
Other children	Netherlands	957	0.0021906334	95th
Other children	Netherlands	447	0.0012610722	95th
Other children	Sweden	1473	0.0014108115	95th
Other children	Czechia	389	0.0019288277	95th
Other children	United Kingdom	651	0.0014637806	95th
Adolescents	Austria	237	0.0006723485	95th
Adolescents	Belgium	576	0.0005009395	95th
Adolescents	Cyprus	303	0.0000696720	95th
Adolescents	Germany	393	0.0009707119	95th
Adolescents	Germany	1011	0.0002198312	95th
Adolescents	Denmark	377	0.0006774272	95th
Adolescents	Spain	651	0.0005887330	95th
Adolescents	Spain	209	0.0005661339	95th
Adolescents	Spain	86	0.0003934021	95th

(Continues)

TABLE A1 (Continued)

Population class	Survey's country	Number of subjects	HRP value	HRP description
Adolescents	Finland	306	0.0002017265	95th
Adolescents	France	973	0.0006376036	95th
Adolescents	Italy	247	0.0001047108	95th
Adolescents	Latvia	453	0.0005963668	95th
Adolescents	Netherlands	1142	0.0005886380	95th
Adolescents	Sweden	1018	0.0003779880	95th
Adolescents	Czechia	298	0.0011912733	95th
Adolescents	United Kingdom	666	0.0000893872	95th
Adults	Austria	308	0.0002668331	95th
Adults	Belgium	1292	0.0004793730	95th
Adults	Germany	10,419	0.0004515567	95th
Adults	Denmark	1739	0.0006363987	95th
Adults	Spain	981	0.0004245298	95th
Adults	Spain	410	0.0005127317	95th
Adults	Finland	1295	0.0004872134	95th
Adults	France	2276	0.0010626625	95th
Adults	Hungary	1074	0.0022262724	95th
Adults	Ireland	1274	0.0001461232	95th
Adults	Italy	2313	0.0000803578	95th
Adults	Latvia	1271	0.0007742746	95th
Adults	Netherlands	2055	0.0004708605	95th
Adults	Romania	1254	0.0009528351	95th
Adults	Sweden	1430	0.0003487847	95th
Adults	Czechia	1666	0.0014516780	95th
Adults	United Kingdom	1265	0.0002502036	95th
Elderly	Austria	67	0.0011315938	95th
Elderly	Belgium	511	0.0006474540	95th
Elderly	Germany	2006	0.0005507172	95th
Elderly	Denmark	274	0.0009455331	95th
Elderly	Finland	413	0.0006589369	95th
Elderly	France	264	0.0012620113	95th
Elderly	Hungary	206	0.0016577441	95th
Elderly	Ireland	149	0.0003178634	95th
Elderly	Italy	289	0.0000664053	95th
Elderly	Netherlands	173	0.0004135454	95th
Elderly	Netherlands	289	0.0004278902	95th
Elderly	Romania	83	0.0006154070	95th
Elderly	Sweden	295	0.0006568300	95th
Elderly	United Kingdom	166	0.0005704285	95th
Very elderly	Austria	25	0.0000431469	75th
Very elderly	Belgium	704	0.0007101792	95th
Very elderly	Germany	490	0.0005460735	95th
Very elderly	Denmark	12	0.0002919343	75th
Very elderly	France	84	0.0013483135	95th
Very elderly	Hungary	80	0.0011606753	95th
Very elderly	Ireland	77	0.0002273517	95th
Very elderly	Italy	228	0.0000560678	95th
Very elderly	Netherlands	450	0.0003819276	95th
Very elderly	Romania	45	0.0005779781	90th
Very elderly	Sweden	72	0.0011697978	95th
Very elderly	United Kingdom	139	0.0009853245	95th

TABLE A2 Chronic dietary exposure per population class, country and survey of consumers (mg/kg bw per day) to salinomycin total residues calculated based on MRLs.

Population class	Survey's country	Number of subjects	HRP value	HRP description
Infants	Bulgaria	523	0.0001262611	95th
Infants	Germany	142	0.0011615451	95th
Infants	Denmark	799	0.0030592219	95th
Infants	Finland	427	0.0000851573	95th
Infants	Italy	9	0.0000003055	50th
Infants	United Kingdom	1251	0.0000888628	95th
Toddlers	Belgium	36	0.0009451083	90th
Toddlers	Bulgaria	428	0.0013120464	95th
Toddlers	Germany	348	0.0013566311	95th
Toddlers	Denmark	917	0.0034613015	95th
Toddlers	Spain	17	0.0003204827	75th
Toddlers	Finland	500	0.0002014140	95th
Toddlers	Italy	36	0.0001441326	90th
Toddlers	Netherlands	322	0.0035655552	95th
Toddlers	United Kingdom	1314	0.0013830439	95th
Toddlers	United Kingdom	185	0.0031144866	95th
Other children	Austria	128	0.0020265999	95th
Other children	Belgium	625	0.0020641276	95th
Other children	Bulgaria	433	0.0017238972	95th
Other children	Germany	293	0.0010431154	95th
Other children	Germany	835	0.0016465425	95th
Other children	Denmark	298	0.0018417514	95th
Other children	Spain	399	0.0017190340	95th
Other children	Spain	156	0.0019119714	95th
Other children	Finland	750	0.0023553356	95th
Other children	France	482	0.0013099882	95th
Other children	Greece	838	0.0001590980	95th
Other children	Italy	193	0.0001796078	95th
Other children	Latvia	187	0.0018870978	95th
Other children	Netherlands	957	0.0026858933	95th
Other children	Netherlands	447	0.0015002999	95th
Other children	Sweden	1473	0.0016501514	95th
Other children	Czechia	389	0.0023657723	95th
Other children	United Kingdom	651	0.0017672507	95th
Adolescents	Austria	237	0.0007674598	95th
Adolescents	Belgium	576	0.0006077690	95th
Adolescents	Cyprus	303	0.0000696720	95th
Adolescents	Germany	393	0.0011860794	95th
Adolescents	Germany	1011	0.0002487173	95th
Adolescents	Denmark	377	0.0008162358	95th
Adolescents	Spain	651	0.0006963589	95th
Adolescents	Spain	209	0.0006780240	95th
Adolescents	Spain	86	0.0004711675	95th
Adolescents	Finland	306	0.0002395432	95th
Adolescents	France	973	0.0007623025	95th
Adolescents	Italy	247	0.0001047108	95th
Adolescents	Latvia	453	0.0007145119	95th
Adolescents	Netherlands	1142	0.0007138700	95th

(Continues)

TABLE A2 (Continued)

Population class	Survey's country	Number of subjects	HRP value	HRP description
Adolescents	Sweden	1018	0.0004498440	95th
Adolescents	Czechia	298	0.0014517224	95th
Adolescents	United Kingdom	666	0.0000930758	95th
Adults	Austria	308	0.0002991234	95th
Adults	Belgium	1292	0.0005765536	95th
Adults	Germany	10,419	0.0005400110	95th
Adults	Denmark	1739	0.0007687185	95th
Adults	Spain	981	0.0004886360	95th
Adults	Spain	410	0.0005955070	95th
Adults	Finland	1295	0.0005745746	95th
Adults	France	2276	0.0012987662	95th
Adults	Hungary	1074	0.0027189821	95th
Adults	Ireland	1274	0.0001624963	95th
Adults	Italy	2313	0.0000803578	95th
Adults	Latvia	1271	0.0009353584	95th
Adults	Netherlands	2055	0.0005603808	95th
Adults	Romania	1254	0.0011510069	95th
Adults	Sweden	1430	0.0004028856	95th
Adults	Czechia	1666	0.0016573759	95th
Adults	United Kingdom	1265	0.0002968219	95th
Elderly	Austria	67	0.0013810233	95th
Elderly	Belgium	511	0.0007733328	95th
Elderly	Germany	2006	0.0006441209	95th
Elderly	Denmark	274	0.0011562597	95th
Elderly	Finland	413	0.0007737541	95th
Elderly	France	264	0.0015478240	95th
Elderly	Hungary	206	0.0019555570	95th
Elderly	Ireland	149	0.0003725985	95th
Elderly	Italy	289	0.0000664053	95th
Elderly	Netherlands	173	0.0004981493	95th
Elderly	Netherlands	289	0.0005073228	95th
Elderly	Romania	83	0.0007169511	95th
Elderly	Sweden	295	0.0008024587	95th
Elderly	United Kingdom	166	0.0006538119	95th
Very elderly	Austria	25	0.0000431469	75th
Very elderly	Belgium	704	0.0008565636	95th
Very elderly	Germany	490	0.0006553551	95th
Very elderly	Denmark	12	0.0003521040	75th
Very elderly	France	84	0.0016487315	95th
Very elderly	Hungary	80	0.0013726363	95th
Very elderly	Ireland	77	0.0002496520	95th
Very elderly	Italy	228	0.0000563481	95th
Very elderly	Netherlands	450	0.0004640843	95th
Very elderly	Romania	45	0.0007082003	90th
Very elderly	Sweden	72	0.0013443370	95th
Very elderly	United Kingdom	139	0.0011951725	95th

TABLE A3 Chronic dietary exposure per population class, country and survey of consumers (mg/kg bw per day) to salinomycin total residues calculated based on chicken and rabbit MRLs.

Population class	Survey's country	Number of subjects	HRP value	HRP description
Infants	Bulgaria	523	0.0004990812	95th
Infants	Germany	142	0.0011635986	95th
Infants	Denmark	799	0.0030720877	95th
Infants	Finland	427	0.0001566755	95th
Infants	Italy	9	0.0000003055	50th
Infants	United Kingdom	1251	0.0002153738	95th
Toddlers	Belgium	36	0.0010414058	90th
Toddlers	Bulgaria	428	0.0019045163	95th
Toddlers	Germany	348	0.0013959250	95th
Toddlers	Denmark	917	0.0035115675	95th
Toddlers	Spain	17	0.0005150540	75th
Toddlers	Finland	500	0.0003365253	95th
Toddlers	Italy	36	0.0002576985	90th
Toddlers	Netherlands	322	0.0035685510	95th
Toddlers	United Kingdom	1314	0.0014516966	95th
Toddlers	United Kingdom	185	0.0031215646	95th
Other children	Austria	128	0.0021310266	95th
Other children	Belgium	625	0.0022497959	95th
Other children	Bulgaria	433	0.0025987398	95th
Other children	Germany	293	0.0010931714	95th
Other children	Germany	835	0.0017001534	95th
Other children	Denmark	298	0.0018421037	95th
Other children	Spain	399	0.0017989011	95th
Other children	Spain	156	0.0020169744	95th
Other children	Finland	750	0.0027271806	95th
Other children	France	482	0.0013856913	95th
Other children	Greece	838	0.0002595078	95th
Other children	Italy	193	0.0002896474	95th
Other children	Latvia	187	0.0019189160	95th
Other children	Netherlands	957	0.0027123846	95th
Other children	Netherlands	447	0.0015462988	95th
Other children	Sweden	1473	0.0016954660	95th
Other children	Czechia	389	0.0024798473	95th
Other children	United Kingdom	651	0.0018306194	95th
Adolescents	Austria	237	0.0008163043	95th
Adolescents	Belgium	576	0.0006562194	95th
Adolescents	Cyprus	303	0.0001270635	95th
Adolescents	Germany	393	0.0012044991	95th
Adolescents	Germany	1011	0.0002655634	95th
Adolescents	Denmark	377	0.0008728750	95th
Adolescents	Spain	651	0.0007252762	95th
Adolescents	Spain	209	0.0007394154	95th
Adolescents	Spain	86	0.0005155678	95th
Adolescents	Finland	306	0.0002544260	95th
Adolescents	France	973	0.0008866259	95th
Adolescents	Italy	247	0.0001626715	95th
Adolescents	Latvia	453	0.0007147973	95th
Adolescents	Netherlands	1142	0.0007595804	95th

(Continues)

TABLE A3 (Continued)

Population class	Survey's country	Number of subjects	HRP value	HRP description
Adolescents	Sweden	1018	0.0004687620	95th
Adolescents	Czechia	298	0.0015816755	95th
Adolescents	United Kingdom	666	0.0001794329	95th
Adults	Austria	308	0.0003971588	95th
Adults	Belgium	1292	0.0006227556	95th
Adults	Germany	10,419	0.0005718416	95th
Adults	Denmark	1739	0.0007850252	95th
Adults	Spain	981	0.0005253548	95th
Adults	Spain	410	0.0005997066	95th
Adults	Finland	1295	0.0006134896	95th
Adults	France	2276	0.0013335511	95th
Adults	Hungary	1074	0.0027797495	95th
Adults	Ireland	1274	0.0002155695	95th
Adults	Italy	2313	0.0001165270	95th
Adults	Latvia	1271	0.0009583072	95th
Adults	Netherlands	2055	0.0006004422	95th
Adults	Romania	1254	0.0015451774	95th
Adults	Sweden	1430	0.0004426870	95th
Adults	Czechia	1666	0.0016777031	95th
Adults	United Kingdom	1265	0.0003690312	95th
Elderly	Austria	67	0.0025480939	95th
Elderly	Belgium	511	0.0008292140	95th
Elderly	Germany	2006	0.0007101664	95th
Elderly	Denmark	274	0.0011651648	95th
Elderly	Finland	413	0.0007737541	95th
Elderly	France	264	0.0015877416	95th
Elderly	Hungary	206	0.0019709015	95th
Elderly	Ireland	149	0.0004559672	95th
Elderly	Italy	289	0.0001158581	95th
Elderly	Netherlands	173	0.0005093344	95th
Elderly	Netherlands	289	0.0005379471	95th
Elderly	Romania	83	0.0009554748	95th
Elderly	Sweden	295	0.0008488628	95th
Elderly	United Kingdom	166	0.0008164312	95th
Very elderly	Austria	25	0.0001258886	75th
Very elderly	Belgium	704	0.0009021340	95th
Very elderly	Germany	490	0.0006711412	95th
Very elderly	Denmark	12	0.0003766844	75th
Very elderly	France	84	0.0016553434	95th
Very elderly	Hungary	80	0.0014315185	95th
Very elderly	Ireland	77	0.0002608400	95th
Very elderly	Italy	228	0.0000891390	95th
Very elderly	Netherlands	450	0.0004725071	95th
Very elderly	Romania	45	0.0010766718	90th
Very elderly	Sweden	72	0.0013623336	95th
Very elderly	United Kingdom	139	0.0011962192	95th