The European Agency for the Evaluation of Medicinal Products
Veterinary Medicines and Inspections

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COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

COLISTIN

SUMMARY REPORT (2)

1. Colistin (CAS: 1066-17-7) is a cyclopeptide antibiotic produced by cultures of *Bacillus polymyxa* var. *colistinus*. In veterinary medicine it is normally used as the sulphate salt for oral preparations and as the methanesulphonate for parenteral administration. It belongs to the polymyxin therapeutic class and is identical to Polymixin E. Colistin is used for the prevention and treatment of diseases caused by sensitive bacteria (e.g. *Salmonella and Escherichia coli*) in rabbits, pigs, poultry, cattle, sheep and goats. It is used in poultry producing eggs for human consumption and cattle, sheep and goats producing milk for human consumption. It is usually administered orally, at 100,000 IU/kg bw/day for 5 to 10 days as a drench or in the feed, drinking water or milk replacer diet. There are also products available for parenteral and intramammary administration.

   Currently, colistin is included in Annex III of Council Regulation (EEC) No. 2377/90 in accordance with the following table:

Pharmacologically active substance(s)	Marker residue	Animal Species	MRLs	Target tissues	Other provisions
Colistin	Colistin	Bovine, ovine, porcine, chicken, rabbits	150 µg/kg 150 µg/kg 150 µg/kg 200 µg/kg	Muscle Fat Liver Kidney	Provisional MRLs expire on 1.7.2002
		Bovine, ovine	50 µg/kg	Milk	
		Chicken	300 µg/kg	Eggs	

2. Additional data were provided in response to the list of questions, further to the establishment of provisional MRLs for colistin, only for bovine, porcine, chickens and rabbits.

3. Colistin is effective primarily against gram-negative microorganisms. It causes disorganisation of the bacterial cell membrane with leakage of intracellular materials and it inhibits bacterial oxidative metabolism. Resistance to colistin is uncommon.

4. The unit of colistin is defined as the minimal concentration which inhibits the growth of *Escherichia coli* 95 I.S.M. in 1 ml broth at pH 7.2. Pure colistin base has been assigned a potency of 1000 µg base activity/mg (30,000 IU/mg). The theoretical potency of colistin sulphate is 800 µg base activity/mg (24,000 IU/mg).

5. No evidence of neurotoxicity was observed in mice following subcutaneous doses of 18 mg/kg b.w. of colistin sulphate. In humans, neurotoxicity is associated with over-dosage or failure to reduce the dose in patients with renal insufficiency. Intravenous administration of 0.5-6.5 mg/kg b.w. of colistin sulphate to anaesthetised dogs produced a dose-related decrease in blood pressure. There have been no reports of an effect on blood pressure in humans, with normal renal function, given normal therapeutic doses of colistin.

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6. In humans, laboratory animals and the target species, colistin sulphate was very poorly absorbed after oral administration. Plasma concentrations after oral administration were usually undetectable. However some limited absorption of colistin from the gastro-intestinal tract has been reported in human children less than 6 months of age. Absorption was much better following intramuscular administration; peak plasma concentrations were obtained approximately 2 hours after dosing. Administration of sodium colistin methanesulphonate resulted in higher blood concentrations than colistin sulphate. In laboratory animals, colistin was bound to plasma protein; the extent of the binding decreased with increasing dose and was higher for colistin sulphate when compared with the sodium methanesulphonate derivative.

7. There was some evidence from a dog study for the formation of a microbiologically-inactive metabolite. However no metabolites of colistin were identified. Excretion was *via* the urine following parenteral administration with no detectable residues found in faeces. Orally-administered colistin was excreted in the faeces and the results of a human study suggested that colistin was "bound" to faeces.

8. Colistin sulphate was of moderate to low acute oral toxicity but was of higher acute toxicity when administered parenterally. The values of the LD_{50} were variable and reflected the differences in potency and purity of the material from the different sources. Acute oral LD_{50} were in the range 452-1366 mg/kg b.w..

9. A repeat-dose study was carried out in which Wistar rats were fed diets containing 0, 40, 200 or 1000 mg/kg feed of colistin sulphate for 26 weeks. There were some changes in organ weights at the top dose level of 1000 mg/kg feed but no corresponding pathological changes. The NOEL was 200 mg/kg feed, equivalent to 12.5 mg/kg b.w. per day.

10. A repeat-dose study was carried out in which Sprague-Dawley rats were fed diets containing "pure" and "feed grade" colistin sulphate corresponding to 0, 2, 40 and 120 mg/kg b.w. per day for 26 weeks. Some changes in organ weights were observed at the top dose level of 120 mg/kg b.w. but there were no corresponding pathological findings. The NOEL was 40 mg/kg b.w. per day. According to the published summary of a 90-day rat study, oral doses of up to 60 mg/kg b.w. per day of colistin sulphate had no adverse effects; in contrast, dose-related nephrotoxicity was observed in rats given daily intramuscular injections of 0.83 - 7.5 mg/kg b.w. per day (expressed as base) of sodium colistin methanesulphonate.

11. According to a published report, no adverse effects on behaviour, growth rate, haematology, clinical chemistry or urinalysis values and no gross- or histo-pathological changes were found in a 90-day study in which dogs were oral doses of 6.67, 20 or 60 mg/kg b.w. per day. There were insufficient details about the experiment to allow firm conclusions to be drawn regarding a NOEL.

12. No classical multigeneration studies were carried out. However there were 2 combined fertility and teratogenicity studies, and 2 further teratogenicity studies, one of which incorporated a littering phase to investigate perinatal and postnatal development of the offspring and the offspring from this study were mated. Consequently all the required end-points were studied. Some of the reproductive toxicity studies were carried out with sodium colistin methanesulphonate which was of lower toxicity and had pharmacokinetics which were different from those of colistin sulphate; consequently it was not possible to extrapolate the NOELs established in these studies directly to colistin sulphate. However it was possible to conclude that colistin did not affect male or female fertility in the rat or the mouse. In addition, colistin was not teratogenic in the rat, the rabbit or the mouse. The NOELs for foetotoxicity and teratogenicity in a rat study with colistin sulphate were greater than 130 mg/kg b.w. per day, the highest dose level administered. When administered parenterally as sodium colistin methanesulphonate, there was evidence of foetotoxicity (delayed ossification) and reduced pup survival at a dose level of 25 mg/kg b.w. per day; 12.5 mg/kg b.w. per day was a NOEL in this study.

13. Colistin sulphate was not mutagenic in *in vitro* bacterial assays for gene mutation, in an *in vitro* assay for gene mutation in mammalian cells nor in an *in vivo* micronucleus test.

14. No carcinogenicity studies were carried out. The absence of such studies was justified by the negative results obtained in the mutagenicity studies and the absence of any structurally-alerting features in the chemical structure of colistin sulphate.

15. A toxicological ADI of 0 - 62.5 µg/kg b.w. per day was calculated by applying a "safety factor" of 200 to the NOEL of 12.5 mg/kg b.w. per day which was established in the 26-week repeat-dose study in Wistar rats. The "safety factor" of 200 was justified because the reporting of the study was not to modern standards.

16. *In vitro* MIC studies showed that gram positive micro-organisms and *Proteus spp*. were not susceptible to colistin sulphate. Of the microorganisms most relevant to the human gut flora, *E. coli* was the most sensitive with an *in vitro* MIC50 of 0.10 µg/ml. A microbiological ADI was calculated as follows:

$$\text{ADI (µg/kg bw)} = \frac{\dfrac{\text{geometric mean } MIC_{50} \text{ x CF2}}{\text{CF1}} \text{ (µg/ml) x daily faecal bolus (150 ml)}}{\text{fraction of an oral dose available for microorganisms} \quad \text{x weight of human (60 kg)}}$$

Based on the above formula, the microbiological ADI can be calculated as follows:

$$\text{ADI} = \frac{\dfrac{0.1 \times 10}{1} \times 150}{0.5 \times 60} = 5 \text{ µg/kg bw i.e.} = 300 \text{ µg/person}$$

The following assumptions were made:

- CF1 = 1 because the MIC_{50} for the most sensitive predominant organism was used;
- CF2 = 10 to correct for the difference in growth conditions between the *in vitro* and *in vivo* situations;
- 150 g was the weight of the daily faecal bolus;
- 0.10 µg/ml is the MIC_{50} for the most sensitive predominant microorganism;
- 0.5 is the fraction of the oral dose available to the bacteria at the distal part of the gastrointestinal tract;
- 60 kg is the human body weight.

17. Six healthy human volunteers were given daily oral doses of 0.45 g colistin sulphate for 3 consecutive days. Faeces were collected before and after treatment. The enterobacteriaceae were eliminated in all volunteers between 24 and 48 hours after treatment started, with the exception of one volunteer carrying *Proteus mirabilis* which persisted throughout the treatment. All 6 volunteers were progressively recolonised by colistin-sensitive enterobacteriaceae in the days following the withdrawal of treatment. With the exception of the *Proteus*-carrier, none of the volunteers was recolonised with colistin-resistant bacteria in the course of the study. The sizes of the group D streptococcal population, the staphylococcal population, yeasts and total anaerobes were not significantly affected by treatment. Because of the limitations of the study, it was not considered appropriate to use the results of the study as a basis for establishment of a microbiological ADI.

18. Published data indicated that colistin was not particularly active against the types of microorganisms used in industrial food processing. For example, the *in vitro* MIC values for Lactobacillus spp were in the range 12.5-100 µg/ml.

19. The microbiological ADI is lower than the toxicological ADI and therefore it was considered to be the most relevant ADI for assessing the risk to consumers. This resulted in an ADI of 5 µg/kg bw, equivalent to 0.3 mg/person.

20. Pharmacokinetic data in the target species confirmed that colistin sulphate was poorly absorbed after oral administration to calves, pigs and rabbits as tissue and serum concentrations were generally undetectable in these species. In chickens, residues in serum were detectable for up to 6 hours after administration in the drinking water.

21. In contrast, residues of colistin were detectable in serum for up to 24 hours after intramuscular or intravenous administration to calves and dairy cows. In calves, bioavailability approached 100% after intramuscular administration. In ewes, peak serum concentrations of 8-20 µg/ml were achieved 2 hours after intramuscular injection.

22. In a non- radiometric residue depletion study, 4 calves (2 males and 2 females) were feed twice daily with replacement milk medicated with colistin equivalent to 100,000 IU (3.33 mg) colistin/kg bw/day for 7 consecutive days. All of the calves were killed 6 hours after the last treatment and their tissues analysed for colistin concentrations by a method based on HPLC (fluorescence) with limits of quantification of 75 µg/kg for liver, muscle and fat and 100 µg/kg for kidney. The colistin concentrations in all edible tissues were below their respective limits of quantification.

    In another study, 20 calves were given replacement milk medicated with colistin equivalent to 114,000 IU colistin/kg bw/day for 15 consecutive days. Four calves, 2 per sex, were killed at 2 hours, 1, 2, 3 and 5 days after treatment. Tissue samples were analysed for colistin concentrations by HPLC and a microbiological assay the limits of quantification of which were both 75 µg/kg in liver, muscle and fat, and 100 µg/kg in kidney. In kidney the mean residue with antimicrobial activity concentrations were 210, 98, 150, less than 100, and less than 100 µg/kg at 2 hours, 1, 2, 3 and 5 days after treatment and respectively. The colistin and the residue with antimicrobial activity concentrations in all other edible tissues were below their respective limits of quantification.

23. Some data were available on the depletion of residues in calves following intravenous injection; highest residues were found in the liver and kidney and were present chiefly as "bound" residues. There was no information concerning residues at the injection site. Only 2 calves were slaughtered at each time point.

24. In a non- radiometric residue depletion study, 4 pigs (2 males and 2 females) were fed twice daily with feed medicated with colistin equivalent to 100,000 IU (3.33 mg) colistin/kg bw/day for 7 consecutive days. All of the pigs were killed 6 hours after the last treatment. Tissue samples were analysed for colistin concentrations by a method based on HPLC(fluorescence) with limits of quantification of 75 µg/kg for liver, muscle and skin plus fat and 100 µg/kg for kidney. The colistin and the residue with antimicrobial activity concentrations in all edible tissues were below their respective limits of quantification.

    In another study, 20 pigs were given feed medicated with colistin equivalent to 114,000 IU colistin/kg bw/day for 15 consecutive days. Four pigs, 2 per sex, were killed at 2 hours, 1, 2, 3 and 5 days after treatment. Tissue samples were analysed for colistin concentrations by HPLC and a microbiological assay the limits of quantification of which were both 75 µg/kg in liver, muscle and skin plus fat, and 100 µg/kg in kidney. The colistin concentrations in all edible tissues were below their respective limits of quantification.

25. In a non- radiometric residue depletion study, 3 chickens (3 males and 3 females) were fed twice daily with feed medicated with colistin equivalent to 100,000 IU (3.33 mg) colistin/kg bw/day for 5 consecutive days. All of the chickens were killed 6 hours after the last treatment and their tissues analysed for colistin concentrations by a method based on HPLC (fluorescence) with limits of quantification of 75 µg/kg for liver, muscle and skin plus fat and 100 µg/kg for kidney. The colistin concentrations in all edible tissues were below their respective limits of quantification.

    In another study, 30 broiler chickens were given feed medicated with colistin equivalent to 114,000 IU colistin/kg bw/day for 15 consecutive days. Six chickens, 3 per sex, were killed at 2 hours, 1, 2, 3 and 5 days after treatment. Tissue samples were analysed for colistin concentrations by HPLC and a microbiological assay the limits of quantification of which were both 75 µg/kg in liver, muscle and skin plus fat, and 100 µg/kg in kidney. The colistin and the residue with

antimicrobial activity concentrations in all edible tissues were below their respective limits of quantification.

26. In a non- radiometric residue depletion study, 6 turkeys (3 males and 3 females) were fed twice daily with feed medicated with colistin equivalent to 100,000 IU (3.33 mg) colistin/kg bw/day for 5 consecutive days. All of the turkeys were killed 6 hours after the last treatment. Tissue samples were analysed for colistin concentrations by a method based on HPLC (fluorescence) with limits of quantification of 75 µg/kg for liver, muscle and skin plus fat and 100 µg/kg for kidney. The colistin concentrations in all edible tissues were below their respective limits of quantification.

27. In a non- radiometric residue depletion study, 12 rabbits (6 males and 6 females) were fed twice daily with feed medicated with colistin equivalent to 100,000 IU (3.33 mg) colistin/kg bw/day for 5 consecutive days. Four rabbits, 2 per sex per time point, were killed 6, 24 and 48 hours after the last treatment. Tissue samples were analysed for colistin concentrations by a method based on HPLC (fluorescence) with limits of quantification of 75 µg/kg for liver, muscle and skin plus fat and 100 µg/kg for kidney. The colistin concentrations in all edible tissues were below their respective limits of quantification.

    In another study, 30 rabbits were given feed medicated with colistin equivalent to 114,000 IU colistin/kg bw/day for 15 consecutive days. Six rabbits, 3 per sex, were killed at 2 hours, 1, 2, 3 and 5 days after treatment. Tissue samples were analysed for colistin concentrations by HPLC and a microbiological assay the limits of quantification of which were both 75 µg/kg in liver, muscle and skin plus fat, and 100 µg/kg in kidney. The colistin and the residue with antimicrobial activity concentrations in all edible tissues were below their respective limits of quantification.

28. Residues in milk following intramuscular administration to dairy cows were detectable for the first 2-6 milkings after treatment. Residues after intramammary infusion were significantly higher but were undetectable by the 7th milking after treatment. Milk from only 5 cows per treatment was used in these experiments. In sheep milk, peak concentrations in milk of 2 µg/ml were found 2 hours after intramuscular administration; approximately 10% of the residues in sheep milk were "bound".

    In new study, 10 cows (5 at a high yielding early stage of lactation and 5 a low-yielding late stage of lactation) were given an intramuscular injection of 10 mg amoxicillin and 25,000 IU of colistin per kg bw per day for 5 consecutive days. The cows were milked twice a day at 12 hour intervals and the colistin concentration in milk determined by an HPLC (fluorescence) based method with a limit of quantification of 10 µg/kg. Between 24 hours after administering the first and last injection the mean colistin concentration in milk was around 50 µg/kg (i.e. in the range 30 to 109 µg/kg). The mean colistin concentrations in milk were 13 (i.e. in the range less than 10 to 33 µg/kg), 8 (i.e. in the range less than 10 to 16 µg/kg) and less than 10 µg/kg (i.e. in the range less than 10 to 12 µg/kg) on days 2, 3 and 4 after treatment respectively.

    In another new study, 8 lactating cows (4 at an early high yielding stage of lactation and 4 at a late low milk yielding stage of lactation) were given feed medicated with colistin equivalent to 100,000 IU colistin/kg bw/day for 10 consecutive days. Cows were milked twice daily at 12 hour intervals and the concentrations of colistin and antimicrobial residue in whole milk were tested by HPLC and a microbiological assay; the limits of quantification of which were both 25 µg/kg. The colistin and the residue with antimicrobial activity concentrations in all milk samples were below their respective limits of quantification.

29. Residues in eggs from hens given colistin sulphate in the drinking water were below the limit of detection of the analytical method. Significant residues were found for up to 8 days in eggs, following intramuscular injection to hens.

    In a new study, 20 laying chickens were given feed medicated with colistin equivalent to a dose of 114,000 I.U. colistin/kg bw/day for 15 consecutive days. Eggs were collected at regular time intervals before during and after treatment and whole eggs (yolk plus white) were analysed by HPLC and a microbiological assay; the limits of quantification of which were both 150 µg/kg. The colistin and antimicrobial residue concentrations in all of the eggs collected on treatment days before treatment and on days 1, 3, 5, 7 and 10 after treatment were less than the limit of quantification.

30. A screening assay based on the antimicrobial inhibition of *Bordetella bronchisseptica* was presented with validation data in support of a detection limit of around 100 µg/kg in porcine edible tissues.

Routine analytical methods based on HPLC (with fluorescence detection) were presented in the ISO 78/2 format. Colistin was measured as the sum of the peak areas for polymyxins E_1 and E_2. These methods had been validated to meet the requirements of Volume VI of the Rules Governing Medicinal Products in the European Community for colistin determination in edible tissues from cattle, pigs, chickens, turkeys, rabbits, chicken's eggs and cow's milk. The limits of quantification of the methods were 75 µg/kg for liver, muscle, and fat (or skin plus fat), 100 µg/kg for kidney, 150 µg/kg in eggs and 10 µg/kg in cow's milk.

Conclusions and recommendations

Having considered that:

- a microbiological ADI of 5 µg/kg bw (i.e. 300 µg/person) was established for colistin,
- the absence of data on the concentration of residues in edible tissues following parenteral administration of colistin,
- residues with antimicrobial activity in the edible tissues of cattle, pigs, chickens, turkeys, rabbits, chicken's eggs and cow's milk following oral administration of colistin depleted so that at 6 hours after treatment the amount of residues likely to be ingested by consumers represents a minor fraction of the microbiological ADI (i.e. 33% made up of less than 15% in edible tissues, less than 5% in eggs and less than 13% in milk),
- the MRLs elaborated are twice the respective limits of quantification of the proposed routine analytical methods,
- validated analytical methods based on HPLC with fluorescence detection for the determination of colistin in the edible tissues of cattle, pigs, chickens, turkeys, rabbits, and hens' eggs and cows' milk were available; the methods were considered to be applicable to the edible tissues, eggs and milk of other species,
- the proposed MRLs for bovine, porcine, rabbits chickens and turkeys were identical; therefore extrapolation of these MRLs to all food producing species could be made, in accordance with the Note for Guidance on Risk Analysis Approach for Residues of Veterinary Medicinal Products in Food of Animal Origin (EMEA/CVMP/187/00-FINAL);

the Committee for Veterinary Medicinal Products recommends the inclusion of colistin in Annex I of Council Regulation (EEC) No 2377/90 in accordance with the following table:

Pharmacologically active substance(s)	Marker residue	Animal Species	MRLs	Target tissues	Other provisions
Colistin	Colistin	All food producing species	150 µg/kg 150 µg/kg 150 µg/kg 200 µg/kg 50 µg/kg 300 µg/kg	Muscle* Fat** Liver Kidney Milk Eggs	

*For fin fish this MRL relates to "muscle and skin in natural proportions".

**For porcine and poultry species this MRL relates to "skin and fat in natural proportions".

Based on these MRLs the daily intake of residue is 183 µg which represents 61% of the microbiological ADI.