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Maximum levels of cross-contamination for 24 antimicrobial active substances in non-target feed. Part 7: *Amphenicols: florfenicol and thiamphenicol*

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Abstract

The specific concentrations of florfenicol and thiamphenicol 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. The FARSC for florfenicol was estimated. However, due to the lack of data, the calculation of the FARSC for thiamphenicol was not possible 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 florfenicol, whilst for thiamphenicol no suitable data for the assessment were available. Uncertainties and data gaps associated to the levels reported were addressed. For florfenicol, it was recommended to perform further studies to supply more diverse and complete data related to the requirements for calculation of the FARSC, whereas for thiamphenicol, the recommendation was to generate the data required to fill the gaps which prevented the FARSC calculation.

Keywords: florfenicol, thiamphenicol, 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 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 amphenicols: florfenicol and thiamphenicol.

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

Amphenicols are derivatives of dichloroacetic acid, with two other components: an aromatic nucleus with an alkyl group in the para position and an aminopropanediol chain, thereby possessing a 2,2-dichloro-*N*-[-1-hydroxy-1-(phenyl) propan-2-yl] acetamide structure.

The chemical structure of thiamphenicol differs from chloramphenicol in having a sulfo-group instead of a nitro-group. Florfenicol has a fluorine atom instead of the hydroxyl group located at C-3 within the structure of chloramphenicol and thiamphenicol. This may allow florfenicol to be less susceptible to deactivation by bacteria with plasmid-borne resistance that involves acetylation of the C-3 hydroxyl group (USP, 2003).

The amphenicols inhibit bacterial protein synthesis by binding to the 50S ribosomal subunit and affecting the activity of the peptidyltransferase enzyme (Fisch and Bryskier, 2005).

Amphenicols are broad spectrum bacteriostatic antimicrobials, active against both Gram-negative and Gram-positive bacteria, including some anaerobes (EMA/CVMP/CHMP, 2019). The spectrum of activity for both florfenicol and thiamphenicol is similar (although thiamphenicol has greater activity against some anaerobes) and includes most important enteric, respiratory and dermal/sepsis-related bacterial pathogens of food animals (Shin et al., 2005), including fish (Samuelsen et al., 1998). Florfenicol is more widely used in feed and water because of lower minimum inhibitory concentration (MIC) values for most important pathogens (other than anaerobes and mycoplasmas), superior pharmacokinetic (PK) characteristics (Ravizzola et al., 1984; Switała et al., 2007) and reduced

[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.

susceptibility to inactivation by chloramphenicol transacetylase enzymes (Fukui et al., 1987; Michel et al., 2003). Each substance will therefore be assessed separately.

1.3.2. Main use[2]

Florfenicol is widely used as a feed additive or water treatment for enteric and respiratory infections in pigs and can also be used in calves or poultry (EMEA/CVMP, 1999). Florfenicol feed premix is also indicated for the treatment of furunculosis caused by susceptible strains of *Aeromonas salmonicida* in salmon, and it is one of the most commonly used antimicrobials in aquaculture (USP, 2003; Romero et al., 2012; Kumar et al., 2018). Injectable formulations are also available for treating individual animals or serious cases in large animals (Papich, 2016; EMA/CVMP/CHMP, 2019).

Thiamphenicol can also be used for the treatment and control of respiratory and intestinal infections in cattle, pigs and poultry by oral or intramuscular administration and is especially effective against anaerobes but is not commonly used for terrestrial food animals within the EU (EMA/ESVAC, 2020). It can also be used topically and by intra-mammary administration in both lactating and dry cows and for intrauterine administration in cows. Thiamphenicol is not permitted for use in laying hens (EMA/CVMP/CHMP, 2019).

1.3.3. Main pharmacokinetic data

1.3.3.1. Florfenicol

The bioavailability of florfenicol administered by the oral route to calves, 2–5 weeks of age, is 89%, at a dose of either 11 or 22 mg/kg (USP, 2003); however, the absorption was widely variable and oral absorption may be reduced when florfenicol is administered mixed with milk replacers. One study reported bioavailability that ranged from 44% to 86% among calves when florfenicol was administered 5 minutes after feeding (Varma et al., 1986).

In pigs, the bioavailability is around 100% in fasted animals (Liu et al., 2003) and no significant difference of the bioavailability between fasted and fed animals was reported (Jiang et al., 2006; De Smet et al., 2018).

In broilers, the oral bioavailability appears to be lower in fed than in fasted animals. In one study, the reported bioavailability was 55% in non-fasted broilers (Afifi and Abo el-Sooud, 1997) whereas reported bioavailability ranged from 87% to 96% in fasted broilers (Shen et al., 2003; Anadón et al., 2008).

For other species, the bioavailability of florfenicol after oral administration was 82% in fasted turkeys (Switała et al., 2007), 83% in fasted horses (McKellar and Varma, 1996), 76% in rabbits (no clear information on the fed or fasted status) (Park et al., 2007) and 96.5–99% in Atlantic salmon at a water temperature around 10°C (Horsberg et al., 1996; USP, 2003).

The bioavailability of florfenicol administered by the oral route is high but the effect of the feed on the bioavailability is uncertain for most species because of the lack of data in fed animals. Only one study suggests that feed could decrease the bioavailability in broilers.

Data on elimination for calves of less than 8 weeks of age suggests that approximately 50% of a 22 mg/kg intravenous dose is excreted unchanged in the urine within 30 h. For adult cattle, approximately 64% of a 20 mg/kg intramuscular dose administered twice, 48 h apart, is excreted as the parent drug in the urine. In horses, 6% of an oral dose is excreted unchanged in the urine within 30 h (USP, 2003). Florfenicol and some metabolites, such as monochloroflorfenicol and florfenicol oxamic acid, are also eliminated via the faecal route (USP, 2003). Quantitative data, especially on the percentage of the parent drug over metabolites, found in large intestines and in faeces is lacking for most species. An enterohepatic recirculation and/or a gastrointestinal secretion from blood to gut lumen of florfenicol is suggested by one study reporting high florfenicol concentrations in caecum or colon after both IM or oral administration (De Smet et al., 2018).

Significant binding of florfenicol to elements within intestinal contents is unlikely, in view of the lack of binding and substantial survival times and high bioactivity associated with contamination of soil (Subbiah et al., 2011). However, there is currently no experimental study supporting this hypothesis.

[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.

1.3.3.2. Thiamphenicol

Amphenicols were shown in one study to be relatively unstable in stored feed (Pietro et al., 2014), which could affect the actual levels presented to animals if cases of long-term administration.

Thiamphenicol is efficiently absorbed from the gastrointestinal tract and it is principally excreted in urine, with only small amounts being found in faeces (Dowling, 2013).

The bioavailability of thiamphenicol after oral administration in milk is around 60% in veal calves and pre-ruminant sheep (Mengozzi et al., 2002) but only of 30% in adult sheep (Abdennebi et al., 1994). Only one short communication reported a low bioavailability of 28% in fasted pigs (Haritova et al., 2002).

In birds, the bioavailability seems high, at least for fasted animals. The bioavailability is of 69% in fasted turkeys, (Switała et al., 2007), 80% in 3-week-old broilers (Ocampo et al., 2000) and higher than 70% in ducks (Tikhomirov et al., 2019).

Thiamphenicol is not readily metabolised in cattle, poultry, sheep (FAO, 1999). In rats, more than 30% of the dose was excreted in the faeces within 75 h (JECFA, 2002). In humans, renal insufficiency prolongs the half-life of thiamphenicol, but hepatic insufficiency, e.g. due to cirrhosis, did not increase the half-life, suggesting that enterohepatic circulation is not likely to be a significant factor that might prolong the presence in the gut (Walter and Heilmeyer, 1975).

No data was found on the possible binding of thiamphenicol to intestinal contents.

1.3.4. Main resistance mechanisms

The most frequently encountered mechanism of bacterial resistance to amphenicols is enzymatic inactivation by acetylation by a variety of types of chloramphenicol acetyltransferases (CATs). CATs can inactivate thiamphenicol but the replacement of the hydroxyl group at C-3 by a fluor residue makes florfenicol resistant to inactivation by CAT enzymes. There are numerous other mechanisms that can inactivate amphenicols, such as *O*-phosphorylation and hydrolytic degradation. A chloramphenicol acetate esterase (*estDL136* gene) identified during a soil metagenomic study, was the first described hydrolytic mechanism capable of inactivating florfenicol (Tao et al., 2012).

However, there are also reports of other resistance mechanisms, such as efflux systems, mutations in the target site genes and permeability barriers conferring resistance to amphenicols. Specific transporters involved in the export of amphenicols by bacterial efflux, including those encoded by *fexA*, *fexB*, *floR, pexA* or *cmlB1* genes, have no known function in normal cell metabolism, but some multidrug transporters play an important role in the excretion of any cytotoxic compound, including amphenicols (Kadlec et al., 2007).

Non-enzymatic resistance mechanisms based on permeability barriers involving alteration in membrane proteins such as porins have been described in various bacteria. The *mar* locus which is present in many Enterobacteriaceae has also been reported to contribute to resistance in *E. coli*. Mutations in the major ribosomal protein gene cluster of *E. coli* and *B. subtilis* as well as in the 23S rRNA gene of *E. coli* are known to confer resistance to amphenicols (Schwarz et al., 2004).

Several publications describe other amphenicol resistance genes in bacteria of animal and human origin, including the ribosomal protection protein genes *optrA* and *poxtA*, the gene encoding RE-CmeABC, a functionally enhanced multidrug efflux pump variant (3–5), and *cfr*, encoding a 23S rRNA methyltransferase. Some of these mechanisms (e.g. *cfr*, *optrA* and *poxtA*) can also confer resistance to antimicrobials of other classes (e.g. lincosamides, oxazolidinones, pleuromutilins and streptogramin A) (Long et al., 2006). Amphenicol resistance genes often occur on MDR plasmids, so usage may co-select for resistance to other clinically relevant antimicrobials, e.g. extended-spectrum cephalosporins or colistin (Du et al., 2020; Wang et al., 2020).

2. Data and methodologies

The data sources and methodology used for this opinion are described in a dedicated document, 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-MICs) 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 amphenicols florfenicol, thiamphenicol: examples

There are numerous publications that report amphenicol resistance in a wide range of bacteria, but very few that report on the response to exposure. Co-resistance to florfenicol and other antimicrobials has been identified in various Enterobacteriaceae, which can increase the spread of multidrug resistance (MDR) and associated virulence or heavy metal tolerance genes (Doublet et al., 2004; Berge et al., 2005; Braibant et al., 2005; Higuera-Llantén et al., 2018; HPRA, 2018). Resistance genes are also being reported from a wider range of bacteria in situations where there is heavy usage (Tang et al., 2020), including *cfr* in LA-MRSA CC398 (Ruiz-Ripa et al., 2021).

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

- The MIC analysis for *Piscirickettsia salmonis*, strains which were cultured in a sub-lethal concentration of florfenicol (0.064 μg/mL) showed a mean increase of one dilution (equivalent to 0.5 μg/mL) when compared with the initial MIC value (Yañez et al., 2014).
- *Salmonella* Enteritidis, *Klebsiella pneumoniae*, *Staphylococcus aureus* and *Listeria monocytogenes* were exposed to a sub-inhibitory concentration of florfenicol (1, 20 μg/mL) for 24 h and 48 h and changes in resistance to human therapeutic antimicrobials were determined. Increases in MIC values for ampicillin, tetracycline, nalidixic acid and meropenem against *Salmonella* and *Klebsiella* were in the range of 20–1,000 μg/mL, 5–62.5 μg/mL, 5–125 μg/mL and 0.05–0.1 μg/mL, respectively, whereas increases in MICs against *Staphylococcus* and *Listeria* were 2.5–10 μg/mL, 2.5 μg/mL, 62.5–500 μg/mL and 0.1–0.2 μg/mL, respectively. Exposure to sub-inhibitory levels of florfenicol was therefore associated with increases in resistance to ampicillin, tetracycline and nalidixic acid ranging from 1.25- to 40-fold compared to unexposed bacteria, with the exception of meropenem (Singh and Bhunia, 2019).
- The minimal selective concentration (MSC) for a strain of *E. coli* was determined by analysing and comparing the growth rates of susceptible and resistant variants at different sub-MICs (0.031, 0.062, 0.125, 0.25, 0.5, 1.0 and 2.0 μg/mL) of florfenicol. The MSC of florfenicol for strain *E. coli* MG1655/pSD11 was determined to be 0.042 μg/mL, which was 1/100 of the MIC value for the isogenic susceptible *E. coli* strain (Zhang et al., 2019).
- After 12 passages at 1/8th of the MIC of a synergistic combination of florfenicol and thiamphenicol, there was no apparent development of resistance for either antimicrobial independently or in combination, with the only changes noted being a doubling in MIC for the combination exposure in the susceptible *Actinobacillus pleuropneumoniae* strain and for thiamphenicol exposure in the resistant *A. pleuropneumoniae* strain after the 9th passage (Rattanapanadda et al., 2019).

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

- Exposure of MDR *S*. Typhimurium isolates to sub-inhibitory concentrations of florfenicol (16 μg/mL) for 30 min during early-log phase resulted in multiple genes associated with attachment and virulence genes located within the *Salmonella* pathogenicity islands being significantly up-regulated. Swimming and swarming motility were decreased due to antimicrobial exposure and florfenicol enhanced the cellular invasion of one isolate. Many of these genes may only be fully expressed *in-vivo* (Holman et al., 2018). However, in another study three clinical bovine *S*. Typhimurium DT104 isolates were exposed to sub-inhibitory concentrations of florfenicol (1 and 5 μg/mL) for 30 min. HEp-2 cellular invasion assays, as well as expression analyses of invasion-

related genes, suggested that the invasiveness of the isolates was not noticeably enhanced after exposure to low levels of florfenicol (Brunelle, 2011).

- The effect of sub-inhibitory concentrations of florfenicol on adherence properties of susceptible and resistant *Staphylococcus aureus* strains was investigated *in vitro*. The susceptible *S. aureus* strain Newman and its resistant derivative carrying the resistance plasmid pSCFS1 from *S. sciuri* were incubated in the presence of 2 μg/mL florfenicol, and the resistant strain was also exposed to 64 μg/mL florfenicol. When grown in the presence of half the strain-specific MIC of florfenicol, both isolates showed significantly increased adherence to HEp-2 cells and to fibronectin-coated microtitre plates (Blickwede et al., 2004).

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 amphenicols under assessment for different animal species followed a two-step approach as described below.

First, the minimal selective concentration (MSC) for a strain of *E. coli* (*E. coli* MG1655/pSD11), which was determined by analysing and comparing the growth rates of susceptible and resistant variants at different sub-MICs of florfenicol was used This was 1/100 of the MIC value of the isogenic susceptible *E. coli* strain (MIC_{test} = 4.25 mg/L and MSC_{test} = 0.0425 mg/L (Zhang et al., 2019) (Table 1).

The predicted minimal selective concentration (PMSC) calculated for florfenicol, using the lowest MIC value available in the EUCAST MIC distribution database (MIC_{lowest}), divided by the MIC_{test}/MSC_{test} factor (as described in 2.2.1.3.1.2 of the Scientific Opinion Part 1; see also the Virtual Issue), was 0.00125 mg/L (Table 1).

No MSC data for thiamphenicol were available.

Table 1: Calculation of the florfenicol and thiamphenicol 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 ($MIC_{lowest}/MIC_{test}/MSC_{test}$)
Florfenicol	4.25	0,0425	100	0.125	0.00125
Thiamphenicol	NA	NA	NA	NA	NA

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

From the PMSC for florfenicol, the FARSC ($FARSC_{intestine}$ and $FARSC_{rumen}$) corresponding to the maximal concentrations in feed was 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 florfenicol:

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

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

With daily faeces being the daily fresh faecal output in kg, I the inactive fraction, F the fraction available, GE 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 intake expressed in kg.

Due to the lack of PMSC for thiamphenicol, FARSC could not be calculated. If PMSC was available, the same procedure would be applied.

Florfenicol

The values of *F*, *I* and *GE* extracted from literature for the calculations of FARSC are summarised in Table 2. There is no value for the bioavailability in adult ruminants. The first set of values (scenario 1) corresponds to the average of published values while scenario 2 corresponds to scenario that would lead to lower FARSC and scenario 3 to the scenario that would lead to higher FARSC. The lowest FARSC (scenario 2) were obtained with lowest published values of *I* (lower inactivation of the drug resulting in greater activity against bacteria), lowest published values of *F* (lower absorption resulting in more drug in the intestines) and predicted highest value of *GE* (greater elimination into the intestines resulting in more drug within the intestines). The estimated FARSC values obtained with these three different sets of values/scenarios for the parameters are shown in Table 3.

Table 2: Predicted minimal selective concentration (PMSC) and pharmacokinetic (PK) values used for the calculation of the Feed Antimicrobial Resistance Selection Concentration ($FARSC_{intestine}$) of florfenicol for the different animal species

Florfenicol data	Scenario #1	Scenario #2	Scenario #3
PMSC (mg/L)		0.00125	
Inactive fraction (*I*)	0	0	0
Bioavailability (*F*) calves	0.86	0.4	0.89
Bioavailability (*F*) pig	0.99[(a)]	0.99[(a)]	–
Bioavailability (*F*) poultry	0.55	0.55	0.96
Bioavailability (*F*) horse	0.83	0.83	–
Bioavailability (*F*) rabbit	0.76	0.76	–
Bioavailability (*F*) salmon	0.97	0.97	0.99[(a)]
Gastrointestinal elimination (*GE*)	0	0.6	0

PMSC: Predicted minimal selective concentration (PMSC). 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. Gastrointestinal elimination (*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 $(1 - F + F \times GE)$, thus $(1 - F)$ in Scenarios 1 and 3.

(a): No calculations were done with the value of $F = 1$ because in this case, the antimicrobial would be totally absorbed, and the gut bacteria would not be exposed to the antimicrobial.

Table 3: The Feed Antimicrobial Resistance Selection Concentration of florfenicol corresponding to the maximum concentration of florfenicol residues in non-target feed that would not develop resistance in the large intestine bacteria ($FARSC_{intestine}$)

Animal category[(a)]	Body weight (kg)[(a)]	Daily Feed Intake (kg DM/animal per day)[(a)]	Daily output of fresh faeces (kg FM/animal per day)[(b)]	FARSC ($\times 10^{-3}$ mg drug/kg feed) Scenario 1	FARSC ($\times 10^{-3}$ mg drug/kg feed) Scenario 2	FARSC ($\times 10^{-3}$ mg drug/kg feed) Scenario 3
Sow lactating	175	5.28	7.7	**182.29**	3.02	–
Piglet (weaned)	20	0.88	0.88	125.00	2.07	–
Pig for fattening	60	2.2	2.64	150.00	2.48	–
Dairy cows	650	20	55.71	–	–	–
Veal calf (milk replacer)	100	1.89	2.36	11.15	1.86	14.19
Cattle for fattening	400	8	18.89	–	–	–
Goat (adult)	60	1.2	1.73	–	–	–
Sheep (adult)	60	1.2	1.47	–	–	–
Chicken for fattening	2	0.158	0.133	2.34	1.35	26.31
Laying hen	2	0.106	0.16	4.19	2.42	47.17

Animal category[(a)]	Body weight (kg)[(a)]	Daily Feed Intake (kg DM/animal per day)[(a)]	Daily output of fresh faeces (kg FM/animal per day)[(b)]	FARSC ($\times 10^{-3}$ mg drug/kg feed) Scenario 1	FARSC ($\times 10^{-3}$ mg drug/kg feed) Scenario 2	FARSC ($\times 10^{-3}$ mg drug/kg feed) Scenario 3
Turkey for fattening	3	0.176	0.109	1.72	0.99	19.35
Horse	400	8	8.33	7.66	1.95	–
Rabbit	2	0.1	0.053	2.76	**0.95**	–
Salmon	0.12	0.0021	0.00238	47.22	2.31	141.67

DM: dry matter: FM: faecal matter; FARSC: Feed Antimicrobial Resistance Selection Concentration.
(a): EFSA FEEDAP Panel (2017), as indicated in Section 2.1.1.3 of the Scientific Opinion Part 1.
(b): Estimated data, obtained as indicated in Section 2.1.1.3.1 of the Scientific Opinion Part 1.

The values of $FARSC_{intestine}$, for the species with available data, ranged in the first scenario using averaged published values from 1.72 $\times$ 10^{-3} mg/kg feed in turkeys for fattening to 182.29 $\times$ 10^{-3} mg/kg feed in lactating sows. From other simulations (scenario 2 and scenario 3) made with a wider range of values for the data used in the calculation, the $FARSC_{intestine}$ could range from 0.99 to 19.35 $\times$ 10^{-3} mg/kg feed in turkeys for fattening, from 0.95 to 2.76 $\times$ 10^{-3} mg/kg feed in rabbits and from 3.02 to 182.3 $\times$ 10^{-3} mg/kg feed in lactating sows. In general, for the different animal species, it ranged from 0.95 to 182.29 $\times$ 10^{-3} mg/kg feed.

For the estimation of FARSC of florfenicol in rumen ($FARSC_{rumen}$), the drug was considered as active in the rumen and I was set to 0. The estimated $FARSC_{rumen}$ values are reported in Table 4.

Table 4: The Feed Antimicrobial Resistance Selection Concentration ($FARSC_{rumen}$) of florfenicol corresponding to the maximum concentration of florfenicol residues in non-target feed that would not develop resistance in the rumen bacteria

Animal category[(a)]	Body weight (kg)[(a)]	Daily Feed Intake (kg DM/animal per day)[(a)]	Volume of rumen content (L)[(b)]	$FARSC_{rumen}$ ($\times 10^{-3}$ mg drug/kg feed)
Dairy cows	650	20	90–180	**5.63**–11.25
Cattle for fattening	400	8	60–120	9.38–**18.75**
Sheep/Goat	60	1.2	9–18	9.38–**18.75**

DM: dry matter; FARSC: Feed Antimicrobial Resistance Selection Concentration.
(a): EFSA FEEDAP Panel (2017), as indicated in Section 2.1.1.3 of the Scientific Opinion Part 1.
(b): Source of data indicated in Section 2.1.1.3 of the Scientific Opinion Part 1.

The values of $FARSC_{rumen}$ ranged, for the different species, from 5.63 to 18.75 $\times$ 10^{-3} mg/kg feed.

Thiamphenicol

The bioavailability of thiamphenicol after oral administration is around 60% in veal calves and pre-ruminant sheep but only of 30% in adult sheep. The bioavailability is higher than 70% in fasted birds. There is no value for the bioavailability in fed pigs or poultry.

No quantitative information is available on the fate of thiamphenicol such as metabolism, or excretion as the parent drug in faeces.

The values of *F, I* and *GE* extracted from literature for the calculations of FARSC are summarised in Table 5.

Table 5: Pharmacokinetic (PK) values used for the calculation of Feed Antimicrobial Resistance Selection Concentration (FARSC) of thiamphenicol for the different animal species

Thiamphenicol data	Scenario #1
Inactive fraction (*I*)	NA
Bioavailability (*F*) pre-weaned (young) ruminants	0.6
Bioavailability (*F*) adult ruminants	0.3
Bioavailability (*F*) poultry	0.7
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. Gastrointestinal elimination (*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 thiamphenicol was not possible.

3.2.1. Associated data gaps and uncertainties

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

i) MSC data: no data for MSC are available for thiamphenicol. MSC is available for florfenicol for *E. coli* (Zhang et al., 2019).
ii) MIC data: no data available in EUCAST for thiamphenicol.
iii) Impact of complexity on determined MSCs: no data determining the microbial community effect on the MSC of florfenicol is available.
iv) Bioavailability: for florfenicol, no data were found for adult ruminants. For other species, the value for bioavailability was extracted from only one or two studies and the selected values can therefore be inaccurate. Moreover, the effect of feed on the bioavailability is uncertain for most species. For thiamphenicol, there is no value for fed animals except for ruminants.
v) Fraction eliminated in gut: several studies suggest some elimination of florfenicol and of some of its metabolites in the gut. However, there are no available quantitative data. An absence of elimination (*GE* = 0) and an elimination of 60% of the absorbed antimicrobial (*GE* = 0.6) by this process were both considered in the calculation of FARSC.
vi) Inactive fraction: No study describing the possibility of inactivation of florfenicol in large intestines or in the rumen was found. Florfenicol was thus considered as fully active in large intestines and in the rumen.

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

3.2.2. Concluding remarks

The FARSC for **florfenicol** (for large intestine and/or rumen in the case of adult ruminants after weaning) ranges from 0.95 to 182.29×10^{-3} mg/kg feed. This large range is the consequence of different bioavailability values between species and of the uncertainty on the extent of gastrointestinal elimination after absorption.

–[3.02–182.29] $\times 10^{-3}$ mg/kg feed for lactating sows
–[2.07–125.00] $\times 10^{-3}$ mg/kg feed for piglets
–[2.48–150.00] $\times 10^{-3}$ mg/kg feed for pigs for fattening
–[1.86–14.19] $\times 10^{-3}$ mg/kg feed for veal calves
–[5.63–11.25] $\times 10^{-3}$ mg/kg feed for dairy cows ($FARSC_{rumen}$, no $FARSC_{intestine}$ was determined)
–[9.38–18.75] $\times 10^{-3}$ mg/kg feed for cattle for fattening, as well as for adult goats and sheep ($FARSC_{rumen}$, no $FARSC_{intestine}$ was determined)
–[1.35–26.31] $\times 10^{-3}$ mg/kg feed for chickens for fattening
–[2.42–47.17] $\times 10^{-3}$ mg/kg feed for laying hens

–[0.99–19.35] × 10^{-3} mg/kg feed for turkeys for fattening
–[1.95–7.66] × 0^{-3} mg/kg feed for horses
–[0.95–2.76] × 10^{-3} mg/kg feed for rabbits
–[2.31–141.67] × 10^{-3} mg/kg feed for salmon

The values for dairy cows, cattle for fattening, sheep and goats only correspond to $FARSC_{rumen}$, because the absence of data on bioavailability for ruminants after weaning prevents the calculation of FARSC corresponding to the maximum concentration of residues in feed that would not develop resistance in the large intestines.

The probability that florfenicol concentrations below the lowest FARSC value for an animal species will confer any enrichment of, and/or selection for, resistant bacteria in the intestine and/or rumen is estimated to be 1–5% (extremely unlikely).

Due to the lack of data on the parameters required to calculate the FARSC for **thiamphenicol**, 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. Florfenicol

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 (see also the Virtual Issue), resulted in 529 papers mentioning florfenicol 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 florfenicol.[4] After removing the reports not matching the eligibility criteria, 20 publications were identified.

3.3.1.2. Evaluation of the studies

The 20 publications identified in the literature search were appraised for suitability for the assessment of the effects of florfenicol 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; see also the Virtual Issue).[5] A total of 13 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 B.1 (Table B.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 florfenicol on growth performance and yield

Seven publications were considered suitable for the assessment of the effects of florfenicol 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 (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 florfenicol used – either unspecified or as any specific commercial preparation – and the concentration(s) applied

[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, other); crustaceans; other animal species.

[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)'.

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 fish

3.3.1.3.1.1. Tilapia

Ferreira et al. (2019) studied the effect of florfenicol added in the water or feed on the performance of tilapia. A total of 390 male Nile tilapia (*Oreochromis niloticus*) juveniles weighting around 1.05 g and with a length of 4 cm, were stoked to 25 aquariums and randomly distributed into three treatments with five replicates (aquarium) per treatment, each replicate containing 130 juveniles. The experimental treatments were the following: (1) basal diet and water without antimicrobial (control), (2) basal diet and antimicrobial supplemented in water (2 mg florfenicol/L) and (3) basal diet supplemented with florfenicol (10 mg/kg feed). Diets were offered *ad libitum* three times a day (8, 12 and 16 h). The study lasted 56 days; the last day fish were weighed and measured, and the following parameters were calculated: biomass (sum of the weight of all fish in the aquarium), the survival and Fulton's condition factor as (weight $\times$ standard length^{-3}) $\times$ 100. Forty minutes after the last biometry all fish were submitted to acute stress and were exposed to air for 12 minutes and then placed in their experimental units. Survival rate and efficacy of the antimicrobial were evaluated by collecting blood for further analysis of cortisol 24 hours' post-stress. Experimental fish were killed and used to determine body chemical composition. The addition of florfenicol to the diet had a positive effect on the feed conversion rate (F:G) (1.64 vs 1.69), feed intake (FI) (57.1 vs 40.8 g) and the survival rate (94.3% vs 87.1%). When florfenicol was added to water, an increase of F:G (from 1.69 to 1.84) was observed. No effect of treatment on standard length, weight gain, biomass, Fulton's condition factor and specific growth rate of tilapia was observed. The 100 % of Nile tilapia juveniles survived to the induced stress, and no differences in plasma cortisol were observed during treatment. Dietary inclusion of 10 mg florfenicol/kg feed promotes growth and survival in Nile tilapia.

In the study of Gaikowski et al. (2013), the safety of florfenicol (Aquaflor® (50% w/w) florfenicol) for tilapia (pure Nile and hybrid tilapia) was assessed following the oral administration of 0, 750, 2,250 and 3,750 mg/kg feed, for 20 days. The study was conducted in 12 replicate tanks (3 per treatment, with 20 fish per tank). The experiment started at 40-days of age of the tilapia (weighing 48 g approximately) after an acclimation period of eight days. Mortality, behavioural changes, feed consumption, body size, gross and microscopic lesions were determined. The gross and microscopic pathological findings were tabulated to determine the frequency and severity of changes. No effect of florfenicol on mortality or morbidity of fish was reported. Feed consumption decreased at the mid and the high concentrations of florfenicol – the groups consumed only 62.5% and 55.3% of the feed offered, respectively. There were dose-dependent reductions of weight gain in the florfenicol-supplemented groups (29.6, 20.7 and 17.6 g, respectively) compared to the control diet (34.5). The study revealed that with increasing concentrations of florfenicol the following effects were observed: increased severity of lamellar epithelial hyperplasia, increased incidence of lamellar adhesions, increased glycogen-type hepatocellular vacuolation in the liver, decreased lymphocytes, increased blast cells, increased individual cell necrosis in the anterior kidney, and tubular epithelial degeneration and mineralisation in the posterior kidney with increasing concentrations of florfenicol. Adverse effects were observed on performance parameters of tilapia at the tested concentrations of 750, 2,250 and 3,750 mg florfenicol/kg feed.

In a third study in tilapia, the effects of florfenicol on the productivity, intestinal bacteria community and non-specific immunity of 192 hybrid tilapia (*Oreochromis niloticus* ♀ $\times$ *Oreochromis aureus* ♂) were investigated (He et al., 2011). A 16-week feeding trial was conducted in a recirculating aquaculture system. Two were the relevant treatments: a control and a treatment consisting of florfenicol (99.8 % purity supplied by Zhejiang, Hisoar Pharmaceutical Co. Ltd., Taizhou, Zheijang, China) supplemented at 20 mg/kg for 16 weeks. For each treatment, four replicates (tanks) were used, each containing 12 fish. Initial body weight (BW) was 46.9 g. Juvenile hybrid tilapias were acclimatised for two weeks in an indoor recirculating system prior to start the experiment. After one day of fasting, fish were randomly distributed into 12 tanks. BW gain, average daily feed intake (ADFI) and gain to feed ratio (G:F) were determined. After 16 weeks of feeding, five fish per tank (20 fish per treatment) were randomly collected and used for the analysis of intestinal microbiota and blood samples to study non-specific immunity indexes. Dietary florfenicol, compared to control, improved BW gain (301–344%) and G:F (from 0.65 to 0.75) while no effect on ADFI or survival was observed. Dietary florfenicol also improved the concentration of the serum complement component. However, a

reduction in the bacterial diversity was observed in the intestinal microbiota of fish fed the diet supplemented with florfenicol. Dietary florfenicol supplementation at the concentration of 20 mg/kg feed showed positive effects on growth performance and feed efficacy in tilapia.

3.3.1.3.1.2. Channel catfish

Gaikowski et al. (2003) assessed the safety of florfenicol (Aquaflor feed premix containing florfenicol 50% w/w) in channel catfish (*Ictalurus punctatus*). A total 240 ten-months-old fish (15–35 g) were used to determine the safety of florfenicol at concentrations of 0, 500, 1,500 or 2,500 mg/kg feed for 20 consecutive days with an acclimatisation period of 14 days. The parameters evaluated included daily mortality, behavioural evaluation (appetite, distribution, flight/fright response), initial and terminal weight and gross and microscopic pathology. The supplementation with up to 2,500 mg florfenicol/kg feed did not show any effect on growth parameters of channel catfish, although there was a negative effect on the feed consumption at the mid and high concentrations (1,500 and 2,500 mg/kg feed). Overall, the inclusion of 1,500 or 2,500 mg florfenicol/kg feed showed adverse effects on the growth-promoting parameters of channel catfish.

Gaunt et al. (2003) (Experiment 1) evaluated the safety of florfenicol for channel catfish. A total of 400 five-month-old channel catfish (*I. puncatus*) were used in the study (BW at start: 47.9–66.5 g). After 6 days of acclimatisation, the fish were distributed between treatments. Four tanks with 20 fish/tank were assigned to one of the five treatments in which florfenicol (Florfenicol Aquaculture Premix (50% Type A Medicated Article), provided by Schering Plough, Animal Health Corporation, Union, New Jersey) was given at concentrations of 0, 400, 800, 1,600 or 4,000 mg/kg feed for 10 days. Fish were observed daily for mortality, signs of toxicity and feeding behaviour. Any fish that died during the study were necropsied and processed for histological examination. All surviving fish were removed from the aquaria (day 11), counted, weighed as a group, euthanaised, necropsied and processed for histological examination. No morbidity or mortality was observed during the study. No differences in weight gain were observed between treatment groups. Palatability data were nearly identical for all treatment groups (data not statistically analysed). Only 6 of the 400 fish were found to have gross lesions during the post-mortem examination. There were no differences in the histopathology of the organs between untreated and treated fish. Dietary florfenicol supplementation at 400, 800, 1,600 and 4,000 mg/kg feed did not show growth-promoting effects in channel catfish.

3.3.1.3.1.3. Yellow perch

Bowker et al. (2013) studied the safety of florfenicol (Aquaflor aquaculture feed premix containing florfenicol 50% w/w; supplied by Merck Animal Health, Summit, New Jersey) for yellow perch (*Perca flavescens*). A total of 180 ten-month-old fish was used; the antimicrobial was administered in feed at 0, 1,500, 4,500 and 7,500 mg/kg feed (calculated from the levels provided in the publication of 0, 15, 45 and 75 mg/kg BW per day; fish were fed at 1 % BW/day) during 20 consecutive days. Yellow Perch fingerlings (7.6 $\pm$ 1.6 cm total length and 5.0 $\pm$ 3.4 g of BW) were stocked into flow-through test tanks (15 fish per tank) and treatments were randomly assigned to tanks in triplicate. Three additional non-study tanks were also stocked with 15 fish each to monitor growth and make weekly adjustments to the amounts of feed administered to test tanks. Feed amounts were also adjusted daily to account for mortality. At the end of the phase, all live fish were collected, measured and weighted, sedated, euthanised and necropsied. Fish were observed daily for mortality and general behaviour. Feeding behaviour was assessed daily by feed consumption and fish aggressivity. No differences in mortality between groups were observed. Fish health and histology assessments revealed no signs or lesions associated with toxicity of florfenicol. No differences were detected on average total length or BW for the four exposure groups. Throughout the study, general fish behaviour was characterised as normal and fish consumed virtually all feed offered. Dietary florfenicol supplementation at 1,500, 4,500 and 7,500 mg/kg feed did not have a growth-promoting effect in yellow perch.

3.3.1.3.2. Study in shrimps

Liu et al. (2014) investigated the effects of feeding white shrimp (*Litopenaeus vannamei)* of initial BW of 10 g diets supplemented with florfenicol or a probiotic (bacterial isolate S12, three treatments) on the productive performance and immune parameters. Five treatments were used, including four replicate tanks per treatment and 40 shrimps per tank. Two were the relevant treatments: a control (no florfenicol or probiotic supplementation) and a treatment consisting of florfenicol (supplied by Keyang Biotechnology, Wuhan, China) supplementation at 3,000 mg/kg feed. The study lasted eight weeks. Growth performance, survival rate and body composition were determined at the end of the

feeding period. The supplementation with 3,000 mg florfenicol/kg feed increased weight gain rate (from 1,342% to 1,434%) and specific growth rate (from 4.77% to 4.87%), and reduced F:G (from 1.12 to 1.10) in comparison with the control diet. Regarding shrimp body composition, the florfenicol-supplemented diet increased the crude lipid content (from 6.44% to 7.20%) and reduced the moisture content (from 76.6% to 74.6%). The study showed a growth-promoting effect of florfenicol in shrimps at 3,000 mg/kg feed.

3.3.1.4. Discussion

From the studies examined, the test item has been described as florfenicol, supplied either from (i) a florfenicol commercial preparation (six studies) or (ii) via an unspecified source (one study). Therefore, an uncertainty on the exact product used/concentration applied has been identified.

A detailed analysis of the uncertainties for florfenicol is included in Appendix A (Table A.2) of this document, and the Section 3.3 of the Scientific Opinion Part 1 (see also the Virtual Issue).

The seven studies considered as suitable for the assessment covered exclusively aquatic animal species: three studies in tilapia (*Oreochromis* spp.), two in channel catfish (*I. punctatus*), one in yellow perch (*P. flavescens*) and one in shrimps (*L. vannamei*)).

In two studies in tilapia, dietary florfenicol supplementation at 10–20 mg/kg feed improved growth performance of the fish (Ferreira et al., 2019, 10 mg florfenicol/kg feed; He et al., 2011, 20 mg florfenicol/kg feed). Another study reported that dietary florfenicol supplementation adversely affected performance of tilapia fingerlings (Gaikowski et al., 2013, 750, 2,250 and 3,750 mg florfenicol/kg feed).

In channel catfish, dietary florfenicol supplementation from 400 to 4,000 mg/kg feed did not affect performance (Gaunt et al., 2003; 400, 800, 1,600 and 4,000 mg florfenicol/kg feed). Another study in Channel catfish showed that dietary florfenicol supplementation at 1,500 and 2,500 mg florfenicol/kg feed adversely affected performance (Gaikowski et al., 2003), although there was no effect at 500 mg florfenicol/kg feed.

In one study in yellow perch, dietary florfenicol supplementation at 15–75 mg/kg bw per day did not affect performance (Bowker et al., 2013), with tested levels of florfenicol being considered to be very high (15–75 mg/kg bw per day).

In shrimps, dietary florfenicol supplementation at 3,000 mg/kg feed improved growth performance (Liu et al., 2014), with the tested level of florfenicol being considered to be very high (3,000 mg/kg feed).

3.3.1.5. Concluding remarks

It is judged 33–66% certain ('about as likely as not') that florfenicol has growth-promoting/increase yield effects in tilapia at concentrations ranging from 10 to 20 mg/kg complete feed (two studies) and in shrimp at a concentration of 3,000 mg/kg complete feed (one study). It is judged 33–66% certain ('about as likely as not') that florfenicol has negative effects of on the performance of tilapia at concentrations ranging from 750 to 3,750 mg/kg complete feed (one study). Likewise, it is judged 33–66% certain ('about as likely as not') that concentrations of 1,500 and 2,500 mg florfenicol/kg complete feed adversely affect the productive performance in channel catfish (one study).

No data are available in the scientific literature showing effects of florfenicol on growth promotion/ increase yield when added (i) to fish feed at concentrations below 10 mg/kg, (ii) to shrimp feed at concentrations below 3,000 mg/kg, or (iii) to feed of any other food-producing animal species or categories.

3.3.2. Thiamphenicol

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 (see also the Virtual Issue), resulted in 183 papers mentioning thiamphenicol 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 thiamphenicol.[4] After removing the reports not matching the eligibility criteria, one publication was identified.

3.3.2.2. Evaluation of the studies

The publication resulted as the outcome of the literature search was appraised for suitability for the assessment of the effects of thiamphenicol on growth or yield of food-producing animals; this appraisal

was performed by checking the study against a series of pre-defined exclusion criteria (Section 2.2.2.2.1 of the Scientific Opinion Part 1; see also the Virtual Issue).[5] The publication of Ueda et al. (1995) was 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 publication and its shortcomings are presented in the Appendix B.2 (Table B.2).

3.3.2.3. Concluding remark

Owing to the lack of suitable data, levels of thiamphenicol 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 florfenicol and thiamphenicol in non-target feed below which there would not be emergence of, and/or selection for, resistance in the large intestines/ rumen?

With regard to florfenicol:

- The Feed Antimicrobial Resistance Selection Concentration (FARSC, for large intestine and/or rumen in the case of adult ruminants after weaning) corresponding to the concentrations of florfenicol 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 ranges, for the different species, from 0.95 to 182.29 $\times$ 10^{-3} mg/kg. This large range is the consequence of different bioavailability values between species and of the uncertainty on the extent of gastrointestinal elimination after absorption.
- For each animal species, the FARSC obtained ranged:
  - [3.02–182.29] $\times$ 10^{-3} mg/kg feed for lactating sows
  - [2.07–125.00] $\times$ 10^{-3} mg/kg feed for piglets
  - [2.48–150.00] $\times$ 10^{-3} mg/kg feed for pigs for fattening
  - [1.86–14.19] $\times$ 10^{-3} mg/kg feed for veal calves
  - [5.63–11.25] $\times$ 10^{-3} mg/kg feed for dairy cows ($FARSC_{rumen}$, no $FARSC_{intestine}$ was determined)
  - [9.38–18.75] $\times$ 10^{-3} mg/kg feed for cattle for fattening, adult goats and sheep ($FARSC_{rumen}$, no $FARSC_{intestine}$ was determined).
  - [1.35–26.31] $\times$ 10^{-3} mg/kg feed for chickens for fattening
  - [2.42–47.17] $\times$ 10^{-3} mg/kg feed for laying hens
  - [0.99–19.35] $\times$ 10^{-3} mg/kg feed for turkeys for fattening
  - [1.95–7.66] $\times$ 10^{-3} mg/kg feed for horses
  - [0.95–2.76] $\times$ 10^{-3} mg/kg feed for rabbits
  - [2.31–141.67] $\times$ 10^{-3} mg/kg feed for salmon
- The probability that florfenicol concentrations below the lowest FARSC value for an animal species will confer any enrichment of, and/or selection for, resistant bacteria in the intestine and/or rumen is estimated to be 1–5% (extremely unlikely).

With regard to thiamphenicol:

- Due to the lack of data on the parameters required to calculate the FARSC for **thiamphenicol**, it is not possible to conclude the assessment 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 florfenicol and thiamphenicol in feed of food-producing animals that have an effect in terms of growth promotion/increased yield?

With regard to florfenicol:

- It is judged 33–66% certain ('about as likely as not') that florfenicol:
  - has growth-promoting/increase yield effects in tilapia at concentrations ranging from 10 to 20 mg/kg complete feed (two studies) and in shrimp at a concentration of 3,000 mg/kg complete feed (one study).
  - has negative effects on the performance of tilapia at concentrations ranging from 750 to 3,750 mg/kg complete feed (one study).
  - adversely affects the productive performance of Channel catfish at concentrations of 1,500 and 2,500 mg florfenicol/kg complete feed (one study).
- No data are available in the scientific literature showing effects of florfenicol on growth promotion/increased yield when added (i) to fish feed at concentrations below 10 mg/kg, (ii) to shrimp feed at concentrations below 3,000 mg/kg, or (iii) to feed of any other food-producing animal species or categories.

With regard to thiamphenicol:

- Owing to the lack of suitable data, levels of thiamphenicol 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 perform further studies to supply more diverse and complete data to reduce uncertainties around the calculation of the FARSC for florfenicol.

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

References

Abdennebi EH, Khales N, Sawchuk RJ and Stowe CM, 1994. Thiamphenicol pharmacokinetics in sheep. Journal of Veterinary Pharmacology and Therapeutics, 17, 12–16. https://doi.org/10.1111/j.1365-2885.1994.tb00515.x

Afifi NA and Abo el-Sooud KA, 1997. Tissue concentrations and pharmacokinetics of florfenicol in broiler chickens. British Poultry Science, 38, 425–428. https://doi.org/10.1080/00071669708418013

Anadón A, Martínez MA, Martínez M, Ríos A, Caballero V, Ares I and Martínez-Larrañaga MR, 2008. Plasma and tissue depletion of florfenicol and florfenicol-amine in chickens. Journal of Agriculture and Food Chemistry, 56, 11049–11056. https://doi.org/10.1021/jf802138y

Aubin J, Gatesoupe FJ, Labbe L and Lebrun L, 2005. Trial of probiotics to prevent the vertebral column compression syndrome in rainbow trout (*Oncorhynchus mykiss* Walbaum). Aquaculture Research, 36, 758–767. https://doi.org/10.1111/j.1365-2109.2005.01280.x

Bengtsson-Palme J and Larsson DGJ, 2016. Concentrations of antibiotics predicted to select for resistant bacteria: proposed limits for environmental regulation. Environment International, 86, 140–149. https://doi.org/10.1016/j.envint.2015.10.015

Berge AC, Epperson WB and Pritchard RH, 2005. Assessing the effect of a single dose florfenicol treatment in feedlot cattle on the antimicrobial resistance patterns in faecal *Escherichia coli*. Veterinary Research, 36, 723–734. https://doi.org/10.1051/vetres:2005027

Blickwede M, Valentin-Weigand P and Schwarz S, 2004. Subinhibitory concentrations of florfenicol enhance the adherence of florfenicol-susceptible and florfenicol-resistant *Staphylococcus aureus*. Journal of Antimicrobial Chemotherapy, 54, 286–288. https://doi.org/10.1093/jac/dkh273

Bowker JD, Ostland VE, Carty D and Bowman MP, 2010. Effectiveness of aquaflor (50% florfenicol) to control mortality associated with *Streptococcus iniae* in freshwater-reared subadult sunshine bass. Journal of Aquatic Animal Health, 22, 254–265. https://doi.org/10.1577/H09-010.1

Bowker JD, Carty D and Bowman MP, 2013. The safety of Aquaflor (50% florfenicol) administered in feed to fingerling yellow perch. North American Journal of Aquaculture, 75, 517–523. https://doi.org/10.1080/15222055.2013.815676

Braibant M, Chevalier J, Chaslus-Dancla E, Pagès JM and Cloeckaert A, 2005. Structural and functional study of the phenicol-specific efflux pump FloR belonging to the major facilitator superfamily. Antimicrobial Agents and Chemotherapy, 49, 2965–2971. https://doi.org/10.1128/aac.49.7.2965-2971.2005

Brunelle BW, 2011. *Salmonella enterica* serovar typhimurium DT104 invasion is not enhanced by sub-inhibitory concentrations of the antibiotic florfenicol. Journal of Veterinary Science and Technology, v. 2:2011 v.2012 no.2011. https://doi.org/10.4172/2157-7579.1000104

Chae W-S, Yoo C-Y, Tutkun L, Kim S and Lee H-J, 2018. Toxicity of a combination of florfrenicol and acetaminophen in pigs administered at overdose. Journal of the Preventive Veterinary Medicine, 42, 182–185. https://doi.org/10.13041/jpvm.2018.42.4.182

Ciprián A, Palacios JM, Quintanar D, Batista L, Colmenares G, Cruz T, Romero A, Schnitzlein W and Mendoza S, 2012. Florfenicol feed supplemented decrease the clinical effects of *Mycoplasma hyopneumoniae* experimental infection in swine in México. Research in Veterinary Science, 92, 191–196. https://doi.org/10.1016/j.rvsc.2011.01.010

De Smet J, Boyen F, Croubels S, Rasschaert G, Haesebrouck F, De Backer P and Devreese M, 2018. Similar gastro-intestinal exposure to florfenicol after oral or intramuscular administration in pigs, leading to resistance selection in commensal *Escherichia coli*. Front Pharmacol, 9, 1265. https://doi.org/10.3389/fphar.2018.01265

Doublet B, Carattoli A, Whichard JM, White DG, Baucheron S, Chaslus-Dancla E and Cloeckaert A, 2004. Plasmid-mediated florfenicol and ceftriaxone resistance encoded by the *floR* and *bla* $_{CMY-2}$ genes in *Salmonella enterica* serovars Typhimurium and Newport isolated in the United States. FEMS Microbiology Letters, 233, 301–305.

Dowling PM, 2013. Chloramphenicol, Thiamphenicol, and Florfenicol. In: Giguère S, Prescott JF and Dowling PM (eds.). Antimicrobial Therapy in Veterinary Medicine. pp. 269–277. https://doi.org/10.1002/9781118675014.ch16

Du Z, Wang M, Cui G, Zu X, Zhao Z and Xue Y, 2020. The prevalence of amphenicol resistance in *Escherichia coli* isolated from pigs in mainland China from 2000 to 2018: a systematic review and meta-analysis. PLoS ONE, 15. https://doi.org/10.1371/journal.pone.0228388

EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), Koutsoumanis K, Allende A, Alvarez-Ordóñez A, Bolton D, Bover-Cid S, Chemaly M, Davies R, De Cesare A, Herman L, Hilbert F, Lindqvist R, Nauta M, Ru G, Simmons M, Skandamis P, Suffredini E, Andersson DI, Bampidis V, Bengtsson-Palme J, Bouchard D, Ferran A, Kouba M, López Puente S, López-Alonso M, Nielsen SS, Pechová A, Petkova M, Girault S, Broglia A, Guerra B, Innocenti ML, Liébana E, López-Gálvez G, Manini P, Stella P and Peixe L, 2021a. Scientific opinion on the maximum levels of cross-contamination for 24 antimicrobial active substances in non-target feed. Part 1: Methodology, general data gaps and uncertainties. EFSA Journal 2021;19(10):6852, https://doi.org/10.2903/j.efsa.2021.6852

EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), Koutsoumanis K, Allende A, Alvarez-Ordóñez A, Bolton D, Bover-Cid S, Chemaly M, Davies R, De Cesare A, Herman L, Hilbert F, Lindqvist R, Nauta M, Ru G, Simmons M, Skandamis P, Suffredini E, Andersson DI, Bampidis V, Bengtsson-Palme J, Bouchard D, Ferran A, Kouba M, López Puente S, López-Alonso M, Nielsen SS, Pechová A, Petkova M, Girault S, Broglia A, Guerra B, Innocenti ML, Liébana E, López-Gálvez G, Manini P, Stella P and Peixe L, 2021b. Scientific opinion on the maximum levels of cross-contamination for 24 antimicrobial active substances in non-target feed. Part 2: Aminoglycosides/aminocyclitols: apramycin, paromomycin, neomycin and spectinomycin. EFSA Journal 2021;19(10):6853, https://doi.org/10.2903/j.efsa.2021.6853

EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), Koutsoumanis K, Allende A, Alvarez-Ordóñez A, Bolton D, Bover-Cid S, Chemaly M, Davies R, De Cesare A, Herman L, Hilbert F, Lindqvist R, Nauta M, Ru G, Simmons M, Skandamis P, Suffredini E, Andersson DI, Bampidis V, Bengtsson-Palme J, Bouchard D, Ferran A, Kouba M, López Puente S, López-Alonso M, Nielsen SS, Pechová A, Petkova M, Girault S, Broglia A, Guerra B, Innocenti ML, Liébana E, López-Gálvez G, Manini P, Stella P and Peixe L, 2021c. Scientific opinion on the maximum levels of cross-contamination for 24 antimicrobial active substances in non-target feed. Part 3: Amprolium. EFSA Journal 2021;19(10):6854, https://doi.org/10.2903/j.efsa.2021.6854

EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), Koutsoumanis K, Allende A, Alvarez-Ordóñez A, Bolton D, Bover-Cid S, Chemaly M, Davies R, De Cesare A, Herman L, Hilbert F, Lindqvist R, Nauta M, Ru G, Simmons M, Skandamis P, Suffredini E, Andersson DI, Bampidis V, Bengtsson-Palme J, Bouchard D, Ferran A, Kouba M, López Puente S, López-Alonso M, Nielsen SS, Pechová A, Petkova M, Girault S, Broglia A, Guerra B, Innocenti ML, Liébana E, López-Gálvez G, Manini P, Stella P and Peixe L, 2021d. Scientific opinion on the maximum levels of cross-contamination for 24 antimicrobial active substances in non-target feed. Part 4: β-Lactams: amoxicillin and penicillin V. EFSA Journal 2021;19(10):6855, https://doi.org/10.2903/j.efsa.2021.6855

EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), Koutsoumanis K, Allende A, Alvarez-Ordóñez A, Bolton D, Bover-Cid S, Chemaly M, Davies R, De Cesare A, Herman L, Hilbert F, Lindqvist R, Nauta M, Ru G, Simmons M, Skandamis P, Suffredini E, Andersson DI, Bampidis V, Bengtsson-Palme J, Bouchard D, Ferran A, Kouba M, López Puente S, López-Alonso M, Nielsen SS, Pechová A, Petkova M, Girault S, Broglia A, Guerra B, Innocenti ML, Liébana E, López-Gálvez G, Manini P, Stella P and Peixe L, 2021e. Scientific opinion on the maximum levels of cross-contamination for 24 antimicrobial active substances in non-target feed. Part 5: Lincosamides: lincomycin. EFSA Journal 2021;19(10):6856, https://doi.org/10.2903/j.efsa.2021.6856

EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), Koutsoumanis K, Allende A, Alvarez-Ordóñez A, Bolton D, Bover-Cid S, Chemaly M, Davies R, De Cesare A, Herman L, Hilbert F, Lindqvist R, Nauta M, Ru G, Simmons M, Skandamis P, Suffredini E, Andersson DI, Bampidis V, Bengtsson-Palme J, Bouchard D, Ferran A, Kouba M, López Puente S, López-Alonso M, Nielsen SS, Pechová A, Petkova M, Girault S, Broglia A, Guerra B, Innocenti ML, Liébana E, López-Gálvez G, Manini P, Stella P and Peixe L, 2021f. Scientific opinion on the maximum levels of cross-contamination for 24 antimicrobial active substances in non-target feed. Part 6: Macrolides: tilmicosin, tylosin and tylvalosin. EFSA Journal 2021;19(10):6858, https://doi.org/10.2903/j.efsa.2021.6858

EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), Koutsoumanis K, Allende A, Alvarez-Ordóñez A, Bolton D, Bover-Cid S, Chemaly M, Davies R, De Cesare A, Herman L, Hilbert F, Lindqvist R, Nauta M, Ru G, Simmons M, Skandamis P, Suffredini E, Andersson DI, Bampidis V, Bengtsson-Palme J, Bouchard D, Ferran A, Kouba M, López Puente S, López-Alonso M, Nielsen SS, Pechová A, Petkova M, Girault S, Broglia A, Guerra B, Innocenti ML, Liébana E, López-Gálvez G, Manini P, Stella P and Peixe L, 2021g. Scientific opinion on the maximum levels of cross-contamination for 24 antimicrobial active substances in non-target feed. Part 8: Pleuromutilins: tiamulin and valnemulin. EFSA Journal 2021;19(10):6860, https://doi.org/10.2903/j.efsa.2021.6860

EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), Koutsoumanis K, Allende A, Alvarez-Ordóñez A, Bolton D, Bover-Cid S, Chemaly M, Davies R, De Cesare A, Herman L, Hilbert F, Lindqvist R, Nauta M, Ru G, Simmons M, Skandamis P, Suffredini E, Andersson DI, Bampidis V, Bengtsson-Palme J, Bouchard D, Ferran A, Kouba M, López Puente S, López-Alonso M, Nielsen SS, Pechová A, Petkova M, Girault S, Broglia A, Guerra B, Innocenti ML, Liébana E, López-Gálvez G, Manini P, Stella P and Peixe L, 2021h. Scientific opinion on the maximum levels of cross-contamination for 24 antimicrobial active substances in non-target feed. Part 9: Polymixins: colistin. EFSA Journal 2021;19(10):6861, https://doi.org/10.2903/j.efsa.2021.6861

EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), Koutsoumanis K, Allende A, Alvarez-Ordóñez A, Bolton D, Bover-Cid S, Chemaly M, Davies R, De Cesare A, Herman L, Hilbert F, Lindqvist R, Nauta M, Ru G, Simmons M, Skandamis P, Suffredini E, Andersson DI, Bampidis V, Bengtsson-Palme J, Bouchard D, Ferran A, Kouba M, López Puente S, López-Alonso M, Nielsen SS, Pechová A, Petkova M, Girault S, Broglia A, Guerra B, Innocenti ML, Liébana E, López-Gálvez G, Manini P, Stella P and Peixe L, 2021i. Scientific opinion on the maximum levels of cross-contamination for 24 antimicrobial active substances in non-target feed. Part 10: Quinolones: flumequine and oxolinic acid. EFSA Journal 2021;19(10):6862, https://doi.org/10.2903/j.efsa.2021.6862

EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), Koutsoumanis K, Allende A, Alvarez-Ordóñez A, Bolton D, Bover-Cid S, Chemaly M, Davies R, De Cesare A, Herman L, Hilbert F, Lindqvist R, Nauta M, Ru G, Simmons M, Skandamis P, Suffredini E, Andersson DI, Bampidis V, Bengtsson-Palme J, Bouchard D, Ferran A, Kouba M, López Puente S, López-Alonso M, Nielsen SS, Pechová A, Petkova M, Girault S, Broglia A, Guerra B, Innocenti ML, Liébana E, López-Gálvez G, Manini P, Stella P and Peixe L, 2021j. Scientific opinion on the maximum levels of cross-contamination for 24 antimicrobial active substances in non-target feed. Part 11: Sulfonamides. EFSA Journal 2021;19(10):6863, https://doi.org/10.2903/j.efsa.2021.6863

EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), Koutsoumanis K, Allende A, Alvarez-Ordóñez A, Bolton D, Bover-Cid S, Chemaly M, Davies R, De Cesare A, Herman L, Hilbert F, Lindqvist R, Nauta M, Ru G, Simmons M, Skandamis P, Suffredini E, Andersson DI, Bampidis V, Bengtsson-Palme J, Bouchard D, Ferran A, Kouba M, López Puente S, López-Alonso M, Nielsen SS, Pechová A, Petkova M, Girault S, Broglia A, Guerra B, Innocenti ML, Liébana E, López-Gálvez G, Manini P, Stella P and Peixe L, 2021k. Scientific opinion on the maximum levels of cross-contamination for 24 antimicrobial active substances in non-target feed. Part 12: Tetracyclines: tetracycline, chlortetracycline, oxytetracycline and doxycycline. EFSA Journal 2021;19(10):6864, https://doi.org/10.2903/j.efsa.2021.6864

EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), Koutsoumanis K, Allende A, Alvarez-Ordóñez A, Bolton D, Bover-Cid S, Chemaly M, Davies R, De Cesare A, Herman L, Hilbert F, Lindqvist R, Nauta M, Ru G, Simmons M, Skandamis P, Suffredini E, Andersson DI, Bampidis V, Bengtsson-Palme J, Bouchard D, Ferran A, Kouba M, López Puente S, López-Alonso M, Nielsen SS, Pechová A, Petkova M, Girault S, Broglia A, Guerra B, Innocenti ML, Liébana E, López-Gálvez G, Manini P, Stella P and Peixe L, 2021l. Scientific opinion on the maximum levels of cross-contamination for 24 antimicrobial active substances in non-target feed. Part 13: Diaminopyrimidines: trimethoprim. EFSA Journal 2021;19(10):6865, https://doi.org/10.2903/j.efsa.2021.6865

EFSA FEEDAP Panel (anel on Additives and Products or Substances used in Animal Feed), Rychen G, Aquilina G, Azimonti G, Bampidis V, Bastos MdL, Bories G, Chesson A, Cocconcelli PS, Flachowsky G, Gropp J, Kolar B, Kouba M, López-Alonso M, López Puente S, Mantovani A, Mayo B, Ramos F, Saarela M, Villa RE, Wallace RJ, Wester P, Anguita M, Galobart J, Innocenti ML and Martino L, 2017. Guidance on the assessment of the safety of feed additives for the target species. EFSA Journal 2017;15:e05021. https://doi.org/10.2903/j.efsa.2017.5021

EMA/CVMP/CHMP (European Medicines Agency/Committee for Medicinal Products for Veterinary Use/Committee for Medicinal Products for Human Use), 2019. Categorisation of antibiotics in the European Union. Answer to the request from the European Commission for updating the scientific advice on the impact on public health and animal health of the use of antibiotics in animals. 12 December 2019. EMA/CVMP/CHMP/682198/2017. Available online: https://www.ema.europa.eu/en/documents/report/categorisation-antibiotics-european-union-answer-request-european-commission-updating-scientific_en.pdf

EMA/ESVAC (European Medicines Agency - European Surveillance of Veterinary Antimicrobial Consumption), 2020. Sales of veterinary antimicrobial agents in 31 European countries in 2018. EMA/24309/2020. Available online: https://www.ema.europa.eu/en/documents/report/sales-veterinary-antimicrobial-agents-31-european-countries-2018-trends-2010-2018-tenth-esvac-report_en.pdf

EMEA/CVMP (European Agency for the Evaluation of Medicinal Products - Committee for Veterinary Medicinal Products), 1999. Florfenicol (extension to chicken). Summary Report (3). EMEA/MRL/589/99-FINAL March 1999. Available online: https://www.ema.europa.eu/en/documents/mrl-report/florfenicol-extension-chicken-summary-report-3-committee-veterinary-medicinal-products_en.pdf

FAO (Food and Agriculture Organization of the United Nations), 1999. Thiamphenicol. Available online: http://www.fao.org/3/w4601e/w4601e0d.htm

Ferreira AL, Amorim MPS, Souza ER, Schorer M, Castro GHF and Pedreira MM, 2019. Probiotic, antibiotic and combinations in Nile tilapia juveniles culture. Anais da Academia Brasileira de Ciencias, 91. https://doi.org/10.1590/0001-3765201920180169

Fisch A and Bryskier A, 2005. Phenicols. In: Bryskier A (ed.). Antimicrobial Agents: Antibacterials and Antifungals. ASM Press, Washington, DC. pp. 925–929.

Fukui H, Fujihara Y and Kano T, 1987. *In vitro* and *in vivo* antibacterial activities of florfenicol, a new fluorinated analog of thiamphenicol, against fish pathogens. Fish Pathology, 22, 201–207.

Gaikowski MP, Wolf JC, Endris RG and Gingerich WH, 2003. Safety of aquaflor (florfenicol, 50% type a medicated article), administered in feed to channel catfish, *Ictalurus punctatus*. Toxicologic Pathology, 31, 689–697. https://doi.org/10.1080/01926230390241828

Gaikowski MP, Wolf JC, Schleis SM, Tuomari D and Endris RG, 2013. Safety of florfenicol administered in feed to tilapia (*Oreochromis* sp.). Toxicologic Pathology, 41, 639–652. https://doi.org/10.1177/0192623312463986

Gaunt P, Endris R, Khoo L, Leard AT, Jack S, Santucci T, Katz T, Radecki SV and Simmons R, 2003. Preliminary assessment of the tolerance and efficacy of florfenicol against *Edwardsiella ictaluri* administered in feed to channel catfish. Journal of Aquatic Animal Health, 15, 239–247. https://doi.org/10.1577/H03-022

Gutiérrez L, Vargas D, Ocampo L, Sumano H, Martinez R and Tapia G, 2011. Plasma concentrations resulting from florfenicol preparations given to pigs in their drinking water. Journal of Animal Science, 89, 2926–2931. https://doi.org/10.2527/jas.2010-3576

Han C, Wei Y, Cui Y, Geng Y, Bao Y and Shi W, 2020. Florfenicol induces oxidative stress and hepatocyte apoptosis in broilers via Nrf2 pathway. Ecotoxicology and Environmental Safety, 191. https://doi.org/10.1016/j.ecoenv.2020.110239

Haritova A, Lashev L and Pashov D, 2002. Pharmacokinetics of thiamphenicol in pigs. Journal of Veterinary Pharmacology and Therapeutics, 25, 464–466. https://doi.org/10.1046/j.1365-2885.2002.00439.x

He S, Zhou Z, Meng K, Zhao H, Yao B, Ringø E and Yoon I, 2011. Effects of dietary antibiotic growth promoter and *Saccharomyces cerevisiae* fermentation product on production, intestinal bacterial community, and nonspecific immunity of hybrid tilapia (*Oreochromis niloticus* female × *Oreochromis aureus* male). Journal of Animal Science, 89, 84–92. https://doi.org/10.2527/jas.2010-3032

Higuera-Llantén S, Vásquez-Ponce F, Barrientos-Espinoza B, Mardones FO, Marshall SH and Olivares-Pacheco J, 2018. Extended antibiotic treatment in salmon farms select multiresistant gut bacteria with a high prevalence of antibiotic resistance genes. PLoS ONE, 13. https://doi.org/10.1371/journal.pone.0203641

Holman DB, Bearson SMD, Bearson BL and Brunelle BW, 2018. Chlortetracycline and florfenicol induce expression of genes associated with pathogenicity in multidrug-resistant *Salmonella enterica* serovar Typhimurium. Gut Pathog, 10, 10. https://doi.org/10.1186/s13099-018-0236-y

Horsberg TE, Hoff KA and Nordmo R, 1996. Pharmacokinetics of florfenicol and its metabolite florfenicol amine in atlantic salmon. Journal of Aquatic Animal Health, 8, 292–301. https://doi.org/10.1577/1548-8667(1996)008<0292:POFAIM>2.3.CO;2

HPRA (Health Products Regulatory Agency), 2018. Flordofen. Licence_VPA10791-006-001_26092018162415. Available online: https://www.hpra.ie/img/uploaded/swedocuments/Licence_VPA10791-006-001_26092018162415.pdf

JECFA (Joint FAO/WHO Expert Committee on Food Additives), 2002. Summary of Evaluations Performed by the Joint FAO/WHO Expert Committee on Food Additives. Thiamphenicol. Available online: http://www.inchem.org/documents/jecfa/jeceval/jec_2265.htm.

Jiang HX, Zeng ZL, Chen ZL, Liu JJ and Fung KF, 2006. Pharmacokinetics of florfenicol in pigs following intravenous, intramuscular or oral administration and the effects of feed intake on oral dosing. Journal of Veterinary Pharmacology and Therapeutics, 29, 153–156. https://doi.org/10.1111/j.1365-2885.2006.00727.x

Kadlec K, Kehrenberg C and Schwarz S, 2007. Efflux-mediated resistance to florfenicol and/or chloramphenicol in *Bordetella bronchiseptica*: identification of a novel chloramphenicol exporter. Journal of Antimicrobial Chemotherapy, 59, 191–196. https://doi.org/10.1093/jac/dkl498

Kumar A, Patyal A and Panda AK, 2018. Sub-therapeutic use of antibiotics in animal feed and their potential impact on environmental and human health: a comprehensive review. Journal of Animal Feed Science and Technology, 6, 15–25. https://doi.org/10.21088/jafst.2321.1628.6118.3

Liu J, Fung KF, Chen Z, Zeng Z and Zhang J, 2003. Pharmacokinetics of florfenicol in healthy pigs and in pigs experimentally infected with *Actinobacillus pleuropneumoniae*. Antimicrobial Agents and Chemotherapy, 47, 820–823. https://doi.org/10.1128/aac.47.2.820-823.2003

Liu H, Li Z, Tan B, Lao Y, Duan Z, Sun W and Dong X, 2014. Isolation of a putative probiotic strain S12 and its effect on growth performance, non-specific immunity and disease-resistance of white shrimp, *Litopenaeus vannamei*. Fish & Shellfish Immunology, 41, 300–307. https://doi.org/10.1016/j.fsi.2014.08.028

Long KS, Poehlsgaard J, Kehrenberg C, Schwarz S and Vester B, 2006. The Cfr rRNA methyltransferase confers resistance to phenicols, lincosamides, oxazolidinones, pleuromutilins, and streptogramin a antibiotics. Antimicrobial Agents and Chemotherapy, 50, 2500–2505. https://doi.org/10.1128/AAC.00131-06

Marien M, Nauwynck H, Duchateau L, Martel A, Chiers K, Devriese L, Froyman R and Decostere A, 2006. Comparison of the efficacy of four antimicrobial treatment schemes against experimental *Ornithobacterium rhinotracheale* infection in turkey poults pre-infected with avian pneumovirus. Avian Pathology, 35, 230–237. https://doi.org/10.1080/03079450600711052

Marien M, Decostere A, Duchateau L, Chiers K, Froyman R, Nauwynck H, Marien M, Decostere A, Duchateau L, Chiers K, Froyman R and Nauwynck H, 2007. Efficacy of enrofloxacin, florfenicol and amoxicillin against *Ornithobacterium rhinotracheale* and *Escherichia coli* O2:K1 dual infection in turkeys following APV priming. Veterinary Microbiology, 121, 94–104. https://doi.org/10.1016/j.vetmic.2006.11.012

McKellar QA and Varma KJ, 1996. Pharmacokinetics and tolerance of florfenicol in equidae. Equine Veterinary Journal, 28, 209–213. https://doi.org/10.1111/j.2042-3306.1996.tb03774.x

Melingen GO and Samuelsen OB, 2011. Feed intake and tissue distribution of florfenicol in cod (*Gadus morhua*) administered feed with different fat contents. Journal of Applied Ichthyology, 27, 57–60. https://doi.org/10.1111/j.1439-0426.2010.01596.x

Mengozzi G, Intorre L, Bertini S, Giorgi M, Secchiari PL and Soldani G, 2002. A comparative kinetic study of thiamphenicol in pre-ruminant lambs and calves. Research in Veterinary Science, 73, 291–295. https://doi.org/10.1016/s0034-5288(02)00110-8

Michel C, Kerouault B and Martin C, 2003. Chloramphenicol and florfenicol susceptibility of fish-pathogenic bacteria isolated in France: comparison of minimum inhibitory concentration, using recommended provisory standards for fish bacteria. Journal of Applied Microbiology, 95, 1008–1015. https://doi.org/10.1046/j.1365-2672.2003.02093.x

Ocampo CL, Sumano LH and Páez ED, 2000. Pharmacokinetic and clinical efficacy of thiamphenicol in poultry. Veterinaria México, 31, 89–93.

Palacios-Arriaga JM, Gutierrez-Pabello JA, Chavez-Gris G and Hernandez-Castro R, 2000. Efficacy of florfenicol premix in weaning pigs experimentally infected with *Actinobacillus pleuropneumoniae*. Revista latinoamericana de microbiologia, 42, 27–33.

Papich MG, 2016. Florfenicol. In: Papich MG (ed.). Saunders Handbook of Veterinary Drugs, 4th Edition. Saunders, St. Louis, W.B. pp. 327–329.

Park BK, Lim JH, Kim MS, Hwang YH and Yun HI, 2007. Pharmacokinetics of florfenicol and its major metabolite, florfenicol amine, in rabbits. Journal of Veterinary Pharmacology and Therapeutics, 30, 32–36. https://doi.org/10.1111/j.1365-2885.2007.00809.x

Pietro WJ, Woźniak A, Pasik K, Cybulski W and Krasucka D, 2014. Amphenicols stability in medicated feed – development and validation of liquid chromatography method. Bulletin of the Veterinary Institute in Pulawy, 58, 621–629. https://doi.org/10.2478/bvip-2014-0095

Rattanapanadda P, Kuo HC, Vickroy TW, Sung CH, Rairat T, Lin TL, Yeh SY and Chou CC, 2019. *In vitro* and *in vivo* synergistic effects of florfenicol and thiamphenicol in combination against swine *Actinobacillus pleuropneumoniae* and *Pasteurella multocida*. Frontiers in Microbiology, 10, 2430. https://doi.org/10.3389/fmicb.2019.02430

Ravizzola G, Carenzi A, Manca N and Peretti P, 1984. *In vitro* antibacterial activity of thiamphenicol. Chemioterapia, 3, 163–166.

Romero J, Feijoó CG and Navarrete P, 2012. Antibiotics in aquaculture–use, abuse and alternatives. In: Carvalho E (ed.). Health and environment in aquaculture., InTech. pp. 159–198.

Ruiz-Ripa L, Bellés-Bellés A, Fernández-Fernández R, García M, Vilaró A, Zarazaga M and Torres C, 2021. Linezolid-resistant MRSA-CC398 carrying the *cfr* gene, and MRSA-CC9 isolates from pigs with signs of infection in Spain. Journal of Applied Microbiology, n/a.. https://doi.org/10.1111/jam.14988

Samuelsen OB, Hjeltnes B and Glette J, 1998. Efficacy of orally administered florfenicol in the treatment of furunculosis in Atlantic salmon. Journal of Aquatic Animal Health, 10, 56–61. https://doi.org/10.1577/1548-8667(1998)010<0056:EOOAFI>2.0.CO;2

Schwarz S, Kehrenberg C, Doublet B and Cloeckaert A, 2004. Molecular basis of bacterial resistance to chloramphenicol and florfenicol. FEMS Microbiology Reviews, 28, 519–542. https://doi.org/10.1016/j.femsre.2004.04.001

Shen J, Hu D, Wu X and Coats JR, 2003. Bioavailability and pharmacokinetics of florfenicol in broiler chickens. Journal of Veterinary Pharmacology and Therapeutics, 26, 337–341. https://doi.org/10.1046/j.1365-2885.2003.00495.x

Shin SJ, Kang SG, Nabin R, Kang ML and Yoo HS, 2005. Evaluation of the antimicrobial activity of florfenicol against bacteria isolated from bovine and porcine respiratory disease. Veterinary Microbiology, 106, 73–77. https://doi.org/10.1016/j.vetmic.2004.11.015

Singh AK and Bhunia AK, 2019. Animal-use antibiotics induce cross-resistance in bacterial pathogens to human therapeutic antibiotics. Current Microbiology, 76, 1112–1117. https://doi.org/10.1007/s00284-019-01744-2

Straus DL, Bowker JD, Bowman MP, Carty D, Mitchell AJ and Farmer BD, 2012. Safety of aquaflor-medicated feed to sunshine bass. North American Journal of Aquaculture, 74, 1–7. https://doi.org/10.1080/15222055.2011.630262

Subbiah M, Mitchell SM, Ullman JL and Call DR, 2011. β-lactams and florfenicol antibiotics remain bioactive in soils while ciprofloxacin, neomycin, and tetracycline are neutralized. Applied and Environment Microbiology, 77, 7255–7260. https://doi.org/10.1128/aem.05352-11

Switała M, Hrynyk R, Smutkiewicz A, Jaworski K, Pawlowski P, Okoniewski P, Grabowski T and Debowy J, 2007. Pharmacokinetics of florfenicol, thiamphenicol, and chloramphenicol in turkeys. Journal of Veterinary Pharmacology and Therapeutics, 30, 145–150. https://doi.org/10.1111/j.1365-2885.2007.00827.x

Tang B, Tang Y, Zhang L, Liu X, Chang J, Xia X, Yang H and Shen Z, 2020. Emergence of fexA in mediating resistance to florfenicols in *Campylobacter*. Antimicrobial Agents and Chemotherapy, 64, e00260–00220. https://doi.org/10.1128/AAC.00260-20

Tao W, Lee Myung H, Wu J, Kim Nam H, Kim J-C, Chung E, Hwang Eul C and Lee S-W, 2012. Inactivation of chloramphenicol and florfenicol by a novel chloramphenicol hydrolase. Applied and Environmental Microbiology, 78, 6295–6301. https://doi.org/10.1128/AEM.01154-12

Tikhomirov M, Poźniak B, Smutkiewicz A and Świtała M, 2019. Pharmacokinetics of florfenicol and thiamphenicol in ducks. Journal of Veterinary Pharmacology and Therapeutics, 42, 116–120. https://doi.org/10.1111/jvp.12714

Ueda Y, Ohtsuki S and Narukawa N, 1995. Efficacy of florfenicol on experimental *Actinobacillus pleuropneumonia* in pigs. Journal of Veterinary Medical Science, 57, 261–265. https://doi.org/10.1292/jvms.57.261

USP (US Pharmacopeia - The Veterinary Medicine Expert Committee on Drug Information), 2003. USP veterinary pharmaceutical information monographs – antibiotics. Journal of Veterinary Pharmacology and Therapeutics, 26 (Suppl 2): 1–271. https://doi.org/10.1034/j.1600-051x.26.s2.1.x

Varma KJ, Adams PE, Powers TE, Powers JD and Lamendola JF, 1986. Pharmacokinetics of florfenicol in veal calves. Journal of Veterinary Pharmacology and Therapeutics, 9, 412–425. https://doi.org/10.1111/j.1365-2885.1986.tb00062.x

Wallgren P, Segall T, Morner AP and Gunnarsson A, 1999. Experimental infections with *Actinobacillus pleuropneumoniae* in pigs - II. Comparison of antibiotics for oral strategic treatment. Journal of Veterinary Medicine, Series B, 46, 261–269. https://doi.org/10.1111/j.0931-1793.1999.0_227.x

Walter AM and Heilmeyer L, 1975. Thiamphenicol. Antibiotika, Fibel, 466–472.

Wang M, Xie X, Wang M, Wu J, Zhou Q and Sun Y, 2020. The bacterial microbiota in florfenicol contaminated soils: the antibiotic resistome and the nitrogen cycle. Environmental Pollution, 259. https://doi.org/10.1016/j.envpol.2019.113901

Yáñez AJ, Valenzuela K, Matzner C, Olavarría V, Figueroa J, Avendaño-Herrera R and Carcamo JG, 2014. Broth microdilution protocol for minimum inhibitory concentration (MIC) determinations of the intracellular salmonid pathogen *Piscirickettsia salmonis* to florfenicol and oxytetracycline. Journal of Fish Diseases, 37, 505–509. https://doi.org/10.1111/jfd.12144

Yilmaz S, 2019. Effects of dietary blackberry syrup supplement on growth performance, antioxidant, and immunological responses, and resistance of Nile tilapia, *Oreochromis niloticus* to *Plesiomonas shigelloides*. Fish & Shellfish Immunology, 84, 1125–1133. https://doi.org/10.1016/j.fsi.2018.11.012

Zhang J, Hu K, Chen Z, Deng H and Huang X, 2019. Effect of plasmid pSD11 on the fitness of *Escherichia coli* in sub-minimum inhibitory concentration of florfenicol. Chinese Journal of Applied & Environmental Biology, 25, 1211–1214. https://doi.org/10.19675/j.cnki.1006-687x.2018.12045

Abbreviations

ADFI	average daily feed intake
AQ	Assessment question
bw	body weight used in toxicity studies
BW	body weight
CAT	chloramphenicol acetyltransferase
DM	dry matter
ESBL	Extended-spectrum-β-lactamases
EUCAST	European Committee on Antimicrobial Susceptibility testing
F	fraction of the antimicrobial that is absorbed from the digestive tract to the blood
F:G	feed to gain
FARSC	Feed Antimicrobial Resistance Selection Concentration
FI	feed intake

FM	fresh matter
GE	fraction of the antimicrobial that is secreted back into the intestinal tract for elimination, after initially being absorbed into the bloodstream
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
MSC	minimal selective concentration
PK	pharmacokinetic(s)
ToRs	Terms of Reference

Appendix A – Uncertainty analysis

A.1. Uncertainty analysis specific for florfenicol

A.1.1. Uncertainties associated to the FARSC calculation

Table A.1: Potential sources of uncertainty identified in the estimation of the maximum concentrations of florfenicol in non-target feed that would not select for antimicrobial resistance in the rumen or large intestines and assessment of the impact that these uncertainties could have on the conclusion

Source or location of the uncertainty	Nature or cause of uncertainty as described by the experts	Impact of the uncertainty on the determination of the Feed Antimicrobial Resistance Selective Concentration (FARSC)
Estimation of PMSC data	Limited MSC data from competition experiments. MSC data is available for *E. coli* only	This limitation was overcome by the PMSC approach. Nevertheless, this could lead to an overestimation of FARSC if a bacterium with a lower MIC is subject to selection.
	Impact of bacterial community complexity on the MSCs values. It is a reasonable assumption to consider that MSCs are similar if the different antimicrobials within a class share an identical mechanism of action and resistance. There is insufficient data to assess the likely impact of complex bacterial communities on resistance selection in a single targeted member of the community, or in any other bacterium that may be present.	If this assumption is not correct, the PMSC, and accordingly the FARSC, could either be over or underestimated, depending on the specific species and the targeted community.
Antimicrobial pharmacokinetic and degradation data	For several animal categories, the value for bioavailability was extracted from only one or two studies and the selected values can therefore be inaccurate. Moreover, the effect of feed on the bioavailability is uncertain for most species.	The complete range of possible individual values for bioavailability was not explored even, though additional simulations were performed. These values could be higher or lower and thus, the FARSC could be over or underestimated.
	Several studies suggest an elimination of florfenicol and of some of its metabolites in the gut. However, there are no available quantitative data.	The complete range of possible values for gastro-intestinal elimination was not explored even though additional simulations were performed. These values could be higher or lower and thus, the FARSC could be over or underestimated.
	No study describing a possible inactivation of florfenicol in large intestines or in the rumen was found. Florfenicol was thus considered as fully active in large intestines and in the rumen	The assumption might lead to underestimation of FARSC if inactivation would occur.

FARSC: Feed Antimicrobial Resistance Selection Concentration; MSC: minimal selective concentration; PMSC: predicted MSC.

A.1.2. Uncertainties associated to the Growth promotion assessment

Table A.2: Potential sources of uncertainty identified in the levels of florfenicol 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 2.2.3 of the Scientific Opinion Part 1 (see also the Virtual Issue), the low number of studies retrieved prevented evidence synthesis.	Underestimation/Overestimation

Appendix B – List of excluded publications and their shortcomings

B.1. Florfenicol

The publications excluded from the assessment of the effects of florfenicol on growth promotion/increased yield following the criteria defined in Section 2.2.2.2.1 of the Scientific Opinion Part 1 (see also the Virtual Issue) are summarised in Table B.1.

Table B.1: Publications not relevant for the assessment of the effects of florfenicol 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 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)
Aubin et al. (2005)	Fish								X	X(1)
Bowker et al. (2010)	Fish						X			
Chae et al. (2018)	Pigs	X								X(2)
Ciprián et al. (2012)	Pigs					X		X		
Gutiérrez et al. (2011)	Pigs							X		
Han et al. (2020)	Poultry							X		
Marien et al. (2006)	Poultry					X		X		
Marien et al. (2007)	Poultry					X		X		
Melingen and Samuelsen (2011)	Fish							X		
Palacios-Arriaga et al. (2000)	Pigs					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 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)
Straus et al. (2012)	Fish							X		X(3)
Wallgren et al. (1999)	Pig				X	X				
Yilmaz (2019)	Fish					X				

(1): Paper with low quality.
(2): Toxicity study (florfenicol used at 3× the maximum recommended concentration).
(3): Toxicity study.

B.2. Thiamphenicol

The publications excluded for the assessment of the effects of thiamphenicol on growth promotion/increased yield following the criteria defined in Section 2.2.2.2.1 of the Scientific Opinion Part 1 (see also the Virtual Issue) are summarised in Table B.1.

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

Author (year)	Species	Excluding criteria								
		Combination of substances administered to the animals	Antimicrobial used different form the one under assessment	Administration 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/	Zootechnical parameters not reported
Ueda et al. (1995)	Pigs				X	X	X			