COLISTIN

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IDENTITY

International Non-proprietary names (INN): Colistin sulphate, Colistimethate sodium (sodium colistin methanesulfonate)

Synonyms Polymyxin E_1 = Colistin A, Polymyxin E_2 = Colistin B, Polymyxin E sulfate = Colistin sulphate, Colistini sulfas, Multimycine, Colymycin, First Guard Sterile Powder

International Union of Pure and Applied Chemistry (IUPAC) Names

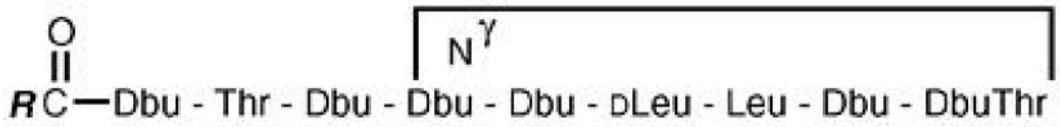
Colistin A: N-[3-amino-1-[[1-[[3-amino-1-[[6,9,18-tris(2-aminoethyl)-3-(1-hydroxyethyl)-12,15-bis(2-methylpropyl)-2,5,8,11,14,17,20-heptaoxo-1,4,7,10,13,16,19-heptazacyclotricos-21-yl]carbamoyl]propyl]carbamoyl]-2-hydroxy-propyl]carbamoyl]propyl]-6-methyl-octanamide

Colistin B: N-[3-amino-1-[[1-[[3-amino-1-[[6,9,18-tris(2-aminoethyl)-3-(1-hydroxyethyl)-12,15-bis(2-methylpropyl)-2,5,8,11,14,17,20-heptaoxo-1,4,7,10,13,16,19-heptazacyclotricos-21-yl]carbamoyl]propyl]carbamoyl]-2-hydroxy-propyl]carbamoyl]propyl]-5-methyl-heptanamide

Colistimethate sodium: pentasodium[3-[[3-(1-hydroxyethyl)-12,15-bis(2-methylpropyl)-2,5,8,11,14,17,20-heptaoxo-6,9,18-tris[2-(sulfonatomethylamino)ethyl]-1,4,7,10,13,16,19-heptazacyclotricos-21-yl]carbamoyl]-3-[3-hydroxy-2-[2-(6-methyloctanoylamino)-4-(sulfonatomethylamino)butanoyl]amino-butanoyl]amino-propyl]aminomethanesulfonate

Chemical Abstract Service Number: Colistin base: CAS 1066-17-7
Colistin sulfate: CAS 1264-72-8
Colistimethate sodium: CAS 8068-28-8

Structural formula of the main components:



Dbu is α ,γ – diaminobutyric acid.
R = 5-methylheptyl (iso-octyl) in colistin A
R = 5-methylhexyl in colistin B

Molecular formula:

Colistin A:	$C_{53}H_{100}N_{16}O_{13}$
Colistin B:	$C_{52}H_{98}N_{16}O_{13}$
Colistimethate sodium:	$C_{58}H_{105}N_{16}Na_5O_{28}S_5$

Molecular weight: (The Merck Index, 2001, European Pharmacopoeia 5.0, 01/2005:0319; European Pharmacopoeia 5.3, 01/2006:320):

Colistin A:	1169.460
Colistin B:	1155.430
Colistimethate sodium:	1749.840

OTHER INFORMATION ON IDENTITY AND PROPERTIES

Pure active ingredient: Colistin (as sulfate or methansulphonate salt)

Appearance: Crystalline powder

Melting point: 215-219 ºC (colistin sulfate)
222-223 ºC (colistimethate sodium)

Solubility: Colistin salts are freely soluble in water. At 20º C, less than 1mL of water is required to dissolve 1 g colistin.
Colistin salts are practically insoluble in ether, acetone and chloroform and slightly soluble in methyl alcohol. At 20º C, more than 10,000 mL of these solvents are required to dissolve 1 g colistin

Optical rotation: Polymyxin $E_1 = [\alpha]_{5461}^{22}$ = -93.3° (2% Acetic Acid)

Polymyxin $E_2 = [\alpha]_{5461}^{22}$ = -94.5° (2% Acetic Acid)

UVmax: 220 nm

RESIDUES IN FOOD AND THEIR EVALUATION

Conditions of Use

An antibiotic originally named "colimycin" was first isolated by Koyama et al, from the broth of *Bacillus polymyxa* var. *colistinus* in 1950 (Koyama et al., 1950). Colistin comprises a multi-component family of polymyxins. It differs from polymyxin B, the other therapeutically used polymyxin, only by one amino acid in position 6 (D-Leucine in colistin, Phenylalanine in polymyxin B). The general structure comprises a cyclic heptapeptide moiety with a straight tripeptide side chain. The peptide contains six L-α,γ-diaminobutyric acid (DAB) residues. The peptides are cyclised through the α-amino and carboxyl groups of the DAB residue in position 4. The linear peptide chain is attached through the γ-amino group of this residue. The N-terminal amino group in the side chain is acylated.

Although colistin was first separated into three components, A, B, and C, in 1953, the basic structure of these components was elucidated in the years 1953-1965 (Suzuki et al., 1963a; Suzuki et al., 1963b; Suzuki et al., 1963c; Studer et al., 1965).

Several (hyphenated) chromatographic techniques and chemical syntheses have been used to analyze the complex composition of these products further and this has resulted in the discovery of a great number of minor components (Thomas et al., 1980; Elverdam et al., 1981; Ikai et al., 1998; Kline et al., 2001; Orwa et al., 2001; Govaerts et al., 2002; Govaerts et al., 2003).

Colistins are highly effective against strains of *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella* spp., *Haemophilus* spp., *Shigella* spp., *Pasteurella* spp., *Brucella* spp., *Aerobacter aerogenes*, and *Bordetella bronchiseptica*. Gram-positive bacteria are generally less sensitive. However, there are sensitive strains of *Staphylococcus* spp., *Bacillus* spp., *Streptococcus pyogenes* and *Corynebacterium* spp. (Storm et al., 1977).

The polymyxin broad-spectrum of activity against Gram-negative bacteria involves binding to lipid A, the anchor for lipopolysaccharide, the main constituent of the outer membrane of these bacteria. The presence on the (positively charged) cyclic heptapeptide of both the tripeptide and the terminal acyl group are considered necessary for the full bactericidal effects of colistin (Nakajima, 1967). Polymyxins containing the longer 6-methyloctanoic acid seem to be more active than the 6-methylheptanoic acid derivatives. Removal of the acyl moiety results in a 30% decrease of the activity. One may assume as a general model that the lipophilic part of the molecule lies on or inserts into the hydrophobic portion of the membrane while the poly-cationic peptide part interacts with membrane polar groups (Pristovšek and Kidrič, 1999). These interactions lead to disruption of the structure and rapid permeability changes of the membranes. Other known effects, e.g., inhibition of respiration, may be secondary to these primary effects. The polymyxins, including colistin, also have important lipopolysaccharide neutralizing effects (Pristovšek and Kidrič, 2001).

Colistin is available as the sulfate salt and as colistimethate sodium. It is administered orally (colistin sulfate) and parenterally (colistin sulfate, colistimethate sodium). A formulation intended for intramammary use is available and, in humans, colistimethate sodium is administered as an aerosol (Li et. al., 2003).

Colistin is used for the prevention and treatment of diseases caused by sensitive bacteria in a variety of species, including cattle, sheep, goats, pigs, poultry and rabbits. In cattle, swine, and poultry, colistin is used to treat digestive diseases caused by *Escherichia coli* and *Salmonella* spp (EMEA, 1995; EMEA, 2002). In humans, colistin is used to treat infections caused by drug-resistant *Pseudomonas aeruginosa* (Li et. al., 2003). According to a very recent review performed by Falagas and Kasiakou (2005), intravenous polymyxin therapy (mostly colistin therapy) has been re-introduced in clinical practice for treatment of infections caused by multi-drug-resistant Gram-negative bacteria.

Colistin is marketed as a single component product and in formulations containing other antimicrobial agents (e.g., various sulfonamides, spiramycin, erythromycin, trimethoprim, neomycin, and oxytetracycline). Formulations containing colistin also are available with antidiarrhoeal agents (e.g., N-butyl scopolamine, kaolin) and vitamins (Meiji Seika Kaisha, Ltd., 1992a).

Dosage

Dosages for colistin are provided in international units (IU) or by weight (mg). An International Standard for Colistin Sulfate has been established and an International Unit defined as the activity contained in 0.00004878 mg of this preparation. The unit was defined

on the basis of a collaborative assay in which nine laboratories from six different countries participated (Lightbown, et al., 1973, WHO Expert Committee on Biological Standardization, 21st Report, 1969). According to the European Pharmacopoeia (5.0, 01/2005:0319), one mg of colistin methanesulfonate should not have a potency less than 11,500 IU.

From the definition of the IU, the material contains 20500 IU/mg. A sponsor has analysed three production batches and five official reference standards, including the WHO standard, for their contents of impurities. Using HPLC, a minor peak in addition to colistin A and B, was detected in all samples. The concentration equivalents ranged from 17.4 to 19.1% for the production batches and was 20.4% for the WHO standard. The European Pharmacopoeia defines colistin sulfate as: "A mixture of the sulfates of polypeptides produced by certain strains of *Bacillus polymyxa* var. *colistinus* or obtained by any other means. It contains a minimum of 77% of the sum of polymyxin E_1, polymyxin E_2, polymyxin E_3, polymyxin $E_{1\text{-}I}$ and polymyxin E_{17}MOA" (Martindale, 1999; Sweetman, 2006).

Doses vary by product and species but generally the daily dose recommended for colistin sulfate is 75,000 IU/kg in poultry and 100,000 IU/kg in the other species (calves, pigs and rabbits). These doses correspond to approximately 3.75-5 mg/kg b.w., respectively. Colistin may be administered in water, milk, complete feed, or by injection. Colistin is administered as 0.01-0.02% of daily milk intake. In water, colistin is administered at 25-50 mg/L. Colistin is administered in complete feed as medicated premixes containing 20 to 40 M IU/100g at a rate of 5-10 kg/tonne (Meiji Seika Kaisha, Ltd., 1992a). Injectable colistin is administered to 1-3-day-old chickens at a dose of 0.2 mg colistin activity/chick (US FDA, 1998)

PHARMACOKINETICS AND METABOLISM

Pharmacokinetics in Humans and Laboratory Animals

In humans, laboratory animals and the target species, colistin sulfate is poorly absorbed following oral administration (Schwartz, 1964; Blood and Radostits, 1989). Plasma concentrations are usually undetectable after oral administration. Enhanced oral absorption is seen in neonatal animals (Schwartz, 1964). Absorption from mucous surfaces and the mammary gland is minimal (Blood and Radostits, 1989). Plasma concentrations were higher following administration of colistimethate sodium than colistin sulfate (Al-Khayyat and Aronson, 1973; Blood and Radostits, 1989).

In humans, intramuscular administration of colistimethate sodium (30 mg base activity) resulted in therapeutic drug concentrations in serum that persisted for 6 hours. A dose of 75 mg colistin base produced peak serum concentrations of 3 µg/mL in 2 hours and detectable serum concentrations for 12 hours. When the dose was increased to 150 mg colistin base, serum concentrations averaged 7.6 mg/mL in 2 hours and persisted for 24 hours (Schwartz, 1964). In patients with cystic fibrosis, intravenous administration of colistin sulfate and colistimethate resulted in mean plasma elimination half-lives ranging from approximately 2 hours for colistimethate sodium to more than 4 hours for colistin (Li et. al, 2003).

Following intramuscular administration of colistin sulfate in dogs, peak plasma concentrations were reported approximately 0.5-1 hour after dosing. Maximum plasma concentrations were 2.8 µg/mL, 7.1 µg/mL and 17.7 µg/mL, respectively, for the 1.1 mg/kg, 2.2 mg/kg and 4.4 mg/kg intramuscular doses. The excretion half-life was approximately 2.7 hours, irrespective of dose. Colistin sulfate had a percent volume of distribution of 33-74%, thus exceeding the extracellular fluid space. Higher doses produced lower distributions. For colistimethate sodium, the volume of distribution was approximately equal to the extracellular space (i.e., 23%). Drug accumulated in liver, kidneys and brain and was mainly present as a bound residue. The renal clearance of colistimethate sodium was 3.85 mL/min/kg, or approximate 79% of the inulin clearance (i.e., 3.85 mL/min/kg for colistimethate sodium vs.

4.91 mL/min/kg for inulin). In dogs, excretion was via the urine following parenteral administration. There were no detectable residues in faeces. Conversely, following oral administration, excretion was via the faeces, often bound to intestinal phospholipids. Following both parenteral and oral administration, colistin is excreted in an inactive form (Al-Khayyat and Aronson, 1973).

Pharmacokinetics in Food Producing Animals

In milk-fed calves, intravenous administration of colistin sulfate, 5 mg/kg, resulted in a peak serum concentration of approximately 16 µg/mL. The volume of distribution was 1.3 L/kg and renal clearance was approximately 3.4 mL/min/kg. Colistin is significantly bound to tissues following an intravenous dose of 5 mg/kg. The excretion half-life was 4-6 hours (Ziv et al, 1982; Blood and Radostits, 1989).

Following intramuscular administration of colistimethate sodium (50,000 IU/kg equivalent to 4 mg/kg) to lactating dairy cows and calves, residues persisted in serum for several hours. In cows, the maximum serum concentrations occurred between 0.5 and 3 hours after dosing. The mean maximum concentration was 60 IU/mL. In calves, peak serum concentrations were reached between 1 and 2 hours after dosing. The mean maximum concentration was 81 IU/mL. The calculated elimination half-life was nearly double in cows compared to calves (i.e., 6.8 hr vs. 4.5 hr). Concentrations in the milk were generally low and were detectable through 2 milkings. Oral administration of colistin sulfate in veal calves resulted in no residues in excess of the limit of detection for the well-diffusion microbiological method (i.e., 1 IU/mL) (Archimbault et al., 1980). In another report, the peak concentration of colistin in serum occurred 2 hours after dosing and colistin was detectable in the serum for only 6 hours after intramuscular administration. Serum concentrations ranged from 0.1 to 1 µg/mL. There were no detectable serum concentrations following oral administration (Escoula et al., 1981).

In ewes, intramuscular administration of colistimethate sodium resulted in higher serum concentrations than did intramuscular administration of colistin sulfate for doses of 7.5 mg/kg and 3.5 mg/kg. Serum protein binding was higher for colistin sulfate than for colistimethate (Ziv and Sulman, 1973). These findings are identical to those determined for dogs (Al-Khayyat and Aronson, 1973).

Plasma protein binding of colistin in cattle and sheep is 40% and 70%, respectively.

In chickens, maximum concentrations of 10.2 µg/mL and 5.7 µg/mL are detected in serum and bile, respectively, approximately 2 hours after an oral dose of 50 mg/kg b.w.. In pigs, concentrations of 1.0 µg/mL and 4.0 µg/mL are detected in serum and bile, respectively, after an oral dose of 25 mg/kg b.w.. Concentrations of 8.3 µg/mL and 9.0 µg/mL are detected in serum and bile, respectively, after an oral dose of 50 mg/kg b.w. (Sato et al., 1972).

Pigs were treated intravenously with colistin sulfate at two doses: 25 mg potency/kg and 50 mg potency/kg. Peak serum concentrations of 1.0 µg/mL and 8.3 µg/mL were reached at 1 hour after administration for the 25 mg/kg and 50 mg/kg doses, respectively. Serum concentrations were undetectable at sampling times thereafter (Sato et al., 1972). In another study, no detectable concentrations of colistin were detected in the serum of gnotobiotic piglets fed colistin sulfate, 40mg/kg, in sterilized milk (Terakado et al., 1972).

In a more recent study, pigs were treated intramuscularly with colistin sulfate at doses of 5.0 mg/kg and 2.5 mg/kg and intravenously at a dose of 2.5 mg/kg. For the intramuscular route of administration, peak plasma concentrations were reached 30 minutes after dosing. The elimination half-life for all doses and routes was approximately 4-4.5 hours and the clearance rate was approximately 3 mL/kg/min (Lin et al., 2005).

Metabolism in Laboratory Animals

Information on the metabolism of colistin in laboratory animal species is limited.

Rats

In an *in vitro* study intended to determine whether low recovery of colistin activity from rat tissue homogenates was due to tissue binding or metabolism, colistin sulfate and colistimethate were added to homogenates of rat kidney and liver tissues, with and without 2 M HCl. In the absence of HCl, only 15% of the added colistin was recovered from the kidney and liver homogenates. Adding equal volumes of 2 M HCl to the homogenates resulted in complete (103%) recovery of the added colistin. Recovery of colistimethate from rat kidney homogenates in the absence of 2M HCl was approximately 81%. Because stability of colistin sulfate had previously been demonstrated in the presence of 2 M HCl, it was concluded that the loss of colistin activity in the homogenates was due to tissue binding rather than metabolism (Al-Khayyat and Aronson, 1973).

Dogs

There are limited data to suggest that dogs can metabolize colistin to a compound devoid of antimicrobial activity (Al-Khayyat and Aronson, 1973). At the doses studied, approximately 67% of the colistin excreted in the urine consisted of antibacterially active colistin whereas 33% consisted of an antibacterially inactive metabolite (Al-Khayyat and Aronson, 1973). The authors note, however, that the tested doses were higher than normal therapeutic doses and may not reflect the importance of metabolism at doses in the therapeutic range. Additionally, there was no attempt made to identify the metabolite.

Metabolism in Food Producing Animals

Cattle

In cattle treated intraruminally with colistin and erythromycin, no colistin activity was detected in rumen fluid (Escoula et al., 1981).

Chickens

In a radiolabelled study, radioactivity in cage droppings was determined following a single subcutaneous administration of colistimethate to day-old chickens. Less than 33% of the administered dose was recovered through 28 days of sampling. In samples collected for the first 12 hours, antimicrobial activity represented less than 0.1% of the total dose. Activity was confirmed to be colistin by HPLC. Subsequent excreta samples had no antimicrobial activity (US FDA, 1998).

TISSUE RESIDUE DEPLETION STUDIES

Radiolabelled Residue Depletion Studies

General

Radiolabelled residue data were not provided for orally administered colistin sulfate in any species.

Limited radiolabelled data are available for injectable colistimethate sodium in day-old chickens (US FDA, 1998). Chicks were treated subcutaneously one time with ^{14}C-colistimethate sodium to provide 0.2 mg colistin potency. Residue concentrations were

comparable for male and female chicks. Total radiolabelled residues were determined at 14, 21 and 28 days after treatment and are summarized in Table 1.

Extraction studies demonstrate that most of the residues in muscle were bound to tissue. While little residue was extracted following acid protease digestion (mean = 1.4%), more than 65% was extracted when the protease treatment was followed by alkaline extraction. When the alkaline extracts were tested for antimicrobial activity, none was detected. In a second study, the antimicrobial activity in enzyme-extracted tissues from treated chickens was compared to antimicrobial activity in enzyme-extracted control tissues. There was no difference in the antimicrobial activity of the tissues from treated and control birds activity (US FDA, 1998).

Table 1: Total radiolabelled residues in chicken tissues following a single subcutaneous administration of ^{14}C-colistimethate sodium at a dose of 0.2 mg colistin potency/chicken

Tissue	Concentration Colistin equivalents (mg/kg)					
	Withdrawal Time (days)					
	14		21		28	
	Males	Females	Males	Females	Males	Females
Liver	0.51	0.52	0.17	0.18	0.07	0.08
Muscle	0.51	0.55	0.23	0.24	0.12	0.14
Skin/fat	0.29	0.32	0.12	0.12	0.07	0.07
Injection site	0.51	0.65	0.21	0.22	0.12	0.12

Residue Depletion Studies with Unlabelled Drug

Cattle

In a study in calves, animals were treated orally for 3 days with colistin sulfate to provide 5 mg potency/kg body weight or 10 mg potency/kg body weight. This represents the highest normal dose and twice the normal dose, respectively. Drug was administered by gastric catheter in 150 mL tap water. Blood samples were collected before administration and at 1, 2, 4, 6, 24, and 72 hours after the final dose. Tissues were collected 72 hours after the final dose. Samples were analyzed using a microbiological assay (test organism = *Bordetella bronchiseptica*, ATCC 4617) with a limit of detection of 0.05 µg potency/g (bile = 0.1 µg potency/g). In a recovery test, recovery of colistin from all tested samples was good with acceptable CVs. None of the tested samples had concentrations of colistin activity above the limit of detection of the assay (Research Institute for Animal Science in Biochemistry and Toxicology, 1990a).

In a more recent study (Meiji Seika Kaisha, Ltd., 1999e), four calves were treated with milk replacer containing colistin to provide 100,000 IU/.kg b.w.. Animals were treated twice daily for 7 days. Drug was mixed in 1/3 of the total volume of milk to be administered morning and evening. Calves were slaughtered 6 hours after the final treatment. Samples of muscle, liver, kidney and fat were collected and kept frozen (- 80ºC) until analyzed. Concentrations of colistin in tissues were determined using a validated HPLC method (see Methods of Analysis, below). Residues were detected in three of four kidney samples. One of the three kidneys contained 139 µg/kg of residues. The study was conducted in compliance with GLP.

Pigs

In a study in pigs, animals were treated orally for 3 days with colistin sulfate to provide 10 mg potency/kg body weight or 20 mg potency/kg body weight. This represents the highest normal dose and twice the normal dose, respectively. Drug was administered by gastric catheter in 150 mL tap water. Blood samples were collected before administration and at 1, 2, 4, 6, 24, and 72 hours after the final dose. Tissues were collected 72 hours after the final dose. Samples were analyzed using the microbiological assay described above. In a recovery test, recovery of colistin from all tested samples was good with acceptable CVs. A low concentration of residual drug was detected in plasma collected 1 hour after the final dose. Thereafter, no detectable drug was found in plasma. None of the tissue samples had concentrations of colistin activity above the limit of detection of the assay (Research Institute for Animal Science in Biochemistry and Toxicology, 1990b).

In a second study in pigs, animals were treated orally for 4 months with colistin sulfate to provide 40 mg potency/kg body weight or 200 mg potency/kg body weight. This represents the highest normal dose and five times the normal dose. Drug was administered in commercial feed. Tissues were collected on the day of drug withdrawal, 1-day following the final dose and 3 days following the final dose. Samples of muscle, fat, liver and kidneys were analyzed using the microbiological assay described above with a limit of detection of 0.03 µg potency/g. In a recovery test, recovery of colistin from all tested samples was acceptable. None of the tissue samples had concentrations of colistin activity above the limit of detection of the assay at any of the withdrawal times (Meiji Seika Kaisha, Ltd., 1978).

In a more recent study (Meiji Seika Kaisha, Ltd., 2000d), four pigs were treated with colistin to provide 100,000 IU/.kg b.w.. Animals were treated twice daily for 5 days. Drug was mixed with a small quantity of feed and given by oral gavage before the morning and evening meals at the recommended dose. Pigs were slaughtered 6 hours after the final treatment. Samples of muscle, liver, kidney and skin+fat were collected and kept frozen (-80ºC) until analyzed. Concentrations of colistin in tissues were determined using the validated HPLC method (see Methods of Analysis, below). Colistin was detected in all samples of liver and of skin and fat and in one sample of kidney, however, no quantifiable residues were found. Individual residue values (integration of the polymyxin E_1 and polymyxin E_2 peaks) are summarized in Table 2. The study was conducted in compliance with GLP.

Table 2: Colistin residues (µg/kg) in tissues of pigs

Withdrawal (hours)	Animal #	Muscle	Liver	Kidney	Skin+Fat
6	1M	<LOD	<LOQ	<LOQ	<LOQ
	2M	<LOD	<LOQ	<LOD	<LOQ
	7F	<LOD	<LOQ	<LOD	<LOQ
	8F	<LOD	<LOQ	<LOD	<LOQ

These samples were subsequently used to validate the microbiological assay (Meiji Seika Kaisha Ltd, 2001). All of the incurred pig samples had residues below the microbiological assay LOQ of 100 µg/kg.

Chickens

In a study (Meiji Seika Kaisha, Ltd., 2000c), six chickens were treated with colistin to provide 100,000 IU/.kg b.w.. Animals were treated twice daily for 5 days. Drug was mixed with a small quantity of feed and given by oral gavage before the morning and evening meals. This is the recommended dose. Chickens were slaughtered 6 hours after the final treatment.

Samples of breast muscle, liver, kidney and skin+fat were collected and kept frozen (-80ºC) until analyzed. Concentrations of colistin in tissues were determined using the validated HPLC method (see Methods of Analysis, below). Colistin was detected in one sample of fat and skin and in three samples of kidney. The kidney of one female contained 184 µg/kg of residues. Individual residue values (integration of the polymyxin E_1 and polymyxin E_2 peaks) are summarized in Table 3. The study was conducted in compliance with GLP.

Table 3: Colistin residues (µg/kg) in tissues of chickens

Withdrawal (hours)	Animal #	Muscle	Liver	Kidney	Skin+Fat
6	1M	<LOD	<LOD	<LOD	<LOQ
	2M	<LOD	<LOD	<LOD	<LOD
	3M	<LOD	<LOD	<LOD	<LOD
	10F	<LOD	<LOD	<LOQ	<LOD
	11F	<LOD	<LOD	<LOQ	<LOD
	12F	<LOD	<LOD	184	<LOD

Turkeys

In a study (Meiji Seika Kaisha, Ltd., 2000f), six turkeys were treated with colistin to provide 100,000 IU/.kg b.w. Animals were treated twice daily for 5 days. Drug was mixed with a small quantity of water and given by oral gavage before the morning and evening meals. This is the recommended dose. Turkeys were slaughtered 6 hours after the final treatment. Samples of breast muscle, liver, kidney and skin+fat were collected and kept frozen (-80ºC) until analyzed. Concentrations of colistin in tissues were determined using the validated HPLC method (see Methods of Analysis, below). Colistin was found in all muscle samples, in five samples of skin and fat, and in one sample of kidney. The kidney of one female animal contained 194.5 µg/kg of residues. Skin and fat of the same animal contained 98 µg/kg. Individual residue values (integration of the polymyxin E_1 and polymyxin E_2 peaks) are summarized in Table 4. The study was conducted in compliance with GLP.

Table 4: Colistin residues (µg/kg) in tissues of turkeys

Withdrawal (hours)	Animal #	Muscle	Liver	Kidney	Skin+Fat
6	1M	<LOQ	<LOD	<LOD	<LOQ
	2M	<LOQ	<LOD	<LOD	<LOQ
	3M	<LOQ	<LOD	<LOD	<LOD
	4F	<LOQ	<LOD	<LOD	<LOQ
	5F	<LOQ	<LOD	194.5	98
	6F	<LOQ	<LOD	<LOD	<LOQ

All the residues were below the LOQ for the method with the exception of one animal in which the kidney sample contained 194.5 µg/kg and the skin+fat sample contained.

Rabbits

In this study (Meiji Seika Kaisha, Ltd., 2000g), twelve rabbits were treated with colistin to provide 100,000 IU/.kg b.w.. Animals were treated twice daily for 5 days. Drug was mixed with a small quantity of water and given by oral gavage before the morning and evening meals. This is the recommended dose. Rabbits were slaughtered in groups of 4 rabbits each at 6, 24 and 48 hours after the final treatment. Samples of muscle, liver, kidney and fat were collected and kept frozen (-80ºC) until analyzed. Concentrations of colistin in tissues were

determined using the validated HPLC method (see Methods of Analysis, below). Colistin residues were found in all fat samples, in four kidney samples and in two muscle samples. All the residues in muscle and liver were below the LOQ for the method. Two kidney samples collected 6 hours after the final treatment contained quantifiable residues. At later sampling times, kidney residues were below the LOQ for the method. Low but quantifiable concentrations were detected in all of the fat collected 6 hours withdrawal. Both fat samples collected from male rabbits at 24 hours withdrawal contained quantifiable residues while the residues in samples collected from female rabbits were below the LOQ. The fat of one male rabbit slaughtered 48 hours after the final dose contained quantifiable residues. Individual residue values (integration of the polymyxin E_1 and polymyxin E_2 peaks) are summarized in Table 5. The study was conducted in compliance with GLP.

Table 5: Colistin residues (µg/kg) in tissues of rabbits

Withdrawal (hours)	Animal #	Muscle	Liver	Kidney	Fat
6	1M	<LOQ	<LOD	1021	85
	2M	<LOQ	<LOD	<LOD	78
	3F	<LOD	<LOD	<LOQ	90
	4F	<LOD	<LOD	239	75
24	5M	<LOD	<LOD	<LOQ	227
	6M	<LOD	<LOD	<LOD	76
	7F	<LOD	<LOD	<LOD	<LOQ
	8F	<LOD	<LOD	<LOD	<LOQ
48	9M	<LOD	<LOD	<LOD	87
	10M	<LOD	<LOD	<LOD	<LOQ
	11F	<LOD	<LOD	<LOD	<LOQ
	12F	<LOD	<LOD	<LOD	<LOQ

All the above discussed tissue residue studies reported by Meiji Seika Kaisha authors were performed using the same lot of colistin sulfate (ACLB 8464). The more recent Virbac milk residue study (Virbac Laboratories, 1997a) used colistin sulfate from the same source (ACLB 7729, 20935 IU/mg).

Residues in Milk and Eggs

Bovine Milk

In a study in lactating dairy cows, animals were treated by intramuscular injection or intramammary infusion. Residue concentrations in milk were determined using a microbiological assay. Residues resulting from intramuscular treatment are summarized in Table 6. Residues resulting from intramammary infusion into all four quarters are summarized in Table 7. Residues resulting from intramammary infusion into a single quarter are summarized in Table 8. Residues in milk following intramammary infusion are higher than residues resulting from intramuscular administration. Notably, when only one quarter was treated, a small residue of colistin was detected in milk collected from the three untreated quarters (Moretain and Boisseau, 1987).

In a more recent study (Virbac Laboratories, 1997a), ten cows were treated intramuscularly with a dose of 100 mg amoxicillin and 250,000 IU colistin per 10 kg b.w. Animals were treated once daily for 5 days. Cows were milked individually, morning and evening. Pooled milk samples from each of the four quarters were mixed and kept frozen (-80ºC) until analyzed. Concentrations of colistin were determined using the validated HPLC method (see Methods of Analysis, below). The study was conducted in compliance with GLP. Residue

values (integration of the polymyxin E_1 and polymyxin E_2 peaks) in morning milk samples are summarized in Table 9.

Quantifiable residues were detected in all milk samples collected during dosing and in the morning milk collected 1-day after the last treatment. Thereafter, the number of samples containing quantifiable residues declined. At three days withdrawal, 3 of 10 milk samples had residues minimally above the LOQ. At four days withdrawal, only 1 of 10 milk samples had residues minimally above the LOQ.

Sheep Milk

Colistin residues were determined in sheep's' milk following a single intramuscular administration of colistin sulfate and colistin methanesulphonate at 3.5 mg/kg and 7.5 mg/kg. Colistin methanesulphonate concentrations in milk were higher than the comparable concentrations produced by colistin sulfate. Peak concentrations of colistin methanesulphonate in milk were approximately 3 and 10µg/mL while peak concentrations of colistin sulfate were approximately 1 and 1.5 µg/mL, for the 3.5 mg/kg and 7.5 mg/kg doses, respectively. Peak concentrations were reached 2-3 hours after treatment. Thereafter, the colistin residues declined, with residues of colistin sulfate declining more rapidly than the residues of colistin methanesulphonate.

Eggs

Laying hens were treated with colistin sulfate orally via drinking water at a dose of 1,000,000 IU/L to provide 90,000 IU/kg body weight for 5 day. A second group was treated once by intramuscular injection, 50,000 IU/kg body weight. Eggs were collected daily during oral treatment and following treatment for both routes of administration. Yolks and albumen were separated and assayed individually using the microbiological assay referenced previously. Albumen samples were heat-treated prior to analysis to remove inherent inhibitory activity native to albumen. Residues also were calculated for whole eggs by considering the relative contribution of yolk and albumen to total egg weight. Results for the intramuscular injection study are summarized in Table 10.

None of the samples from chickens treated orally contained detectable residues of colistin. The report notes, however, that low concentrations of colistin in albumen may reflect partial destruction of colistin due to the heating or inclusion of colistin in the coagulated albumen following heat treatment (Roudaut, 1989).

Table 6: Residues of colistin (IU/mL) in milk of cows treated by intramuscular injection [(a)]

Compound	Formulation	Milkings								
		-2	-1	1	2	3	4	5	6	7
Colistin sulfate	40,000,000 IU/40 mL; aq. sol.	2.73	3.34	3.91	2.28	1.54	0.47	0.16	-	-
Colistin sulfate	25,000,000 IU/100 mL; oil susp.	2.31	3.83	4.61	2.21	0.96	0.29	-	-	-
Colistin sulfate	25,000,000 IU/100 mL; oil susp.	2.38	3.68	4.99	2.80	1.53	0.86	0.27	0.14	-
Colistimethate sodium	25,000,000 IU; sol. powder	1.21	1.58	1.74	0.88	-	-	-	-	-

(a) three injections of 25,000 IU/kg after three consecutive milkings

Table 7: Residues of colistin (IU/mL) in milk of cows treated by intramammary infusion into all four quarters

Compound	Formulation	Milkings								
		-2	-1	1	2	3	4	5	6	7
Colistin sulfate [(b)]	500,000 IU/3g; gel	88.1	75.4	139.5	15.5	5.40	1.19	1.08	0.59	-
Colistin sulfate [(c)]	100,000 IU/10 mL; gel	22.5	18.1	28.0	5.8	1.46	0.55	0.10	-	-
Colistin sulfate [(d)]	125,000 IU/250 mL; aq. sol.	-	-	25.8	2.93	1.29	0.12	-	-	-

(b) 3 administrations of 500,000 IU/quarter after three consecutive milkings
(c) 3 administrations of 100,000 IU/quarter after three consecutive milkings
(d) 1 administrations of 125,000 IU/quarter

Table 8: Residues of colistin (IU/mL) in milk of cows treated by intramammary infusion into a single quarter

Compound	Formulation	Milkings													
Withdrawal sampling time		-2	-1	1	2	3	4	5	6	7	8	9	10	11	12
Colistin sulfate (b)	500,000 IU/3g; gel														
Front quarter treated		93.1	115.0	138.8	54.9	18.1	7.11	4.64	3.05	2.02	1.38	0.81	0.55	0.17	ND
Three non-treated quarters		0.42	0.62	0.65	0.52	ND	-	-	-	-	-	-	-	-	-
Rear quarter treated		75.0	70.0	86.7	22.6	6.10	2.98	1.71	0.85	0.30	ND	-	-	-	-
Three non-treated quarters		0.60	0.62	0.57	ND	-	-	-	-	-	-	-	-	-	-
Colistin sulfate (c)	100,000 IU/10 mL; gel														
Front quarter treated		26.2	21.5	33.0	12.3	4.38	1.32	0.25	ND	-	-	-	-	-	-
Three non-treated quarters		ND	ND	ND	-	-	-	-	-	-	-	-	-	-	-
Rear quarter treated		18.0	20.5	17.5	5.70	1.98	0.98	0.19	ND	-	-	-	-	-	-
Three non-treated quarters		ND	ND	ND	-	-	-	-	-	-	-	-	-	-	-

(b) 3 administrations of 500,000 IU/quarter after three consecutive milkings
(c) 3 administrations of 100,000 IU/quarter after three consecutive milkings
ND not detectable

Table 9: Residues (µg/kg) in cows' milk following intramuscular administration

Milking	1F	2F	3F	4F	5F	6F	7F	8F	9F	10F	Mean±SD
Pre-treatment	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
D2 morning	48	47	54	35	33	31	59	48	42	32	42.9±9.8
D3 morning	55	46	94	38	47	37	37	47	62	40	50.3±17.4
D4 morning	72	55	109	44	47	28	48	51	58	53	56.5±21.6
D5 morning	48	44	94	44	42	21	43	23	49	41	44.9±19.8
1-day withdrawal	45	30	81	31	70	26	78	36	74	36	50.7±22.3
2 days withdrawal	25	11	33	12	11	<LOQ	10	<LOQ	<LOQ	14	16.6±8.9
3 days withdrawal	13	<LOQ	16	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	12	<LOQ	13.7±2.1
4 days withdrawal	<LOQ	<LOQ	12	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	12

<LOQ: <10 µg/kg

Table 10: Residues of colistin (IU/g) in albumen, yolk and whole eggs after intramuscular injection of 50,000 IU/kg body weight

Tissue/Matrix	Time after injection (days)	Mean ± SD
Albumen	1	<6
	2	<6
Yolk	1	<3
	2	
	3	12.5±4.2
	4	12.0±3.6
	5	11.2±3.9
	6	9.0±3.0
	7	5.7±2.3
	8	<3
Whole egg	1	
	2	3.38±1.20
	3	3.81±1.12
	4	4.00±1.27
	5	3.57±1.25
	6	2.93±0.82
	7	1.72±0.69

In a recent study (Meiji Seika Kaisha, Ltd., 2000h), fifteen laying hens were treated with colistin to provide 100,000 IU/.kg b.w.. Animals were treated twice daily for 5 days. Drug was mixed with a small quantity of feed and given by oral gavage before the morning and evening meals. This is the recommended dose. Eggs were collected from treated hens on treatment (Day 3), on the day of treatment withdrawal (Day 5), and following a 1-day withdrawal (Day 6). Yolk and albumen were mixed for each egg and samples were kept frozen (-80ºC) until analyzed. Eggs were analysed individually. Concentrations of colistin in eggs were determined using the validated HPLC method (see Methods of Analysis, below). No detectable residues of colistin were found in any eggs at any of the sampling times. The study was conducted in compliance with GLP.

METHODS OF ANALYSIS

Several methods have been used to monitor residues of colistin in tissues.

Microbiological Assay: A microbiological assay (test organism = *Bordetella bronchiseptica*, ATCC 4617) has been used in serum (Archimbault et al., 1980; Escoula et al., 1981), in tissues of calves (Virbac Laboratories, 1992; Research Institute for Animal Science in Biochemistry and Toxicology, 1990a) and pigs (Research Institute for Animal Science in Biochemistry and Toxicology, 1990b; Meiji Seika Kaisha, Ltd., 1978), eggs (Roudaut, 1989) and milk (Moretain and Boisseau, 1987; Meiji Seika Kaisha, Ltd., 1992b). In a more recent study in pigs, a microbiological assay using *Escherichia coli*, CMCC (B) 4413, is reported to be an effective alternative to the *B. bronchiseptica* assay (Lin et. al., 2005).

In the most recent validation of the *B. bronchiseptica* microbiological assay (Meiji Seika Kaisha, 2001), tissue residues from an oral residue depletion study in pigs were analysed with the microbiological assay after they had been analysed with a validated HPLC assay. In muscle, the study demonstrates accuracy of 90.1-114.7% for samples having concentrations from 100 µg/kg to 1000 µg/kg. The overall recovery (all tested tissues) is 113.9% with a standard deviation of 43%. For the tested tissues, the LOQ is considered 100 µg/kg, the lowest value tested. All of the incurred pig samples had residues below the microbiological assay LOQ of 100 µg/kg. In the HPLC assay, the

residue concentrations were less than 75 µg/kg for muscle, liver and skin/fat and less than 100 µg/kg for kidney.

High Performance Liquid Chromatography Assay: A validated HPLC with fluorescence detection has been used in tissue residue studies in cattle (Meiji Seika Kaisha, Ltd., 1999e; Meiji Seika Kaisha, Ltd., 1999b), pigs (Meiji Seika Kaisha, Ltd., 2000d; Meiji Seika Kaisha, Ltd., 1999a), chickens (Meiji Seika Kaisha, Ltd., 2000c; Meiji Seika Kaisha, Ltd., 1999c), turkeys (Meiji Seika Kaisha, Ltd., 2000f; Meiji Seika Kaisha, Ltd., 2000b), rabbits (Meiji Seika Kaisha, Ltd., 2000g; Meiji Seika Kaisha, Ltd., 2000a), milk (Virbac Laboratories, 1997a; Virbac Laboratories, 1997b) and eggs (Meiji Seika Kaisha, Ltd., 2000e; Meiji Seika Kaisha, Ltd., 1999d). Samples are deproteinated with trichloroacetic acid, and extracted with acid methanol. The extract is passed through a C_{18} cartridge prior to derivatization. The samples are derivatized with o-phthalaldehyde for fluorescence detection. A column-switching program is used to adsorb fluorescent derivative onto the first analytical column (end-capped RP18, 5µm, 8 x 4 mm) using an acetonitrile/phosphate buffer mobile phase at a flow rate of 0.6 mL/min. The derivatives are eluted onto the second analytical column (end-capped RP18, 5µm, 125 x 3 mm) using an acetonitrile/phosphate buffer mobile phase at a flow rate of 1.0 mL/min. The column temperature is 35ºC. Detection is accomplished with an excitation wavelength of 340 nm and an emission wavelength of 440 nm. Polymyxin E_2 elutes between 12.0 and 14.3 minutes. Polymyxin E_1 elutes between 15.1 and 18.1 minutes. Quantitation is achieved by integration of the two polymyxin peaks.

***LOQ*:** In the species for which validation data are provided, the method has an LOQ of 75 µg/kg for muscle, liver and fat (or skin/fat, as appropriate) and 100 µg/kg for kidney. The LOQ for milk is 10 µg/kg. The LOQ for eggs is 150 µg/kg.

***LOD*:** The LOD varies by tissue and species and is summarized in Table 11.

***Linearity*:** In the species for which validation data are provided, the method has an linear range of 75-300 µg/kg for muscle, liver and fat (or skin/fat, as appropriate) and 100-400 µg/kg for kidney. The linear range for milk is 10-1000 µg/kg. The linear range for eggs is 150-600 µg/kg.

***Precision*:** The precision varies by tissue and species and is summarized in Table 12.

Table 11: Method LODs, (µg/kg) by species and tissue.

Species	Muscle	Liver	Kidney	Fat (or Skin/Fat, as appropriate)	Milk	Eggs
Cattle	60	45	45	32	3	NA*
Pig	51	30	49	45	NA	NA
Chickens	29	48	72	25	NA	47
Turkeys	6	33	49	31	NA	NA
Rabbits	34	58	50	30	NA	NA

*NA=not applicable

Table 12: Method precision(%) by species and tissue

Species	Muscle	Liver	Kidney	Fat (or Skin/Fat, as appropriate)	Milk	Eggs
Cattle	6.4-6.5	5.5	6.9	11.7-12.1	4.3-13.6	NA*
Pig	5.5-6.1	9.7-13.1	8.6-8.7	8.7-10.6	NA	NA
Chickens	6.3-7.2	5.6-5.9	7.6	9.4-11.1	NA	5.5-6.0
Turkeys	6.0%	10.6	3.9-9.0	7.9-11.5	NA	NA
Rabbits	11.3-12.1	4.6-5.4	7.3-11.6	7.6-11.4	NA	NA

*NA=not applicable

***Accuracy*:** The accuracy varies by tissue and species and is summarized in Table 13.

***Recovery*:** Acceptable recovery was demonstrated for the method.

Table 13: Method accuracies (%), by species and tissue

Species	Muscle	Liver	Kidney	Fat (or Skin/Fat, as appropriate)	Milk	Eggs
Cattle	96.9-103.8	98.0-102.5	95.4-103.9	96.0-105.6	98-110	NA*
Pig	98.9-100.9	98.4-101.1	94.0-104.2	96.2-104.6	NA	NA
Chickens	94.2-107.8	93.8-105.7	96.0-105.3	93.6-106.4	NA	92.6-106.8
Turkeys	98.9-101.0	94.6-104.8	98.5-101.8	94.0-107.5	NA	NA
Rabbits	95.6-103.2	96.1-104.4	96.7-103.1	97.0-103.5	NA	NA

*NA=not applicable

The method uses readily available reagents and materials and can be implemented easily under normal laboratory conditions.

In a recent study, the microbiological assay (Meiji Seika Kaisha, 2001) was used to assess residues in pig tissues previously analysed with the HPLC assay (Meiji Seika Kaisha, Ltd., 2000d). For tissues derived from pigs treated orally with colistin, both assays demonstrate the absence of detectable residues.

The Committee was aware that suitable microbiological assays are available for screening but these methods were not submitted for evaluation.

APPRAISAL

Colistin has not been previously reviewed by the Committee. Colistin is a cyclopeptide (polymyxin) antibiotic with activity primarily against the Gram-negative organisms. Colistin is available as the sulfate salt and as colistimethate sodium. It is administered orally (colistin sulfate) and parenterally (colistin sulfate, colistimethate sodium). Colistin is used for the prevention and treatment of diseases caused by sensitive bacteria in a variety of species, including cattle, sheep, goats, pigs, poultry and rabbits. In cattle, pig, and poultry, colistin is used to treat digestive diseases caused by *Escherichia coli* and *Salmonella* spp.

Colistin is poorly absorbed following oral administration. Limited radiolabelled data are available following subcutaneous administration in day-old chickens. Metabolism is minimal and antimicrobial activity is low in incurred tissues.

In unlabelled residue studies, most tissues contain residues below the LOQ for the methods used at the sampling times employed. There are low but detectable residues in cows' milk following treatment with injectable and intramammary formulations. Detectable residues are found sporadically in other tested tissues in various species. No residues are detected in the eggs from treated hens following oral administration but low concentrations were found when colistin was administered intramuscularly.

Colistin A + B can serve as the marker residue for colistin. It represents the most significant residue and corresponds to the major microbiologically active residues of concern. Since the proportion of the two components also depends on the purity and composition of the drug used, it is not reasonable to select a single chemical entity as the marker residue. In addition to a microbiological assay, a highly specific fluorescence HPLC method is validated to measure residues of colistin (as Colistin A + B) in tissues, milk, and eggs.

In the species for which the HPLC method has been validated, the method has an LOQ of 75 μg/kg for muscle, liver and fat (or skin/fat, as appropriate) and 100 μg/kg for kidney. The LOQ for milk is 10 μg/kg. The LOQ for eggs is 150 μg/kg. The method has a linear range of 75-300 μg/kg for muscle, liver and fat (or skin/fat, as appropriate) and 100-400 μg/kg for kidney. The linear range for milk is 10-1000 μg/kg. The linear range for eggs is 150-600 μg/kg. Precision and accuracy, which are species and tissue specific, are acceptable.

The HPLC method is suitable for monitoring residues of colistin in milk and tissues and is considered practicable as it uses readily available reagents and materials and can be implemented easily under normal laboratory conditions. The microbiological assay, while not specific for colistin, is suitable as a screening test.

MAXIMUM RESIDUE LIMITS

In recommending MRLs for colistin, the Committee considered the following factors:

- Residues of colistin following oral administration generally were below the LOQ for the method of analysis in most tissues of most species, even at very short withdrawal periods. Low but quantifiable residues were detected in the fat of orally treated rabbits and in eggs of hens treated by intramuscular injection. Quantifiable residues of colistin also were found in cows' milk following intramammary infusion and intramuscular injection.

- Colistin A+B is considered a suitable marker residue in tissues, eggs and milk and represents approximately 80% of the microbiologically active residues. This information is incorporated in the calculation of the intake estimates to ensure they correctly reflect residues of microbiological concern.

- The validated HPLC method, used to measure residues of colistin in the more recently conducted studies submitted for the Committee's review, is suitable for monitoring residues for regulatory purposes. The assay measures colistin A (polymyxin E_1) and colistin B (polymyxin E_2).

- The MRLs recommended for all edible tissues in all species and for hens' eggs are twice the LOQ for the HPLC method. Because detectable residues were found in these tissues, the theoretical intake values for all the edible tissues are included in the calculation of the Theoretical Maximum Daily Intake (TMDI).

- The MRL recommended for cows' milk takes into consideration the potential use of colistin by both the intramuscular and intramammary routes of administration.

- An ADI of 0-7 µg/kg of body weight was established by the Committee based on a microbiological endpoint. This ADI is equivalent to up to 420 µg for a 60 kg person.

The Committee recommended MRLs for colistin in cattle, sheep, pigs, chickens, turkeys, and rabbits of 150 µg/kg in liver, muscle and fat (including skin + fat, where applicable), and 200 µg/kg in kidney, 300 µg/kg in hens' eggs, and 50 µg/kg in cows' milk, determined with the HPLC assay as the sum of polymyxin E_1 (colistin A) and polymyxin E_2 (colistin B).

The MRLs recommended would result in a theoretical maximum daily intake of 229 µg or 55% of the ADI, based on the model daily food intake of 300 g muscle, 100 g liver, 50 g each of kidney and fat, 100 g eggs, and 1.5 kg of milk.

Tissue	MRL	Standard Food Basket	Microbiological activity	TMDI
Muscle	150 µg/kg	0.3 kg	0.8	56 µg
Liver	150 µg/kg	0.1 kg	0.8	19 µg
Kidney	200 µg/kg	0.05 kg	0.8	13 µg
Fat*	150 µg/kg	0.05 kg	0.8	9 µg
Milk	50 µg/kg	1.5 kg	0.8	94 µg
Eggs	300 µg/kg	0.1 kg	0.8	38 µg
TMDI				229 µg

* Skin + Fat, where applicable

The 66th meeting of the Committee agreed to apply the principle of using median residue concentrations to better estimate long-term (chronic) exposures to residues. Estimated daily intake (EDI) values were determined using median residue values for each tissue from each food-producing species for which data were available. Where residue values were below the LOD or LOQ of the validated method, values of ½ the LOD and ½ the LOQ, respectively, were used in the calculations. As with the calculations of the TMDIs, all EDI calculations incorporate an adjustment to account for the fact that colistin A+B represents only 80% of the microbiological activity. The resulting EDI values represent 4% (for chickens) to 9% (for cattle) of the ADI.

An EDI, using the highest median values from among the tissues and food-producing species, was calculated. This EDI represents 14% of the ADI.

Tissue	Median	Standard Food Basket	Microbiological activity	EDI
Muscle (turkey)	38 µg/kg	0.3 kg	0.8	14.3 µg
Liver (pigs)	38 µg/kg	0.1 kg	0.8	4.8 µg
Kidney (rabbits)	145 µg/kg	0.05 kg	0.8	9.1 µg
Fat*(rabbits)	82 µg/kg	0.05 kg	0.8	5.1 µg
Milk (cattle)	11 µg/kg	1.5 kg	0.8	20.6 µg
Eggs (chickens)	24 µg/kg	0.1 kg	0.8	3.0 µg
EDI				56.9 µg

* Skin + Fat, where applicable

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