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Maximum levels of cross-contamination for 24 antimicrobial active substances in non-target feed. Part 5: *Lincosamides: lincomycin*

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Abstract

The specific concentrations of lincomycin in non-target feed for food-producing animals, below which there would not be an effect on the emergence of, and/or selection for, resistance in bacteria relevant for human and animal health, as well as the specific antimicrobial concentrations in feed which have an effect in terms of growth promotion/increased yield were assessed by EFSA in collaboration with EMA. Details of the methodology used for this assessment, associated data gaps and uncertainties, are presented in a separate document. To address antimicrobial resistance, the Feed Antimicrobial Resistance Selection Concentration (FARSC) model developed specifically for the assessment was applied. However, due to the lack of data on the parameters required to calculate the FARSC, it was not possible to conclude the assessment until further experimental data become available. To address growth promotion, data from scientific publications obtained from an extensive literature review were used. Levels of lincomycin in feed that showed to have an effect on growth promotion/increased yield were reported. It was recommended to carry out studies to generate the data that are required to fill the gaps which prevented the calculation of the FARSC for lincomycin.

Keywords: lincomycin, antimicrobial resistance, sub-inhibitory concentration, Feed Antimicrobial Resistance Selection Concentration (FARSC), growth promotion, yield increase, food-producing animals

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1. Introduction

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

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

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

Lincosamides, together with the structurally unrelated antimicrobials, macrolides and streptogramins are usually grouped into a single family, the macrolides, lincosamides and streptogramins (MLS) family (Schwarz et al., 2016). This classification is justified by a similar, although not identical, mechanism of action. The mode of action of MLS family is via protein synthesis inhibition by binding to the 50S ribosomal subunit. Relevant lincosamides include clindamycin and lincomycin.

Chemically lincomycin consists of a non-canonical amino acid linked to a sugar with clindamycin being a chlorinated derivative. Lincosamides bind to the 50S subunit of the ribosome near the peptidyl transferase centre (sharing overlapping binding sites with macrolides) and cause premature peptidyl-tRNA release (Spížek and Řezanka, 2017).

Lincosamides are active mainly against Gram-positive bacteria (e.g. staphylococci and streptococci) although not in enterococci, Mycoplasma, anaerobes and protozoans, and the two most widely used clinically are lincomycin and its derivative clindamycin, both of which are used in veterinary medicine. For human infections, only clindamycin is used because of the adverse toxic effects of lincomycin.

[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.

1.3.2. Main use[2]

Lincomycin is often used in combination with spectinomycin for treatment of Gram-positive and anaerobic respiratory and enteric infections in livestock. This includes *Serpulina* (formerly *Brachyspira) hyodysenteriae* (causing dysenteria), *Mycoplasma hyopneumoniae* (causing pneumonia), *M. hyosynoviae* (arthritis), *Brachyspira pilosicoli* (colitis), *Lawsonia intracellularis* (ileitis) and associated enteropathogens (e.g. *Escherichia coli*) in pigs, and *Mycoplasma gallisepticum, Avibacterium paragallinarum* (infectious coryza) and *E. coli* in poultry. The main administration route for the above is oral except for respiratory infections in large animals, where the primary administration route is intramuscular injection. Lincomycin in combination with neomycin can also be used for intramammary treatment of staphylococcal, streptococcal and mycoplasma mastitis in cattle (Guardabassi et al., 2008). In ruminants, lincomycin may also be used against *Staphylocccus aureus* associated arthritis, and as a topical treatment of foot lesions in cattle (Guardabassi et al., 2008).

1.3.3. Main pharmacokinetic data

Lincomycin is rapidly but incompletely absorbed when administered orally to animals (Papich, 2017). The oral absorption of lincomycin was lower in fed than fasted animals. In fed pigs, the oral bioavailability was found to be 41 $\pm$ 23% (Nielsen and Gyrd-Hansen, 1998).

Lincomycin is eliminated unchanged or in the form of various metabolites in bile and urine (Giguère, 2013). After absorption, approximately 50% of lincomycin is metabolised in the liver of pigs but high concentrations of the active form are observed in the intestine. A published study showed that 17% of lincomycin was found as the parent drug in the faeces of pigs after oral administration (Hornish et al., 1987).

Compared to the parent compound, none of lincomycin metabolites were found to have had any significant antimicrobial activity. Both *N*-desmethyl and lincomycin sulfoxide have 15–100 times less antimicrobial activity than the parent lincomycin. There was no evidence that the remaining metabolites have any antibacterial activity (EMEA, 1998).

1.3.4. Main resistance mechanisms

Lincosamides share several resistance mechanisms with macrolides and streptogramins B, often generating cross-resistance between these drug classes, so-called MLS_B resistance (Schwarz et al., 2016). As for macrolides, a common resistance mechanism is by methylation of 23S rRNA by methyl transferases encoded by the large family of different *erm* genes carried on plasmids and transposons. In addition, the CFR 23S rRNA methylase can provide resistance to lincosamides and several other antimicrobials such as oxazolidinones, phenicols, pleuromutilins and streptogramin A (Shen et al., 2013). Similarly, mutations in 23S rRNA and ribosomal proteins L4 and L22 can result in reduced susceptibility to one or more of the MLS_B antimicrobials. The ABC-F proteins (encoded by the *vga* and other genes) confer resistance by ribosome protection and are thought to act by binding to antimicrobial-inhibited ribosomes and promote dissociation of the drug from its binding site. In addition, resistance to lincosamides can be conferred by a number of different efflux genes. Finally, the *lnu* genes encode lincosamide nucleotidyltransferases which enzymatically inactivates lincosamides and reduce their activity (Roberts, 2004, 2008; Spížek and Řezanka, 2017; Feßler et al., 2018).

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).

[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.

3. Assessment

3.1. Introduction

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

3.1.1. Resistance development/spread due sub-MIC concentrations of lincosamides including lincomycin: examples

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

- No relevant studies have been found regarding sub-inhibitory effects of lincosamides on resistance selection.

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

- Horizontal gene transfer can be stimulated by lincosamides as shown by the induction of Tn*916* transfer in *E. faecalis* at sub-inhibitory drug concentrations. Thus, at concentrations 10-fold below the MIC of lincomycin and clindamycin, HGT was increased by about three orders of magnitude (Scornec et al., 2017).
- With regard to virulence-associated factors, lincomycin at sub-inhibitory levels (1/32 of MIC) could stimulate biofilm formation about fivefold in *S. suis* (Waack and Nicholson, 2018), which could potentially increase persistence and virulence.
- Studies on other licosamides showed that sub-MIC concentrations of clindamycin, similarly to the macrolides, have been shown to reduce expression of extracellular proteins in several bacterial species, including Panton-Valentine leucocidin, α-haemolysin and protein A in *S. aureus* (Herbert et al., 2001; Otto et al., 2013; Campbell et al., 2019; Hu et al., 2019), streptolysin S and M protein production in *Streptococcus pyogenes* (Shibl and Al-Sowaygh, 1979; Gemmell et al., 1981) and lipase production by *Cutibacterium* (formerly *Propionibacterium*) spp. (Unkles and Gemmell, 1982).

In summary, our understanding of the sub-MIC effects of lincosamides, including lincomycin, is very limited except for a few studies showing effects on virulence-associated functions and one study showing strong stimulation of HGT by lincosamides at concentrations 1/10 of MIC.

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 lincomycin for different animal species, if suitable data were available, would follow a two-step approach as described below:

The first step would be the calculation of the predicted minimal selective concentration (PMSC) for lincomycin as indicated in Table 1. However, no minimal selective concentration (MSC) data required to do the calculations are available for this substance.

Table 1: Calculation of lincomycin 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})
Lincomycin	NA	NA	NA	0.5	NA

MIC: minimum inhibitory concentration; MSC: minimal selective concentration; MSC_{test}: MSC experimentally determined; MIC_{lowest}, lowest MIC data for lincomycin calculated based on data from the EUCAST database as described in Bengtsson-Palme and Larsson (2016), see Methodology Section 2.2.1.3.1.1 in the Scientific Opinion Part 1 (EUCAST database https://mic.eucast.org/search/ last accessed 15 May 2021); NA: not available.

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

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

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

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

The oral bioavailability of lincomycin was around 41 ± 23% in pigs. However, the low level of absorption through the gut wall is not the only explanation for this bioavailability since only 17% of lincomycin was recovered as the parent drug in the faeces of pigs after oral administration. Hepatic first-pass effect (hepatic metabolism) should also contribute to limit the bioavailability.

For the calculations, the factor $(1 - F + F \times GE)$, reflecting the fraction available for microorganisms was considered equal to 0.17 in pigs.

The potential inactivation of lincomycin by binding to intestinal contents is not described.

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

Lincomycin data	Scenario #1
Inactive fraction (I)	NA
Fraction of the dose available for intestinal microorganisms corresponding to $(1 - F + F \times GE)$ in pigs	0.17

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.

There are no quantitative data on the fate of lincomycin for other species and no proposal for PK parameter values was done.

3.2.1. Associated data gaps and uncertainties

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

i) MSC data: no data for MSC are available.
ii) MIC data: data available only for few bacterial species in EUCAST database.

iii) Bioavailability: only bioavailability data for pigs was found. No data were available for other species.
iv) Fraction eliminated in gut: several studies suggest an elimination of lincomycin as parent drug and inactive metabolites. However, there are no quantitative data except for pigs (in only one study) to consider this process.
v) Inactive fraction: no data on the possible binding of lincomycin in digestive tract are available.
vi) Ruminants: no PK data are available for lincomycin administered to adult ruminants by oral route.

3.2.2. Concluding remarks

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

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

3.3.1. Lincomycin

3.3.1.1. Literature search results

The literature search, conducted according to the methodology described in Section 2.2.2.1 of the Scientific Opinion 'Part 1: Methodology, general data gaps and uncertainties' (see also the Virtual Issue), resulted in 399 papers mentioning lincomycin 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 lincomycin.[4] After removing the reports not matching the eligibility criteria, 46 publications were identified.

3.3.1.2. Evaluation of the studies

The 46 publications identified in the literature search were appraised for suitability for the assessment of the effects of lincomycin on growth or yield of food-producing animals; this appraisal was performed by checking each study against a series of predefined exclusion criteria (see Section 2.2.2.2.1 of the Scientific Opinion 'Part 1: Methodology, general data gaps and uncertainties'; see also the Virtual Issue).[5] A total of 37 publications were not considered suitable for the assessment because of several shortcomings identified in the design of the study or in the reporting of the results. The list of excluded publications and their shortcomings are presented in Appendix A.1 (Table A.1).

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

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

Nine publications were considered suitable for the assessment of the effects of lincomycin on growth and yield performance in food producing animals. The effects of the administration of the antimicrobial on the endpoints described in Section 2.2.2.2.2 of the Scientific Opinion 'Part 1: Methodology, general data gaps and uncertainties' (see also the Virtual Issue) were evaluated. The selected publications and the effects on the relevant endpoints are described below. The summary of the studies includes the description of the source of lincomycin used – either as the base or as any

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

[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)'.

specific form/commercial preparation – and the concentration(s) applied as reported in each study; where a specific compound has been used, the calculation of the concentration applied to the base substance is provided.

3.3.1.3.1. Study in pigs

In the study of Biehl et al. (1985), a total of 120 pigs for fattening (unspecified breed, both sexes) weighing ca. 20 kg were distributed in six pens in groups of 20 animals allotted by weight and sex and allocated to three dietary treatments (40 pigs/treatment). The basal diets were either not supplemented or supplemented with different treatments. Two were the relevant treatments: a control and a treatment consisting of lincomycin (unspecified form) supplemented at a concentration of 44 mg/kg feed. The study lasted 30 days. Mortality and health status were checked every day. Animal weight and cumulative feed intake (FI) were recorded weekly. Weights and feed to gain ratios (F:G) were calculated on day 30 (end of experiment) and on day 90 (marketing). The study also assessed the effect of treatment on health-related parameters: serological tests (Aujeszky's disease, influenza, *Mycoplasma hyopneumoniae*), nasal swabs for *Bordetella bronchiseptica* and faecal swabs for *Salmonella* Typhimurium. At the end of the trial, the pigs treated with lincomycin showed, compared to the control group, an improvement of average daily gain (ADG) (640 g vs 581 g) and F:G (2.5 vs 2.66). The treated animals showed no differences with control in regard to health-related parameters. Dietary lincomycin supplementation (at 44 mg/kg feed) had a growth-promoting effect in pigs for fattening.

In the study of Harvey et al. (1995), a total of 36 barrows (Yorkshire × American Landrace × Hampshire, weaned at 28–32 days of age) were allocated to six dietary treatments and distributed in three pens (replicates) per treatment, in groups of two animals. Two were the relevant treatments obtained from a basal diet (starter) which was either not supplemented (control) or supplemented with lincomycin (unspecified form) at a concentration of 220 mg/kg feed. The study lasted 28 days. Mortality and health status were checked daily. Animals' weight and FI were recorded weekly. At the end of the trial, animals were bled for haematologic (red blood cells, mean cell volume, haematocrit, haemoglobin, mean cell haemoglobin concentration (MHC) and leucocytes), immunologic (lymphoblastogenesis stimulation index and blastogenic response to phytohaemagglutinin) and serum biochemical (alkaline phosphatase (ALP), gamma glutamyl transferase (GGT), albumin, calcium, cholesterol, glucose, phosphorus, iron, triglycerides, urea nitrogen and iron-binding capacity) measurements. Additionally, 24 animals (4 animals/treatment) were slaughtered and the weight of liver, left kidney, spleen and heart was recorded, and specimens from each organ were examined microscopically. No effect of dietary supplementation of lincomycin on any of the endpoints measured was identified. Dietary lincomycin supplementation at 220 mg/kg feed did not have growth-promoting effects in weaned piglets.

3.3.1.3.2. Studies in poultry

In the study of Buresh et al. (1986), a total of 1,120 one-day-old turkeys (Nicholas Large White) were distributed in 10 experimental groups (112 animals/treatment), each including 14 pens with four males and four females. The starter diets were either not supplemented or supplemented with different treatments. Three were relevant treatments: control and two treatment which consisted on the supplementation of lincomycin (unspecified form) at a concentration of 4 mg/kg feed, with or without supplementation with methionine 0.18%. The study lasted 21 days. General health status was checked throughout the study. Animal weight and cumulative feed intake were recorded weekly and F:G was calculated at the end of the experiment. At the end of the trial, the poults treated with lincomycin without methionine supplementation showed an increase in weight gain (340.2 g vs 319.9 g) and improved F:G (1.29 vs 1.36) compared to the corresponding control group. Similar results were obtained in the group supplemented with lincomycin and 0.18% methionine: increases in average weight gain (498.5 g vs 475.4 g) and improved F:G (1.23 vs 1.30). Dietary lincomycin supplementation (at 4 mg/kg feed) had a growth-promoting effect in turkeys for fattening.

In the study of Feighner and Dashkevicz (1987), a total of 224 male chickens for fattening (Arbor Acres × Peterson) day-old were distributed in eight dietary treatments (28 chicks/treatment), each including four replicate groups of seven birds. The starter diets were either not supplemented or supplemented with different treatments. Two were relevant treatments: a control and a treatment which consisted on the supplementation of lincomycin (unspecified chemical form; purchased from Sigma) at a concentration of 4 mg/kg feed. The study lasted 9 days. General health status was

checked throughout the study. Animal weight and cumulative FI were recorded at day 5 and 9, F:G was calculated at the end of the experiment. Ileal contents were sampled from five randomly chosen birds per group; the ileal homogenates were assayed for cholyltaurine hydrolase (bacterial bile-acid transforming) activity. At the end of the trial, the chicks treated with lincomycin showed an increase of weight gain (+ 28.0%) and improved F:G (−17.5%) compared to the control group. In the unsupplemented control group, the average weight gain was 136.4 g, and the average F:G was 1.27 while the absolute figures for the lincomycin group were not given. Dietary lincomycin supplementation (at 4 mg/kg feed) had a growth-promoting effect in chickens for fattening.

In the study of Henry et al. (1987), a total of 144 male 1-day-old chickens for fattening (Arbor Acres × Peterson) were distributed in five dietary treatments. Two were the relevant treatments: a control and a treatment consisting of lincomycin (unspecified form) supplemented at a concentration of 4 mg/kg feed. The control group included 24 animals (four pens of six birds each) while the supplemented group included 30 animals (five pens of six birds each). Since the main aim of the study was to investigate the antimicrobial-induced modulation of trace element deposition, the FI of the supplemented group was restricted to 90 % of controls (that were fed *ad libitum*), in order to avoid a confounding effect of greater feed intake in antimicrobial-fed animals. The study lasted 21 days. General health status was checked throughout the study. Animal weight and cumulative FI were recorded weekly and F:G was calculated at the end of the experiment. At termination, the right tibia, liver and both kidneys were sampled to analyse the contents of manganese, copper, iron and zinc; the weight of the entire empty intestinal tract was measured and expressed per 100 g body weight. At the end of the trial, the chicks treated with lincomycin (and with restricted feed intake) showed no differences with controls in regard to growth performance. The relative intestine weight was lower in supplemented animals (2.32% vs 2.84%) and the supplementation with lincomycin increased manganese deposition in tibial bone (7.7 vs 6.2 mg/kg bone dry matter (DM)), compared to the control. Dietary lincomycin supplementation (at 4 mg/kg feed) did not have a growth-promoting effect on the performance of male chickens for fattening; only increased manganese absorption was identified.

In the study of Patel and McGinnis (1985), a total of 560 one-day-old unsexed chicks (breed unspecified) were distributed in seven dietary groups. All groups included 80 animals (4 pens of 20 birds each). The starter diets differed in composition (control, addition of guar meal), addition of hemicellulase and were either not supplemented (two groups) or supplemented with lincomycin (unspecified form) (five groups) at a concentration of 3.3 mg/kg feed. The assessment only considered controls and groups supplemented with lincomycin without hemicellulase or guar meal. The study lasted 54 days. General health status was checked throughout the study. Animal weight, cumulative FI and F:G were recorded at week 2, 4 and 7. At day 54, four chickens/pen were slaughtered and the following parameters were evaluated: fat pad/live weight, fat pad/weight and carcass weight/live weight. The chicks treated with lincomycin showed no differences compared with controls fed the basal diet with respect to body weight at different sampling times, gain/feed ratio or carcass parameters. Dietary lincomycin supplementation (at 3.3 mg/kg feed) did not have a growth-promoting effect in chickens for fattening.

In the study of Proudfoot et al. (1990), a total of 800 chicks (Arbor Acres; unspecified age) were distributed in four dietary treatments. All groups included 200 animals in two replicate trials, each with 100 animals/treatment (50 animals in 2 pens). The starter diets were either not supplemented (control) or supplemented with lincomycin (unspecified form). Three lincomycin-supplemented groups were included: (i) lincomycin added to feed at concentration of 2.2 mg/kg feed, (ii) lincomycin added in drinking water to provide the equivalent to 2.2 mg/kg feed and (iii) lincomycin added in drinking water to provide the equivalent to 1.1 mg/kg feed. The study lasted 42 days. Cumulative mortality, weight gain, and F:G were recorded at day 21 and 42. Financial indices, calculated as return from sale minus breeding cost were calculated at termination. Starting from week 2, on a weekly basis, one bird per pen was killed and the histomorphology of the intestinal wall (duodenum, ileum, caecum) was assessed. The paper did not provide figures for the tested parameters, but only the assessment of treatments as source of variation together with other factors. The different treatments did not influence mortality indices, weight or financial indices at the end of the experiment. Dietary lincomycin supplementation (at 2.2 mg/kg feed) did not have a growth-promoting effect in chickens for fattening.

In the study of Stutz and Lawton (1984), Experiments 4 and 5, a total of 168 and 120 two-day-old male chickens for fattening (Hubbard) were allocated to six and four dietary treatments, respectively, and distributed in six (control) or three (experimental) pens per treatment, in groups of eight birds per pen. The basal diet based on maize and soybean meal was either not supplemented (control) or

supplemented with different treatments. In both experiments, two were the relevant treatments: a control and a treatment consisting of lincomycin (unspecified form) supplementation at a concentration of 55 mg/kg feed (Experiment 4) or of 4.4 mg/kg feed (Experiment 5). Both experiments lasted 8 days (from day 3 to day 11 of age). In both experiments, body weight (BW) and cumulative FI were recorded and F:G calculated at the end of each experiment. At the end of both experiments, 32 chickens (control) or 16 chickens (lincomycin treatment) were slaughtered for relative ileal weight determination, whereas ileal digesta from 12 chickens (control) or 6 chickens (lincomycin treatment) were used for enumeration of *C. perfringens*. At the end of Experiment 4, the birds treated with lincomycin at 55 mg/kg feed, compared to the control group, showed higher ADG (142 vs. 126 g/day), and an improved F:G (1.27 vs. 1.38), and had decreased relative ileum weight (1.24 vs. 1.46% BW) and lower *C. perfringens* count (2.3 vs. 3.5 $\log_{10}$/g digesta). At the end of Experiment 5, the birds treated with lincomycin at 4.4 mg/kg feed, compared to the control group, showed higher ADG (146 vs. 135 g/day), and an improved F:G ratio (1.28 vs 1.35), and had decreased relative ileum weight (1.32 vs. 1.55% BW) and lower *C. perfringens* count (1.6 vs 3.0 $\log_{10}$/g digesta). Dietary lincomycin supplementation at 4.4 and 55 mg/kg feed had a growth-promoting effects in chickens for fattening.

In the study of Sun et al. (2005), a total of 2,496 one-day-old chickens for fattening (Cobb 500) were distributed in four dietary treatments. Each group included 624 animals in 13 replicate pens (48 animals/pen). Four types of diets were used: starter (1–14 days), grower (15–28 days), finisher I (29–35 days) and finisher II (36–49 days). Two were the relevant treatments: a control and a treatment which consisted on the supplementation of lincomycin (unspecified form) at the concentration of 2 (starter diet) and 4 (grower diet) mg/kg feed. The study lasted 49 days, and lincomycin was administered only up to day 28. Mortality, weight gain, FI and F:G were recorded weekly starting from day 15. Also, starting from week 2, one bird per pen was euthanised and the morphology of the intestinal tract (duodenum, ileum, caecum) was evaluated. The lincomycin-treated group showed a reduced overall mortality compared to the control group (7.6% vs 12.0%) and final body weight gain was greater (2,736 g vs 2,650 g). The cumulative F:G at days 35 and 49 of the study was lower than in controls (1.73 vs 1.96 and 1.96 vs 2.00, respectively) and similarly cumulative ADG was improved at days 35 and 49 (49.0 vs 50.4 and 54.1 vs 55.8 g, respectively). Dietary lincomycin supplementation (at 4 mg/kg feed) had a growth-promoting effect in chickens for fattening; however, the positive effect was found from 7 days after the withdrawal of lincomycin from the diet.

3.3.1.4. Discussion

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

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

3.3.1.4.1. Pigs

Two studies in pigs were identified as suitable for the assessment, one in weaned barrows (Harvey et al., 1995) and one in pigs for fattening (Biehl et al., 1985). In the assessed studies, treatments contained groups of animals treated with only one lincomycin concentration and did not allow dose-related effects to be assessed. The lower concentration 44 mg/kg feed used in pigs for fattening (Biehl et al., 1985) showed a positive effect on performance parameters (F:G, WG) after 30 days of supplementation; however, the effect did not carry over after lincomycin withdrawal before slaughter (90 days). The second study tested higher concentration (220 mg/kg feed) in weaned barrow, but the results did not show any effect on growth performance).

3.3.1.4.2. Poultry

From the studies retrieved from the literature, seven of them reporting the effects of the oral administration of lincomycin on growth promotion/increased yield in poultry were considered suitable for the current assessment. Those studies covered only two animal species: chickens for fattening (six studies; one of them with two experiments) and turkey poults (one study). In all the studies, the treatments contained groups of animals treated with only one lincomycin concentration in feed and did not allow to assess any dose-related effects.

In six studies in chickens for fattening, dietary lincomycin supplementation at 2–55 mg/kg feed was tested. A positive effect on growth performance was found only in three studies (Stutz and Lawton, 1984; Feighner and Dashkevicz, 1987; Sun et al., 2005). In the first study (Sun et al., 2005), two

concentrations at different stages of life were used: 2 mg in starter and 4 mg/kg feed in grower diet. The positive effect on growth performance was found from 35 days (7 days after lincomycin withdrawal from the diet). This is a positive effect of lincomycin added to the feed of chickens for fattening during the first 28 days, but it is questionable if the effect was due only to the dose tested in the grower diet (4 mg/kg) or if the 2 mg/kg in the starter diet impacted also on the result; due to the fact that the effect was found from 5 weeks of age, it can be reasonably assumed that only the highest concentration tested in grower chicks (4 mg/kg) caused the observed effect.

The other studies (Stutz and Lawton, 1984; Feighner and Dashkevicz, 1987), which used 4, 4.4 and 55 mg/kg feed, had some limitations as there were a small number of animals in experimental groups (28 chicks) and the short duration of the experiment (8–9 days); nevertheless, a positive effect on growth performance was detected in all three experiments. The other three studies (Patel and McGinnis, 1985; Henry et al., 1987; Proudfoot et al., 1990) tested similar concentrations (4, 3.3 and 2.2 mg/kg feed, respectively), but the results did not show any effect on growth performance.

In one study in turkey poults, dietary supplementation at 4 mg/kg feed improved growth performance of turkey for fattening.

3.3.1.5. Concluding remarks

It is judged 33–66% certain ('about as likely as not') that lincomycin has growth-promoting/ increase yield effects in pigs for fattening at the concentration of 44 mg/kg complete feed (one study), in chickens for fattening at concentrations ranging from 4 to 55 mg/kg feed (three studies) and in turkeys for fattening at the concentration of 4 mg/kg complete feed (one study).

No data are available in the scientific literature showing effects of lincomycin on growth promotion/ increase yield when added (i) to pig feed at concentrations below 44 mg/kg, (ii) to poultry feed below 4 mg/kg or (iii) to feed of any other food-producing animal species or categories.

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 lincomycin in non-target feed below which there would not be emergence of, and/or selection for, resistance in the large intestines/rumen?

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

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

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

- It is judged 33–66% certain ('about as likely as not') that lincomycin has growth-promoting/ increase yield effects in pigs for fattening at the concentration of 44 mg/kg complete feed (one study), in chickens for fattening at concentrations ranging from 4 to 55 mg/kg feed (three studies) and in turkeys for fattening at the concentration of 4 mg/kg complete feed (one study).
- No data are available in the scientific literature showing effect of lincomycin on growth promotion/increased yield when added (i) to pig feed at concentrations below 44 mg/kg, (ii) to poultry feed below 4 mg/kg or (iii) to feed of any other food-producing animal species or categories.

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

5. Recommendations

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

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Abbreviations

ADG	average daily gain
ALP	alkaline phosphatase
BW	body weight
bw	body weight used in toxicity studies
DM	dry matter
EUCAST	European Committee on Antimicrobial Susceptibility testing
F	fraction of the antimicrobial that is absorbed from the digestive tract to the blood
FARSC	Feed Antimicrobial Resistance Selection Concentration
F:G	feed to gain ratio
FI	feed intake
GE	fraction of the antimicrobial that is secreted back into the intestinal tract for elimination, after initially being absorbed into the bloodstream
GGT	gamma glutamyl transferase
HGT	horizontal gene transfer
I	fraction of the antimicrobial present in the digestive tracts that would be inactive on the microbiota
MIC	minimum inhibitory concentration
MIC_{lowest}	minimal 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
MHC	mean cell haemoglobin concentration

MSC	minimal selective concentration
MLS_B	macrolides, lincosamides, streptogramin B
MSC	minimal selective concentration
PK	pharmacokinetic(s)
PMSC	predicted MSC
rRNA	ribosomal ribonucleic acid
tRNA	transfer ribonucleic acid

Appendix A – List of excluded publications and their shortcomings

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

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

Author (year)	Species	Excluding criteria								
		Combination of substances administered to the animals	Antimicrobial used different from the one under assessment	Administration via route different 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)
Alexopoulos et al. (2006)	Pigs				X		X			
Amezcua et al. (2007)	Pigs		X							X(1)
Bains (1974)	Poultry	X	X		X		X			X(2)
Choi et al. (2011)	Pigs	X						X		X(2)
DeGeeter et al. (1976)	Pigs					X		X		
George et al. (1977)	Poultry	X			X	X		X		
Goren et al. (1988)	Poultry	X			X	X			X	
Guaragni et al. (2020)	Poultry					X		X		
Hamdy (1974)	Pigs				X	X	X			
Hamdy and Blanchard (1969)	Poultry				X	X	X		X(3)	
Hamdy et al. (1976)	Poultry	X			X	X	X			

Author (year)	Species	Excluding criteria								
		Combination of substances administered to the animals	Antimicrobial used different from the one under assessment	Administration via route different 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)
Hamdy et al. (1979)	Poultry	X			X	X	X			
Hamdy et al. (1982)	Poultry	X			X	X	X			
Han et al. (2011)	Pigs	X						X		
Jordan et al. (1998)	Poultry				X	X				
Mateusen et al. (2002)	Pigs				X		X	X		
McOrist et al. (2000)	Pigs	X			X		X	X		
Milbradt et al. (2014)	Poultry				X			X		
Namkung et al. (2004)	Pigs				X			X		
Oliveira et al. (2017)	Pigs									X(2)
Ortiz et al. (1995)	Poultry	X			X	X				
Patel et al. (1980)	Poultry	X						X		X(4)
Sahu et al. (2017)	Poultry							X		
Sandhu and Dean (1980)	Poultry	X				X		X		
Schildknecht et al. (1980)	Poultry	X			X	X		X	X	
Silva et al. (2017)	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)
Stipkovits et al. (2001)	Pigs	X			X	X		X		
Sun et al. (2009)	Pigs				X	X		X		
Suthongsa et al. (2017)	Pigs							X		
Tang et al. (2005)	Pigs				X	X				
Tsiloyiannis et al. (2001)	Pigs	X					X			
Wang et al. (2016)	Poultry and Pigs		X							X(5)
Wang et al. (2015)	Poultry				X	X		X		
Wheelhouse et al. (1985)	Poultry				X	X		X		
Winkelman et al. (2001)	Pigs				X	X		X		
Yin et al. (2008)	Pigs							X		
Zwirzitz et al. (2019)	Pigs	X								X(6)

(1): Study not relevant: it assays fermented whey inoculated with lactic acid bacteria.
(2): Absence of a negative control group without antimicrobial.
(3): No mention made of statistical analyses in Methods, Results or Tables.
(4): Interactions among coccidiostasts, methionine and lincomycin, with no untreated control group.
(5): The paper is review on antimicrobial peptides, just mentioning lincomycin.
(6): Insufficient/unproper replication.

Appendix B – Table of uncertainties

Uncertainties associated to the growth promotion assessment

Table B.1: Potential sources of uncertainty identified in the levels of lincomycin 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