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Maximum levels of cross-contamination for 24 antimicrobial active substances in non-target feed.

Part 2: *Aminoglycosides/aminocyclitols: apramycin, paromomycin, neomycin and spectinomycin*

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Abstract

The specific concentrations of apramycin, paromomycin, neomycin and spectinomycin 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 for these antimicrobials, it was not possible to conclude the assessment until further experimental data become available. To address growth promotion, data from scientific publications obtained from an extensive literature review were used. Levels in feed that showed to have an effect on growth promotion/increased yield were reported for apramycin and neomycin, whilst for paromomycin and spectinomycin, no suitable data for the assessment were available. It was recommended to carry out studies to generate the data that are required to fill the gaps which prevented the calculation of the FARSC for these four antimicrobials.

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Keywords: apramycin, paromomycin, neomycin, spectinomycin, antimicrobial resistance, growth promotion, food-producing animals

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1. Introduction

The European Commission requested 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–I – 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 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 aminoglycosides/aminocyclitol substances: apramycin, neomycin, paromomycin and spectinomycin.

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 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

Aminoglycosides are a class of antimicrobials first described in 1943, with the discovery of streptomycin (Schatz et al., 1944). Several other members of the class either also originated from *Streptomyces* (named with the suffix '-mycin') or from *Micromonospora* spp. (suffix '-micin') or semi-synthetic derivatives were introduced over the intervening years (Krause et al., 2016). Aminoglycosides consist of amino sugars and are commonly divided into four groups based on the identity of the aminocyclitol moiety. These groups are (1) derivatives containing the aminocyclitol streptidine (e.g. streptomycin and dihydrostreptomycin), (2) derivatives containing the aminocyclitol streptamine (e.g. spectinomycin), (3) derivatives containing a 4,5-disubstituted deoxystreptamine moiety (e.g. neomycin and paromomycin) and (4) derivatives containing a 4,6-disubstituted deoxystreptamine moiety (e.g. gentamicin, kanamycin, amikacin and tobramycin) (EMA/CVMP, 2018). A variety of amino and hydroxyl substitutions adorn the core structure of aminoglycosides. These groups have a direct influence on the mechanisms of action and susceptibility to various aminoglycoside-modifying enzymes (AMEs) of each aminoglycoside. Aminoglycosides are inhibitors of protein synthesis and exert their effect by binding to the bacterial ribosome, specifically to the 30S subunit (Bryan and Kwan, 1983). This is an energy-dependent, and often irreversible, process, resulting in impairment of the elongation of the nascent chain, disrupting the proof-reading process (Melançon et al., 1992). This leads to mistranslated

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.

proteins being inserted into, e.g. the cell membrane, causing altered permeability and loss of integrity (Busse et al., 1992). The exact binding and steps affected by the aminoglycosides vary slightly by substance (Mingeot-Leclercq et al., 1999) and, in this context, spectinomycin is of particular importance. Spectinomycin does not induce mRNA misreading like most other aminoglycosides and is therefore generally not bactericidal (Sparling and Davis, 1972). Another important feature of aminoglycosides is that they require active uptake into the cells (Bryan and Kwan, 1983), likely attributable to the relatively large size of these molecules (Chung et al., 1985). Transport across the cytoplasmic membrane requires energy from the electron transport system in an oxygen-dependent process. Due to this mechanism of uptake of aminoglycosides, which requires respiration, anaerobic bacteria are intrinsically resistant (Bryan et al., 1979).

The aminoglycosides have broad-spectrum activity essentially active against Gram-negative bacteria (cocci, coccobacilli and bacilli), given that they are aerobes or facultative anaerobes, and against staphylococci. They are also active against Gram-positive bacilli such as *Listeria monocytogenes*, *Corynebacterium diphtheriae* and *Bacillus anthracis*. However, they are inactive against obligate anaerobic bacteria and against streptococci and pneumococci.

Studies have shown differences in the spectrum of activity (e.g. spectinomycin has low activity in *P. aeruginosa*), minimum inhibitory concentration (MIC) values and resistance mechanisms of apramycin, neomycin, paromomycin and spectinomycin (Mingeot-Leclercq et al., 1999; Avent et al., 2011; Krause et al., 2016). Therefore, the FARSC assessments will be done separately.

1.3.2. Main use²

The main use of the aminoglycosides apramycin, neomycin, paromomycin and spectinomycin differs slightly, but is generally indicated for treatment of bacterial enteritis caused by *Escherichia coli*, *Enterobacter* spp., *Klebsiella* spp., *Salmonella enterica* in ruminants, pigs and poultry.

Apramycin is also used for the treatment of *E. coli* septicaemia in poultry and colibacillosis in rabbits (Suarez et al., 2012). The use of spectinomycin is also reported in respiratory infections, for example, to reduce losses from *Mycoplasma gallisepticum* and *M. synoviae* in chickens and *M. bovis* in cattle. However, the primary use of spectinomycin is probably for pigs, where it is used for treatment and metaphylaxis against dysentery caused e.g. by *Brachyspira hyodysenteriae* or porcine proliferative enteropathy caused by *Lawsonia intracellularis* and other pathogens. These treatments are commonly used in combination with lincomycin. Paromomycin is approved for gastrointestinal infections caused by *E. coli* in pigs and calves, and against *Cryptosporidium parvum* infection in calves. The main administration route for the above is oral except for respiratory infections, where the primary administration route is by intramuscular injection.

1.3.3. Main pharmacokinetic data

Aminoglycosides/aminocyclitols are very poorly absorbed from the gastrointestinal tract because of their high polar and cationic nature.

The bioavailability of apramycin is very low; about 4% in pigs (Dai et al., 2017) and 2% in broilers (Afifi and Ramadan, 1997). Very low serum concentrations were also described after oral administration to calves (Ziv et al., 1985).

The bioavailability of neomycin ranges from 0.01% to 1.27% in calves (Pedersoli et al., 1994).

A higher bioavailability was reported for spectinomycin in one publication with values ranging from 11.8% and 26.4% after oral administration of 50 and 100 mg/kg body weight (bw), respectively, in fasted broilers (Abu-Basha et al., 2007).

No data are available for the bioavailability of paromomycin after oral administration in target species.

The aminoglycosides are not inactivated in the intestine and are eliminated unchanged after oral administration (EMA/CVMP, 2013, 2018; Papich and Rivière, 2017). After oral administration of radiolabelled neomycin, about 90% of the radioactivity was recovered in faeces and 70–80% was present as parent neomycin (EMA/CVMP, 2013).

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.

However, aminoglycosides can bind to faeces and become inactive. The binding of neomycin to faecal content is of 85% for rats (Hazenberg et al., 1986), from 83% to 98% for humans (Hazenberg et al., 1984) and 75% for dogs (Wagman et al., 1974). Paromomycin was also found to bind at 70% to dog faeces (Wagman et al., 1974).

1.3.4. Main resistance mechanisms

Four main mechanisms exist for resistance to aminoglycosides/aminocyclitols, of which the two-first listed are the clinically most important: (i) mutations altering either ribosomal rRNA or proteins, (ii) inactivation of the antimicrobial by modification, (iii) enzymatic modification of the ribosome and (iv) decreased uptake of the substance (Shaw et al., 1993; Davies and Wright, 1997; Krause et al., 2016).

Inactivation of the aminoglycoside compounds themselves encompasses a wide diversity of resistance genes with different mechanisms, many of them transferrable between bacteria (Davies and Wright, 1997). Three major categories of resistance genes that perform aminoglycoside modifications exist: ATP-dependent O-phosphorylation by phosphotransferases (*aph* genes), ATP-dependent O-adenylation by nucleotidyltransferases (*ant* or *add* genes) and acetyl CoA-dependent N-acetylation by acetyltransferases (*aac* genes). These genes exist in hundreds of variants and have been mobilised to plasmids that can be horizontally transferred between bacteria.

The enzymatic modification of the ribosomal RNA (16S rRNA) is carried out by rRNA methylases (e.g. ArmA, RmtA, RmtB and RmtC), which can confer high levels of resistance. Some of the rRNA methylases confer resistance to specific aminoglycosides, while other confer resistance to a range of compounds (Davies and Wright, 1997; Doi et al., 2016).

Resistance through decreased uptake of the antimicrobial is a common mechanism in *P. aeruginosa* (Mingeot-Leclercq et al., 1999); while this mechanism generally results in intermediate (moderate) levels of resistance only, it is significant as the mechanism applies broadly to most or all aminoglycosides. The *arn* lipopolysaccharide (LPS) modification locus and the PhoPQ two-component system have been implied in *P. aeruginosa* resistance (Tierney and Rather, 2019). For some aminoglycosides, active efflux of the antimicrobial has also been observed (Edgar and Bibi, 1997; Hayashi et al., 1997; Poole, 2005).

Importantly, novel aminoglycoside resistance mechanisms are still being discovered (Böhm 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 subminimum inhibitory concentrations, Sub-MIC) may have different effects on bacterial antimicrobial resistance evolution, properties of bacteria and in animal growth promotion. Some examples, including the 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 aminoglycosides including apramycin, neomycin, paromomycin and spectinomycin: examples

Comparatively little work has been done on how aminoglycoside exposure contributes to the development of antimicrobial resistance. Particularly, there is almost a complete lack of studies systematically investigating resistance selection and transmission associated with neomycin and spectinomycin. Resistance mechanism patterns among Enterobacteriaceae have been found to correlate with changing aminoglycoside usage in geographic regions or even in local hospitals (Miller et al., 1997). Resistance genes to aminoglycosides are, as mentioned above, also fairly mobile between bacteria and the spread of resistance from the animal sector (exposed to e.g. apramycin) to

humans is suspected to be a main driver of clinical resistance development (Chaslus-Dancla et al., 1989; Johnson et al., 1995).

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

- There are no relevant data on the effect of sub-MIC levels of neomycin and spectinomycin, and very limited data on apramycin. Nearly all studies on paromomycin resistance development have been done when the drug is used as an antiparasitic drug in *Leishmania* spp. Hendrickx et al. (2014, 2015) could show that, by exposing *L. donovani* and *L. infantum* to sublethal concentrations of paromomycin (below 150 µM), resistance developed consistently within just a few generations.
- Apramycin resistance plasmids increase the fitness of *E. coli* carriers also in the absence of any antimicrobial exposure, suggesting that carriage of, at least the *aac(3)-IV* resistance gene on a natural *E. coli* plasmid, is generally not associated with a high fitness cost to bacteria (Yates et al., 2006).
- Sub-MIC exposure (1/4 of MIC) to apramycin (also to some macrolides as tilmiosin and tylosin) reduces the growth rate of *Pasteurella multocida*, *P. haemolytica*, *Bordetella bronchiseptica* and *Actinobacillus pleuropneumoniae*, indicating that resistant strains may have a fitness advantage at sublethal exposure levels as they would outcompete sensitive ones (Diarra et al., 1999).

Sub-MIC concentrations of other aminoglycosides have also shown these effects:

- It is conceivable that high-level resistance to gentamicin can be achieved through accumulation of several mutations, each conferring moderate or low resistance, based on experimental evolution experiments (Ibacache-Quiroga et al., 2018).
- Gullberg et al. (2011) showed that 1 µg/mL streptomycin (1/4 of the MIC) was sufficient to select for streptomycin resistance in *E. coli*.
- Kanamycin at 2/3 of the MIC (470 ng/mL) selected for a multiresistance plasmid (pUUH239.2) in *E. coli* (Gullberg et al., 2014).
- Klümper et al. (2019) noted a minimal selective concentration (MSC) of gentamicin around 0.2 µg/mL in the absence of a microbial community and around 10 µg/mL in the presence of a microbial community. In the same study, they retrieved an MSC around 0.3 µg/mL for kanamycin without a microbial community and around 5 µg/mL with the community.
- *E. coli* exposed to streptomycin displayed an increased mutation rate which might result in resistance selection starting at 0.5 µg/mL, then increasing with concentrations up to the highest tested (5 µg/mL) (Ren et al., 1999). The increasing effect on mutation rate appears to be maintained also at higher concentrations (Balashov and Humayun, 2002).

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

- Exposure to sublethal levels of spectinomycin (0.5 µg/mL) increased the rate of HGT in *Klebsiella pneumoniae* (Acosta et al., 2020).
- Sub-MIC exposure to neomycin, paromomycin, as well as amikacin, gentamicin, kanamycin and netilmicin reduced biofilms of *Pseudomonas aeruginosa* and also had an attenuating effect on quorum sensing-mediated virulence (Khan et al., 2020).
- Overall, there is an apparent lack of systematic studies on the effects of the aminoglycosides under assessment (apramycin, neomycin, paromomycin and spectinomycin) on antimicrobial resistance development and horizontal gene transfer. Sub-MIC exposure to different aminoglycosides has shown effects on both mutational resistance emergence, mutation rate and horizontal gene transfer rates at concentrations down to 0.5 µg/mL for streptomycin.

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 four aminoglycosides 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 apramycin, neomycin, paromomycin and spectinomycin, as indicated in Table 1. However, no MSC data required to do the calculations are available for those antimicrobials.

Table 1: Calculation of apramycin, neomycin, spectinomycin and paromomycin 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})
Apramycin	NA	NA	NA	NA	NA
Neomycin	NA	NA	NA	0.125	NA
Spectinomycin	NA	NA	NA	2	NA
Paromomycin	NA	NA	NA	NA	NA

MIC: minimum inhibitory concentration. MSC: minimal selective concentration. MSC_{test}: MSC experimentally determined. MIC_{lowest}: lowest MIC data for neomycin and spectinomycin calculated based on data from the EUCAST database as described in Bengtsson-Palme and Larsson (2016), see Methodology Section 2.2.1.3.1.1 in the [Scientific Opinion Part 1](#). No MIC data for apramycin nor paromomycin in the EUCAST database (EUCAST database <https://mic.eucast.org/search/> last accessed 15 May 2021); NA: not available.

Due to the lack of PMSC, no FARSC could be calculated. If PMSC was available, the FARSC (FARSC_{intestine} and FARSC_{rumen}) corresponding to the maximal concentrations in feed would be calculated for each species from the equations below (for details, see Section 2.2.1.3.2 of the [Scientific Opinion Part 1](#); see also the [Virtual Issue](#)):

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

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

With daily faeces being the daily fresh faecal output in kg, *I* the inactive fraction, *F* the fraction available, expressed in kg. *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 reported oral bioavailability was 4% in pigs and 2% in broilers for apramycin, from 0.01% to 1.27% in calves for neomycin, from 11.8% and 26.4% in fasted broilers for spectinomycin. There are no data for these antimicrobials in other species nor are there for paromomycin.

Except for the higher values reported in one publication for spectinomycin in broilers, the bioavailability is systematically lower than 5% and in agreement with the fact that aminoglycosides are not absorbed.

Thus, *F* was considered equal to 0 for aminoglycosides whatever the species. One other scenario was proposed with a higher bioavailability of 20%.

An extensive binding of several aminoglycosides to faecal contents of dogs, humans and rats was reported. There were no data available for the food-producing species, and therefore, several possible values were considered (75% for the average of the binding, 50% for the minimum and 90% for the maximum).

The values of *F* and *I* extracted from literature for the calculations of FARSC are summarised in Table 2. The first set of values (scenario 1) corresponds to the average of published values while scenario 2 corresponds to a scenario that would lead to lower FARSC and scenario 3 to a scenario that would lead to higher FARSC.

Table 2: Pharmacokinetic (PK) values used for the calculation of Feed Antimicrobial Resistance Selection Concentration (FARSC) of the aminoglycosides/aminocyclitols under assessment for the different animal species

Aminoglycoside/aminocyclitols data	Scenario #1	Scenario #2	Scenario #3
Inactive fraction (<i>I</i>)	0.75	0.5	0.9
Bioavailability (<i>F</i>)	0	0	0.2

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. The fraction remaining in the digestive tract and that could be available for the bacteria is equal to $(1 - F)$.

Due to the absence of MSC and other PK data, the estimation of the FARSC for apramycin, paromomycin, neomycin and spectinomycin was not possible.

3.2.1. Associated data gaps and uncertainties

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

- i) MSC data: no data for MSCs are available for those substances.
- ii) MIC data: not available in EUCAST for apramycin nor paromomycin.
- iii) Impact of complexity on determined MSCs: no data determining the community effect on the MSC of the substances under assessment. For other aminoglycosides: a single study shows that kanamycin and gentamicin may have increased MSCs in the presence of a microbial community (Klümper et al., 2019).
- iv) Bioavailability: for many species, quantitative data are not available even if aminoglycosides/aminocyclitol substances are considered as not absorbed from the digestive tract.
- v) Inactive fraction: no data on the binding of aminoglycosides in digestive tract of food-producing species are available.

3.2.2. Concluding remarks

Due to the lack of data on the parameters required to calculate the FARSC for apramycin, neomycin, paromomycin spectinomycin, 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. Apramycin

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 227 papers mentioning apramycin and any of the food-producing animal species considered³ and any of the performance parameters identified as relevant for the assessment of the possible growth-promoting effects of apramycin.⁴ After removing the reports not matching the eligibility criteria, 34 publications were identified.

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.

3.3.1.2. Evaluation of the studies

The 34 publications identified in the literature search were appraised for suitability for the assessment of the effects of apramycin 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 23 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 apramycin on growth performance and yield

Eleven publications were considered suitable for the assessment of the effects of apramycin 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 apramycin used – either as the base or as any specific form/commercial preparation – and the concentration(s) applied as reported in each study; where a specific compound has been used, the calculation of the concentration applied to the base substance is provided.

3.3.1.3.1. Studies in pigs

In the study of Hossain et al. (2016), a total of 140 crossbred healthy weaned pigs ((Yorkshire × Duroc) × Landrace; 21 days of age, BW 5.7 kg) were used in a 6-week experiment to determine the effect of rice bran extract (0 and 100 mg/kg feed) and apramycin (unspecified form) supplementation (0 and 165 mg/kg feed). The experiment included two phases (from 1 to 2 weeks, and from 3 to 6 weeks). Pigs were randomly allotted to one of the four treatments in a randomly complete block design with seven replicates (pen) per treatment with five pigs (three gilts and two barrows) per pen, and two of these involved relevant treatments. Individual pig BW and feed refusals were recorded at 2 and 6 weeks to calculate average daily gain (ADG), average daily feed intake (ADFI) and growth efficiency. The incidence of diarrhoea was recorded and scored three times per day throughout the study, and a cumulative score per diet and day was then assessed. At 2 and 6 weeks, faecal samples from two pigs per pen were collected directly from the rectum and used to determine *Lactobacillus* spp., *E. coli* and *Salmonella* spp. An indigestible marker (chromium oxide) was added to feed to assess faecal nutrient digestibility (dry matter (DM), nitrogen (N) and energy). On weeks 2 and 6, two pigs from each pen (one gilt and one barrow) were randomly selected and blood samples collected to determine immunoglobulin and biochemical parameters (cholesterol, glucose, red blood cells, white blood cell and lymphocyte counts). No effect of apramycin was detected on diarrhoea score. Regarding the effect of apramycin supplementation on performance, pigs fed diets supplemented with apramycin had improved ADG during phase 2 (515 vs 508 g/day) and overall (422 vs 391 g/day), although the treatment did not affect their feed intake (FI). Moreover, apramycin improved the gain to feed ratio (G:F) during phase 1 (0.83 vs 0.81) and overall (0.70 vs 0.65). Dietary supplementation with apramycin reduced faecal *E. coli* counts at 2 and 6 weeks (6.19 vs 6.28 and 6.28 vs 6.33 log₁₀ CFU/g) and *Salmonella* spp. counts at 6 weeks (2.39 vs 2.47 log₁₀ CFU/g). Feeding apramycin improved the digestibility (both at 2 and 6 weeks) of DM (82.9% vs 80.2% and 83.1% vs 80.2%, respectively) and N digestibility (83.8% vs 80.7% and 83.5% vs 79.2%, respectively). No interaction between rice bran extract and apramycin supplementation was observed for any of the studied parameters, except for DM digestibility and total cholesterol at week 6. Dietary apramycin supplementation at 165 mg/kg feed had growth-promoting effects in weaned piglets.

The study by Lei et al. (2018) was conducted to investigate the effects of dietary supplementation with a phytogenic feed additive and a positive control containing apramycin (150 mg/kg feed) on growth performance, nutrient digestibility, blood characteristics and faecal scores in weaned pigs. A total of 150 pigs ((Landrace × Yorkshire) × Duroc; BW 8.0 kg) were randomly assigned to treatments

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)'.

with five pens per treatment each including six pigs. The study lasted 42 days in three phases (1–7 days, 8–22 days and 22–42 days). Two were the relevant treatments obtained from a basal diet which was either not supplemented (control) or supplemented with apramycin (unspecified form) at a concentration of 150 mg/kg feed. Individual BW and feed consumption on a pen basis was measured at the beginning and at days 7, 21 and 42 to calculate ADG, ADFI and G:F. Pigs received the diet including an indigestible marker (chromium oxide) from 3 to 7 days, 17–21 and 38–42 days and on days 7, 21 and 42 faeces samples were collected from at least three pigs per pen to determine DM and N digestibility. On days 7, 21 and 42, two pigs (one gilt and one barrow) per pen were blood sampled and red and white blood cell counts, and immunoglobulin (IgG) were determined. Additionally, subjective faeces consistency per pen were recorded daily (from days 1 to 7). During the whole treatment period, apramycin supplementation increased ADG (525 vs 495 g/day). A higher nutrient digestibility of N at 21 days was observed in pigs receiving apramycin supplementation treatments (82.2% vs 79.2%). Regarding blood parameters, a higher number of red blood cells at 7 days was observed in pigs receiving apramycin (6.1 vs 5.2×10^6 red blood cells/ μL). Dietary apramycin supplementation at 150 mg/kg feed had growth-promoting effects in weaned piglets.

In the study of Orban et al. (1996), Experiments 1 and 2, the effect of feeding apramycin sulfate (34 mg/kg feed) on growth performance and changes on intestinal microbial population of weaned piglets was evaluated. In each experiment, a total of 192 weaned pigs (unspecified breed/genotype; 96 barrows and 96 gilts, 33 days of age, BW 7.3 and 10 kg, respectively) were allocated to four dietary treatments, distributed in six pens per treatment (with eight pigs each) and fed the experimental diets for 32 days after weaning. Two were the relevant treatments obtained from a basal diet which was either non-supplemented (control) or supplemented with 34 mg apramycin sulfate/kg feed (corresponding to 28.9 mg apramycin/kg feed). Body weight and FI were measured weekly and G:F calculated. At the end of the 32-day experimental period, six pigs from each treatment were killed and faecal bacterial populations (coliforms, lactobacilli, bifidobacteria) were enumerated. There was no effect of dietary treatment on growth performances or intestinal microbiota. Dietary apramycin sulfate supplementation at 34 mg/kg feed (corresponding to 28.9 mg apramycin/kg feed) did not have a growth-promoting effect in weaned piglets.

In the study of Yan et al. (2011), the effect of an herb extract mixture including a treatment with apramycin (as a positive control), on growth performance, nutrient digestibility, blood characteristics and faecal noxious gas content in growing pigs was evaluated. A total of 100 crossbred pigs ((Landrace \times Yorkshire) \times Duroc; BW 27.5 kg) were allocated to five dietary treatments, with five replicate pens per treatment (with four pigs each, two gilts and two barrows). Two were the relevant treatments obtained from a basal diet which was either not supplemented (control) or supplemented with apramycin (unspecified form) at a concentration of 30 mg/kg feed. The study lasted 6 weeks. Body weight was measured at the beginning and the end (6 weeks) of the experimental period, and feed consumption was recorded on a pen basis during the experiment to calculate the ADG, ADFI and G:F. Chromium oxide was added as indigestible marker to the diet for 7 days prior to faecal collection at the sixth week to determine nutrient digestibility. Faecal samples were collected at random from at least two pigs per pen at the end of the study. Two pigs were randomly selected from each pen and blood was collected to determine red blood cells, white blood cells and lymphocyte counts. For analysis of faecal ammonia and sulfur hydrogen, faeces were collected from two pigs per pen on the last 2 days of the experiment and subjected to fermentation for 5 days. Pigs fed diets supplemented with apramycin showed a higher ADG (738 vs 693 g/day). Apramycin supplementation increased the percentage of lymphocytes. Pigs fed apramycin supplemented diet showed decreased ammonia emission on the third day of the fermentation period. Dietary apramycin supplementation at 30 mg/kg feed improved growth promotion of pigs for fattening.

In the study of Yan et al. (2012a), the effect of a bacteriophage supplementation, including a treatment with apramycin, on growth performance, nutrient digestibility, blood characteristics and faecal microbial shedding in growing pigs was evaluated. A total of 144 pigs ((Duroc \times Yorkshire) \times Landrace; half barrows and half gilts, BW 28.8 kg) were allotted to four dietary treatments and distributed in nine replicate pens per treatment (with four pigs each). Two were the relevant treatments, obtained from a basal diet which was either not supplemented (control) or supplemented with apramycin (unspecified form) at a concentration of 500 mg/kg feed. The experiment lasted 6 weeks. Pigs' weight was measured at the beginning and the end of the experimental period, feed consumption was recorded on a pen basis during the experiment to calculate ADG, ADFI and G:F. Chromium oxide was added as indigestible marker to the diet for 7 days prior to faecal collection at the sixth week to determine nutrient digestibility. Faecal samples were

collected at random from at least two pigs per pen at the end of the study. Two pigs were randomly selected from each pen and blood was collected to determine red blood cells, white blood cells and lymphocyte counts. At the end of the experiment, faecal samples were collected from the rectum from two randomly selected pigs from each pen and used for *E. coli* and *Lactobacillus* and *Salmonella* counts. The severity of diarrhoea was assessed from all pigs and was scored, during the period of 5–10 days. A higher DM (80.1% vs 77.4%) and energy digestibility (79.2% vs 76.6%) was observed in pigs fed apramycin. A reduction of *E. coli* (6.00 vs 6.55 log₁₀ CFU/g) and *Salmonella* (2.57 vs 3.62 log₁₀ CFU/g) was observed in treated pigs. Dietary apramycin supplementation at 500 mg/kg feed had a growth-promoting effect in pigs for fattening.

In the study of Yan et al. (2012b), the effect of a dietary herb mixture and apramycin supplementation on growth performance, nutrient digestibility, blood characteristics and faecal microbial shedding in weanling piglets was evaluated. A total of 144 weaned piglets ((Landrace × Yorkshire) × Duroc, weaned at 21 day; BW 7.6 kg) were randomly allocated to four treatments with nine replicate pens per treatment (with four pigs each, two barrows and two gilts). Treatments followed a 2 × 2 factorial design with two levels of herbal extract (HE: 0 and 250 mg/kg) and apramycin (0 or 30 mg/kg; unspecified form). Two were the relevant treatments: HE/Apramycin: 0/0 mg/kg and HE/Apramycin 0/30 mg/kg. A three-period feeding programme (0–1 week, 2–3 weeks and 4–6 weeks) was followed. BW was measured at the beginning and the end (6 weeks) of the experimental period, and feed consumption was recorded on a pen basis during the experiment to calculate the ADG, ADFI and G:F. Chromium oxide was added as indigestible marker at the beginning of the first (day 1), third (day 15) and sixth (day 36) week, to determine nutrient digestibility. Fresh faecal samples were collected at random from at least two pigs per pen at the end of the first (day 7), third (day 21) and sixth week (day 42) to determine the apparent digestibility of DM, N and energy. At the beginning of the experiment, two pigs were randomly selected from each pen and blood was collected to determine red blood cells, white blood cells and lymphocyte counts. At day 42, faecal samples were collected from the rectum from two pigs randomly selected from each pen, pooled and used to quantify *E. coli* and *Lactobacillus*. Antimicrobial supplementation during the whole fattening period (from 0 to 6 weeks) reduced ADFI (764 vs 787 g/day) and increased G:F (0.667 vs 0.626). Apramycin supplementation increased the DM digestibility at day 7 (0.814 vs 0.794) and 42 (0.826 vs 0.794), N digestibility at day 7 (0.825 vs 0.789) and 21 (0.827 vs 0.800), and energy digestibility at day 21 (0.824 vs 0.792). No effect of apramycin on blood parameters and faecal *E. coli* and *lactobacilli* counts was observed, except for an increase in red blood cells at day 21 and lymphocytes at day 42. Dietary apramycin supplementation at 30 mg/kg feed showed growth-promoting effects in weaned piglets.

In the study of Yoon et al. (2012a), the effect of antimicrobials on growth performance, apparent total tract digestibility, faecal and intestinal microflora and intestinal morphology of weaned pigs was evaluated. A total of 240 weaning pigs ((Landrace × Yorkshire) × Duroc; half males and half females, BW 6.2 kg at 21 days) were randomly allotted to four treatments with four replicate pens per treatment (with 15 pigs each). Two were the relevant treatments obtained from a basal diet which was either not supplemented (control) or supplemented with apramycin (unspecified form) at a concentration of 1,500 mg/kg feed. A two-feeding programme was applied (from 1 to 14 days and from 15 to 28 days post-weaning). The pigs were weighed individually at the start of the trial and on days 14 and 28 and feed consumption was calculated at the end of each phase, and the ADFI and feed conversion ratio (F:G) calculated. To evaluate the effect of the dietary treatments on the apparent digestibility of energy and nutrients, indigestible chromium oxide was added to diets from days 8 to 14 and 22 to 28. Faecal samples were collected from each pen on the last 3 days of each phase to determine apparent nutrient digestibility. Additionally, faecal samples were collected on days 14 and 28 to measure bacterial counts. To study the effect of dietary treatments on the microflora of ileum and caecum digesta, two pigs from each pen were selected and killed on day 28. During the overall period (1–28 days), a higher ADG (357 vs 311 g/day) and a lower F:G (1.48 vs 1.63) were observed in pigs fed apramycin-supplemented diets. A higher apparent digestibility of DM, crude protein (at 14 and 28 days) and energy (on day 28) were reported. Dietary apramycin reduced the count of coliforms in faeces (days 14 and 28), ileum (day 28) and caecal (day 28) contents. Dietary apramycin supplementation at 1,500 mg/kg feed showed growth-promoting effects in weaned piglets.

In the study of Zhang and Kim (2014), the effect of levan (mixture of oligosaccharides) and apramycin supplementation on growth performance, nutrient digestibility and faecal DM content in weaned pigs was evaluated. A total of 144 weaned pigs ((Yorkshire × Landrace) × (Hampshire × Duroc); 18 days of age, BW 5.5 kg) were used in a 28-day trial. Pigs were randomly allocated into four treatments with two levels of

apramycin (0 or 165 mg/kg; unspecified form) and two levels of levan (0 or 1 g/kg) with six replicates (with six pigs each, half gilts and half barrows). The experiment included two-feeding programme (1–14 days and 15–28 days). Individual BW and feed consumption per pen were monitored on days 14 and 28 to determine ADG, ADFI and G:F ratio. From days 8 to 14 and days 22 to 28, chromium oxide was added to diets as an indigestible marker for the determination of the apparent total tract digestibility of DM, N and gross energy. Then, on days 14 and 28, faecal samples were collected from two pigs per pen per rectum. No mortality was observed during the experiment, and no interactive effects between levan and apramycin were observed. In the whole feeding period, apramycin increased ADG (400 vs 358 g/day) and G:F (0.726 vs 0.665). Apramycin increased the coefficient of total tract apparent digestibility of DM, N and gross energy, as well as the faecal dry matter content, compared to non-medicated diets, at 14 days, but no effect was observed at 28 days. Dietary apramycin supplementation at 165 mg/kg feed showed growth-promoting effects in weaned piglets.

In the study of Zhao et al. (2012), the effect of dietary fructan, mannan oligosaccharides and apramycin on growth performance, nutrient digestibility, blood profile and diarrhoea score in weaned pigs was evaluated. A total of 150 weaned piglets ((Yorkshire × Landrace) × Duroc; 21 days of age, BW 7.2 kg) were randomly allotted to five dietary treatments, with three replicates with 10 pigs each (five gilts and five barrows) per treatment. Two were the relevant treatments, obtained from a basal diet which was either not supplemented (control) or supplemented with apramycin (unspecified form) at a concentration of 100 mg/kg feed. The study lasted 28 days. Individual body weight was recorded at the beginning and on days 14 and 28 and feed consumption was recorded on a pen basis during the experiment to calculate ADG, ADFI and G:F. During days 8–14 and during days 22–28, pigs were fed diets including chromium oxide as indigestible marker to determine apparent nutrient digestibility for DM and N (faecal samples collected from at least two pigs in each pen via rectal massage). On day 14, blood samples were collected from two pigs per pen and used to determine IgG, white blood and red blood cells and lymphocyte counts. Subjective diarrhoea scores were recorded daily (on a pen basis) from day 10 to day 17 and scored. A higher ADFI was observed in pigs fed apramycin during the overall experimental period (543 vs 476 g/day). Feeding apramycin increased the apparent total tract digestibility of DM and N at day 14 (84.4% vs 78.7% and 81.3% vs 77.3%, respectively) and that of DM at day 28 (77.4% vs 76.8%). Dietary apramycin supplementation at 165 mg/kg feed had growth-promoting effects in weaned piglets.

In the study of Zhao and Kim (2015), the effect of dietary supplementation of apramycin on growth performance, nutrient digestibility, faecal noxious gas emission, faecal microflora and diarrhoea score in weaned pigs was evaluated. A total of 168 weaned piglets (Yorkshire × Landrace) × Duroc; 28 days of age, BW 7.9 kg) were used in a 28-day experiment. Pigs were randomly allotted to four dietary treatments, with seven pens per treatment (with six pigs, three gilts and three barrows per pen). Two were the relevant treatments obtained from a basal diet which was either not supplemented (control) or supplemented with apramycin (unspecified form) at a concentration of 100 mg/kg feed. Individual pig BW was recorded at the beginning, days 14 and 28 of the experimental period and feed consumption was recorded on a pen basis during the experiment to calculate ADG, ADFI and G:F. From days 8 to 14 and from 22 to 28 days, pigs were fed a diet including chromium oxide as an indigestible marker to determine apparent nutrient digestibility for DM and N (faecal samples collected from at least two pigs per pen via rectal massage). Urine was collected in a bucket via a funnel below the cage. A pooled faecal sample per pen was used to determine the viable counts of *E. coli* and *Lactobacillus*. Apramycin supplementation increased the ADG during the overall period (439 vs 400 g/day). Apramycin diets increased the apparent digestibility of N (day 14: 0.799 vs 0.776, and day 28: 0.802 vs 0.783) and energy (day 28: 0.815 vs 0.807). A reduction in faecal *E. coli* counts (6.52 vs 7.13 log₁₀ CFU/g) occurred. A reduction in faecal emission of ammonia (7.3 vs 9.4 to 7.3 mg/kg) and total mercaptans (2.8 vs 3.7 to 2.8 mg/kg) was observed in pigs fed apramycin. Dietary apramycin supplementation at 100 mg/kg feed had growth-promoting effects in weaned piglets.

In the study of Zhou et al. (2015), the effect of fermented ginkgo biloba and a positive control including apramycin on growth performance, nutrient digestibility, serum biochemical parameters and immune function in weaned piglets was evaluated. A total of 120 castrated piglets (Duroc × Landrace × Yorkshire, weaned at 21 days, average initial BW 7.6 kg) were randomly allocated to five dietary treatments with six replicates (pens) each, consisting in four pigs (two gilts and two barrows) in a randomised complex block design according to their BW and sex. Two were the relevant treatments, obtained from a basal diet which was either not supplemented (control) or supplemented with apramycin (unspecified form) at a concentration of 30 mg/kg feed. Individual pig BW and the feed consumed were recorded weekly for calculating ADG, FI and G:F. The apparent total

tract digestibility of DM, N and gross energy was determined using chromic oxide as inert indicator. Pigs were fed diets including the indigestible marker from days 36 to 42, and fresh faecal samples were collected from the rectum of two pigs randomly selected from each pen from days 39 to 42. On the last day of the experiment, two pigs were randomly selected from each pen and blood samples were collected and used to determine biochemical parameters and serum immunoglobulins and immune cells. Apramycin supplementation increased final BW (29.6 vs 28.2 kg), ADG (521 vs 491 g/day), and G:F (0.683 vs 0.635). Additionally, apramycin increased the apparent digestibility of DM (82.1% vs 78.9%), N (82.3% vs 79.1%) and gross energy (82.3% vs 79.3%). Regarding blood biochemical parameters, supplementation with apramycin increased total protein, albumin, alkaline phosphatase (ALP), glucose, haemoglobin, total iron, total iron binding capacity, superoxide dismutase, glutathione superoxidase dismutase and reduced blood urea N and malondialdehyde. Immunoglobulins and lymphocyte CD4:CD8, B-cells and MCH also increased with apramycin supplementation. Dietary apramycin supplementation at 30 mg/kg feed improved growth promotion of weaned piglets.

3.3.1.4. Discussion

From the studies examined, the test item has been described as (i) 'apramycin sulfate' (one study) or (ii) 'apramycin' (unspecified form; ten studies). Therefore, for the case (ii), an uncertainty on the exact product used/concentration applied has been identified.

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

The eleven studies considered as suitable for the assessment covered only two animal categories within pigs; i.e. weaned piglets (nine studies) and pigs for fattening (two studies). In all studies, treatments contained groups of animals treated with only one apramycin concentration and did not allow to assess any dose-related effects.

In eight studies in weaned piglets, dietary apramycin supplementation at concentrations ranging between 30 and 1,500 mg/kg feed had growth-promoting/increase yield effects in piglets (Yan et al. (2012b) and Zhou et al. (2015), 30 mg/kg feed; Zhao et al. (2012) and Zhao and Kim (2015), 100 mg/kg feed; Lei et al. (2018), 150 mg/kg feed; Zhang and Kim (2014) and Hossain et al. (2016), 165 mg/kg feed; Yoon et al. (2012a), 1,500 mg/kg feed). Another study in weaned piglets reported that dietary apramycin sulfate supplementation did not affect the performance of pigs (Orban et al., 1997, 34 mg/kg feed, corresponding to 28.9 mg apramycin/kg feed).

In two studies in pigs for fattening, dietary apramycin supplementation at 30–500 mg/kg feed had growth-promoting/increase yield effects in pigs (Yan et al., 2011, 30 mg apramycin/kg feed; Yan et al., 2012a, 500 mg apramycin/kg feed).

3.3.1.5. Concluding remarks

It is judged 66–90% certain ('likely') that apramycin has growth-promoting/increase yield effects in weaned piglets at concentrations ranging from 30 to 1,500 mg/kg complete feed (eight studies), and in pigs for fattening at concentrations ranging from 30 to 500 mg/kg complete feed (two studies).

No data are available in the scientific literature showing effects of apramycin on growth promotion/increased yield when added (i) to weaned piglets feed at concentrations below 30 mg/kg, (ii) to pigs for fattening feed at concentrations below 30 mg/kg or (iii) to feed of any other food-producing animal species or categories.

3.3.2. Neomycin

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](#), resulted in 738 papers mentioning neomycin and any of the food-producing animal species considered³ and any of the performance parameters identified as relevant for the assessment of the possible growth-promoting effects of neomycin.⁴ After removing the reports not matching the eligibility criteria, 64 publications were identified.

3.3.2.2. Evaluation of the studies

The 64 publications identified in the literature search were appraised for suitability for the assessment of the effects of neomycin 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](#)).⁵ A total of 60 publications were not considered suitable for the assessment because of several shortcomings identified in their design or in the reporting of the results. The list of excluded publications and their shortcomings are presented in Appendix A.2 (Table A.2).

The publications considered suitable for the assessment are described and assessed in Section 3.3.2.3.

3.3.2.3. Assessment of the effects of neomycin on growth performance and yield

Four publications were considered suitable for the assessment of the effects of neomycin 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 neomycin used – either as the base or as any specific form/commercial preparation – and the concentration(s) applied as reported in each study; where a specific compound has been used, the calculation of the concentration applied to the base substance is provided.

3.3.2.4. Studies in pigs

In the study of Hu et al. (2014), a total of 360 weaned piglets ((Duroc × (Landrace × Yorkshire)); 26-day-old, BW 7.14 kg) were allocated to five dietary treatments and distributed in four pens per treatment, in groups of 18 pigs (both genders 1:1) per each pen. Two diets based on maize and soybean meal (Phase 1 diet until 14 days of experiment and Phase 2 diet afterwards, until 28 days of experiment) were either not supplemented (control) or supplemented with different treatments. Two were the relevant treatments: a control and a treatment consisting of neomycin sulfate supplementation in feed at 120 mg/kg feed (corresponding to 81.6 mg neomycin/kg feed). Health status was assessed every day. Animal BW and FI were recorded on days 1, 15 and 29, and ADG and G:F calculated. Faecal score was recorded twice a day. On day 28, faecal samples were collected by rectal massage from five pigs per group for bacteria counts. At the end of the study (28 days), the pigs treated with neomycin sulfate at 120 mg/kg feed, compared to the control group, showed significantly higher ADG (330 vs 299 g), improved G:F (0.612 vs 0.567) and lower diarrhoea incidence (2.94% vs 6.53%). As compared to control group, in the faecal samples of piglets treated with neomycin sulfate, a higher proportion of lactobacilli (28.8% vs 15.5% of total bacteria) and lower of *E. coli* (0.87% vs 5.25% of total bacteria) was found. Dietary neomycin sulfate supplementation at 120 mg/kg feed (corresponding to 81.6 mg neomycin/kg feed) had a growth-promoting effect in weaned piglets.

In the study of Hu et al. (2015), a total of 360 weaned piglets ((Duroc × (Landrace × Yorkshire)); 26-day-old, body weight 7.66 kg) were allocated to five dietary treatments, and distributed in four pens per treatment, in groups of 18 pigs (both genders 1:1) per each pen. Two diets (Phase 1 diet until 14 days of experiment and Phase 2 diet afterwards, until 28 days of experiment) were either not supplemented (control) or supplemented with different treatments. Two were the relevant treatments: a control and a treatment consisting of neomycin sulfate supplementation at 100 mg/kg feed (corresponding to 68 mg neomycin/kg feed). Mortality and health status were not mentioned. Animal BW and FI were recorded on days 1, 15 and 29, and ADG and G:F were calculated. Faecal score was recorded twice a day. On day 27, blood was collected from five pigs per group for haematology analysis. On day 28, faecal samples were collected by rectal massage from five piglets per group for bacteria counts. At the end of the study (28 days), the pigs treated with neomycin sulfate at 100 mg/kg feed, compared to the control group, showed significantly higher daily body weight gain (350 vs 312 g), improved G:F ratio (0.621 vs 0.564) and improved diarrhoea incidence (3.6% vs 6.6%). However, neomycin sulfate at the level of 100 mg/kg feed had no effect on blood haematology and faecal bacteria counts. Dietary neomycin sulfate supplementation at 100 mg/kg feed (corresponding to 68 mg neomycin/kg DM) had a growth-promoting effect in weaned piglets.

3.3.2.5. Studies in poultry

In the study of Amal et al. (2013), a total of 160 5-day-old, unsexed Ross 308 chickens for fattening were allocated to five dietary treatments and distributed in four pens per treatment, in groups of eight birds per pen. One basal diet was either not supplemented (control) or supplemented with different treatments. Two were the relevant treatments: a control and a treatment consisting of neomycin supplementation (unspecified form) at 16 mg/kg feed. Mortality and health status were assessed, and mortality was negligible (< 0.5% per treatment). Animal weight and cumulative FI were recorded weekly and body weight gain and feed to gain ratio F:G calculated. At the end of the experiment (42 days), blood samples were collected from all animals before slaughter to determine plasma metabolites (protein, cholesterol, urea, enzyme activities (ALP, aspartate transaminase (AST)) and minerals). At the end of the trial, chicks were fasted overnight and slaughtered, eviscerated and used to determine carcass characteristics. The birds treated with neomycin at 16 mg/kg feed, compared to the control group, showed significantly higher final BW (1,606 vs 1,511 g), higher cumulative weight gain (1,533 vs 1,440 g) and higher FI (3,289 vs 3,260 g). Dietary neomycin supplementation at 16 mg/kg feed had a growth-promoting effect in chickens for fattening.

In the study of Sugiharto et al. (2017), a total of 192 11-day-old male Lohman MB-202 chickens for fattening were allocated to four dietary treatments and distributed in six pens per treatment, in groups of eight birds per pen. One basal diet based on maize and soybean meal was either not supplemented (control) or supplemented with different treatments. Two were the relevant treatments: a control and a treatment consisting of neomycin supplementation (unspecified form) at 3 mg/kg feed. Mortality and health status were not specified. Animal BW and cumulative FI were recorded at days 10, 17 and 23 and body weight gain and F:G calculated. Twenty-four chickens with the same weight (age 28 days) were separated into individual cages to collect excreta for 2 days and measure utilisation of DM and organic matter as well as N retention. At the end of the experiment (day 23), chickens were slaughtered to sample and weight internal organs, carcass, breast, thigh, drumstick, wing muscles as well as to measure the pH, drip loss and chemical composition of breast. At the end of the trial, the birds treated with neomycin at 3 mg/kg feed, compared to the control group, showed significantly lower abdominal fat percentage (1.12% vs 1.88%) and higher crude protein content in breast (23.1% vs 22.6%). There was no effect of neomycin on the performance and on any of the carcass characteristics except the abdominal fat proportion. There was no effect of neomycin on digestibility and retention of nutrients. Dietary neomycin supplementation at 3 mg/kg feed did not have growth-promoting effects in chickens for fattening.

3.3.2.6. Discussion

From the studies examined, the test item has been described as (i) 'neomycin sulfate' (two studies) or (ii) 'neomycin' (unspecified form; two studies). Therefore, for the case (ii), an uncertainty on the exact product used/concentration applied has been identified.

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

3.3.2.6.1. Pigs

Only two studies were considered as suitable for the assessment of the effects on neomycin in pigs, covering only one animal category, i.e. weaned piglets. The studies contained groups of animals treated with only one neomycin concentration and did not allow the assessment of any dose-related effect.

In two studies in weaned piglets, dietary neomycin supplementation at 68–81.6 mg/kg feed had growth-promoting/increase yield effects (Hu et al. (2015), 100 mg neomycin sulfate/kg feed (corresponding to 68 mg neomycin/kg feed); Hu et al. (2014), 120 mg neomycin sulfate/kg feed (corresponding to 81.6 mg neomycin/kg feed)).

3.3.2.6.2. Poultry

Only two studies were considered as suitable for the assessment of the effects of neomycin in poultry, covering only one animal species and category, i.e. chickens for fattening. The studies contained groups of animals treated with only one neomycin concentration and did not allow the assessment of any dose-related effect.

Neomycin at 16 mg/kg feed promoted growth performance in chickens for fattening (Amal et al., 2013). In another study, Sugiharto et al. (2017) showed that neomycin at 3 mg/kg feed had no effect on growth performance of chickens for fattening.

3.3.2.7. Concluding remarks

It is judged 33–66% certain ('about as likely as not') that neomycin has growth-promoting/increase yield effects in weaned piglets at the concentrations ranging from 68 to 81.6 mg/kg complete feed (two studies) and in chickens for fattening at the concentration of 16 mg/kg complete feed (one study).

No data are available in the scientific literature showing effect of neomycin on growth promotion/increased yield when added (i) to weaned piglets feed at concentrations below 68 mg/kg, (ii) to chickens for fattening feed at concentrations below 16 mg/kg feed or (iii) to feed of any other food-producing animal species or categories.

3.3.3. Paromomycin

3.3.3.1. 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 72 papers mentioning paromomycin and any of the food-producing animal species considered³ and any of the performance parameters identified as relevant for the assessment of the possible growth-promoting effects of paromomycin.⁴ After removing the reports not matching the eligibility criteria, four publications were identified.

3.3.3.2. Evaluation of the studies

The four publications identified in the literature search were appraised for suitability for the assessment of the effects of paromomycin 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](#); see also the [Virtual Issue](#)).⁵ None of the publications was considered suitable for the assessment because of several shortcomings identified in their design of the study or in the reporting of the results. The list of excluded publications and their shortcomings are presented in Appendix A.3 (Table A.3).

3.3.3.3. Concluding remark

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

3.3.4. Spectinomycin

3.3.4.1. 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 292 papers mentioning spectinomycin and any of the food-producing animal species considered³ and any of the performance parameters identified as relevant for the assessment of the possible growth-promoting effects of spectinomycin.⁴ After removing the reports not matching the eligibility criteria, 15 publications were identified.

3.3.4.2. Evaluation of the studies

The 15 publications identified in the literature search were appraised for suitability for the assessment of the effects of spectinomycin 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](#); see also the [Virtual Issue](#)).⁵ None of the publications was considered suitable for the assessment because of several shortcomings identified in their design or in the reporting of the results. The list of excluded publications and their shortcomings are presented in Appendix A.4 (Table A.4).

3.3.4.3. Concluding remark

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

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

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

AQ2. Which are the specific concentrations of apramycin, neomycin, paromomycin and spectinomycin in feed of food-producing animals that have an effect in terms of growth promotion/increased yield?

With regard to apramycin:

- It is judged 66–90% certain ('likely') that apramycin has growth-promoting/increase yield effects in weaned piglets at concentrations ranging from 30 to 1,500 mg/kg complete feed (eight studies), and in pigs for fattening at concentrations ranging from 30 to 500 mg/kg complete feed (two studies).
- No data are available in the scientific literature showing effects of apramycin on growth promotion/increased yield when added (i) to weaned piglets feed at concentrations below 30 mg/kg, (ii) to pigs for fattening feed at concentrations below 30 mg/kg, or (iii) to feed of any other food-producing animal species or categories.

With regard to neomycin:

- It is judged 33–66% certain ('about as likely as not') that neomycin has growth-promoting/increase yield effects in weaned piglets at concentrations ranging from 68 to 81.6 mg/kg complete feed (two studies) and in chickens for fattening at a concentration of 16 mg/kg complete feed (one study).
- No data are available in the scientific literature showing effects of neomycin on growth promotion/increased yield when added (i) to weaned piglets feed at concentrations below 68 mg/kg, (ii) to chickens for fattening feed at concentrations below 16 mg/kg feed or (iii) to feed of any other food-producing animal species or categories.

With regard to paromomycin:

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

With regard to spectinomycin:

- Owing to the lack of suitable data, levels of spectinomycin 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. Recommendation

To carry out studies to generate the data that are required to fill the gaps which have prevented calculation of the FARSC for apramycin, neomycin, paromomycin and spectinomycin.

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Abbreviations

ADFI	average daily feed intake
ADG	average daily gain
ALP	alkaline phosphatase
AMEs	aminoglycoside-modifying enzymes
ATP	adenosine triphosphate
AQ	Assessment question
AST	aspartate transaminase
bw	body weight used in toxicity studies
BW	body weight
CFU	colony forming units
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
F:G	feed conversion ratio or feed to gain ratio
FARSC	Feed Antimicrobial Resistance Selection Concentration
FI	feed intake
G:F	gain to feed ratio
GE	fraction of the antimicrobial that is secreted back into the intestinal tract for elimination, after initially being absorbed into the bloodstream
HE	herbal extract
I	fraction of the antimicrobial present in the digestive tracts that would be inactive on the microbiota
LPS	lipopolysaccharide
MCH	mean corpuscular hemoglobin
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 _{sus}	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
MRL	maximum residues limit
MSC	minimal selective concentration
PK	pharmacokinetic
PMSC	predicted MSC
rRNA	ribosomal ribonucleic acid
ToRs	terms of reference

Appendix A – List of excluded publications and their shortcomings

A.1. Apramycin

The publications excluded from the assessment of the effects of apramycin on growth promotion/increase 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 A.1.

Table A.1: Publications not relevant for the assessment of the effects of apramycin 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)
Ahmed et al. (2013)	Pigs					X	X			
Ahmed et al. (2014a)	Pigs					X	X			
Ahmed et al. (2014b)	Pigs					X	X			
Andreotis et al. (1980)	Pigs						X			
Bovera et al. (2012)	Rabbits	X								X ⁽¹⁾
Cracknell et al. (1986)	Poultry	X					X			
Dritz et al. (1993)	Pigs	X							X	X ⁽¹⁾
Guo et al. (2004)	Poultry				X		X			
Hahn et al. (2006)	Pigs	X								
Hampson and Murdoch (2003)	Pigs						X		X	
Hong et al. (2004)	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)
Hu and McDougald (2002)	Poultry				X	X				
Jacquier et al. (2014)	Rabbits	X								
Jin et al. (2008a)	Pigs	X								
Jin et al. (2008b)	Pigs	X								
Jin et al. (2009)	Pigs	X								
Kim et al. (2005)	Pigs						X		X	
Kim et al. (2015)	Pigs				X	X	X	X		X ⁽²⁾
Kyriakis (1989)	Pigs						X			
Prieto et al. (2014)	Pigs	X								
Saives et al. (2008)	Rabbits						X			
Umesha et al. (2010)	Poultry					X	X			
Yoon et al. (2012b)	Pigs								X	

(1): No negative control.

(2): Only 1 replicate pen/treatment.

A.2. Neomycin

The publications excluded from the assessment of the effects of neomycin 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 A.2.

Table A.2: Publications not relevant for the assessment of the effects of neomycin 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)
Abeer et al. (2019)	Poultry				X	X	X			
Al-Sagan and Abudabos (2017)	Poultry	X			X	X	X			
Berge et al. (2005)	Ruminants	X								
Berge et al. (2009)	Ruminants	X								
Bergstrom et al. (2007a)	Pigs	X								
Bergstrom et al. (2007b)	Pigs	X								
Bergstrom et al. (2007c)	Pigs	X								
Dennis et al. (2019)	Ruminants	X								
Donovan et al. (2002)	Ruminants	X								
Dritz et al. (1993)	Pigs	X							X	X ⁽¹⁾
Dritz et al. (2002)	Pigs	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)
Fomenky et al. (2017)	Ruminants	X								
Frantz et al. (2004)	Pigs	X								
Gargallo and Zimmerman (1980)	Pigs		X ⁽²⁾					X		
Goetsch and Owens (1986a)	Ruminants	X	X ⁽²⁾							
Goetsch and Owens (1986b)	Ruminants	X	X ⁽²⁾							
Gottlob et al. (2004)	Pigs	X								
Gottlob et al. (2005a)	Pigs	X								
Gottlob et al. (2005b)	Pigs								X ⁽³⁾	
Gottlob et al. (2005c)	Pigs								X ⁽³⁾	
Gottlob et al. (2006a)	Pigs								X ⁽³⁾	
Gottlob et al. (2006b)	Pigs	X								
Gottlob et al. (2007)	Pigs								X ⁽³⁾	
Greenfield et al. (1973)	Poultry	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)
Heinrichs et al. (2003)	Ruminants	X								
Heinrichs et al. (2009)	Ruminants	X								
Hildabrand et al. (2004)	Pigs	X								
Jesse et al. (1988)	Pigs	X								
Kareem et al. (2015)	Poultry	X								
Keegan et al. (2003)	Pigs	X								
Keegan et al. (2005)	Pigs	X								
Kehoe and Carlson (2015)	Ruminants	X								
Kim et al. (2011)	Ruminants								X	
Le Marrett et al. (2000)	Poultry				X	X ⁽⁴⁾	X			
Lee et al. (2011)	Pigs	X								
LeMieux et al. (2003)	Pigs	X								
Loh et al. (2010)	Poultry	X								
May et al. (2012)	Pigs	X								
Mukhtar et al. (2013)	Poultry									X ⁽⁵⁾

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)
Neill et al. (2004)	Pigs	X								
Neill et al. (2005)	Pigs	X								
Neill et al. (2006)	Pigs	X								
Onifade (1997)	Poultry	X								
Onifade and Babatunde (1997)	Poultry	X								
Pereira et al. (2019)	Poultry	X								
Quigley III and Drew (2000)	Ruminants	X			X	X				
Quigley III et al. (1997)	Ruminants	X			X	X				X ⁽⁶⁾
Rosyidah et al. (2011)	Poultry	X								
Salaheen et al. (2017)	Poultry	X								
Sarker et al. (2010)	Ruminants								X	
Shelton et al. (2009)	Pigs	X								
Shields et al. (2010)	Ruminants	X								X ⁽⁶⁾
Shrimpton et al. (1958)	Poultry	X								X ⁽⁷⁾

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)
Simon et al. (2016)	Poultry	X						X ⁽⁸⁾		X ⁽⁹⁾
Sulabo et al. (2007)	Pigs	X								
Tang et al. (2015)	Pigs				X	X				
Thanh et al. (2009)	Poultry	X							X	
Touchburn and Nestor (1971)	Poultry	X							X	
Williams (1985)	Poultry	X								
Yen et al. (1987)	Pigs								X	

- (1): No negative control.
 (2): Cannulated animals.
 (3): Antimicrobial administered via water for drinking and data on water consumption not available.
 (4): Naturally challenged with litter from turkey flocks with colibacillosis.
 (5): The study shows the same results as Amal et al. (2013) and has been considered as a repetition.
 (6): Additional antimicrobial therapy.
 (7): Antimicrobial was administered 24 h before death as a mean to reduce carcass spoilage.
 (8): Only BW reported.
 (9): The authors investigated the immune response of multi-drugs treated (dysbiotic) animals.

A.3. Paromomycin

The publications excluded from the assessment of the effects of paromomycin 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 A.3.

Table A.3: Publications not relevant for the assessment of the effects of paromomycin 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	Zotechnical parameters not reported	Insufficient reporting/statistics	Other (indicate)
Bleyen et al. (2009)	Poultry				X	X				
Fayer and Ellis (1993)	Ruminants					X				
Hafez et al. (2010)	Poultry					X				
Hu and McDougald (2004)	Poultry				X	X				

A.4. Spectinomycin

The publications excluded from the assessment of the effects of spectinomycin 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 A.4.

Table A.4: Publications not relevant for the assessment of the effects of spectinomycin 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)
Bains (1974)	Poultry	X			X		X			X ⁽¹⁾
Berge et al. (2009)	Ruminants	X			X		X			
George et al. (1977)	Poultry	X			X	X				
Goren et al. (1988)	Poultry				X	X			X	
Hamdy (1974)	Pigs				X	X	X			
Hamdy and Blanchard (1969)	Poultry				X	X	X		X ⁽²⁾	
Hamdy et al. (1976)	Poultry	X			X	X	X			
Hamdy et al. (1979)	Poultry	X			X	X	X			
Hamdy et al. (1982)	Poultry	X			X	X	X			X ⁽¹⁾⁽³⁾
Jordan et al. (1998)	Poultry	X			X	X				
Kolar and Seymour (1974)	Poultry								X ⁽⁴⁾	

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)
McOrist et al. (2000)	Pigs	X			X		X	X		
Ortiz et al. (1995)	Poultry	X			X	X				
Tsiloyiannis et al. (2001)	Pigs	X					X			
Zwirzitz et al. (2019)	Pigs	X								X ⁽⁵⁾

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

(2): No mention made of statistical analyses in Methods, Results or Tables.

(3): Additional subcutaneous administration of spectinomycin to all birds.

(4): Antimicrobial administered via water for drinking and data on water consumption not available.

Appendix B – Table of uncertainties

Uncertainties associated to the Growth promotion assessment

Table B.1: Potential sources of uncertainty identified in the levels of apramycin 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 Scientific Opinion Part 1 (see also the Virtual Issue), although meta-analysis was not applicable to the studies retrieved, evidence synthesis was done, since: <ul style="list-style-type: none"> • 8 studies showing consistent (positive) results in a comparable range of concentrations were available in weaned piglets. The uncertainty resulting in the process of evidence synthesis was based on 9 studies, 8 showing positive effect, 1 showing no effects; • 2 studies showing consistent (positive) results in a comparable range of concentrations were available in pigs for fattening. Consistency of results across categories (i.e. piglets and pigs for fattening) would reduce the uncertainty in the conclusions for both categories. 	The extent of the underestimation or overestimation on the levels which shown growth-promoting effect is modulated by the consistency of the results.

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