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Maximum levels of cross-contamination for 24 antimicrobial active substances in non-target feed. Part 8: *Pleuromutilins: tiamulin and valnemulin*

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Abstract

The specific concentrations of tiamulin and valnemulin in non-target feed for food-producing animals, below which there would not be an effect on the emergence of, and/or selection for, resistance in bacteria relevant for human and animal health, as well as the specific antimicrobial concentrations in feed which have an effect in terms of growth promotion/increased yield were assessed by EFSA in collaboration with EMA. Details of the methodology used for this assessment, associated data gaps and uncertainties, are presented in a separate document. To address antimicrobial resistance, the Feed Antimicrobial Resistance Selection Concentration (FARSC) model developed specifically for the assessment was applied. However, due to the lack of data on the parameters required to calculate the FARSC, it was not possible to conclude the assessment until further experimental data become available. To address growth promotion, data from scientific publications obtained from an extensive literature review were used. Levels in feed that showed to have an effect on growth promotion/ increased yield were reported for tiamulin, while for valnemulin no suitable data for the assessment were available. It was recommended to carry out studies to generate the data that are required to fill the gaps which prevented the calculation of the FARSC for these two antimicrobials.

Keywords: tiamulin, valnemulin, antimicrobial resistance, sub-inhibitory concentration, growth promotion, yield increase, food-producing animals

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1. Introduction

The European Commission requested the European Food Safety Authority (EFSA) to assess, in collaboration with the European Medicines Agency (EMA), (i) the specific concentrations of antimicrobials resulting from cross-contamination in non-target feed for food-producing animals, below which there would not be an effect on the emergence of, and/or selection for, resistance in microbial agents relevant for human and animal health (term of reference 1, ToR1), and (ii) the levels of the antimicrobials which have a growth promotion/increase yield effect (ToR2). The assessment was requested to be conducted for 24 antimicrobial active substances specified in the mandate.[1]

For the different substances (grouped by class if applicable)[1], separate scientific opinions included within the 'Maximum levels of cross-contamination for 24 antimicrobial active substances in non-target feed' series (Scientific Opinions Part 2 - Part 13, EFSA BIOHAZ Panel, 2021b-l – see also the Virtual Issue; for practical reasons, they will be referred as 'Scientific Opinion Part X' throughout the current document) were drafted. They present the results of the assessments performed to answer the following questions: Assessment Question 1 (AQ1), which are the specific antimicrobial concentrations in non-target feed below which there would not be emergence of, and/or selection for, resistance in the large intestines/rumen, and AQ2: which are the specific antimicrobial concentrations in feed of food-producing animals that have an effect in terms of growth promotion/increased yield. The assessments were performed following the methodology described in Section 2 of the Scientific Opinion 'Part 1: Methodology, general data gaps and uncertainties' (EFSA BIOHAZ Panel, 2021a, see also the Virtual Issue). The present document reports the results of the assessment for the pleuromutilins: tiamulin and valnemulin.

1.1. Background and Terms of Reference as provided by the requestor

The background and ToRs provided by the European Commission for the present document are reported in Section 1.1 of the Scientific Opinion "Part 1: Methodology, general data gaps and uncertainties" (see also the Virtual Issue).

1.2. Interpretation of the Terms of Reference

The interpretation of the ToRs, to be followed for the assessment is in Section 1.2 of the Scientific Opinion "Part 1: Methodology, general data gaps and uncertainties" (see also the Virtual Issue)

1.3. Additional information

1.3.1. Short description of the class/substance

Pleuromutilin is a natural antimicrobial substance produced by the fungus *Clitophilus scyphoides*. Pleuromutilins are diterpene antimicrobial agents that comprise a tricyclic structure with a five-, six- and eight-membered ring and eight stable chiral centres, as well as a glycolic ester moiety forming the side chain also regarded as an extension at position C14 (Schwarz et al., 2016; Paukner and Riedl, 2017). Tiamulin has a chemical structure similar to that of valnemulin (EMEA/CVMP, 1998; EMA/CVMP, 2017). These are semi-synthetic derivatives of pleuromutilin used exclusively in veterinary medicine. Tiamulin was approved for use in veterinary medicine in 1979, followed by valnemulin in 1999. Retapamulin was the only pleuromutilin approved for topical use for humans since 2007 (Novak, 2011). However, in 2019, the U.S. Food and Drug Administration approved lefamulin, a newly developed pleuromutilin, to treat adults with community-acquired bacterial pneumonia due to its activity against *Staphylococcus aureus*, *Streptococcus pneumoniae* and atypical bacteria (Andrei et al., 2019; WHO, 2021; Zhanel et al., 2021).

Pleuromutilins are antibacterial agents that inhibit protein synthesis and act by binding to the bacterial 50S ribosome at the peptidyl transferase centre and interfere with peptide bond formation (van Duijkeren et al., 2014; Schwarz et al., 2016). They are active against Gram-positive bacteria such as streptococci and staphylococci, anaerobic bacteria and mycoplasma (Giguère et al., 2013).

[1] Aminoglycosides: apramycin, paromomycin, neomycin, spectinomycin; Amprolium; Beta-lactams: amoxicillin, penicillin V; Amphenicols: florfenicol, thiamphenicol; Lincosamides: lincomycin; Macrolides: tilmicosin, tylosin, tylvalosin; Pleuromutilins: tiamulin, valnemulin; Sulfonamides; Polymyxins: colistin; Quinolones: flumequine, oxolinic acid; Tetracyclines: tetracycline, chlortetracycline, oxytetracycline, doxycycline; Diaminopyrimidines: trimethoprim.

The spectrum of activity is similar, however valnemulin has greater activity (lower MIC) than tiamulin (EMEA/CVMP, 1998; EMA/CVMP, 2017; Pringle et al., 2012; Paukner and Riedl, 2017). Also due to the different pharmacokinetics (see Section 1.3.3) they will be assessed separately.

1.3.2. Main use[2]

Tiamulin and valnemulin have been used for decades in veterinary medicine for the control of respiratory and intestinal infections in different animal species, especially in pigs and to a lesser extent in poultry and rabbits. Tiamulin is available as an oral solution, a powder for medication in drinking water, medicated feed premixes and as an injectable formulation for pigs and valnemulin is available as oral powder and premixes for feed (van Duijkeren et al., 2014).

Tiamulin is authorised and available in most European Union (EU) member states. Tiamulin is indicated in pigs notably for the treatment and metaphylaxis of swine dysentery (*Brachyspira hyodysenteriae*), treatment of colitis due to *Brachyspira pilosicoli*, treatment of ileitis (*Lawsonia intracellularis*) and treatment of enzootic pneumonia (*Mycoplasma hyopneumoniae*). In chickens, veterinary medicinal products containing tiamulin are currently approved for the treatment and prevention of chronic respiratory disease caused by *Mycoplasma gallisepticum* and *Mycoplasma synoviae*; for turkeys for the treatment and prevention of infectious sinusitis and air-sacculitis caused by *M. gallisepticum*, *M. meleagridis* and *M. synoviae*; and for rabbits for the reduction of mortality due to epizootic rabbit enteropathy when associated with infections by *Clostridium perfringens* susceptible to tiamulin (van Duijkeren et al., 2014; EMA/CVMP, 2014).

Valnemulin is authorised centrally as a premix for medicated feeding stuff in pigs for the treatment and prevention of swine dysentery, the treatment of clinical signs of porcine proliferative enteropathy (ileitis), the prevention of clinical signs of porcine colonic spirochaetosis (colitis) and for the treatment and prevention of swine enzootic pneumonia. In rabbits, valnemulin is indicated for the reduction of mortality during an outbreak of epizootic rabbit enteropathy (EMA/CVMP, 2006).

1.3.3. Main pharmacokinetic data

Tiamulin

The absolute bioavailability of tiamulin after oral administration cannot be determined due to very high toxicity after intravenous administration.

Tiamulin undergoes extensive metabolism in the liver and is excreted in the bile, the remainder is excreted via the kidney. In laying hens, broilers and turkeys, over 15 metabolites were detected in tissue extracts after oral administrations but most of the residue was accounted for by 4 metabolites (EMA/CVMP, 2017).

In pigs following oral administration of radiolabelled tiamulin, approximately 35% of the dose were eliminated in urine and 65% in faeces and over 15 metabolites were detected in the liver, but no individual metabolite accounted for more than 5% of the total residues. The 6-desmethyltiamulin accounted less than 1% of the total residue in bile and urine samples and had 67% of the antibacterial activity of tiamulin. Other metabolites had an activity relative to tiamulin lower than 3.3% (EMA/CVMP, 2017).

Valnemulin

The bioavailability of valnemulin is reported to be 74.4% in fasted broiler chickens (Wang et al., 2011). A short communication reported a bioavailability of 52.6% in broilers with no information on the fed or fasted status (Sun et al., 2017). In pigs, a study suggested a bioavailability of 59% but the confidence on this value is low due to the fact that the animals receiving treatment by oral and intravenous routes were not comparable (Yuan et al., 2015).

Valnemulin is excreted rapidly mainly via the bile and faeces. After oral administration to pigs, 11 metabolites were found in bile representing around 60% of the residues. Only 2 of these metabolites (representing 4.4% of the identified metabolites) retained an antimicrobial activity of approximately 70% that of valnemulin (EMEA/CVMP, 1998).

[2] Antimicrobials are currently used in food-producing animal production for treatment, prevention and/or metaphylaxis of a large number of infections, and also for growth promotion in non-EU countries. In the EU, in future, use of antimicrobials for prophylaxis or for metaphylaxis is to be restricted as addressed by Regulation (EU) 2019/6 and use in medicated feed for prophylaxis is to be prohibited under Regulation (EU) 2019/4.

1.3.4. Main resistance mechanisms

Resistance to pleuromutilins, including tiamulin and valnemulin, derives from chromosomal mutations in the 23S rRNA and *rplC* genes. These chromosomal mutations emerge slowly and in a stepwise fashion and are not yet identified to be transferred horizontally. For instances, tiamulin resistance in *B. hyodysenteriae* has been demonstrated to develop in a stepwise manner both *in vitro* and *in vivo*. These observations suggest that several mutations are needed to achieve high levels of resistance (Karlsson et al., 2001; Paukner and Riedl, 2017). In most cases, the MICs of valnemulin follow those of tiamulin but with a few dilution steps lower (EMEA/CVMP, 1998; EMA/CVMP, 2017; Pringle et al., 2012; Paukner and Riedl, 2017).

Data on resistance mechanisms to pleuromutilins in mycoplasma are scarce but it has been shown that a single mutation of the 23S rRNA gene increases tiamulin and valnemulin MICs. A combination of two or three mutations is needed to confer high levels of resistance (Gautier-Bouchardon, 2018; Bokma et al., 2020). Usually, the mutants are cross-resistant to lincomycin, chloramphenicol and florfenicol and some mutants also to erythromycin, tilmicosin and tylosin (Li et al., 2010).

Transferable resistance genes conferring resistance to pleuromutilins have been identified and located on plasmids or transposons like the *vga* genes and the *cfr* gene. However, the mechanism of antimicrobial resistance varies in different bacterial species (Feßler et al., 2018).

The *vga*(A) gene codes for an ABC-F protein that mediates resistance by protecting the ribosome against lincosamides, pleuromutilins and streptogramin A antibiotics. *vga* genes has been reported in meticillin-resistant *S. aureus* (MRSA) notably in a specific livestock-associated MRSA clone ST398 that has emerged worldwide, especially in swine but has also been identified in MRSA ST398 isolates from broilers (van Duijkeren et al., 2014).

The gene *cfr* was the first gene that conferred combined resistance to phenicols, lincosamides, oxazolidinones, pleuromutilins and streptogramin A antimicrobial agents (Long et al., 2006; Vester, 2018). The *cfr* gene has been detected in several bacterial species including staphylococci, enterococci and *Escherichia coli* from food animals (Schwarz et al., 2000; Witte and Cuny, 2011; Liu et al., 2013; Zhang et al., 2014). This gene is of global concern as it is often located on plasmids and can be spread between bacterial species and genera (Shen et al., 2013).

Also, the enterococcal ABC transporter gene *lsa*(E), that confers resistance to pleuromutilins, lincosamides and streptograminA, has also been detected in meticillin-susceptible *Staphylococcus aureus* (MSSA) and MRSA, suggesting exchange of this gene between *Enterococcus* spp. and *S. aureus* (Li et al., 2014).

2. Data and methodologies

The data sources and methodology used for this opinion are described in a dedicated document, the Scientific Opinion the Scientific Opinion 'Part 1: Methodology, general data gaps and uncertainties' (see also the Virtual Issue).

3. Assessment

3.1. Introduction

As indicated in the Scientific Opinion 'Part 1: Methodology, general data gaps and uncertainties' (see also the Virtual Issue), exposure to low concentrations of antimicrobials (including sub-minimum inhibitory concentrations, sub-MIC) may have different effects on bacterial antimicrobial resistance evolution, properties of bacteria and in animal growth promotion. Some examples including emergence of, and selection for, antimicrobial resistance, mutagenesis, virulence and/or horizontal gene transfer (HGT), etc. for the antimicrobials under assessment are shown below.

3.1.1. Resistance development/spread due to sub-MIC concentrations of pleuromutilins including tiamulin and valnemulin: examples

3.1.1.1. Effects of sub-MIC concentrations on selection for resistance and mutagenesis

- Few studies were identified on effects of sub-MIC levels of pleuromutilins on selection of resistance. Generally, pleuromutilins have shown a low potential for resistance development *in vitro* as identified for tiamulin and valnemulin in *Brachyspira* spp., *Mycoplasma* spp., *S. aureus* and *E. coli*. It has been demonstrated that spontaneous mutation frequencies are

low (< 10^9) and resistance developed slowly in a stepwise manner with multiple mutations required to cause high-level resistance. Mutations in 23S rRNA, *rplC*, and *rplD* genes encoding the large ribosomal proteins L3 and L4, have been identified as the primary resistance mechanism *in vitro* (Paukner and Riedl, 2017).

- *In vitro* development of tiamulin resistance was investigated in two *B. pilosicoli* and two *B. hyodysenteriae* strains. The four strains became resistant to tiamulin after several passages on agar containing tiamulin in increasing concentrations. The resistance emerged slowly. Three of the strains that went through more than 60 passages increased their MIC for tiamulin from 0.031 to 0.25 to more than 128 μg/mL. The tiamulin MIC for one *B. hyodysenteriae* strain that went through 29 passages increased from 0.0125 to 4 μg/mL. One *B. pilosicoli* strain developed cross-resistance to valnemulin; with a MIC increase from 0.25 to more than 64 μg/mL. The valnemulin MIC for one *B. hyodysenteriae* strain increased from 0.031 to 32 μg/mL. Valnemulin MIC was not determined for the *B. hyodysenteriae* strain that only went through 29 passages. For the second *B. pilosicoli* strain, the valnemulin MIC increased from 0.031 to 4 μg/mL (Karlsson et al., 2001).
- Sulyok et al. (2017) performed *in vitro* studies on selection of antimicrobial-resistant mutants on the three different strains of *M. bovis* with low MIC. The methodology for the selection of resistance was carried out by serial passages in broth medium containing sub-inhibitory concentrations (increasing in twofold dilutions from 0.039 to 10 μg/mL). The culture containing the highest antimicrobial concentration with detectable growth was used to inoculate another antimicrobial dilution panel for the following passage series. Significant increase in MICs of the tested strains were identified for tiamulin (from 0.078 and 0.156 to 0.625 and 10 μg/mL) and for valnemulin (from < 0.039 and 0.039 to 10 μg/mL). Passages were performed until MIC values reached > 64 μg/mL for pleuromutilins. Resistant mutants where then passaged on free-medium in order to determine if the resistant phenotype was stable without selection pressure. Tiamulin- and valnemulin-resistant mutant strains were successfully obtained after passages 2–3 and passages 3–14, respectively. All tiamulin-resistant mutant strains showed cross-resistance to florfenicol (MIC 32 μg/mL) and elevated lincomycin MICs (4–16 μg/mL). One tiamulin-resistant strain became resistant to all the tested 50S inhibitors except tylosin. The development of valnemulin resistance strongly differed among the strains: one strain became resistant after only 3 passages, whereas the two other strains tested needed 10 and 14 passages. It appears that after five passages on antimicrobial-free medium, the valnemulin MIC value (0.078 μg/mL) for the mutant strain decreased. Tiamulin-resistant mutant strains evolved rapidly also (in two to five steps) and the number of mutations was correlated with the number of passages needed for the evolution of resistance.

3.1.1.2. Effects of sub-MIC concentrations on horizontal gene transfer and virulence

- No relevant studies were identified.

3.2. ToR1. Estimation of the antimicrobial levels in non-target feed that would not result in the selection of resistance: Feed Antimicrobial Resistance Selection Concentration (FARSC)

As explained in the Methodology Section (2.2.1.3) of the Scientific Opinion 'Part 1: Methodology, general data gaps and uncertainties' (see also the Virtual Issue), the estimation of this value for these two pleuromutilins for different animal species, if suitable data were available, would follow a two-step approach as described below:

The first step would be the calculation of the predicted minimal selective concentration (PMSC) for valnemulin and tiamulin as indicated in Table 1. However, no MSC data required to do the calculations is available for those substances.

Table 1: Calculation of the valnemulin and tiamulin predicted minimal selective concentration (PMSC)

Antimicrobial (all values in mg/L)	MIC_{test}	MSC_{test}	MIC_{test}/MSC_{test} ratio	MIC_{lowest}	Predicted MSC (PMSC) for most susceptible species ($MIC_{lowest}/MIC_{test}/MSC_{test}$)
Valnemulin	NA	NA	NA	NA	NA
Tiamulin	NA	NA	NA	0.25	NA

MIC: minimum inhibitory concentration; MSC: minimal selective concentration; MSC_{test}: MSC experimentally determined; MIC_{lowest}: lowest MIC data for tiamulin calculated based on data from the EUCAST database as described in Bengtsson-Palme and Larsson (2016), see Methodology Section 2.2.1.3.1.1 in the Scientific Opinion Part 1. No data for valnemulin in the EUCAST database (EUCAST database https://mic.eucast.org/search/ last accessed 15 May 2021); NA: not available.

Due to the lack of PMSC, no FARSC could be calculated. If PMSC was available, the FARSC ($FARSC_{intestine}$ and $FARSC_{rumen}$) corresponding to the maximal concentrations in feed would be calculated for each species from the equations below (for details, see Section 2.2.1.3.2 of the Scientific Opinion Part 1 (see also the Virtual Issue)), by including specific values for the pleuromutilins under assessment.

$$FARSC_{intestine}\ (\text{mg/kg feed}) = \frac{\text{PMSC} \times \text{daily faeces}}{(1-I) \times (1-F+F \times GE \times \text{daily feed intake})}$$

$$FARSC\ (\text{mg/kg feed}) = \frac{\text{PMSC} \times \text{volume of rumen}}{(1-I) \times \text{daily feed intake}}$$

With daily faeces being the daily fresh faecal output in kg, I the inactive fraction, F the fraction available, GE is the fraction of the antimicrobial that is secreted back into the intestinal tract for elimination, after initially being absorbed into the bloodstream, and daily feed intake being the daily dry-matter feed intake expressed in kg.

Tiamulin

No data are available for bioavailability of tiamulin.

It was demonstrated that in pigs following oral administration of radiolabelled tiamulin, 65% of the radioactivity was found in faeces. This radioactivity was mainly associated to metabolites described as inactive but without any available quantitative data.

No data are available for other species.

Due to the absence of MSC and other PK data, the estimation of the FARSC for tiamulin was not possible.

Valnemulin

The bioavailability of valnemulin is 74.4% in broilers. The data for other species are lacking or is uncertain. No data are available on the fate of valnemulin after absorption and especially on the metabolism or on excretion in intestines after absorption (Table 2).

Table 2: Pharmacokinetic (PK) values used for the calculation of Feed Antimicrobial Resistance Selection Concentration (FARSC) of valnemulin for broilers

Valnemulin (broilers)	Scenario #1
Inactive fraction (*I*)	NA
Bioavailability (*F*) poultry	0.75
Gastrointestinal elimination (*GE*)	NA

Inactive fraction (*I*) is the fraction of antimicrobial that would not have any activity on bacteria. Bioavailability (*F*) is the fraction of antimicrobial that is absorbed from the digestive tract to the blood. *GE* is the fraction of the antimicrobial that is secreted back into the intestinal tract for elimination, after initially being absorbed into the bloodstream. The fraction remaining in the digestive tract and that could be available for the bacteria is equal to $(1 - F + F \times GE)$. NA: not available.

In pigs, valnemulin was described to be extensively metabolised after oral administration. By considering that 4.4% of the metabolites have an antimicrobial activity of approximately 70% that of valnemulin, the percentage of the dose available for intestinal microorganisms would be around 3% (4.4 multiplied by 0.7). Thus, the value for $(1 - F + F \times GE)$ was set at 0.03 for pigs (Table 3).

No data are available for other species.

Table 3: Pharmacokinetic (PK) values used for the calculation of Feed Antimicrobial Resistance Selection Concentration (FARSC) of valnemulin for pigs

Valnemulin (pigs)	Scenario #1
Inactive fraction (I)	NA
Fraction of the dose available for intestinal microorganisms corresponding to $(1 - F + F \times GE)$ in pigs	0.03

Inactive fraction (I) is the fraction of antimicrobial that would not have any activity on bacteria. Bioavailability (F) is the fraction of antimicrobial that is absorbed from the digestive tract to the blood. GE is the fraction of the antimicrobial that is secreted back into the intestinal tract for elimination, after initially being absorbed into the bloodstream. The fraction remaining in the digestive tract and that could be available for the bacteria is equal to $(1 - F + F \times GE)$. NA: not available.

Due to the absence of MSC and other PK data, the estimation of the FARSC for valnemulin was not possible.

3.2.1. Associated data gaps and uncertainties

With regard to the uncertainties and data gaps described in the Scientific Opinion Part 1 (Sections 3.1 and 3.3; see also the Virtual Issue), we identified the following for the pleuromutilins under assessment:

i) MSC data: no data for MSCs are available.
ii) MIC data: MIC data only exist for few bacterial species for tiamulin and are not available for valnemulin in EUCAST database (accessed on 15 May 2021).
iii) Bioavailability: for tiamulin, no data are available. For valnemulin, there is no value for fed animals.
iv) Fraction eliminated in gut: several studies suggest an elimination of tiamulin and valnemulin as inactive metabolites. However, there are no quantitative data to consider this process except for valnemulin in pigs.
v) Inactive fraction: no data on the possible binding of tiamulin or valnemulin in digestive tract are available.
vi) Ruminants: no data are available for tiamulin or valnemulin administered to ruminants.

3.2.2. Concluding remarks

Due to the lack of data on the parameters required to calculate the FARSC, it is not possible to conclude the ToR1 assessment until further experimental data are available.

3.3. ToR2. Specific antimicrobials concentrations in feed which have an effect in terms of growth promotion/increased yield

3.3.1. Tiamulin

3.3.1.1. Literature search results

The literature search, conducted according to the methodology described in Section 2.2.2.1 of the Scientific Opinion 'Part 1: Methodology, general data gaps and uncertainties' (see also the Virtual Issue), resulted in 273 papers mentioning tiamulin and any of the food-producing animal species considered[3] and any of the performance parameters identified as relevant for the assessment of the

[3] Ruminants: growing and dairy (cattle, sheep, goats, buffaloes); pigs: weaned, growing and reproductive; equines; rabbits; poultry: chickens and turkeys for fattening, laying hens, turkeys for breeding, minor avian species (ducks, guinea fowl, geese, quails, pheasants, ostrich); fish: salmon, trout, other farmed fish (seabass, seabream, carp); crustaceans; other animal species.

possible growth-promoting effects of tiamulin.[4] After removing the reports not matching the eligibility criteria, 69 publications were identified.

3.3.1.2. Evaluation of the studies

The 69 publications identified in the literature search were appraised for suitability for the assessment of the effects of tiamulin on growth or yield of food-producing animals; this appraisal was performed by checking each study against a series of pre-defined exclusion criteria (see Section 2.2.2.2.1 of the Scientific Opinion 'Part 1: Methodology, general data gaps and uncertainties'; see also the Virtual Issue).[5] A total of 61 publications were not considered suitable for the assessment because of several shortcomings identified in the design of the study or in the reporting of the results. The list of excluded publications and their shortcomings are presented in Appendix A.1 (Table A.1).

The publications considered suitable for the assessment are described and assessed in Section 3.3.1.3.

3.3.1.3. Assessment of the effects of tiamulin on growth performance and yield

Eight publications were considered suitable for the assessment of the effects of tiamulin on growth and yield performance in food producing animals. The effects of the administration of the antimicrobial on the endpoints described in Section 2.2.2.2.2 of the Scientific Opinion 'Part 1: Methodology, general data gaps and uncertainties' (see also the Virtual Issue) were evaluated. The selected publications and the effects on the relevant endpoints are described below. The summary of the studies includes the description of the source of tiamulin used – either as the base or as any specific form/commercial preparation – and the concentration(s) applied as reported in each study; where a specific compound has been used, the calculation of the concentration applied to the base substance is provided.

3.3.1.3.1. Studies in pigs

In the study of Cai et al. (2018), a total of 100 finishing pigs (Duroc × (Landrace × Large White)), with initial mean body weight of 51.1 kg were distributed in 25 pens in groups of 4 animals and allocated to 5 dietary treatments (20 pigs/treatment). Two basal diets (from 1 to 35 days and from 36 to 70 days) were either not supplemented or supplemented with different treatments. Two were the relevant treatments: a control and a treatment consisting of tiamulin (unspecified form) supplemented at the concentration of 500 mg/kg feed. The study lasted 70 days. Body weight (BW) and feed intake (FI) were recorded on days 0, 35 and 70 to calculate average daily weight gain, average daily feed intake (ADFI) and gain to feed ratio (G:F). From day 64 to day 70, chromium oxide (0.2%) was added to the diet to determine the apparent total tract digestibility (dry matter (DM), nitrogen (N) and gross energy). On day 70, fresh faecal samples were collected from at least two pigs per pen to assess digestibility coefficients and *E. coli* and *Lactobacillus* spp. colony counts. On days 35 and 70, samples of blood were collected from 10 pigs per treatment. At the end of the trial, the pigs treated with tiamulin showed, compared to the control group, higher average daily weight gain (881 vs. 805 g) and improved G:F (0.366 vs. 0.326). An increase in relative count of lymphocytes (62.4% vs. 52.9%), a decrease in faecal counts of *E. coli* (6.4 vs. 6.7 $\log_{10}$ CFU/g) and an increase of *Lactobacillus* spp. (7.0 vs. 6.4 $\log_{10}$ CFU/g) were seen in tiamulin-treated pigs compared to the control. Dietary tiamulin supplementation at 500 mg/kg feed had a growth-promoting effect in pigs for fattening.

In the study of Cho and Kim (2015a), a total of 120 weaned piglets (Duroc × (Yorkshire × Landrace)) weaned at 21 days (7.95 kg BW) were distributed in 24 pens in groups of 5 animals and

[4] (i) Intake-related parameters: feed intake, feed/gain ratio, feed efficiency, feed intake/milk yield, feed intake/egg mass; (ii) Weight-related parameters: body weight, body weight gain; (iii) Carcass-related parameters: carcass weight, carcass yield, carcass chemical composition, relative weight of the (different sections of) intestine; (iv) Milk or egg production/quality: milk yield, fat/protein yield, egg production/laying rate, egg weight, egg mass; (v) Digestibility/utilisation of nutrients: utilisation of some nutrients (e.g. DM, Ca, P), digestibility; (vi) Health-related parameters: reduction of morbidity and/or mortality; (vii) Herd/flock related parameters; (viii) Other endpoints: e.g. intestinal morphological characteristics (villi height/width), changes in microbiota.

[5] The following exclusion criteria were applied: 'Combination of substances administered to the animals', 'Antimicrobial used different from the one under assessment', 'Administration via route different from oral', 'Use of the antimicrobial with a therapeutic scope', 'Animals subjected to challenges with pathogens', 'Animals in the study sick or not in good health, Zootechnical parameters not reported', 'Insufficient reporting/statistics', 'Other (indicate)'.

allocated to 4 dietary treatments (30 pigs/treatment). Two basal diets (1–14 days, 15–42 days) were either not supplemented or supplemented with different treatments. Two were the relevant treatments: a control and a treatment consisting of tiamulin (unspecified form) supplemented at the concentration of 500 mg/kg feed. The study lasted 42 days. BW and FI were recorded at 1, 15 and 42 days of trial and average daily weight gain and (F:G) calculated. Samples of blood were collected from 10 pigs per treatment on days 14 and 42. On days 8–14 and 36–42, chromium oxide (0.2%) was added to the diet to determine the apparent total tract digestibility (DM, N and gross energy). On days 12–14 and 40–42, fresh faecal samples were collected from two pigs per pen to determine digestibility coefficients and *E. coli* and *Lactobacillus* spp. counts. At the end of the trial, the piglets treated with tiamulin showed, compared to the control group, higher average daily weight gain (516 vs. 488 g), ADFI (882 vs. 839 g) and higher nitrogen faecal apparent digestibility (85.4% vs. 81.8%). Dietary tiamulin supplementation at 500 mg/kg feed had a growth-promoting effect in weaned piglets.

In the study of Cho and Kim (2015b) a total of 125 weaned piglets (Duroc × (Landrace × Yorkshire)), weaned at 21 days (6.76 kg BW) were distributed in 25 pens in groups of 5 animals and allocated to 5 dietary treatments (25 pigs/treatment). Three basal diets (0–7 days, 8–21 days, 22–42 days) were either not supplemented or supplemented with different treatments. Two were relevant treatments: a control and a treatment consisting of tiamulin (unspecified form) supplemented at the concentration of 39 mg/kg feed. The study lasted 42 days. Animal weight and FI were recorded at 1, 7, 14 and 42 days of the experiment and average daily gain and F:G calculated. Samples of blood were collected from 10 pigs/treatment at the beginning and end of the experiment. On days 14–21 and 35–42 of the experiment, chromium oxide (0.2%) was added to the diet to determine the apparent total tract digestibility (DM, N and gross energy). On days 7, 21 and 42 of trial, fresh faecal samples were collected from two pigs in each pen to determine digestibility coefficients (days 21 and 42), faecal moisture and faecal pH (days 7, 21 and 42). At the end of the trial, the piglets treated with tiamulin showed, compared to the control group, higher average daily weight gain (478 vs. 449 g) and improved G:F (0.709 vs. 0.672). Apparent digestibility coefficients showed, at 42 days, compared to the control group, a positive effect on DM (81.1% vs. 80.7%) and N (81.2% vs. 80.2%) digestibility. Dietary tiamulin supplementation at 39 mg/kg feed had a growth-promoting effect in weaned piglets.

In the study of Cromwell and Stahly (1985), a total of 244 pigs were used in two experiments. In the first trial, 100 pigs (Hampshire × Yorkshire; initial BW 15 kg) were distributed in 20 pens in groups of 5 animals and allocated to 5 dietary treatments (20 pigs/treatment). In the second trial, 144 pigs (Duroc × Yorkshire; initial BW 11 kg) were distributed in 24 pens in groups of 6 animals and allocated to 6 dietary treatments (24 pigs/treatment). Basal diets (grower and finisher in trial 1; starter, grower and finisher in trial 2) were either not supplemented or supplemented with different treatments. In trial 1 (duration 65 days up to 58 kg BW), there were four relevant treatments: a control (0 mg tiamulin/kg feed) and three treatments consisting of tiamulin (unspecified chemical form; Dynamutilin provided by E.R Squibb & Sons, Inc., Princeton, NJ, USA) supplemented at 11, 22 or 44 mg/kg feed. In trial 2 (duration 73 days up to 56 kg BW) there were five relevant treatments: a control (0 mg tiamulin/kg feed) and four treatments consisting of tiamulin supplemented at 2.75, 5.50, 11 or 22 mg/kg feed. In both trials, after tiamulin withdrawal (day 65 in trial 1 and day 73 in trial 2) animals received a non-medicated basal diet until the end of experiment (95 kg live weight, corresponding to day 112 in the first trial and to day 126 in the second one). Animal weight and FI were recorded to calculate average daily weight gain and F:G. At the end of the administration in trial 1 (58 kg, 65 days), the addition of tiamulin resulted in a quadratic improvement of average daily weight gain (590, 679, 679, 714 g at 0, 11, 22, 44 mg/kg feed, respectively) and in a linear effect for F:G (2.97, 2.97, 2.87, 2.84 at 0, 11, 22, 44 mg/kg feed, respectively). A cubic effect was noted for average daily weight gain also after tiamulin withdrawal. At the end of the trial 2 (56 kg, 73 days), the addition of tiamulin to the diet resulted in quadratic improvements in average daily weight gain (583, 604, 605, 641, 619 g at 0, 2.75, 5.50, 11, 22 mg/kg feed, respectively) and F:G (2.77, 2.76, 2.65, 2.65, 2.63 at 0, 2.75, 5.50, 11, 22 mg/kg feed, respectively). Owing to the lack of pair-wise statistical comparisons between groups, it is not possible to derive the minimum concentration in which tiamulin may have an effect in growth performance. However, the data showed dose-related effects of tiamulin suggesting that a concentration of 11 mg tiamulin/kg feed would have an effect on improving daily weight gain (trial 1), and a concentration of 5.5 mg tiamulin/kg feed would have an effect on improving F:G (trial 2) of pigs for fattening.

In the study of Lei et al. (2018), a total of 140 weaned piglets ((Yorkshire × Landrace) × Duroc)) with an average weight of 6.37 kg were distributed in 28 pens in groups of 5 animals (3 females and 2 males) and allocated to four dietary treatments (35 pigs/treatment). The basal diets were either not

supplemented or supplemented with different treatments. Two were the relevant treatments: a control and a treatment consisting of tiamulin (unspecified form) supplemented at the concentration of 39 mg/kg feed. The study lasted 42 days. The general health status was checked throughout the study. Animal weight, FI and G:F were measured weekly. Total tract apparent digestibility (using chromium oxide 0.2%) was assessed for DM, N and gross energy in 2 pigs/pen on days 7, 21 and 42; in the same pigs, faecal score, moisture and pH were also assessed. Faecal lactic acid bacteria and coliform bacteria counts were assessed in 2 pigs/pen on day 42. Blood samples were collected from 2 pigs/pen on days 1, 21 and 42; concentrations of red blood cells (RBC), white blood cells (WBC), lymphocytes and IgG in serum were measured. At the end of the trial, and from day 22 onwards the pigs treated with tiamulin showed, compared to the control group, an improvement of daily weight gain (481 vs. 433 g), improved G:F (0.703 vs. 0.629) and on day 42 coliform bacteria counts in faeces were reduced (6.05 vs. 6.32 log CFU/g). Dietary tiamulin supplementation at 39 mg/kg feed had a growth-promoting effect in weaned piglets.

In the study of Nitikanchana et al. (2012) a total of 1,313 pigs for fattening (PIC 1050 × 337) with an average BW of 22.2 kg, sex unspecified, were distributed in 40 pens in groups of 31–33 animals/pen and allocated to four dietary treatments (approx. 320 pigs/treatment). The basal diet was either not supplemented or supplemented with different treatments. Two were the relevant treatments: a control and a treatment consisting of tiamulin (unspecified chemical form; Denagard, Novartis Animal Health, Greensboro, NC, USA) supplemented at a concentration of 35 mg/kg feed. The study lasted 35 days and comprised two parts: from day 1 to day 15 the animals were fed with tiamulin-supplemented diet; after then and until day 35 the animals were fed an unsupplemented-tiamulin diet. The general health status was checked throughout the study. Average daily weight gain, animal weight, ADFI and G:F were calculated separately for the supplemented period, unsupplemented period and the total experimental period of 35 days. At the end of supplemented phase there was greater average daily weight gain (0.676 vs. 0.648 kg) and improved F:G (1.56 vs. 1.82) in the tiamulin-supplemented group, compared to control. At the end of unsupplemented phase of the treatment group average weight gain was lower (0.871 vs. 0.921 kg) and F:G higher (2.19 vs. 2.05) in the supplemented group in comparison with the control. Evaluation of overall study period did not show any differences in growth performance. Dietary tiamulin supplementation at 35 mg/kg feed had a growth-promoting effect in pigs for fattening.

In the study of Serpunja et al. (2018), a total of 120 weaned piglets ((Yorkshire × Landrace) × Duroc) with an average weight of 8.4 kg, 21 days of age (sex unspecified) were distributed in 20 pens in groups of 6 animals and allocated to 4 dietary treatments (30 pigs/treatment). The basal diets were either not supplemented or supplemented with different treatments. Two were the relevant treatments: a control and a treatment consisting of tiamulin (unspecified chemical form; provided by Novartis AG, Basel, Switzerland) supplemented at the concentration of 39 mg/kg feed. The study lasted 42 days. The general health status was checked throughout the study. BW and weight gain, FI, and G:F were measured at days 21 and 42. Total tract apparent digestibility (using chromium oxide 0.2%) was assessed for DM, N and gross energy in 2 pigs/pen on days 21 and 42. Faecal moisture and pH were assessed in 5 pigs/group (1/pen) on day 7, 21 and 42. Faecal counts of *Lactobacillus* spp., *Salmonella* spp. and *E. coli* were assessed in 2 pigs/pen on day 42. Blood samples were collected from 2 pigs/pen on day 7, 21 and 42, and concentrations of RBC, WBC and lymphocytes were measured. At the end of the trial the pigs treated with tiamulin showed, compared to the control group, an improvement of daily weight gain (385 g vs. 342 g), improved G:F (0.74 vs. 0.63), higher digestibility of nitrogen (81.8% vs. 78.5%) and increased Lactobacillus counts (8.21 vs. 7.43 $\log_{10}$ CFU/g). Dietary tiamulin supplementation at 39 mg/kg feed had a growth-promoting effect in weaned piglets.

3.3.1.3.2. Study in poultry

In the study of Cai et al. (2015), a total of 765 one-day-old chickens for fattening (Ross 308), with an average BW of 49 g, were distributed in 45 pens in groups of 17 animals and allocated to five dietary treatments (153 chicks/treatment). The basal diets (starter and grower) were either not supplemented or supplemented with different treatments. Two were the relevant treatments: control and a treatment consisting of tiamulin (unspecified form) supplemented at the concentration of 1,000 mg/kg feed. The study lasted 28 days. From day 22–28, chromium oxide (0.2%) as an indigestible marker was added to the diets to determine apparent total tract digestibility (DM, N and gross energy). BW and FI were recorded on days 0, 14, 28 and F:G calculated. At the end of the experiment, nine birds per treatment were slaughtered and the weights of the liver, spleen, bursa of Fabricius, abdominal fat, breast muscle and gizzard were recorded. Meat quality (colour, pH and drip

loss of breast muscle) was measured. At day 28, excreta samples were collected from each cage and pooled to count *E. coli* and *Lactobacillus* spp. colonies. At the end of the trial, tiamulin-treated birds showed, compared to the control group, a higher digestibility coefficient for DM (78.49% vs. 75.52%). Dietary tiamulin supplementation at 1,000 mg/kg feed improved DM digestibility in chickens for fattening.

3.3.1.4. Discussion

From the studies examined, the test item has been described as 'tiamulin' (unspecified form; eight studies). Therefore, an uncertainty on the exact product used/concentration applied has been identified.

A detailed analysis of the uncertainties for tiamulin is included in Appendix B (Table B.1) of this document, and the Section 3.3. of the Scientific Opinion Part 1 (see also the Virtual Issue).

3.3.1.4.1. Pigs

The seven publications considered as suitable for the assessment covered two pigs' categories: four studies were performed in weaned piglets and three studies in pigs for fattening. One study in pigs tested different concentrations of tiamulin which allows assessment of concentration related effects.

In weaned piglets, dietary tiamulin supplementation at 39 mg/kg feed (Cho and Kim, 2015b; Lei et al., 2018; Serpunja et al., 2018) or 500 mg/kg feed (Cho and Kim, 2015a) showed growth-promoting effects in piglets.

In three studies in pigs for fattening, dietary tiamulin supplementation at 5.5 and 11 mg/kg feed (Cromwell and Stahly, 1985, two experiments), 35 mg/kg feed (Nitikanchana et al., 2012) and 500 mg/kg feed (Cai et al., 2018) improved growth performance of pigs.

3.3.1.4.2. Poultry

One study in chickens for fattening (Cai et al., 2015) was identified; this study used tiamulin at 1,000 mg/kg feed with a growth-promoting effect.

3.3.1.5. Concluding remarks

It is judged 50–66% certain that tiamulin has growth-promoting/increase yield effects in weaned piglets at concentrations ranging from 39 to 500 mg/kg complete feed (four studies) and in pigs for fattening at concentrations ranging from 5.5 to 500 mg/kg complete feed (three studies). It is judged 33–66% certain ('about as likely as not') that tiamulin has growth-promoting/increase yield effects in chickens for fattening at a concentration of 1,000 mg/kg complete feed (one study).

No data are available in the scientific literature showing effects of tiamulin on growth promotion/increase yield when added (i) to weaned piglets feed at concentrations below 39 mg/kg, (ii) to pigs for fattening feed below 5.5 mg/kg feed, (iii) to chickens for fattening feed below 1,000 mg/kg or (iv) to feed of any other food-producing animal species or categories.

3.3.2. Valnemulin

3.3.2.1. Literature search results

The literature search, conducted according to the methodology described in Section 2.2.2.1 of the Scientific Opinion 'Part 1: Methodology, general data gaps and uncertainties' (see also the Virtual Issue), resulted in 25 papers mentioning valnemulin and any of the food-producing animal species considered[3] and any of the performance parameters identified as relevant for the assessment of the possible growth promoting effects of valnemulin.[4] After removing the reports not matching the eligibility criteria, five publications were identified.

3.3.2.2. Evaluation of the studies

The five publications identified in the literature search were appraised for suitability for the assessment of the effects of valnemulin on growth or yield of food-producing animals; this appraisal was performed by checking each study against a series of pre-defined exclusion criteria (see Section 2.2.2.2.1 of the Scientific Opinion 'Part 1: Methodology, general data gaps and uncertainties'; see also the Virtual Issue).[5] None of the publications was considered suitable for the assessment because of several shortcomings identified in their design or in the reporting of the results. The list of excluded publications and their shortcomings are presented in Appendix A.2 (Table A.2).

3.3.2.3. Concluding remark

Owing to the lack of suitable data, levels of valnemulin in feed which may have a growth promotion/production yield effect in any food-producing animal species could not be identified.

4. Conclusions

ToR1: to assess the specific concentrations of antimicrobials resulting from cross-contamination in non-target feed for food-producing animals, below which there would not be an effect on the emergence of, and/or selection for, resistance in microbial agents relevant for human and animal health.

AQ1. Which are the specific concentrations of tiamulin and valnemulin in non-target feed below which there would not be emergence of, and/or selection for, resistance in the large intestines/rumen?

- Due to the lack of data on the parameters required to calculate the Feed Antimicrobial Resistance Selection Concentration (FARSC) corresponding to the concentrations of those antimicrobials in non-target feed below which there would not be expected to be an effect on the emergence of, and/or selection for, resistance in microbial agents relevant for human and animal health, it is not possible to conclude until further experimental data are available.

ToR2: to assess which levels of the antimicrobials have a growth promotion/increase yield effect.

AQ2. Which are the specific concentrations of tiamulin and valnemulin in feed of food-producing animals that have an effect in terms of growth promotion/increased yield?

With regards to tiamulin:

- It is judged 50–66% certain that tiamulin has growth-promoting/increased yield effects in weaned piglets at concentrations ranging from 39 to 500 mg/kg complete feed (four studies) and in pigs for fattening at concentrations ranging from 5.5 to 500 mg/kg complete feed (three studies).
- It is judged 33–66% certain ('about as likely as not') that tiamulin has growth-promoting/increased yield effects in chickens for fattening at a concentration of 1,000 mg/kg complete feed (one study).
- No data are available in the scientific literature showing effect of tiamulin on growth promotion/increase yield when added (i) to weaned piglets feed at concentrations below 39 mg/kg, (ii) to pigs for fattening feed below 5.5 mg/kg feed, (iii) to chickens for fattening feed below 1,000 mg/kg or (iv) to feed of any other food-producing animal species or categories.

With regards to valnemulin:

- Owing to the lack of suitable data, levels of valnemulin in feed which may have a growth promotion/production yield effect in any food-producing animal species could not be identified.

The results from these assessments for the different animal species are summarised in Annex F (Tables F.1 and F.2) of EFSA BIOHAZ Panel, 2021a – Scientific Opinion 'Part 1: Methodology, general data gaps and uncertainties' (see also the Virtual Issue).

5. Recommendations

To carry out studies to generate the data that are required to fill the data gaps which have prevented calculation of the FARSC for tiamulin and valnemulin.

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Abbreviations

ADFI	average daily feed intake
AQ	assessment question
bw	body weight used in toxicity studies
BW	body weight
CFU	colony forming unit
EUCAST	European Committee on Antimicrobial Susceptibility testing
F	fraction of the antimicrobial that is absorbed from the digestive tract to the blood
FARSC	Feed Antimicrobial Resistance Selection Concentration
FI	feed intake
GE	fraction of the antimicrobial that is secreted back into the intestinal tract for elimination, after initially being absorbed into the bloodstream
F:G	feed to gain ratio
G:F	gain to feed ratio
HGT	horizontal gene transfer
I	fraction of the antimicrobial present in the digestive tracts that would be inactive on the microbiota
MIC	minimum inhibitory concentration
MIC_{lowest}	minimum inhibitory concentration of the most susceptible species/strain included in the EUCAST database for a certain antimicrobial used to calculate the PMSC (see below)
MIC_{res}	minimum inhibitory concentration of the resistant strain
MIC_{susc}	minimum inhibitory concentration of the susceptible strain
MIC_{test}	minimum inhibitory concentration of the susceptible isolate used in the competition experiments to calculate the MSC
MRSA	meticillin-resistant *Staphylococcus aureus*
MSC	minimal selective concentration
MSSA	meticillin-susceptible *Staphylococcus aureus*
PK	pharmacokinetic(s)
PMSC	predicted MSC
RBC	red blood cells
rRNA	ribosomal ribonucleic acid
ToRs	terms of reference
WBC	white blood cells
WHO	World Health Organization

Appendix A – List of excluded publications and their shortcomings

A.1. Tiamulin

The publications excluded from the assessment of the effects of tiamulin on growth promotion/increased yield following the criteria defined in Section 2.2.2.2.1 of the Scientific Opinion 'Part 1: Methodology, general data gaps and uncertainties' (see also the Virtual Issue) are summarised in Table A.1.

Table A.1: Publications not relevant for the assessment of the effects of tiamulin on growth promotion/increase yield and excluding criteria

Author (year)	SPECIES	Excluding criteria								
		Combination of substances administered to the animals	Antimicrobial used different from the one under assessment	Administration via route different form oral	Use of the antimicrobial with a therapeutic scope	Animals subjected to challenges with pathogens	Animals in the study sick or not in good health	Zootechnical parameters not reported	Insufficient reporting/ statistics	Other (indicate)
Abecia et al. (2007a)	Rabbits							X	X	X(1)
Abecia et al. (2007b)	Rabbits				X			X		X(2)
Badiola et al. (1994)	Poultry				X					
Bónai et al. (2008)	Rabbits	X								
Burch (1982)	Pigs						X		X	
Burch (1984)	Pigs						X			
Burch et al. (1986)	Pigs	X			X		X		X	
Burch et al. (2006)	Pigs	X			X		X			
Delic et al. (2018)	Pigs								X	
Devi et al. (2015)	Pigs	X				X				
Duttlinger et al. (2019)	Pigs	X								
El-Ghany and Abd El-Gha (2009)	Poultry				X		X			
Francisco et al. (1996)	Pigs								X	X(3)
Haj and Ben (2008)	Rabbits									
Hampson et al. (2002)	Poultry				X	X				
Han et al. (2011)	Pigs	X						X		

Author (year)	SPECIES	Excluding criteria								
		Combination of substances administered to the animals	Antimicrobial used different from the one under assessment	Administration via route different form oral	Use of the antimicrobial with a therapeutic scope	Animals subjected to challenges with pathogens	Animals in the study sick or not in good health	Zootechnical parameters not reported	Insufficient reporting/ statistics	Other (indicate)
Han and Thacker (2009)	Pigs	X								
Han and Thacker (2010)	Pigs	X								
Hinz and Rottmann (1990)	Poultry				X	X				
Hong et al. (2012)	Pigs	X								
Hsu et al. (1983)	Pigs					X				
Islam et al. (2008)	Poultry	X								X(4)
Jacquier et al. (2014)	Poultry	X								
Jeong and Kim (2015)	Pigs								X	
Johnson and Lay (2017)	Pigs	X								X(4)
Jordan and Knight (1984)	Poultry				X	X			X	
Jordan et al. (1991)	Poultry				X	X				
Jordan et al. (1998)	Poultry				X	X				
Keegan et al. (2005)	Pigs	X								
Kiarie et al. (2018)	Pigs	X								
Kovacs et al. (2009)	Rabbits	X								
Lee et al. (2011)	Pigs	X								
Lee et al. (2009)	Pigs	X						X		
Lehel et al. (1995)	Poultry	X							X	
Lessard et al. (2014)	Pigs					X		X		
Meingassner et al. (1978)	Poultry	X			X	X		X	X	

Author (year)	SPECIES	Excluding criteria								
		Combination of substances administered to the animals	Antimicrobial used different from the one under assessment	Administration via route different form oral	Use of the antimicrobial with a therapeutic scope	Animals subjected to challenges with pathogens	Animals in the study sick or not in good health	Zootechnical parameters not reported	Insufficient reporting/ statistics	Other (indicate)
O'Connor et al. (1979)	Pigs				X	X		X	X	
Oliver et al. (2014)	Pigs	X				X		X		
Papadomichelakis et al. (2011)	Rabbit							X		
Park et al. (2018)	Pigs							X		X(5)
Patterson et al. (2019)	Pigs	X				X		X		
Puls et al. (2019a)	Pigs	X						X		
Puls et al. (2019b)	Pigs	X					X	X		
Ricketts et al. (1991)	Poultry	X						X	X	
Roberts et al. (2011)	Pigs				X		X	X		
Rueff et al. (2019)	Pigs	X			X					X(4)
Schuhmacher et al. (2006)	Poultry							X		
Stephens and Hampson (2002)	Poultry			X	X	X		X		X(4)
Stipkovits et al. (1992)	Poultry	X				X		X	X	
Stipkovits et al. (1999)	Poultry	X								
Stipkovits et al. (2001)	Pigs	X			X	X		X		
Stipkovits et al. (2003)	Pigs				X		X	X	X	
Stipkovits et al. (2004)	Poultry				X	X		X		
Vieira et al. (2010)	Poultry	X						X		

Author (year)	SPECIES	Excluding criteria								
		Combination of substances administered to the animals	Antimicrobial used different from the one under assessment	Administration via route different form oral	Use of the antimicrobial with a therapeutic scope	Animals subjected to challenges with pathogens	Animals in the study sick or not in good health	Zootechnical parameters not reported	Insufficient reporting/ statistics	Other (indicate)
Wallgren et al. (1999a)	Pigs				X	X		X		
Wallgren et al. (1999b)	Pigs				X	X				
Walsh et al. (2007)	Pigs	X								
Walter et al. (2000)	Pigs	X			X		X	X		
Walter et al. (2001)	Pigs				X	X				
Woodward et al. (2015)	Poultry				X	X		X		
Zakeri and Kashefi (2011)	Poultry				X		X	X		

(1): The paper is aimed at exploring the effect on the microbiota of litters of TIA treated rabbit does at 100 mg/kg.
(2): The paper is aimed at exploring the effects on microbiota of rabbit does at different feeding levels and receiving or not TIA at 100 mg/kg.
(3): Piglets receiving tiamulin were previously treated with enrofloxacin and controls did not.
(4): No negative control.
(5): The paper deals with the effect of different substances on transport stress- related consequences in piglets.

A.2. Valnemulin

The publications excluded from the assessment of the effects of valnemulin on growth promotion/increase yield following the criteria defined in Section 2.2.2.2.1 of the Scientific Opinion 'Part 1: Methodology, general data gaps and uncertainties' (see also the Virtual Issue) are summarised in Table A.2.

Table A.2: Publications not relevant for the assessment of the effects of valnemulin on growth promotion/increased yield and excluding criteria

Author (year)	SPECIES	Excluding criteria								
		Combination of substances administered to the animals	Antimicrobial used different from the one under assessment	Administration via route different form oral	Use of the antimicrobial with a therapeutic scope	Animals subjected to challenges with pathogens	Animals in the study sick or not in good health	Zootechnical parameters not reported	Insufficient reporting/ statistics	Other (indicate)
Cunha et al. (2017)	Rabbit	X					X		X	X(1)
Dip et al. (2015)	Rabbit						X			
Jordan et al. (1998)	Poultry				X	X				
Stipkovits et al. (2001)	Pigs	X			X	X		X		
Tzika et al. (2009)	Pigs						X			

(1): Absence of a control group without antimicrobial.

Appendix B – Table of uncertainties

Table B.1: Potential sources of uncertainty identified in the levels of tiamulin in feed which have growth promotion/increase yield effect and assessment of the impact that these uncertainties could have on the conclusion

Source of the uncertainty	**Nature or cause of uncertainty**	**Impact of the uncertainty on the conclusion on the level (s) which have growth promotion/increase yield effect**
Form(s) of antimicrobial used	The specific form of the antimicrobial used in the study (as the '(free) base' substance, its salts or specific products/ formulations containing the base substance) has not been clearly described in several publications. In summarising the results, the concentrations have been reported as for 'base' substance when the form of the antimicrobial is not specified (conservative assumption)	Underestimation of the concentration which may have shown growth-promoting effect
Evidence synthesis and integration	As described in Section 2.2.1 of the Scientific Opinion Part 1 (see also the Virtual Issue), although meta-analysis was not applicable to the studies retrieved, evidence synthesis was done, since: • Four studies showing consistent (positive) results in a comparable range of concentrations were available in weaned piglets. The uncertainty resulting in the process of evidence synthesis was based on four studies all showing positive effect; • Three studies showing consistent (positive) results in a comparable range of concentrations were available in pigs for fattening. Consistency of results across categories (i.e. piglets and pigs for fattening) would reduce the uncertainty in the conclusions for both categories. For cattle chicken for fattening, the low number of studies retrieved prevented evidence synthesis	The extent of the underestimation or overestimation on the levels which shown growth-promoting effect is modulated by the consistency of the results