GENERAL CONSIDERATIONS

Note: Only the general considerations on risk assessment principles discussed and adopted by the Committee at its 66th meeting which are considered pertinent for the reading of the monographs and for future assessments of veterinary drug residues are provided here.

Considerations related to the terms of reference of JECFA

Criteria for compounds to come on the JECFA agenda

The Committee considered the current criteria established by Codex for veterinary drugs to be evaluated by JECFA. These criteria are:

> In order to be placed on the CCRVDF priority list for the development of an MRL, the candidate veterinary drug, when used in accordance with good veterinary practices, should meet some, but not necessarily all, of the following criteria:
>
> 1. Use of the drug will have potential to cause public health and/or trade problems;
> 2. Drug available as commercial product;
> 3. Commitment that a dossier will be available.

The Committee considered that the process of prioritization of veterinary drugs for evaluation by Codex and the process of risk assessment of the veterinary drug by JECFA would be greatly improved by adherence to these criteria and provision of the information to the JECFA Secretariat.

Issues relating to data availability

In reaching its conclusions on ADIs and MRLs, the Committee evaluates the available data, including those submitted by the sponsor and those identified in a search of the open literature. The Committee's decisions depend on consideration of the primary data. Limited reliance is placed on summary or review data alone, if not supported by relevant primary data. On a number of occasions, limited or at times no data are available for evaluation of compounds on the meeting agenda. Hence, in these instances, the Committee is unable to complete its evaluation because of significant gaps in the database. On such occasions, the Committee will identify the critical gaps and will suggest those additional data that should enable the evaluation to be concluded. The Committee is concerned that even after a reasonable time interval, appropriate data are not being either generated or submitted to the Committee. It is important to note that JECFA is not a regulatory body and has no means to compel data submission. Hence, possible strategies to help resolve these issues were sought.

The Committee proposes that two lists of veterinary drugs of public health concern be introduced. These lists would include the following categories of veterinary drugs:

i) Veterinary drugs for which significant concerns had been identified, either because of incomplete information or pending resolution of a problem identified in the evaluation;

ii) Veterinary drugs for which the significant concerns noted in i) were not addressed, despite requests for data to resolve the outstanding issues. It is recommended that these compounds should not be used in food-producing animals until outstanding data are provided and evaluated by JECFA.

Compounds would remain in category i) for a specified period and then either would be removed from the list because of resolution of the concerns or would be moved to category ii). The Committee recommends that CCRVDF take an active role in establishing and supporting such lists and should emphasize the need for Codex members and commercial entities to fulfil their responsibility in submitting relevant data in a timely manner.

Information on approved uses

Assessment of efficacy is not within the mandate of the Committee. However, since one of the criteria for scheduling a compound for JECFA evaluation is that the veterinary product containing the active compound is currently registered by a national or regional authority, confirmation of its authorization, including approved dosages and conditions of use, should be provided in the data submission.

Risk–benefit comparison

The Committee recognizes that CCRVDF may use risk–benefit considerations in prioritizing compounds for evaluation. The number of veterinary drugs available and approved for certain therapeutic indications is very limited, and there is general concern that loss of a compound may have significant impact on food animals and derived products. Consideration of the relative benefit provided by the availability of such a drug is outside the scope of the Committee, which has neither the mandate nor the expertise to address such questions. Hence, JECFA will continue to restrict its considerations to the human health risks of the compound.

Considerations related to flexibility in the scientific process of JECFA risk assessments

The Committee discussed the rapid developments in science typified by the fields of genomics, proteomics, analytical chemistry, mathematical modelling and toxicological testing, together with the need to be able to bring to bear the most appropriate tools in the evaluation of veterinary drugs. The Committee recognized the continued need for flexibility in its approach and the importance of balancing this flexibility with consistency. The Committee also recognized that some of these new tools and technologies may require validation.

JECFA risk assessment should not be tied to specific approaches. JECFA will continue to apply the necessary flexibility to bring to bear the most appropriate science and risk assessment techniques.

A decision-tree approach in the evaluation of veterinary drugs by JECFA

The Committee recommended that the JECFA Secretariat convene a working group to develop a general decision-tree for the evaluation of veterinary drugs, which would identify different options for hazard identification, hazard characterization and exposure assessment. The proposed approach will then be discussed at the next JECFA meeting dedicated to the assessment of veterinary drugs. The decision-tree would be anticipated to provide a tool to assist in assessing different options in the evaluation of the veterinary drug, including the determination of a “traditional” ADI and recommended MRL. The decision-tree is envisioned as a flexible document that will be adapted to advancement in science and in response to the nature of the compounds under evaluation. The working group will be expected to develop possible branches to the decision-tree to make use of the best science available. Other options that may be considered are the use of a threshold of toxicological concern as an alternative to an ADI and recommendations for analytical methods for the detection of residues of the drug in the absence of a formal MRL.

Expression of the ADI and derivation of the MRL

CCRVDF at its 15th session discussed rounding practices when establishing ADIs and recommending MRLs for veterinary drug residues and requested JECFA to comment on certain practices suggested by CCRVDF.

The Committee considered the expression of the ADI at its thirty-sixth meeting in 1990. The Committee decided to express the ADI numerically to only one significant figure. If an ADI is calculated from a NOEL that has more than one significant figure, the ADI would therefore be rounded to one significant figure, consistent with accepted rounding procedures.

In the past, JECFA has applied its rounding practice to the derivation of ADIs for 25 veterinary drugs, resulting in 14 ADIs that have been rounded down and 11 ADIs that have been rounded up. Most of the veterinary drugs that have been reviewed by JECFA resulted in a calculated ADI of one significant figure without rounding.

The present Committee noted that the recommendation from CCRVDF in its report of the 15th session suggests a misunderstanding of the relationship between the ADI and the derivation of the MRL.

One of the functions of JECFA is to establish health-based guidance values for residues of veterinary drugs, most often an ADI. The ADI is an output of a risk assessment of the compound, following application of the first two steps of the risk assessment paradigm: hazard identification and hazard characterization. As such, it represents a health-based guidance value, where exposure is considered to represent a negligible risk to consumers if it does not exceed this value. The ADI has a number of uses in risk assessment and risk management, only one of which is in helping to derive the recommended MRLs.

The MRL and the ADI are separate outputs of the risk assessment process and serve different purposes.

The ADI is derived from the NOEL or lowest-observed-effect level (LOEL) from the appropriate toxicological studies, using a safety factor. Given that there are assumptions and uncertainties in deriving the ADI, such as the use of safety factors, the use of a range of doses in toxicological studies and normal biological variation, it is more meaningful to express the ADI to only one significant figure to avoid any inference of inappropriate precision.

The general rounding rule for mid-way values (x.5) is to round up, in line with common convention (see, for example, Australian Standard AS 2706-2003 (reference 23)). Examples for rounding to one significant figure are as follows: 1.25 becomes 1, 0.73 becomes 0.7 and 1.5 becomes 2.

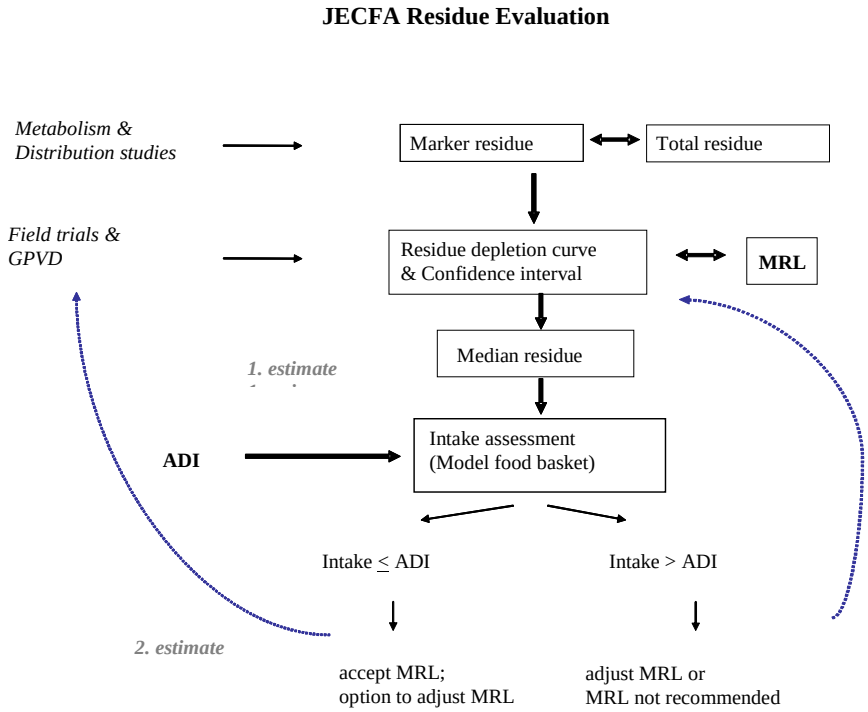
The MRL recommendation procedure is an iterative process. The MRL is not derived directly from the ADI. If the ADI is based on toxicological end-points, all residues of toxicological relevance are considered; if the ADI is based on microbiological end-points, all residues of microbiological relevance are considered. The MRL recommendation procedure also takes into account the conditions of use (e.g. use of the veterinary drug according to good practice in the use of veterinary drugs, or GPVD) and the residues that result from such use (e.g. residue depletion studies). It also considers results of radiolabelled residue studies, the bioavailability of bound residues, the identification of target tissues and a marker residue, the availability of practical analytical methods, estimated exposure resulting from recommended MRLs and consideration of extension of the MRLs to tissues, eggs and milk of other species.

The initial consideration in recommending an MRL is whether it is sufficiently protective of human health. If the use of the veterinary drug yields an estimated intake of veterinary drug residues consistent with the ADI, the recommended MRLs may then be adjusted accordingly when taking into account the other factors noted above. As a general principle, the Committee will not normally recommend an MRL that results in residue levels that lead to dietary intake exceeding the ADI based on toxicological or microbiological considerations.

To protect consumers in all segments of the population, historically the Committee has based its recommendations on intakes estimated using a conservative model diet consisting of 300 g of muscle, 100 g of liver, 50 g of kidney and fat, 1.5 kg of milk and 100 g of eggs. Previously, the Committee estimated intakes by using MRLs to derive a theoretical maximum daily intake (TMDI). At the current meeting, the Committee modified this procedure and is now using the median residue levels to derive an estimated daily intake (EDI) to better reflect estimates of chronic (lifetime) exposure (see section 2.4.1).

Figure 1 is an update of the figure prepared during the Bilthoven MRL workshop (reference 24).

Figure 1: The JECFA residue evaluation process



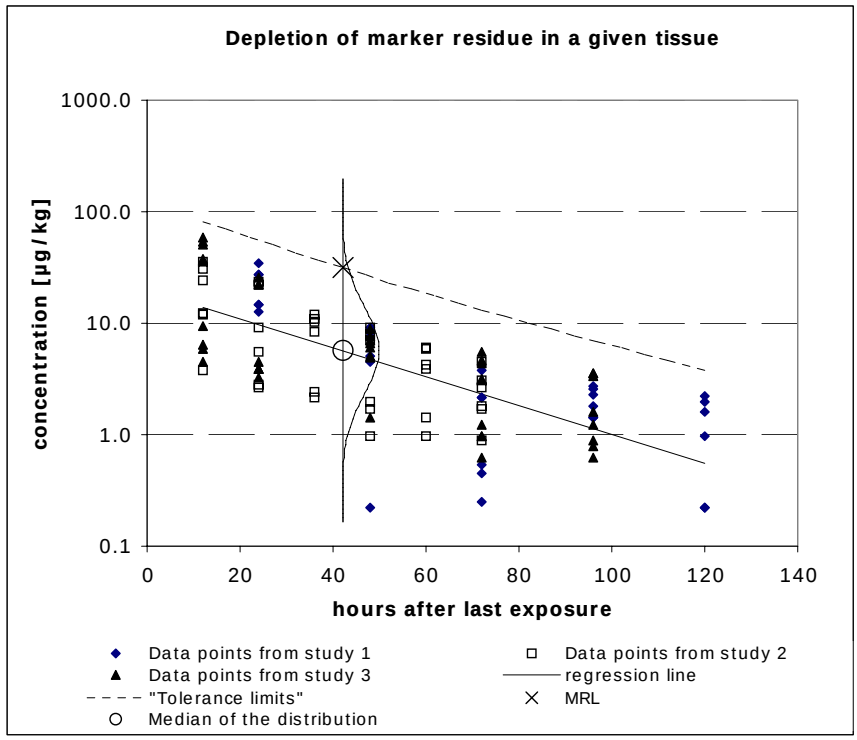
The Committee confirmed that the rounding practices used in expressing the ADI are scientifically and mathematically sound. In addition, since the ADI is not directly used in the derivation of the MRL, the JECFA rounding practices have no direct consequence on the MRL.

New procedure for estimating chronic dietary intakes

The estimation of long-term (chronic) dietary intakes of residues of veterinary drugs by the Committee was in the past closely linked to the determination of the MRLs recommended by the Committee. The Committee used a calculated figure of total residue of toxicological or microbiological concern, the "theoretical maximum daily intake" (TMDI), for comparison with the ADI. The new procedure uses the same formula as used previously for the calculation of the TMDI, including factors such as the ratio of marker to total residue concentrations, the only exception being that the median concentration replaces the MRL as the point estimate of the residue concentration in the formula.

The MRL and the median concentration are derived from the same time point of the depletion data of the marker residue. The MRL is a point on the curve describing the upper one-sided 95% confidence limit over the 95th percentile. The median is the corresponding point on the regression line for the same time point. Both figures are obtained from a statistical evaluation of the data (see Figure 2).

Figure 2: Explanation of the relationship between MRL and the median concentration used for the calculation of the estimated daily intake (EDI)



In developing this new calculation procedure, the present Committee concluded that the TMDI was no longer the most suitable estimate of chronic intake, because the MRL was a single concentration representing the estimated upper limit of a high percentile of the distribution of marker residue present in a given tissue of the treated animals. The Committee concluded that it was not realistic to use an extreme value of the distribution in a scenario describing chronic intakes. In such a scenario, all concentrations of the distribution of residues should be considered. The median concentration represents the best point estimate of a central tendency over a prolonged period of time, because the concentrations of residues in a given tissue consumed varies from day to day, as reflected in the distribution. Therefore, the Committee decided to use the median of the residue distribution to substitute for the MRL in the intake estimate. The new estimate of intake is called “estimated daily intake” (EDI). In calculating the median from an array of results, including values below the limit of quantification (LOQ) or below the limit of detection (LOD), half of the respective limit is used for the calculation of median concentrations of residues.

COLISTIN

First draft prepared by
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and
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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

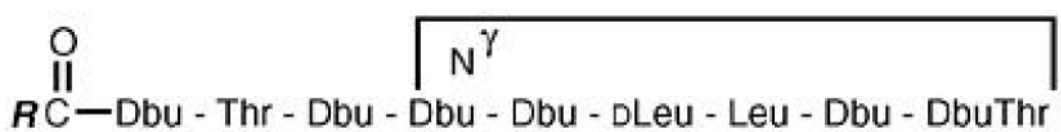
Colistin A: N-[3-amino-1-[[1-[[3-amino-1-[[6,9,18-tris(2-aminoethyl)-3-(1-hydroxyethyl)-12,15-bis(2-methylpropyl)-2,5,8,11,14,17,20-heptaoxo-1,4,7,10,13,16,19-heptazacyclotricos-21-yl]carbamoyl]propyl]carbamoyl]-2-hydroxy-propyl]carbamoyl]propyl]-6-methyl-octanamide

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

Polymyxin $E_2 = [\alpha]^{22}_{5461}$ = -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-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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ERYTHROMYCIN

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IDENTITY

Chemical names International Union Pure and Applied Chemistry (IUPAC) name: 6-(4-dimethylamino-3-hydroxy-6-methyl-tetrahydropyran-2-yl)oxy-14-ethyl-7,12,13-trihydroxy-4-(5-hydroxy-4-methoxy-4,6-dimethyl-tetrahydropyran-2-yl)oxy-3,5,7,9,11,13-hexamethyl-1-oxacyclotetradecane-2,10-dione)

C.A.S. number 114-07-8

Synonyms and abbreviations

Mixture of macrolide antibiotics, the main component (erythromycin A) being (3R, 4S, 5S, 6R, 7R, 9R, 11R, 12R, 13S, 14R)-4-[(2,6-dideoxy-3-C-methyl-3-O-methyl-α-L-*ribo*-hexopyranosyl)oxy]-14-ethyl-7,12,13-trihydroxy-3,5,7,9,11,13-hexamethyl-6-[3,4,6-trideoxy-3dimethylamino-β-D-*xylo*-hexopyranosyl)-oxy]oxacyclotetradecane-2,10-dione

Structural formula See next page.

Molecular formula $C_{37}H_{67}NO_{13}$
Molecular weight **Erythromycin** A: Mz = 734; Erythromycin B: Mz = 718; Erythromycin C: Mz = 720

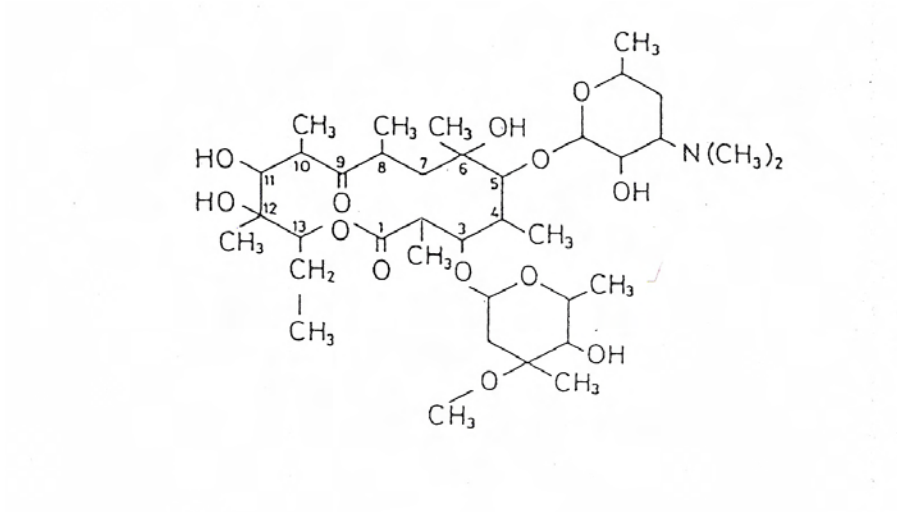
OTHER INFORMATION ON IDENTITY AND PROPERTIES

Appearance White or slightly yellow powder or colorless or slightly yellow crystals

Degree of purity Erythromycin B ≤ 5.0%
Erythromycin C ≤ 5.0%
Any impurity ≤ 3.0%
Sum of impurities ≤ 7.0%

Qualitative composition of impurities
N-demethyl-erythromycin A, anhydroerythromycin A, erythromycin A enol ether, pseudoerythromycin A enol ether, erythromycin E, erythromycin F.

Description of physical properties
Slightly hygroscopic, slightly soluble in water but less soluble at higher temperatures, freely soluble in alcohol, soluble in methanol, sensitive to light.



Three erythromycins are produced during fermentation. Erythromycin A and B contain the same sugar moieties, desoxamine and cladinose (3-O-methylmycarose). They differ in position 12 of the aglycone, erytronolide, A having a hydroxyl group. Erythromycin C contains desoxamine and the same aglycone present in A, but differs by the presence of mycarose instead of cladinose (Merck Index). The principal product is erythromycin A with small proportions of B and C.

INTRODUCTION

Erythromycin was first reviewed by the Committee in 1968. No ADI was established but acceptable levels of residues were defined in milk (0-0.04 mg/ml), meat and eggs (0-0.3 mg/kg) (FAO/WHO, 1969).

Pharmacokinetic and metabolic studies in experimental animals, target animals and humans were evaluated, including original studies in calves and chicken and three new non radiolabelled residue depletion studies in chickens, two in laying hens and one in turkeys and the description and validation of the analytical methods used.

Conditions of use

General

Erythromycin, a macrolide antibiotic is effective *in vitro* against *Mycoplasma*, Gram positive *Coci*, *Neisseria*, some strains of *Haemophilus*, *Corynebacterium*, *Listeria*, *Pasteurella mutocida*, *Brucella*, and *Treponemes. Proteus*, *Pseudomonas* and *E.Coli* are relatively resistant to the drug. In veterinary medicine, this compound is used for the treatment of clinical and subclinical mastitis in lactating cows, for the treatment of infectious diseases due to erythromycin sensitive bacteria (cattle, sheep, swine, poultry) and for the treatment of chronic diseases due to mycoplasma in poultry (EMEA, 2000). In Europe, for broiler chickens and laying hens, the most often recommended dose (as erythromycin base) is 20 mg/kg/day. Uses and dose ranges are presented in Table 1.

Table 1: Formulation and dose ranges of erythromycin

Formulation	Country	Dosage
Erythromycin thiocyanate Oral powder 5 g per 100 g	Belgium France Germany	20 mg / kg / day for 5 days
	Netherlands	20 mg / kg / day for 5 to 7 days
	International zone*	5 g of erythromycin in 100 liter of drinking water for 3 to 5 days
Erythromycin thiocyanate Oral powder 5.1 g per 100 g	Greece	Curative treatment: 2-4 g of product / liter of drinking water Preventive treatment: 1-2 g of product / liter of drinking water
Erythromycin thiocyanate Oral powder 10 g per 100 g	France	20 mg / kg / day for 3 days
	Algeria	10 g of erythromycin / 100 liter of drinking water for 3 to 6 days
Erythromycin thiocyanate Oral powder 16.5 g per 100 g	Ireland and United Kingdom	25.5 mg / kg / day for 1 to 5 days
Erythromycin thiocyanate Oral powder 20 g per 100 g	France	20 mg / kg / day for 3 days
	Greece	50 mg / kg / day for 3 days
	Spain	130 mg erythromycin / liter of drinking water or 0.65 g erythromycin / liter of water For CRD: treatment for 5 days. For infectious coriza and sinusitis: treatment for 7 days
	International zone*	20 g of erythromycin in 200 liter (prevention) or 100 liter (treatment) of drinking water for 3 to 5 days
	International zone*	5 g of erythromycin per liter of drinking water for 1 to 5 days
Erythromycin Phosphate Oral powder 29.6 g per 100 g	Ireland and United Kingdom	25.5 mg / kg / day for 1 to 5 days

* Countries other than the European Union and United States

PHARMACOKINETIC AND METABOLIC STUDIES

Absorption

Erythromycin base is destroyed by gastric acid, except if administered with a protective enteric coating. Acidic media degrades erythromycin rapidly to form derivates with little antimicrobial activity. Erythromycin stearate is more stable, however *in vitro* studies have demonstrated that erythromycin stearate dissolves in gastric acid, retains only 2% antibiotic activity and is rapidly destroyed (DiSanto and Chodos, 1981; Periti et al., 1989; Martindale, 1989). The major site of absorption in rat, dogs and humans is the small intestine. Erythromycin is only slightly absorbed from the stomach. In man, absorption occurs mainly in the duodenum (Anderson et al., 1959; Huber, 1977).

Humans

In humans, erythromycin is rather slowly absorbed after oral administration. Peak serum concentrations occur 1 to 6.3 hours after dosing and vary from 0.1 to 4.8 µg/ml, depending on the

formulation, and the coating of erythromycin administered. The oral absorption is less than 50% and erythromycin is degraded by gastric acid. It is absorbed in the small intestine as erythromycin base (DiSanto and Chodos, 1981; Griffith and Black, 1970; Burrows, 1980).

The oral bioavailability of unprotected erythromycin base and salts is less than 50 % of the dose. Food reduces the absorption of erythromycin (Griffith and Black, 1970; Burrows, 1980). Wilson and Van Boxtel (1978) observed that erythromycin propionate and stearate were better absorbed before rather than after breakfast.

Laboratory animals

In laboratory animals, erythromycin is rather slowly absorbed after oral administration in laboratory animals (except rats). Oral administration of propionyl erythromycin (25 mg/kg) in rats did not produce high peak serum concentrations (<0.1µm/ml). However, the maximum serum concentration was reached rapidly (1 hour after administration). At the end of six hours fo11owing oral administration, only a trace of antibiotic activity was found in rat serum (Anderson et al., 1959).

Calves

In calves, 2 hours after a single intramuscular treatment of 5 mg erythromycin/kg b.w., the mean highest concentration in plasma (0.652 µg/m1) was reached. Twelve hours after treatment, the concentration of erythromycin in serum was about 0.22 µg/ml. After repeated intramuscular treatments of 5 mg erythromycin/kg bw/day for 5 days, no accumulation phenomenon was observed (Report PK 5251/E-00).

Chickens

In chickens, 30 minutes after the beginning of a repeated administration of erythromycin via drinking water at a dose of 25,000 IU/kg b.w./day for 3 days (approximate1y 27 mg/kg b.w.), the average serum levels ranged from 0.11 to 0.22 µg/ml. After the last administration, serum levels declined to approximately 0.04 µg/ml (Report PK 8400/E-00).

Distribution

Plasma protein binding

In humans, erythromycin is highly bound to plasma proteins. The extent of protein binding is >74% *in vitro* and >90% *in vivo* (Wilson and Van Boxtel, 1978). Erythromycin undergoes a relatively low extent of binding to bovine serum proteins (37-43%) (Baggot and Gingerich, 1976).

Milk protein binding

Studies show that antibiotics are bound only to a minor extent to milk proteins. However, the unbound fraction of erythromycin may be decreased because it may be bound to milk casein. Erythromycin is <25% bound to dry udder secretion and to dry udder tissue homogenates (Ziv, 1980).

Serum levels

According to Wilson and Van Boxtel (1978), dose levels of 250 mg of erythromycin base or erythromycin stearate in adults produce similar peak serum concentrations (0.4 µg/ml) within 2-4 hours after oral administration. In cattle, after intramuscular administration of erythromycin in cattle, peak serum concentrations are maintained for several hours and then decline slowly. The l2-hour levels are about 25% of peak concentration (Burrows, 1980).

The elimination half-life ($T_{½}$) of erythromycin following intravenous injection of a single dose in different species is shown in Table 2.

Table 2: Elimination half-life of erythromycin following intravenous injection

Species	Dose	$T_{½}$	References
Cow	12.5 mg/kg	3.16 ± 0.44 hours	Baggot and Gingerich,1976
Dog	-	1 hour	Burrows, 1980
Man	100 mg	1.02 ± 0.17 hours	Wilson and Van Boxtel, 1978

Tissue distribution

Animal studies indicate that erythromycin is well distributed in the body and tissue levels (e.g. liver, spleen, kidneys, and lungs) are general1y higher than serum levels and persist longer (Wilson and Van Boxtel, 1978).

Humans

In humans, erythromycin is distributed to various tissues and fluids. About 10% of erythromycin is estimated to cross the placenta and fetal blood levels are no higher than 10% (usually closer to 2%) of those present in normal circulation. An estimated 0.1% of a daily dose appears in breast milk in pregnant women (Wilson and Van Boxtel, 1978).

Rats

In rats given 100 mg erythromycin base per kg bw orally, erythromycin is concentrated in the liver, sub maxillary glands, spleen, adrenals, lungs and kidneys two hours after administration. Large amounts are also found in the thymus, skin, muscle, reproductive organs and heart (Lee et al., 1953).

Twenty hours after an intravenous treatment of 10 mg erythromycin (N-methyl-^{14}C-erythromycin, 8 µCi) to rats, about 37-43% of the administered dose is recovered in the intestinal tract plus feces, 27.2 - 36.1% in the urine, and 21-29% in the expired air. It is rapidly metabolized in the liver, mainly through a demethylation process, and excreted in the bile as des-N-methyl-erythromycin, the major metabolite, present only in the bile and in the intestinal contents of rats. The isotopic methyl group is eliminated in the expired air as $C0_2$ (Lee et al., 1953)

Cattle

After intravenous administration, erythromycin is widely distributed in cows. The apparent vo1ume of distribution (Vd) is 0.8 1iter/kg. Tissue concentrations are higher than serum concentrations and erythromycin concentrations in milk of lactating cows (the dose fraction recovered in milk is 3.8%. At 6 hours, the percent of the dose of erythromycin in the central and tissue compartments were 6 and 19%, respectively, with 75% of the dose eliminated (Wilson and Van Boxtel, 1978; Ziv, 1980a). Compared to adult cows, a larger apparent volume of distribution and a higher body clearance rate were determined in calves (Burrows, 1980).

In lactating cows, erythromycin was well distributed in the body and mean erythromycin concentrations in renal cortex, muscle and liver varied from 0.09 to 0.14 µg/g tissue, 16 hours after a single intramammary application of 1200 mg erythromycin base. The highest concentration was observed in the liver (Nouws and Ziv, 1979).

Five hours after intramuscular administration of erythromycin anhydrate (8.3 mg/kg bw) in cows, renal, muscle and liver concentrations were 0.11 to 0.92 µg/g, with the highest values in the liver. At a dose of 9 mg/kg, concentrations were less than 0.03 to 0.06µg/g; 67 hours after intramuscular

injection of erythromycin base, the renal cortex concentration was 0.1 µg/g following a dose of 17.5 mg/kg (Nouws and Ziv, 1979).

Metabolism

The metabolism of erythromycin has been studied in different animal species and in humans. (Lee et al, 1956a; Lee et al, 1956b; Wilson and Van Boxtel, 1978; Pineau et el., 1990; Tsubaki and Ichikawa, 1985). These studies show that erythromycin is rapidly metabolized in the liver, mainly through an N-demethylation process in both rats and dogs and in the liver microsomal system of rabbits. Collectively, these studies strongly suggest that the metabolism of erythromycin by N-demethylation occurs in all species tested. Des-N-methyl-erythromycin is the major metabolite and the only microbiologically active metabolite of erythromycin. However, the antimicrobial activity is presumably low and the only form of erythromycin known to be active *in vivo* is the free base. It is excreted in the bile and eliminated through the faeces. Only erythromycin was found in the liver and the absence of des-N-methyl-erythromycin indicates that it is excreted in the bile immediately after erythromycin demethylation. It is absorbed from the intestinal tract but the very minute amount of des-N-methyl-erythromycin available in the body may explain its absence from urine.

The hepatic cytochrome P-450 isozymes that catalyse erythromycin demethylation in rat are highly similar to the form of liver cytochrome P-450 present in rabbit, hamster, gerbil and mouse and this may also extend to humans since human liver contains a protein equivalent to the rat cytochrome P-450. Similarly, a high degree of similarity was found between the ovine cytochrome P-450 involved in N-demethylation of erythromycin and the form isolated in rabbits. This suggests that an equivalent form of these liver cytochrome P-450 isozymes, with similar catalytic activities, is present in the species tested. In cattle a form of cytochrome P-450 isozyme exhibiting a high catalytic activity for N-demethylation was found; this activity was not measured for erythromycin but for other substrates having a N-methyl group structure.

Excretion

Renal excretion

In humans, the portion of an erythromycin dose excreted in the urine varies from 0.02 to 20% and the elimination half-live may be prolonged in renal disease. However, except complete renal failure, renal impairment has only a minor impact on the pharmacokinetics of erythromycin (Wilson and Van Boxtel, 1978).

Urinary excretion of erythromycin accounts for approximately 10% of an administered oral or IM dose (Burrows, 1980). Twenty hours after administration of isotopic erythromycin in rats, 27 to 36% of the radioactivity was recovered in the urine (Lee et al., 1956a).

Faecal Excretion

In humans, 15% of an administered dose was excreted in the bile (Griffith and Black, 1970).

In rats, erythromycin and its metabolites are excreted mainly by way of bile, but in part, also by direct passage through the intestinal wall (Baggot and Gingerich, 1976). Two hours following intravenous injection of isotopic erythromycin, 15.1% of the dose was excreted in the bi1e (Lee et al, 1956b). Twenty hours following intravenous injection of isotopic erythromycin, 37-43% of the radioactivity is recovered in the intestinal tract plus faeces (Lee et al. 1956a). An enterohepatic recirculation may also contribute to the high concentrations of erythromycin in faecal samples (Kroboth et al., 1982).

Pharmacokinetic studies in calves and poultry

Calves

A 1988 study (Report PK 5251/E-00) was performed in calves to determine:

- The pharmacokinetics and bioavailability of erythrocin (erythromycin thiocyanate) injectable following single and multiple IM administrations
- Pulmonary levels after a single IM administration of erythromycin
- Residues of erythromycin in tissues after multiple IM administration of injectable erythrocin

Erythromycin concentrations were assayed by a microbiological method on agar medium using *Micrococcus Luteus* as the sensitive organism (LOD: 0.02 IU/ml in serum; 0.16IU/g in all tissues).

Erythromycin was administered as a single intravenous injection to five calves at a dose of 5 mg erythromycin activity/kg. The study demonstrated a large apparent volume of distribution (Vd area, or Vdβ 1.95 l/kg), a short mean residence time (MRT 2.36 h) and an efficient ability of the organism to remove the drug (Cl 0.77 l/kg/h). As a second experiment in the same assay, a single intramuscular injection of a dose of 5 mg erythromycin activity/kg was administered to seven calves. Good bioavailability was observed (F 95%). Compared to the intravenous route, the elimination half-life and volume of distribution was apparently increased. This could be related to a slow-rate of absorption from the injection site.

In another study, three calves received five consecutive intramuscular injections in the neck or gluteal muscle at a dose of 5 mg/kg at 24-h intervals. No accumulation was observed. The peak concentrations observed after each injection were similar to the C_{max} values obtained after a single injection**.**

In a third study, ten calves received a single intramuscular injection in the neck or into the gluteal muscle alternatively at a dose of 5 mg/kg erythromycin during five days. Drug levels in lungs were always higher than in serum. These data are consistent with the well-known higher tissue than serum concentrations of erythromycin.

In the fourth study, ten calves received a single intramuscular injection in the neck or into the gluteal muscle alternatively at a dose of 5 mg/kg erythromycin during five days. Liver, kidney, muscle non-injection sites, the last three injection sites and fat tissues were collected at different withdrawal times after the last injection. Five days after the last injection, the tissues were free of antibiotic residues except some injection sites. Seven days after the end of the treatment, all tissues, including injection sites were negative. One calf slaughtered ten days after the last injection confirmed these results.

Poultry

A study (Report PK 8400/E-00) was performed in poultry to determine:

- Pulmonary and blood levels of erythromycin after a drinking water medication for three consecutive days
- Residues of erythromycin in chicken tissues after administration by oral route in drinking water for three consecutive days
- Residues of erythromycin in whole hen eggs after a drinking water medication for seven consecutive days

In the first study, 168 adult broilers were given erythromycin thiocyanate in their drinking water for 3 consecutive days. The treated water was changed every day but the water consumption during treatment was not measured. Assuming that the water consumption was similar during the treatment period and before treatment, where water consumption was 235m1/chicken on average, the dose

could be estimated to be 25 mg/kg/day. Blood and lung samples (2-10 birds/sampling time) were collected before the first administration and at:

- 30 min, lh, 2h, 3h, 4h, 6h, 8h, 9h and 24h on day 1 and 2 of medication
- 30 min, 2h, 3h, 4h, 6h, 8h, 9h and 24h on day 3 of medication
- 3h, 4h, 6h. 8h, 9h and 12h after the end of medication

The microbiological method using *Micrococcus Luteus* as the sensitive organism was used to determine blood and lung levels (LOD: 0.02 IU/ml of serum; 0.2 IU/g tissue). Similar but low serum values were noted during the whole treatment period (0.03-0.2 IU/ml, on average), although large variations were observed between broilers, possibly due to differences in individual water consumption. Higher pulmonary (0.5-1 IU/g) than serum levels were observed during the three days treatment, with maximum levels occurring 4 - 12 hours after each changing of medicated water and an elimination time of eight hours. In samples collected 12 hours after the end of the medication, pulmonary levels were below the limit of detection.

In the second study, 15 adult broilers received one dose of erythromycin thiocyanate estimated to be 25 mg/kg/day by the oral route in their drinking water for 3 consecutive days with the treated water changed every 24 hours. At 3, 5, 7 and 10 days after the end of the treatment, the chickens were slaughtered and liver, muscle, fat and skin tissues were collected. The microbiological method noted above was used (LOD: 0.2 IU/g tissue). In all cases the amounts of erythromycin in chicken tissues were below the detection limits 3 days after the end of the treatment.

In the third study, 40 laying hens received the same treatment as the broilers in the previous experiment for seven consecutive days. Five eggs were collected at 3, 5 and 7 days during treatment. Erythromycin was determined in the whole egg with the microbiological method (LOD: 0.06 µg/ml whole egg). Concentration levels of the drug were below the detection limits six days after the end of the treatment.

RESIDUE DEPLETION STUDIES IN TARGET ANIMALS AND IN HENS' EGGS

No radiolabelled study was performed. The following new original residue depletion studies with unlabelled erythromycin were performed in poultry:

Table 3: Residues depletion studies with erythromycin in target animals and eggs

Species	Dose	Study number
Chicken	20 mg / kg / day for 3 days	MPK/5814/9812 MPK/Erythromycin/9957
	20 mg / kg / day for 8 days	MPK/5814/0301
	50 mg / kg / day for 3 days	MPK/210H1/0148
Laying hens	20 mg / kg / day for 3 days	MPK/5814/9908 MPK/Erythromycin/9961
	20 mg / kg / day for 7 days	MPK/5814/0417
Turkey	20 mg / kg / day for 3 days	MPK/5814/0225

The maximum recommended therapeutic dose is 20/mg/kg/day. The route of administration, the dose and the species are those intended for therapeutic use. These studies were performed in accordance with GLP and the European Community guidelines 87/18/EEC and 88/320/EEC, including all supplements published up to the day of the corresponding study start.

Residue Depletion Studies in Chicken

- Chickens treated for three consecutive days at the maximum recommended dose (MPK/5814/9812 and MPK/Erythromycin/9957)

A residue study was performed in order to assess the depletion of erythromycin and its metabolites (N-desmethyl erythromycin A) in edible tissues of broiler chickens after repeated administration. The test product was erythromycin thiocyanate 20% oral powder. Thirty six chickens (18 males and 18 females plus 6 extra birds to replace any that might become ill and not meeting the inclusion criteria were selected for the 7 day acclimatization period) were treated by oral administration of 20 mg/kg/day of erythromycin for 3 consecutive days. All birds belonged to the same strain, TR 551, and were healthy when they received the treatment. For the entire duration of the study, the experimental broiler chickens were in premises at temperature and hygrometry ranging from 16 - 20 ºC and 46 - 76%, respectively. Animals were kept in individual cages and all animals were exposed to an alternating cycle of illumination (12 hours of light followed by 12 hours of darkness). Animals received an individual daily ration of maize, soya, sunflower and wheat supplemented with vegetables and had free access to water. They weighed 0.9±0.1 kg at the beginning of the treatment. Six animals per sampling time were slaughtered at 1 day, 2 days, 3 days, 4 days and 5 days after the end of treatment. Individual edible tissues collected from animals were: 100g of pectoral muscle, 100g of liver, 100g sample of kidneys and 100g of fat and skin in natural proportions.

A specific HPLC method coupled to a mass spectrometer detection system (LC/MS/MS) was used to determine erythromycin A, B and C and N-desmethyl-erythromycin-A in chicken tissues. Antimicrobial activity was measured by a microbiological plate assay. Erythromycin B and C were not detected in all samples, therefore only erythromycin A and N-desmethyl-erythromycin-A were reported. Mean concentrations of erythromycin A measured by both methods were below the LOD or the LOQ for all tissues at 1, 2, 3, 4 and 5 days after the end of the treatment (LOQ: 100 µg/g for all edible tissues and for both methods; LODs for the LC/MS/MS method were: 25 µg/kg for kidney, 30 µg/kg for liver, 3 µg/kg for muscle and 5 µg/kg for skin + fat; the LOD for the microbiological method was 50 µg /kg for all tissues). Mean concentrations of N-desmethyl-erythromycin A are presented in the following table.

Table 4: Mean concentrations of N- desmethyl-erythromycin A in edible tissues of broiler chickens receiving 20mg/kg/day for 3 days

Slaughter time after the end of treatment	Muscle (µg/kg)		Fat/skin (µg/kg)		Liver (µg/kg)		Kidney (µg/kg)	
	HPLC	Micro	HPLC	Micro	HPLC	Micro	HPLC	Micro
1 day	< LOD	< LOD	< LOQ	< LOD	< LOD	< LOD	< LOD	< LOD
2 days	< LOD	< LOD	< LOD	< LOD	282*	< LOD	< LOD	< LOD
3 days	< LOD	< LOD	< LOD	< LOD	163*	< LOD	< LOD	< LOD

Note: * one value

Results also show that only small concentrations of N-desmethyl-erythromycin A were measured by HPLC in liver up to 3 days in only two individual samples after the end of the treatment. For days 4 and 5, concentrations of the metabolite were under the LOD. Because the depletion rate of the residue was very rapid in all edible tissues, it was not possible to provide any correlation between both methods in broiler chickens.

Following the selection of Erythromycin A as the residue marker in edible tissues of broiler chickens, the following additional residue depletion studies were performed in the broiler chickens with different doses.

- Chickens treated for eight consecutive days at the maximum recommended dose (MPK/5814/0301)

Thirty six broiler chickens (18 males and 18 females plus 6 extra birds to replace any that might become ill and not meet the inclusion criteria) were selected for the acclimatization period (10 days) before the beginning of the treatment. The animals had an approximate age of 8 weeks at start of treatment and a mean weight of 1794g (1423-2154g). They were fed *ad libitum* with a pelleted concentrate ration and had free access to water. Chickens were treated by oral administration of 20% erythromycin A thiocyanate via drinking water at 20 mg/kg/day of erythromycin for 8 consecutive days. Six animals per sampling time were slaughtered at 12 hours, 1, 2, 3 and 4 days after the last administration. Individual edible tissues were collected from animals as follows: 400g sample of breast/leg muscle, entire liver, both kidneys and 200g sample of fat and skin in natural proportions.

The concentrations of residues in edible tissue were analyzed with the same LC/MS/MS validated method as the above reported study. Mean concentrations of erythromycin A were below the LOD or the LOQ for all tissues at 12 hours, 1, 2, 3, and 4 after the end of the treatment. The study demonstrated that, whatever the duration of administration, the 20 mg/kg b.w. dose of erythromycin, leads to concentrations of residues in edible tissues below the limit of quantification.

- Chickens treated for five consecutive days at two and one-half times the maximum recommended dose (MPK/210H1/0148)

A total of 36 broiler chickens (18 males and 18 females plus 6 extra birds to replace any might become ill and not meet the inclusion criteria) were selected for the acclimatization period (10 days) before the beginning of the treatment. The study was performed with a dose of 50 mg/kg body weight. The test product was a powder containing 5.5% erythromycin thiocyanate. The animals had an approximate age of 6 weeks at start of treatment and a weighed 800-1000g. They were fed *ad libitum* with a commercial feed and had free access to water. Chickens were treated by oral administration via drinking water for five consecutive days. Six animals per sampling time were slaughtered at 6h, 10h, 24h, 2, 5 and 7 days after the end of treatment. Individual edible tissues collected from animals were: 200g of muscle, entire liver, both kidneys and 40g of fat and skin in natural proportions. Erythromycin A was assayed with the same validated LC/MS/MS method as in the two previous studies. The results are presented in Table 5.

Table 5: Mean concentrations of Erythromycin A in edible tissues of chickens treated with 50mg/kg/day for 5 days

Sampling time after the end of treatment (hours)	Muscle (µg/kg)	Fat/skin (µg/kg)	Liver (µg/kg)	Kidney (µg/kg)
6	133 ± 16	131 ± 35	3220 ± 2080	308 ± 170
10	< LOQ	< LOQ	1760 ± 2840	185 ± 79
24	< LOD	< LOQ	631 ± 393	< LOD

Note: < LOQ: below the quantification limit (100 µg/g for all edible tissues);
< LOD: below the detection limit (25 µg/kg for kidney, 30 µg/kg for liver, 3 µg/kg for muscle and 5 µg /kg for skin + fat).

Concentrations of erythromycin were measurable in all tissues at six hours after the end of the treatment but only in liver and kidney at ten hours after the end of the treatment (the concentrations in muscle and fat/skin were less than the LOQ). Residues at one day after the end of treatment were only found in liver; concentrations in other tissues were below the LOD or LOQ. At day 2 or further times post-treatment, concentrations in all tissues were below the LOQ or the LOD. The study demonstrates that the administration of doses higher than the recommended during consecutive days does not result in accumulation of residues.

Residue Depletion Studies in Laying Hens

- Laying hens treated for three consecutive days at the maximum recommended dose (MPK/5814/9908 and MPK/erythromycin/9961)

A residue study was performed in eggs from laying hens that received a repeated administration for three days. A total of 40 laying hens were selected for the acclimatization period (14 days) before the beginning of the treatment, 30 were selected for treatment according to individual egg production. From these, 25 laying hens were selected for sampling, keeping 5 animals to replace any that might become ill. All birds belonged to the same strain and were healthy when they received the treatment; no animal had received any treatment ten days before the beginning of the study. The laying hens were treated by oral administration of 20 mg/kg/day of erythromycin thiocyanate 20% oral powder. The laying hens weighed 2.1 ± 0.2 kg and were approximately 8 months at the beginning of the treatment. They were fed *ad libitum* with a commercial feed and had free access to water. All eggs produced were collected daily during the treatment and ten days after the end of the treatment. The production ranged from 19-26 eggs per day and 13-17 eggs per laying hen over the 16-day experimental period. Control samples were collected three days before the beginning of the treatment period. The total antimicrobial activity of residues was also determined with the microbiological plate assay. Test samples were a mixture of albumen and yolk of each egg at each time point. Only Erythromycin A and N-desmethyl-erythromycin-A were detected. Results are reported in the following table.

Table 6: Mean concentrations of erythromycin A and N-desmethyl-erythromycin A in eggs of laying hens treated with 20mg/kg/day for 3 days

Sampling time	Mean concentration erythromycin A (µg/kg)		Mean concentration N-desmethyl-erythromycin A (µg/kg)	Ratio HPLC / microbiological method for erythromycin A	Ratio HPLC / microbiological method for desmethyl-erythromycin A
	HPLC	Micro			
Beginning of treatment					
Day 0	109 ± nc	194 ± nc	< LOD	0.56	-
Day 1	78 ± 12	158 ± 76	< LOD	0.46	-
Day 2	83 ± 24	198 ± 76	< LOD	0.33	-
End of treatment					
1 day post treatment	57 ± 6	221 ± 80	97 ± nc	0.23	-
2 day post treatment	71 ± 6	207 ± 59	120 ± nc	0.25	0.76
3 day post treatment	54 ± nc	118 ± 21	69 ± nc	-	-
4 day post treatment	< LOQ	< LOD	64 ± nc	-	-
5 day post treatment	< LOQ	< LOD	< LOD	-	
Mean				0.33	0.76

Note: <LOD: below the limit of detection (0.92 µg/kg for the HPLC method and 50 µg/kg for the microbiological method); <LOQ: below the limit of quantification (50 µg/kg for the HPLC method and 100 µg/kg for the microbiological method); nc: non calculated

Erythromycin A could only be quantified in 25% of eggs at day 1 and 12.5% of the eggs at day 2. Concentrations of erythromycin A after the end of the treatment measured by LC/MS/MS were near the LOQ of the method (50 µg/kg). N-desmethyl-erythromycin A had high concentrations but it has a very low antimicrobial activity. Both compounds were below the LOQ (50 µg/kg) six days after the end of treatment. Erythromycin A was identified as the marker residue for eggs.

- Laying hens treated for seven consecutive days at the maximum recommended dose (MPK/5814/0417)

Residues in eggs from laying hens were analyzed after repeated administration for seven days. The hens weighed 1.6 ± 0.16 kg and were approximately 23 weeks at the beginning of the treatment. Twenty laying hens were treated by oral administration of 20 mg/kg/day of erythromycin thiocyanate 20% oral powder. All birds belonged to the same strain and were healthy when they received the treatment after an acclimatization period of 21 days. The laying hens were fed *ad libitum* with wheat and a pelleted concentrated ration and had free access to water. Eggs were collected daily beginning five days before the start of the treatment, during the treatment and unti1 28 days after the last treatment. Ten eggs per day were randomly selected for sampling at each time point coming from the first ten hens having regularly laid one egg per day and throughout all study. Samples were a mixture of albumen and yolk of each egg selected at each time point. Only erythromycin A was measured in eggs with the LC/MS/MS method used in the above studies. The results are presented in Table 7.

Table 7: Mean concentrations of erythromycin A in eggs of laying hens treated with 20 mg/kg/day for seven days.

Sampling time	Mean concentration (µg/kg)
Beginning of treatment	
Day 1	< LOD
Day 2	< LOQ
Day 3	< LOQ
Day 4	72 ± 11.1
Day 5	135 ± nc
Day 6	59 ± 7.1
Day 7	68 ± 18.3
End of treatment	
1 day post treatment	59 ± 0.7
2-8 days post treatment	< LOQ
9-21 days post treatment	< LOD

Note: < LOD: below the limit of detection (0.9 µg/kg);
< LOQ: below the limit of quantification (50 µg/kg);
nc: not calculated

Erythromycin could be quantified only 1 day after the end of the treatment. After this time, the concentrations of the residues were below the LOQ (50µg/kg). The study demonstrated that, whatever the duration of the administration, the administration of 20mg/kg b.w./day results in concentrations of erythromycin in eggs no higher that those found with fewer days of administration.

Residue Depletion Studies in Turkeys

- Treatment for three consecutive days at the maximum recommended dose (MP/5814/0225)

Thirty four white turkeys were treated by oral administration of 20 mg/kg/day of erythromycin thiocyanate 20% oral powder via drinking water for 3 consecutive days. All animals belonged to the same strain and were healthy when they received the treatment. Animals received a daily ration of commercial concentrated feed and had free access to water. They weighed 2289±309g at the beginning of the treatment. Turkeys were selected for the acclimatization period (14 days) before the beginning of the treatment. Six animals per sampling time were collected at 3, 4, 5, 6 and 8 days after treatment (1, 2, 3, 4 and 6 days after the last dose), 4 birds used as controls were sampled at six days after the end of the treatment. Individual edible tissues collected from animals were: whole liver, both kidneys, 400g of pectoral muscle, and approximately 20-50g of fat+skin in natural

proportions. The LC/MS/MS assay method for the detection of residues in turkeys was adapted from the chicken method. Three days after the end of the treatment, residues were detectable only in liver (166±64 µg/kg). At this time, residues were measured only in one sample of kidney, muscle and fat. After four days post-treatment, all concentrations of residues were below the LOQ or the LOD in all tissues. The results are presented in Table 8.

Table 8: Mean concentrations of erythromycin A in edible tissues of turkeys treated with 20mg/kg/day for three days.

Sampling time after treatment	Muscle (µg/kg)	Fat/skin (µg/kg)	Liver (µg/kg)	Kidney (µg/kg)
3 days	266*	318*	166 ± 63.6	424*
4, 5,6 and 8 days	< LOQ	< LOQ	< LOQ	< LOD

Note: * only one value;< LOQ: below the quantification limit (100 µg/kg for all tissues);
< LOD: below the detection limit (4 µg/kg for skin/fat and 3 µg/kg for all other tissues).

METHODS OF ANALYSIS

Erythromycin A, B and C and N-methyl-erythromycin A by a LC/MS/MS method

Chicken (MPK/5814 /9812 and validation in MPK/erythromycin/9957).

***Extraction procedure for muscle, liver and kidney*:** 1 g thawed tissue was shaken with 1 ml of double distilled water using a vortex mixer. Nine ml of acetonitrile was added and the mixture vortexed for an additional 20 seconds. The mixture was shaken for 10 minutes using a mechanical shaker. After decantation, 100µl of the supernatant was added to 300µl of the dilution solution and 15µl was injected into the chromatographic system.

Extraction procedure for skin/fat: 1 g frozen tissue was shaken with 20 ml of acetonitrile for 20 seconds using a vortex mixer followed by 10 minutes using a mechanical linear shaker. After decantation, 1 ml of supernatant and 1 ml of double distilled water and 2 ml of n-hexane were added. The mixture was shaken 10 minutes using a mechanical shaker, centrifuged at 2500 rpm for 5 minutes, n-hexane was decanted off and 15 µl of solution were injected onto the chromatographic system.

***Chromatographic procedure*:** 15 µl was injected onto the column using the autosampler. The column used was an RP 18ec 30 x 4 mm, 5 µm. The elution was done under isocratic conditions with acetonitrile/double distilled water 50:50 v/v with 1% formic acid. The retention times were 2.7 min for erythromycin A, 3.1 min for erythromycin B, 2.0 min for erythromycin C and 2.4 min for N-desmethyl-erythromycin A. Detection was done by mass-mass using a quadripole instrument with an electrospray source in the positive ionization mode. Multiple Reaction Monitoring (MRM) was applied and the following transition ions were monitored 734.6 →576.6 for erythromycin A, and 718.6 →565.2 for erythromycin B, 720.6 →576.5 for erythromycin C, and 720.5 →562.2 for N-desmethyl-erythromycin A. No internal standard was used. Quantification of both products was done after interpolation of unknown sample peak areas against the theoretical concentrations of calibration curves. Results were expressed as free erythromycin A and N-desmethyl-erythromycin A (µg/kg). The method was validated using the following criteria consistent with internationally recognized guidelines.

***Specificity*:** The method was found specific from endogenous compounds, erythromycin B and C, N-desmethyl-erythromycin A, tylosin, tilmicosin and spiramycin.

***Linearity*:** The linearity of detector response was assessed in the four tissues matched with standard solutions using 100-5000 µg/kg. The correlation coefficients were 0.9996, 0.9993, 0.9998 and

0.9993 for erythromycin A in muscle, liver, kidney and skin/fat respectively and 0.9979, 0.9987, 0.9992 and 0.9976 for N-methyl-erythromycin A in the same tissues, respectively**.**

***Stability*:** Stability was demonstrated at a concentration of 2000 µg/kg after 3 freeze-thaw cycles.

The within-day precision, day-to-day precision, accuracy, recovery rate, LOQ and LOD are presented in the following tables.

Table 9: Validation parameters for erythromycin A in chicken tissues

Erythromycin A	Muscle	Kidney	Fat / skin	Liver
Concentration (µg/kg)	100 - 5000	100 - 5000	100 - 5000	100 - 5000
Within-day precision (%)	1.95 - 7.57	2.30 - 7.24	2.51 - 7.80	2.89 - 8.19
Day-to-day precision (%)	2.28 - 7.57	2.30 - 7.24	2.51 - 9.36	4.31 - 13.6
Accuracy (E%)	$-1.5 \leq E\% \leq +2.0$	$-2.0 \leq E\% \leq +2.4$	$-7.0 \leq E\% \leq +10.1$	$-3.3 \leq E\% \leq +3.0$
Recovery rate (%)	> 98.9	> 92.1	> 87.4	> 98.1
LOQ (µg/kg)	100	100	100	100
LOD (µg/kg)	3	25	5	30

Table 10: Validation parameters for N-desmethyl-Erythromycin A in chicken tissues

N-desmethyl-Erythromycin A	Muscle	Kidney	Fat / skin	Liver
Range (µg/kg)	100 - 5000	100 - 5000	100 - 5000	100 - 5000
Within-day precision (%)	2.56 - 6.37	1.95 - 7.19	2.61 - 9.33	2.48 - 6.85
Day-to-day precision (%)	2.62 - 7.15	1.95 - 7.19	2.61 - 9.33	3.86 - 9.47
Accuracy (E%)	$-3.0 \leq E\% \leq +3.4$	$-1.2 \leq E\% \leq +3.8$	$-4.0 \leq E\% \leq +10.4$	$-4.2 \leq E\% \leq +4.0$
Recovery rate (%)	> 99.1	> 96.8	> 81.7	> 97.3
LOQ (µg/kg)	100	100	100	100
LOD (µg/kg)	3	25	24	48

Turkey (MPK/5814/0225)

The analytical method was adapted from the chicken method described above. It was validated using the criteria noted above. The method was partially validated as noted below. The retention time was approximately 3.6 min for erythromycin A.

***Specificity*:** The method was found specific from endogenous compounds, erythromycin B and C, N-desmethyl erythromycin A, tylosin, tilmicosin and spiramycin.

***Linearity*:** The linearity of detector response was assessed in the four tissues matched with standard solutions in the range of 100-5000 µg/kg. The correlation coefficients were 0.9998, 0.9997, 0.9988 and 0.9993 for erythromycin A in muscle, liver, kidney and skin/fat. The within-day precision, accuracy, recovery rate, LOQ and LOD are presented in Table 11.

Table 11: Validation parameters for erythromycin A in turkey tissues

Erythromycin A	Muscle	Liver	Kidney	Fat / skin
Range (µg/kg)	100 - 5000	100 - 5000	100 - 5000	100 - 5000
Within-day precision (%)	1.1 - 2.3	1.6 - 4.6	1.1 - 2.2	4.9 - 11.2
Accuracy (E%)	-5.0 - 6.5	-8.0 - 8.4	-6.8 - 0.2	-3.3 - 8.8
LOQ (µg/kg)	100	100	100	100
LOD (µg/kg)	3	3	3	4

Eggs (MPK/ 5814/9908 and validation in MPK/erythromycin/9961)

Extraction procedure*:* 1g frozen mixed egg was shaken with 1 ml of double distilled water for 20 seconds using a vortex mixer. Nine ml of acetonitrile were added and the mixture was shaken for 20 seconds using a vortex mixer and for 10 minutes using a mechanical linear shaker. After decantation, 500 µl of supernatant were added to 500 µl of acetonitrile, 1 ml of double distilled water and 2 ml of n-hexane. The mixture was shaken 10 minutes using a mechanical shaker, centrifuged, the n-hexane was decanted off and 10 µl of solution were injected onto the chromatographic system.

Chromatographic procedure**:** 15 µl were injected onto a RP 18ec 30 x 4 mm, 5 µm column. The elution was obtained under isocratic conditions with acetonitrile/double distilled water 50:50 v/v with 1% formic acid. The retention times were 3 min for erythromycin A and 2.5 min for N-desmethyl-erythromycin A. Detection was done by mass-mass using a quadripole instrument with a turbo ionspray source in the positive ionization mode. MRM was applied and the following transition ions were monitored 734.6→158.0 for erythromycin A and 720.5 →144.2 for N-desmethyl-erythromycin A. No internal standard was used. Quantification of both products was done after interpolation of unknown sample peak areas against the theoretical concentrations of calibration curves. Results were expressed as free erythromycin A and N-desmethyl-erythromycin A.

Specificity**:** The method was found specific from endogenous compounds, erythromycin B and C, N-desmethyl erythromycin A, tylosin, tilmicosin and spiramycin.

Linearity**:** The linearity of detector response was assessed matched with standard solutions in the range of 50-5000 µg/kg. The correlation coefficients were 0.9993 and 0.9997 for erythromycin A and N-methyl-erythromycin A respectively.

Stability**:** The stability of erythromycin A and N-desmethyl-erythromycin A was evaluated at the concentration level of 2000 µg/kg after 3 freeze-thaw cycles. The mean concentration was approximately 104% of the mean reference value. Long term stability was evaluated in both compounds in a mixed frozen egg stored at -80 °C for 1 month at the concentration level of 2000 µg/kg. The mean concentration of the pool was 102% and 104% of the nominal value of erythromycin A and N-methyl-erythromycin A, respectively.

The within-day precision, day-to-day precision, accuracy, recovery rate, LOQ and LOD are presented in Table 12.

Table 12: Validation parameters for erythromycin A and N-desmethyl-erythromycin A in eggs

	Erythromycin A	N-desmethyl-Erythromycin A
Range (µg/kg)	50 - 5000	50 - 5000
Within-day precision (%)	1.76 - 9.87	1.10 - 7.79
Day-to-day precision (%)	1.76 - 11.87	1.11 - 7.79
Accuracy (E%)	$-5.0 \leq E\% \leq +7.8$	$-1.3 \leq E\% \leq +2.0$
Recovery rate (%)	> 82.6	> 71.9
LOQ (µg/kg)	50	50
LOD (µg/kg)	0.92	2.2

Microbiological assay method for erythromycin in chicken tissues and eggs

A microbiological method was used for the assay of erythromycin A and its related metabolites with potential microbiological activity in edible tissues (muscle, kidney, liver and fat/skin) of chicken (MPK/erythromycin/9957) and eggs (MPK/Erythromycin/9961).

***Extraction procedure*:** 1 g of test sample was vortexed with 4 ml of acetonitrile, placed in an ultrasonic bath for five minutes and on a liner shaker for ten minutes. The extract was centrifuged at 7500g for 10 minutes and the supernatant transferred into a disposable tube. Two wells of each Petri dish were filled with this mixture.

***Petri dishes*:** Plates were prepared (diameter 100mm) using agar medium and *Microccocus Luteus ATCC9341* as test organism. For each point, two plates with two replicates per plates were used. The diameter of bacterial growth inhibition was the measured response. Amounts of erythromycin were calculated by interpolating the inhibition diameter into the standard curve. The method has been validated according to the following criteria.

***Specificity*:** The method shows specificity between erythromycin and endogenous component of the tissues. Three different matrixes are tested under experimental conditions for each tissue. No inhibition zone was observed for any tissue.

***Linearity in chicken tissues*:** The linearity of detector response was assessed in the four tissues matched with standard solutions in the range from 100 to 2000µg/kg. The correlation coefficients were 0.9994, 0.9513, 0.9571 and 0.9969 for erythromycin A in muscle, liver, kidney and skin/fat, respectively. Other validation parameters are presented in the following tables for each tissue.

***Linearity in eggs*:** The linearity of detector response was assessed matched with standard solutions in the range from 100 to 2000µg/kg. The correlation coefficient was 0.9567 as average of 9 standard curves. Other validation parameters are presented in the following tables.

Table 13: Validation parameters of the microbiological method for erythromycin in chicken
Table 13-A

Validation parameters for kidney							
Concentrations (µg/kg).	100	200	500	1000	1500	2000	Overall
Recovery rate (%) n=9	85.5	86.5	88.2	85.1	84.8	86.1	86.0
Repeatability, Cv_r% n=3 per day	5.9	9.4	7.8	10.8	5.7	6.0	-
Intermediate precision, CV_r %/ 3 days	12.9	12.8	13.5	10.8	5.7	10.5	-
Accuracy: difference (% value)	-5.11	3.78	7.07	4.02	-1.90	-4.27	0.60
Limit of Detection (LOD)				50 µg/kg			
Limit of Quantification (LOQ)				100 µg/kg			
- = not calculated							

Table 13-B

Validation parameters for fat + skin							
Concentrations (µg/kg).	100	200	500	1000	1500	2000	Overall
Recovery rate (%) n=9	101.5	102.9	100.0	97.5	97.1	96.6	99.2
Repeatability Cv_r% n=3 per day	3.7	5.6	4.6	7.5	6.9	6.7	-
Intermediate precision, CV_r %/ 3 days	3.7	5.6	5.4	7.5	6.9	6.7	-
Accuracy: difference (% value)	0.22	-0.22	-1.13	4.99	-3.44	0.67	0.18
Limit of Detection (LOD)				50 µg/kg			
Limit of Quantification (LOQ)				100 µg/kg			
- = not calculated							

Table 13-C

Validation parameters for muscle							
Concentrations (µg/kg).	100	200	500	1000	1500	2000	Overall
Recovery rate (%) n=9	94.1	94.0	93.6	92.6	93.1	95.9	93.9
Repeatability Cv_r% n=3 per day	9.4	13.3	7.9	4.6	7.3	4.5	-
Intermediate precision, CV_r%/ 3 days	9.8	13.3	7.9	4.6	7.3	6.2	-
Accuracy: difference (% value)	-3.22	5.22	2.53	-2.00	-1.45	1.06	0.36
Limit of Detection (LOD)				50 µg/kg			
Limit of Quantification (LOQ)				100 µg/kg			
- = not calculated							

Table 13-D

Validation parameters for liver							
Concentrations (µg/kg).	100	200	500	1000	1500	2000	Overall
Recovery rate (%) n=9	86.6	83.7	88.5	87.8	87.8	88.8	87.2
Repeatability, Cv_r% n=3 per day	5.2	7.6	7.0	8.8	6.1	6.3	-
Intermediate precision, CV_r %/ 3 days	9.2	7.9	13.3	8.8	6.2	6.3	-
Accuracy: difference (% value)	0.22	-5.72	9.47	4.04	1.26	-6.51	0.46
Limit of Detection (LOD)				50 µg/kg			
Limit of Quantification (LOQ)				100 µg/kg			
- = not calculated							

Table 14: Validation parameters of a microbiological assay method for erythromycin in eggs

Validation parameters							
Concentrations (µg/kg).	100	200	500	1000	1500	2000	Overall
Recovery rate (%) n=9	87.5	93.5	94.2	93.4	92.4	92.8	92.3
Repeatability, Cvr% n=3 per day	2.9	2.6	4.7	3.9	4.3	4.6	-
Intermediate precision, CVr%/ 3 days	5.0	4.5	5.1	5.7	4.5	4.6	-
Accuracy : difference (% value)	-4.89	3.11	3.87	5.77	-2.21	-4.59	0.18
Limit of Detection (LOD) :				50 µg/kg			
Limit of Quantification (LOQ) :				100 µg/kg			
- = not calculated							

Wang, et al. (2005) published a method for determination of five macrolide antibiotic residues in eggs (spiramycin, tilmicosin, oleandomycin, erythromycin and tylosin) using liquid chromatography/electrospray ionization tandem mass spectrometry. Data acquisition under MS/MS was achieved by multiple reaction monitoring of two or three fragment ion transitions for both quantification and confirmation. A full experimental design was used to study the measurement uncertainty arising from intermediate precision and trueness or proportional bias. The overall recoveries of spiramycin, tilmicosin, oleandomycin, erythromycin and tylosin at fortified levels of 60, 100, 200 and 300 µg/kg were 96.8, 98.2, 98.3, 98.8 and 95.4, respectively. The method detection limits ($S/N \geq 3:1$) of five macrolides were <1.0 µg/kg).

APPRAISAL

Erythromycin is an old drug. It was first reviewed by the Committee in 1968. No ADI was established but acceptable levels of residues were defined in milk (0-0.04 mg/ml) and meat (0-0.3 mg/kg). Erythromycin is a mixture of three compounds produced during fermentation. The main product is erythromycin A with small portions of B (≤ 5%) and C (≤ 5%). In veterinary medicine, erythromycin is used for the treatment of clinical and subclinical mastitis in lactating cows, for the treatment of infectious diseases due to erythromycin sensitive bacteria (cattle, sheep, swine, and poultry) and for the treatment of chronic diseases due to mycoplasma in poultry. The maximum recommended therapeutic dose in veterinary use is 20/mg/kg/day as erythromycin base.

Data from pharmacokinetic and metabolic studies in experimental and target animals and humans were submitted for evaluation by the Committee together with two earlier residue studies in calves and chickens. Three new non radiollabeled residue depletion studies in chickens, laying hens and turkeys treated with erythromycin and the description and validation of the analytical procedures employed were provided.

Erythromycin is rather slowly absorbed in humans, rats, cattle and chicken with some differences related to the mode of administration (IM, IV and oral), the salt form and the coating of the administered compound. Protein binding is variable ranging from 90% in man to 38-45% in cattle. The major site of absorption is rats, dogs and humans is the small intestine. Erythromycin is only slightly absorbed in the stomach. The tissue concentrations are higher than in serum and persist longer. Erythromycin is mainly excreted in the faeces through the bile, 37 to 43 % of the dose was recovered in the intestinal tract plus faeces of rats. Urinary excretion ranged from 10 to 36 % in different species (human, rats and dogs).

Erythromycin is rapidly metabolized in the liver, mainly through an N-demethylation process in a variety of species of rodents, ruminants and humans. N-desmethyl-erythromycin was the major metabolite and the only microbiologically active metabolite of erythromycin. However, its antimicrobial activity is low and the only form of erythromycin known to be active *in vivo* is erythromycin free base.

Two studies were performed in 1988 in calves and poultry using erythromycin thiocyanate. In poultry, the residues of erythromycin were determined in chicken tissues after administration by the oral route in drinking water for 3 consecutive days. In a similar study the residues of erythromycin in whole eggs were determined following medication administered to laying hens for 7 consecutive days in drinking water. The concentration of the erythromycin in chicken tissues declined to values below the limit of detection three days after the end of treatment and six days after the end of treatment in whole eggs.

There was no radiolabelled study reported, however, four new residue depletion studies with unlabelled erythromycin were performed in poultry. The route of administration, the dose and the species are those intended for therapeutic use.

The residues with microbiological activity were measured in chickens and laying hens by a microbiological plate assay using agar medium and *Micrococcus luteus* ATCC9341 as test organism (LOD: 50 µg/kg for all chicken tissues and eggs; LOQ: 100 µg/kg for all chicken tissues and eggs). The concentration of erythromycin A and its metabolites were simultaneously assayed using a LC/MS/MS method. For chickens, erythromycin A, B and C, the LOD are as follows: 25 µg/kg for kidney, 30 µg/kg for liver, 3 µg/kg for muscle and 5 µg/kg for skin + fat and the LOQ is 100 µg/kg in all tissues. For N-desmethyl-erythromycin A, the LOD are as follows: 25 µg/kg for kidney, 48 µg/kg for liver, 5 µg/kg for muscle and 24 µg/kg for skin + fat and the LOQ is 100µg/kg in all tissues. For eggs, the erythromycin LOQ is 50 µg/kg and the LOD is 0.92 µg/kg. For turkey, the erythromycin LOQ is 100 µg/kg for all tissues and the LOD is 4 µg/kg for skin/fat and 3 µg/kg for all other tissues.

In the chicken studies, all tissue residue depletion results showed that from day 1 to day 3 after the end of the treatment period, low concentrations of erythromycin A and N-desmethyl-erythromycin A were measured in only a few liver samples. Prolonged treatment for up to eight days resulted in the same tissue residue concentration characteristics. In eggs, during the three days of treatment, mean concentrations of erythromycin A ranged from 109 µg/kg (day 1) to 83 µg/kg (day 3) measured by the LC/MS/MS method. Taking into account the standard deviation, there are no significant differences in residue concentration between days. The egg residues of erythromycin after the end of the treatment were 57±6 µg/kg, 71± 6 µg/kg and 54± nc µg/kg for days 1, 2 and 3, respectively.

Results show that erythromycin A and its metabolite N-desmethyl-erythromycin A were the major compounds observed (the ratio erythromycin/total active microbiological metabolites was 0.33 and the ratio of N-desmethyl-erythromycin/total active microbiological metabolites was 0.75). Erythromycin could only be quantified in 25% of eggs at day 1 and 12.5% of the eggs at day 2. Concentrations of erythromycin after the end of the treatment measured by LC/MS/MS were near the LOQ (50 µg/kg). N-desmethyl-erythromycin was present at higher concentrations but had very low antimicrobial activity. Both compounds were below the LOQ at 6 days after the end of treatment. Erythromycin A was identified as the marker residue for eggs.

Tissue residue depletion studies in turkeys yielded similar results. The LC/MS/MS assay method was adapted from the chicken method. At three days after the end of the treatment, residues were detected only in liver (166±64 µg/kg). At this time, residues were measured only in one sample of kidney, muscle and fat. Four days post-treatment, all concentrations of residues were below the LOQ or the LOD.

The 66th meeting of the Committee agreed to apply a new approach to estimate chronic (long term) exposure using the median residue concentration of residues, using the standard food basket, in addition to the historically used theoretical maximum daily intake calculation based on the MRL. For numerical values reported below the LOD and the LOQ, one-half the analytical limit was applied to each, respectively, for estimating daily exposure concentrations. For erythromycin, the summary of the median residue concentrations in chickens, turkeys and eggs are presented in the following tables, derived from using individual values in all studies using an Excel spreadsheet.

Table 15a: Median residues in chicken and turkey tissues (µg/kg)

Tissue & Time	Chicken			Turkey			
	12 hours	1 day	2 days	1 day	2 days	3 days	4 days
Muscle	1.5	1.5	1.5	50	50	1.5	1.5
Liver	15	15	15	50	50	15	15
Kidney	12.5	12.5	12.5	50	12.5	12.5	12.5
Fat+Skin	50	2.5	2.5	50	25.8	2.5	2.5

Table 15b: Median residues in chicken eggs (µg/kg)

Time (days)											
0 day	1 day	2 days	3 days	4 days	5 days	6 days	7 days	8 days	9 days	10 days	11days
0.45	25	25	25	25	25	25	41	25	25	25	25

The LC/MS/MS analytical methods provided are applicable for the determination of residues of erythromycin in chicken and turkey tissues (muscle, fat, kidney and liver) and eggs. The method was validated.

MAXIMUM RESIDUE LIMITS

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

- The marker residue is erythromycin A. Metabolites exhibited little or no microbiological activity.
- Only MRLs in poultry tissues and eggs were considered.
- Residue studies in bovine and ovine tissues suggest mean ratios of marker residue to total residues for tissues to be 0.7 for muscle and kidney, 0.5 for liver and 0.85 for fat. The ratio was not always available for all species and when it was, it was only available based on few individual data. From submitted studies, the marker residue to total residues for eggs was estimated to be 0.33.
- Residue depletion studies generated very limited numbers of residue concentrations above the limit of quantification for all studies in chickens, turkeys and eggs.
- A validated LC/MS/MS method is available with a limit of quantification of 100 µg/kg for all tissues and 50 µg/kg for eggs. The limit of quantification of the microbiological method is 100 µg/kg for all tissues and eggs.
- For residue concentrations reported below the LOD and the LOQ, one-half the analytical limit was applied to each, respectively, for estimating daily exposure concentrations.
- The ADI for erythromycin A was 0 – 0.7 µg/kg body weight, equivalent to 42 µg per person per day.

Noting the factors noted above, the Committee recommended MRLs of 100 µg/kg for chicken and turkey muscle, liver, kidney and fat/skin and 50µg/kg for eggs at the limit of quantification of the LC/MS/MS method, measured as erythromycin A.

Applying the MRLs and the standard food basket, the theoretical maximum daily intake is 55 µg, equivalent to approximately 130% of the ADI. 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. Median residue values were determined using an Excel® spreadsheet program. 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. Applying the highest estimated median residue concentrations in turkeys (50 µg/kg in all tissues) – the median residue concentrations in turkey tissues were higher than the corresponding median residue concentrations in chicken tissues - and eggs (41µg/kg), the estimated daily intake is 29.1 µg/kg, equivalent to approximately 69% of the ADI. Results are shown in Table 16.

Table 16: Estimated daily intake

Tissue	Median intake (µg/kg)	Standard Food Basket (kg)	Daily intake (µg)
Muscle	50	0.3	15
Liver	50	0.1	5
Kidney	50	0.05	2.5
Fat	50	0.05	2.5
Eggs	41	0.1	4.1
Total			29.1

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FLUMEQUINE

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ADDENDUM

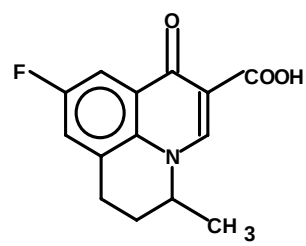
To the monographs prepared by the 48th, 54thand 60th meetings of the Committee and published in the FAO Food and Nutrition Papers 41/10, Rome 1998; 41/13, Rome 2000; and 41/15, Rome 2003

IDENTITY

Chemical name: 9-Fluoro-6,7-dihydro-5-methyl-1-oxo-1H,5H-benzo[ij]quinolizine-2-carboxylic acid

Synonyms: R-802, Apurone

Structural formula:



Molecular formula: $C_{14}H_{12}NFO_3$

Molecular weight: 261.26

OTHER INFORMATION ON IDENTITY AND PROPERTIES

Pure active ingredient: flumequine

Appearance: white microcrystalline powder

Melting point: 253-255ºC

Solubility: Soluble in aqueous alkaline solutions and alcohol; insoluble in water

Optical rotation: Produced and used as a racemic mixture

BACKGROUND

Flumequine has previously been evaluated by the Committee at the 42nd, 48th, 54th, 60th and 62nd meetings. At its 42nd meeting, the Committee (WHO, 1995) was unable to establish an ADI for flumequine due to the lack of adequate information on the toxicological and microbiological hazards of flumequine and residue data were not provided for evaluation.

The 48th meeting of the Committee (WHO, 1998) established an ADI of 0-30 μg per kg of body weight based on the overall NOEL for hepatotoxicity of 25 mg per kg of body weight per day observed in a 13-week study in CD-1 mice and a safety factor of 1000. MRLs for flumequine of 500 μg/kg for muscle, 1000 μg/kg for liver, 3000 μg/kg for kidney and 1000 μg/kg for fat in cattle, expressed as parent drug, were recommended. Temporary MRLs of 500 μg/kg for muscle, 1000 μg/kg for liver, 3000 μg/kg for kidney and 1000 μg/kg for fat, expressed as parent drug, were recommended in sheep, chickens and pigs. The Committee also recommended a temporary MRL of 500 μg/kg for trout muscle (including normal proportions of skin), expressed as parent drug.

The 54th meeting of the Committee (WHO, 2001) evaluated data from studies conducted with radiolabelled flumequine in pigs, sheep, chickens and trout. Based on these studies, the Committee recommended MRLs of 500 μg/kg for muscle and liver, 3000 μg/kg for kidney and 1000 μg/kg for fat in cattle, pigs, sheep and chickens. The Committee also recommended an MRL of 500 μg/kg for trout muscle, including skin in natural proportions.

The 60th meeting of the Committee (WHO, 2003) evaluated new studies which further elucidated the mechanism of flumequine-induced hepatotoxicity in mice. Based on these studies, the Committee noted the possibility that flumequine induces tumours in the mouse liver by a mechanism that includes genotoxic effects. The Committee therefore withdrew the ADI that had been established at the 48th meeting and withdrew the MRLs that had been recommended at the 54th meeting.

The 62nd meeting of the Committee (WHO, 2004) reconsidered three short-term studies in mice that had been evaluated at the 60th meeting. The Committee also evaluated a new study conducted with flumequine in rat liver cells *in vivo* to assess unscheduled DNA synthesis. The Committee concluded that the available data supported a non-genotoxic, threshold-based mechanism for tumour formation by flumequine in the mouse liver. As a result, the Committee re-established the ADI of 0-30 μg/kg body weight that had originally been established for flumequine at its 48th meeting. The MRLs that had been recommended at its 54th meeting and withdrawn at its 60th meeting were also re-established.

The 62nd meeting of the Committee also recommended a temporary MRL of 500 μg/kg for muscle of black tiger shrimp (*Penaeus monodon*) based on the evaluation made at the 60th meeting. The MRL was temporary and the following information was requested by 2006:

(i) A detailed description of a regulatory method, including its performance characteristics and validation data

The 60th meeting also evaluated residue depletion studies in shrimp. Flumequine was administered to shrimps at a rate of 12 mg/kg of body weight as a single intramuscular injection, or as a single forced oral dose, or as medicated feed for 5 consecutive days. Of the three methods of drug delivery, only medicated feed is practical for large-scale use in shrimp production. Flumequine delivery in feed resulted in the lowest concentration of residues in shrimp muscle, between 29.8 and 45.5 μg/kg of muscle during treatment, and depleting to less than the LOQ at 96 hours after withdrawal of medicated feed. Based on these data, dosing with 12 mg of flumequine per kg of body weight as medicated feed, the Committee considered it is unlikely to demonstrate efficacy against bacterial diseases in shrimp (FAO,

2003). In order to address this concern, the 62nd meeting, which re-established the ADI for flumequine, requested by 2006, information on the approved dose for treatment of black tiger shrimp and the results of the residue studies conducted at the recommended dose.

No new information was provided for evaluation by 2006.

Conditions of Use

Flumequine is a fluoroquinolone with antimicrobial activity against Gram-negative organisms. Fluoroquinolones specifically inhibit topoisomerase II (also referred to as DNA gyrase), an enzyme that controls the supercoiling of bacterial DNA by catalyzing the cleavage/reunion of the two strands in the DNA molecule. Binding of fluoroquinolones to DNA gyrase disrupts enzyme activity, resulting in rapid cell death. The bactericidal action of fluoroquinolones is rapid and concentration-dependent. However, activity is inhibited at very high concentrations through direct inhibition of RNA synthesis, which can be antagonized by protein synthesis inhibitors and RNA synthesis inhibitors (Maddison and Watson, 2002).

Flumequine is used in the treatment of enteric infections in food animals and in the treatment of bacterial infections in farmed fish. Flumequine also has limited use in humans for the treatment of urinary tract infections.

METHODS OF ANALYSIS

The 48th meeting of the Committee (FAO, 1998) considered an HPLC method with fluorescence detection for the analysis of flumequine and 7-hydroxyflumequine in the tissues of cattle, pigs, chickens, sheep, and trout. The method had not been properly validated for regulatory purposes. A similar analytical method, which had been validated and was suitable for regulatory purposes, was considered by the 54th meeting.

The 60th meeting of the Committee (FAO, 2003) considered an HPLC method with fluorescence detection for quantifying residues of flumequine in the muscle of black tiger shrimps. The Committee requested that detailed information on a regulatory method including method performance characteristics and method validation be submitted for review.

The present Committee considered two analytical methods for the determination of flumequine residues in shrimps. The first was a method submitted by the government of Thailand (ACFS, 2006) in response to a request by the 60th meeting of the Committee, and re-submitted for consideration by the 66th meeting. The second was a method reported by Pfenning et al. (1996) in the published literature.

Thailand submitted the method of Samuelsen (1990) modified for quantifying flumequine residues in shrimp. The tissue samples are mixed with anhydrous sodium sulfate and extracted twice with ethyl acetate. The sample mixture is centrifuged, and the organic phase removed, filtered and evaporated to dryness under nitrogen at 50-55°C. The residue is reconstituted in mobile phase for HPLC analysis using a C18 column with fluorescence detection (excitation 327 nm; emission 369 nm). A mobile phase of 0.1 M oxalic acid/acetonitrile/methanol (in a ratio, by volume, of 60:30:10) is applied at a flow rate of 1 mL/min.

A single laboratory validation study was conducted to demonstrate method performance. *Linearity* of calibration curves was demonstrated for concentrations of flumequine of 0.5-30 μg/kg (r = 0.9997) and 100-2000 μg/kg (r = 0.9999). *Accuracy* (recovery) was demonstrated at 5, 50 and 500 μg/kg. *Precision* (repeatability within laboratory) was demonstrated at 5, 50

and 500 μg/kg. The numerical performance characteristics for the method are summarized below.

Table 1: Performance characteristics of the liquid chromatographic assay for flumequine residues in shrimp muscle

Performance Characteristic	Shrimp Muscle
LOD (μg/kg)	1
LOQ (μg/kg)	5
Accuracy (%)	80-120
Precision (% CV)	5-7

The method submitted by the government of Thailand has been satisfactorily validated in a single laboratory study and is suitable for regulatory purposes.

Pfenning et al. (1996) reported a method for quantifying flumequine and nalidixic, oxolinic and piromidic acids in salmon and shrimp. The method is the same as that described by Munns et al. (1995) for analyzing residues of flumequine and nalidixic, oxolinic and piromidic acids in catfish. The method determines piromidic acid using liquid chromatography with UV detection, and flumequine, nalidixic acid and oxolinic acid using liquid chromatography with fluorescence detection (325 nm excitation, 360 nm emission). The analysis uses a polymer column in a 46^{o} C oven. The mobile phase is 0.02 M phosphoric acid/acetonitrile/tetrahydrofuran (in a ratio, by volume, of 72:16:12). The identity of all four quinolones present as residues in muscle of salmon and shrimp was confirmed by gas chromatography/mass spectrometry (GC/MS).

Sample preparation involves homogenizing the tissue with acetone, evaporating the acetone and then defatting the extract with hexane and extracting the quinolone residues into chloroform. The extract is further purified by first partitioning into base and then back-extracting from a solution acidified to pH 6.0. The final residue is evaporated to dryness and dissolved in mobile phase for analysis.

Recovery determinations were conducted in composites of shrimp muscle at fortification concentrations of 5, 10, 20, 40 and 80 μg/kg of each quinolone. Average recoveries and relative standard deviations for shrimp, which represent an average of five concentrations for each of the four analytes, were from 81.3 to 91.2% and from 7.3 to 10.7%, respectively. The identity of all four analytes was confirmed in extracts of shrimp muscle fortified at 10 μg/kg by determination of decarboxylated quinolones by GC/MS. Recoveries and relative standard deviations for the determination of flumequine in shrimp muscle are presented in Table 2.

Table 2: Recovery of flumequine from fortified composites of shrimp muscle

Amount added, μg/kg	Mean (n=6), %	SD	RSD, %
5.23	108	11.6	10.8
10.46	94.4	5.82	6.2
20.92	79.4	7.46	9.4
41.84	87.1	3.82	4.4
83.68	87.0	10.5	12.0

No interference resulted from compounds co-eluting with flumequine at its retention time of approximately 12.8 minutes. The analysis of shrimp muscle for flumequine by this method presented no particular problems.

The liquid chromatography method reported by Pfenning et al. (1996) for quantifying flumequine in the muscle of shrimp is validated and suitable for regulatory purposes.

APPRAISAL

The 62nd meeting of the Committee requested information to allow consideration to be given to making the temporary MRL of 500 μg/kg for flumequine in muscle of black tiger shrimp (*Penaeus monodon*) permanent. The results of a single laboratory validation study of the modified Samuelson method were acceptable and the method was considered to be suitable for regulatory purposes. The Committee also considered a published report describing an analytical method for the determination of flumequine in shrimp (Pfenning et al., 1996). This method was similarly validated and considered suitable for regulatory purposes. These data satisfy the provision of new information in respect of a regulatory method as requested by the 62nd meeting.

The 60th meeting evaluated residue depletion studies in shrimp. Based on the available data, dosing with 12 mg of flumequine per kg of body weight as medicated feed is unlikely to demonstrate efficacy against bacterial diseases in shrimp (FAO, 2003). In order to address this concern, the 62nd meeting, which re-established the ADI for flumequine, requested additional information on the approved dose for the treatment of black tiger shrimps and the results of residue studies conducted at the recommended dose. No new information was provided for evaluation.

The present Committee noted the limited availability of approved veterinary drugs for the treatment of aquaculture species. This concern has been addressed by the Joint FAO/WHO Technical Workshop on Residues of Veterinary Drugs Without ADI/MRL, which convened in Bangkok in August 2004 (FAO/WHO, 2004). In the absence of data, the Committee could not apply any procedures to extrapolate between species. However, the Committee thought it appropriate to assign the temporary MRL for flumequine to the muscle of all freshwater and marine species of shrimp based on known species similarities.

MAXIMUM RESIDUE LIMITS

The Committee considered the following factors in recommending MRLs:

- An ADI of 0 – 30 μg per kg of body weight based on a toxicological endpoint, which results in a maximum daily intake of 1,800 μg for a 60 kg person.
- Flumequine is the marker residue in shrimp muscle.
- A validated liquid chromatography method with a LOQ of 0.5 μg/kg for flumequine in shrimp muscle is available and suitable for regulatory purposes.
- Information on the approved dose for the treatment of black tiger shrimp and the results of residue studies conducted at the recommended dose had been requested but no new data had been provided.
- The availability of veterinary drugs for the treatment of diseases in aquaculture species is limited.

Based on the above considerations, the Committee recommended maintaining the temporary MRL of 500 μg/kg for muscle of the black tiger shrimp (*Penaeus monodon*) and recommended a temporary MRL to all freshwater and marine species of shrimp. The theoretical maximum daily intake (TMDI) of flumequine residues accounts for 92% of the upper bound of the ADI (FAO, 2000). No suitable data were available to calculate an estimated daily intake (EDI) value. The Committee confirmed its previous request for

information on the approved dose for the treatment of diseases in shrimps and the results of residue depletion studies conducted at the recommended dose. This information is requested by the end of 2008.

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MELENGESTROL ACETATE

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ADDENDUM
to the monograph and its addendum prepared by the 54th, 58th and 62nd meetings of the Committee and published in FAO Food and Nutrition Papers 41/13, 41/14 and 41/16, respectively.

Melengestrol acetate (MGA) is an orally active progestogen. It is used to improve feed conversion efficiency, promote growth and suppress estrus in female beef cattle fed for slaughter. The range of approved doses is 0.25 to 0.50 mg/heifer daily. MGA is fed for the duration of the fattening/finishing period, usually 90 to 150 days. The Committee previously evaluated MGA at the 54th, 58th and 62nd meetings (FAO, 2000; FAO, 2002; FAO, 2004, respectively). An ADI of 0-0.03 μg/kg of body weight was established based on hormonal activity. At the 62nd meeting of the Committee, MRLs of 8 μg/kg for fat and 5 μg/kg for liver in cattle were recommended, considering the new studies for which sufficient material could be isolated to identify and measure the relative progestational activity of all metabolites from treated animals compared to parent drug. The Secretariat was made aware of an error in the calculation of the MRLs and placed MGA on the agenda for the purpose of recalculating the MRLs.

The 62nd meeting reported the structure and progestogenic activity of the major metabolites of MGA. The progestogenic activity of the metabolites relative to MGA ranged from 0.09% to 12%. The percent of the total progestogenic activity attributable to MGA and to its metabolites was estimated from the percent of the total radioactive residue attributable to MGA and to its metabolites, and by assuming the relative progestogenic potency of all metabolites of MGA was 12%. The present Committee used percent median tritium-labelled MGA data from the 54th meeting when estimating the percent of total progestogenic activity attributable to MGA and to its metabolites in fat, liver, muscle and kidney (Table 1).

Table 1: Conversion of total radioactivity to progestogenic activity for MGA-related residues in tissues

Tissue	% of total radioactive residue attributable to:		% of total progestogenic activity attributable to[b]		
	MGA[a]	MGA metabolites	MGA	MGA metabolites	Sum of progestogenic residues (%)
Fat	86	14	$\frac{86 \times 1 \times 100}{86 + (0.12 \times 14)}$	$\frac{14 \times 0.12 \times 100}{86 + (0.12 \times 14)}$	98.08 + 1.92 = 100
Liver	30	70	$\frac{30 \times 1 \times 100}{30 + (0.12 \times 70)}$	$\frac{70 \times 0.12 \times 100}{30 + (0.12 \times 70)}$	78.12 + 21.88 = 100
Muscle	40	60	$\frac{40 \times 1 \times 100}{40 + (0.12 \times 60)}$	$\frac{60 \times 0.12 \times 100}{40 + (0.12 \times 60)}$	84.75 + 15.25 = 100
Kidney	34	66	$\frac{34 \times 1 \times 100}{34 + (0.12 \times 66)}$	$\frac{66 \times 0.12 \times 100}{34 + (0.12 \times 66)}$	81.11 + 18.89 = 100

[a] % Median ^{3}H-MGA data from 54th meeting of the Committee.

[b] The % of progestogenic activity of MGA-related residues is calculated by applying a weighting factor of 1 to MGA and of 0.12 (corresponding to the relative potency of the metabolite of MGA with the highest progestogenic activity) to all MGA metabolites in fat, liver, muscle and kidney, respectively.

The present Committee reconsidered data submitted to the 54th meeting. The Committee recommended that the MRLs should be derived from the 99th percentile of MGA concentrations in perirenal fat, collected either by biopsy or upon slaughter within short intervals after cessation of treatment, of feedlot animals treated with the highest recommended dose. The 54th meeting of the Committee had obtained this information by evaluating eight studies involving 370 animals treated at different doses (FAO, 2000). The majority of the animals (n=199) in two studies had been treated with the highest recommended dose of 0.5 mg MGA per heifer daily. The 99th percentile derived for MGA residues in perirenal fat for these animals was 18.5 µg/kg. There was relatively good agreement between this value and the 99th percentile value of 16.3 µg/kg for MGA residues in perirenal fat for all study animals derived by dose-response interpolations and dose extrapolations.

The median concentration of the marker residue obtained from a study with three animals treated with tritium-labelled MGA was 6.6 µg/kg for fat, 3.6 µg/kg for liver, 0.2 µg/kg for muscle and 0.6 µg/kg for kidney (Table 2). The proportion of the concentration found in the other three tissues, compared with perirenal fat that contains the highest residues, was derived from this study and was approximately 1:1.8 for liver, 1:33 for muscle and 1:11 for kidney.

MAXIMUM RESIDUE LIMITS

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

- The parent drug, MGA, is the marker residue.
- Fat, which contains the highest residue, is the most suitable target tissue for the purpose of monitoring the residues of MGA.
- The median concentrations of the marker residue were 6.6 µg/kg for fat, 3.6 µg/kg for liver, 0.2 µg/kg for muscle and 0.6 µg/kg for kidney.
- The conversion of marker residue to total residue was based on the fraction of the total progestogenic activity attributable to the marker residue; this fraction was 0.98 for fat, 0.78 for liver, 0.85 for muscle and 0.81 for kidney.
- A validated analytical method previously identified is available and suitable for routine monitoring.
- The established ADI is 0-0.03 µg/kg bw, equivalent to 0-1.8 µg for a 60 kg person.

On the basis of the above considerations, the Committee recommended MRLs in cattle of 18 µg/kg in fat, 10 µg/kg in liver, 1 µg/kg in muscle and 2 µg/kg in kidney, expressed as MGA. The TMDI corresponding to these MRLs is 2.7 µg or 150 % of the upper bound of the ADI. The Estimated Daily Intake (see Table 2) is 0.9 µg or 50% of the upper bound of the ADI.

Table 2: Estimated daily intake of MGA residues

Tissue	Median MGA[a] (µg/kg)	Fraction of total progestogenic activity attributable to marker residue	Total residue (µg/kg)	Standard Food Basket (kg)	Intake of residues (µg)
Fat	6.62	0.98	6.8	0.05	0.34
Liver	3.60	0.78	4.6	0.1	0.46
Muscle	0.20	0.85	0.24	0.3	0.07
Kidney	0.61	0.81	0.75	0.05	0.04
EDI					0.9

[a] Marker residue MGA

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RACTOPAMINE HYDROCHLORIDE

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ADDENDUM
to the Ractopamine Hydrochloride residue monograph prepared by the 62nd meeting of the Committee and published in FAO Food and Nutrition Paper 41/16, Rome 2004

The 62nd meeting of the Committee reviewed ractopamine hydrochloride and established an ADI of 0-1.0 μg per kg of body weight, equivalent to 0-60 μg for a 60 kg person, and recommended MRLs for edible tissues of pigs and cattle, expressed as ractopamine base: muscle, 10 μg/kg; liver, 40 μg/kg; kidney, 90 μg/kg; and fat, 10 μg/kg. The recommended MRLs result in a theoretical daily maximum intake of 50 μg, or 84% of the upper bound of the ADI, based on the JECFA model diet of 300 g of muscle, 100 g of liver and 50 g each of kidney and fat.

In considering these recommendations at its 15th Session in 2004, CCRVDF noted that the ADI recommended by JECFA had been rounded down resulting in a lower ADI than had been established by some member states (CAC, 2005). After further discussion concerning the JECFA policy on rounding the ADI to one significant figure, the CCRVDF agreed that JECFA should be requested to consider setting the MRLs using the calculated value obtained by dividing the NOEL by the safety factor and to recommend MRLs for ractopamine hydrochloride based on this result, not the one significant figure rounded value (0-1 μg per kg b.w.). The present Committee confirmed that the rounding practices used in expressing the ADI are scientifically and mathematically sound (see General considerations, page 2).

The present Committee reviewed the MRLs recommended at the 62nd meeting. The MRLs recommended for liver and kidney of pigs and cattle by the 62nd Committee were based primarily on the large set of available data from the pig studies, supported by the smaller number of studies and data for cattle. While these MRLs should accommodate the majority of situations involving slaughter within 12 hours of last treatment for both pigs and cattle, the available data for cattle suggest that the residue distribution between liver and kidney of cattle may not be the same as that in pigs.

The relationship between marker and total residues in cattle liver and kidney appears to be dose-dependent, so that the amount of marker residue in cattle liver may be as high as 80-85% of the marker residue found in kidney from the same animal. In one study (Study ABC-0408, 1989), conducted to assess relationships between marker and total residues, the mean residues found in livers and kidneys from 3 animals sacrificed 12 hours after the last treatment were 36 μg/kg and 43 μg/kg, respectively. This is higher than the residues ratio found in the cattle liver and kidney in other studies, where residues in liver varied from 32% of those found in kidney (Smith and Shelver, 2002) to 45% at 12 hours post-treatment (Study T4V739301, 1995). These studies were summarized in the FAO monograph prepared by the 62nd meeting of the Committee (FAO, 2004). No new data were provided for consideration at the present meeting of the Committee.

The study conducted at a dose equivalent to 45 mg/kg in feed by rumen insertion of a capsule containing the drug (Dalidowicz et al, 1987) was the only study where ractopamine parent

residues were detected in muscle and fat (0.02 and 0.01 mg/kg, respectively). Residues of parent ractopamine were not detected in the other studies where tissues were tested. Total radiolabelled residues were 0.01 to 0.05 mg/kg in muscle and fat at 12 hrs post-administration and depleted rapidly to non-detectable within a few days, even with administration above the recommended dose. Therefore, the recommendation of MRLs for muscle and fat of cattle based on the limit of quantitation (LOQ) was considered appropriate. The studies conducted at the recommended dose did not provide evidence that residues in liver and kidney of cattle would exceed the recommended MRLs for those tissues.

Pooled data from the depletion studies in pigs reviewed by the 62nd meeting of the Committee were evaluated using an Excel®-based worksheet. The data covering the time span from 12 to 120 hours after the last exposure used by the 62nd Committee in recommending the MRLs, were subjected to further statistical evaluations. Figures 1 and 2 show the data points, the linear regression line and a dotted line of the tolerance limits[1].

The Committee considered that the data available for residue depletion in cattle were not suitable for evaluation using the Excel® worksheet, due to the various dose rates and modes of administration used in the studies. A residue depletion study with unlabelled ractopamine administered in feed at the recommended maximum dose is required for the Committee to conduct this evaluation to reconsider the MRLs for cattle.

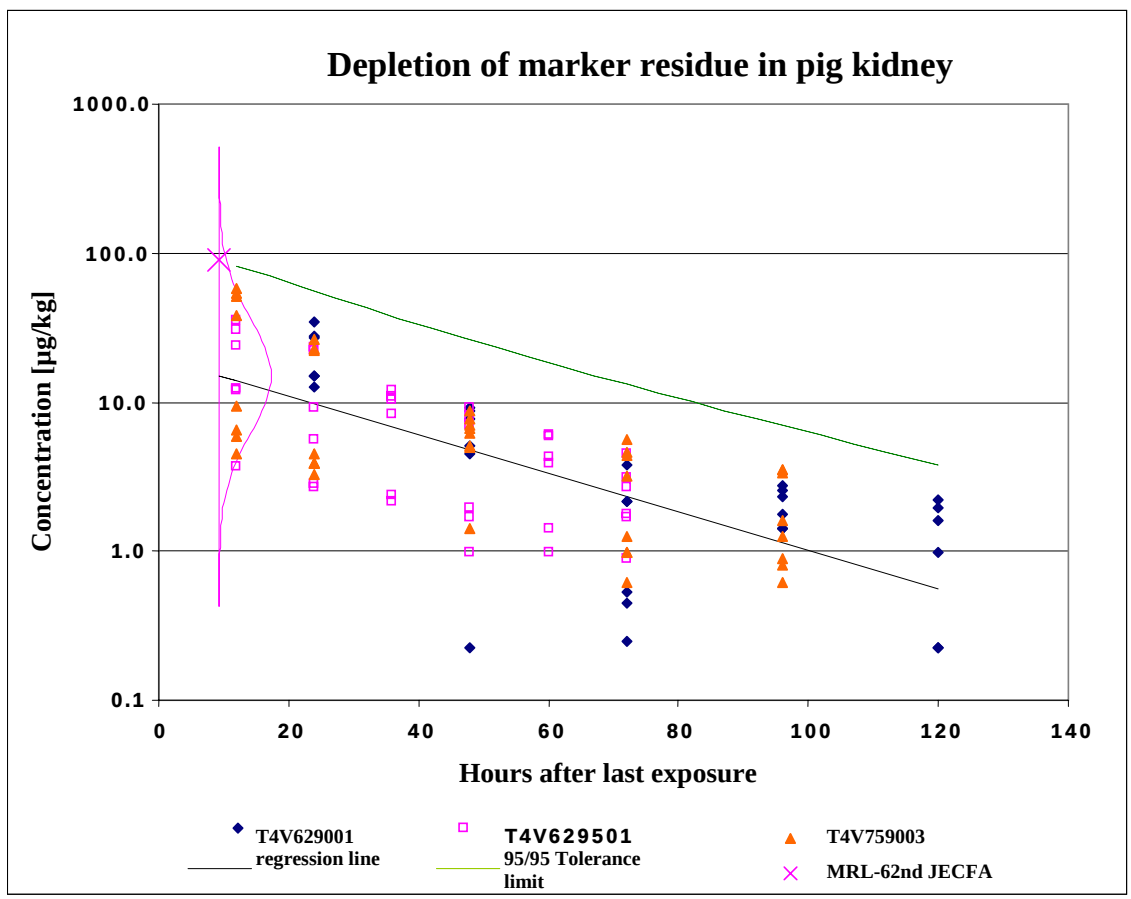

Figure 1: Depletion of marker residue in kidneys of ractopamine-treated pigs

[1] The upper limit of the one-sided 95 % confidence interval over the 95th percentiles is used throughout and referred to as "tolerance limit".

The MRLs recommended by the 62nd JECFA are shown as a point "×" on the line showing the tolerance limits. The MRL for kidney is 90 µg/kg. The exact depletion time to reach this point is 9.324 hours. At this time point the distribution of marker residue concentrations between -4 and +4 standard deviations is shown in Figure 1. It has a median value of 14.97 µg/kg. The residue content corresponding to +4 standard deviations is 520 µg/kg; the content corresponding to -4 standard deviations is 0.431µg/kg, indicating that the residue contents in kidney show a high variability. The MRL for liver is 40 µg/kg. The exact time to reach this value is 2.39 hours. Figure 2 shows the distribution of residue contents at this time point. The median concentration is 9.8 µg/kg. The numerical value corresponding to +4 standard deviations is 151 µg/kg; the content corresponding to -4 standard deviations is 0.640 µg/kg. The concentrations in liver are lower than those measured in kidney and show a slightly smaller variability. The MRL in kidney determines the minimum depletion time to comply with both MRLs. The statistical approach using the Excel®-based worksheet would lead to approximately the same MRL recommendations made by the 62nd meeting of the Committee.

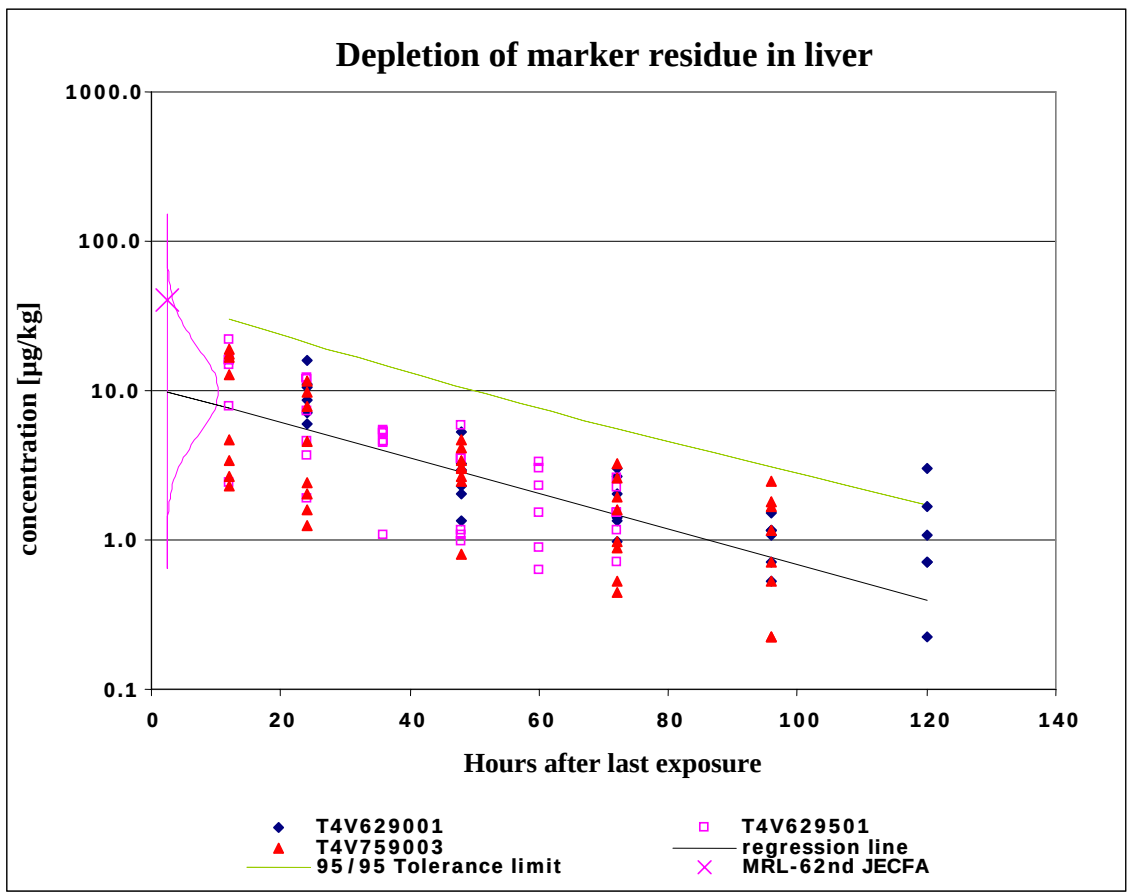

Figure 2: Depletion of marker residue in liver of pigs treated with ractopamine

The distribution of residue contents at time point "×" were used by the present Committee for testing different approaches to intake estimation. Based on one of the recommendations of the *Workshop on MRLs for Pesticides and Veterinary Drugs* held 7-10 November, 2005, in Bilthoven, The Netherlands, in the framework of the ongoing project "*Updating the Principles and Methods of Risk Assessment*", the Committee considered the median value of the distribution of ractopamine residue concentrations (from which the MRL is derived) for

the calculation of conservative estimates of long-term (chronic) intakes. Different approaches were tested to investigate the variability of the estimate, taking account of the variability of the ratio between the marker residue and the total residue as functions of animal species, dose and time. After review of the results, the median value of the distribution of residue concentrations (from which the MRL is recommended) was used as a new point estimate instead of the recommended MRL. The calculations are based on the time (9.3 h) estimated to obtain 95% of ractopamine residues in kidney below the MRL of 90 µg/kg recommended for this tissue (Figure 1). The median residue concentrations calculated at this time were: liver, 8 µg/kg, kidney, 15 µg/kg, muscle, 5.0 µg/kg and fat 2.5 µg/kg. Using this approach, the data-derived factors for the transformation of marker residue concentrations to total residue concentrations were: muscle, 1.0; liver, 6.3; kidney, 3.1; fat, 1.0.

Applying this analysis to recommended MRLs, the Committee concluded that based on the available data the MRLs recommended by the 62nd Committee for liver and kidney of pigs and cattle remained appropriate. The Committee affirmed the MRLs recommended at the 62nd meeting of the Committee. Based on the median values and the data-derived factors, the corresponding daily intake estimate is 9.0 µg (see Table 1).

Table 1: Estimated Daily Intake of Ractopamine Residues from median value obtained from data for tissues from pigs

Food Item	Median (µg/kg)	Standard Food Basket (kg)	MR/TR[1]	EDI (µg)
Muscle	5.00	0.300	1.00	1.5
Liver	8.14	0.100	6.29	5.1
Kidney	14.97	0.050	3.05	2.3
Fat	2.50	0.050	1.00	0.1
Total:				9.0

[1] MR = marker residue (parent drug); TR = total residues

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TRICLABENDAZOLE

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ADDENDUM
to the monograph prepared by the 40th meeting of the Committee
and published in FAO Food & Nutrition Paper 41/5

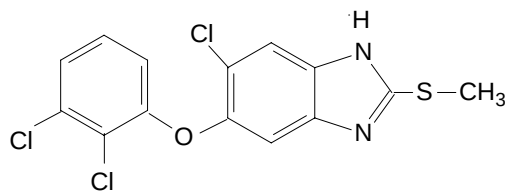
IDENTITY

Chemical name: 5-Chloro-6-(2,3-dichlorophenoxy)-2-methlythio-1H-benzimidazole {International Union of Pure and Applied Chemistry, or IUPAC, name};

Chemical Abstracts Service (CAS) number: 68786-66-3.

Synonyms: Triclabendazole (common name); CGA 89317, CGP 23030; proprietary names Fasinex®, Fascinex®, Soforen®, Endex®, Combinex®, Parsifal®, Fasimec®, Genesis™ Ultra.

Structural formula:



Molecular formula: $C_{14}H_9Cl_3N_2OS$

Molecular weight: 359.66

OTHER INFORMATION ON IDENTITY AND PROPERTIES

Pure active ingredient: triclabendazole

Appearance: White crystalline solid

Melting point: 175-176°C (Merck), α-modification; 162 °C (β-modification)

Solubility: Soluble in tetrahydrofuran, cyclohexanone, acetone, *iso*-propanol, *n*-octanol, methanol; slightly soluble in dichloromethane, chloroform, toluene, xylene, ethyl acetate; insoluble in water, hexane.

RESIDUES IN FOOD

The 40th meeting of the Committee established an ADI of 0 – 3 µg/kg body weight based on a long-term study in mice with a NOEL of 0.27 mg/kg body weight per day and a safety factor of 100. The Committee recommended MRLs in cattle and sheep. In cattle, the MRLs expressed as triclabendazole equivalents were: muscle and fat, 200µg/kg; liver and kidney, 300µg/kg. For sheep, MRLs for muscle, liver, kidney and fat were 100µg/kg. The marker residue was identified as 5-Chloro-6-(2', 3'-dichlorophenoxy)-benzimidazole-2-one. The 40th Committee concluded that more accurate estimates of total residues in edible tissues and the ratio of total residue concentrations to marker residue concentrations would be required before the MRLs for

triclabendazole in sheep could be reconsidered. The 15th Session of CCRVDF (Codex Committee on Residues of Veterinary Drugs in Foods) requested a new evaluation. Data were provided for cattle and sheep.

Conditions of Use

Triclabendazole is a benzimidazole anthelmintic used for the control of liver fluke, *Fasciola hepatica* and *Fasciola gigantica*, in sheep, goats and cattle. It is related both by chemical structure and pharmacological activity to other benzimidazole compounds, such as fenbendazole and thiabendazole.

Dosage

Triclabendazole is applied to cattle as a drench at 12 mg/kg body weight or as a pour-on at 30 mg/kg body weight. The recommended therapeutic oral dose for cattle is 12 mg/kg body weight and for sheep and goats is 10 mg/kg body weight. Veterinary advice differs from country to country regarding the interval for a possible repeat treatment, but typically at 8-10 week intervals during the fluke season, or at 5 to 6 week intervals when acute or sub-acute infection is present (FAO, 1993; EMEA, 1997).

PHARMACOKINETICS AND METABOLISM

The evaluation by the 40th Committee, that addressed studies using ^{14}C-labelled triclabendazole in rats, rabbits, dogs, sheep, goats, cattle and pigs, considered that the absorption, distribution, metabolism and excretion of triclabendazole were qualitatively similar in both laboratory animals and food-producing animals. The biotransformation and excretion of triclabendazole was very rapid, with two major metabolic pathways identified: oxidation of the methylthiol group initially to a sulfoxide and subsequently to a sulfone metabolite, and 4-hydroxylation of the dichlorophenoxy ring (Figure 1). Five identified metabolites - triclabendazole, sulfoxide, sulfone, ring-hydroxylated metabolites and keto-triclabendazole accounted for approximately 40-60% of the administered dose, with quantitative differences in relative proportions of metabolites being observed between species. Faecal excretion of triclabendazole and its metabolites accounted for a principal portion of the dose.

Laboratory Animals

Rats

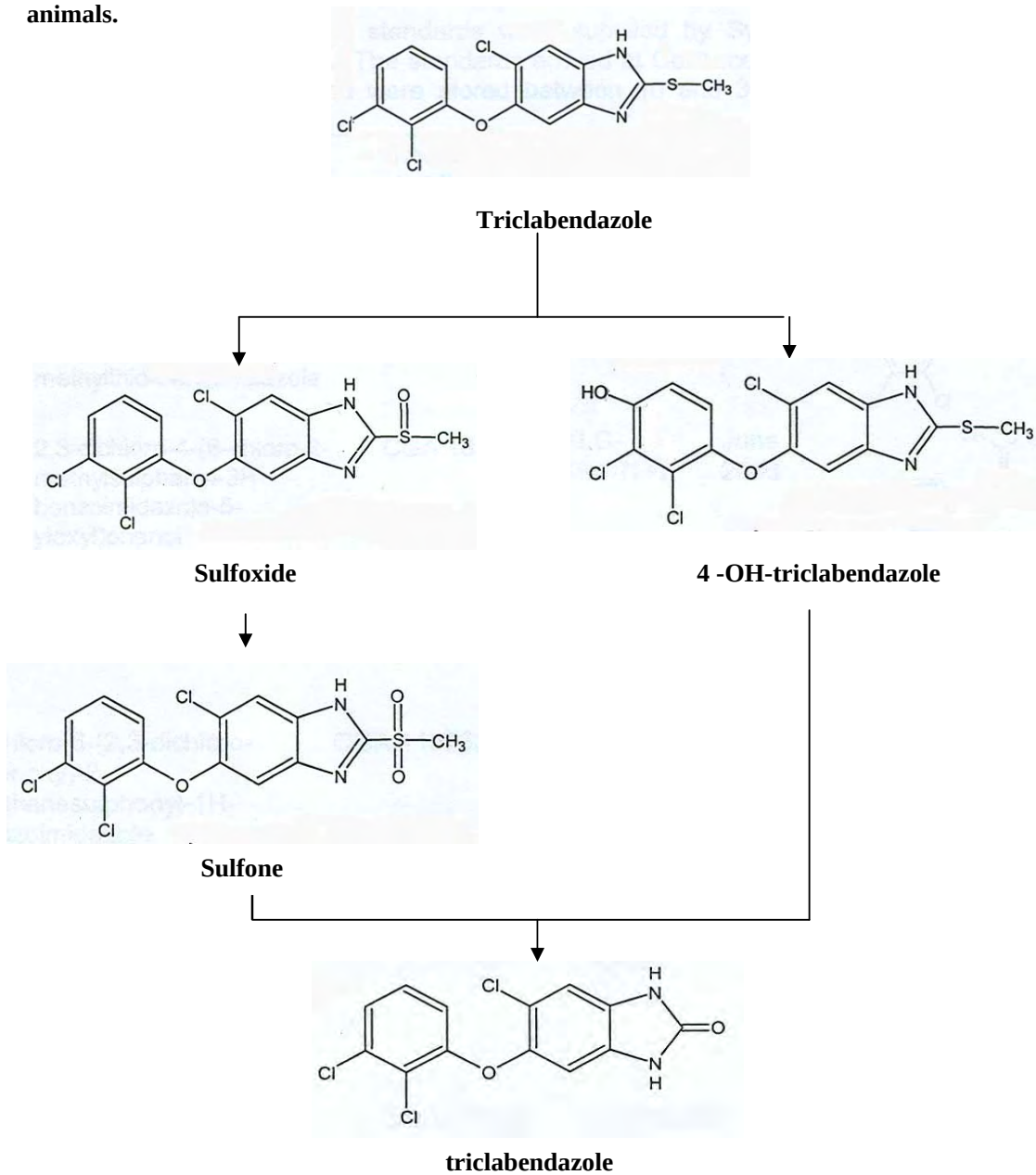
The absorption and disposition of ^{14}C-triclabendazole was investigated in male rats following a single intravenous administration of 1 mg/kg body weight and by oral administration at different doses: single doses of 1, 10 and 80 mg/kg body weight, respectively; 10 daily doses of 1 mg/kg body weight (Study DM23/1991, 1996). Peroral absorption of triclabendazole was almost complete at the lower doses (1 mg/kg body weight) and decreased to about 50% at higher doses (10 mg/kg body weight). Within 48 hours, 87-92% of a 1 mg/kg dose (oral or intravenous administration) was excreted in urine and faeces. The major route of excretion was via the faeces, accounting for 82-85% of the dose. Maximum concentration of radioactivity in plasma was observed 8 hours after oral administration, irrespective of the dose levels. Concentrations of radioactivity measured at different periods after the administration indicated wide distribution of triclabendazole metabolites in rat tissues following oral and intravenous treatment.

In a subsequent GLP study, twelve male rats were given a single oral gavage dose of 12 mg/kg body weight ^{14}C-triclabendazole (Study 1969/024, 2004). Recovery of radioactivity was quantified in the excreta and expired carbon dioxide collected daily from four rats for 10 days. Blood and tissues were collected at necropsy on days 10 (6 rats) and 28 (6 rats) for determination of radioactivity. The mean recovery in faeces from rats within 10 days after dosing was 96%, with a

mean recovery of 8% in urine. Less than 0.01% was detected in exhaled air. The major elimination of radioactivity in the faeces suggests that biliary excretion is significant in rats. Distribution of radioactivity in tissues (liver, kidney and muscle) showed the highest residue in kidney after 10 days and in kidney and muscle after 28 days. Chromatographic analysis of the radioactive extracts from excretions and tissues of rats killed on day 10 showed 4-12 metabolite fractions, none of which could be identified by comparison with the available reference standards.

In a GLP study of bioavailability of triclabendazole residues, male rats received a single dose of ^{14}C-triclabendazole either orally by gavage or by intravenous administration or by addition to powdered diet or by dietary dose of ^{14}C-triclabendazole derived residues from cattle tissues (Study 1969/026, 2004). Groups of rats which received ^{14}C-triclabendazole derived residues from cattle tissues were given lyophilized liver, muscle and kidney from cattle that had been killed 28 days after receiving a single oral dose of 12 mg/kg body weight (Study 1969/023, 2004).

Figure 1: Proposed metabolic pathway of triclabendazole in laboratory and food-producing animals.



The maximum concentration of radioactivity in blood from rats after dietary administration of ^{14}C-triclabendazole was found at 4 hours after gavage and 4.8 hours after dose in powdered diet, while the T_{max} was 12 and 6 hours in the groups administered lyophilised muscle and liver containing incurred residues of triclabendazole. Measurements of the area under the blood concentration time curve showed marked differences in the bioavailability of triclabendazole and triclabendazole-derived residues. The bioavailability of triclabendazole was 71.5% when given by gavage and 67.6% powdered diet, but reduced to 6.4% and 9.8% for triclabendazole- derived residues from lyophilized cattle muscle and liver, respectively.

The bioavailability of ^{14}C-triclabendazole-derived residues in tissues collected from cattle and sheep was reported in two additional GLP studies using bile duct-cannulated male rats (Study 017AM04, 1995; Study 1969/025, 2004). In the first study (Study 017AM04, 1995), tissues containing triclabendazole residues were obtained from cattle that received a single oral dose of 12 mg/kg body weight (Study 380/214-1011, 1994) and sheep that received a single oral dose of 10 mg/kg body weight (Study 380/215-1011, 1994) respectively; and were terminated 28 days post-dosing. The lyophilized tissues were orally administered to rats as diet mixture for 24 hours. The observed amount of radioactivity into systemic circulation after the administration, the sum of the % of dose in urine, bile, tissues and carcass, defined the bioavailability. The bioavailability was 14% and 7% in kidney, 9% and 8% in liver, 4% and 5% in muscle for cattle and sheep, respectively. Similar to the bioavailability study using the areas under plasma concentration time curve (AUC) (Study 1969/026, 2004), the bioavailability of triclabendazole-derived residues from the lyophilized tissues was very low compared to the concentrations of triclabendazole observed following oral dosing to rats. The administered dose was almost completely eliminated within 48 hours, predominantly in faeces. The rats were killed 48 hrs after the initial administration and residues in tissues were determined by radioactivity. The residues were considered negligible and were only detected in livers and kidneys at concentrations less than the limit of quantification, 0.002 mg/kg.

In the second new GLP study of residue bioavailability (Study 1969/025, 2004), rats were offered, over a 24 hour period, lyophilized liver, muscle or kidney containing ^{14}C triclabendazole residues from a cow dosed with triclabendazole at 12 mg/kg body weight and terminated at 28 days post administration (Study 1969/023, 2004). The bioavailability (defined as the % of dose in urine, bile, tissues and carcass) was 20%, 3.3% and 18% from liver, kidney and muscle, respectively. The mean recovery of radioactivity was > 90% with the majority eliminated in faeces (> 80%). Recovery in bile was 3-19% and in urine was < 1% of the administered dose. Absorbed residues did not depot in tissue of rats, as determined at 72 hours after dosing. Concentrations of radioactivity in tissues were very low or below the limit of quantification.

Dogs

A non-GLP study was reported in which 2 male dogs received doses of 0.5, 5 and 40 mg/kg body weight orally and 0.5 mg/kg body weight intravenously of ^{14}C-triclabendazole (Study DM23/1991, 1996). The concentrations of ^{14}C-triclabendazole-related residues in blood and plasma following oral administration showed that approximately 35-53% of a 0.5 mg/kg dose was absorbed at the lower doses, and the absorption decreased to about 25% at higher doses. In plasma, radioactivity attained the maximum concentration 8-24 hours after oral administration. Elimination of triclabendazole was almost exclusively *via* faeces. 79% and 54%, respectively, of the radioactivity associated with 0.5 mg/kg intravenous and oral dose of triclabendazole was recovered in faeces. Overall recovery in faeces and urine accounted for 80% of the intravenous dose and only 55% of the oral dose after 168 hours. Highest recovery of radioactivity in the excreta (90% of the dose) was found in the high dose of oral administration (40 mg/kg).

Food Producing Animals

Cattle

Studies considered by the 40th meeting of the Committee indicated rapid metabolism of triclabendazole following intravenous administration in cattle. The sulfoxide metabolites reached maximum concentrations in blood 4 hours after treatment, with terminal half-life of approximately 13 hours. The sulfone metabolites were produced more slowly with maximum plasma concentrations occurring 32 hours after dosing and terminal elimination half life of 40 hours (Study 86/12/1099, 1986; FAO, 1993).

In a new GLP study, two ruminating calves, one male and one female, were each given a single oral dose of ^{14}C triclabendazole 12 mg/kg body weight (Study 380/214-1011, 1994). Excretion of radioactivity was measured in urine and faecal samples collected at 24 hour intervals for 7 days following administration. A small portion of the administered radioactivity was found in urine (2.2%), with excretion primarily in faeces (76%). As 4% of the dose was found in the faeces at 7 days post-dosing, it was considered that faecal elimination may not have been complete. Distribution of radioactivity was extensive into tissues at 28 days post-dosing. Highest concentrations of radioactivity were detected in liver (0.461 mg equivalents/kg), followed by muscle (0.33-0.35 mg equivalents/kg) and kidney (0.195 mg equivalents/kg). Only very low residues were detected in fat (0.05 - 0.08 mg equivalents/kg).

Further investigation of the urinary and faecal metabolites was conducted in another GLP study (Study 017AM02, 1995). Four metabolites were detected in the urine but could not be identified with the reference standards. However, no sulfate or glucuronide conjugates were detected in the urine. Eight metabolite fractions were detected in the faecal extracts, with the most polar fractions displaying a similar chromatographic behavior to the fractions found in urine. Parent triclabendazole was the major fraction, accounting for 17% of the dose. The other metabolites identified in the faecal extracts were 5-chloro-6-(2,3-dichloro-phenoxy)-2-methanesulfinyl-1H-benzimidazole, 5-chloro-6-(2,3-dichloro-phenoxy)-2-methanesulfonyl-1H-benzimidazole, 2,3-dichloro-4-(6-chloro-2-methylsulfanyl-3H-benzimidazole-5-yloxy)-phenol and 2,3-dichloro-4-(6-chloro-2-methanesulfonyl-3H-benzimidazole-5-yloxy)-phenol, representing, respectively, 3%, 5%, 4% and 3% of the dose.

In a subsequent GLP study (Study 1969/023, 2004); the distribution, metabolism and excretion of ^{14}C triclabendazole was investigated in a ruminating male calf. Following a single oral dose of 12 mg/kg body weight, urine and faeces were collected for 10 days and the animal was killed 28 days after dosing. Similar to the previous studies (Study 380/214-1011, 1994; Study 017AM02, 1995), faecal elimination predominated, accounting for 78.2%, and urine contained only 3.4% of the dose. Triclabendazole was the major metabolite in faeces, with smaller amounts of sulfone, sulfoxide and hydroxylated metabolites. The majority of urinary metabolites were more polar than triclabendazole, including small amounts of 5-chloro-6-(2,3-dichloro-phenoxy)-2-methanesulfinyl-1H-benzimidazole, 5-chloro-6-(2,3-dichloro-phenoxy)-1,3-dihydro-benzimidazole-2-one, 2,3-dichloro-4-(6-chloro-2-methanesulfonyl-3H-benzoimidazol-5-yloxy)-phenol, 2,3-dichloro-4-(6-chloro-2-methylsulfanyl-3H-benzimidazole-5-yloxy)-phenol and 2,3-dichloro-4-(6-chloro-2-methanesulfinyl-3H-benzimidazole-5-yloxy)-phenol. At 28 days after dosing, levels of triclabendazole residues measured by concentrations of radioactivity were highest in liver (0.283 mg equivalents/kg) and muscle (0.209 mg equivalents/kg). The residues in kidney were lower (0.163 mg equivalents/kg) and much lower residue amounts were detected in fat (0.026 mg equivalents/kg). The systemic levels of radioactivity in the blood (0.070 mg equivalents/kg) and plasma (0.051 mg equivalents/kg) were generally lower than in tissues.

The studies demonstrated that triclabendazole was rapidly absorbed following administration of oral therapeutic dose to cattle (12 mg/kg body weight) and principally excreted *via* faeces. The major metabolites in faeces were similar to those of rats. Residue concentrations in tissues 28 day

after dosing were highest in liver followed by muscle and kidney. The residues in fat were negligible compared to the other tissues.

Sheep

The absorption, distribution and excretion studies of triclabendazole in sheep and goats reported in the previous evaluation by the Committee indicated a similar metabolism of triclabendazole in sheep and goats to other species tested (FAO, 1993). The orally administered dose was absorbed effectively and metabolized rapidly. The same metabolites were excreted by sheep and goats, and the elimination was nearly complete by 10 days post dosing. More than 90% of the administered dose was recovered in faeces, 2-3% in urine and 0.5% in milk. Disposition of triclabendazole residues in sheep and goat tissue indicated by the amount of radioactivity was similar to that observed in cattle. At 28 days after dosing, liver contained the highest concentration, followed by muscle and kidney, whereas radioactivity amounts in fat tissue were comparatively minor.

New GLP studies of triclabendazole metabolism in ruminating sheep demonstrated a corresponding metabolic pattern to the previous studies. Following a single oral administration of ^{14}C triclabendazole at nominal dose level (10 mg/kg body weight) to a male and a female sheep, the average recovery of radioactivity was 85% of dose at 7 days post-dosing (Study 380/215-1011, 1994). The primary route of excretion was in faeces, accounting for 77% of the administered dose. Absorption of triclabendazole, as indicated by radioactivity in plasma, was highest at 8 hours (19.59 mg equivalents/kg) and decreased to 10.05 mg equivalents/kg at 48 hours post-dosing. Distribution of radioactivity in tissues at 28 days post-dosing revealed highest levels in muscle (0.237-0.306 mg equivalents/kg), followed by liver (0.237 mg equivalents/kg) and kidney (0.198 mg equivalents/kg); while substantially lower levels were detected in fat tissue (0.02 mg equivalents/kg).

Chromatographic analysis of the excreta revealed 11 metabolite fractions in the faecal extracts and 5 metabolite fractions in urine (Study 017AM03, 1995). Only 5 components in faecal extracts co-chromatographed with the reference standards and corresponded to sulfoxide, sulfone, hydroxylated metabolites and unchanged triclabendazole. The major metabolic pathways in sheep were oxidation to the sulfoxide, with further oxidation to the sulfone, and hydroxylation of the dichlorophenyl moiety, essentially the same as found in cattle. As with the metabolite pattern observed for excretion in cattle (Study 380/214-1011, 1994; Study 017AM02, 1995; Study 1969/023, 2004), unchanged triclabendazole recovered from faecal samples was a dominant metabolite fraction, accounting for 16-17% of the dose. Metabolism and elimination in sheep was similar to that observed in the rat.

TISSUE RESIDUE DEPLETION STUDIES

Radiolabelled Residue Depletion Studies

Cattle

In a pilot GLP study to investigate depletion of total residues of triclabendazole, two ruminating cows (7-month old heifers) each received a single oral dose of ^{14}C-triclabendazole at 12 mg/kg body weight (Study 400-05, 1991). The cows were sacrificed at 28 and 42 days, respectively, following drug administration and liver, kidney and composite muscle and fat samples were analyzed by combustion analysis to determine total residues in each tissue. Limits of determination were 0.002 mg/kg for liver, 0.003 mg/kg for kidney and muscle and 0.007 mg/kg for fat. The total residues found in tissues from each animal are given in Table 1.

Table 1: Total residues of ^{14}C-triclabendazole in tissues of cows after treatment with a single oral dose (12 mg/kg body weight) of ^{14}C-triclabendazole

Days post-treatment	Total ^{14}C-triclabendazole residues in tissues (Mean±SD, mg/kg)			
	muscle	liver	kidney	fat
28	0.131 ± 0.017	0.241 ± 0. 013	0.106 ±0.016	0.013 ± 0.003
42	0.097 ± 0.007	0.093 ± 0.009	0.069 ± 0.011	<0.008 ± 0.001

Tissues collected from the cow sacrificed at 28 days following drug administration were subsequently analyzed after approximately 5 months frozen storage at -20°C (Study 400-05A, 1992). Tissue samples (20 g) were extracted sequentially with 3 40-mL portions of methanol, followed by 3 portions of ethyl acetate. Recoveries of radiolabelled residues were low (liver, 13.8%; kidney, 4.7%; muscle, 4.7%; fat, 0.0%), so further characterization of the extracted residue was not attempted.

In a subsequent GLP study, liver, kidney and muscle tissues from a single male calf sacrificed 28 days following administration of ^{14}C-triclabendazole, 12 mg/kg body weight, were analyzed to determine the relationship between the residues measured as keto-triclabendazole and the total residues (Study 1969/023, 2004). Residues measured as keto-triclabendazole accounted for 24% of the total residue in liver, 27% of the total residue in kidney and 32% of the total residue in muscle at 28 days post-administration.

Tissues collected from two ruminating calves slaughtered 28 days after administration of a single oral dose of ^{14}C-triclabendazole, 12 mg/kg body weight (Study 380/214-1011, 1994), were analyzed to determine both keto-triclabendazole residues and total radiolabelled residues (Study 132/94, 1995). The results of the keto-triclabendazole analyses were not corrected for recovery in this study, conducted in compliance with GLP. Residues measured as keto-triclabendazole accounted for 13% of the total residue in liver, 21% of the total residue in kidney and 31% of the total residue in muscle at 28 days post-administration. After correcting the residues measured as keto-triclabendazole for recovery using the mean recoveries reported in the study for muscle, liver and kidney fortified at 0.1 mg/kg, the corrected percentages of marker-to-total residues were: muscle, 42%; liver, 19%; kidney, 24%.

Sheep

Muscle and liver tissues collected from two sheep slaughtered 28 days after administration of a single oral dose of ^{14}C-triclabendazole, 10 mg/kg body weight (Study 380/215-1011, 1994), were analyzed to determine both keto-triclabendazole residues and total radiolabelled residues (Study 132/94, 1995). Concentrations of keto-triclabendazole (not corrected for recovery) accounted for 29% of the total residues in muscle and 17% of the residues in liver. After correction for analytical recovery, residues measured as keto-triclabendazole account for 39% and 24%, respectively, of the total residues in muscle and liver.

Residue Depletion Studies with Unlabelled Drug

Cattle

Three residue studies were reported in the monograph prepared by the 40th Committee (FAO, 1993) in which cattle received a single oral dose of 12 mg/kg body weight triclabendazole. In these studies, residues were measured as 5-chloro-6-(2',3'-dichlorophenoxy)-benzimidazole-2-one and converted to triclabendazole equivalents using a conversion factor of 1.0913. Two animals were slaughtered at each time point in the first two studies, while four cattle were slaughtered at each time point in the third study. The first and third studies included time points from 2 - 28 days,

while the second study was extended to 42 days. The studies demonstrated a consistent depletion profile, although the residue concentrations varied at the same or equivalent time points in the studies. In general, highest residues were observed in liver and kidney at times up to 7 days following treatment, while residues in fat were near or below the detection limits of the analytical methods used (0.03-0.06 mg/mg) at 14 days post-treatment. Residue concentrations in muscle tissue were lower than those in liver and kidney at 7-14 days post-treatment, but were similar at later time points. It was not stated whether these studies were conducted in compliance with GLP.

A new study was conducted in compliance with GLP in which 24 cattle (168 - 367 kg) were treated orally with triclabendazole at 18 mg/kg body weight, with a repeat treatment 28 days following the initial dose (Study Y03/49, 2004). Six animals were slaughtered at each of the 14, 28, 42 and 56 days sampling times following the second treatment. Samples of muscle (tenderloin), liver, kidney and renal fat were collected and analyzed for residues of keto-triclabendazole using a liquid chromatography method with a limit of quantification of 0.05 mg/kg. Results were corrected for analytical recovery. As in the earlier studies (FAO, 1993), highest residues were observed in kidney and liver at 14 days post-treatment, while mean residues in fat were <0.10 mg/kg. At 42 days post-treatment, residues in muscle and liver were similar, approximately 2.5 times higher than those in kidney, while residues were only detectable in muscle and liver at 56 days post-treatment (Table 2).

Table 2: Residues of triclabendazole measured as keto-triclabendazole in cattle tissues following two oral treatments (28 days between treatments) with triclabendazole (Fasinex®-10%) at 18 mg/kg body weight

Time following second treatment(days)	Residues of triclabendazole measured as keto-triclabendazole (Mean±SD, mg/kg)			
	Muscle	Liver	Kidney	Fat
14	0.238 ± 0.027	0.979 ± 0.235	0.656 ± 0.265	0.089 ± 0.029
28	0.14 ± 0.027	0.365 ± 0.083	0.129 ± 0.020	<0.050 - 0.062[a]
42	0.127 ± 0.021	0.228 ± 0.054	0.066 ± 0.015	<0.050
56	0.091 ± 0.015	0.102 ± 0.033	<0.050	N.A.[b]

[a] Only one of 6 fat samples contained residues at >0.050 mg/kg. [b] Not analyzed.

Sheep

Three residue depletion studies in which sheep were orally dosed with triclabendazole (single dose of 10 mg/kg body weight or 15 mg/kg body weight) were reported in the monograph prepared for the 40th meeting of the Committee (FAO, 1993). Residues depleted to below detection limits in fat (0.03 mg/kg) within 14-21 days after administration, but remained detectable in liver, kidney and muscle for 28 days. Highest residues were reported in liver at 21 days post-treatment, but results were similar in liver, kidney and muscle at 28 days post-treatment. It was not stated whether these studies were conducted in compliance with GLP.

A GLP study was provided to the current meeting of the Committee in which 24 lambs (29-42 kg body weight) received a single oral dose of 0.2 mL, equivalent to 10 - 13 mg/kg of triclabendazole body weight (Study Y04/22, 2004). Six animals were slaughtered at each of the time points 14, 28, 42 and 56 days post-treatment. Two control animals did not receive the treatment and were slaughtered prior to the first group of treated animals. Samples of liver, kidney, muscle and fat were collected from each animal and analyzed for residues of keto-triclabendazole by liquid chromatography, with results corrected for analytical recovery. Samples of some tissues were not analyzed from the later collection dates as all samples of these tissues collected at the previous date had been below the limit of quantification. Most persistent residues were found in muscle and were detectable in 3 of 6 animals at 56 days post-treatment. Residues were not detected in liver or kidney samples at 42 days post-treatment, or in fat samples at 14 days post-treatment.

Table 3: Residues of triclabendazole measured as keto-triclabendazole in sheep tissues following a single oral treatment with triclabendazole (Fasinex®-5 %) at 10 - 13 mg/kg b.w.

Days Post-Treatment	Triclabendazole residues measured as keto-triclabendazole (Mean±SD mg/kg)			
	Muscle	Liver	Kidney	Fat
Control	<0.05	<0.05	<0.05	<0.05
14	0.154 ± 0.030	0.429 ± 0.073	0.242 ± 0.031	<0.05
28	0.112 ± 0.030	0.158 ± 0.037	0.096 ± 0.022	N.A.
42	0.065 ± 0.014	<0.05	<0.05	N.A.
56	0.054 ± 0.003[a]	N.A[b]	N.A.	N.A.

[a] Average for muscle from 3 animals with detectable residues. [b] Not analyzed.

METHODS OF ANALYSIS FOR RESIDUES IN TISSUES

Analytical methods for triclabendazole residues reviewed in the monograph prepared for the 40th meeting of the Committee were based on reversed phase liquid chromatography with UV-detection (FAO, 1993). The method used in the depletion studies considered by the 40th Committee measures the hydrolyzable residues of triclabendazole after oxidation to keto-triclabendazole, or 5-chloro-6-(2',3'-dichlorophenoxy)-benzimidazole-2-one, designated as the marker residue. Analytical recoveries were reported for tissues from cattle and sheep. A factor of 1.0913 was applied to convert the marker residue to triclabendazole equivalents. Several methods from the open literature were also noted in the monograph. However, validation of the methods reported was not to contemporary standards.

A report on the validation of an analytical method for the determination of triclabendazole residues in cattle and sheep tissues (liver, kidney, muscle, fat) was reviewed by the present Committee (Study V03/57, 2004). This is an up-dated version of a method considered by the 40th Committee. Tissues are initially digested with hot alkali solution to release bound residues, then acidified, cooled and extracted with dichloromethane. For fatty tissues, an additional step to remove lipids by hexane - acetonitrile partitioning is included. The extract is evaporated to dryness, then taken up in ethanol:glacial acetic acid (1:1) and heated following addition of hydrogen peroxide to oxidize the residues to keto-triclabendazole (the marker residue, identified as 5-chloro-6-(2',3'-dichlorophenoxy)-benzimidazole-2-one in the report of the 40th Committee). After a further partitioning step and evaporation to remove acetic acid, the residues are dissolved in dichloromethane, loaded on an anion exchange solid phase extraction cartridge and eluted with isopropyl alcohol/ dichloromethane (12% v/v).

The dried eluate is dissolved in acetonitrile and injected into the liquid chromatograph, with separation on a reversed phase (C-18) column and UV-detection at 296 nm. Quantification is by external standard curve. Performance characteristics determined for the method are summarized in Table 4. It should be noted that the limits of detection and quantification are based on estimates from calibration curves. The lowest concentration at which accuracy and recovery were tested and demonstrated to meet acceptable performance criteria was 0.050 mg/kg triclabendazole equivalents (0.046 mg/kg keto-triclabendazole).

No endogenous substances present in extracts produced a response in excess of the limit of quantification for keto-triclabendazole in any tissue. Other benzimidazole drugs, such as fenbendazole, thiabendazole and albendazole were not detected. It was noted that the detection wavelength of 296 nm limits potential interferences. The metabolites of triclabendazole, triclabendazole sulphoxide and triclabendazole sulphone, were detected, as was parent drug triclabendazole, but are fully separated by the chromatography conditions used in the method. It should be noted that these compounds would normally be oxidized to keto-triclabendazole during

the analysis. A confirmatory method was proposed which uses a phenyl liquid chromatography column as an alternative liquid chromatography system. Limits of quantification were higher than for the original method and the information obtained does not provide sufficient evidence for structural confirmation.

Table 4: Summary of validation study results for analysis of triclabendazole residues by liquid chromatography in various edible tissues

Species	Edible Tissue	Limit of Detection[a] (mg/kg)	Limit of Quantification[b] (mg/kg)	Mean Recovery (%)	Repeatability[c] (%)
Cattle	Muscle	0.012	0.036	81-100	2.1-8.5
	Liver	0.024	0.074	84-87	1.7-9.6
	Kidney	0.020	0.058	89-97	2.8-8.9
	Fat	0.007	0.020	78-90	10.1-6.1
Sheep	Muscle	0.014	0.041	80-102	3.1-5.2
	Liver	0.008	0.024	90-102	2.3-4.3
	Kidney	0.012	0.034	89-93	5.0-6.5
	Fat	0.015	0.042	79-102	10.0-6.8

[a] Based on mean response of blank, plus 3 standard deviations
[b] Based on mean response of blank, plus 10 standard deviations
[c] Within run, measured at 0.050, 0.100 and 0.200 mg/kg

Further validation of the method for analysis of sheep and cattle tissues was provided in a subsequent report (Study V05/24, 2005). These studies demonstrated no background interferences, confirmed that precision was ≤15% at concentration > 0.10 mg/kg and demonstrated the stability of the residues under freeze/thaw conditions.

The method has also been extended to the analysis of tissues from goats (study Y04/51, 2004). Results, given in Table 5, are based on analysis of three replicates at each of three concentrations for the three tissues tested (muscle, liver, kidney).

Table 5: Recovery and precision for determination of keto-triclabendazole residues in goat tissues

Tissue	Concentration keto-triclabendazole(mg/kg)					
	50		100		100[a]	
	Recovery (%)	CV (%)	Recovery (%)	CV (%)	Recovery (%)	CV (%)
Muscle	99	2.2	102	2.2	95	4.1
Liver	110	13	97	3.4	91	11
Kidney	98	1.7	85	2.7	85	5.4

[a] Fortified samples, analyzed after storage at room temperature for 16-24 hours.

The stability of residues of triclabendazole in cattle tissues, measured as keto-triclabendazole, was determined using incurred tissues from two animals collected in Study Y03/49 (Study V03/57, 2004a). Three replicates of each tissue were analyzed prior to storage and then at 1.5, 3 and 6.5 months after storage in a freezer room that was maintained at a temperature ranging from a maximum average of -8^{o}C to a minimum average of -22^{o}C over the time period of the study. The average results, corrected for recovery, given in Table 6, demonstrate that the residues remain essentially stable during this time period, with some decrease (maximum 33 %) being seen at the final time point.

Table 6: Stability of incurred triclabendazole residues in cattle tissues under typical conditions of frozen storage

	Residues measured as keto-triclabendazole, corrected for analytical recovery (mg/kg)		
	0 months (pre-storage)	2 months	4 months
Muscle 1	0.17 ± 0.01	0.15 ± 0.00	0.16 ± 0.01
Muscle 2	0.13 ± 0.01	0.12 ± 0.00	0.11 ± 0.006
Kidney 1	0.25 ± 0.013	0.27 ± 0.01	0.24 ± 0.00
Kidney 2	0.17 ± 0.01	0.15 ± 0.02	0.15 ± 0.00
Liver 1	0.47 ± 0.02	0.41 ± 0.04	0.41 ± 0.05
Liver 2	0.34 ± 0.01	0.27 ± 0.02	0.28 ± 0.02

A similar study of residue stability in sheep tissues under typical conditions of frozen storage was undertaken using incurred muscle, liver and kidney samples from two animals from Study Y04/22 (Study Y04/22, 2004a). Three replicates of each tissue were analyzed prior to storage and after 2 and 4 months of frozen storage. The storage temperature varied from -5°C to -21°C during the period of storage. As with the cattle tissues, there was minimal change in the residue concentrations during the period of storage (Table 7).

Table 7: Stability of incurred triclabendazole residues in sheep tissues under typical frozen storage conditions

	Residues measured as keto-triclabendazole, corrected for analytical recovery, (mg/kg)			
	0 months (pre-storage)	1.5 months	3 months	6.5 months
Muscle 1	0.23 ± 0.01	0.24 ± 0.00	0.21 ± 0.00	0.19 ± 0.02
Muscle 2	0.25 ± 0.01	0.24 ± 0.02	0.20 ± 0.01	0.17 ± 0.02
Kidney 1	0.48 ± 0.03	0.42 ± 0.01	0.43 ± 0.05	0.36 ± 0.02
Kidney 2	0.47 ± 0.03	0.47 ± 0.06	0.44 ± 0.04	0.41 ± 0.02
Liver 1	0.85 ± 0.04	0.80 ± 0.02	0.78 ± 0.15	0.70 ± 0.04
Liver 2	0.75 ± 0.04	0.76 ± 0.04	0.81 ± 0.05	0.62 ± 0.01

APPRAISAL

The previous evaluation by the 40th meeting of the Committee addressed studies using ^{14}C-labelled triclabendazole in rats, rabbits, dogs, sheep, goats, cattle and pigs. The absorption, distribution, metabolism and excretion of triclabendazole were qualitatively similar in both laboratory animals and food producing animals. Following an oral dose of ^{14}C-labelled triclabendazole, the biotransformation and excretion of triclabendazole was very rapid. Absorbed triclabendazole which entered the circulation was rapidly metabolized *via* two major pathways: oxidation of the methyl thiol group initially to a sulfoxide and later to a sulfone metabolite, and 4-hydroxylation of the dichlorophenoxy ring. Five identified metabolites, triclabendazole, sulfoxide, sulfone, ring-hydroxylated metabolites and keto-triclabendazole accounted for approximately 40-60% of the administered dose. Quantitative differences in relative proportions of metabolites were observed between species. Elimination of triclabendazole was nearly complete within 10 days after administration. Faecal excretion of triclabendazole and its metabolites accounted for the principal portion of the dose, with only minor elimination *via* urinary excretion.

New studies confirmed the findings in the report of the 40th Committee that absorption is rapid and is not dose-dependent in rats after oral dosing, reaching a maximum plasma concentration 8 hrs post-dose. The studies also confirmed that elimination is rapid, with approximately 90% of the dose eliminated through urine and faeces within 48 hrs, primarily through faeces (>80%). Studies in which rats were fed lyophilized tissues containing incurred residues of triclabendazole demonstrated that the bioavailability of these residues was low (<20%)

The biotransformation of triclabendazole is also rapid in dogs, with the sulfoxide and sulfone metabolites accounting for approximately all of an administered oral dose in plasma. Following oral administration, approximately 35-53% of a 0.5 mg/kg dose was absorbed, with a decrease to about 25% at higher doses. Maximum concentration in plasma occurred 8-24 hours after oral administration and elimination of triclabendazole was almost exclusively *via* faeces.

Previous studies have indicated rapid metabolism of triclabendazole following intravenous administration in cattle, with the sulfoxide metabolite achieving maximum concentrations in blood 4 hours after treatment, with terminal half-life of approximately 13 hours. Maximum plasma concentrations of the sulfone metabolite were observed 32 hours after dosing, with terminal elimination half-life of 40 hours.

The new studies confirmed that absorption and elimination were rapid in cattle and that, as in rats and dogs, elimination is primarily in faeces. The major metabolites in cattle faeces were similar to those of rats. Residue concentrations in tissues 28 day after dosing were highest in liver followed by muscle and kidney. The residue in fat was negligible compared to other edible tissues.

The studies of absorption, distribution and excretion of triclabendazole reviewed by the 40th Committee indicated that the metabolism of triclabendazole in sheep and goats was similar to that seen in other species tested. At 28 days after dosing with ^{14}C- triclabendazole, liver contained the highest residue concentrations, followed by muscle and kidney, whereas residues were relatively negligible in fat tissue. The results of new studies of triclabendazole metabolism in ruminating sheep were consistent with the metabolic pattern seen in the previous studies. Faecal elimination accounted for 77% of the administered dose. Distribution of radioactivity in tissues at 28 days post-dosing revealed highest total residues in muscle, followed by liver and kidney.

Three residue depletion studies in cattle considered by the 40th Committee demonstrated that at 28 days following a single oral dose of 12 mg/kg body weight, average residue concentrations were 0.12 mg/kg in liver, 0.07 mg/kg in kidney, 0.11 mg/kg in muscle and 0.05 mg/kg in fat, representing, respectively, 50%, 66%, 84% and >100% of the total residues in these tissues.

Two new GLP studies using ^{14}C-triclabendazole by oral administration at 12 mg/kg body weight provided additional information on the relationship between the marker and the total residues in cattle. In the first study, two ruminating calves were slaughtered 28 days after administration of a single oral dose of ^{14}C-triclabendazole, 12 mg/kg body weight. Residues measured as keto-triclabendazole, corrected for analytical recovery, accounted for 24% of the total residue in liver, 19% of the total residue in kidney and 42% of the total residue in muscle at 28 days post-administration. Subsequently, residues were measured as keto-triclabendazole in tissues from a single calf killed 28 days after a single oral dose of ^{14}C-triclabendazole, 12 mg/kg body weight. Residues measured as keto-triclabendazole accounted for the following percentages of the total residue in each tissue: liver 24%, kidney 27% and muscle 32%. These studies demonstrated that the relationships of marker to total residue calculated from information available to the 40th meeting of the Committee over-estimated the proportion of total residue present as marker residue.

In muscle and liver tissues collected from two sheep slaughtered 28 days after administration of a single oral dose of ^{14}C-triclabendazole, 10 mg/kg body weight, residues measured as keto-triclabendazole, corrected for analytical recovery, accounted for 39% of the total residues in

muscle and 24% of the residues in liver. These results are consistent with those found in incurred tissues from cattle and with the relationships reported for sheep by the 40th Committee.

Based on the results of the new studies in cattle and sheep, factors for conversion of recovery-corrected residues measured as keto-triclabendazole to total residues are 4 for liver, 3.7 for kidney and 2.5 for muscle. No conversion factor was considered necessary for fat, given the limited distribution of residues in that tissue.

The new GLP studies in cattle and sheep gave results consistent with the distribution and depletion studies reviewed by the 40th Committee. Unlike the earlier studies, however, the new GLP study in cattle was conducted under conditions of repeat treatment, 28 days subsequent to the initial treatment, using a dose 1.5 times the recommended treatment. Highest residues measured as keto-triclabendazole were found in liver at all time points (14, 28, 42, 56 days), but residues in muscle were similar to those found in liver at 56 days after the second treatment. Residues were below the detection limit of 0.05 mg/kg in kidney at 56 days and were only detectable in fat at 14 days post-treatment. There were no detectable residues in fat at the later timepoints when residues in other tissues were approaching the MRLs. This eliminated residues in fat from inclusion in the calculation of theoretical maximum daily intake.

The three residue depletions studies with sheep orally dosed with triclabendazole (single dose of 10 mg/kg body weight or 15 mg/kg body weight) considered by the 40th Committee demonstrated that residues depleted to below detection limits in fat (0.03 mg/kg) within 14-21 days after administration, but remained detectable in liver, kidney and muscle for 28 days. Highest residues were reported in liver at 21 days post-treatment, but results were similar in liver, kidney and muscle at 28 days post-treatment. In the new GLP study considered by the present Committee, sheep received a single oral dose of 10-13 mg/kg body weight. Most persistent residues were in muscle, detectable in muscle from 50% of the animals at 56 days post-treatment. Residues were not detected in liver or kidney samples at 42 days post-treatment or in fat samples at 14 days post-treatment.

The results of the studies demonstrate that while liver is the tissue in which highest residues are initially found, particularly for cattle within approximately 4 weeks of treatment, muscle is also a suitable target tissue and is the preferred target tissue at longer times following treatment.

A suitable validated liquid chromatographic method was available for regulatory use to detect and quantify residues as keto-triclabendazole. However, a confirmatory method which meets contemporary criteria in many countries, such as one based on LC/MS, was not provided for review.

The median residues calculated for the residues represented by an MRL established at the 95/95 limit, reflecting the normal distribution in a group of animals in which the residues are below the MRL in 95% of the treatment group, were also considered as an alternative approach to estimation of a dietary intake. The median residue concentrations calculated from the data were: muscle, 88.5 μg/kg; liver, 99.5 μg/kg; kidney, 25 μg/kg; fat, 12.5 μg/kg. The value for fat is a default value intermediate between the LODs of the proposed regulatory method for fat of cattle and sheep and was selected to reflect that there were no detectable residues in fat when residues in liver and muscle are approaching the MRLs. Using the median residues approach, an estimate of dietary intake of residues from fat is included in the estimated daily intake calculation. Based on the median values, the corresponding daily intake estimate is 121.7 μg.

MAXIMUM RESIDUE LIMITS

In recommending MRLs, the Committee took into account the following factors:

- The marker residue is keto-triclabendazole.
- The appropriate target tissue is muscle.
- A validated analytical method is available for analysis of triclabendazole residues in edible tissues of cattle, sheep and goats.
- The bioavailability of incurred triclabendazole residues in tissues fed to rats did not exceed 20%.

- The factors calculated for conversion of marker-to-total residue in cattle tissues by the 40th meeting of the Committee, based on the then available studies, have been demonstrated to be incorrect by data from the more recent GLP studies which provided both total residue and marker residue concentrations from the same tissues.

- The factors to convert from marker to total residue , derived from mean results of the new GLP studies in cattle and sheep, are 4.3 for liver, 4.2 for kidney and 2.7 for muscle of cattle and sheep, calculated at 28 days after a single administration at the recommended dose. When multiple doses are used, these factors are sufficiently conservative to apply to the various timepoints where residues are at the MRLs. The same factors are applicable to goats, based on the available information. A factor was required for kidney as detectable residues were present below the LOQ at the timepoint used for calculation of the MRLs for liver and muscle, requiring their inclusion in the intake estimate. Due to the rapid depletion of residues in fat, a factor to convert marker to total residues was not required as the intake estimate based on one-half the LOQ of the analytical method provides a conservative estimate.

- Maximum residue limits for liver and muscle of cattle, sheep and goats were based on the mean residue concentrations plus 3 standard deviations from the new GLP studies in cattle and sheep. The time point for which the MRLs for cattle were calculated is 56 days following the second treatment at 1.5 times the recommended dose. Residues deplete more rapidly in cattle following a single treatment at the recommended dose. These MRLs will be achieved in sheep at a time point intermediate between 28 and 42 days after a single treatment at the recommended dose.
- Maximum Residue Limits for kidney and fat were based on twice the limit of quantification.
- An ADI of 0-3 μg per kg of body weight was established by the 40th meeting of the Committee, equivalent to 0-180 μg for a 60 kg person (WHO, 1993).

On the basis of the above considerations, the Committee recommended the following MRLs for edible tissues of cattle, sheep and goats, expressed as the marker residue, keto-triclabendazole.

Muscle	150 μg/kg
Liver	200 μg/kg
Kidney	100 μg/kg
Fat	100 μg/kg

The MRLs recommended above would result in a theoretical daily maximum intake of 230 μg, based on a daily food intake of 300 g of muscle, 100 g of liver, 50 g each of kidney and fat, or 128% of the upper bound of the acceptable daily intake. An estimate of residue intake calculated as the TMDI is tabulated as follows:

Table 8: Theoretical Maximum Daily Intake (TMDI) of Triclabendazole Residues

Food Item	MRL (μg/kg)	Standard Food Basket (kg)	MR/TR[1]	TMDI (μg)
Muscle	150	0.300	2.7	121.5
Liver	200	0.100	4.5	90
Kidney	100	0.050	3.7	18.5
Fat	100	0.050	1	-
Total:				230.0

[1] MR = marker residue (parent drug); TR = total residue

The Committee also calculated long term (chronic) exposure an estimated median daily intake, using the median values from the residue data used in recommending the MRLs. The median value of the distribution of residue concentrations from which the MRL is derived is used as new point estimate instead of the MRL. The Committee considers that this represents a more realistic estimate of intake, consistent with the approach to intake calculations used by the Joint FAO/WHO meeting on Pesticide Residues (JMPR). The median residue concentrations calculated from the data are: muscle, 88.5 μg/kg; liver, 99.5 μg/kg. kidney, 25 μg/kg; fat, 25 μg/kg. In the absence of quantifiable residues in kidney and fat at the time point where the MRLs were recommended, a value equal to one-half the LOQ was assigned as a conservative estimate of intake from kidney and fat. The median residue concentrations above would result in an estimated daily intake of 121 μg, based on a daily food intake of 300 g of muscle, 100 g of liver, 50 g each of kidney and fat, or 67% of the upper bound of the acceptable daily intake. Estimates of the residue intake calculated as the EDI are tabulated in Table 9.

Table 9: Estimated Daily Intake of triclabendazole residues

Tissue	Median residue concentration (μg/kg)	Standard Food Basket (kg)	MR/TR[1]	EDI (μg)
Muscle	88.5	0.300	2.7	71.7
Liver	99.5	0.100	4.3	42.8
Kidney	25	0.050	4.2	5.2
Fat	25	0.050	1	1.3
Total:				121.0

[1] MR = marker residue (parent drug); TR = total residue

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