

COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

COLISTIN

SUMMARY REPORT (1)

1. Colistin is a cyclopeptide antibiotic produced by cultures of *Bacillus polymyxa* var. *colistinus*. 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 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, as a drench or in the feed, drinking water or milk replacer diet. There are also products available for parenteral and intramammary administration.
2. Colistin is effective primarily against gram-negative micro-organisms. It causes disorganisation of the bacterial cell membrane with leakage of intracellular materials and it inhibits bacterial oxidative metabolism. Resistance to colistin is uncommon.
3. 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).
4. 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.
5. 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 under 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.
6. 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.
7. Colistin sulphate was of moderate to low acute oral toxicity but was of higher acute toxicity when administered parenterally. The values of the LD₅₀ were variable and reflected the differences in potency and purity of the material from the different sources. Acute oral LD₅₀ were in the range 452 to 1366 mg/kg b.w..

8. A repeat-dose study was carried out in which Wistar rats were fed diets containing 0, 40, 200 or 1000 ppm of colistin sulphate for 26 weeks. There were some changes in organ weights at the top dose level of 1000 ppm but no corresponding pathological changes. The NOEL was 200 ppm, equivalent to 12.5 mg/kg b.w. per day.
9. 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.
10. 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.
11. 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 >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.
12. 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.
13. 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.
14. 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.
15. *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:

$$\text{Upper limit of ADI} = \frac{0,1 \times 10}{1 \times 150} \div \frac{0,5 \times 60}{} = 5 \mu\text{g/kg b.w. per day.}$$

The following assumptions were made:

- 0.10 µg/ml is the MIC50 for the most sensitive predominant microorganism
 - 150 g is the weight of the daily faecal bolus
 - CF1 = 1 because the MIC50 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
 - 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.
16. 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.
 17. 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.
 18. Pharmacokinetic data in the target species confirmed that colistin sulphate was poorly absorbed after oral administration to calves, pigs and rabbits 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.
 19. 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.
 20. Residues in edible tissues after oral administration to calves, pigs, rabbits and chickens were usually below the limit of detection of the analytical method. These studies were not in accordance with the requirements of Volume VI of the "Rules Governing Medicinal Products in the European Community" in terms of numbers of animals and birds slaughtered per time point. There were no residues depletion studies in sheep or goats.
 21. 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.
 22. 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".
 23. 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.

24. Taking into account the pattern of residues depletion and the capabilities of the analytical method, the following provisional MRLs were elaborated:

Pharmacologically active substance	Marker residue	Animal species	MRLs	Target tissues	Other provisions
Colistin	Colistin	Bovine, ovine, porcine, rabbits, chickens	150 µg/kg 200 µg/kg 150 µg/kg 150 µg/kg	Liver Kidney Muscle Fat	Provisional MRLs expire on 01/07/2000
		Bovine, ovine	50 µg/kg	Milk	
		Chickens	300 µg/kg	Eggs	

It was not possible to elaborate MRLs for goat meat or milk because there were no residues depletion data and no comparative pharmacokinetic data which would have permitted extrapolation from another species.

Based on these MRLs, it was calculated that consumer intake of residues from the consumption of 0.5 kg meat tissues, 1.5 litres milk and 0.1 kg eggs would be 182.5 µg/day. This represented 60% of the upper limit of the ADI of 0 - 300 µg/day, calculated above.

25. The proposed routine analytical method for the determination of residues was a microbiological assay based on *Bordetella bronchiseptica* ATCC 4617 as the indicator organism. The following Limits of Quantification were claimed:

Muscle	67 µg/kg
Kidney	100 µg/kg
Liver	67 µg/kg
Fat	67 µg/kg
Milk	25 µg/kg
Eggs	200 µg/kg for albumen; 100 µg/kg for yolk

Data on accuracy and precision were provided for milk and meat tissues but not for eggs. The method was not acceptable because specificity was not evaluated. A specific routine analytical method, validated in accordance with Volume VI of the "Rules Governing Medicinal Products in the European Community", was required.

LIST OF QUESTIONS

1. A specific routine analytical method, validated in accordance with Volume VI of the “Rules Governing Medicinal Products in the European Community”, should be provided.
2. Information should be provided on the relationship between the concentrations of residues in tissues, milk and eggs, determined by a specific method, with the likely concentrations determined by the non-specific microbiological method.
3. Residues depletion data should be provided for each major indicated species using the numbers of animals or birds specified in Volume VI of the “Rules Governing Medicinal Products in the European Community”.
4. A scientific justification should be provided for the absence of residues depletion data for the “minor” indicated species.

This information should be provided to the CVMP by 1 July 1999.