TYLOSIN

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IDENTITY

International nonproprietary name: Tylosin (INN-English)

European Pharmacopoeia name:
(4R,5S,6S,7R,9R,11E,13E,15R,16R)-15-[[(6-deoxy-2,3-di-O-methyl-β-D-allopyranosyl)oxy]methyl]-6-[[3,6-dideoxy-4-O-(2,6-dideoxy-3-C-methyl-α-L-ribo-hexopyranosyl)-3-(dimethylamino)-β-D-glucopyranosyl]oxy]-16-ethyl-4-hydroxy-5,9,13-trimethyl-7-(2-oxoethyl)oxacyclohexadeca-11,13-diene-2,10-dione

IUPAC name: 2-[12-[5-(4,5-dihydroxy-4,6-dimethyl-oxan-2-yl)oxy-4-dimethylamino-3-hydroxy-6-methyl-oxan-2-yl]oxy-2-ethyl-14-hydroxy-3-[(5-hydroxy-3,4-dimethoxy-6-methyl-oxan-2-yl)oxymethyl]-5,9,13-trimethyl-8,16-dioxo-1-oxacyclohexadeca-4,6-dien-11-yl]acetaldehyde

Other chemical names: 6S,1R,3R,9R,10R,14R)-9-[((5S,3R,4R,6R)-5-hydroxy-3,4-dimethoxy-6-methylperhydropyran-2-yloxy)methyl]-10-ethyl-14-hydroxy-3,7,15-trimethyl-11-oxa-4,12-dioxocyclohexadeca-5,7-dienyl}ethanal

Oxacyclohexadeca-11,13-diene-7-acetaldehyde,15-[[(6-deoxy-2,3-dimethyl-b-D-allopyranosyl)oxy]methyl]-6-[[3,6-dideoxy-4-O-(2,6-dideoxy-3-C-methy-a-L-ribo-hexopyranosyl)-3-(dimethylamino)-b-D-glucopyranosyl]oxy]-16-ethyl-4-hydroxy-5,9,13-trimethyl-2,10-dioxo-[4R-(4R*,5S*,6S*,7R*,9R*,11E,13E,15R*,16R*)]-

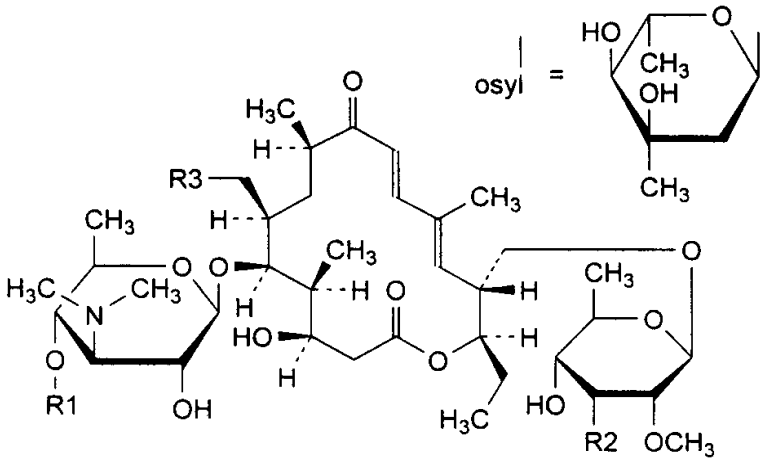
Synonyms: AI3-29799, EINECS 215-754-8, Fradizine, HSDB 7022, Tilosina (INN-Spanish), Tylan, Tylocine, Tylosin, Tylosine, Tylosine (INN-French), Tylosinum (INN-Latin), Vubityl 200

Chemical Abstracts System number: CAS 1401-69-0

Structural formula:

Tylosin is a macrolide antibiotic representing a mixture of four tylosin derivatives produced by a strain of Streptomyces fradiae (Figure 1). The main component of the mixture (> 80%) is tylosin A (M_r = 916; McGuire, et al., 1961). Tylosin B (desmycosin, M_r = 772; Hamill, et al., 1961), tylosin C (macrocin, M_r = 902; Hamill and Stark, 1964) and tylosin D (relomycin, M_r = 918; Whaley, et al., 1963) may also be present. All four components contribute to the potency of tylosin, which is not less than 900 IU/mg, calculated with reference to the dried substance (European Pharmacopoeia, 2004). Relative antimicrobial activities of tylosin derivatives are: tylosin A – 1.0, tylosin B – 0.83, tylosin C – 0.75 and tylosin D – 0.35 (Teeter and Meyerhoff, 2003).

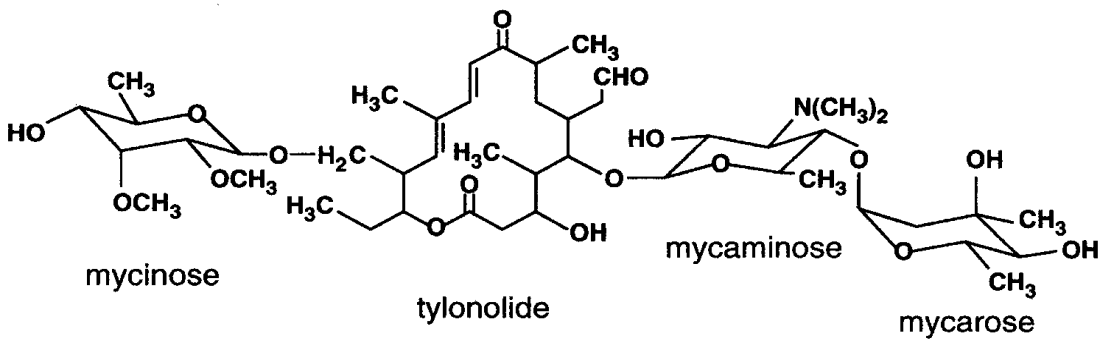
Figure 1: Chemical structure of tylosin.



Name	Mol. Formula	R1	R2	R3
tylosin A	$C_{46}H_{77}NO_{17}$	osyl	OCH_3	CHO
tylosin B	$C_{39}H_{65}NO_{14}$	H	OCH_3	CHO
tylosin C	$C_{45}H_{75}NO_{17}$	osyl	OH	CHO
tylosin D	$C_{46}H_{79}NO_{17}$	osyl	OCH_3	CH_2OH

Tylosin A contains a polyketide lactone (tylactone) substituted with three 6-deoxyhexose sugars (Figure 2). The addition of D-mycaminose to the aglycone is followed by concurrent ring oxidation at C-20 and C-23 (to generate the tylonolide moiety) and substitution with L-mycarose and 6-deoxy-D-allose. Bis-O-methylation of the latter generates mycinose and completes the biosynthesis of tylosin (Baltz, et al., 1983; Baltz and Seno, 1988).

Figure 2: Chemical structure of tylosin A.



Other pharmacologically active compounds, i.e., lactenocin, demecinosyl-tylosin (DMT) and O-mycaminosyl-tylonolide (OMT) have been isolated from fermentation media or aqueous commercial samples containing tylosin. In solutions for injections containing tylosin, an alkaline degradation product called tylosin aldol (TAD) has also been detected. Two epimers of this product, TAD1 and TAD2, as well as isotylosin A (isoTA) have been isolated (Paesen, et al., 1995abc). More recently, two photoreaction products of tylosin in water, isotylosin A alcohol (isoTA1) and isotylosin A aldol (isoTA2) have been identified (Hu and Coats, 2007; Hu, et al., 2008).

Molecular formula: $C_{46}H_{77}NO_{17}$

Molecular weight: 916.1

OTHER INFORMATION ON IDENTITY AND PROPERTIES

Pure active ingredient: The main component of the mixture (> 80%) is tylosin A

Appearance: An almost white or slightly yellow crystalline powder

Melting point: 128-132°C

Solubility: 5 mg/ml (water 25°C), soluble in lower alcohols, esters, ketones, chlorinated hydrocarbons, benzene, ether, acetone, chloroform

UV Absorption: UV_{max} at 282 nm; Extinction coefficient ($E_{1\ cm}$ 1%) is 245 at 282 nm

Stability: Solutions are stable at pH 4-9 (maximum stability at pH 7);
Below pH 4 tylosin B (desmycosin) is formed as a result of acid hydrolysis, as occurs in honey;
In neutral and alkaline pH, tylosin aldol (TAD) is formed together with polar degradation products of unknown identity;
When tylosin solution is exposed to daylight, two photodegradation products, isotylosin A alcohol (isoTA1) and isotylosin A aldol (isoTA2), are formed

pKa: 7.73

log P (octanol-water): 1.63

RESIDUES IN FOOD AND THEIR EVALUATION

Tylosin was first evaluated by the Committee at the twelfth meeting (FAO/WHO, 1969). At that meeting, the Committee concluded that tylosin used in animal feed or in veterinary medicine should not give rise to detectable residues in edible products of animal origin. No ADI was established. Tylosin was subsequently evaluated at the thirty-eighth meeting of the Committee (FAO/WHO, 1991). Because of deficiencies in the toxicological and microbiological data, the Committee was not able to establish an ADI or recommend MRLs for tylosin. Before reviewing the compound again, the Committee requested the following information:

1. Detailed information from the reported reproduction and teratogenicity studies.
2. Studies designed to explain the positive result that was obtained in the mouse lymphoma genotoxicity assay in the absence of metabolic activation.
3. Studies designed to test the hypothesis that the increased incidence of pituitary adenomas in male rats after the administration of tylosin is a consequence of the greater rate of bodyweight gain in these rats.
4. Studies from which a NOEL for microbiological effects in humans can be determined.
5. Additional studies of residues in eggs using more sensitive analytical methods.
6. Additional information on microbiologically active metabolites of tylosin.
7. Studies on the contribution of the major metabolites of tylosin to the total residues in edible tissues of cattle and pigs.

In 2005, the 15th Session of the Codex Committee of Residues of Veterinary Drugs in Food (CCRVDF) requested that information on tylosin be submitted for evaluation by the sixty-sixth meeting of the Committee. However, none of the requested information was provided. In the absence of submitted information and in the light of a large number of scientific articles on tylosin appearing in

the open literature since the thirty-eighth meeting of the Committee, a comprehensive review of the available information in the published literature concerning analytical methods, pharmacokinetics and tissue residues of tylosin in different animal species was carried out (Lewicki, 2006). However, the sixty-sixth meeting of the Committee did not critique the review as it considered published information alone was not suitable for conducting an evaluation of the compound. Tylosin was included on the agenda for the seventieth meeting of the Committee, as a result of a request from the 17th Session of the CCRVDF. Data as requested at the thirty-eighth meeting were provided for evaluation by the present meeting of the Committee.

Conditions of use

Tylosin is active against Gram-positive bacteria, mycoplasma and certain Gram-negative bacteria. Macrolide antibiotics are bacteriostatic compounds that reversibly bind to the 23S rRNA in the 50S ribosome subunit and inhibit mRNA-directed protein synthesis. They also stimulate the dissociation of peptidyl-tRNA from ribosomes during translocation. The precise mechanism of action has not been fully elucidated and many theories exist (Zhanel, et al., 2001; Gaynor and Mankin, 2005). It has been suggested that 16-membered-ring macrolides inhibit protein synthesis by blocking elongation of the peptide chain, but the 14- and 15-membered-ring macrolides are only potent inhibitors of mRNA-directed peptide synthesis (Retsema and Fu, 2001). It was also demonstrated that the 16-membered-ring macrolides (carbomycin, spiramycin and tylosin) inhibit peptidyl transferase, and the presence of mycarose was correlated with peptidyl transferase inhibition. However, tylosin B did not inhibit peptidyl transferase (Poulsen, et al., 2000). Results of comparative antibacterial evaluation of tylosin A and tylosin B showed that both compounds have almost identical antibacterial activity. In the same study, tetrahydro-desmycosin and dihydro-desmycosin showed decreased antimicrobial activity (Iveković, et al., 2003). Moreover, 4'-deoxy-10,11,12,13-tetrahydro-desmycosin, a derivative of tetrahydro-desmycosin, retained the antibacterial spectrum of tylosin with some improvement against tylosin-sensitive *Staphylococc*i and *Haemophilus influenzae* (Narandja, et al., 1995).

Tylosin is registered exclusively for veterinary use in several countries, primarily for use in the chronic respiratory disease (CRD) complex in chickens and infectious sinusitis in turkeys caused by *Mycoplasma gallisepticum*. Tylosin is also used to treat swine and bovine respiratory diseases and swine dysentery.

Dosage

Tylosin and its phosphate and tartrate salts are used in cattle, pigs and poultry for the treatment of infections caused by organisms sensitive to tylosin. Tylosin may be administered to calves orally in the drinking water, milk or milk replacer, at a daily dose of 10-40 mg/kg bw and to cattle by intramuscular injection at a dose of 5-20 mg/kg bw per day. In pigs, tylosin may be administered in the drinking water at a daily dose of 5-25 mg/kg bw; in the feed at a dose of 3-7 mg/kg bw per day; or by intramuscular injection at a dose of 5-20 mg/kg bw per day. In poultry, tylosin is used primarily in the treatment of chronic respiratory disease complex in chickens and infectious sinusitis in turkeys caused by *Mycoplasma gallisepticum*. It may be administered in the drinking water (0.5 g per litre) at a dose equivalent to 75 mg/kg bw per day; in addition, it may also be administered by intramuscular injection at a dose of 20-120 mg/kg bw per day (Plumb, 2002; Giguère, 2006). Tylosin is also approved for emergency use in the control of American foulbrood of honey bees at a dose of 200 mg/hive in 20g confectioners/powdered sugar once weekly for three weeks (FDA, 2005).

PHARMACOKINETICS AND METABOLISM

Tylosin is a highly lipid soluble, weak organic base (pKa = 7.73) that readily forms salts and esters. Available forms of tylosin are: tylosin base, tylosin tartrate and tylosin phosphate (McFarland, et al., 1997; European Pharmacopoeia, 2004). It is slightly to moderately bound to plasma proteins (30-47%) and is widely distributed in body fluids and tissues (Burrows, 1980). The volume of distribution (V_d) of tylosin is from 1-14.6 l/kg in various animal species. Although the comparative pharmacokinetics of

tylosin in animals is poorly described in the scientific literature, reviews on tylosin pharmacokinetics are available (Wilson, 1984; WHO, 1991). Allometric relationships between tylosin total body clearance (Cl_B) and animal body weight have also been presented (Lewicki, 2006).

Absorption

Laboratory Animals

Rats

Tylosin is rapidly absorbed following oral administration to rodents. After a single oral dose of 50mg/kg bw of tylosin base or tylosin tartrate to rats, peak serum concentrations of tylosin of ≤1.0 µg/ml were seen after 1-2 hours. Within 7 hours, serum concentrations decreased to less than the limit of detection (LOD = 0.10 µg/ml) of the microbiological assay (WHO, 1991). Similar results were obtained in rats after intragastric administration of a solution of tylosin base. After a single dose of 20, 50 or 100 mg/kg bw of tylosin base, peak serum concentrations (about 0.5-1.1µg/ml) appeared after 2 hours (Kietzmann, 1985). When rats were given water mixed with a commercially available preparation of tylosin base (final concentration about 71 µg/ml), the bioassay of serum after 1-10 days of continuous medication revealed no detectable tylosin concentrations (< 0.1 µg/ml), while lung tissue contained 3.93-18.14 µg of tylosin/g (Carter, et al., 1987).

In rats, the reported V_d of 2.2 l/kg (Duthu, 1985) was similar to the values of V_d calculated for other animal species (for a review, see Lewicki, 2006). The elimination of tylosin from plasma is rapid in rats. Duthu (1985) reported a plasma elimination half-life ($t_{1/2}$) of 0.4 hour after intravenous administration and Cl_B of 86 ml/min/kg. A similar Cl_B of 70.9 ml/min/kg was observed for tylosin in mice (Cacciapuoti, et al., 1990).

Dogs

In dogs receiving tylosin orally by capsule at a dose of 1, 10 or 100 mg/kg bw/day for 8 days, tylosin blood concentrations determined 2 hours after the last dose ranged from < 0.15 µg/ml to 9. 5µg/ml (WHO, 1991). In another study, dogs receiving 25 or 100 mg/kg bw of tylosin base orally by capsule daily for 29 days demonstrated peak serum concentrations of 1.4-2.7 µg/ml at 2 hours after dosing at 25 mg/kg bw/day, and peak serum concentrations of 2.7-4.6 µg/ml at 2-5 hours after dosing at 100 mg/kg bw/day (WHO, 1991). In a separate study, there was no evidence of tylosin accumulation in the serum after 2 years of continuous administration of tylosin base in the diet (Anderson et al., 1966).

The magnitude and duration of tylosin blood levels following twice daily intramuscular injections with Tylocine Injection (tylosin 50mg/ml) was determined in Beagle dogs (van Duyn and Kline, Undated-a). Tylosin was administered at a dose rate of 11 mg/kg bw (5 mg/lb bw) 12-hourly for one day. Tylosin was rapidly absorbed after each injection producing two very similar blood level curves during the 24-hour period. Tylosin serum levels peaked at 1.9 and 1.7 µg/ml, respectively, at approximately 2 hours post-injection before declining to approximately 0.1 µg/ml at 10 hours.

A single intramuscular injection of 11 mg tylosin per kg bw was given to each of five dogs weighing 5-9 kg. Blood samples were collected at 0.5, 1, 2, 4, 6, 8, 10, 12, 24 and 48 hours after injection and the serum was assayed for microbiological activity. The mean concentrations of tylosin in blood were 2.5 µg/ml at 0.5 hour; 2.5 µg/ml at 1 hour; and 2.3 µg/ml at 2 hours after dosing. By 10 hours after dosing, the tylosin blood level had declined to about. 0.1 µg/ml, the detection limit of the assay (van Duyn, et al., Undated).

In a study by van Duyn and Kline (Undated-b), six dogs were each given two intramuscular injections of 11 mg tylosin per kg bw 12 hours apart. Samples of blood were collected and assayed for tylosin activity at 2-hourly intervals up to 24 hours after the first injection. The peak blood concentrations of

tylosin were 1.9 µg/ml and 1.7 µg/ml, occurring approximately 2 hours after each injection. Tylosin was not detected after about 12 hours.

Weisel and coworkers (1977) investigated the pharmacokinetics of tylosin in dogs after a single intravenous dose of tylosin. These authors reported values of 1.7 l/kg for V_d, 21.9 ml/min/kg for Cl_B, and 0.9 hour for plasma $t_{1/2el}$.

Food Producing Animals

Cattle

Peak blood concentrations of tylosin in cows were reached in 2-4 hours following intramuscular injection of tylosin base in 50% propylene glycol, or an aqueous solution of the tartrate salt (Sauter, et al., 1962; Gingerich, et al., 1977). In calves receiving tylosin base at a dose of 17.6mg/kg bw, peak concentrations of tylosin ranging from 2.07 to 2.3 µg/ml were observed 2 hours after intramuscular injection (van Duyn and Folkerts, 1979). Intratracheal administration of tylosin base at a dose of 25 mg/kg bw to calves resulted in peak serum concentrations of 5.2-5.8 µg/ml tylosin 1 hour after dosing. With intramuscular and subcutaneous injections, peak concentrations of 2.7-4.7 and 1.25-1.8 µg/ml, respectively, were reached 2 and 8 hours after injections (Hjerpe, 1979). In a separate study, peak serum concentrations of tylosin occurred about 5-6 hours after intramuscular injection of cattle, with systemic bioavailability of 70-80% of the administered dose (Ziv and Sulman, 1973; Baggot, 1978). The absorption of tylosin base following intramuscular injection was 17% and 94% complete after 7 hours and 24 hours, respectively (Nouws and Ziv, 1977a).

Kiorpes (1993) reported the relative bioavailability of Tylan® 200 Injection when administered subcutaneously and intramuscularly to cattle, using a cross-over study design. Two groups comprising six animals of each sex were administered 17.6 mg/kg bw Tylan® 200 Injection either subcutaneously or intramuscularly for five consecutive days. Serum samples were assayed for tylosin antimicrobial activity using a validated microbiological method; the limit of quantitation (LOQ) of the analytical method was approximately 0.1 mg/l. Following subcutaneous administration, tylosin was more slowly absorbed and attained a C_{max} of 0.89 mg/l, approximately one-half the C_{max} of 1.80 mg/l obtained after intramuscular administration. The time to reach maximum concentration (T_{max}) was 1 hour and 4.1 hours for intramuscular and subcutaneous administration, respectively, and the mean $t_{1/2}$ values were 6.9 hours and 16.2 hours, respectively. The bioavailability of tylosin following subcutaneous administration was 113% relative to intramuscular administration (Kiorpes, 1993).

The pharmacokinetics of tylosin in calves has also been reported by Abdul-Karim (2006a). Blood plasma concentrations after intravenous (10 mg/kg bw) and oral administration by gavage (20 mg/kg bw twice daily for five days) were determined by LC-MS/MS (the LOQ of the analytical method was 5 µg/kg) and the pharmacokinetic parameters were presented. Following the intravenous dose, C_0 was 16.9 µg/ml; V_{dss} was 3.49 l/kg; Cl_B was 23.0 ml/min/kg; $AUC_{0\text{-}24h}$ was 7.4 µg·h/ml; and $t_{1/2el.}$ was 10.9 hours. While recognising that estimates were based on limited data, oral bioavailability of tylosin in water is very low (about 1.7%). In the same study, the kinetics of tylosin plasma concentration were evaluated in cattle after a single intravenous injection (10 mg/kg bw) and intramuscular injections for three consecutive days (10 mg/kg bw per day). Pharmacokinetic parameters determined for the intravenous dose were: C_0 = 31.3 µg/ml; V_{dss} = 2.01 l/kg; Cl_B = 10.0 ml/min/kg; $AUC_{0\rightarrow\infty}$ = 16.8 µg·h/ml; and $t_{1/2el.}$ = 12.7 hours. Pharmacokinetic parameters determined for the final intramuscular dose were: C_{max} = 2.1 µg/ml; T_{max} = 2.6 hours; $t_{1/2el.}$ = 16.6 hours; and $AUC_{0\rightarrow\infty}$ = 18.4µg·h/ml. The bioavailability of tylosin following intramuscular administration was 110% relative to intravenous administration (Abdul-Karim, 2006a).

The administration of tylosin base as a single intravenous or intramuscular injection to cattle at a dose of 4.6-7.3 mg/kg bw has been reported (Nouws and Ziv, 1977b; Nouws and Ziv, 1979). Tylosin concentrations in bile were 59.1 µg/ml (i.v.) and 56.3 µg/ml (i.m.) at 7 hours after injection; 35.1 µg/ml (i.m.) at 24 hours after injection; and 12.1 µg/ml (i.m.) at 31 hours after injection. The

bile:serum concentration ratios were 296:1 (i.v.) and 62:1 (i.m.) at 7 hours after injection; 100:1 (i.m.) at 24 hours after injection; and 48:1 (i.m.) at 31 hours after injection. These ratios were much lower than the range of 1230-3780:1 reported for the dog (WHO, 1991). In the cattle study, tylosin concentrations in urine were 29.7 µg/ml (i.v.) and 41.7 µg/ml (i.m.) at 7 hours after injection; 12.9 µg/ml (i.m.) at 24 hours after injection; and 17.7 µg/ml (i.m.) at 31 hours after injection (Nouws and Ziv, 1977b; Nouws and Ziv, 1979).

In general, lipophilic weak bases such as tylosin readily pass from plasma to milk, which has a lower pH than plasma. This was confirmed in several experiments in different ruminant species. In cows receiving a single intravenous injection of tylosin tartrate at a dose of 20 mg/kg bw, peak concentrations of tylosin in milk (about 10 µg/ml) were observed 4 hours after injections; corresponding plasma concentrations of tylosin were about. 3.5 µg/ml. Lower peak values (about. 6 µg/ml) were observed in cows' milk 6 hours after a single intramuscular injection of tylosin tartrate at the same dose. When tylosin base was administered to cows intramuscularly at a dose of 12.5 mg/kg bw 12 hourly for 48 hours, the concentration of tylosin in milk peaked at about 7 µg/ml after 60 hours and then rapidly decreased to 1.5 µg/ml at 72 hours. Milk:serum concentration ratios corrected for differences in protein binding ranged up to about 20:1 (Gingerich, et al., 1977). Similar milk:serum concentration ratios up to 17.5:1 were observed in cows after a single intramammary infusion of 200 mg of tylosin/quarter. When mastitic cows received repeated intramuscular injections of tylosin base at a dose of 10 mg/kg bw every 12 hours for 5 days, concentrations of tylosin in milk steadily increased up to 18 µg/ml on day 5 after the onset of therapy (El-Sayed, et al., 1986).

Pigs

When pigs were administered tylosin tartrate orally at 30 mg/kg bw, tylosin activity was detected in plasma 10 minutes after dosing, with the maximum concentration of 2.4 µg/ml occurring approximately 1.5 hours later. A comparison of the blood AUCs following i.v. and p.o. administration provided an estimate of biological availability of 22.5%. When tylosin as the granulated phosphate was administered orally to pigs at a dose of 110 mg/kg bw, tylosin serum activity peaked 1 hour after dosing (average 17.8 µg/ml) and was not detectable (< 0.1 µg/ml) 24 hours after dosing. Similar results were obtained after the oral administration of tylosin phosphate in water at a dose rate of 50mg/kg bw Tylosin concentrations were detected in serum from 10 minutes to 8 hours after dosing and peaked 1 hour after dosing at 8.5 µg/ml (WHO, 1991). The results of a comparative residue study in pigs suggest that absorption of tylosin phosphate from the alimentary tract is comparable to that of tylosin tartrate (Iritani, et al., 1975).

Following intramuscular injection of pigs with tylosin base in 50% propylene glycol, or with an aqueous solution of the tartrate salt at a dose of 2.5-5 mg/kg bw, peak blood concentrations of tylosin were reached within 0.5-2 hours. Moreover, the results demonstrated that tylosin activity persisted in blood for up to 14 hours with the base, but only up to 8 hours with the tartrate salt (Sauter, et al., 1962). When pigs received tylosin base at a dose of 10 mg/kg bw by intramuscular injection, peak plasma concentrations of tylosin (0.4-1.9 µg/ml) were reached after 0.3-3 hours and bioavailability was 95% (Prats, et al., 2002a). Following a single intramuscular injection of pigs with a commercial mixture of tylosin and florfenicol (FTD-inj®) at doses of 2.5 or 10 mg/kg bw (tylosin) and 5 or 20 mg/kg bw (florfenicol), the C_{max} of tylosin was 1.3 µg/ml occurring at 2.4 hours for the low dose and 2.7 µg/ml occurring at 2.57 hours for the high dose. The $t_{1/2el}$ was 3.9 hours and 3.0 hours for the low and high doses, respectively (Kim, et al., 2008).

Tylosin levels in serum and lung tissue were measured in pigs following a single intramuscular dose of 17.6mg tylosin per kg bw as Tylan® 50. Tylosin was rapidly absorbed producing measurable serum and lung concentrations within 2 hours after the injection. Peak concentrations of 14.0 mg/kg and 2.0 µg/ml were observed in lung and serum, respectively, at 4 hours. Tylosin was not detected in serum after 12-24 hours but persisted in lung tissue for 48 hours (van Duyn and Johnson, Undated). In a similar experiment, tylosin was measured in serum and lung tissue of pigs following administration of a single intramuscular injection of Tylan® 50 at 8.8 mg/kg. Peak concentrations of tylosin occurred 2

hours after the injection and were 5.7mg/kg in lung tissue and 2.0 µg/ml in serum. Tylosin was detectable in serum for less than 12 hours and in lung tissue for 36-48 hours (van Duyn, Undated).

In another study comparing tylosin concentrations in lung and serum, pigs were injected intramuscularly with Tylan® 200 at a rate of 10 mg/kg bw for five consecutive days. The activity of tylosin residues was determined in sera and lung tissue by microbiological assay. Peak serum activity (1.7 µg/ml) occurred in pigs 2 hours after dosing and declined over 4 and 6-hours to 0.6 and 0.4 µg/ml, respectively. No activity was detected in sera sampled at 12 and 72 hours after dosing. Peak tylosin activity (5.8 mg/kg) in lung occurred 2 hours after injection. Tylosin activity in lung then declined, being below the LOQ of 0.1 mg/kg at 12 and 72 hours (Cochrane and Thomson, 1990).

Pratts and coworkers (2002a) reported the values of 4.5 hours for plasma $t_{1/2el.}$, 26.8 ml/min/kg for Cl_B and 14.6 l/kg for V_d for tylosin after single intravenous administration to healthy pigs. These authors also reported $t_{1/2el}$ of tylosin exceeding 24 hours when tylosin base was administered intramuscularly to pigs at a dose of 10 mg/kg bw.

Chicken/Poultry

When broiler chickens weighing 720 g received a single dose of 50 mg tylosin (as tylosin tartrate) per bird by stomach intubation, tylosin activity was detected in serum after 0.5 hour. Maximum serum concentrations of 0.6-4.0 µg/ml occurred after 2 hours, and serum concentrations were negligible after 24 hours. Following oral dosing of chickens weighing 2 kg at 1, 2, and 3 hours with 50 mg tylosin, maximum serum concentrations of about 0.3 µg/ml resulted at 4 hours after the last dose. Serum concentrations declined thereafter and were negligible at 24 hours after dosing (WHO, 1991). Similar results were obtained in chickens receiving a single oral dose of 10mg/kg bw of tylosin tartrate. A maximum plasma concentration of 1.2 µg/ml was observed 1.5 hours after tylosin administration and the oral bioavailability of tylosin was 30-34% in this study (Kowalski, et al., 2002). Ziv (1980) reported that chickens drinking water medicated with tylosin tartrate at rates of 500 and 700 mg/l for 48 hours had average serum concentrations of tylosin of 0.12 and 0.17 µg/ml, respectively. In this study, maximum concentrations of 0.2-0.3 µg/ml occurred after 24 hours (Ziv, 1980). In an oral bioequivalence study, two commercial products containing tylosin tartrate were compared on the basis of serum tylosin concentrations in 5- and 7-week old broilers and 9-month old layers. The birds were dosed with drinking water medicated with 750 mg tylosin tartrate/litre for 5 days. The rolling average tylosin concentration in serum approximated 0.20 µg/ml for each of the two commercial products (Ziv and Risenberg, 1991). In contrast to results obtained from pigs (Iritani, et al., 1975), tylosin phosphate was not as well absorbed as tylosin tartrate from the alimentary tract in chickens. No tylosin was detected in blood or muscle of chickens fed a diet containing tylosin phosphate up to 1500 mg/kg for eight weeks (Yoshida, et al., 1973).

In a more recent GLP-compliant study, the pharmacokinetics of tylosin in broiler chickens was investigated. Tylosin A was administered intravenously at 25 mg tylosin activity/kg bw as Tylan® Soluble; orally by gavage as an aqueous solution at 25 mg tylosin activity/kg bw as Tylan® Soluble; or orally by gavage in a feed slurry at 25 mg tylosin activity/kg bw as Tylan® Premix (Lacoste, 2003). The dose rates used in these studies conform to the recommended daily doses for chickens. Pharmacokinetic analysis of plasma concentration-time data for intravenous administration of Tylan® Soluble gave values for AUC and AUMC of approximately 7.1µg·h/ml and 35.8 µg·h^2/ml, respectively. The calculated mean residuence time (MRT) approximates 5.0 hours. The first phase $t_{1/2}$ calculated using α approximates 0.16 hour; the second phase $t_{1/2}$ calculated using β approximates 1.26 hours; and the terminal elimination phase $t_{1/2}$ calculated using γ approximates 35 hours. Pharmacokinetics analysis of plasma concentration-time data obtained after oral administration of tylosin yielded the following values: C_{max} = 0.4 µg/ml, T_{max} = 2 hours (Tylan® Soluble); and C_{max} = 0.2 µg/ml, T_{max} = 2 hours (Tylan® Premix). The absolute oral bioavailability calculated from the AUC_{total} and corrected from mean administered doses was approximately 11% for Tylan® Soluble and approximately 7% for Tylan® Premix (Lacoste, 2003).

A recent study assessed the kinetics of tylosin plasma concentrations in chickens after intravenously administering 10 mg/kg bw; orally administering 74 mg/kg bw per day in drinking water; and orally administering 92.5 mg/kg bw per day as a premix (Abdul-Karim, 2006b). The pharmacokinetic parameters were similar to those reported by Lacoste (2003). The bioavailability of tylosin in drinking water and as a feed premix was 3 and 8%, respectively. With intravenous doses, the initial mean plasma concentration (C_0) was 8.7 µg/ml, the mean Cl_B was 136ml/min/kg, and the mean V_{dss} was 8.6 l/kg (Abdul-Karim, 2006b).

Distribution

As mentioned above, tylosin is widely distributed in body fluids and tissues. Tissue:plasma concentration ratios of tylosin are reported to be 2.05:1 in cows and 2.5:1 in goats (Baggot and Gingerich, 1976; Atef, et al., 1991). In cows, the reported V_d of 1.1-2.27 l/kg (Ziv and Sulman, 1973; Baggot and Gingerich, 1976; Gingerich, et al., 1977; Cester, et al., 1993) is similar to that for sheep and goats. However, higher values of V_d for tylosin of 2.48-5.68 l/kg were reported for young calves (Burrows, et al., 1983; Burrows, et al., 1986).

Cattle

Tylosin base was administered intramuscularly to cows at a dose of 6.8-7.3 mg/kg bw The ratios of tissue:serum concentrations for tylosin measured 7-31 hours after treatment were 35.2:1 in kidney cortex, 13.9:1 in kidney medulla and 5.7:1 in liver. At 24 hours, tylosin concentrations were 35 µg/ml in bile, 13 µg/ml in urine, < 0.4 µg/ml in plasma and < 0.4 mg/kg in muscle (Nouws and Ziv, 1977b; Nouws and Ziv, 1979). When calves less than 3 weeks of age received a single intramuscular injection of tylosin base at a dose of 17.6 mg/kg bw, tylosin concentrations in lung for 24 hours after dosing ranged from 4.5 to 15.7 mg/kg, with a lung AUC_{48h}: plasma AUC_{48h} ratio of 16.6:1 (van Duyn and Folkerts, 1979). Tylosin base was administered at a dose of 10 mg/kg bw by intramuscular injection 12 hourly on three occasions to six-week old calves with pneumonia. The tylosin tissue:serum concentration ratios measured two hours after the last dose were 2.0:1 for pneumonic lung, 1.6:1 for nonpneumonic lung, 2.1:1 for liver and 2.6:1 for kidney. The highest tylosin concentration (about 3.3 mg/kg) was found in kidney, while the lowest concentration (< 0.5 mg/kg) occurred in muscle and cerebrospinal fluid (Burrows, et al., 1986).

Chickens

The distribution of tylosin in chickens has been reported and compared to other species the V_d of 0.69 l/kg in chickens is generally lower. The plasma $t_{1/2el.}$ of tylosin after single intravenous administration in healthy chickens is reportedly 0.5 hour (Kowalski, et al., 2002). When 5-7-week old chickens received 100 or 250 mg tylosin (as tartrate)/kg bw orally, maximum tylosin concentrations in urine of < 100 µg/ml at the 25 mg/kg dose, and > 1400 µg/ml at the 250 mg/kg dose, occurred 2-4 hours after dosing. Urinary concentrations of tylosin declined rapidly thereafter (WHO, 1991).

Metabolism

Rats

The metabolism of tylosin occurs primarily in the liver of rats (and other animal species). The major routes of biotransformation of tylosin are reduction, O-demethylation at the mycinose moiety, N-demethylation at the mycaminose substituent, and a combination of reduction and N-demethylation. Approximately 99% of the metabolic residues in rats was excreted in the faeces, comprising the following metabolites (expressed as a % of total ^{14}C-residues): tylosin D (10%), tylosin A (6%), and tylosin C and dihydrodesmycosin (DDM; 4%); no tylosin B was identified in the metabolic profile for rats (Table 1). Only 1% of the metabolic residues of tylosin are excreted in the urine of rats (Sieck, et al., 1978a).

In a more recent study, Fischer strain 344 rats were dosed orally by gavage with 10 mg ^{14}C-tylosin/kg bw once daily for four days (Kennington and Donoho, 1994). At four hours after the last dose, the rats were euthanized and liver and kidney were taken for assay. Liver had a mean residue of 90 µg of tylosin equivalents/kg. Analysis of an organic extract of the tissue by direct flow ionspray-mass spectrometry (ISP-MS) revealed the presence of multiple metabolites including tylosin A, tylosin D and DDM. Inconclusive evidence for the presence of cysteinyl-tylosin A residues in liver was also presented. In a separate study involving eight rats, about. 95% of the radioactivity was excreted in the faeces. The major radioactive components were tylosin D and DDM; low levels of tylosin C, the seco-acid of tylosin A, the seco-acid of tylosin D and desmethyl-dihydrodesmycosin were also present (Kennington and Donoho, 1994).

Elimination

Cattle

Tylosin excretion has also been studied following the intramuscular administration of ^{14}C-tylosin at a dose of 17.6 mg/kg bw daily for three days to two young Holstein calves weighing approximately 150kg (Kennington, et al., 1994a). Excretion in urine and faeces accounted for 48% of the administered radioactivity up to the time of slaughter (4 hours after the third and final dose). Approximately 20% of the excreted radioactivity was found in urine and 80% in faeces. Tylosin A (30%), tylosin C (25%), tylosin D (11%), and desmethyl-tylosin D (11%) were found in faecal extracts, while cysteinyl-tylosin A accounted for 70% of the total radioactivity in urine (Kennington et al., 1994a).

Tylosin is rapidly eliminated from blood in cattle with Cl_B ranging from 23.7 - 42.2 ml/min/kg in young calves and from 7.4 - 8.7 ml/min/kg in cows; $t_{1/2el.}$ of tylosin after a single intravenous administration in healthy animals ranged from 1.0 to 2.4 hours in young calves, and from 1.6 - 2.8 hours in cows. Slightly longer $t_{1/2el.}$ values of 2.2-3.2 hours were reported for tylosin after intramuscular administration (for a review, see Lewicki, 2006).

Pigs

In pigs receiving ^{14}C-labelled tylosin, 99% of the metabolic residues are excreted in faeces and 1% was excreted in urine (FAO, 1991). The principal components of the excreted residues (expressed as % of total ^{14}C-residues) were tylosin D (33%), dihydrodesmycosin (DDM; 8%) and tylosin A (6%). In addition, at least ten minor metabolites of tylosin representing 5% or less of the total residues were isolated in excreta. No tylosin B was identified in the metabolic profile (FAO, 1991).

In a GLP-compliant study, three pigs were dosed with ^{14}C-tylosin at a rate of 220 mg/kg in feed for 5 days. Approximately 94% of the radioactivity was excreted in faeces and 6% was excreted in urine (Kennington et al., 1994b). Tylosin D and dihydrodesmycosin (DDM) accounted for about 43% and 44% of the total radioactivity in faeces, respectively, from two of the pigs. Faeces from the third animal contained the seco-acid of tylosin D as the major component (approximately 56%) and tylosin D as a minor component (approximately 6%).

TISSUE RESIDUE DEPLETION STUDIES

Radio-labelled Residue Depletion Studies

Cattle

The tissue distribution and excretion of ^{14}C-tylosin has been studied in cattle following intramuscular administration (Kennington, et al., 1994a). Two Holstein calves of approximately 150 kg bw were treated once daily for three days with intramuscular injections of ^{14}C-tylosin at a dose of 17.6 mg/kg bw. Four hours after the last dose, the calves were slaughtered and tissues, bile and excreta were taken

for analysis. The mean total residues of tylosin (expressed as mg of tylosin equivalents/kg [mg equiv/kg]) measured 4 hours after slaughter were 25.2 mg equiv/kg (liver), 47.8 mg equiv/kg (kidney), 2.9 mg equiv/kg (muscle), 1.5 mg equiv/kg (fat), 11.1 mg equiv/kg (lung), 2.5 mg equiv/kg (skin) and 77.2 mg equiv/kg (bile). In liver, tylosin A was the main component of the residue present. Other major metabolites in liver and kidney included tylosin D, tylosin C, and cysteinyl-tylosin A (Table 1). Lung and fat tissues were fractionated for metabolic profiling; tylosin A and cysteinyl-tylosin A were identified as the major metabolites in both tissues. By comparison, tylosin A was the only significant residue present in muscle. When these tissue samples were analyzed by HPLC with UV detection, the mean residues of tylosin A were 2.6 mg/kg (liver), 7.0mg/kg (kidney), 0.7 mg/kg (muscle) and 0.9 mg/kg (fat), which corresponded to 11%, 14%, 25% and 62% of the total residues in the respective tissues. From microbiological assay results, it was calculated that tylosin A represented 37%, 31% and 70% of the microbiologically active residues present in kidney, liver and muscle, respectively. Residues (expressed as % of total radioactivity) present in faecal extracts were tylosin A (30%), tylosin C (25%), tylosin D (11%) and desmethyl-tylosin D (11%). Urine contained cysteinyl-tylosin A as the major metabolite (69% of the total radioactivity).

Pigs

The total radioactive tissue residues that result from feeding tylosin to pigs at a feeding rate of 110 mg/kg bw twice daily were investigated (Table 1; Sieck, et al., 1978a,b). In two more recent studies, three crossbred castrated male pigs of approximately 17 kg bw were fed ^{14}C-tylosin at a dose rate of 220 mg/kg in feed for five days (Kennington, et al., 1994a). Four hours after the last dose, the pigs were slaughtered and tissues and bile were taken for assay. The mean total residues of tylosin, expressed in µg of tylosin equivalents/kg, were 450 (liver), 460 (kidney), 70 (muscle), 50 (fat), 170 (lung) and 70 (skin). Residues of tylosin A were not detected when these samples were analysed by HPLC with UV detection; the method LOQ was 50 µg/kg in all tissues. Tylosin A accounted for 12% and 8% of the total radioactive residue in liver and kidney, respectively. Smaller amounts of tylosin D (10% of the total radioactive residue in liver; 6% in kidney), DDM (5% in liver; 4% in kidney) and cysteinyl tylosin A, which readily converts to tylosin A, were also present (Table 1).

Chickens

The disposition of ^{14}C-tylosin in the edible tissues of laying hens was studied for up to 7 days after three consecutive days of *ad libitum* access to drinking water medicated with 0.53 g ^{14}C-tylosin/l (Marth, et al., 2000). Samples of liver, kidney, muscle, skin with adhering fat, and abdominal fat were collected from each of four animals sacrificed at intervals of 0, 2, 5 and 7 days after withdrawal of the medicated water. Excreta were collected daily from the group of animals sacrificed 5 days after the medicated water had been withdrawn. Total radioactive residues for tissues from two birds, one sacrificed on day zero and the other on day 5 after the medicated water was withdrawn, were 20- to 30-fold higher than for the other birds in the respective groups. The observed difference could not be attributed to a clinical or physiological abnormality. Total radioactive residues in liver for the four birds at zero withdrawal (4 hours) were 14.0, 1.0, 0.5 and 0.5 mg equivalents of ^{14}C-tylosin per kg of tissue. The mean total radioactive residue in liver declined to less than 0.1 mg of tylosin equivalents/kg by 7 days after withdrawal. By comparison, the mean total residue in kidney decreased to below 0.1 mg of tylosin equivalents/kg by 2 days after withdrawal, and in skin with adhering fat and in abdominal fat to below 0.1 mg of tylosin equivalents/kg at all sampling times. Some 66-89% of the radioactivity in liver samples from the two high-residue birds was extractable and selected extracts were characterized by HPLC/ESI-MS-MS. Tylosin A was the principal component of the residue in liver, accounting for approximately 16% of the total residue. In excreta, radioactive residues at zero withdrawal (4 hours) ranged from 360 to 940 mg/kg and by 5 days withdrawal time, radioactive residues had declined to 11 mg/kg. Tylosin D was confirmed as the single most abundant residue at 9% of the total radioactive residue in excreta; tylosin A and the seco-acid of tylosin D were present at lower levels (Table 1). Evidence for possible N-demethylation at the mycaminose substituent was also obtained (Marth, et al., 2000).

In another study, Marth and coworkers (2001) investigated the disposition of tylosin in broiler chickens given 0.53 g ^{14}C-tylosin/l of drinking water for three days. Samples of liver, kidney, muscle, skin with adhering fat, abdominal fat and bile were collected from each of six animals at 0, 2, 5 and 7 days after withdrawal of the medicated water. The mean total radioactive residue in liver declined from 0.7 mg of tylosin equivalents/kg at day 0 to less than 0.1 mg of tylosin equivalents/kg by day 5 after withdrawal. In kidney, the mean total radioactive residue decreased to below 0.1 mg of tylosin equivalents/kg by day 5, and in muscle, skin and abdominal fat residues was < 100 µg of tylosin equivalents/kg at all time points. The liver extract contained multiple radioactive components indicating extensive metabolism; however, tylosin D was the only residue detected by HPLC/MS/MS (ESI) on account of the low residue concentrations and reduced assay sensitivity due to matrix effects. Although traces of nonpolar radioactive material were present indicating the presence of radioactivity in the tylosin A region, the radioactivity and UV signals were < LOQ of 50 µg/kg for the HPLC method. With kidney, a pooled chloroform extract was analyzed by HPLC using flow scintillation analysis; however, the quantity of radioactive residue was too low to characterize. The distribution of radioactive residues in edible tissues was in the following rank order (highest to lowest concentration): liver>kidney>skin with adhering fat> muscle.

Liver is the most appropriate target tissue because it has higher and slower depleting residues than other tissues. The data were not sufficient to define a marker residue but based on the data from other studies, the most practical marker residue is tylosin A (Marth, et al., 2000).

The data indicate that the major biotransformation products in liver are likely to result from reduction. Evidence was also found for possible demethylation and a combination of reduction and N-demethylation of tylosin. In excreta collected during the final 24 hours of dosing, tylosin A and tylosin D were the most abundant residues, accounting for 29% and 12% of the total radioactive residue, respectively. Other identified radioactive residues were each less than 10% of the total radioactive residue. These moieties included 20-dihydrodesmycosin and desmycosin. Radioactive residues in excreta accounted for at least 69% (mean) of the dose by day 7 after withdrawal of the medicated drinking water (Marth, et al., 2001).

Eggs

The distribution, metabolic fate and residue depletion of ^{14}C-tylosin in the edible tissues, eggs, and the excreta of laying hens were studied for up to 7 days withdrawal after three consecutive days of *ad libitum* access to drinking water medicated with 0.53 g ^{14}C-tylosin per litre (Burnett, et al., 1999). Eggs were collected daily from all birds throughout the dosing period and from hens after withdrawal of the medicated drinking water and prior to sacrifice. Total radioactive residues for whole eggs from 2 of the 16 treated birds at zero-day withdrawal were 1.6 to 1.7 mg equivalents tylosin A per kg of egg, while the residue ranged from 0.11 to 0.25 mg equivalents per kg for eggs collected from the remaining 14 birds. This difference was not ascribed to any clinical or physiological observation. Residues in albumen were highest in samples taken on the last day of treatment (mean of 0.4 mg equivalents of tylosin/kg) and on the following day (mean of 0.4 mg equivalents of tylosin/kg). Mean residues in albumen on day 1 and 2 after withdrawal of the medicated water were 0.16 and 0.04 mg equivalents of tylosin/kg, respectively, and were not detected (the LOD of the analytical method was 0.02 mg equivalents of tylosin/kg) in most of the eggs collected at later time points. Maximum residues in yolk occurred in eggs collected 2 and 3 days after withdrawal of the medicated water (mean values of 0.34 and 0.34 mg equivalents of tylosin/kg, respectively) and residues in yolk declined to 0.19 and 0.07 mg equivalents of tylosin/kg at 4 and 5 days, respectively, after withdrawal of the medicated water. Residue concentrations in whole eggs were highest in eggs collected on the last day of treatment (mean of 0.33 mg equivalents of tylosin/kg) and in eggs collected on the following day (mean of 0.36 mg equivalents of tylosin/kg). Thereafter, mean residues in whole eggs depleted to 0.19, 0.13, 0.13 and 0.07 mg equivalents of tylosin/kg at 1, 2, 3 and 4 days after withdrawal of the medicated water, respectively.

In the study above, approximately 78-89% of the radioactivity from albumin and yolk samples of the two high-residue birds was extractable and selected extracts were characterized by HPLC/ESI-MS-MS. A majority of the extracted radioactivity eluted with the polar material near the reversed phase HPLC void volume, indicating a marked change in polarity from the parent compound and the presence of multiple components. Metabolites found at lower concentrations in these samples were N-desmethyl-tylosin A, dihydro-tylosin A (tylosin D), N-desmethy-dihydro-tylosin A, and O-desmethyl-tylosin A (demethylated on the mycinose moiety). The remainder of the radioactivity was predominantly polar materials, eluting early in the chromatograms. In whole eggs, tylosin A was the most abundant of the identified residues and accounted for about 17% of the total radioactive residue. Tylosin was not detected in low-residue eggs (the LOD of the analytical method was about 0.02mg/kg). This study also indicates that the primary biotransformation routes for tylosin are reduction, O-demethylation at the mycinose moiety, N-demethylation at the mycaminose substituent, and a combination of reduction and N-demethylation (Table 1). The metabolites in eggs were present at lower concentrations than the parent compound and included N-desmethyl-tylosin A, tylosin D, N-desmethyl-dihydro-tylosin A, and O-desmethyl-tylosin A (Burnett, et al., 1999).

Table 1: **Summary of studies on tylosin radiolabelled metabolism in animals**.

Animals	Source	Residue/Metabolite	References
Rats	Faeces	Tylosin D*, Tylosin A, Tylosin C, Dihydrodesmycosin (DDM)	Sieck, et al., 1978a
Rats	Faeces Liver	Tylosin D*, DDM*, Tylosin A, Tylosin C, Seco-acid of Tylosin A, Seco-acid of Tylosin D, Desmethyl-DDM Tylosin A, Tylosin D, DDM, Cysteinyl-Tylosin A	Kennington and Donoho, 1994
Pigs	Liver	DDM*, Tylosin A, 3-4 others not identified	Sieck, et al., 1978b; see also FAO (1991)
Pigs	Liver Faeces	DDM, + 3 others Tylosin D*, Tylosin A, DDM, + 10 others (each < 5%)	Sieck, et al., 1978a; see also FAO (1991)
Pigs	Liver Faeces	Tylosin A, DDM, Tylosin A, Tylosin D, DDM, minor metabolites including T-1	Mertz, et al., 1982; see also FAO (1991)
Cattle	Liver/Kidney	Tylosin A*, Tylosin C*, Tylosin D*,	Kennington, et al., 1994a

Animals	Source	Residue/Metabolite	References
	Faeces Urine	Cysteinyl-Tylosin A*, Tylosin A*, Tylosin C*, Tylosin D*, Desmethyl-Tylosin D*, Cysteinyl-Tylosin A*	
Pigs	Liver and Kidney Faeces	Tylosin A, Tylosin D, DDM, Cysteinyl-Tylosin A Seco-acid of Tylosin D*, Tylosin D	Kennington, et al., 1994b
Chickens	Eggs	Tylosin A*, N-Desmethyl-Tylosin A, Tylosin D, N-Desmethyl-dihydro-Tylosin A, Tylosin C	Burnett, et al., 1999
Chickens	Liver Excreta	Tylosin A* Dihydro-Tylosin A Tylosin A, Tylosin D, Seco-acid of Tylosin D	Marth, et al., 2000
Chickens	Liver Excreta	Tylosin D, + others not identified, Tylosin A*, Tylosin D*, 20-Dihydrodesmycosin, Tylosin B (Desmycosin)	Marth, et al., 2001
Turkeys	Liver	tylosin D (50-250 µg/kg)	Montesissa, et al., 1999
* - more than 10% of the total residue			

Residue Depletion Studies with Unlabelled Drug

Several residue studies administered different formulations of tylosin to various animal species; however, most of these studies have been previously reviewed by the Committee and are reported elsewhere (FAO, 1991). Therefore residue studies published up to 1990 have, in general, not been included in the present monograph.

Cattle

Using a crossover study design with a 21 day washout period, Tylan® 200 Injection was administered intramuscularly or subcutaneously to twelve cattle at a dose of 17.6 mg/kg bw for 5 consecutive days (Thomson and Moran, 1994). The animals were slaughtered 21 days after the last treatment and samples of liver and kidney were collected. No residues of tylosin A in liver or kidney were detected by HPLC with UV detection (the LOD of the analytical method was 20 µg/kg).

Luperi and Villa (1999) investigated the tissue depletion of tylosin in dairy cattle. Six groups of 4 cows each received 0.05 ml/kg bw per day (10 mg tylosin/kg bw) of Tylan® 200 Injection by intramuscular administration once daily for four consecutive days. The animals were slaughtered 7, 14, 21, 28, 35 and 42 days after the last dose. Samples of kidney, liver, abdominal fat, muscle, udder and injection site tissue were collected for analyses by HPLC-UV. The LOQ for the method was reported as 50µg tylosin/kg in all tissues but it could not be confirmed based on the information provided. Tylosin residues were quantifiable in all kidney samples and in one udder sample collected 7

days after the last treatment. Residues of tylosin found at the injection sites were quantifiable in all animals sacrificed at 7 and 14 days and in two animals sacrificed 21 days after the last treatment; mean concentrations were 1620 µg/kg (day 7), 205 µg/kg (day 14) and 30 µg/kg (day 21). Tylosin concentrations were < LOQ in all other tissues from 7 days after the last treatment (Table 2).

Table 2: Group means and CV% of Tylosin residues (µg/kg) in tissues from dairy cattle dosed intramuscularly with 10mg tylosin/kg bw daily for four consecutive days.

Group	Withdrawal period	Untreated Muscle	Liver	Kidney	Abdominal fat	Injection site
1	7 days	< LOD	< LOD	73.7 (37.7)	< LOD	1620 (49.4)
2	14 days	< LOD	< LOD	7.8* (75.1)	< LOD	205 (65.8)
3	21 days	< LOD	< LOD	< LOD	< LOD	30.4 (118.2)
4	28 days	< LOD	< LOD	< LOD	< LOD	< LOD
5	35 days	< LOD	< LOD	< LOD	< LOD	< LOD
6	42 days	< LOD	< LOD	< LOD	< LOD	< LOD

Pigs

The depletion of tylosin and sulphadimidine residues was investigated following oral administration of Tylan® Sulpha Premix to pigs (Grassetti and Villa, 2001a). Four groups of pigs (2 males and 2 females per group) received the test item in feed at the nominal rate of 200 mg/kg tylosin and 200mg/kg sulphadimidine for 21 consecutive days. The mean daily dose of each active ingredient received by the four groups of pigs was 9.6, 9.8, 9.4 and 9.9 mg/kg bw per day. Animals were killed after withdrawal periods of 5, 8, 11 and 14 days and samples of kidneys, liver, muscle, and skin with adhering fat were analyzed by HPLC-UV. The LOQ of the analytical method for tylosin was 50 µg/kg for all tissues. Tylosin levels were below the limits of detection (LOD = 2.3 µg/kg for kidney, 6.0 µg/kg for liver, 4.7 µg/kg for muscle, 1.9µg/kg skin with fat, respectively) in all tissues at all time points. However, based on the information supplied the claimed values for LOQ and LOD could not be verified.

Pratts and coworkers (2002b) investigated the depletion of tylosin residues in pigs. Sixteen pigs were assigned to four groups (each n = 4) and administered tylosin base by intramuscular injection at a dose of 10 mg/kg bw once daily for 5 days. The groups of animals were sacrificed at 3, 7, 10 or 14 days after the last treatment. The highest concentration of tylosin residues was found at the injection site at 3 days (110-2500 µg/kg) and 7 days (100-4100 µg/kg) after the last treatment. Residues at the injection site depleted to below the LOQ (50 µg/kg) of the HPLC assay at 10 and 14 days after the last dose. Tylosin residues in other tissues declined at a faster rate compared to injection sites Results are summarized in Table 3.

Table 3: Tylosin residues (µg/kg) in tissues from pigs dosed intramuscularly with 10mg tylosin base/kg bw daily for five days (Prats, et al., 2002b).

Animals	Withdrawal (days)	Residue concentration (µg/kg) measured by an HPLC assay*				
		Inj. site (Muscle)	Muscle	Liver	Kidney	Skin + fat
group 1	3	440	50	70	120	84
	3	110	60	80	< 50	101
	3	1260	< 50	< 50	< 50	66
	3	2540	< 50	80	< 50	78
group 2	7	120	< 50	< 50	< 50	460
	7	310	100	< 50	110	< 50
	7	100	< 50	< 50	70	56
	7	4100	< 50	< 50	< 50	< 50
group 3	10	< 50	< 50	< 50	< 50	< 50
	10	< 50	< 50	< 50	< 50	< 50
	10	< 50	< 50	< 50	< 50	< 50
	10	< 50	< 50	< 50	< 50	< 50
group 4	14	< 50	< 50	< 50	< 50	< 50
	14	< 50	< 50	< 50	< 50	< 50
	14	< 50	< 50	< 50	< 50	< 50
	14	< 50	< 50	< 50	< 50	< 50
* HPLC assay LOQ = 50 µg/kg						

Chickens

Walker, et al. (2007) investigated the depletion of tylosin residues in muscle, liver, kidney, and skin with fat of broiler chickens following oral administration of Tylan® Soluble in drinking water at a rate of 500 mg tylosin/l. Based on water consumption and body weight data for the 5-day treatment period, the mean daily dose of tylosin was estimated at about 105 mg/kg bw per day. Groups of chickens (3 males and 3 females per group) were euthanized at 0, 12, 24 and 48 hours after withdrawal of the medicated water and samples of liver, kidney, muscle, and skin with fat were collected. The samples were analyzed for tylosin A (marker residue) using a validated HPLC-MS-MS method. The method LOQ was 50 µg/kg for all tissues. Residues of tylosin in muscle, liver, kidney and skin/fat were less than 100 µg/kg at 0 hours and approached, or were less than, 5 µg/kg (the LOD of the method) at 12 hours and 24 hours after the medicated water had been withdrawn.

Milk

In an early study, cows received intramuscular injections of tylosin at a dose of 17.6 mg/kg bw daily for five days. Tylosin residues in milk measured by a microbiological plate assay (with a sensitivity limit of 25µg/kg) at 0, 48, 72, 84 and 96 hours after the last injection were 750, 350, 140, 80 and 50µg/kg. Residues were not detected in milk samples collected at 108-144 hours following the last injection of tylosin (FAO, 1991).

A separate study reported the depletion of tylosin activity from milk after the intramuscular injection of tylosin (Matsuoka and Johnson, 1976). Five lactating cows (four cows in early lactation and one cow in late lactation) were injected once daily for 3 days at a dose of 10 mg/kg bw. Tylosin residues were below the assay sensitivity of 50 µg/kg by 48 hours after the last injection.

Another study was performed to determine the level of tylosin A residues in the milk of dairy cows following intramuscular administration of Tylan® 200 (Moran, et al., 1990). Six cows weighing 562 to 820 kg were administered 10 mg tylosin/kg bw intramuscularly for 3 days. Milk was collected twice

daily from 1 day prior to treatment to 5 days after the last treatment and analyzed for tylosin A using a validated HPLC method with ultraviolet detection. The highest concentrations of tylosin A residues were observed in milk during treatment with mean tylosin A concentrations of 1.1 mg/kg, 1.5 mg/kg and 1.4 mg/kg on days 1, 2, and 3, respectively. Concentrations of tylosin A were less than LOQ (50 µg/kg) at the afternoon milking on day 3 post-treatment and less than the LOD (20 µg/kg) at the morning milking on day 4 post-treatment.

More recently, tylosin residues were determined in the milk of 12 cows on two farms (Curtis, 1999). The cows were fed tylosin phosphate at a dose rate of 200 mg/cow/day for 17 days. Milk samples were assayed for tylosin by HPLC with UV detection (LOQ was 50 µg/kg) on days -1, 0 (initial access to medicated feed), 1, 2, 3, 4, 5, 7 and 17. Tylosin residues were not quantifiable in any milk samples.

Five high-yielding and five low-yielding dairy cows were treated intramuscularly for 5 consecutive days with Tylan® 200 at a dose of 10 mg tylosin/kg bw (Keukens, 1996). Morning and evening milk was collected from individual animals, starting at day 0 immediately prior to the first dose and continuing until 12 days after the last dose. Milk samples were analyzed for tylosin using an HPLC method with UV detection. The method LOQ and LOD were 25 µg/kg and 10 µg/kg, respectively. The maximum concentration in milk ranged from 1.3 to 2.6 mg/kg in the evening milk on day four of treatment. Tylosin residues in all samples were less than 50 µg/kg from day 3 after the last dose. There is no conclusive evidence that the marker residue was correctly quantified in this study. Dudriková and Lehotský (1998) measured tylosin residues in cows' milk by HPLC (Sokol, et al., 1996). Cows were treated with tylosin base at 10 mg/kg bw once daily for 5 days. The residues in milk are similar to that reported by Keukens (1996), and declined slowly to 30 µg/kg five days after the last treatment.

Nagy, et al. (2001) investigated tylosin residues in the milk of ewes. Tylosin was administered intramuscularly at a dose of 10 mg/kg bw once daily for 5 days. Milk residues were not detected 2 days after the last dose. Data are summarized in Table 4.

Table 4: Tylosin residues in milk (Dudriková and Lehotský, 1998; Nagy, et al., 2001).

Sampling time of experiments (hours)	Residue concentration (μg/l) measured by an HPLC assay*	
	Cows 10 mg/kg bw once daily for 5 days	Ewes 10 mg/kg bw once daily for 5 days
0◄	-	-
12	2220	630
24◄	1080	130
36	870	1822
48◄	690	470
60	1560	1650
72◄	1790	260
84	3760	1050
96◄	1650	160
108	1190	900
120(1)	1210	140
132	1010	31
144(2)	290	-
156	280	-
168(3)	160	-
180	50	-
192(4)	30	-
204	100	-
216(5)	30	-
228	30	-
240(6)	-	-

*- limit of detection = 10 μg/l; ◄- time of tylosin injection; (1-6)- days after the last injection

Eggs

Tylosin residues in eggs and their distribution between albumen and yolk have been studied by Kan and Petz (2000). Residues in albumen reflect plasma concentrations, and the time needed to achieve a constant concentration was 2-3 days. Drug residues in yolk reflect plasma concentrations during the 10 days of rapid yolk growth. Depending on the length and timing of the exposure relative to yolk growth, residue concentrations in yolk can increase, remain constant or decrease. In general, drug residues in yolk require exposure for about 8-10 days to reach a constant concentration and depletion from yolk generally takes about 10 days (Kan and Petz, 2000). In laying hens that received tylosin at a dose of 500 g/ton of feed for 14 days, tylosin concentrations in whole eggs, measured every 2 days, reached equilibrium of 40-60μg/kg from 4-14 days after the start of treatment. Tylosin transfer rate from the diet of laying hens to eggs (i.e. the ratio of drug intake to the drug content of eggs) was only 0.005% (Furusawa, 2001). Similar transfer rates for whole eggs were reported in laying hens that had received tylosin tartrate in drinking water at a dose of 500 mg/l for 5 days (0.007%), or tylosin phosphate in feed at a dose of 400 g/ton for 7 days (0.009%) (Roudaut and Moretain, 1990).

Differences in the distribution of residue between albumen and yolk were observed in experiments comparing tylosin and other macrolide antibiotics (Roudaut and Moretain, 1990). After exposure of laying hens to tylosin tartrate in drinking water (500 mg/l for 5 days), only one hen excreted detectable residues into albumen and yolk as measured by a microbiological assay. Maximum concentrations were 0.66 mg/kg and 1.7 mg/kg in albumen and yolk, respectively. In hens receiving tylosin tartrate in drinking water (1000 mg/l for 5 days), residues of tylosin above the detection limit of the bioassay (150 μg/kg) were seen in whole eggs for up to 5 days after withdrawal of the medicated water. However, tylosin residues in albumen were detected only during the first day after withdrawal of the

medicated drinking water (Table 5). In a more recent study, where laying hens received tylosin tartrate in drinking water at a dose of 0.05%, no tylosin residues were detected in yolk samples during the 7 days on treatment or the subsequent 3 days after treatment ceased (McReynolds, et al., 2000).

Table 5: Tylosin residues (µg/kg) in eggs from hens administered tylosin tartrate in the drinking water (Roudaut and Moretain, 1990).

Days on feed	Albumen	Yolk	Whole egg
1	< 150 (ND-750)	ND	- (520)
2	190 (ND-3060)	< 200 (ND-510)	160 (0-890)
3	210 (ND-980)	< 200 (ND-1320)	200 ((0-1090)
4	250 (ND-1340)	250 (ND-1800)	250 (0-1480)
5	220 (ND-870)	400 (ND-2480)	260 (0-1350)
Days after withdrawal			
1	230 (ND-830)	650 (ND-2990)	370 (0-1460)
2	ND	470 (ND-2390)	140 (0-710)
3	-	390 (ND-2000)	130 (0-630)
4	-	< 200 (ND-1280)	80 (0-423)
5	-	< 200 (ND-660)	- (190)
6	-	< 200 (ND-230)	-
7	-	ND	-
Limit of detection of microbiological assay = 150 µg/kg (albumen) and 200 µg/kg (yolks); ND - not detected; data in parentheses = analytical range			

The residue depletion profiles of tylosin in eggs were investigated in laying hens after oral administration of Tylan® G250 in the diet (Grassetti and Villa, 2001b). Twenty-four laying hens received tylosin phosphate at an inclusion rate of 800 mg/kg in feed for 5 consecutive days. Tylosin residue levels were determined in eggs produced the day before dosing to 5 days after dosing ceased. The HPLC method with UV detection was not specific for tylosin A; the LOQ was 50 µg/kg and the LOD was 13 µg/kg for tylosin residues. One egg collected on the fifth day of dosing contained a residue of 75 µg tylosin/kg. Tylosin residues in all other eggs were less than the LOQ of the method.

In a separate study, eggs were collected daily from seventeen chickens offered water medicated with Tylan® Soluble to provide 500 mg tylosin activity/l for 3 days (Warren, 1998). Tylosin residues in 12 eggs selected at random each day were determined by HPLC with UV detection; the method was not specific for tylosin A. During the treatment period, only 4 of 36 eggs collected during the treatment period contained residues exceeding the LOQ (50 µg tylosin/kg) of the method. After withdrawal of the medicated water, no residue concentrations exceeded the LOQ and the majority of eggs contained residues below the LOD (10 µg tylosin/kg).

King and Walker (2007) investigated the depletion of tylosin residues in eggs following the administration of drinking water medicated with Tylan Soluble at a concentration of 500 mg tylosin/l for 5 consecutive days to twenty-two laying hens. This study was designed to meet the requirements of the EMEA/CVMP Guideline 036/95: "Note for guidance: Approach towards harmonisation of withdrawal periods" (EMEA, 1996). The mean daily dose of tylosin calculated from water consumption and body weight data during the 5-day treatment period was about 92 mg tylosin/kg bw (dose range was 86.9 to 96.7 mg tylosin/kg bw). Eggs were collected daily from each bird and individually homogenised prior to analysis for marker residue (tylosin A) using a validated HPLC method (Adam, et al., 2007). The mean concentration of tylosin was < LOQ on all days. The highest concentration of tylosin was 117 µg/kg in an egg at day 2 after initiation of treatment; the concentration of tylosin was below LOQ after day 6. Two reports have been published that describe findings consistent with those of King and Walker (Furusawa, 2001; Hamscher, et al., 2006). In these studies, hens were medicated with feed containing 500 mg/kg or 1500 mg/kg of tylosin. Residues in

eggs determined by HPLC and HPLC-ESI-MS-MS were near or above the LOD (60µg/kg) during treatment, declining immediately when the medicated feed was withdrawn (Hamscher, et al., 2006).

Honey

Information in the scientific literature concerning residues of tylosin in honey is scant. Feldlaufer and coworkers (2006) reported residue depletion studies conducted in the USA which determined the incurred residues of tylosin in honey resulting when tylosin tartrate was applied as a dust in confectioner's sugar to honeybee colonies. Each colony comprised approximately 40,000 worker bees. In order to maximize the likelihood of residues being detected, tylosin treatments were applied during the honey flow. One hive was an untreated control; two hives were treated on three occasions with 200 mg or 1000 mg of tylosin (total of 600 mg and 3000 mg of tylosin) over a two-week period. Honey was sampled during and after tylosin administration. Tylosin residues in honey from brood chambers and supers were measured by microbiological assay. Results are shown in Table 6. Honey samples collected from supers following the treatment of colonies with the target therapeutic dose of 600 mg of tylosin/hive contained 160 µg of tylosin equivalents/kg of honey at 3 weeks after the last treatment.

Table 6: Mean concentrations (mg/kg) of tylosin in brood chamber and surplus honey (Feldlaufer, et al., 2006).

Treatment (mg/hive)	0 day (on treatment)	7 days after final treatment	14 days after final treatment	21 days after final treatment
Brood chamber				
0	-	0.12 (0.03, 0.3*)	0.0 (0.0, 0.06*)	0.0 (0.0, 0.03*)
600	-	1. 5 (0.7, 3.5*)	0.5 (0.2, 1.0*)	0.4 (0.2, 0.9*)
3000	-	5.6 (2.2, 17.5*)	4.5 (1.9, 13.4*)	2.0 (0.9, 4.9*)
Super				
0	0.05 (0.0, 0.2*)	0.0 (0.0, 0.06*)	0.0 (0.0, 0.07*)	0.05 (0.0, 0.2*)
600	1.3 (0.6, 3.1*)	0.4 (0.2, 0.9*)	0.3 (0.1, 0.7*)	0.2 (0.05, 0.4*)
3000	8.7 (3.2, 34.3*)	3.6 (1.5, 9.9*)	2.5 (1.1, 6.3*)	1.6 (0.7, 3.9*)
* (lower, upper 95% confidence limits- rounded values)				

Nalda and coworkers (2006) reported a study in honey which investigated residues of tylosin A, B, C and D. This trial was conducted in Spain and samples were collected in spring when the honey flow is adequate for attaining high residues in honey. Fifteen beehives with comparable bee populations and health status were selected and assigned to three groups. One group was fed a placebo; a second group was administered a sugar mixture containing 200 mg/kg of tylosin (identified in the study as 201 to 205); and a third group was administered a sugar mixture containing 400 mg/kg of tylosin (identified in the study as 401 to 405). One month after the sugar mixture had been consumed, honey was collected from the brood chambers and analysed with a validated HPLC-ESI-MS-MS method. Honey from beehives treated with tylosin contained residues of tylosin A, B, C and D with tylosin A accounting for more than 80% of the total residue. Residues of tylosin in honey were not correlated to the applied dose. For example, honey from a hive treated with 200mg/kg had higher concentrations of residues than some beehives treated with 400 mg/kg of tylosin. This could be attributed to the different social behaviour of beehives, food storage, etc. The data demonstrate that residues of tylosin B, which accounts for approximately 6-12% of the total residue, and tylosin C and tylosin D collectively account for approximately 15% of tylosin residues in honey. Nalda and coworkers (2006) suggested that further field experiments are necessary to optimise the dosage such that residues of tylosin in honey may be reduced.

Table 7: Residues (µg/kg) of tylosin A, B, C and D in honey samples from treated beehives (Nalda, et al., 2006).

Sample	Tylosin A (TA)	Tylosin B (TB)	TA:TB ratio	Tylosin C (TC)	Tylosin D (TD)	Total (%TA)
Placebo	< LOD	< LOD	-	< LOD	< LOD	< LOD
200 mg of tylosin per kg of sugar mixture *						
201	1230	90	13.7	< LOD	110	1430 (86)
202	1030	100	10.3	< LOD	110	1240 (83)
203	600	70	8.6	< LOD	20	690 (87)
204	870280	16	54.4	< LOD	30	1060 (82)
205		410	10.4	70	180	4940 (87)
400 mg of tylosin per kg of sugar mixture *						
401	1550	230	6.7	10	80	1870 (83)
402	3740	310	12.1	20	140	4210 (89)
403	500	70	7.1	< LOD	10	580 (86)
404	2110	330	6.4	20	90	2550 (83)
405	5730	700	8.2	80	210	6720 (85)
Limit of detection (LOD) for the HPLC-ESI-MS method: 2 (TA), 3 (TB), 2 (TC) and 2 (TD) µg/kg; * - no information was provided regarding the length of time that had elapsed between the application of the tylosin formulation and the sampling time						

In a Canadian experiment, Thompson and coworkers (2007) used a slightly modified version of a previously reported HPLC-ESI-MS-MS method (Thompson, et al., 2005) for determining tylosin A and B in honey. The hives in the study were healthy, single brood chamber colonies containing approximately 30,000 adult honeybees. Twenty colonies with similar populations of brood and adult bees were identified and randomly assigned to five treatment groups. Treatments contained varying amounts of tylosin tartrate as Tylan® Soluble 100GM in two different formulations. One formulation consisted of either 0 mg or 300 mg of tylosin tartrate mixed with 20 g of confectioner's sugar. The second formulation consisted of the antibiotic incorporated into a 100g pollen patty. Each patty consisted of 40% milled pollen, 20% soy flour and 40% (v/v) sucrose syrup, mixed into a moist kneadable texture, to which 300, 900 or 1500 mg tylosin tartrate was added. During September 2004, treatments were applied to the top bars of the brood chambers on three occasions at weekly intervals. Colonies in the sugar dusting treatments received, in total, either 0 mg or 900 mg of tylosin tartrate whereas colonies treated with pollen patties received, in total, 900, 2700 or 4500 mg of tylosin tartrate. These treatment rates exceeded the hypothesized total target dose of 600 mg tylosin tartrate per colony and were chosen to examine the concentration of residue carried over to the following year. For residue determination, 15 g samples of newly deposited honey were collected from colonies in July 2005, approximately 1 week after the start of the summer honey flow. Samples were stored at -20^{o}C prior to analysis (Thompson, et al., 2007).

Table 8: Residues (µg/kg) of tylosin A and tylosin B in incurred honey after 294 days of withdrawal [a] (Thompson, et al., 2007).

Replicate	Source [b]	Tylosin A (TA)	Tylosin B (TB)	TA:TB ratio
Sugar dust: 900 mg of tylosin per colony				
1	Brood chamber	114	97	1.2
2		62	44	1.4
3		11	10	1.1
4		ND [c]	ND	-
1	Super	179	150	1.2
2		46	31	1.2
3		32	32	1.0
4		ND	ND	-
Pollen patty [d]: 2700 mg of tylosin per colony				
1	Brood chamber	19	22	0.9
2		80	60	1.3
3		16	13	1.2
4		28	24	1.2
1	Super	29	33	0.9
2		64	48	1.3
3		ND	ND	-
4		ND	ND	-
Pollen patty [d]: 4500 mg of tylosin per colony				
1	Brood chamber	77	60	1.3
2		23	14	1.6
3		16	13	1.2
4		16	17	0.9
1	Super	ND	ND	-
2		ND	ND	-
3		23	19	1.2
4		6	7	0.9

[a] – colonies were treated on three successive occasions, 7 days apart, during September 2004 and were sampled in July 2005; withdrawal period is calculated from date of last application to sample collection;
[b] – honey samples from brood chambers and supers were from the same colonies within treatments and replicates; supers contain honey normally extracted for human consumption;
[c] – ND, non-detectable (limit of detection of HPLC-ESI-MS-MS method: 0.4 µg/kg (tylosin A) and 1.1 µg/kg (tylosin B); practical limit of quantitation: 5 µg/kg (tylosin A); 5 µg/kg (tylosin B);
[d] – values for colonies treated with a total of 900 mg of tylosin tartrate formulated in pollen patties are not listed because no residues were detected in honey taken from brood chambers or supers

Tylosin A degrades to tylosin B in an acidic medium such as honey and studies into the stability of tylosin residues in honey during storage have been performed using HPLC. Kochansky (2004) reported the conversion of tylosin to tylosin B with a half-life of approximately 4 months during storage at 34°C. The stability of tylosin A in honey matrices has also been investigated by spiking a series of replicate honey samples with tylosin A and storing them in the dark at -20°C and 20°C. Samples were analyzed at 2-weekly intervals for a period of 16 weeks; no appreciable degradation of tylosin A was observed when stored at -20°C. Over the same period of time, approximately 20% of tylosin A degraded to tylosin B when stored at ambient temperature (Thompson, et al., 2007).

Honey samples drawn from bee colonies treated with a commercial formulation of tylosin were analyzed for the presence of both tylosin A and tylosin B. Though the formulation of tylosin in sugar dustings greatly increased the propensity and concentration of tylosin A and tylosin B within incurred honey samples, a relatively consistent ratio (from 0.9 to 1.6) of tylosin A to tylosin B was observed

across all samples irrespective of treatment and source of honey. Accordingly, for samples with detectable residues, the ratio of tylosin A to tylosin B was similar, with an overall average 1.2:1 (Table 8). This suggests that after a prolonged withdrawal period (294 days in this study), the contributions of tylosin A and its primary breakdown product, tylosin B, are of comparable importance in terms of antimicrobial load (Thompson, et al., 2007).

METHODS OF ANALYSIS

A validated analytical method for the quantitation of the marker residue in target animal tissues is necessary for enforcement of MRLs and is required as part of the information for the evaluation of veterinary drug residues (FAO, 2000). Many different analytical methods (screening or confirmatory) have been described for tylosin and other macrolide antibiotics in the open literature between 1985 and 2005. These methods are reportedly suitable for quantifying tylosin and/or its degradants and metabolites in aqueous solutions and fermentation media, animal feeds, environmental samples and excreta. A number of methods for the detection of tylosin or other macrolides in biological fluids and animal tissues have also been published. Microbiological assays that lack specificity and are not suitable for identifying the exact nature of an antibiotic residue, are commonly used for screening samples for tylosin residues. More specific methods, such as liquid chromatography coupled with ultraviolet (LC-UV) detection, have been proposed for the determination of tylosin residues in animal tissues. Gas chromatography coupled to mass spectrometry (GC-MS) has been described as a confirmatory method for tylosin residue analysis. Several other methods based on a combination of liquid chromatography with mass spectrometry (HPLC-MS) and tandem mass spectrometry (HPLC/MS/MS) have been reported for quantitation and confirmation of tylosin residues in animal tissues.

Apart from clearly described liquid scintillation counting methods used in ^{14}C-tylosin residue studies (Kennigton and Donoho, 1994; Kennington et al., 1994a; Kennington et al., 1994b; Burnett et al., 1999; Marth et al., 2000; Marth et al., 2001), several HPLC or HPLC/MS/MS methods for tylosin A (or other tylosin factors/metabolites) residue analysis were provided for evaluation by the Committee. Only those which are validated are discussed below.

An analytical method was provided for determining tylosin A in chicken whole eggs (Adam, et al., 2007). The analytical method includes homogenization with methanol/acetonitrile/0.1 M ascorbic acid followed by centrifugation. Tylosin is then isolated from the supernatant using C18 Solid Phase Extraction (SPE). The purified sample is evaporated to dryness and reconstituted for further HPLC separation on a phenyl stationary phase, and UV detection at 280 nm. Quantification of tylosin is as factor A. Analytical recoveries ranged from 74–87% with coefficients of variation of 3.6–9.6%. Intra-day and inter-day accuracy at MRL level was 78–80% and precision (intra-day and inter-day) in the 5–9% range. The method LOQ for tylosin A was claimed to be 50µg/kg for whole eggs; however, critical analysis of the information provided suggested the LOQ is likely to be above 100 µg/kg. Similarly, the LOD for tylosin A was claimed to be 4 µg/kg for whole eggs but on the basis of the information provided, the LOD is likely to approximate 50 µg/kg. The method is not acceptable for measuring tylosin residues at or below a concentration of 100 µg/kg but is acceptable for measuring higher concentrations.

A validated HPLC/MS/MS method with electrospray ionization is available for determining residues of tylosin A in the edible tissues of chickens and in eggs (Roberts, 2007). The analytical method involves extraction from tissue and eggs by homogenizing with acidified acetonitrile followed by centrifugation. The supernatant is diluted with acetonitrile/water and then analysed by HPLC with detection by tandem mass spectrometry (HPLC/MS/MS) operating in the selected reaction monitoring (SRM) mode. Acceptable specificity, sensitivity, linearity, precision, recovery and accuracy were demonstrated for the method. Analytical recoveries ranged from 85 to 103% with coefficients of variation of 5–10%. The method LOQ was 50 µg/kg for liver, kidney, muscle and skin with fat and 100 µg/kg for eggs. The LOD of the analytical method was 5 µg/kg for all tissues and eggs. The ion chromatograms and other information provided confirmed the claimed performance characteristics.

Intra-day and inter-day accuracy at a potential MRL level was in the 85–102% range, and intra-day and inter-day precision was in the 5–10% range. The assay specificity was acceptable as attested by the quality of the signals on the ion chromatograms provided. Assay linearity was shown to be acceptable in the fortified matrix range of 10 to 500 µg/kg after extraction and dilution. Although there were no significant matrix effects in any matrix, the inclusion of an internal standard would correct for any matrix effect during the electrospray ionization process. This method could be extended to other matrices and is a suitable analytical method for regulatory use with residues in the edible tissues of cattle, pigs, chickens, milk and eggs.

A microbiological assay was provided for analysis of edible tissues of cattle and pigs; however, the method was not appropriately validated. In honey, a validated method is required for the analysis of residues of tylosin A plus tylosin B (see section Appraisal below); however, a suitable method was not available for review.

New methods for tylosin analysis have appeared recently in the open literature. These include methods for detecting tylosin and/or its metabolites and degradation products in aqueous solutions (Song, et al., 2007; Hu, et al., 2008) and animal feeds (Peng and Bang-Ce, 2006; González de la Huebra, et al., 2007; Vincent, et al., 2007). New methods for the detection of tylosin and/or other macrolides in biological fluids and animal tissues have also been published (García-Mayor, et al., 2006; Hamscher, et al., 2006; Tang, et al., 2006; Wang, et al., 2006; Litterio, et al., 2007) (Table 9).

Table 9: Overview of the newest HPLC or HPLC/MS/MS methods for residues of tylosin in foods of animal origin.

Method of detection	Matrix	Compounds detected	LOD [1,2] (µg/kg)	Reported Validation Status [3]	Reference
HPLC-UV, PDA	Sheep: Milk	Tylosin Erythromycin Oleandomycin Roxithromycin Josamycin Spiramycin Ivermectin	24.1 [4]	Yes	García-Mayor, et al., 2006
HPLC-ESI-MS-MS	Bovine: Milk	Tylosin Spiramycin Tilmicosin Oleandomycin Erythromycin	0.06	Yes	Wang, et al., 2006
HPLC-ESI-MS-MS	Laying hens: Eggs	Tylosin	1 [4]	Yes	Hamscher, et al., 2006
HPLC-ESI-MS-MS screening method	Animal muscle	Tylosin A + 4 macrolides + 6 fluoroquinolones + 3 other	0.1	Yes (only for screening)	Tang,et al.,2006

ESI - electrospray ionisation;
[1] - limit of detection (LOD);
[2] - for multiresidue methods only a value for tylosin was specified;
[3] - declared by authors for the time of publication;
[4] - limit of quantitation (LOQ)

Several analytical methods (including optical SPR biosensor screening assay) concerning tylosin residues in honey were presented (Thompson, et al., 2003; Benetti, et al., 2004; Wang, 2004; Caldow, et al., 2005; Thompson, et al., 2005; Nalda, et al., 2006; Thompson, et al., 2007; Hammel, et al., 2008) (Table 10).

Table 10: Overview of the HPLC/MS or HPLC/MS/MS methods for residues of tylosin in honey.

Method of detection	Compounds detected	LOD [a,b] (μg/kg)	Reported Validation Status [c]	Reference
HPLC-API-MS	Tylosin Lincomycin	10	Yes	Thompson, et al., 2003
HPLC-ESI-MS-MS	Tylosin	Not specified Ccα = 2.6 μg/kg [d] Ccβ = 4.4 μg/kg [d]	Yes	Benetti, et al.,2004
HPLC-ESI-MS-MS	Tylosin Spiramycin Tilmicosin Oleandomycin Erythromycin	0.01 LOC = 0.4 μg/kg [e]	Yes	Wang, 2004
HPLC-ESI-MS-MS	Tylosin Lincomycin	2	Yes	Thompson, et al., 2005
HPLC-PDA-ESI-MS	Tylosin A Tylosin B Tylosin C Tylosin D	2 3 2 2	Yes	Nalda, et al.,2006
HPLC-ESI-MS-MS	Tylosin A Tylosin B	0.4 1.1	Yes	Thompson et al., 2007
HPLC-ESI-MS-MS screening method	Tylosin + 24 antibiotics + 17 sulphonamides	48	Yes (only for screening)	Hammel, et al.,2008

API - atmospheric pressure ionisation; ESI - electrospray ionisation;
[a] - limit of detection (LOD);
[b] - for multiresidue methods only a value for tylosin was specified;
[c] - declared by authors for the time of publication;
[d] – in the 2002/657/EC European decision Ccα and Ccβ replace the LOD and LOQ;
[e] - limit of confirmation (LOC)

APPRAISAL

Tylosin is an old drug with a long history of use. It was first evaluated at the twelfth meeting of the Committee in 1968 when it was concluded that tylosin used in animal feed or in veterinary medicine should not give rise to detectable residues in edible products of animal origin. No ADI was established. The drug was subsequently evaluated at the thirty-eighth meeting of the Committee. At that meeting, the Committee was not able to establish an ADI due to deficiencies in the toxicological and microbiological data submitted. Information addressing the deficiencies identified by the thirty-eighth meeting of the Committee was requested for evaluation by the sixty-sixth meeting of the Committee but none was provided. New data addressing the deficiencies were made available for the present meeting of the Committee to evaluate.

Tylosin is a macrolide antibiotic produced by fermentation from a strain of the soil microorganism, *Streptomyces fradiae*. It is a mixture of four compounds. The main product is tylosin A (> 80%) and

the minor components are tylosin B, C and D, which may be present in varying amounts. It is active against Gram-positive bacteria, Mycoplasma and certain Gram-negative bacteria. Tylosin and its phosphate and tartrate salts are registered exclusively for veterinary use in several countries. Tylosin is used primarily in the chronic respiratory disease (CRD) complex in chickens and infectious sinusitis in turkeys caused by *Mycoplasma gallisepticum*. It is also used to treat swine and bovine respiratory diseases and swine dysentery, and other infections caused by organisms sensitive to tylosin.

Tylosin is a highly lipid soluble, weak organic base (pKa = 7.73) that readily forms salts and esters. It is slightly to moderately bound to plasma proteins (30-47%). Tylosin is widely distributed in body fluids and tissues with a V_d which ranges from 1–14.6 l/kg in different animal species. From a residue perspective, the distribution of tylosin is highly dependent on the route of administration. When administered by injection, tylosin residues are generally highest and most persistent in kidney with the exception of injection site residues. By contrast, residue concentrations following oral administration are generally higher in liver than in other tissues. The concentration of tylosin residues observed after oral administration is generally lower than after injectable administration.

The biotransformation of tylosin has been studied in rats, chickens, pigs and cattle and the comparative metabolism was shown to be qualitatively similar for these species. Tylosin is principally metabolized in the liver resulting in four major metabolites and several minor metabolites in most species. The primary biotransformation routes for tylosin are reduction, O-demethylation at the mycinose moiety, N-demethylation at the mycaminose substituent and a combination of reduction and N-demethylation. Tylosin A is the most abundant residue in rats, chickens, pigs and cattle while a major metabolic pathway is the reduction of tylosin A to tylosin D.

A radiometric study was available in calves treated intramuscularly daily with ^{14}C-tylosin/kg. Tylosin A accounted for approximately 11% (liver), 15% (kidney), 25% (muscle) and 62% (fat) of the total residues and represented 31% (liver), 37% (kidney) and 70% (muscle) of the microbiologically active residues present. Residues of tylosin A in liver and kidney were less than 20 µg/kg in cattle receiving the same dose of unlabelled tylosin/kg daily for five consecutive days intramuscularly or subcutaneously at 21 days after the last dose.

A radiometric study was conducted in pigs using ^{14}C-tylosin in feed at an inclusion rate of 220 mg/kg. Tylosin A accounted for 12.3% of the total residues in liver and 7.6% in kidney. The mean total residues of tylosin (µg of tylosin equivalents/kg) were 450, 460, 70, 50 and 70 in liver, kidney, muscle, fat and skin, respectively. Tylosin A was not detected in any sample (LOQ was 50 µg/kg).

Residue depletion studies in cattle and pigs indicated that tissue residues of tylosin were generally low to non-detectable following the oral route of administration and depleted rapidly with predictable kinetics following intramuscular injection. High concentrations of tylosin residues were found at the injection sites in both dairy cattle and pigs. Residues at the injection site depleted to below the LOQ (50 µg/kg) by 10 days after the last dose.

Radiometric studies were conducted in laying hens and broiler chickens. In laying hens, total radioactive residues in liver at zero day withdrawal were 13.7, 1.0, 0.5 and 0.5 mg equivalents of ^{14}C-tylosin/kg. The mean total radioactive residue in liver declined to less than 0.1 mg of tylosin equivalents/kg by 7 days after withdrawal and in kidney decreased to below 0.1 mg of tylosin equivalents/kg by 2 days after withdrawal. Residues in skin with adhering fat and in abdominal fat were below 0.1 mg of tylosin equivalents/kg at zero day withdrawal. In broilers, the mean total radioactive residue in liver declined from 0.7 mg of tylosin equivalents/kg at zero day withdrawal to less than 0.1 mg of tylosin equivalents/kg by 5 days withdrawal; in kidney, to less than 100 µg of tylosin equivalents/kg by 5 days withdrawal; in muscle, skin with adhering fat and abdominal fat to less than 100 µg of tylosin equivalents/kg at zero day withdrawal. In a residue depletion study broiler chickens receiving tylosin in drinking water for five days, the residues of tylosin A in liver, kidney, muscle and skin with adhering fat were less than 100 µg/kg at 0 hours and at or below 5 µg/kg at 12 hours after the medicated water had been withdrawn.

Residue depletion studies were performed in cows' milk. When tylosin phosphate was included in the feed at a rate of 200 mg/cow/day, tylosin residues were not quantifiable (LOQ 50 µg/kg) in any milk samples collected during treatment. In a second study cows were treated intramuscularly with 10mg tylosin/kg bw for 5 days. The maximum concentration in milk was 1.3 to 2.6 mg/kg on the fourth day of treatment. Concentration of tylosin residues in all samples was less than 50 µg/kg from day 3 after the last dose. In a third study, dairy cows were administered the same intramuscular dose for 3 days. The highest concentrations of tylosin A residues were observed in milk during treatment with mean tylosin A concentrations of 1.1 -1.5 mg/kg on days 1, 2 and 3. Concentrations of tylosin A were less than the LOQ (50 µg/kg) at the afternoon milking on day 3 post-treatment and less than the LOD (20 µg/kg) at day 4 post-treatment.

One radiometric study and three depletion studies with unlabelled tylosin were performed in eggs. In the radiolabelled study, variable results were obtained. The residues in whole eggs were 108 – 245 µg tylosin equivalents/kg in 14 of 16 birds but negligible residues in the other 2. Residue concentrations in whole eggs were highest in eggs collected on the last day of treatment. Mean residues in whole eggs depleted over four days after withdrawal of the medicated water. Tylosin A was the most abundant of the residues in whole eggs and at the highest concentration of tylosin equivalents, and accounted for approximately 17% of the total radioactive residues. Tylosin A was not detected in eggs produced by the other 14 birds (LOD 20 µg/kg). In a residue depletion study with unlabelled drug at an inclusion rate of 800mg/kg in feed for 5 consecutive days only one egg collected on the fifth day of dosing contained a measurable residue of 75 µg tylosin/kg. The concentration of residues in all other eggs was less than the method LOQ (50 µg/kg). In residue depletion studies with unlabelled drug in the drinking water of laying hens with 500 mg tylosin activity per litre for 3 or 5 days, only 4 of 36 eggs collected during the treatment period contained residues above the LOQ (50 µg tylosin/kg). After withdrawal of the medicated water, no residue concentrations exceeded the LOQ with the majority of eggs having residues below the LOD (10 µg/kg). In the last study, the mean concentration of tylosin A in whole eggs was less than the LOQ of the method (50 µg/kg).

Tylosin A was identified as the marker residue for tylosin in the tissues of chickens, pigs and cattle as well as in milk and eggs. Tylosin A represents the most significant residue and corresponds to the major microbiologically active residue of concern. A validated HPLC/MS/MS method with electrospray ionization is available for determining residues of tylosin A in the edible tissues of chickens and eggs, and could be extended to other matrices. This method is suitable for regulatory use to detect and quantify residues of tylosin A.

As distinct from mammalian and avian tissues, tylosin B is a major end product in honey resulting from the conversion of tylosin A to tylosin B in acidic media such as honey. The conversion accounts for the ratio of tylosin A concentration/tylosin B concentration varying as a function of time. Tylosin B contributes significantly to the antimicrobial activity of tylosin residues in honey, requiring that both tylosin A and tylosin B are taken into account when considering dietary intake of residues. This implies that tylosin A is not a suitable marker for residues of tylosin in honey, unlike the situation with chickens, pigs, cattle, milk and eggs. In the absence of a suitably validated method for quantifying the microbiological activity of residues of tylosin A and tylosin B in honey, it is not appropriate to recommend a MRL for tylosin in honey.

MAXIMUM RESIDUE LIMITS

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

- An ADI of 0-30 µg/kg bw based on a microbiological endpoint was established by the seventieth meeting of the Committee, equivalent to 0-1800 µg for a 60-kg person.
- The marker residue is tylosin A and represents approximately 100% of the microbiologically active residues, except in honey. This information is incorporated in the calculation of the intake estimates to ensure that they correctly reflect residues of microbiological concern.

- Liver and muscle are suitable target tissues.
- A validated analytical method is available for analysis of tylosin A residues in edible tissues of chickens and in eggs, and could be extended to the edible tissues of cattle and pigs and to milk.
- The MRLs for all edible tissues of cattle, pigs and chickens were based on the data provided.
- The MRL for eggs was based on the highest value of tylosin A concentration observed.
- The MRL for milk was based on twice the LOQ.

On the basis of the above considerations, the Committee recommended the following MRLs for edible tissues of cattle, pigs and chickens, expressed as the marker residue, tylosin A: muscle, 100 µg/kg; liver, 100 µg/kg; kidney, 100 µg/kg; fat, 100 µg/kg (cattle and pigs); and skin/fat, 100 µg/kg (chickens). The Committee also recommended a MRL for milk of 100 µg/kg and a MRL for eggs of 300 µg/kg, both expressed as the marker residue, tylosin A.

ESTIMATION OF DAILY INTAKE

The sixty-sixth meeting of the Committee agreed to apply a new approach to estimate chronic exposure to residues of veterinary drugs in food. However, the Estimated Daily Intake (EDI) for tylosin A was not estimated because there were insufficient quantitative data points to calculate the median values for residues in food animal tissues. Using the model diet and the microbiological activity of tylosin A as 100% of the microbiological activity of the residue, the recommended MRLs would result in an intake of 230 µg, which represents 13% of the upper bound of the ADI (1800 µg for a 60-kg person) (Table 11).

Table 11: Estimation of daily intake of tylosin A residues.

Tissue	MRL (µg/kg)	Standard food basket (kg)	Microbiological Activity[2]	Daily intake (µg)
Muscle	100	0.3	100%	30
Liver	100	0.1	100%	10
Kidney	100	0.05	100%	5
Fat[1]	100	0.05	100%	5
Milk	100	1.5	100%	150
Eggs	300	0.1	100%	30
1. In chickens: skin/fat. 2. Microbiological activity of Tylosin A				
			Total daily intake	230
			Upper limit of the microbiological ADI	1800
			% of ADI	12.8

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